UNIVERSITAT DE BARCELONA

Noves estratègies per a l'anàlisi directa de compostos orgànics per espectrometria de masses

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NOVES ESTRATÈGIES PER A L’ANÀLISI DIRECTA DE COMPOSTOS ORGÀNICS PER ESPECTROMETRIA DE MASSES

Memòria presentada per tal d’optar al títol de Doctora per la

Universitat de Barcelona per na

Raquel Seró Llor

Directora de la tesi:

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Barcelona, setembre de 2019

La **Dra. Encarnación Moyano Morcillo**, catedràtica de Química Analítica del Departament d'Enginyeria Química i Química Analítica de la Universitat de Barcelona,

FA CONSTAR:

que la present memòria titulada "*Noves estratègies per a l'anàlisi directa de compostos orgànics per espectrometria de masses*" ha estat realitzada sota la meva direcció per la Sra. **Raquel Seró Llor** en el Departament d'Enginyeria Química i Química Analítica de la Universitat de Barcelona, i que tots els resultats presents són fruit de les experiències realitzades per l'esmentada doctoranda.

I perquè així consti, expedeixo i signo el present certificat.

Barcelona, setembre de 2019

Dra. Encarnación Moyano Morcillo

Tots mirem des del mateix lloc, tanmateix
veiem coses diferents, i així ho expliquem,
convençuts que és la realitat. Car hem de tenir
en compte que el paisatge no canvia

Anònim

AGRAIMENTS

En primer lloc agrair a la Dra. Encarnación Moyano, l'interès mostrat en aquesta Tesi, el seguiment i supervisió continus dins i fora del laboratori i, sobretot, la motivació i recolzament, i la paciència!, amb la que em trobo en deute. També els ànims i la confiança. Reconec l'empenta, la insistència i l'entusiasme que transmets en el camp de la investigació, i també molt més enllà, la passió ben dirigida que, al meu veure, m'ha resultat imprescindible per arribar a saber tot el que ara sé.

El meu agraïment a la Dra. Maria Teresa Galceran, pels consells i mestratge, per la inspiració i l'orientació i per l'esperit treballador, que m'ha estimulat des del primer dia. Pel seu recolzament i ajut desinteressats. Amb aquest treball vull participar en el reconeixement que et demostra la comunitat científica amb llur admiració i confiança.

Una consideració als Drs. F. Javier Santos i Oscar Núñez per l'acolliment i ajut que m'heu brindat. Sempre recordaré els bons moments viscuts amb vosaltres al laboratori i que, malauradament, m'escau de qualificar de curts. Gràcies per l'actitud despresa, la col·laboració amb idees generals i de síntesi, els consells i la generositat.

La meva gratitud als companys de laboratori del grup CECEM, Juanfra, Ane, Guillem, Noemí, Sergio, Nerea i del Departament de Química Analítica, Alejandro, Clara, Gema, Laura, Montse, Sara, Roger, per regalar-me la vostra amistat i cooperació, perquè la vostra manera de treballar em deixa un testimoni humà de convivència que va més enllà de la simple relació professional. Un record fraternal als companys que ara ja no hi són però que sempre tindré presents.

Gràcies a la Universitat de Barcelona per concedir-me la beca que m'ha permès realitzar aquesta tesi.

Agraeixo a les persones que s'han dedicat a llegir aquesta tesi i el que decideixin esmerçar en reflexionar-hi. Sempre estaré oberta a les seves valoracions i suggeriments.

Cal esmentar com d'essencial han estat la comprensió, la paciència i els ànims rebuts dels amics. Gràcies per seguir sent els meus amics després de tants anys.

I, sobretot, als meus pares, pel seu exemple en el treball i en la vida, pels valors que m'han transmès i pel recolzament i la confiança, sempre incondicional, que m'han prestat.

Una menció especial al padrí *Antonio*, que ara ja no està al meu costat però que sempre m'acompanya. Un home savi i comprensiu. Les seves paraules, que traspuaven un amor que es feia sentir, han esdevingut, per a mi, una lliçó de vida que sempre servaré en el meu cor.

A tots ells, i als que hagi pogut obviar involuntàriament, moltes gràcies.

ÍNDEX

RESUM

Avui dia, l'espectrometria de masses és una eina indispensable en molts laboratoris analítics que treballen en els camps d'aplicació d'alimentació, medi ambient o d'anàlisi forense, així com en el camp de l'anàlisi clínica per al diagnòstic de malalties. Les excel·lents prestacions d'aquesta tècnica en termes de sensibilitat i de selectivitat així com la gran quantitat d'informació estructural que se n'obté, faciliten la identificació inequívoca d'una gran varietat de compostos orgànics en tot tipus de matrius. En aquest context, l'avenç i el desenvolupament de mètodes analítics ràpids, senzills i versàtils que permetin donar una resposta gairebé immediata al problema analític que es planteja són de gran interès per millorar la productivitat d'aquests laboratoris i per facilitar la presa de decisions diagnòstiques. És per aquest motiu que aquesta tesi està centrada en un nou grup de tècniques d'ionització a pressió atmosfèrica anomenat *Ambient Ionization Mass Spectrometry* (*Ambient MS*), les quals possibiliten l'anàlisi directa de tot tipus de mostres sense o amb una mínima manipulació i permeten obtenir informació molecular rellevant, gairebé en temps real. La revisió bibliogràfica realitzada en aquesta tesi, basada en els principis, les característiques i les aplicacions de les tècniques *Ambient MS* més consolidades, ha posat de manifest el seu potencial i les grans perspectives de futur, tot i que la seva aplicació en els laboratoris analítics és, encara avui, minsa.

En aquesta tesi s'ha estudiat l'aplicabilitat de la tècnica d'ionització per desorció per electroesprai (DESI), pionera del grup de tècniques *Ambient MS*, combinada amb l'espectrometria de masses d'alta resolució, per a l'anàlisi de compostos sospitosos i desconeguts en matrius d'elevada complexitat. D'una banda, s'ha establert un mètode d'escombratge per a la identificació de drogues veterinàries en pinsos com a conseqüència d'una contaminació creuada i, de l'altra, s'ha caracteritzat un producte fitosanitari sospitós d'haver estat adulterat i del qual se'n desconeixia la seva naturalesa química. S'han avaluat diferents estratègies de manipulació ràpides (< 5 min) de les mostres mitjançant les quals s'han resolt els problemes de contaminació del sistema instrumental del DESI provocats per la textura pulverulenta de les matrius de pinso i s'ha disminuït el temps d'assecatge de la mostra líquida fitosanitària analitzant un paper de filtre impregnat amb la mostra. També s'han estudiat els paràmetres de la font DESI que afecten d'una forma més crítica a l'eficàcia d'extracció i d'ionització dels compostos de la superfície de les matrius estudiades. Així, s'ha avaluat la sensibilitat del mètode en funció de la composició i cabal del dissolvent, de la pressió del gas de nebulització, de la configuració dels paràmetres geomètrics (angle i distància de l'esprai envers la superfície) i del mode d'anàlisi de la superfície (estàtic o dinàmic). En aquesta mateixa línia, s'han optimitzat la resolució i el temps d'acumulació

dels ions a la C-Trap en l'analitzador híbrid Q-Orbitrap per assolir una molt bona exactitud en la mesura de la massa, així com una sensibilitat i una selectivitat adequades tant per a la identificació de les drogues veterinàries a uns nivells inferiors als establerts a la legislació, com per a la caracterització dels compostos detectats en el producte fitosanitari. Amb el propòsit d'interrogar de forma ràpida i senzilla la gran quantitat d'informació espectral obtinguda en l'anàlisi de les mostres emprant els mètodes DESI-HRMS desenvolupats, s'han avaluat diferents estratègies de tractament de les dades per facilitar la identificació dels compostos en les mostres, com són l'ús de bases de dades per a la identificació de compostos sospitosos o l'anàlisi del defecte de massa de Kendrick per a la detecció de compostos desconeguts.

També en aquesta tesi s'han modificat i adaptat dues tècniques *Ambient MS* basades en l'ús de substrats mostrejadors com a sondes generadores de l'esprai, per a la resolució de dos problemes analítics molt diferents. Per una banda, s'ha avaluat la possibilitat de substituir les agulles metàl·liques per hisops mèdics en la *touch spray* (TS) amb el propòsit de desenvolupar un mètode que permeti l'assistència en les proves de resecció de tumors (gliomes) mitjançant el mostreig i l'anàlisi directa del teixit viu. Per altra banda, s'ha incorporat una làmpada de Criptó al disseny original del *paper spray* (PS) per establir un mètode basat en la fotoionització a pressió atmosfèrica (PS-APPI) que permeti la determinació ràpida de compostos per- i polifluoroalquilats neutres (PFASs), que presenten problemes per ionitzar-se pel mecanisme d'electroesprai, en productes d'impregnació. En ambdós casos s'ha avaluat la millor configuració del disseny TS i PS-APPI (disposició de l'estri, tipus de capil·lar d'entrada a l'espectròmetre de masses, mode de subministrament del dissolvent) per a la seva implementació al laboratori. A més, l'optimització dels paràmetres operacionals ha posat de manifest que la composició del dissolvent i el potencial d'ionització aplicat en TS influeixen en l'estabilitat de l'electroesprai generat a la punta de l'hisop mentre que, en PS-APPI, aquests paràmetres afecten a la resposta dels ions generats en la fase gas pel mecanisme d'APPI. L'estudi dels perfils fosfolipídics obtinguts en l'anàlisi de les mostres de teixit emprant el mètode TS-MS ha permès discriminar entre teixit sa i teixit tumoral. A més, les diferències en la resposta de dos oncometabòlits, el *N*-acetil aspartat (NAA) i el 2-hidroxiglutarat (2HG), han possibilitat establir qualitativament el grau d'infiltració del glioma en el teixit cerebral sa i si hi ha, o no, una mutació del tumor del gen isocitrat deshidrogenasa (IDH). Finalment, el mètode PS-APPI-HRMS desenvolupat per a la determinació de PFAS ha mostrat bons paràmetres de qualitat i s'ha aconseguit arribar a la sensibilitat necessària per determinar-los en productes d'impregnació. A més, l'aplicació del mètode desenvolupat a l'anàlisi de mostres reals ha permès detectar la presència d'alguns PFASs a uns nivells de concentració de l'ordre dels mg L^{-1}.

ABSTRACT

Nowadays, mass spectrometry is an indispensable tool in many analytical laboratories and application fields for food, environmental, forensic analysis and for clinical diagnosis. The excellent performance in terms of sensitivity and selectivity of this technique, as well as the structural information obtained from mass spectral data facilitate the identification of a large variety of organic compounds in many types of matrices. In this context, the development of fast, simple, versatile and real-time analytical methods is highly attractive for high-throughput laboratories and for providing information upon which health-care decisions can be based. Thus, this thesis is focused on a new family of mass spectrometric techniques called Ambient Ionization Mass Spectrometry (Ambient MS), which enable the direct analysis of samples with minimal or without any sample manipulation prior to analysis, providing relevant molecular information data almost in-real time. The published book chapters included in this thesis, which are based on the fundamentals, characteristics and approaches of the most relevant Ambient MS techniques, have clearly demonstrated their great potential for the screening of a large number of compounds and future perspectives. Nevertheless, most of the new Ambient MS applications published until now are still homemade prototypes for proof-of-concept studies. Thus, more work needs to be done for the development of real routine analytical methods.

In the present thesis, the applicability of desorption electrospray ionization (DESI), one of the first introduced Ambient MS techniques, combined with high-resolution mass spectrometry (HRMS) has been evaluated for non-target analysis and for the identification of unknown compounds in complex samples. In this study, a DESI-HRMS screening method has been developed for the identification of veterinary drugs in cross-contaminated feedstuffs. Moreover, the potential of DESI-HRMS has been tested for the characterization of an unknown complex sample suspected of being an adulterated phytosanitary product. Several fast sample manipulation strategies (< 5 min), which allowed preventing contamination and the DESI system carry-over due to the dusty texture of feed samples, have been evaluated. Furthermore, a simple and fast sample manipulation procedure, a filter paper impregnated with the phytosanitary product, has been used to reduce the drying time of the sample before the DESI analysis. In addition, DESI working conditions, which affect both extraction and ionization efficiency of desorbed compounds from sample surface, have been optimized. Among them, DESI solvent composition and flow rate, nebulizing gas pressure, geometrical parameters (nebulization capillary angle and tip distance to the sample surface) and DESI sampling mode (static or scanning) have been critical to obtain the best sensitivity.

Furthemore, Q-Orbitrap mass resolution and C-Trap injection time have been optimized to achieve accurate mass measurements and enough sensitivity and selectivity for the identification of veterinary drugs below legislated levels, but also for the characterization of unknown compounds detected in the phytosanitary product. For the reliable identification of non-target and unknown compounds in analyzed samples, several approaches such as custom-made databases or Kendrick mass defect analysis, have been applied to interrogate mass spectral raw data.

In this thesis, modified touch spray (TS) and paper spray (PS) techniques have also been evaluated for clinical diagnosis and for the analysis of non-polar/low polar compounds, respectively. A TS-MS method using commercial medical swabs has been developed to provide relevant diagnostic molecular information for brain cancer. Swabs are envisioned as a tool for *in vivo* sampling of brain tissue and for the subsequent ionization occurring directly from the swab tip during intraoperative surgical assessment. Moreover, PS has been combined with atmospheric pressure photoionization (PS-APPI) for the direct determination of neutral per- and polyfluorinated alkyl substances (PFASs) in waterproof impregnation sprays. The best TS and PS-APPI assembly configurations have been evaluated (spray probe position, MS inlet shape and length, solvent supply mode) for their implementation in the laboratory. Operational working parameters optimization have shown that solvent composition and spray voltage in TS are critically involved to the generation of a stable cone-jet electrospray plume. Instead, the effect of these parameters in PS-APPI has been more related to the response of the ions generated into the gas-phase by APPI. Phospholipid profiles detected by swab TS-MS tissue analysis have allowed to discriminate between cancer and normal tissues. Moreover, measurements of *N*-acetylaspartate (NAA) and 2-hydroxyglutarate (2HG) oncometabolites have been evaluated to qualitatively assess tumor infiltration grade and mutation status of the isocitrate dehydrogenase (IDH) gene. Finally, PS-APPI-HRMS method quality parameters have demonstrated the good performance of the developed method for the quantification of neutral PFASs in waterproof impregnation sprays using internal standard calibration method. The analysis of raw waterproof impregnation sprays have also demonstrated the good performance of the developed PS-APPI-HRMS method, revealing the presence of several neutral PFASs up to mg L^{-1} levels.

ABREVIATURES I ACRÒNIMS

2HG	2-hidroxiglutarat
Ambient MS	*Ambient ionization mass spectrometry*
APCI	Ionització química a pressió atmosfèrica (*atmospheric pressure chemical ionization*)
API	Ionització a pressió atmosfèrica (*atmospheric pressure ionization*)
APPI	Fotoionització a pressió atmosfèrica (*atmospheric pressure photoionization*)
ASAP	*Atmospheric solids analysis probe*
CAS	*Chemical abstracts service*
CI	Ionització química (*chemical ionization*)
CUSA	*Cavitron ultrasonic surgical aspirator*
D	Dopant
DADP	Diperòxid de diacetona
DAPCI	*Direct atmospheric pressure chemical ionization*
DAPPI	*Desorption atmospheric pressure photoionization*
DART	Anàlisi directa en temps real (*direct analysis in real time*)
DBDI	*Dielectric barrier discharge ionization*
DDA	*Data dependent acquisition*
DEP	Sondes d'exposició directa (*direct exposure probe*)
DESI	Ionització per desorció per electroesprai (*desorption electrospray ionization*)
DIA	*Data independent acquisition*
DIP	Sondes d'inserció directa (*direct insertion probe*)
EASI	*Easy ambient sonic-spray ionization*
EESI	*Extractive electrospray ionization*
EI	Ionització electrònica (*electron ionization*)
ESI	Ionització per electroesprai (*electrospray ionization*)
ESP	Espiramicina
FA	Alcohol fluorat (*fluorinated alcohol*)
FAB	Bombardeig ràpid d'àtoms (*fast atom bombardment*)

FD	Desorció per camp (*field desorption*)
FI	Ionització per camp (*field ionization*)
FIA	Anàlisi per injecció en flux (*flow injection analysis*)
FIB	*Fast ion bombardment*
FT-ICR	Ressonància ciclotrònica d'ions amb transformada de Fourier (*Fourier transform ion cyclotron resonance*)
FOSA	fluorooctanosulfonamida
FOSE	Fluorooctanosulfonamido-etanol
FS	Fosfatidilserina
FTOH	Fluorotelòmer alcohol
FWHM	*Full width at half maximum*
GC	Cromatografia de gasos (*gas chromatography*)
HCD	*High-energy collision dissociation*
HRMS	Espectrometria de masses d'alta resolució (*high resolution mass spectrometry*)
HS-DBDI	*Head space-dielectric barrier discharge ionization*
ICP-OES	Espectrometria d'emissió òptica per plasma acoblat inductivament
IDH	Isocitrat deshidrogenasa
IM	*Ion mobility*
IT	Trampa d'ions (*ion trap*)
IT-TOF	Trampa d'ions - temps de vol
IUPAC	Unió Internacional de Química Pura i Aplicada
KM	Massa de Kendrick
KMD	Defecte de massa de Kendrick
LAPPI	*Laser-ablation atmospheric pressure photoionization*
LC	Cromatografia de líquids (*liquid chromatography*)
LESA	*Liquid extraction surface analysis*
LIT	Trampa d'ions lineal (*linear ion trap*)
LIT-Orbitrap	Trampa d'ions lineal - Orbitrap
LMJ-SSP	*Liquid microjunction - surface sampling probe*
LOD	Límit de detecció

LOQ	Límits de quantificació
LTP	*Low temperature plasma*
MALDI	Ionització per desorció amb làser assistida per matriu (*matrix assisted laser desorption ionization*)
MLOD	Límit de detecció de mètode (*method limit of detection*)
MLOQ	Límit de quantificació de mètode (*method limit of quantification*)
MON	Monensina
MRM	*Multiple reaction monitoring*
MS	*Mass spectrometry*
MS/HRMS	Espectrometria de masses en tàndem en alta resolució (*high resolution tandem mass spectrometry*)
MS/MS	Espectrometria de masses en tàndem (*tandem mass spectrometry*)
MSI	*Mass spectrometry imaging*
MS^n	Fragmentació en etapes successives (*Multiple-stage mass spectrometry*)
NAA	*N*-acetil aspartat
NAR	Narasina
NKM	Massa nominal de Kendrick (*Nominal Kendrick mass*)
PC	Fosfatildilcolina
PEG	Polietilenglicol
PESI	*Probe electrospray ionization*
PFAS	Substàncies per- i polifluoroalquilades
PFOA	Àcid perfluorooctanoic
PFOS	Sulfonat de perfluorooctà
POC	*Point of care*
PS	*Paper spray*
PTFE	Politetrafluoroetilè
Q	Quadrupol
Q-Orbitrap	Quadrupol - Orbitrap
QqQ	Triple quadrupol
Q-TOF	Quadrupol - temps de vol
Q-trap	Quadrupol - trampa d'ions

REIMS	*Rapid evaporative ionization mass spectrometry*
RNKM	Residual de la massa nominal de Kendrick (*Reminder of nominal Kendrick mass*)
RSD	Desviació estàndard relativa (%) (*relative Standard deviation*)
TATP	Triperòxid de triacetona
TCP	*Tumor control probability*
TD	Desorció tèrmica
TIA	Tiamulina
TIC	Corrent total d'ions (*total ion current*)
TIL	Tilosina
TOF	Temps de vol (*time-of-flight*)
TS	*Touch spray*
UHPLC	Cromatografia de líquids d'ultra elevada eficàcia (*ultra-high performance liquid chromatography*)
UV	Ultravioleta
WADA	Agència mundial d'antidopatge (W*orld Anti-Doping Agency*)

OBJECTIUS I ESTRUCTURA

OBJECTIUS I ESTRUCTURA

L'objectiu d'aquesta Tesi ha estat l'estudi de les tècniques *Ambient Ionization Mass Spectrometry* i l'avaluació de tres d'aquestes tècniques per al desenvolupament de metodologies analítiques ràpides i selectives basades en l'anàlisi directa per espectrometria de masses que ajudin a millorar el rendiment dels laboratoris analítics.

Aquest objectiu general es pot desglossar en una sèrie d'objectius concrets que donen cos al treball inclòs en aquestes memòria i que són els següents:

- Desenvolupar mètodes d'anàlisi directa per espectrometria de masses emprant la tècnica d'ionització per desorció per electroesprai (DESI) per a l'anàlisi ràpida i eficaç de mostres d'elevada complexitat. Aquests mètodes han de tenir la capacitat de permetre la detecció i la identificació simultània d'un ampli nombre de compostos orgànics per poder ser emprats com a mètodes de cribratge en laboratoris analítics.

- Estudiar l'aplicabilitat de l'ús de la DESI amb l'espectrometria de masses d'alta resolució per implementar mètodes d'anàlisi de compostos sospitosos (*non-target*) i desconeguts i avaluar estratègies pel tractament de les dades obtingudes que permetin la ràpida identificació i caracterització d'aquests compostos. En concret, es pretén escollir problemes analítics que permetin posar de manifest la capacitat de la tècnica DESI-HRMS.

- Estudiar la possibilitat d'utilitzar tècniques *Ambient MS* basades en l'ús de substrats mostrejadors com a sondes generadores d'esprai per a l'anàlisi de mostres que requereixen un mostreig mínimament invasiu, com per exemple, per a l'anàlisi *in vivo* de teixit biològic.

- Avaluar la possibilitat de combinar l'APPI amb tècniques *Ambient MS* basades en la desorció assistida per potencial per tal d'ampliar el camp d'aplicació d'aquestes tècniques.

Per tal d'assolir aquests objectius, la present memòria s'ha estructurat en tres apartats:

- Una *Introducció* on es comenta la importància de les fonts d'ionització basades en l'anàlisi directa per espectrometria de masses (*Ambient MS*) per al desenvolupament de mètodes analítics ràpids i, en la qual, s'hi s'inclouen dos capítols intitulats

"*Introduction to Ambient Mass Spectrometry Techniques"(Chapter 1, pp. 3-35)* del llibre *"Ambient Mass Spectrometry Techniques in Food and Environment" (editorial CRC Press, 2019)* i "*Ambient Ionisation-High Resolution Mass Spectrometry: Environment, Food, Forensic and Doping Analysis*" *(Chapter 9, pp. 51-88)* del llibre *"Application of Time-of-Flight and Orbitrap Mass Spectrometry in Environmental, Food, Doping and Forensic Analysis" (Elsevier, Series: Comprehensive Analytical Chemistry, 2016, vol. 71)*. En aquestes publicacions s'hi discuteixen els principis i les característiques de les tècniques *Ambient MS* més consolidades, se'n fa una classificació de les diferents tècniques, es comenten les seves similituds i diferències en base al seu disseny i configuració i s'inclou una revisió bibliogràfica de les metodologies desenvolupades més rellevants en diferents camps d'aplicació. Aquesta introducció també conté una actualització bibliogràfica on es discuteixen les aplicacions *Ambient MS* més recents relacionades amb els camps d'aplicació del medi ambient, de l'alimentació, de l'anàlisi forense i del control antidopatge així com per a la diagnosi clínica.

- El *Capítol 2* està dedicat a l'estudi i avaluació de la tècnica d'ionització per desorció per electroesprai (DESI) per al desenvolupament de metodologies ràpides basades en l'anàlisi directa per espectrometria de masses de compostos sospitosos i desconeguts en matrius complexes. En aquest capítol hi consta: una introducció, on es comenten les principals característiques de la tècnica DESI i els avantatges que presenta l'espectrometria de masses d'alta resolució en aquest tipus de metodologies, un apartat on s'inclou el treball experimental realitzat i un tercer apartat de discussió de resultats. El treball experimental corresponent a aquest capítol està recollit en dos articles científics intitulats "*Desorption electrospray ionization-high resolution mass spectrometry for the screening of veterinary drugs in cross-contaminated feedstuffs*", publicat a la revista *Analytical and Bioanalytical Chemistry (2015, 407:7369-7378), i "Desorption electrospray ionization-high resolution mass spectrometry for the analysis of unknown materials: The phytosanitary product case*", publicat a la revista *Analyst (2019, 194:350-356)*.

- El *Capítol 3* està dedicat a estudiar la *touch spray* (TS) i el *paper spray* (PS) per a la resolució de dos problemes analítics específics. El primer dels treballs inclosos en aquest capítol forma part de la investigació realitzada durant l'estada formativa de 5 mesos a la Universitat de Purdue i els resultats del qual es recullen en l'article

científic "*Analysis of human gliomes by swab touch spray-mass spectrometry: application to intraoperative assessment of surfical margins and presence of oncometabolites*", publicat a la revista *Analyst (2017, 142:4058-4066)*. Els resultats del segon treball es troben recollits en l'article científic intitulat "*Paper Spray-Atmospheric Pressure Photoionitzacion-High Resolution Mass Spectrometry for the Direct Analysis of neutral Fluorinated Compounds in Waterproof Impregnation Sprays*", que s'ha enviat per a la seva publicació a la revista *Analytical and Bioanalyical Chemistry.*

- Finalment s'inclouen les conclusions obtingudes de la realització d'aquesta Tesi, així com la bibliografia corresponent.

CAPÍTOL 1

Introducció

Les activitats que l'ésser humà desenvolupa dia a dia tenen conseqüències que transcendeixen de l'àmbit local o regional en el qual s'exerceixen fins a afectar al funcionament global del nostre planeta. L'impacte humà (in)voluntari, sovint influenciat per motius econòmics i socials, contribueix a la formació, a l'ús i a l'emissió d'un gran nombre de contaminants o substàncies nocives que provoquen efectes adversos en el medi ambient i en l'ecosistema que ens envolta, en la qualitat dels productes alimentaris que consumim i, en conseqüència, al benestar de les persones i a la seva qualitat de vida. D'aquestes problemàtiques sorgeix la necessitat de realitzar un seguiment de les activitats que es troben englobades en els camps d'aplicació del medi ambient, de l'alimentació o de l'anàlisi forense, entre d'altres, i que impliquen la monitorització d'un nombre cada vegada més gran de compostos en un volum de mostres considerable. En aquest escenari, és de gran interès el desenvolupament de mètodes ràpids, senzills i automatitzats que permetin la detecció, la identificació i/o la quantificació d'un gran nombre de compostos mitjançant tècniques analítiques que ofereixin una elevada sensibilitat i selectivitat. Aquests requeriments es troben directament relacionats amb el desig d'augmentar la productivitat dels laboratoris de control, emprant mètodes més universals que permetin determinar diverses famílies de compostos sense incrementar i, fins i tot, disminuir el cost de l'anàlisi. En camps d'aplicació com l'anàlisi clínica, aquests requisits estan també relacionats amb la necessitat d'obtenir un diagnòstic en el mínim temps de resposta. Així, en general, es requereixen mètodes ràpids, senzills, versàtils, que no siguin específics d'un únic tipus de problema, malaltia o pacient, i/o que permetin l'anàlisi *in-situ* en aquells casos en què és primordial obtenir una resposta immediata. En aquest context, l'avenç i el desenvolupament de noves tècniques d'anàlisi instrumental tenen un paper molt important en el progrés i en l'evolució de noves metodologies analítiques que possibilitin assolir aquests objectius.

Avui dia, l'espectrometria de masses (MS) és una tècnica d'anàlisi instrumental gairebé indispensable en molts laboratoris analítics, principalment per la seva capacitat per identificar inequívocament una gran varietat de compostos (molècules orgàniques, sals inorgàniques, complexos organometàl·lics, biomolècules, etc.) i una gran sensibilitat, selectivitat i rapidesa. Ara bé, la majoria de mètodes analítics basats en l'anàlisi per MS inclouen tractaments de mostra (extracció dels anàlits, procediments de neteja dels extractes o de preconcentració) seguits d'una etapa de separació cromatogràfica per, finalment, detectar, identificar i/o quantificar els compostos per MS. Tant l'eliminació d'interferències

en les etapes del tractament de la mostra com la separació que té lloc durant la cromatografia, contribueixen a disminuir l'efecte que els components de la matriu de la mostra poden tenir en l'etapa d'ionització dels compostos i faciliten la interpretació dels espectres de masses que s'obtenen (Uclés et al., 2017). Per contra, aquestes dues etapes són les que afecten més negativament al temps i al cost de l'anàlisi, l'increment dels quals es veu directament influït pel grau de complexitat de la matriu que es pretén analitzar.

Com la gran majoria de tècniques instrumentals, l'espectrometria de masses ha sofert avenços significatius al llarg dels anys, que han permès desenvolupar mètodes analítics enfocats tant a la monitorització, a la quantificació i al control de compostos coneguts o legislats com a la identificació de compostos orgànics desconeguts en tot tipus de matrius. Els analitzadors de masses de baixa resolució continuen essent molt utilitzats en els laboratoris analítics, principalment en configuracions com el triple quadrupol (QqQ), la trampa d'ions en la seva versió lineal (LIT) així com el quadrupol-trampa d'ions (Q-trap), que permeten dur a terme experiments d'espectrometria de masses en tàndem (Holcapek et al., 2012). La capacitat de filtració, així com la velocitat d'escombratge i l'eficàcia d'emmagatzematge d'aquests analitzadors han millorat substancialment durant la darrera dècada, fent-los més ràpids, sensibles i selectius. Els analitzadors de triple quadrupol presenten una elevada sensibilitat i selectivitat i són molt adequats per a la monitorització i per a l'anàlisi quantitativa de compostos d'interès, especialment quan es treballa en el mode d'adquisició de monitorització de reaccions múltiples (MRM). No obstant això, els analitzadors de QqQ presenten certes limitacions quan s'aborda la determinació d'un nombre molt elevat de compostos ja que, segons els criteris descrits pels protocols europeus (EU directive, 1996, 2002), calen un mínim de dues transicions (un ió precursor i dos ions producte) per confirmar la presència d'un compost. A més, la necessitat de seleccionar prèviament aquestes transicions en el mode de treball de MRM implica que el seu ús quedi restringit a l'anàlisi de compostos diana (*target analysis*). Per a la monitorització de compostos sospitosos emprant analitzadors de QqQ, els modes d'escombratge d'ions precursors o de pèrdua de neutres són útils per a la l'anàlisi de compostos anàlegs que presenten ions producte o fragments neutres característics, tot i que impliquen que s'hagi de realitzar un escombratge d'ions (*full scan)*. No obstant això, s'ha de tenir en compte que els modes de treball en *full scan* en analitzadors quadrupolars són menys sensibles ja que, durant l'escombratge en el quadrupol, els ions són enviats de manera seqüenciada al detector, cosa que implica que el percentatge d'ions que s'envia per a cada valor de *m/z* sigui inferior al

100%. A diferència del quadrupol, la trampa d'ions és un analitzador polsat que emmagatzema paquets d'ions en trajectòries d'oscil·lació estables, els quals són enviats al detector en provocar la inestabilitat d'aquestes trajectòries. Els sistemes de trampa d'ions permeten l'escombratge d'ions (*full scan)* amb bona sensibilitat i, per tant, són més idonis per a la monitorització de compostos sospitosos.

Tot i els avantatges que presenten els sistemes analitzadors que s'acaben de comentar, el fet que treballin a baixa resolució implica que no sigui possible diferenciar o evitar les interferències isobàriques (mateixa massa nominal), que són especialment freqüents quan s'analitzen mostres amb matrius complexes que no han estat sotmeses a processos d'extracció i de neteja exhaustius. En aquest context, els analitzadors d'alta resolució, com ho són el temps de vol (TOF) o l'Orbitrap, permeten adquirir l'espectre de masses complet (*full scan*) amb una precisió en la mesura de la massa de fins a 0.0001 Da i, en la majoria dels casos, amb una sensibilitat i selectivitat equiparables als sistemes de QqQ (MRM) o de IT (escombratge d'ions producte). L'exactitud en la massa que proporcionen els analitzadors d'alta resolució permet realitzar una identificació temptativa de la fórmula molecular, reduint així el nombre de possibles candidats per un determinat ió (*m/z*), i fa possible la separació de compostos isobàrics. A més, l'elevada sensibilitat i selectivitat que proporcionen els analitzadors de masses d'alta resolució actuals en l'adquisició dels espectres de masses en *full scan* possibilita l'anàlisi retrospectiva de mostres, especialment útil per a la identificació de compostos sospitosos i/o desconeguts (Agüera et al., 2017; Lehotay et al., 2015). D'altra banda, els analitzadors híbrids d'alta resolució com el Q-TOF, el Q-Orbitrap o el IT-Orbitrap, permeten dur a terme experiments d'espectrometria de masses en tàndem, on l'ió precursor se selecciona en baixa resolució (al quadrupol o a la trampa d'ions) i la seva fragmentació en una cel·la de col·lisió genera els ions producte que són mesurats posteriorment en alta resolució. La informació estructural que s'obté és útil per a la caracterització de compostos desconeguts i permet disminuir la possibilitat d'obtenir falsos positius (Knolhoff et al., 2015). Avui dia, els nous sistemes d'espectrometria de masses disposen de modes d'escombratge que combinen diferents modes d'adquisició en el mateix cicle, com ara el *data dependent acquisition* (DDA) o el *data independent acquisition* (DIA), que permeten abordar l'anàlisi de compostos sospitosos o desconeguts d'una manera menys dirigida (García-Reyes et al., 2017).

Les altes prestacions dels analitzadors moderns d'alta resolució, juntament amb l'ús d'eines per al post-processament de dades (bases de dades online, programari que permet obtenir

espectres de tàndem virtuals o anàlisi estadística multivariant) i la utilització de fonts d'ionització a pressió atmosfèrica, s'han aprofitat per a desenvolupar mètodes directes d'anàlisi per MS en els que no es fa ús de cap separació cromatogràfica prèvia (Knolhoff et al., 2015), però també han permès simplificar, o fins i tot eliminar, etapes del tractament de mostra que, en molt casos, poden ser discriminants (alguns compostos de la mostra poden perdre's durant aquesta etapa). Aquest últim aspecte és especialment rellevant quan es pretenen desenvolupar mètodes en els que és necessari monitoritzar un gran ventall de substàncies, com per exemple per a l'autenticació de productes alimentaris, per al diagnòstic de malalties o per al cribratge de compostos sospitosos, entre d'altres.

El terme "anàlisi directa per espectrometria de masses" fa referència a l'anàlisi de les mostres en el seu estat natiu sense o amb una mínima manipulació prèvia a la seva anàlisi per MS (Byliński et al., 2017). Així doncs, amb l'eliminació de les etapes de tractament de mostra i de separació cromatogràfica, a més del tipus d'analitzador de masses utilitzat, els factors principals que poden influir en la sensibilitat, la selectivitat i la rapidesa dels mètodes d'anàlisi directa per MS són: el sistema d'introducció de la mostra, les condicions en què opera la font d'ionització i el mecanisme d'ionització emprats. Pel que fa a la introducció de la mostra, es pot fer servir qualsevol sistema que sigui compatible tant amb la font d'ionització utilitzada com amb el tipus de matriu que es pretenen analitzar. Així, per aquelles fonts d'ionització que operen en condicions d'alt buit, com ara la ionització electrònica (EI), la ionització química (CI) o la ionització/desorció per camp (FI/FD), el sistema d'introducció ha de contemplar la transferència de la mostra des d'unes condicions a pressió atmosfèrica a l'alt buit. La forma més tradicional d'introduir directament els anàlits a les fonts EI, CI, FI o FD són les sondes d'inserció directa (DIP) i d'exposició directa (DEP), on la mostra és portada a la zona d'alt buit, a través de diversos compartiments i d'un buit diferencial, per a la seva volatilització, sublimació o desorció prèvia a l'etapa d'ionització (Hoffmann et al., 2005). Tanmateix, les estratègies d'ionització directa que ofereixen les fonts EI, CI i FI/FD estan limitades a l'anàlisi de compostos suficientment volàtils i/o tèrmicament estables, ja que la ionització té lloc en la fase gas. Amb la introducció del bombardeig ràpid d'àtoms (FAB) l'any 1981 (Barber et al., 1981) s'aconsegueix, per primera vegada, l'anàlisi de molècules polars, làbils i d'elevat pes molecular, com ara els pèptids, els polisacàrids, els nucleòtids o els biopolímers. Avui dia, aquesta tècnica s'ha substituït per la ionització per desorció amb làser assistida per matriu (MALDI), atès que és una tècnica més sensible que permet ionitzar molècules d'elevat pes molecular (fins a 100,000 Da) (Karas et

al., 1988). A més, amb la tècnica MALDI és possible escanejar teixits i cèl·lules biològiques, al llarg dels eixos x i y, per crear mapes topogràfics (imatges) de la superfície estudiada en funció de la seva composició molecular (Edwards et al., 2017). En ambdues tècniques, la mostra s'ha de sotmetre a mètodes de preparació on es mescla amb una matriu (sòlida o líquida viscosa) per tal d'afavorir el procés de desorció/ionització. La mostra-matriu es porta a un receptacle en condicions d'alt buit on s'irradia amb partícules energètiques (àtoms/ions), en el cas del FAB/FIB, o amb fotons (làser), en la MALDI, per a la generació d'ions que són dirigits cap a l'analitzador de masses que, en el cas de la font MALDI, sol ser un TOF (Hoffmann et al., 2005). Ara bé, els espectres que s'obtenen utilitzant aquestes fonts d'ionització són complexos si es té en compte que els ions produïts provenen tant de la mostra d'interès com de la matriu utilitzada per a la desorció/ionització. A més, no existeix cap protocol eficaç per escollir la matriu més adient o el procediment al qual s'ha de sotmetre la mostra abans de l'anàlisi, els quals afecten directament a la reproductibilitat del mètode.

La introducció de les fonts d'ionització a pressió atmosfèrica (API), l'electroesprai (ESI), la ionització química a pressió atmosfèrica (APCI) o la fotoionització a pressió atmosfèrica (APPI), ha permès simplificar considerablement el procés d'introducció de la mostra. Emprant aquestes tècniques, la mostra es pot introduir directament a la font d'ionització en forma d'una dissolució que és arrossegada per una fase mòbil i que forma un esprai de microgotes carregades, en l'ESI, i neutres, en l'APCI i l'APPI. Aquest esprai es genera, a pressió atmosfèrica, per nebulització assistida pneumàticament (N_2) i, en el cas de l'ESI, aplicant un potencial a l'agulla. En la font ESI, la ionització té lloc en la fase líquida, on l'anàlit generalment es protona o desprotona via equilibris àcid/base, i els ions passen a la fase gas per evaporació iònica a partir de les microgotes carregades que es troben enriquides en ions positius o negatius segons la polaritat emprada. En el cas de l'APCI i l'APPI, la ionització es produeix en fase gas mitjançant reaccions ió-molècula iniciades en aplicar un elevat potencial a un elèctrode corona (APCI) o gràcies a les interaccions amb els fotons emesos per una làmpada ultraviolada de buit (APPI) (Mellon, 2003). Ara bé, per dur a terme l'anàlisi directa emprant aquestes fonts API, és necessari que la mostra sigui líquida. Així doncs, no és possible l'anàlisi directa de matrius sòlides sense realitzar, prèviament a la ionització, un tractament a la mostra per tal d'obtenir una solució o un extracte. Una de les estratègies més simples, robustes i automatitzades per al desenvolupament de mètodes d'anàlisi directa emprant les fonts API és l'anàlisi per injecció en flux (FIA), en la qual se sol utilitzar la instrumentació pròpia dels sistemes cromatogràfics, principalment el

mostrejador automàtic, l'injector i la bomba, per introduir una quantitat coneguda d'extracte o matriu líquida que és arrossegada per una fase mòbil cap a la font d'ionització (Alechaga et al., 2015; Mol et al., 2014). L'ESI és considerada la font d'ionització més "suau", i genera ions o grups d'ions (clúster isotòpic) en els quals es manté la informació de la composició elemental del compost. Aquesta ionització és aplicable a un ampli ventall de compostos de polaritat mitjana-alta, sempre que presentin diversos grups funcionals ionitzables (per obtenir la molècula protonada o desprotonada) en la seva estructura química, o que sigui possible la formació d'adductes amb components de la solució (amoni, ions de metalls alcalins, bases conjugades amb acetat o formiat). Tanmateix, per a l'anàlisi de compostos menys polars o sense grups fàcilment ionitzables en fase líquida, són més adequades les fonts APCI i APPI. D'altra banda, el mecanisme d'ionització d'ESI permet la generació d'espècies múltiplement carregades, a diferència de l'APCI i l'APPI, la qual cosa fa possible ampliar l'anàlisi a molècules d'elevat pes molecular (Mellon, 2003). Per contra, l'ESI és més sensible que l'APCI o l'APPI als efectes de disminució (supressió) o d'augment (*enhancement*) en l'eficàcia d'ionització, que són produïts per la presència d'altres substàncies que coelueixen amb els anàlits amb els quals competeixen durant el procés d'ionització.

A principis del segle XXI, sorgeix un nou concepte d'ionització en el panorama de les tècniques analítiques que "revoluciona" les possibilitats de l'anàlisi directa per espectrometria de masses. Es tracta d'un nou grup de tècniques d'ionització anomenades *ambient*, terme anglès que es podria traduir com "ionització en el seu propi entorn", i que s'inclouen en un nou apartat en espectrometria de masses anomenat *"ambient ionization mass spectrometry"* (*Ambient MS*). Amb l'*Ambient MS*, es fa realitat la possibilitat d'obtenir, gairebé de forma instantània, informació espectral de qualsevol objecte, simplement mantenint-lo davant de l'espectròmetre de masses. Les primeres tècniques que van encunyar aquest terme, la ionització per desorció per electroesprai (DESI) (Takáts et al., 2004) i l'anàlisi directa en temps real (DART) (Cody et al., 2005), van ser desenvolupades l'any 2004 pel Dr. R. G. Cooks, qui també va introduir el terme *Ambient MS*, i el Dr. R. B. Cody, respectivament. L'*Ambient MS* engloba aquelles tècniques d'ionització a pressió atmosfèrica que permeten la desorció i la ionització directa dels anàlits en el seu entorn natiu, ja sigui en estat sòlid, líquid o gasós, amb una mínima o nul·la manipulació de la mostra, prèvia a l'anàlisi per MS. A partir de la introducció de la DESI i la DART, el nombre de tècniques *Ambient MS* desenvolupades s'ha vist augmentat considerablement; els articles de

revisió més actuals ja en classifiquen més de 80, les quals difereixen, principalment, en la combinació de les etapes d'extracció i de desorció dels anàlits de la mostra i en el mecanisme d'ionització (Javanshad et al., 2017; Monge et al., 2013). Una de les característiques principals de les tècniques *Ambient MS* és que el processament de la mostra (extracció/desorció) es produeix *in situ*, és a dir, durant l'anàlisi, en la mateixa etapa en què es produeix la ionització o just immediatament abans. Així, es tracta de tècniques molt ràpides (uns segons), atès que només cal realitzar una preparació prèvia mínima o nul·la de la mostra. D'altra banda, l'ús de dissolvents o d'altres reactius és mínim, la qual cosa disminueix el cost de les anàlisis i afavoreix una recerca química sostenible. Com en la MALDI, algunes d'aquestes tècniques, com ara la DESI, també permeten la generació d'imatges de la superfície de la mostra segons la seva composició química (*imaging*), però amb l'avantatge de que no és necessari l'ús de matrius per desorbir i ionitzar els anàlits. A més, la simplicitat del disseny de les fonts *Ambient MS*, així com la versatilitat que ofereix treballar en condicions de pressió atmosfèrica, han permès modificar l'enfocament del desenvolupament de noves metodologies analítiques. Així, enlloc d'incidir en estudiar noves estratègies per adequar la mostra al sistema instrumental, es pot avaluar la possibilitat d'adaptar la tècnica al tipus de problema analític que es presenta. Això es pot fer de forma ràpida i relativament senzilla, ja sigui fent petites modificacions en una determinada tècnica *Ambient MS* ja establerta o implementant-ne una de nova sense la necessitat d'optimitzar tractaments de mostra o d'acoblar múltiples tècniques (LC-MS, GC-MS, FIA-MS, etc.), més costoses. Ara bé, cal remarcar que les tècniques *Ambient MS* també tenen limitacions que, en molts casos, afecten a la sensibilitat, a la representativitat i a la reproductibilitat del mètode, dificultant el desenvolupament de mètodes d'anàlisi quantitativa. A més, per a l'anàlisi de matrius molt complexes, les mesures de massa exacta i la selectivitat que aporta l'acoblament d'aquestes tècniques a l'HRMS no són suficients per obtenir resultats d'una qualitat suficient, essent necessari, en aquests casos, l'ús d'estratègies de manipulació simples prèvies a l'anàlisi.

1.1 TÈCNIQUES D'IONITZACIÓ *AMBIENT* EN ESPECTROMETRIA DE MASSES

En aquesta introducció s'inclouen dues publicacions incloses en dos llibres, apartats 1.1.1 i 1.1.2, on es discuteixen els principis, les característiques i les aplicacions de les tècniques *Ambient MS* en els camps de l'alimentació, el medi ambient, l'anàlisi forense i el control antidopatge. A la *Publicació I* (apartat 1.1.1) es descriuen els principis de les tècniques més consolidades i se'n fa una classificació segons el tipus de processament al qual se sotmet la mostra durant l'anàlisi, el mecanisme d'ionització de la tècnica i el processament de la mostra, simultani o just abans de la ionització. A més, s'hi discuteixen les similituds i les diferències, pel que fa al disseny, de les diferents tècniques *Ambient MS,* es discuteixen les estratègies de manipulació que s'han proposat per a l'anàlisi de mostres molt complexes així com per superar les limitacions d'algunes d'aquestes tècniques per a l'anàlisi quantitativa. La *Publicació II* (apartat 1.1.2), tot i que també hi ha una classificació de les tècniques *Ambient MS* i conté un apartat d'aplicacions que il·lustren l'aplicabilitat i la utilitat d'aquestes tècniques, se centra principalment en les característiques que aporta l'espectrometria de masses d'alta resolució al desenvolupament de mètodes *Ambient MS* per resoldre els problemes relacionats amb la matriu, com són la presència d'interferències isobàriques o els problemes de sensibilitat. Per últim, a l'apartat 1.2 s'inclou una actualització bibliogràfica on es presenten les recents aplicacions publicades a la literatura relacionades amb el desenvolupament de mètodes *Ambient MS* en els camps d'aplicació en els quals aquestes tècniques tenen una major rellevància, les estratègies d'anàlisi més utilitzades i les seves perspectives de futur.

1.1.1 PUBLICACIÓ I

Introduction to Ambient Mass Spectrometry Techniques

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En el llibre *Ambient Mass Spectrometry Techniques in Food and the Environment*. Editat per, Leo M. L. Nollet, Basil K. Munjanja. (2019) CRC Press. ISBN 9781138505568

CHAPTER 1

Introduction to Ambient Mass Spectrometry Techniques

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CONTENTS

INTRODUCTION

Cooks and coworkers introduced the term "ambient ionization" in mass spectrometry (MS) in 2004 (Takáts et al. 2004). This term grouped those emerging desorption/ionization techniques that operated at atmospheric pressure and were able to subject an object for its immediate mass spectral analysis by handling it in front of the interface without the need of sample preparation or sample pretreatment. However, nowadays, it would be more accurate to say that these methods frequently do not require other sample preparation than the sample processing that takes place during the analysis (Venter et al.

2014; Monge et al. 2013). To classify an ionization source in this new group of ambient MS techniques, it should meet the following requirements:

- The ability to perform ionization of compounds in the open air on objects of unusual shape or size, not typically amenable to direct MS analysis.
- The capability to perform direct surface analysis, avoiding time-consuming sample preparation steps typically required in MS-based chemical analysis of solid samples.
- The easiness to be swappable in any mass spectrometer equipped with an atmospheric pressure interface.
- The capacity to generate ions without significant in-source fragmentation, which would simplify the mass spectral data interpretation and the compound identification when analyzing complex samples.

The two first introduced ambient mass spectrometry (ambient MS) techniques were desorption electrospray ionization (DESI) (Takáts et al. 2004) and direct analysis in real time (DART) (Cody, Laramee, and Durst 2005), which boosted the development of a growing number of new ambient MS methods and a large number of acronyms. Table 1.1 summarizes the ambient MS techniques mostly used in food and environmental analysis. A number of excellent books and book chapters (Domin and Cody 2015; Gross 2017; Moyano and Galceran 2015; Kauppila and Vaikkinen 2014; Sero, Nunez, and Moyano 2016), review articles (Ding and Duan 2015; Monge et al. 2013; Espy et al. 2014; Cooks and Mueller 2013; Badu-Tawiah et al. 2013; Cody 2013; Javanshad and Venter 2017; Snyder et al. 2016; Klampfl and Himmelsbach 2015; Kauppila and Kostiainen 2017; Venter et al. 2014), and tutorials (Huang et al. 2011; Bodzon-Kulakowska et al. 2014), which have already been published under the topic of "ambient ionization," can be recommended for a further in-depth study of ambient MS technique fundamentals and state of the art. The ambient MS techniques developed until now combine different desorption methods with different ionization techniques, thus making their classification very challenging. Some authors have classified ambient MS techniques based only on the ionization mechanism (spray or jet ionization, electric discharge ionization, and gas-, heat- or laser-assisted desorption/ionization), although more thorough classifications have been made based on the intrinsic extraction/desorption/ionization mechanisms involved in these techniques (Black, Chevallier, and Elliott 2016). However, these divisions are sometimes debatable, especially when several of these mechanisms concur in the same technique.

Ambient MS techniques can offer advantageous characteristics to analytical laboratories working in the field of food and environmental analysis (Kauppila and Vaikkinen 2014; Chen et al. 2017; Nielen et al. 2011; Black, Chevallier, and Elliott 2016; Luo et al. 2017; Porcari et al. 2016). For instance, real-time and *in situ* analysis, low sample requirements with little sample invasion, fast and high-throughput analysis, minimal or no sample prior preparation, small or no use of organic solvents, and relatively low matrix effects are some of the ambient MS characteristics that can be attractive for food and environmental applications. These features allow facing some requirements such as workload, turnaround time, and cost per sample frequently demanded by modern analytical laboratories. Table 1.2 summarizes some of the most recent ambient MS applications in food and environmental analysis. As can be seen, most of these applications used DESI and DART, probably because they were the first techniques developed, and they have been more widely studied than others introduced later. Furthermore, the availability of commercial devices for both DESI and DART, which can be coupled to many mass

TABLE 1.1 Acronyms and Primary References for Ambient MS Techniques Used in Environmental and Food Analysis

Abbreviation	Name	First Reference
DAPCI	Desorption atmospheric pressure chemical ionization	Song and Cooks (2006)
DAPPI	Desorption atmospheric pressure photoionization	Haapala et al. (2007)
DART	Direct analysis in real time	Cody, Laramee, and Durst (2005)
DBDI	Dielectric barrier discharge ionization	Na, Zhao, et al. (2007)
DESI	Desorption electrospray ionization	Takáts et al. (2004)
DICE	Desorption ionization by charge exchange	Chan et al. (2010)
EASI	Easy ambient sonic-spray ionization	Haddad, Sparrapan, and Eberlin (2006)
EESI	Extractive electrospray	Chen, Venter, and Cooks (2006)
ELDI	Electrospray-assisted laser desorption ionization	Shiea et al. (2005)
FAPA	Flowing atmospheric pressure afterglow	Andrade et al. (2008)
LAESI	Laser ablation electrospray ionization	Nemes and Vertes (2007)
Leaf spray	Leaf spray	Liu et al. (2011)
LESA	Liquid extraction surface analysis	Kertesz and Van Berkel (2010)
LMJ-SSP	Liquid microjunction surface sampling probe	Berkel, Sanchez, and Quirke (2002)
LTP	Low-temperature plasma	Cotte-Rodríguez and Cooks (2006)
MALDESI	Matrix-assisted laser desorption electrospray ionization	Sampson, Hawkridge, and Muddiman (2006)
Nano-DESI	Nano-desorption electrospray ionization	Roach, Laskin, and Laskin (2010b)
ND-EESI	Neutral desorption extractive electrospray	Chen, Wortmann, and Zenobi (2007)
PADI	Plasma-assisted desorption ionization	Ratcliffe et al. (2007)
PESI	Probe electrospray ionization	Hiraoka et al. (2008)
PS	Paper spray	Wang et al. (2010)
REIMS	Rapid evaporative ionization mass spectrometry	Schäfer et al. (2009)

Bold italics acronyms: Commercially available Ambient MS techniques.

spectrometer brands, may also affect the increase of their use. The simplicity of ambient MS methods allows the easy modification of the already existing ones to adapt them to both the sample type and the analytical problem. For this reason, many prototypes, home-made new approaches, are continuingly being introduced, and most of them have been explored for their application to target analysis of organic contaminants in food and environmental samples, food fraud, food authentication, and screening of emerging pollutants, among others. Although most of the characteristic aspects of ambient MS techniques can be very attractive and "revolutionary," one should be aware of intrinsic limitations. For instance, the detection of a compound largely depends on the ionization

TABLE 1.2 Applications of Ambient MS Methods in Environmental and Food Analysis

Ambient MS Technique	Mass Analyzer	Sample	Scope of the Analysis	References
Food Analysis				
DESI	IT, Q-LIT, QqQ, miniature MS	Fruits, vegetables, cereals, olive oil, fish, meat, wine, and coffee	Analysis of pesticides and lipids, food authentication, and food profiling	Cajka, Riddellova, Zomer, et al. (2011), Gerbig and Takáts (2010), Garcia-Reyes, Jackson, et al. (2009), Schurek et al. (2008), Mainero-Rocca et al. (2017), Berchtold et al. (2013), Hartmanova et al. (2010), Joyce et al. (2013), Montowska et al. (2014), Zhou et al. (2008), B. Li et al. (2011)
EASI	Q, Q-TOF, FT-ICR	Meat and vegetable oils	Analysis of lipids, authentication, and lipid profiling	Porcari et al. (2016)
PS	Q, IT, QqQ	Fruits, vegetables, coffee, meat, milk, olive oil, and sport drinks	Analysis of pesticides, drugs, and foodstuffs contaminants; authentication; metabolomics; and food profiling	Evard et al. (2015), Garrett, Rezende, and Ifa (2013), Guo et al. (2017), Zhang and Lee (2013)
Leaf spray	Q-Orbitrap	Plants	Discrimination analysis	Pereira et al. (2017)
LESA	IT	Meat	Proteomics and peptidomic analysis	Montowska et al. (2015)
DART	QqQ, TOF, Orbitrap, QTOF, LIT-Orbitrap	Fruits, vegetables, cereals, sweets, fish, meat, and beverages	Analysis of pesticides, xenobiotics, mycotoxins, phytohormones, melamine, and caffeine; authentication; metabolomics; screening; and characterization	Avula et al. (2015), Kalachova et al. (2011), Crawford and Musselman (2012), Farré, Picó, and Barceló (2013), Novotná et al. (2012), Hrbek et al. (2014), Yang et al. (2015), Danhelova et al. (2012), Lojza et al. (2012), Rajchl et al. (2013), Luo et al. (2017)
DAPPI	QTOF, IT	Fruits	Analysis of pesticides and screening of contaminants	Räsänen et al. (2014), Suni et al. (2012), Luosujärvi et al. (2010)
DAPCI	IT	Fruits	Screening and discrimination analysis	Yang et al. (2009)

(*Continued*)

TABLE 1.2 (*Continued*) Applications of Ambient MS Methods in Environmental and Food Analysis

Ambient MS Technique	Mass Analyzer	Sample	Scope of the Analysis	References
LTP	IT, miniature MS	Fruits, vegetables, wine, coffee, milk, and fish	Analysis of pesticides, melamine, and volatile compounds, and authentication analysis	Garcia-Reyes, Mazzoti, et al. (2009), Gerbig et al. (2017)
EESI	Q-LIT, Q-TOF	Fruits, vegetables, olive oil, beverages, and cheese	Analysis of lead complexes, screening, and authentication	Zhang et al. (2017), Bai et al. (2012), Sun et al. (2015)
REIMS	Q-TOF	Meat	Authentication/classification	Verplanken et al. (2017)
ELDI	IT	Fungus	Determination of chemical surface composition	Huang et al. (2012)
Environmental Analysis				
DESI, nano-DESI	IT, Orbitrap, LIT-Orbitrap	Consumer goods, clams, air, plants, soil, aerosols, and water particles	Analysis of pesticides, phthalates, toxins, and PAHs	Ewing et al. (2015), Gerbig et al. (2015), Cain et al. (2014), Boone et al. (2015), Mattarozzi et al. (2016)
PS	IT, QqQ, Orbitrap	Water	Analysis of amines, malachite green, crystal violet, and their metabolites	Jjunju et al. (2016), Fang et al. (2016)
LAESI	IT, Q-Orbitrap	Mussels	Analysis of domoic acid	Beach et al. (2016)
DART	TOF	Lake water and aerosols	Analysis of pesticides and PAHs	X. Wang et al. (2014), S. Zhou, Forbes, and Abbatt (2015)
DAPCI	IT	Soil	Analysis of plasticizers	N. N. Wang et al. (2014)
DBDI	IT, Q-LIT, LIT-Orbitrap	Spiked water	Analysis of pesticides and drugs	Mirabelli, Wolf, and Zenobi (2016)
EESI	IT	Water	Analysis of malachite green and tetrabromobisphenol A	Tian et al. (2014), Fang et al. (2016)

mechanism as well as on the nature of the analyte to yield ions under the concrete ionization mechanism. Therefore, not a single ionization method, especially when is used under one set of conditions, can deliver ions of all constituents in a complex sample. However, all ionizable compounds on the sample surface will contribute to the generation of complex mass spectral data. On the other hand, ambient ionization is generally a soft ionization that yields ions with low internal energy, which suffer no or little fragmentation in the atmospheric pressure region. Mono-charged and multiple-charged ions, as well as adduct ions, can be expected from those ambient MS methods shearing electrospray-like ionization mechanisms, while plasma-based techniques only will generate mono-charged ions and radical molecular ions. Under this scenario, the quality of the results would depend on the degree of information that the mass spectrometer can provide. Ambient MS techniques have been coupled to low-resolution mass analyzers (quadrupole and ion traps) mainly for the analysis of target compounds. However, the complexity of mass spectral data and the application of ambient MS techniques for the screening of complex samples require the use of MS instruments capable of acquiring data at high-resolution and/or performing tandem MS experiments. High-resolution MS instruments are required to overcome interference problems, avoid overlapping isotope clusters, and provide high-quality mass spectral information to identify both the chemical formula and the chemical structure. Today, highly sensitive and selective instruments, such as linear ion trap (LIT), time of flight (TOF), Orbitrap and hybrid instruments such as triple quadrupole (QqQ), quadrupole-TOF (Q-TOF), quadrupole-Orbitrap or LIT-Orbitrap, have demonstrated to provide the necessary performance to design reliable methods with minimal sample preparation, thus facilitating high throughput analysis.

This chapter aims to provide a general picture of ambient MS techniques used for the analysis of organic compounds in environmental and food samples. Classification and a brief description of the ambient MS methods used in these fields as well as the most important and critical features of the desorption/ionization techniques are included.

AMBIENT MS TECHNIQUES

What really differentiates ambient MS from other direct MS techniques is that the sample processing takes place in real time and proximal to ionization. Among the ambient MS techniques, the difference relies on the way of how sample processing is combined with well-known ionization mechanisms. For instance, electrospray ionization (ESI), sonic-spray ionization (SSI), gas-phase ion–molecule reactions, and photochemical ionization have been coupled in real time with sample processing steps such as liquid extraction, thermal desorption, and laser ablation, among others. The classification of ambient MS techniques in subclasses is not easy and differs depending on the reviews published until now, with a certain degree of overlap. In addition, it is sometimes difficult to distinguish between a new ambient MS technique and the simple rebranding of an already reported ambient ionization source. Despite the difficulties to categorize the ambient MS techniques, the approximations of Venter et al. (2014) and Monge et al. (2013) seem to provide the most logical arrangement since both the sample processing and the desorption/ionization mechanisms are taken into account. Venter et al. (2014) classified ambient MS techniques based on the predominant sample processing method: liquid extraction (spray desorption, liquid junction, and substrate spray), thermal desorption, and laser ablation. On the other hand, the classification of Monge et al. (2013) is based on the intrinsic desorption/ionization mechanism of surface ambient MS techniques. They are classified into one-step and two-step

techniques: one-step technique where desorption works in parallel with the ionization and two-step technique where desorption/ablation is followed by a secondary independent ionization step. It must be mentioned that these authors do not include paper spray (PS) and extractive electrospray ionization (EESI) in their classifications. In this work, we propose a classification (Table 1.3) based on the number of steps necessary to desorb/ionize the analytes from the sample (Monge et al. 2013), but also considering the sample processing that takes place in each technique (Venter et al. 2014). Thus, this classification allows including PS and EESI, which are classically considered as ambient MS techniques.

One-Step Ambient MS Techniques

As mentioned earlier, in this group of techniques, sample processing occurs in parallel with the ionization mechanism. Liquid–solid extraction, thermal desorption, or chemical sputtering occurs in the same space and time than the ionization of compounds desorbed from the sample surface.

Solid–Liquid Extraction

Those one-step ambient MS techniques having liquid–solid extraction and liquid–liquid extraction (LLE) as the most important sample processing steps can be further divided into two subgroups depending on how the extraction product is presented to the ionization event: spray desorption or liquid microjunction. DESI (Takáts et al. 2004) is the most well-known spray desorption ambient MS technique (Figure 1.1), in which the sample processing starts by generating a charged spray plume through pneumatically assisted electrospray of a solvent. This charged spray plume is directed onto the sample surface to create

TABLE 1.3 Classification of Ambient MS Techniques Used in Environmental and Food Analysis

Techniques	Sample Processing		Ionization	Ambient MS Techniques
One step	Liquid–solid extraction	Spray desorption	ESI	DESI, EESI
			Sonic spray	EASI
		Liquid microjunction	ESI	LMJ-SSP, LESA Nano-DESI
	Thermal/chemical desorption		Plasma based	DART, DAPCI, FAPA, LTP, DBDI, PADI,
			Photoionization	DAPPI
Two step	Liquid–solid extraction	Spray desorption	ESI	ND-EESI
		Substrate spray	ESI	PESI
		Ultrasonic nebulization	APPI	EAPPI
	Laser desorption/ ablation	Laser ablation	ESI	LAESI (ELDI)
		Matrix-assisted laser desorption	ESI	MALDESI
Three step	Solid–liquid extraction	Substrate spray	ESI	PS
Others	Thermal ablation/chemical ionization			REIMS

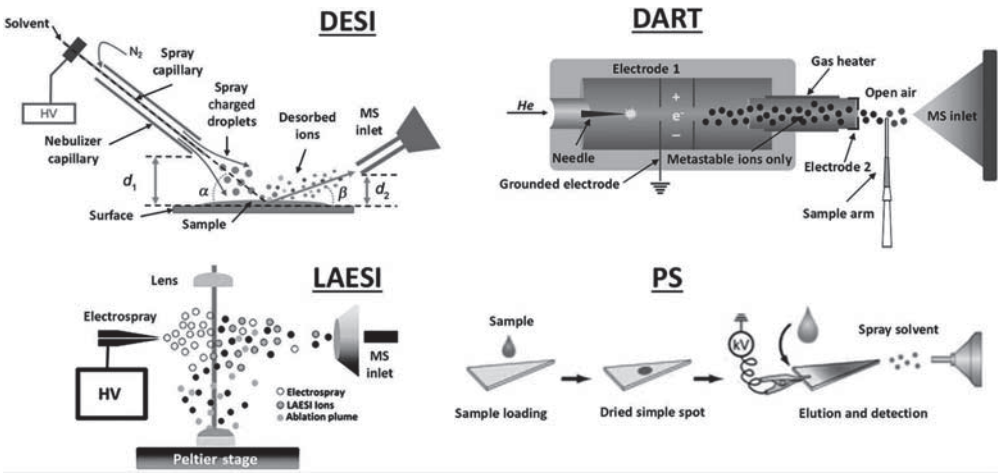

FIGURE 1.1 **(See color insert after page 124.)** Schematic illustrations of some commercially available ambient MS techniques: DESI (Reprinted with permission from reference (Sero, Nunez, and Moyano 2016), Copyright (2016) Elsevier.), DART and LAESI (Reprinted with permission from reference (Stopka et al. 2014), Copyright (2014) Royal Society of Chemistry.), and PS (Reprinted with permission from reference (Wang et al. 2010), Copyright (2010) Wiley-VCH.).

a microlocalized liquid layer where compounds are dissolved/extracted. Finally, after the impact of subsequent arriving primary charged droplets on the surface, the liberation of secondary droplets containing analytes is produced, thus generating analyte ions through traditional ESI mechanisms. Contrary to DESI, easy ambient sonic-spray ionization (EASI) (Haddad, Sparrapan, and Eberlin 2006) operates free of high voltage, in spite of sharing the same practical setup (nebulizing gas flow and polar solvent systems). The nebulizer gas flows at sonic speed (2–5 times higher than DESI) coaxially to a solvent flow, which generates charged droplets through a statistical imbalance of charge. Although DESI is commercially available for its installation in several mass spectrometers, EASI is still a homemade device. However, DESI commercial ionization source can be adapted to perform EASI analysis with little custom readjustments. Regarding EESI (Chen, Venter, and Cooks 2006), the main difference when comparing with DESI and EASI is the use of two orthogonal sprays: the first spray contains charged droplets generated by the electrospray of a solvent, and the second spray can be either a neutral aerosol produced from a liquid sample or a gas stream containing volatile compounds (Figure 1.2). In this technique, collisions between neutral (containing analytes) and solvent charged droplets produce the extraction of analytes and subsequently the ionization takes place through ESI mechanisms.

Regarding liquid microjunction-based techniques, a solvent is delivered through a capillary to form a semistatic liquid junction where analytes are dissolved/extracted from the sample surface. The extracting liquid is transferred to a position where analytes can be ionized by well-known ionization mechanisms, typically by electrospray. Techniques such as liquid microjunction surface sampling probe (LMJ-SSP) (Berkel, Sanchez, and Quirke 2002), nano-DESI (Roach, Laskin, and Laskin 2010b), and liquid extraction surface analysis (LESA) (Kertesz and Van Berkel 2010) belong to this group of ambient MS methods (Figure 1.2). The LMJ-SSP, already commercialized as flowprobe™, uses two concentric tubes to supply the extraction solvent and to suction out the extracting liquid

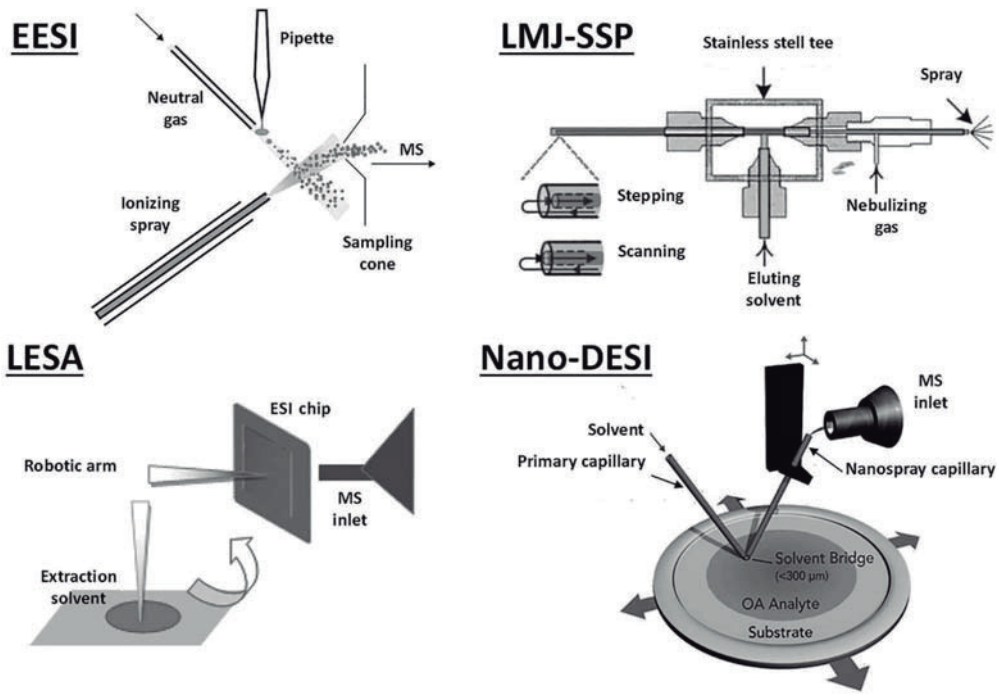

FIGURE 1.2 **(See color insert after page 124.)** Schematic views of some one-step spray-based setup techniques: EESI (Reprinted with permission from reference (X. Li et al. 2011), Copyright (2011) Nature America, Inc.), LMJ-SSP (Reprinted and adapted with permission from reference (Berkel, Sanchez, and Quirke 2002), Copyright (2002) American Chemical Society.), LESA (Reproduced with permission from reference (Montowska et al. 2014), Copyright (2014) American Chemical Society.), and nano-DESI (Reprinted with permission from reference (Roach, Laskin, and Laskin 2010a), Copyright (2010) American Chemical Society.).

phase with analytes, which are further ionized by ESI or atmospheric pressure chemical ionization (APCI). Nano-DESI operates more similar to LMJ-SSP than to DESI, since the lack of nebulizing gas removes the momentum transfer of incoming droplets to the liquid film. Instead, a solvent delivered by a fused silica capillary dissolves the compounds on the sample surface and forms a solvent bridge with a self-aspirating nanospray emitter. Regarding LESA, which is fully automated, microliquid extraction from a solid surface is combined with a nano-electrospray, thus being considered an adaptation of the infusion nano-ESI automated device (Nanomate).

Thermal and Chemical Desorption

The thermal-assisted desorption is the most common way to remove analytes from the sample surface before gas-phase ionization through plasma-base techniques and atmospheric pressure photoionization (APPI). However, other mechanisms such as sputtering, where the sample surface is bombarded with high-energy species generated in atmospheric pressure plasmas, can also contribute to remove compounds with very low or no vapour pressure.

The plasma-based techniques falling into this group rely on an electrical discharge involving metastable and reactive charged species, which interact, directly or indirectly,

through proton- and charge-transfer reactions with thermally/chemically desorbed analytes. Despite the different type of atmospheric pressure plasma used, such as coronas and dielectric barrier discharges (DBDs) and direct current (dc) and radio frequency (rf) glows, the design of the plasma source and how the plasma interacts with the sample allow classifying these techniques. In DART (Cody, Laramee, and Durst 2005), flowing atmospheric pressure afterglow (FAPA) (Andrade et al. 2008), and desorption atmospheric pressure chemical ionization (DAPCI) (Song and Cooks 2006), the plasma is generated in a physically and/or electrically isolated region from the sample introduction zone. Regarding DART, the plasma species generated by a dc corona-to-glow discharge are electrically filtered and heated before their interaction with the sample (Figure 1.1). Therefore, metastable species, formed in the discharge supporting gas (He or N_2), interact with gas-phase water molecules to generate protonated water clusters by Penning ionization, which participate in proton-transfer reactions with the thermal desorbed analytes. In the FAPA source, the dc atmospheric pressure glow discharge is generated in a sealed discharge cell, and plasma species are transported into the open air producing an afterglow discharge (Figure 1.3). Although FAPA source seems to be similar to DART, it behaves quite differently in practice, which is due to the differences in the source design and the operating conditions that will be discussed later. In DAPCI, a dc corona discharge ionizes gas-phase solvent vapours, as in APCI, producing reagent species in a heated chamber, and the flowing gas transfers the reagent ions onto the sample surface inducing "chemical sputtering" of adsorbed analytes (Figure 1.4).

By contrast, in low-temperature plasma (LTP) (Cotte-Rodríguez and Cooks 2006) and dielectric barrier discharge ionization (DBDI) (Na et al. 2007), the plasma is generated in the sample region, and all reactive species are used for ionization of compounds (Figure 1.3). In these techniques, a "cold" (~30°C) nonequilibrium plasma is generated by

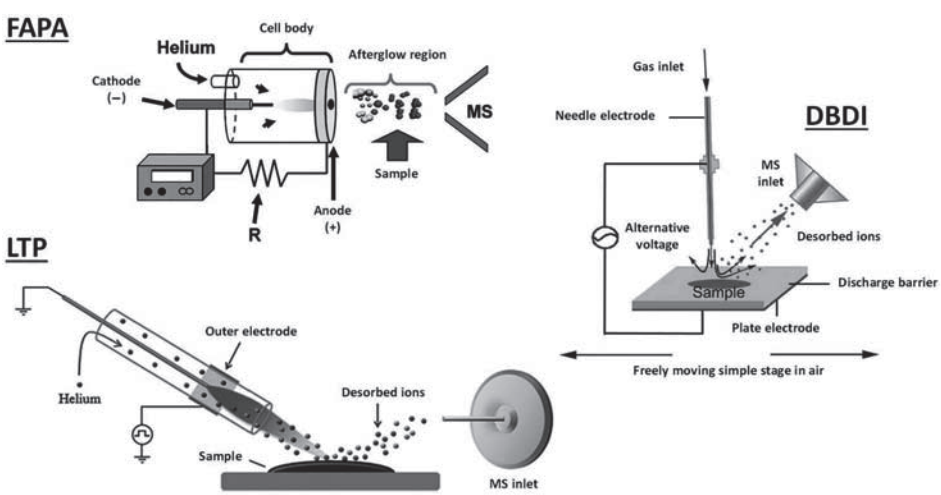

FIGURE 1.3 **(See color insert after page 124.)** Schematics of some one-step plasma-based techniques: FAPA (Reprinted with permission from reference (Shelley, Wiley, and Hieftje 2011), Copyright (2011) American Chemical Society.), DBDI (Reprinted with permission from reference (Na, Zhang, et al. 2007), Copyright (2007) John Wiley & Sons, Ltd.), and LTP (Reprinted with permission from reference (Benassi et al. 2013), Copyright (2013) John Wiley & Sons, Ltd.).

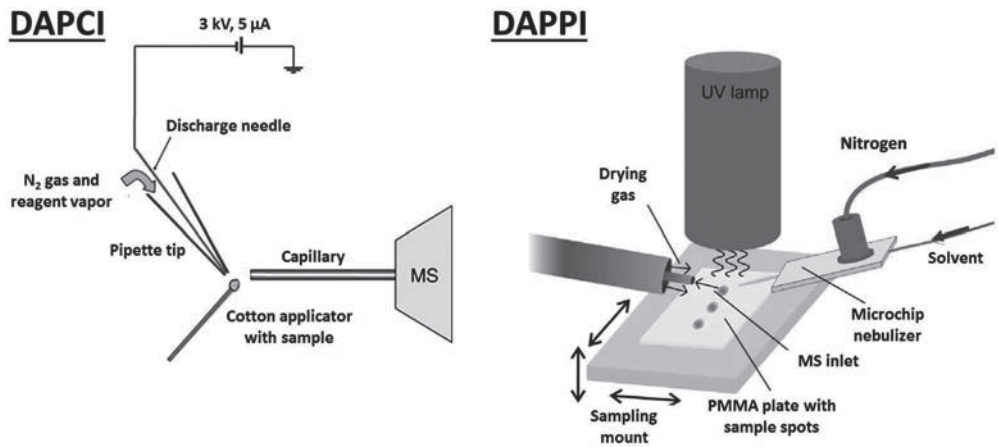

FIGURE 1.4 **(See color insert after page 124.)** Schematic illustrations of DAPCI (Reprinted with permission from reference (Song and Cooks 2006), Copyright (2006) John Wiley & Sons, Ltd.) and DAPPI (Reprinted with permission from reference (Haapala et al. 2007), Copyright (2007) American Chemical Society.).

a DBD, sustained by an inert gas, so no damage to the surface due to heating is expected. Moreover, plasma-assisted desorption ionization (PADI) (Ratcliffe et al. 2007) uses an rf glow discharge plasma in direct contact with the sample to desorb/ionize target analytes. As in LTP, the plasma plume in PADI operates at room temperature, which favors the use of both techniques for the analysis of thermally labile compounds.

Finally, DAPPI (Haapala et al. 2007) (Figure 1.4) is the only non-plasma-based technique included in this group, in which analytes thermally/chemically desorbed are ionized by APPI mechanisms. Thus, a heated mix containing the carrying gas and solvent vapours (dopant) is directed onto the sample surface. The analytes are desorbed by the hot vapour, after which the ionization is produced by ultraviolet (UV) radiation involving photoionization, charge-transfer reactions, and ion–molecule reactions with solvent/dopant species.

Two-Step Ambient MS Techniques

In the ambient MS techniques included in this group, the sample is nebulized, and/or analytes are desorbed/extracted through thermal desorption, mechanical ablation, or laser desorption/ablation in a first step that is followed by an independent secondary ionization step.

Liquid–Solid Extraction

The extraction atmospheric pressure photoionization (EAPPI) (Liu et al. 2016) is a two-step technique that uses an ultrasonic nebulizer for sample processing (extraction, nebulization, and vaporization). The generated aerosol is transported by a carrier gas to the photoionization region (vacuum UV lamp), while mixing with a gaseous dopant. By contrast, in probe electrospray ionization (PESI) (Hiraoka et al. 2008) (Figure 1.5), which involves a solid sampling electrospray probe, a needle is inserted into a sample to pick up or to coat the needle surface with sample material before positioning it close to the mass spectrometer inlet. Finally, the ionization step is performed by applying a high voltage to the needle probe to induce an electrospray.

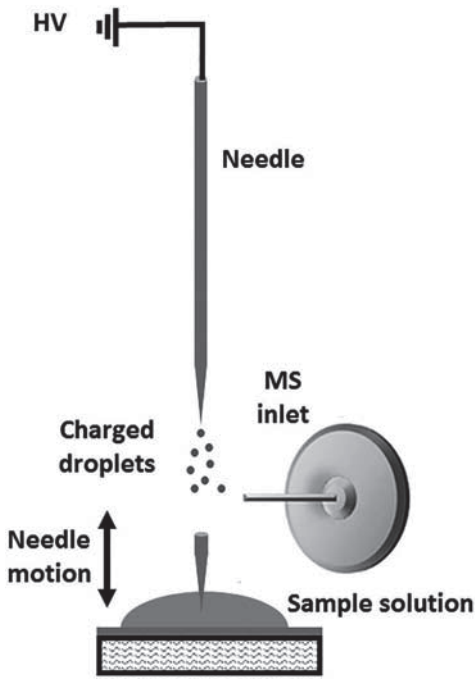

FIGURE 1.5 Schematic setup of PESI.

Laser Desorption/Ablation

This group of techniques uses an infrared (IR) or UV laser to produce the desorption or ablation of analytes from a surface with and without a matrix. The sample surface is broken into small pieces by the sudden delivery of energy. The fine clusters or particles are dispersed by the expanding plume, which is subsequently merged with an electrospray plume or a plasma stream depending on the ionization source used in the second step. One advantage of not being the sample in direct contact with the ionizing plume is that desorption and ionization can be optimized independently.

The first technique that coupled laser sampling to an ESI source was electrospray-assisted laser desorption ionization (ELDI) (Shiea et al. 2005). In ELDI, a pulsed nitrogen laser is used, while in laser ablation electrospray ionization (LAESI) (Nemes and Vertes 2007) (Figure 1.1), which is commercially available, the desorption/ablation takes place applying a mid-IR laser. By contrast, matrix-assisted laser desorption electrospray ionization (MALDESI) (Sampson, Hawkridge, and Muddiman 2006) uses an IR or UV laser to excite an exogenous matrix that cocrystallizes with the analyte, while applying a voltage to the stainless-steel target plate.

Three-Step Ambient MS Techniques

In this classification, a three-step ambient MS group has been proposed to include PS as an ambient MS technique, since it incorporates two sampling/processing steps and the ionization as a third step. In PS (Wang et al. 2010; Liu et al. 2015), a prepared

liquid sample is spotted onto a paper triangle and a solid-phase extraction (SPE) and/or chromatographic separation occurs when delivering a solvent and applying a high voltage to the paper substrate (Figure 1.1). The extracting solvent transports the extracted compounds to the apex of the triangle paper, whereas the different interaction of sample components with the paper substrate could produce their spatial/temporal separation. Electrospray is the driven force that pulls the solvent to the apex of the paper triangle, and also it is responsible for compound ionization through ESI mechanisms. Leaf (Liu et al. 2011, 2015) is an ambient MS method sharing similar principles with PS. However, in this technique, the plant material serves both as a substrate and as a sample. For instance, plant materials having a natural sharp shape (e.g., bean sprout) can be analyzed directly by leaf spray, while a small nick cut with a sharp tip has to be made to different samples. As in PS, the spray solvent is supplied on the pointy plant material to extract the endogenous compounds and to transport them to the tip. Finally, by applying a high voltage directly on the plant material, a spray of charged droplets is generated at the tip through ESI mechanisms.

Other Ambient MS Techniques

Rapid Evaporative Ionization Mass Spectrometry

Rapid evaporative ionization mass spectrometry (REIMS) is an ambient MS technique based on other principles for desorption or ionization that cannot be classified in any of the previous categories. Originally, this technique was developed for the real-time identification of tissues during surgical interventions (Schäfer et al. 2009), although it has recently been proposed as a new analytical approach for direct analysis of meat samples (Balog et al. 2016; Verplanken et al. 2017). In REIMS, surgical blades are used to ablate tissues in order to produce aerosols and charged species by the heat dissipated during the electrosurgical process, being subsequently transported to the MS inlet. The ionization mechanism proposed for REIMS is quite similar to that taking place in both APCI and thermospray ionization in filament-off mode (Schäfer et al. 2009). The desorption of neutral molecules is followed by gas-phase ionization via proton transfer reactions with the ionized water molecules as in APCI.

PHYSICAL DESIGNS AND EXPERIMENTAL APPROACHES

As mentioned earlier, in ambient MS analysis, sample processing and ionization usually take place in a single platform near the inlet of the mass spectrometer. In this context, geometrical parameters, solvent and/or gas characteristics and flow rate, plasma formation approaches, surface properties, solubility, and temperature are important variables that critically affect the performance of the techniques.

Probe Assembly Configurations

In one-step spray desorption-based techniques (Table 1.3) such as DESI, EASI, and EESI, the geometrical configuration and operational parameters are important aspects to be considered (Weston 2010). For instance, when tuning DESI source, the response depends on the incident and the collection angles, the sample spot-to-MS inlet distance, as well as on

both the tip-to-surface and the MS orifice-to-surface heights (Figure 1.1). Recommended parameters for DESI typically are within the range of 45°–60° and 5°–30° for incident and collection angles, respectively, 2–3 mm for the sample spot-to-MS inlet distance, and 1–3 mm and ~1 mm for the tip-to-surface and the MS orifice-to-surface heights, respectively (Monge et al. 2013). The liquid sample in EESI (Figure 1.2) is sprayed into an electrospray plume positioned orthogonally to the sample spray, being the distance and the angle between both sprays critical to guarantee an efficient overlapping. The geometrical configuration of liquid microjunction-based techniques is quite different, and the sampling probe rests on the surface of the sample for longer time than in spray-based ones. The differences among these techniques are due to the way of delivering and removing the extracting solvent from the sample surface. As can be seen in Figure 1.2, in LMJ-SSP, the coaxial capillaries are positioned perpendicularly (100–300 μm) above the sample. The outer capillary supplies the solvent to form the liquid junction and extracts compounds from the sample surface, whereas the inner capillary pulls the solution that contains the analytes to the ionization source. In contrast to the vertical arrangement of LMJ-SSP, in nano-DESI, the two fused silica capillaries that form the liquid microjunction are positioned at an angle above the sample surface (Figure 1.2). The primary capillary continuously supplies the solvent to create and maintain the liquid bridge, whereas the secondary capillary, an electrospray emitter, aspirates and ionizes the desorbed analytes. Regarding LESA (Figure 1.2), a robotic arm picks up a conductive disposable pipette tip and moves it above to a well containing the extracting solvent, which is aspirated by immersing the pipette tip into it. Afterwards, the pipette tip is positioned above the surface spot to form the liquid microjunction by dispensing a specific volume of solvent on the surface to extract the compounds. The latter liquid phase is aspirated back into the tip and positioned in one of the emitters of a chip-based nano-ESI for the ionization. The main advantages of LESA are the fully automatization and the use of disposable tips, which prevent contamination and carryover.

As mentioned before, method performance of plasma-based techniques is more affected by the physical design of the plasma source and the kind of plasma–sample interaction than by geometrical configurations (Ding and Duan 2015). Thus, in LTP and PADI, the plasma is generated in the sampling zone, whereas in DART, FAPA, and DAPCI, the plasma is isolated from the sample ionization region. The DART source (Figure 1.1) consists of two chambers through which the DART gas flows. In the first region, a dc corona-to-glow discharge (1–5 kV) generates the plasma, which is filtered by one or several perforated plate electrodes and heated before passing through a final grid electrode to interact with compounds thermally desorbed from the sample surface. This design allows the selective removal of ionic species from the heated plasma gas (typically He), thus only electronically excited metastable atoms arrive to the outside region. In DART, the sample is placed near the source outlet with minimum disturb of the gas flow to the MS inlet, being geometries for direct desorption and for transmission commercially available. By contrast, in DAPCI, the plasma species are transferred to the sample region without any filtration (Figure 1.4). This ambient source is built by simple modifications of the conventional APCI source, where gaseous reagent ions, generated by atmospheric corona discharge, are sprayed through a pipette tip and impacted on the solid sample, thus producing the desorption and subsequent ionization of analytes. In FAPA, LTP, and DBDI, a two-electrode configuration with different power supplies, electrode shape, and discharge cells are used. For instance, FAPA operates in a current controlled glow discharge regime (~25 mA) applying a direct voltage (typically a few hundred volts) to a pin-to-plate or pin-to-capillary electrodes in a sealed discharge

cell that physically separates the source from the sample (Figure 1.3). Unlike DART, FAPA does not use any electrode to filter plasma species before interacting with the sample. Moreover, no external heating is needed in FAPA, since the temperature of the plasma stream is high enough (>200°C) for an efficient desorption of the analytes. Regarding ambient MS methods based on DBD, at least one dielectric layer has to be placed in between the electrodes, being necessary a high ac voltage to transport the current through the discharge gap. Typically, amplitudes of 1–100 kV and frequencies of a few Hz to MHz are commonly used. The difference between DBDI and LTP lays on the geometrical configuration. In DBDI source, a glass slide is inserted between a plate electrode and a needle electrode to function as dielectric material and sample plate (Figure 1.3). When ac high voltage (5–10 MHz) is applied between the electrodes, an LTP consisting of numerous transient micro-discharges is ignited, and analytes placed in the surface plate are desorbed and ionized. On the other hand, LTP uses a glass tube (typically, ~4 mm inner diameter and ~6 mm outer diameter) as dielectric material to physically separate the ring outer electrode from the metal pin internal grounded electrode, which is directly in contact with the flowing gas (Figure 1.3). The generated plasma interacts with the sample, thus desorbing and ionizing analyte molecules. Since thermal desorption processes clearly play a major role in plasma-based techniques, temperature becomes a critical parameter to improve sensitivity. As commented before, FAPA does not use additional heating since the heat generated in the source is enough to desorb analytes from the sample surface. By contrast, DART uses an external heating to desorb semi-volatile and low-volatile compounds. In fact, a selective detection of thermal-dependent compounds can be performed in DART by ramping the temperature of the discharge gas to produce the thermal fractionation of the compounds in the sample surface. PADI is another "proximal" plasma-based technique, where high-energy plasma species interact directly with the sample. Hence, an rf glow discharge is set at the end of a pin electrode, which comes in direct contact with the sample, that works as ground electrode.

Some thermal desorption techniques are not plasma-based. Among them, DAPPI has increased its popularity in recent years because it can be applied for the analysis of low-polarity compounds (Kauppila and Kostiainen 2017). In this technique, a heated microchip is used to deliver both a narrow jet of vaporized solvent (dopant) and a nebulizer gas onto the sample surface, thus achieving surface temperatures up to 350°C for the desorption (Figure 1.4). Ionization is initiated by photons emitted by a vacuum UV lamp, typically a krypton discharge lamp that emits 10 eV photons, although dopants such as toluene, acetone, or anisole (typically added at 10 μL/min) are required to ionize those compounds with ionization energies below that of the photons.

For the two-step laser-based desorption/ablation techniques, the key parameters affecting their figures of merit are the characteristics of the lasers used for ablation or desorption of the analytes from the sample surface, the ionization method, the use of matrix, and the geometrical configuration (Javanshad and Venter 2017). Both UV and IR lasers are used to generate the plume of particles, clusters, and free molecules, which are subsequently ionized in a separated step. For instance, UV nitrogen lasers, mainly at 337 nm operating at 10 MHz with a pulse length of 4 ns and a pulse energy of 20 μJ, are used in ELDI. Although, in most of the techniques as for instance in LAESI, IR lasers typically tuned at 2,940 nm with pulses of 5 ns duration at 2–20 Hz and pulse energy between 100 and 52 mJ are used (Figure 1.1). As regards the ionization step, the most popular is electrospray ionization, which is used in techniques such as LESI, LAESI, and in MALDESI where an exogenous matrix is used to improve ionization. Although both

reflection and transmission approaches are used in laser-based techniques, most of the geometrical implementations use reflective geometries with incident laser beams hitting perpendicularly the sample. However, other incident angles such as 45° with respect to the sample have also been used in some cases. In the less-used inverted geometry (transmission mode), the laser beam from the backside illuminates the sample, and the plume of desorbed material exits from the front side in the opposite direction of laser application.

Solvents, Gases, and Sample Devices

In liquid extraction-based methods, a solvent is always used, and its selection is a key aspect to be considered. In spray desorption techniques such as DESI, a spray of solvent charged droplets is used to perform the desorption/extraction of compounds and to ionize the analytes, whereas in EESI and in liquid microjunction techniques, the solvent extraction takes place in line with an electrospray emitter. Solvent composition affects both the initial spray droplet size that depends on both the surface tension and the dielectric constant of the solvent, as well as on the capacity of solubilizing sample component (Javanshad and Venter 2017). In general, organic/aqueous binary mixtures containing 50%–80% of methanol or acetonitrile are used to dissolve analytes. Moreover, the solvent system has to be electrically conductive and typically, when working in positive ion mode, acetic or formic acid (0.1%–0.5%) is used to increase ionization efficiency. DESI is generally indicated for polar compounds that can be easily ionized by controlling the pH of the solvent mixture. Even so, low polar and hydrophobic compounds have also been analyzed using nonpolar solvents such as toluene (Green et al. 2010). In this case, the solvent is ionized by electrochemical oxidation at the metal spray needle interface with the subsequent ionization of analytes by charge-transfer processes. As regards flow velocities, both sheath gas and liquid solvent flow rates, which are interdependent, affect the performance of the technique. A threshold velocity of gas flow is required for an efficient production of small secondary droplets, but values above the optimum generate a spray focused in a small spot, reducing the amount of desorbed material. This effect can be compensated by increasing the solvent flow rate. Typical gas pressure values (N_2) ranging from 120 to 150 psi and solvent flow rates from 2 to 10 µL/min are used to maximize the ion signal (Bodzon-Kulakowska et al. 2014). In nano-DESI, no sheath gas is needed since the flow rate for the liquid junction is governed by electrospray-induced flow generated by the nano-spray tip and additionally assisted by the vacuum in the MS inlet. In LMJ-SSP, typically suction flows of 10 µL/min are used by controlling inner and outer capillary diameters and gas flow. This technique can work in two operating modes: stepping and scanning. In stepping mode, a single spot of the sample is analyzed at a time, whereas in scanning mode, the liquid microjunction is dragged across the surface obtaining one- and two-dimensional images. In this last case, a thorough control of flow rate is needed since low flow rates worse the spatial resolution, while high flow rates and scanning speed decrease the signal intensity.

Today, the reduction of gas consumption in miniaturized plasma-based ion sources is an attractive feature for their implementation in mass spectrometer instruments for fieldwork. The discharge gases most frequently used are helium, nitrogen, argon, or air at flow rates that depend on the technique, ranging from <1.5 L/min in pin-to-capillary FAPA sources to ~3 L/min for DART. Although higher flow rates can increase signal

responses, they generally produce strong turbulences that worse reproducibility (Ding and Duan 2015).

In ambient MS, the addition of specific reagents can be used to enhance selectivity and sensitivity for the detection of certain compounds, thus increasing the range of chemicals accessible by a particular method. This is typically done by transforming the analyte to a permanent charged product or to a product that is easy to ionize. The most popular reactive ionization-based technique is reactive DESI (Wu et al. 2009). In this case, a derivatizing agent is added to the spray solvent, and chemical reactions occur in the short timescale of the solvent extraction/ionization/detection process (Laskin and Lanekoff 2016). Reactive DESI has been used for the detection of reaction intermediates that have lifetimes of the order of milliseconds (Girod et al. 2011). The addition of reagents to the solvent has also been used in most of the techniques based on electrospray ionization such as PS, EESI, or ELDI in order to improve ionization of compounds that are not easy to ionize (Venter et al. 2014). Similarly, in thermal desorption-based methods, reagent and solvent vapours can be added to modulate ionization by increasing differences in proton affinities or by generating other reactive species (Venter et al. 2014). For instance, *in situ* silylation or methylation has been performed in DART by depositing the reagent onto the sampling device placed in the heated gas stream or by adding the reagent to the discharge gas through a T-junction as in LTP.

It is currently assumed that one of the main characteristics of ambient MS techniques is their ability to analyze solid samples in its native form, as for instance, the analysis of fruit peels by simply holding them with a pair of tweezers in the plasma of a DART source. However, samples are sometimes deposited onto auxiliary sampler devices, such as glass slides and fused capillaries. The nature and characteristics of these holders can affect the performance of the methods. For instance, in DESI, nonconductive surfaces are typically used to prevent neutralization of the charged droplets at the surface. Moreover, surface wettability must be taken into account, since it affects both spray–surface and analyte–surface interactions. Several materials such as glass and paper, and polymers such as polytetrafluoroethylene (PTFE) and polymethyl methacrylate are used to spot liquid samples on them for the DESI analysis. In general, the best results are achieved with the nonpolar PTFE surfaces probably because the lower interaction with the analytes helps the desorption from the sample (Wiley et al. 2015). In DART, commercially available sampling devices included clamp-like tweezers or fingers to fix the sample (fabrics, disks, swabs, etc.) and holders for glass capillaries when analyzing liquids and powdered samples (Cody and Dane 2015). ELDI and LAESI use stainless steel target plates although silver or gold plates are recommended to prevent the formation of metal–analyte adducts. Several ambient MS techniques can work in transmission mode by using transmissive/porous materials. In techniques such as DESI, DART, and DAPPI, a screen mesh that supports or embeds the sample is placed in front of the DESI tip, the DART plasma, or the microchip in DAAPI. Afterwards, the plasma or heated solvent passes through the screen mesh to desorb compounds and, in some techniques, to ionize analytes. In this mode, the material type affects the coating of the sample onto the strand surface and both metallic and polymeric meshes, such as PEEK, are used as sampling substrates (Ding and Duan 2015). By contrast, laser ablation techniques use a transparent sample holder generally a glass slide or a fused silica capillary. In this case, the laser is transmitted through the sample holder and the plume of desorbed material exits in the opposite direction where the laser is applied (Monge et al. 2013).

Regarding substrate spray techniques, samples or sample substrates having sharp tips (such as sticks, paper triangles, and needles) are used. In PS, a metal clip, connected to a dc high voltage (3–5 kV) power supply, holds a triangular paper placed in front of the MS inlet (Figure 1.1). The characteristics of the paper substrate and the solvent have an important effect on the efficiency of the method. Paper substrate (filter paper, glass fiber paper, silanized paper, chromatography paper, etc.) influences the interaction between the analyte and the paper, and the triangle tip corner angle (30°–45°) has a high impact on the generation of electrospray. Moreover, the nature of the solvent affects both the extraction efficiency and the ESI. Generally, PS applications use methanol/water mixtures, although less-polar solvents can also be used to extract less-polar analytes (Liu et al. 2015). By contrast, PESI uses a solid needle to pick the sample, which is directly ionized by electrospray from the sharp tip of the probe (Hiraoka et al. 2008). One of the advantages of working with a solid needle, in front of other techniques that use capillary tubes, is that clogging is prevented. Disposable stainless steel acupuncture needles with a tip diameter below 1 μm are generally used, although other kinds of conductive needles and even surface-coated wires for selective detection of specific compounds have also been used. The solid needle probe is mounted in a system that allows driving it up and down and adjusting it to a position just touching the biological or liquid sample. The sample volume picked up depends on the size of the needle, the hydrophobicity of needle surface, the dipping depth, and the viscosity of the sample. After the sample collection, the needle is moved to the upper position located in front of the MS inlet, and a high voltage (~2 kV) is applied to generate the electrospray plume (Figure 1.5). Although for biological samples the small amount of water content (~pL) is enough for the ESI, the use of auxiliary spray solvents has also been proposed to improve ionization. Needles have also been used for the direct spray of tissue samples in MS analysis biopsy, and this strategy could be applied to the analysis of meat/fish tissues (Monge et al. 2013). In this case, the spray solvent wets the tissue sample held in the needle tip, and a high voltage directly applied on the metal needle generates charged droplets containing compounds extracted from the tissue. Another technique that has been used for the analysis of tissue materials is REIMS, where a high-frequency electric current is applied to surgical blades to produce the ablation of tissue material and to lead to the formation of an aerosol that contains charged species (Schäfer et al. 2009). In a typical setup, ions and aerosols created during electrosurgery are aspirated by a Venturi air jet pump, which allows transporting the thermally generated ions to the mass spectrometer for analysis.

Ambient MS Imaging

One interesting application of ambient ionization MS techniques is mass spectrometry imaging (MSI) (Laskin and Lanekoff 2016). MSI enables to acquire spatial information and identification of chemical compounds distributed on a sample surface. Although secondary ion mass spectrometry and matrix-assisted laser-desorption ionization have been extensively used for MSI, today ambient MS is also applied taking advantage of its ability to analyze a surface at atmospheric pressure with any pretreatment. Techniques such as DESI, EASI, LMJ-SSP, nano-ESI, PESI, DAPPI, and LTP, and those based in laser desorption combined with ESI or with chemical ionization have been developed. For data acquisition, small areas (pixels) of the sample surface are scanned individually and sequentially, and an automated moving stage is used to assure the reproducibility of the scan velocity. Recorded mass spectra are converted to 2D images by using adequate image software. Important

characteristics of these techniques are as follows: spatial resolution, sensitivity, and ability to perform depth profiling. Lateral resolution mainly depends on the spot size of the ionizing agent although it is also limited by operating conditions. However, it must be taken into consideration that when working at high spatial resolution, a high acquisition time is required to overcome the decrease in sensitivity. In DESI, the characteristics of the sprayer (solvent, flow rate, nebulizer gas pressure, and tip dimensions) are important to obtain a high-quality MS image. At standard conditions, lateral resolutions of 150–250 μm are obtained although values as low as 40 μm can be found if the distance between the sprayer and the surface is decreased to 400 μm (Kertesz and Van Berkel 2008). Resolution of LMJ techniques is mainly determined by the size of the capillaries and the ability to control the distance between the sample and the probe. In LMJ-SSP, resolution values from 500 to 1,000 μm are obtained (Laskin and Lanekoff 2016), while for nano-DESI, where two independent small diameter capillaries are used, better resolutions (100–150 μm) are typically found and values down to 12 μm have been obtained using pulled capillaries (Laskin et al. 2012). The size of the pipette tip is the main reason why LESA spatial resolution is worse than in other liquid extraction-based ambient MS techniques (~1 mm). In PESI, a good spatial resolution (60 μm) can be obtained by controlling the diameter of the needle probe, the space in between two adjacent holes, and the depth of penetration of the needle. In PESI imaging, an auxiliary solvent sprayer is typically used to rewet the sample adhered to the needle to facilitate the electrospray process. For DART and related thermal desorption-based techniques, the ability to generate spatially resolved information is very limited, and lateral resolutions are typically of 1 mm. The best results, resolutions of 200 μm, are obtained with LTP focusing the plasma onto a small area of the sample surface. One advantage of LTP is that depth profiling can be performed simply by increasing the ablation time of the plasma torch. Ablation can also be easily obtained using lasers and the coupling of laser ablation to electrospray for ionization of the neutrals ablated from the sample provide IMS techniques with the best lateral resolution and with the additional advantage of being able to perform *in situ* depth profiling, thus providing a three-dimensional (3D) reconstruction of molecular distributions. The most popular techniques are LAESI and ELDI using nanosecond lasers, mid-IR and UV, respectively. In LAESI, a lateral resolution of 250 μm is typically obtained, although this value can be reduced to 50 μm when using an optical fiber to deliver the laser light. The ablated plume generated either in transmission or reflection mode can also be captured into a single drop or a continuous flow of solvent in a probe similar to LMJ-SSP procedure, thus providing very good spatial resolutions (50 μm) in transmission mode (Laskin and Lanekoff 2016). The use of femtosecond mid-IR lasers allowed to achieve very good lateral resolutions down to 10 μm although focusing of the laser is needed to obtain reproducible results (Sarkar et al. 2009).

SAMPLE HANDLING

Advantages of ambient MS techniques such as short analysis time (in seconds) and operational simplicity made these techniques highly attractive for high-throughput applications, which quickly led to the development of new strategies to adapt the ambient MS technique to specific samples and analytical needs. Although the original objective of ambient MS techniques is to perform real-time analysis without additional sample preparation, with the exception of the sample processing that takes place during the analysis, sample manipulations are sometimes needed. However, this sample handling should be minimal, simple, and fast to be compatible with the philosophy of ambient MS methods.

Before the analysis of food and environmental samples with ambient MS techniques, fast and simple manipulation procedures are often necessary when dealing with matrices such as solids, non-smooth surfaces, semisolids, or viscous samples. For instance, powdered samples such as powdered milk and spices have to be solvent-extracted or solvent-agglomerated before the analysis with gas-assisted nebulization techniques such as DESI and DART. These simple strategies might prevent the carryover contamination by blowing the sample, which could let to false-positive results in the analysis of subsequent samples (Nielen et al. 2011; Zhang et al. 2015; Yang et al. 2009). For solid, high viscosity or gelatinous semisolid samples (i.e., oils and jelly), a simple extraction or a sample dissolution is enough before their analysis by ambient MS techniques that are more suitable for liquid samples such as PS or EESI (Zhang, Cooks, and Ouyang 2012; Garrett, Rezende, and Ifa 2013; Huang et al. 2014; Liu et al. 2016). Moreover, trituration and homogenization of solid samples are sometimes recommended to guarantee the representativeness of the analyzed sample (Sero et al. 2015).

Another question to be considered is that the surface analysis of solid samples only reveals what is present on the sample surface, which may be a handicap for some food and environmental analytical purposes. For instance, when analyzing leaves with some ambient MS techniques, the plant cuticle, a wax protective layer on the upper epidermis of the leaf, prevents the desorption/ionization of compounds in inner layers of leaf. For this reason, the removal of wax layer by washing the leaf surface with nonpolar solvents (i.e., hexane or chloroform) and/or cryosectionation of plant tissues to detect analytes coming from inner layers of leaves are frequent procedures before the ambient MS analysis. Other interesting procedures that can be used are imprinting strategies, where plant tissues are pressed (imprinted) on a substrate such as tape, thin-layer chromatography (TLC) plate, or porous Teflon to transfer the compounds from the plant to the substrate for a later ambient MS analysis or imaging (Laskin and Lanekoff 2016; Janfelt 2015). Thermal imprinting has also been used for the EASI-MS lipid profiling of meat, fats, and fish for food authentication (Porcari et al. 2016). In this strategy, the fatty tissue is wetted with a small amount of solvent, and the surface is heated before imprinting into a piece of paper.

The use of a minimal sample manipulation/treatment is also justified when sample components cause severe matrix effects and difficult matrix interferences unavoidable via high-resolution MS or tandem MS. To overcome matrix interferences, fast separation strategies have also been applied in combination with ambient MS methods. In DART, the temperature of plasma gas can be varied in time to favor a thermal separation of compounds of different volatilities (Edison et al. 2011). Furthermore, one important drawback of ambient MS methods relies in their sensitivity. The use of chromatographic substrates in ambient MS analysis has been proposed for a simple sample purification to improve limits of detection (LODs) of compounds to be identified in complex samples. TLC has been used as off-line technique for the purification of sample extracts before the ambient MS analysis. Other chromatographic techniques such as gel-permeation chromatography and reversed-phase chromatography have also been used to separate the components (Chen et al. 2017; Hajslova, Cajka, and Vaclavik 2011; Lu et al. 2017; Black, Chevallier, and Elliott 2016).

Most of ambient MS methods suffer from high LODs, which is not a problem for most applications such as food fraud and food profiling/authentication, but it is an important disadvantage when the analytical objective is the detection of analytes at trace levels in complex samples such as in food safety and in environmental analysis. For this reason, more extensive and time-consuming sample preparation protocols, typically

used for more traditional analytical techniques, have been found to be necessary in some qualitative and quantitative applications. For instance, dispersive SPE procedures such as QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) protocols improved LODs and limits of quantitation when screening and quantifying trace levels of pesticides and mycotoxins in food samples by DESI, LTP, and DBDI-MS (Chen et al. 2017; Kauppila and Vaikkinen 2014). By using these sample treatment strategies, the good sensitivity and reproducibility of the method demonstrated that ambient MS techniques can easily be used as an alternative for high-throughput pesticide residue analysis (Porcari et al. 2014; Mattarozzi et al. 2016; Mirabelli, Wolf, and Zenobi 2016). On the other hand, to determine low levels of analytes in liquid food samples, LLE, SPE, liquid-phase microextraction, and solid-phase microextraction (SPME) procedures have been established, thus allowing the cleanup of the extracts and the preconcentration of analytes (Cajka, Riddellova, Tomaniova, et al. 2011; Chen et al. 2017). These extraction procedures have been used for monitoring/screening studies and quantitative analysis of target compounds taking advantage of sensitivity improvement (up to 700-fold) and matrix effect reduction. The distinctive future of SPME is that the fiber is frequently used as a probe in ambient MS analysis. In this context, three different strategies have been applied. In one of them, the SPME fiber is directly analyzed with spray-based ambient MS techniques by applying a high voltage and a solvent to the SPME probe, thus inducing an electrospray at the tip of the fiber to ionize the analytes via ESI mechanisms. The second strategy is based on the surface desorption/ionization of analytes by the impact of charged solvent species via DESI and LTP on the SPME fiber surface. Finally, the third strategy is based on desorption of the analytes through nebulization, laser desorption, laser ablation, thermal evaporation, or thermal desorption from SPME probe and a later ionization such as DART. The developments of highly selective adsorbents towards target compounds, novel coating materials for improvement of both extraction and ionization efficiencies, and new coupling strategies to enhance hyphenation properties are highly encouraging (Fang et al. 2016).

APPLICABILITY AND METHOD PERFORMANCE

Today, ambient MS techniques have been applied for a wide range of applications in food and environmental analysis, and the easiness of implementation and the use of ambient MS systems for on-site analysis by nonexpert users bring a shift in MS applications for routine analysis in quality control laboratories. Furthermore, the new developments in miniaturization of mass spectrometers, designed for the on-site analysis, can be a valuable alternative to current in-lab MS analysis operations.

Food quality and safety assurance in food supply chain are crucial for public health and world sustainability. Most of the food products available in supermarkets are susceptible or have already been exposed to some form of food fraud, which increase the concern for authorities and food industry. As can be seen in Table 1.2, the application of many ambient MS techniques to the food supply chain, such as production, processing, storage, or transportation, has been proven that ambient MS techniques can play a key role in this field, thus providing important information about food components (e.g., lipids, hormones), contaminants (e.g., pesticides, veterinary drugs, mycotoxins), authenticity, and traceability (Vaclavik et al. 2009; Black, Chevallier, and Elliott 2016). Moreover, ambient MS techniques have also been applied for the rapid analysis of contaminants present in environmental samples such as polycyclic aromatic hydrocarbons (PAHs), volatile

organic compounds, and perfluorinated compounds in soil and environmental water (Chen et al. 2017; Luosujärvi et al. 2010). However, the need for detecting analytes at trace levels makes the direct analysis by ambient MS techniques less attractive, and sample manipulation strategies are usually required.

As mentioned earlier, two of the most powerful characteristics of ambient MS techniques are the ability for high-throughput analysis and the capacity to deliver a wealth of chemical information with unprecedented easiness. These main characteristics make these techniques very attractive for the screening of targeted and untargeted compounds, which is one of the main applications of ambient MS in food and environmental analysis. The screening of pesticides, phenolic compounds, or triacylglycerol compounds in several food samples has been successfully achieved (Deng et al. 2017; Black, Chevallier, and Elliott 2016). However, one of the problems when performing the direct analysis of fruits, vegetables, and plants is the high variability of the sample surface composition from unit to unit, which can be a problem regarding sample representativeness. An alternative to surface analysis, porous cotton, or polyester swabs/disks damped with a solvent was used to extract target compounds such as pesticides from the fruit surface by swabbing the surface of multiple fruit pieces of a sample batch before ambient MS analysis (Edison et al. 2011; Edison, Lin, and Parrales 2011). Moreover, signals between repeated measurements of a given sample often show high fluctuation during the ambient MS surface analysis, but the correct use of ions ratio as well as the normalization of the responses by using internal standards can correct the poor repeatability observed. Although this relatively poor reproducibility can be acceptable for screening analysis, it may be critical for quantitative analysis as well as for the comparison between groups of a high number of samples for fingerprinting approaches such as food authentication or food traceability.

In order to improve LODs, high-resolution mass analyzers (i.e., TOF and Orbitrap) have been used taking advantage of both the high sensitivity and the selectivity of these instruments. This approach allows achieving LODs below the maximum residue levels established for a large group of compounds in food safety and fulfilling the legislation in environmental analysis. However, efforts are still devoting to detect analytes at trace levels when dealing with highly complex samples (biological, environmental, food, or even small organisms), being sample manipulation strategies necessary in order to obtain satisfactory LODs. The choice of the sample manipulation largely depends on the amount of matrix interferences, the sensitivity of the compounds of interest, and the selectivity required for the analytical objective.

In the last 5 years, the use of ambient MS techniques in metabolomic studies has exponentially increased due to its potential for providing a wealth chemical information, for its high capacity to identify potential markers and for its capability to classify samples based on the ambient MS profile as fingerprint (Clendinen, Monge, and Fernández 2017). Targeted and untargeted approaches have been explored for plant biology and agricultural studies, DESI, leaf spray, DART, LAESI, and PESI being some of the used ambient MS techniques. Good results were obtained for most of these techniques for classification and discrimination analysis using multivariate statistical approaches such as principal component analysis. It must be mentioned that laser-based techniques such as LAESI were found to be challenging for plant metabolomics since the ablation process of the material was highly affected by the water content of the sample, so that the data obtained must be carefully evaluated in order to get reliable conclusions (Etalo et al. 2015). Regarding foodomics, much attention has been given to food quality and safety as well as to genetically modified foods. By far, DART-MS has been the most used ambient

MS technique in this field, and it has been implemented within targeted metabolomics workflows, including metabolomics fingerprinting for beer origin recognition, authentication of milk products, differentiation between organic and conventional fruits, or for olive oil authenticity assessment. However, untargeted approaches are still a challenge, and the extraction efficiency of metabolites of interest needs a further improve for a better sensitivity. Furthermore, sample preparation protocols that involve metabolite extraction or sample grinding into powder before analysis have a drastic impact on the compounds that can be detected.

Regarding quantitative analysis, accurate methods are necessary to ensure that the concentration of the contaminants detected in environmental and food safety analysis does not exceed the maximum legislated levels to survey both the risk on human health and the environmental quality standards. There is still some debate about if ambient MS techniques are suitable for quantification and if they are able to guarantee the absence of false-positive and false-negative results. Among the quantification methods developed with ambient MS techniques, those applied to liquid-based samples showed the best accurate quantification results (Räsänen et al. 2014; Black, Chevallier, and Elliott 2016; Chen et al. 2017). This fact is related to the intrinsic difficulty to obtain reliable results via direct surface analysis, and most of the limitations are related to the representativeness of the sample with respect to the entire sample batch. Moreover, the use of internal standards does not allow solving this problem since it is not possible to guarantee the uniform spiking on the sample surface. In this context, sample manipulation is needed in order to get accurate and reproducible quantitative results and opens the possibility for matrix-matched calibration when dealing with complex samples.

CONCLUDING REMARKS

Considering the continuous developments regarding the technology field, predicting the directions of ambient MS techniques in food and environmental applications is still a challenging task. However, the attractive features of these techniques such as real time and *in situ* analysis, low sample requirements, and high throughput and minimal sample preparation will clearly boost the expansion and the use of ambient MS in environmental and food analytical laboratories.

Today, most of the new ambient MS applications published in food and environmental analysis are still homemade prototypes for proof-of-concept studies. Thus, more work needs to be done to understand which ambient MS techniques have the capabilities for the development of real routine analytical methods. Until now, DESI, DART, EESI, PS, and LAESI have shown the best capabilities to scope the demands for the modern analytical laboratories. Furthermore, the continuous effort on the automatization and robotization of the initial prototypes will improve the reproducibility necessary for some important food applications such as food authenticity and food profiling. On the other hand, it is clearly demonstrated that ambient MS techniques are very attractive for the screening of a large number of compounds. Thus, the development of multimode and/or hybrid ambient MS techniques would allow concurring different ionization mechanisms in order to expand the simultaneous detection of a broader spectrum of chemical species, obtaining additional chemical information of a sample in a single experiment. Although no additional sample manipulation than the *in situ* sample processing is necessary in most applications, the matrix complexity is still a challenge. Hence, the combination of ambient MS techniques with well-established simple and fast

sample pretreatment procedures may be necessary for reducing both the matrix interferences and matrix effects. The combination of ambient MS techniques with in line and/or *in situ* sample processing procedures should be considered to overcome these problems. Moreover, the need to detect contaminants at trace levels and the accurate surface quantification remains some of the most important difficulties in ambient MS analysis. Finally, the development of hand-portable mass spectrometers using ambient ionization sources would allow greatly enhancing the *in situ* analysis of food and environmental samples directly in the field.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support received from Spanish Ministry of Economy and Competitiveness under the project CTQ2015-63968-C2-1-P and from the Agency for Administration of University and Research Grants (Generalitat de Catalunya, Spain) under the project 2017SGR-310. Raquel Sero is grateful to the University of Barcelona for an Ajuts de Personal Investigador predoctoral en Formació (APIF) grant.

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1.1.2 PUBLICACIÓ II

Ambient Ionisation-High Resolution Mass Spectrometry: Environmental, Food, Forensic and Doping Analysis

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En el llibre *Applications of Time-of-Flight and Orbitrap Mass Spectrometry in Environmental, Food, Doping and Forensic Analysis*. Editat per, S. Pérez, P. Eichhorn, D. Barceló. (2016) 71:51-88. Elsevier

Chapter 3

Ambient Ionisation—High-Resolution Mass Spectrometry: Environmental, Food, Forensic and Doping analysis

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Chapter Outline

Comprehensive Analytical Chemistry, Vol. 71. http://dx.doi.org/10.1016/bs.coac.2016.01.003

1. INTRODUCTION

Mass spectrometry (MS) is nowadays a powerful and a wide-range technique used in many analytical and bioanalytical laboratories. This technique combines the high sensitivity, selectivity and speed with the capability to analyse a wide range of molecules and mixtures, from relatively small and thermally stable organic molecules to almost all types of organic compounds, inorganic salts, organometallic complexes, biomolecules and even supramolecular entities and biological species (viruses and bacteria). Although most MS systems need less than 1 s to produce ions and to acquire the signal, many currently used MS-based analytical methods often take several hours to complete the qualitative and/or quantitative analysis of complex samples. For conventional MS-based analytical techniques such as gas chromatography−mass spectrometry (GC−MS) and liquid chromatography−mass spectrometry (LC-MS) long and tedious sample treatment protocols and time-consuming chromatographic separations are generally required before the MS analysis. Therefore, sample preparation process and chromatographic separation became the bottleneck of many routine laboratories thus constraining the analytical efficiency of MS-based methods. Additionally, sample treatments and chromatographic analyses introduce a certain level of compound discrimination not desirable in screening analysis.

'Direct analysis' refers to the analysis of samples in their native state with little or no sample preparation. Nevertheless, before the MS analysis, samples must be placed into the vacuum system or ions must be efficiently transferred from atmospheric pressure into the vacuum system of the mass analyser. Solid samples can be directly analysed by techniques such as secondary ion mass spectrometry (SIMS), fast atom bombardment, or matrix-assisted laser desorption ionisation (MALDI). Gaseous samples can be directly ionised by electron ionisation or chemical ionisation and in liquid samples, although they can be accomplished by the latter methods, ions can be generated and transferred into the vacuum interfacing atmospheric pressure ionisation (API) sources. However, API sources are not able to perform the direct analysis of samples under ambient conditions and frequently require sample preprocessing.

At the begining of XXI century, a new group of ionisation techniques has been developed making real the analysis of almost anything instantaneously. Nowadays, we can get useful information from almost everything with minimal effort just by holding an object in open air in front of a mass spectrometer and recording the mass spectra in real time. These group of techniques have been named 'ambient ionisation' techniques [1] and they comprise a large and a growing family of sampling/ionisation sources that allow the generation of ions under ambient conditions at atmospheric pressure and in their ambient states with minimal or no sample preparation or chromatographic separation prior to analysis. However, it must be pointed out that with ambient ionisation

techniques, the sample processing takes place in situ during the analysis, so it would be more precise to say that ambient ionisation techniques frequently require 'no additional sample preparation' [2]. Over the past 10 years, many distinct ambient techniques have been introduced differing in the way that the variety of sample processing methods and ionisation mechanisms are combined or implemented [3–7] and their classification is still a controversial subject. Different authors have attempted to categorise ambient ionisation techniques mostly on the basis of their dominant desorption and/or evaporation principles (spray, aerosol, laser, plasma, heating, or acoustic radiation), but there is much overlapping of very closely related techniques and the tendency to create a new acronym every time a new variant is added. With the objective to simplify and to rationalise the classification, it has been proposed to categorise ambient MS techniques on the basis of the parent ionisation method from which they have been developed [8], or even taking into account the intrinsic desorption/ionisation mechanism [9]. Table 1 summarises the acronyms and the primary reference for some representative ambient techniques. They have been simply grouped into three wide categories: spray-, plasma-, and laser-based techniques.

Among the ambient ionisation MS techniques, desorption electrospray ionisation (DESI) [1] and direct analysis in real time (DART) [18] are definitely the two pioneer ones that prompted the development and broad applications of a large number of ambient ionisation methods (Table 1). Nevertheless, sonic-spray ionisation (SSI) [14] and secondary electrospray ionisation (SESI) were introduced c. 10 and 4 years previously and sporadic reports describing ambient MS approaches appeared earlier. A full and detailed revision about ambient ionisation MS techniques has been published in 2015 by Marek and Cody [3]. This book includes basic concepts and terminology, as well as applications and theory providing a comprehensive treatise devoted to this subject.

Ambient MS techniques can easily be coupled to most types of mass spectrometers equipped with atmospheric pressure interfaces and without any modification to the ion optics or the vacuum interface. Nevertheless, the information that we can obtain from ambient ionisation techniques depends on the information content that the mass spectrometer can provide. The complexity of the mass spectra obtained from the raw sample, without the need for sample treatment and chromatographic separation, makes necessary to rely on the capabilities of the MS system. Initially, ambient MS techniques were coupled to low-resolution mass analysers (ion traps and quadrupoles) demonstrating the applicability of these techniques, mainly for target analysis [4,8,9]. Nevertheless, the complexity of mass spectra obtained from complex samples and the application of these ionisation techniques to the screening of non-target compounds and unknown substances require MS instruments able to acquire data at high resolution and/or to perform tandem MS experiments to extract the maximum information. The ability to filter highly probably isobaric

TABLE 1 Acronyms and Primary Reference of Some Representative Ambient Mass Spectrometry Techniques

Acronym	Name	First Reference
Spray-Based Techniques		
DESI	Desorption electrospray ionisation	[1]
DAPPI	Desorption atmospheric pressure photoionisation	[10]
EASI	Easy ambient sonic-spray ionisation	[11]
PSI	Paper spray ionisation	[12]
SESI	Secondary electrospray ionisation	[13]
SSI	Sonic-spray ionisation	[14]
Plasma-Based Techniques		
ASAP	Atmospheric pressure solids analysis probe	[15]
APTDI	Atmospheric pressure thermal desorption/ ionisation	[16]
DAPCI	Desorption atmospheric pressure chemical ionisation	[17]
DART	Direct analysis in real time	[18]
DBDI	Dielectric barrier discharge ionisation	[19]
LTP	Low-temperature plasma	[20]
PADI	Plasma assisted desorption/ionisation	[21]
Laser-Based Techniques		
ELDI	Electrospray-assisted laser desorption ionisation	[22]
LAESI	Laser ablation-electrospray ionisation	[23]
MALDESI	Matrix-assisted laser desorption electrospray ionisation	[24]

interferences and to provide accurate mass values and accurate isotope patterns makes feasible the unequivocal identification of compounds from MS data obtained without the benefit of sample treatments and/or previous chromatographic separations. Ambient MS techniques have been successfully coupled to mass spectrometers equipped with different high-resolution mass analysers such as time of flight (TOF), Orbitrap, ion cyclotron resonance (ICR) as well as hybrid instruments such as quadrupole-TOF, linear trap-Orbitrap, and quadrupole-Orbitrap [4,18,20,25–28]. High-sensitivity full-scan mode, high

mass resolving power and capabilities for tandem MS experiments are the main characteristics desirable for those mass analysers to be coupled with ambient techniques.

Because ambient ionisation is a rapidly growing topic within the MS field, new publications describing new ambient ionisation methods are continuously appearing and widening the application areas. For this reason, this chapter presents a snapshot of the field and focuses on the most relevant ambient ionisation techniques within the scope of this book. The application section includes several interesting selected publications to illustrate the usefulness and applicability of ambient ionisation methods coupled to high-resolution MS (ambient HRMS) for environmental, food, doping, and forensic analysis.

2. AMBIENT IONISATION METHODS

This section briefly describes some relevant ambient MS techniques to provide a basic knowledge and the fundamentals needed to understand the ionisation mechanisms and the setup configurations.

2.1 Desorption Electrospray Ionisation

DESI is the first ambient ionisation method that was introduced in 2004 by R.G. Cooks et al. [1,17] and it made possible the concept of open-air surface analysis under ambient conditions. The basis of DESI is unquestionably electrospray ionisation (ESI) in combination with a built-in sampling step based on solvent extraction. In DESI, a solvent or solvent mixture is electrosprayed, under strong pneumatic assistance, onto a sample surface at an impact angle α. The mist composed of charged microdroplets, ionic clusters, and gas-phase solvent ions, generated during the electrospray process and driven by the high-velocity gas stream, receive sufficient energy to impact onto the sample surface. Three mechanisms of ion formation have been proposed for DESI [2,29] and one of these mechanisms will predominate depending on the experimental conditions and the solvent—analyte pair. The first one described as 'droplet pick-up' involves the initial wetting of the surface by the first arriving microdroplets and analyte dissolution into them. Later, the splashing caused on the arrival of subsequent charged droplets results in the emission of secondary electrically charged microdroplets that contain dissolved analyte. These small droplets assist in the formation of dry ions by ion evaporation or via charge-residue model as occurs in the standard ESI experiments. Finally, analyte ions are transported away from the surface at an angle β (Fig. 1). The evaporation of the solvent and Coulomb fission will generate ions by processes analogous to conventional ESI. Finally, the nearby mass spectrometer inlet inhales a portion of the mist of the secondary electrospray. The second mechanism, described as 'condensed phase charge transfer', supposes the analyte ion desorption to occur by a type of chemical

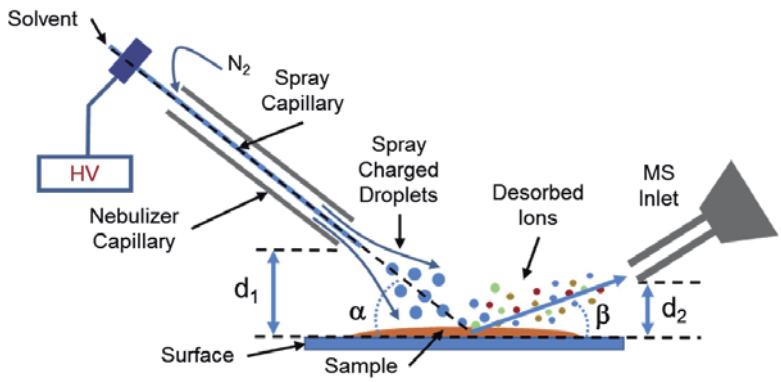

FIGURE 1 Schematic of the DESI-MS interface.

sputtering. The ions bombarding the surface can induce chemical reactions where electrons, protons, or other small ions are transferred from the impacting microdroplets to the sample surface leading to the formation of volatile erosion products. The third mechanism, 'gas phase charge transfer', proposes ion formation after volatilization or desorption of neutral species from the surface into the gas phase. The ionisation may occur via proton/electron transfer or ion−molecule reactions at atmospheric pressure.

Similarly to ESI, DESI is able to desorb/ionise compounds ranging from small molecules to large molecules. The charged microdroplets pick up proteins and other large biomolecules from the surface, ionise them, and transport them to the mass spectrometer. In addition, gas-phase solvent ions in the spray protonate or react with analyte molecules on the surface thus generating ions from compounds that have low desorption energies, including volatile and semivolatile compounds, low-polarity molecules of smaller size, low-molecular-weight polar compounds, and certain inorganic ions. DESI is a soft ionisation technique that causes minimum fragmentation yielding low-energy intact quasi-molecular ions. In positive-ion mode, molecules are mainly ionised by proton transfer yielding protonated molecules $[M+H]^+$, but sodium $[M+Na]^+$, potassium $[M+K]^+$ or ammonium adducts $[M+NH_4]^+$ are also common. Moreover, radical molecular ion $[M]^{+\bullet}$ formation also occurs for particular analytes and positively charged molecules $[M]^+$ can be also present in the gas phase via ion evaporation for compounds with a permanent charge such as quaternary ammonium salts. Analogously, in negative-ion mode the $[M-H]^-$ predominates, but radical ions due to electron transfer $[M]^{-\bullet}$ and adducts such as $[M+Cl]^-$ or $[M+CH_3COO]^-$ can also be observed. Finally, large molecules with multiple ionizing sites can yield multiply charged ions quite often.

DESI introduces new parameters to the ESI experiments (Fig. 1). The relative position of the emitter vs the sample surface and the mass spectrometer inlet (α, d_1, β, d_2) has a direct effect on the ionisation process and the

sensitivity of the method. DESI solvent composition and analyte solubility in the DESI solvent (also called spray solvent) have an important effect on both desorption and transfer of analytes from the surface to the mass spectrometer, thus affecting the electrospray droplet formation (size and charge of primary droplets), the focus of the spray, as well as the solvent extraction and ESI of the analyte. Sample surface (substrate) also plays a crucial role in DESI performance since the DESI process involves the landing and the release of charged particles on/from the surface. The fundamental features of the solid surface, including its chemical composition and texture, severely affect the energy and charge transfer processes and consequently the ionisation efficiency in DESI. Thus, several important parameters such as limit of detection, signal stability, carryover and reproducibility of the DESI method can be influenced by the nature of the surface [30].

Reactive DESI

Cooks and collaborators have introduced a variant to DESI, termed 'reactive DESI'. This new ionisation method is based on adding reagents to the DESI solvent that can derivatise analytes in order to enhance desorption and/or ionisation [31]. By this strategy, chemical selectivity in DESI can be greatly increased, particularly for the less polar analytes and the weaker acid/base compounds. Reactive DESI has allowed the analysis of many compounds that are difficult to ionise such as steroids, triacylglycerol species, and saturated hydrocarbons [32–34], by converting them into charged products or derivative products that can be efficiently ionised. The primary reagent-containing droplets impact onto the sample surface and secondary droplets are emitted containing a distribution of both reagent and analyte, allowing reactions to occur in the short times between dissolution of the analyte and drying of the droplets in the inlet capillary. It has been described that reactions often occur at accelerated rates in the confined droplets and they may be favoured by the large change in some physicochemical properties; for instance, pH can greatly decrease in positive-ion mode as a result of the voltage applied and the decrease in the droplet size, thus promoting acid/base-catalysed chemistry [35]. As an example, some reactions that have been used in reactive DESI methods are shown in Fig. 2. Phenylboronic acid has been used to recognise *cis*-diol functionalities [36], while boric acid has been demonstrated to produce a selective DESI signal in their reaction with phosphonate esters commonly used as chemical warfare agents [37]. Two other reactions have been demonstrated to be useful for the DESI analysis of ketosteroids. One of them involves the addition of hydroxylamine in the spray solvent, which becomes protonated during the electrospray process and can attack the carbonyl of the steroid [34]. The other one is similar to the first except that the hydrazine, Girard reagent T (GT), was used to attack the carbonyl with the advantage of incorporating a permanent charge in the analyte molecule increasing sensitivity as a result of not having to ionise the derivative product

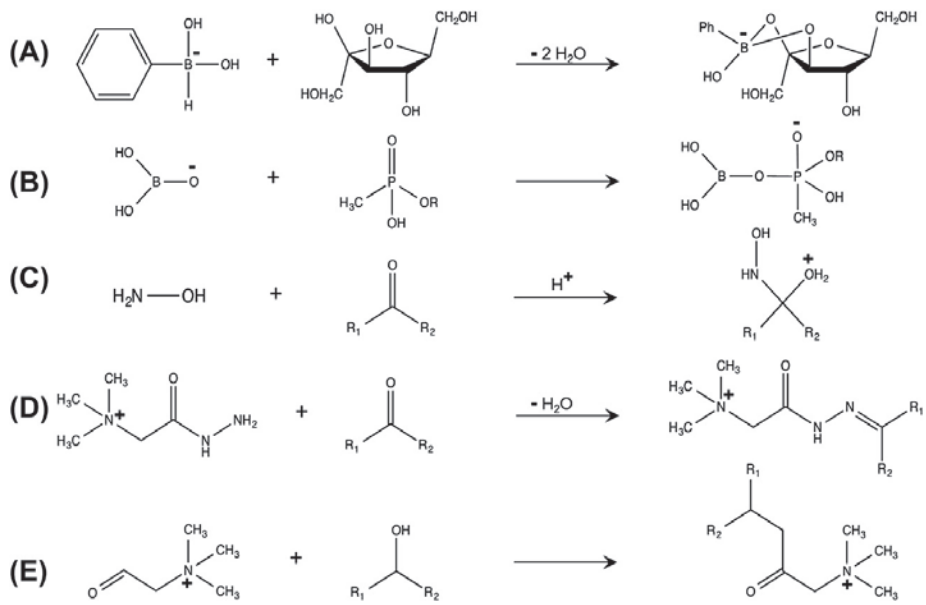

FIGURE 2 Chemicals reactions used in reactive DESI-MS.

[38]. Furthermore, betaine aldehyde has also been used to carry out nucleophilic attack of the alcohol functional group in cholesterol, which is difficult to ionise but can be easily detected with reactive DESI [31]. Finally, another reactive DESI approach that does not require covalent bond formation is through metal adduction, especially useful for those analytes that easily bind to charged metal ions. For instance, adducting reagents have been doped into the DESI solvent for various applications such as chloride and trifluoroacetate for explosives [39], $AgNO_3$ for the analysis of various unsaturated lipids and fatty acids [40], as well as ammonium for triglycerides [26].

DESI Imaging

Historically, SIMS and MALDI have been used for MS imaging with a spatial resolution of less than 100 nm with SIMS and at just a few micrometres with MALDI-MS. Nowadays, one of the most interesting applications of DESI is the two-dimensional (2D) mapping of chemical entities from a surface (DESI-MS imaging). Although DESI provides a lower spatial resolution (as low as 30 μm) than SIMS or MALDI, the advantage of imaging with DESI-MS stems from the ability to analyse a surface with no pre-treatment at atmospheric pressure preventing interferences from matrix ions, as occurred with MALDI-MS, and reducing sample contamination. Additionally, the non-destructive nature of DESI imaging allows the use of the interrogated sample for other studies without concern of any information loss. Applications of DESI-MS imaging have ranged from plant, animal and human tissues to human fingerprints for forensics [41–43].

Nano-DESI

Nano-DESI, a DESI-related technique, is based on a liquid micro-junction approach to ambient surface analysis [44] that uses two fused silica capillaries connected by a solvent bridge formed on the sample surface (Fig. 3). It involves a solid—liquid extraction mechanism as part of the desorption process, and does not employ a nebulising gas. While one capillary pumps the solvent onto the surface at an angle, the self-aspirating 'nanospray' capillary is positioned at an angle orthogonal to the surface and near the MS inlet. Solvent composition becomes a key factor in the sensitivity and selectivity of this ionisation method, allowing the efficient extraction of particular classes of compounds [27].

2.2 Other Spray-Based Ionisation Techniques

Extractive Electrospray Ionisation

Extractive electrospray ionisation (EESI) is a variant of SESI (Fig. 3) proposed to solve one of the limitations of DESI, to handle the most volatile molecules [45]. Because of quick evaporation of volatile compounds from the surface, DESI or related techniques have difficulties in picking up the molecules from the surface and then ionise/transport them to the mass spectrometer. In EESI, a vapour or a fine spray of neutral droplets containing the analyte molecules is dispersed into the stream of charged droplets produced by ESI. Then, molecules are incorporated into the charged droplets and become ionised via ESI-like process with the advantage of less important ion suppression due to matrix effects. This ionisation technique has been successfully used for the analysis of trace amounts of organic compounds in complex matrices including urine, food and polluted water [25,45].

Paper Spray Ionisation (PSI)

Paper spray ionisation, introduced for the first time in 2009 by Cooks et al. [12], is described as a three-step process: extraction of chemicals from the deposited sample, transport of the extracted analytes by the solvent spray and the generation of the charged droplets by a mechanism similar to ESI [46]. The original setup is extremely simple (Fig. 3). A triangular paper substrate is held in a metal clip that is connected to a DC high-voltage power supply and placed in front of the inlet of a mass spectrometer. The sample is deposited onto the paper triangle to form a sample spot and then a spray solvent wets the paper substrate. Finally, when the DC high voltage is applied, charge droplets are generated at the tip of the paper triangle carrying the analytes extracted from the deposited sample towards the mass spectrometer inlet. The paper substrate plays an important role in the solvent—substrate and substrate—sample interactions for the chemical extraction that allows a simple and fast but effective sample purification in real time prior to the spray ionisation. This

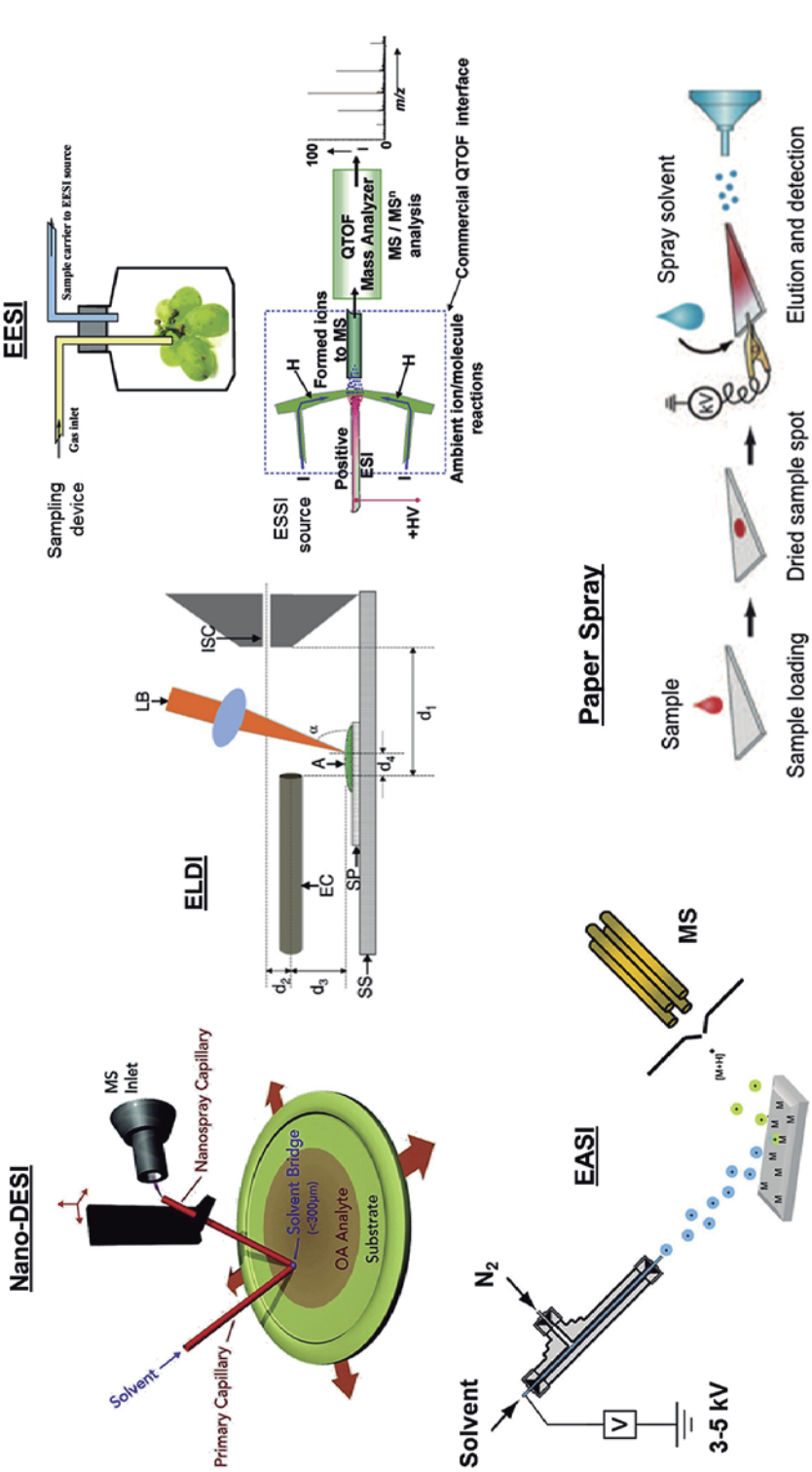

FIGURE 3 Schematic of some spray-based ambient MS interfaces: Nano-DESI *(Reproduced with permission from reference P. Roach, J. Laskin, A. Laskin, Anal Chem. 82 (2010) 7979–7986. Copyright (2010) American Chemical Society.)*, EESI *(Reproduced with permission from reference H. Chen, Y. Sun, A. Wortmann, H. Gu, R. Zenobi, Anal Chem. 79 (2007) 1447–1455. Copyright (2007) American Chemical Society.)*, ELDI *(Reproduced with permission from reference J. Shiea, M.Z. Huang, H.J. Hsu, C.Y. Lee, C.H. Yuan, I. Beech, J. Sunner, Rapid Commun. Mass Spectrom. 19 (2005) 3701–3704. . Copyright (2005) John Wiley & Sons, Ltd.)*, EASI *(Reproduced with permission from reference R.M. Alberici, R.C. Simas, G.B. Sanvido, W. Romão, P.M. Lalli, M. Benassi, I.B.S. Cunha, M.N. Eberlin, Anal Bioanal. Chem. 398 (2010) 265–294. Copyright (2010) Springer-Verlag.)* and paper spray *(Reproduced with permission from reference R.M. Alberici, R.C. Simas, G.B. Sanvido, W. Romão, P.M. Lalli, M. Benassi, I.B.S. Cunha, M.N. Eberlin, Anal Bioanal. Chem. 398 (2010) 265–294. Copyright (2010) Wiley-VCH.)*.

ionisation technique has been successfully used for the qualitative and quantitative analysis of a wide range of compounds from complex samples in applications fields including food safety, public safety, forensics, and biomedicine.

Easy ambient Sonic-Spray Ionisation (EASI)

Easy ambient sonic-spray ionisation (EASI), introduced in 2006 by Eberlin et al., uses SSI as the root technique sharing with it all the advantageous features [8,11,47]. In EASI (Fig. 3), in contrast to DESI, the need for a high-voltage power supply has been eliminated. Simply spraying a solvent pneumatically assisted by a gas, high-flow-rate charge droplets are produced. This sonic spray forms a stream of bipolar (±) charged droplets that bombard the sample surface producing efficient desorption/ionisation of the analyte molecules at ambient conditions. Although in EASI desorption and ionisation occur in solution, as in ESI and SSI, positive and negative ions are generated simultaneously, often from the same analyte. This fact makes possible to obtain more mass spectral information in a single run taking advantage of the polarity switching capabilities available in some instruments. In addition, improved signal-to-noise ratios can be observed with EASI compared with other ambient ionisation techniques, which may be due to the low concentration of solvent components on the droplets that may reduce solvent noise and due to the extreme softness of the ionisation process that does not produce in-source fragmentation, thermal degradation, electrochemical transformations or oxidation interferences [48].

EASI has been used in a number of applications including pharmaceutical degradation studies, MS fingerprinting certification and quality of olive oil from different geographic regions, identification of illicit drugs, document authenticity, etc. Recently, a new variant of EASI (V-EASI) has been developed for the analysis of liquid samples [49]. In the V-EASI the Venturi effect caused by the N_2 or air sonic flow is used to self-aspirate liquid samples into the standard EASI sprayer assembly. This strategy has been applied for the analysis of antioxidant additives in gasoline samples identifying the additive 'Santoflex' in some of the samples analysed by V-EASI coupled to Fourier transform ion cyclotron resonance (FT-ICR) MS.

Electrospray-Assisted Laser Desorption Ionisation (ELDI)

Electrospray-assisted laser desorption ionisation (ELDI) is an ionisation technique that combines laser desorption and post-ionisation by electrospray for the rapid analysis of solid materials under ambient conditions (Fig. 3) [22]. In fact, in MALDI more neutrals than ions are usually released from the sample layer and in ELDI these neutrals are desorbed close to an ESI plume, wherein the neutrals are then ionised by ion—molecule reactions. The standard conditions of ESI for ELDI involve a solvent mixture, flow rate and spray

voltage similar to that used in DESI. The ultraviolet (UV) pulsed nitrogen laser is adjusted to irradiate at an angle of ∼45 degrees the sample deposited onto a metallic insulated substrate, while spraying to force the post-ionisation of analytes. The mass spectra are characteristic of electrospray, including adducts and multiple charged ions. ELDI has been applied to the analysis of peptides and proteins, to detect chemicals on different surfaces and also to identify compounds on thin layer chromatography (TLC) plates.

2.3 Direct Analysis in Real Time

DART is the ambient ionisation source contemporary of DESI introduced by R.B. Cody in 2005 [18]. It is a form of atmospheric pressure chemical ionisation (APCI) where the initial ion formation step involves Penning ionisation. The ionisation occurs as the result of chemical reactions in gas phase between two neutral atoms or molecules at collision energies below the threshold energy for ionisation [50,51]. Fig. 4 shows a schematic diagram of DART ionisation source, which typically consists of two chambers aligned consecutively through which a noble gas, usually helium, flows before entering the ambient atmosphere to interact with the sample. In the first section, a glow discharge is initiated when the gas is exposed to a high electric field between the needle electrode and a first electrode. The transient highly energetic species (ions, electrons and excited atoms) existing in the cold plasma may

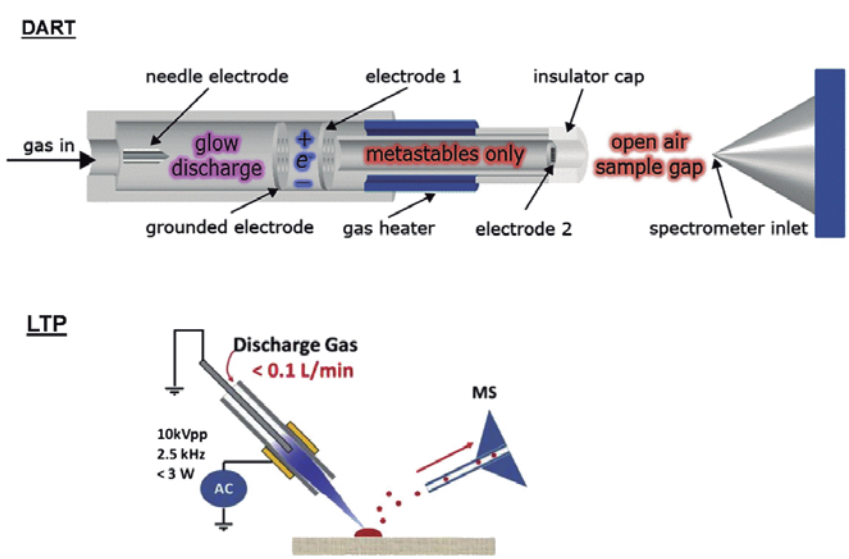

FIGURE 4 Schematic of the configurations of DART *(Reproduced with permission from reference R.B. Cody, Wikipedia. https://commons.wikimedia.org/wiki/File:DART_ion_source_schematic.png, (last accessed 07.09.15) from JEOL USA Inc.)* and LTP probe *(Reproduced with permission from reference J.D. Harper, N.A. Charipar, C.C. Mulligan, X. Zhang, R.G. Cooks, Z. Ouyang, Anal Chem. 80 (2008) 9097−9104. Copyright (2008) American Chemical Society.).*

recombine resulting in the formation of metastable species. Downstream perforated electrodes and an exit grid electrode are used to prevent ion—ion and ion—electron recombination. A heated chamber adjusts the temperature of the gas stream to help in the thermal desorption (TD) of less-volatile substances from the sample surface. Finally, the ionising neutral gas containing metastable species is directed towards the sample surface at an angle suitable for its reflection into the mass spectrometer inlet. DART is a soft ionisation method that generates ions with relatively low internal energy; nevertheless, it is a small-molecule technique and it is not suitable for the analysis of large biomolecules such as proteins, since multiple charge ions are not observed in DART mass spectra. In general, DART forms deprotonated molecules in negative-ion mode and protonated and/or ammoniated molecules in positive DART, alkali metal adduct ions being much less frequently observed.

The majority of DART applications have used helium as the DART gas for Penning ionisation, since the long-lived helium triplet state 2^3S_1 has an internal energy (19.8 eV), which is clearly above the ionisation energy (IE) of any potentially relevant molecule. Helium gas atoms are effectively energised in glow discharge to yield metastable helium atoms He* (2^3S_1). Then, these metastable species interact with neutral molecules, resulting in the formation of radical ions and electrons (Table 2) if the neutral molecules have IEs lower than the internal energy of the metastable species (He*). Since the ionisation takes place at atmospheric pressure, atmospheric gases play a key role in the ionisation mechanism. A cascade of gas-phase reactions is initiated to produce reagent ions that in the vicinity of the surface sample interact with analyte molecules. For such a process, it is a prerequisite to have analyte molecules present in the gas phase, so helium gas is heated to an adjusted temperature to provide sufficient energy for evaporation and TD of compounds. The concentration of analyte molecules in the gas phase does not need to be high and it is enough to lift the molecules off the surface, thus, even compounds with very low vapour pressure, such as low-mass polymers or fullerenes, can be analysed by DART-MS [51]. It must be taken into account that thermal degradation can limit the maximum temperature applicable to thermolabile compounds. Nevertheless, in some occasions to reduce temperature can potentially be an advantage when analysing small molecules immersed in high-mass matrices. Additionally, TD alone can deliver gas-phase ions by charge separation under some circumstances such as rapid evaporation or thermally stable salts. This phenomenon is exploited in atmospheric pressure TD ionisation (APTDI) [16].

The concentration of He* in the DART gas interacting with samples is rapidly reduced due to the favourable generation of nitrogen ions that strongly compete with other molecules resulting in low yields of direct Penning ionisation of these molecules. Nevertheless, the nitrogen ions initiate a cascade of chemical reactions to ionise the gas-phase molecules. In positive-ion mode, the primary water radical ions effectively undergo reactions leading to the formation of protonated water and water clusters that may then act as reagent ions

TABLE 2 DART Gas-Phase Chemical Reactions

Penning Ionization	
$He^* + N_2 \rightarrow He + N_2^{+\bullet} + e^-$	$He^* + O_2 \rightarrow He + O_2^{+\bullet} + e^-$
$He^* + H_2O \rightarrow He + H_2O^{+\bullet} + e^-$	$He^* + M \rightarrow He + M^{+\bullet} + e^-$
Atmospheric gas molecules ionization	
$N_2^{+\bullet} + N_2 \rightarrow N_4^{+\bullet}$	$H_3O^+ + nH_2O \rightarrow [(H_2O)_n + H]^+$
$N_4^{+\bullet} + H_2O \rightarrow 2N_2 + H_2O^{+\bullet}$	$H_2O^{+\bullet} + NH_3 \rightarrow NH_4^+ + OH^\bullet$
$H_2O^{+\bullet} + H_2O \rightarrow H_3O^+ + OH^\bullet$	$[(H_2O)_n + H]^+ + NH_3 \rightarrow NH_4^+$
$O_2 + e^- \rightarrow O_2^{-\bullet}$	
Analyte molecules ionization	
Positive-ion formation	*Negative-ion formation*
$M + [(H_2O)_n + H]^+ \rightarrow [M + H]^+ + nH_2O$	$M + O_2^{-\bullet} \rightarrow [M + O_2]^{-\bullet}$
$M + NH_4^+ \rightarrow [M + NH_4]^+$	$[M + O_2]^{-\bullet} \rightarrow M^{-\bullet} + O_2$
$M + N_4^{+\bullet} \rightarrow 2N_4 + M^{+\bullet}$	$M + e^- \rightarrow M^{-\bullet}$
$M + O_2^{+\bullet} \rightarrow O_2 + M^{+\bullet}$	$MX + e^- \rightarrow M^- + X^\bullet$
$M + O_2^{+\bullet} \rightarrow O_2 + [M - H]^+ + R^\bullet$	$MH + e^- \rightarrow [M - H]^- + H^\bullet$

for analyte positive-ion generation by APCI mechanism. These ionic water species can undergo proton transfer reactions with analyte molecules (M) having proton affinities higher than that of water (691 kJ/mol) and water dimers (808 kJ/mol) to yield $[M+H]^+$ ions. Additionally, analyte molecular ions ($M^{+\bullet}$) can either be generated by Penning ionisation with He* or more probably by charge transfer with other gas-phase ions. The ratio of $M^{+\bullet}$ to $[M+H]^+$ ion formation depends less on the atmospheric water content and mainly on such analyte properties as IE and proton affinity (PA). Low IE will favour $M^{+\bullet}$ ion formation, whereas high PA will promote $[M+H]^+$ ion formation. Sometimes, both ionic species can be observed simultaneously causing distorted isotopic patterns due to the superimposition of both ion signals, a disadvantage for mass spectrum interpretation.

The presence of ammonia in the gas phase makes possible the formation of ammonium adducts for moderately polar analytes. For instance, it has been observed that $[M+NH_4]^+$ ions are predominant when ionizing poly(ethylene glycol)s, ketones, triacylglycerols or polysiloxanes [7]. The origin of NH_4^+ in the gas phase may either be due to its ubiquitous presence as impurity of both the sample and the LC-MS system or because of its generation from traces of ammonia in the laboratory atmosphere, which may be from chemicals or even

from human breath that may contain trace amounts of this compound [52]. The relatively high PA (853.6 kJ/mol) of ammonia ensures the efficient ionisation of trace levels by proton transfer in the gas phase.

The presence of $O_2^{+\bullet}$ in the DART background mass spectrum may be also responsible for the observation of $M^{+\bullet}$ and $[M-H]^+$ ions. Molecular ions ($M^{+\bullet}$) can also be generated via charge exchange when $O_2^{+\bullet}$ ions interact with analyte molecules. Nevertheless, these molecular ions can be self-reactive for hydride/alkyl abstraction reactions under appropriate DART conditions thus yielding $[M-H]^+$ or $[M-R]^+$ ions, but because of the relatively slow kinetics of this phenomenon it is possible the simultaneous presence of both ions in the mass spectra [50,51].

In negative-ion mode, thermal electrons are assumed to generate reagent ions. The electrons may be produced by Penning ionisation of neutral gas molecules or by reaction of the excited atoms with surfaces such as the exit electrode. Moreover, because the main gaseous neutral species is nitrogen, the electrons emitted upon $N_2^{+\bullet}$ ions generation are of relevance. Taking into account this scenario, it is understandable that electron capture becomes the main ionisation mechanism in negative-ion mode for molecules with a high electron affinity. Atmospheric oxygen is the preferred species to undergo the initial electron capture to form $O_2^{-\bullet}$ reagent ions. This radical ion can form adducts with analyte molecules $[M+O_2]^{-\bullet}$, which may either prevail as such or dissociate to create radical anions $M^{-\bullet}$. Additionally, depending on the nature of the analyte, direct electron capture by the analyte, dissociative electron capture, deprotonation by dissociation or reaction with a base, or even anion attachment are also possible. Finally, it must be taken into account that in addition to $O_2^{-\bullet}$ other background ions, $NO_2^{-\bullet}$ and $CO_3^{-\bullet}$, can be generated from atmospheric components and, depending on trace of solvents, CN^-, Cl^-, OH^-, among others, can also be formed. All these ions may form adducts and/or reaction products with analyte molecules.

Dopant-Assisted DART

To influence ion formation some authors have recommended the introduction of volatile additives (dopants) into the DART gas stream. Water can be considered as a dopant in positive-ion mode, but its additional delivery into the DART source is unnecessary because the water content is high enough in the ambient environment. In contrast, the concentration level of ammonia in the ambient environment is much lower and the additional delivery of this additive has been demonstrated to enhance the DART ionisation of polar compounds with relatively low proton affinities, such as peroxides and esters [18,53]. The introduction of dopants can be achieved by opening a bottle with the volatile additive or holding a cotton swab wetted with the dopant substance nearby the DART source. Using this strategy, anion adducts such as $[M+Cl]^-$ and $[M+$trifluoroacetate$]^-$ can be observed in negative-ion mode for nitroglycerine, ethylene glycol dinitrate (EGDN), hexahydro-1,3,5-trinitro-1,3,5-triazine,

pentaerythritol tetranitrate, and octohydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine when adding dichloromethane or trifluoroacetic acid as dopant [18]. This is advantageous for the detection of these explosives that do not produce molecular ions under the standard DART conditions. Furthermore, introducing a deuterated dopant such as D_2O into the DART gas stream can easily allow carrying out hydrogen/deuterium exchange experiments usually used in structural elucidation. This last strategy has been used for the detection of melamine in contaminated pet food [54]. Furthermore, the combination of H/D exchanges in the DART source with tandem MS and accurate mass measurements has been used to examine ion structures for fragments of caffeine, theophylline, and theobromine [5].

2.4 Low-Temperature Plasma

The low-temperature plasma (LTP) probe is based on the generation of a plasma using an annular dielectric barrier discharge configuration (Fig. 4) [20]. A discharge gas at low flow rate (<500 mL/min) and a high-voltage AC are used to sustain the plasma in an ambient environment. The temperature of the sampling torch can be adjusted over a wide range and can be as low as 30°C avoiding damages of the sample substrates (skin of human fingers, fabrics on luggage, etc.). The ionisation mechanism involved in the LTP includes TD, chemical sputtering and surface reactions simultaneously occurring during the desorption/ionisation process. In the past few years, great progress has been made in the elucidation of the LTP mechanism by means of plasma spectroscopic diagnostics [55]. These studies indicated that metastable helium atoms (He*) play a central role as in other plasma ionisation techniques. The He* atoms are involved in the direct Penning ionisation process of analytes, but also act as indirect precursors for the formation of other highly excited helium species involved in the ionisation pathways. The helium dimer ion (He_2^+) seems to be the dominant positive ion in the plasma and it serves to carry energy from the discharge into the afterglow region in the open atmosphere and it interacts with the atmospheric nitrogen to generate N_2^+ ion via charge transfer, which is an important reagent ion and the key reaction intermediate for the formation of other gas-phase reagent ions such as protonated water clusters.

This technique has been demonstrated to be applicable for the direct analysis of a wide range of chemicals from complex samples, especially for small organic molecules with low to moderate polarity [56], but not for ionic analytes. Nevertheless, the molecular weight of analytes is also critical, maybe due to the difficulty in desorbing intact large molecules from condensed-phase samples. The analytical performance of the original LTP setup is adequate for many semi- and non-volatile compounds, such as trinitrotoluene (TNT) and cocaine, but increasing source temperature could facilitate sampling ionisation. For instance, in the analysis of atrazine the signal intensity of $[M+H]^+$

was improved (up to two orders of magnitude) when applying a thermal assistance of 150°C in the LTP probe [20]. Finally, it must be pointed out that the implementation of LTP probe for sampling analysis is relatively easy, being the relative positions of the LTP probe, the sample and the MS inlet not critical for a proper analytical performance. These favourable characteristics of the LTP probe make possible the development of quantitative and qualitative analytical applications in the fields of public safety, food safety, product quality control and forensics.

3. AMBIENT MASS SPECTROMETRY APPLICATIONS

3.1 Environmental

Environmental analysis usually requires highly sensitive techniques because of the low concentration levels of environmentally relevant contaminants. Nowadays, the high sensitivity attainable with TOF and especially with Orbitrap mass analysers makes it possible to find applications of environmental interest employing ambient MS techniques in combination with HRMS such as the analysis of volatile organic compounds and atmospheric aerosols [57–60]. For instance, Roach et al. [57] proposed the use of nanospray DESI (nano-DESI) combined with HRMS with a linear ion trap-Orbitrap for the detailed, molecular-level chemical characterization of organic aerosols (OA) collected in laboratory and field experiments. Atmospheric aerosols interact with incoming solar radiation and can modify cloud properties resulting in significant impacts on climate, air quality, and human health. The characterization of aerosols is of important interest because of the vast uncertainty on the composition and atmospheric chemistry of OA, which are either emitted as primary OA from a variety of sources including industrial processes, combustion of fossil fuels, and biomass burning or formed as a result of gas-to-particle partitioning in atmospheric physicochemical processes that from secondary OA. The stable signals achieved using nano-DESI, where the analyte is desorbed into a solvent bridge formed between two capillaries and the analysis surface, make it possible to obtain very high-quality HRMS data for both laboratory-generated and field-collected OA using a very small amount of material (lower than 10 ng) and without any sample treatment. Characterizing the molecular composition and chemical transformation of OA is a major challenge in atmospheric aerosol research. The authors conducted ageing experiments and examined an aged (brown) limonene secondary OA sample using ESI, DESI, and nano-DESI, showing that larger oligomers can be observed with greater intensity by both nano-DESI and DESI than they were by ESI. These differences were attributed to the decomposition of chemically labile oligomeric species during either the extraction process or the subsequent residence time in the ionisation solvent. This suggested that the solvent residence time of OA in a nano-DESI-HRMS experiment was sufficiently short to preserve these labile chemical bonds. Later, the same research group proposed

the use of reactive nano-DESI coupled to an Orbitrap mass analyser for the analysis of secondary organic aerosol produced through ozonolysis of limonene [58]. The authors used the selectivity of the Girard reagent T (GT) towards carbonyl compounds to examine the utility of reactive nano-DESI for the chemical analysis of complex organic mixtures. For that purpose, 1−100 μM GT solutions were used as the working solvents for reactive nano-DESI analysis.

Recently, Tao et al. [60] also proposed nano-DESI-HRMS (with a mass resolution of 100,000 full width half maximum (FWHM) using an Orbitrap mass analyser) for the molecular characterization of organosulphates in OA from Shanghai and Los Angeles urban areas. A diverse mixture of oxygenated hydrocarbons, organosulphates, organonitrates, and organics with reduced nitrogen were detected in the Los Angeles samples. A majority of the organics in the Shanghai samples were detected as organosulphates.

Ambient MS techniques have also been proposed for the characterization and analysis of crude oil components [27,61]. For instance, nano-DESI-HRMS using a linear ion trap-Orbitrap has also been proposed for the first time for the chemical characterisation and analysis of the polar constituents of liquid petroleum crude oil samples [27]. The analysis was performed in both positive and negative ionisation modes. The results demonstrated that nano-DESI analysis efficiently ionises petroleum constituents. Water-soluble compounds were predominantly observed as sodium adducts in nano-DESI spectra indicating that addition of sodium to the solvent may be a viable approach for the efficient ionisation of water-soluble crude oil constituents. This is an interesting example showing that HRMS is mandatory to resolve the highly complex liquid petroleum crude oil extracts. In this case, the authors employed a linear ion trap-Orbitrap with a mass resolution of 100,000 FWHM.

Environmental water applications of ambient MS techniques have also been described in the literature. In these cases, in order to attain the low limits of detection required for environmental water analysis both preconcentration approaches and the sensitivity of HRMS instruments are required. For instance, Strittmatter et al. [62] described the analysis of wastewater samples by the direct combination of C18/strong cation exchange mixed-mode thin-film microextraction (MFTE) and DESI-HRMS. Both techniques make the analytical workflow simpler and faster, hence their combination enables considerably shorter analysis time compared to traditional LC-MS approaches. The authors validated the method using carbamazepine and triclosan as typical examples for pharmaceuticals and personal care product components, which draw increasing attention as wastewater-derived environmental contaminants. The results obtained by MFTE in combination with DESI-HRMS were compared to those of parallel LC-MS determinations showing good agreement within a concentration range three orders of magnitude wide. Although several matrix effects were observed with wastewater samples, limits of detection down to nanogram per litre level were achieved. In this example, the use of an

Orbitrap mass spectrometer was ideal for screening purposes and led to the detection of various different personal care product components in wastewater treatment plant effluents, including beta-blockers, non-steroidal anti-inflammatory drugs, and UV filters.

Another interesting environmental water application of ambient MS techniques is the determination of organic UV filters by stir bar sorptive extraction in combination with DART-TOF HRMS analysis [63]. Because of the low concentrations of UV filters expected in environmental water samples, 250 mL of water was preconcentrated with polydimethylsiloxane (PDMS) stir bar sorptive extraction for 4 h, and the PDMS stir bars were exposed to the DART stream without any separation elution step. To demonstrate the suitability of DART-HRMS with a TOF instrument, the authors used a test set of seven organic UV filters, namely, benzophenone-3 (BP-3), ethylhexyl dimethyl *p*-aminobenzoate, 4-tbutyl-4′-methoxydibenzoylmethane, homomethylsalicylate, 2-(ethylhexyl) salicylate, octocrylene (OC), and 4-methylbenzylidene camphor. In the first step, standard solutions of the targeted analytes prepared in methanol were investigated in order to determine optimum parameters for the DART-HRMS analysis (see spectrum in Fig. 5a). Limits of detection in the range 20—40 ng/L (depending on the analyte) with acceptable precisions (5—30% relative standard deviation (RSD) values at 500 ng/L level) were reported. The analysis of a real lake water sample, collected at an area used for leisure activities, was carried out and reported contamination with BP-3 and OC (Fig. 5b). The authors compared the results obtained with the developed DART-HRMS method with a confirmatory analysis using TD-GC-MS, obtaining comparable results for the two detected UV filters, and showing that DART-TOF-MS is a rapid screening method for environmental water contamination.

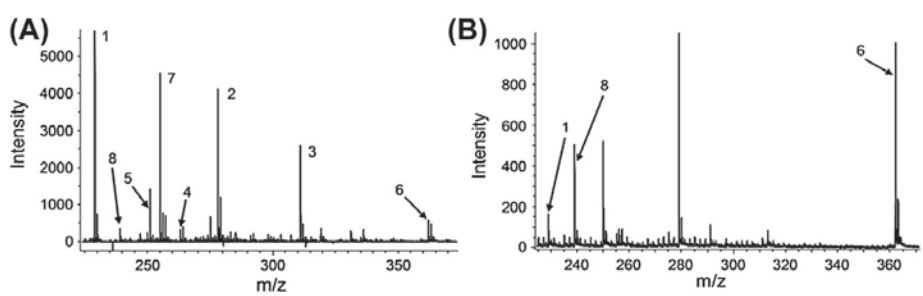

FIGURE 5 (A) DART-TOF-MS spectrum of deionised water spiked with a mixture containing seven UV filters (each 700 ng/L) and the internal standard (400 ng/L). Peaks: (1) BP-3, (2) ethylhexyl dimethyl *p*-aminobenzoate, (3) 4-tbutyl-4′-methoxydibenzoylmethane, (4) homomethylsalicylate, (5) 2-(ethylhexyl) salicylate, (6) OC, (7) 4-methylbenzylidene camphor, (8) benzyl cinnamate (BC, internal standard). (B) DART-TOF-MS spectrum of a real lake water sample taken during the summer season. Peaks: (1) BP-3, (6) OC, (8) BC. *Reproduced with permission from reference M. Haunschmidt, C.W. Klampfl, W. Buchberger, R. Hertsens, Anal Bioanal. Chem. 397 (2010) 269—275. Copyright (2010) Springer.*

3.2 Food

The quality of food products is an issue of great interest in our society. For this reason, the development of new methods focused on the analysis but also in the characterization, classification and authentication of food products has increased dramatically [64,65]. Nowadays, there is a growing necessity for applications in food able to cope with a large number of analytes in very complex matrices. The new analytical procedures demand sensitivity, robustness and high resolution within an acceptable analysis time. Moreover, the possibility of analysing multiple compounds for target and non-target screening, such as multi-residue methods in various matrices, minimizing sample manipulation is also demanded [66]. In this context, ambient MS techniques appear as a powerful tool for high-throughput analysis of food products minimizing sample treatment as much as possible [67,68]. Due to the complexity of food matrices the enhanced resolution and accurate mass measurements attainable with HRMS instrumentation is frequently a requirement.

Among the most common ambient MS techniques, DART in combination with HRMS either using TOF or Orbitrap mass analysers is the most frequently employed one in the analysis of food matrices. DART-HRMS has been proposed for food safety analysis for the determination of pesticides and fungicides in fruits and vegetables [69,70] and mycotoxins in cereal samples [71], as well as in food quality control analysis for the determination of caffeine in coffee products [72] or the rapid control of Chinese star anise fruits and teas for the presence of neurotoxic anisatin [73], among other applications. For example, Vaclavik et al. [71] proposed DART-HRMS using an Orbitrap mass analyser for the rapid quantitative determination of multiple mycotoxins isolated from wheat and maize by a modified QuEChERS procedure. The lowest calibration levels estimated for the respective targeted analytes ranged from 50 to 150 μg/kg. Quantitative analysis was performed either with the use of matrix-matched calibration or by employing commercially available ^{13}C-labelled internal standards for deoxynivalenol, nivalenol and zearalenone mycotoxins. Good recoveries (100−108%) and repeatabilities (RSD <6.9%) were obtained at a concentration level of 500 μg/kg by isotope dilution procedure. Regarding matrix-matched calibration, recoveries and repeatabilities were in the range 84−118% and 7.9−12.0% (RSD), respectively. The authors validated DART-Orbitrap-MS trueness for deoxynivalenol and zearalenone in wheat/maize by the analysis of certified reference materials, achieving good agreement also with data validated by ultra-high performance liquid chromatography-time of flight mass spectrometry (UHPLC-TOF MS) analysis.

Edison et al. [69] proposed DART-Orbitrap-MS as a rapid screening method for pesticides in order to streamline the processing of products for food safety and control analysis. For that purpose, foam swabs were used to recover multi-class mixtures of pesticides from the surfaces of apples, kiwis, peaches and tomatoes, and the swabs were analysed by DART-Orbitrap-MS.

This simple sample treatment in combination with DART-HRMS allowed expanding the types of food commodities to be analysed in food safety control laboratories. The authors observed that whilst smooth-skinned products, such as apples, maintained a high detection rate for the targeted pesticides even when 10 apples were swabbed with one foam disk, commodities with rougher surfaces, such as peaches, suffered a decrease in detection rate when 10 peaches were swabbed with one foam disk. So, in order to maintain consistency across sampling process, a composite size of three units was then selected. Several classes of pesticides such as thiocarbamates, phenylamides and organochlorine pesticides were easily detected in the analysed samples. A similar approach was later used by Kern et al. [74] who employed an accurate mass fragment library based on DART-Orbitrap-MS data for the rapid analysis of pesticides on food products after swabbing them with foam disks.

DART-HRMS techniques have also been widely employed for metabolomic analysis of food commodities to achieve characterization, classification and authentication in the prevention of frauds [75—79]. In this area, the high resolution and accurate mass measurements achieved with HRMS instrumentation is an indispensable tool to obtain fingerprinting spectra that can be easily processed by chemometric techniques. An interesting example is the one reported by Cajka et al. [75] describing the application of DART-TOF-MS for the chicken meat metabolomic studies aiming at the retrospective control of feed fraud. DART-TOF-MS was used as a tool for differentiation between chickens fed by feed that contained 5—8% (w/w) of chicken bone meal (a banned component) and those representing a reference group, ie, grown otherwise under the same conditions. A simultaneous (all-in-one) extraction procedure using a water and cyclohexane mixture able to isolate both polar and non-polar metabolites in a single extraction step was employed. After extraction, DART-HRMS spectra were obtained. Fig. 6A shows the DART-TOF-MS fingerprints of a chicken muscle extract. Fingerprinting data was then chemometrically processed by principal component analysis (PCA) and orthogonal partial least squares discriminant analysis. As an example, Fig. 6B shows the PCA two-score plots of the polar and non-polar extracts obtained from chicken muscles. Differentiation of chicken muscle according to diet (feed with and without the addition of chicken bone meal) was feasible employing DART-TOF-MS fingerprints of polar as well as non-polar extracts. Additional experiments conducted after 6 months confirmed the classification potential of DART-TOF-MS analysis to detect frauds.

Similar approaches were used by the same research group employing DART-TOF-MS for fish metabolomics aimed to assess the response of dietary supplementation [76], for olive oil quality and authenticity assessment [78], and for beer origin recognition [79], as well as DART-Orbitrap-MS fingerprinting in the prediction of acrylamide formation in biscuits [80] or in the authentication of milk and milk-based food products [77], among other examples. For instance, the use of DART-Orbitrap-MS in this last application allowed the authentication

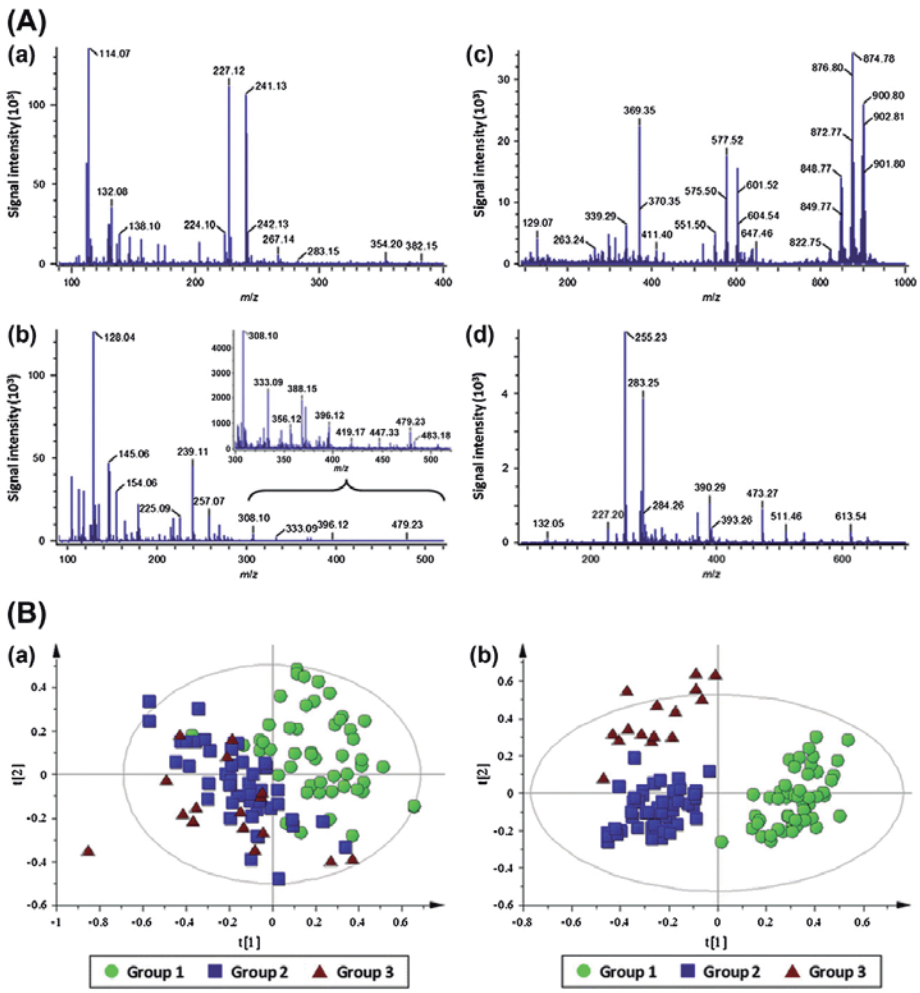

FIGURE 6 (A) DART-TOF-MS fingerprints of the chicken muscle extracts: (a) polar extract, DART(+); (b) polar extract, DART(−); (c) non-polar extract, DART(+); dopant: ammonia; (d) non-polar extract, DART(−). (B) A two-score plot of PCA: (a) polar extracts (chicken muscle); (b) non-polar extracts (chicken muscle). (group 1 chicken fed with the feed with the addition of chicken bone meal; group 2 chicken fed with the feed without the addition of chicken bone meal; group 3 chicken fed with the feed without the addition of chicken bone meal (experiment repeated after 6 months)). *Reproduced with permission from reference T. Cajka, H. Danhelova, M. Zachariasova, K. Riddellova, J. Hajslova, Metabolomics 9 (2013) 545−557. Copyright (2013) Springer.*

of milk and dairy products in several scenarios: (1) discrimination among milks obtained from various farm animal species (cow, goat and sheep), (2) discrimination between cows' milk produced in conventional and organic farming, and (3) detection of vegetable oil added to milk-based products (soft cheese). These examples show that DART in combination with HRMS and chemometric analysis is a powerful tool for characterization, classification and authentication of food.

Martínez-Villalba et al. [81] demonstrated the applicability of DART-Orbitrap-MS for the high-throughput determination of antiparasitic veterinary drugs in feed and food. The combination of an analysis time of less than 1 min per sample and the possibility to acquire accurate masses under HRMS makes the DART-HRMS technique an effective tool for rapid qualitative screening of antiparasitic veterinary drugs. The results obtained by the authors in this work demonstrated the feasibility of this approach to quantify the targeted analytes at levels down to 1 mg/kg for benzimidazolic compounds in milk samples and 0.25 mg/kg for coccidiostats in chicken feed.

Although many DESI applications in food analysis have been described in the literature [82], only few of them have been carried out in combination with HRMS (DESI-Orbitrap-MS). For instance, DESI-HRMS has been proposed for the analysis of anionic oligosaccharides [83] and to study natural polysaccharide interactions with a model drug in a controlled release system [84]. Nano-DESI in combination with a quadrupole-time of flight (q-TOF) mass analyser was employed by Hartmanova et al. [85] for the fast profiling of anthocyanins in wine. In this work, the acidification of samples in combination with an acidic DESI solvent (methanol:water 75:25 with 0.2% formic acid) were essential for obtaining good-quality mass spectra. The authors applied the proposed method to obtain nano-DESI-HRMS profiles of main anthocyanins in two vintages and three cultivars wine samples. The obtained results were in agreement with anthocyanins isolated by solid-phase extraction (SPE) and analysed by LC-MS, but the nano-DESI-HRMS method offered high selectivity and accurate mass measurements that can contribute to anthocyanin identification in screening analyses.

Recently, Seró et al. [28] developed a DESI-HRMS method for the screening of veterinary drugs in cross-contaminated feedstuffs. The reliable detection was performed working at high mass resolution (70,000 FWHM) using an Orbitrap mass analyser. To evaluate the applicability of the developed DESI-HRMS method, 50 feed samples were analysed in order to detect those samples suspected of being cross-contaminated by veterinary drugs. Feed samples were screened and the acquired mass spectral raw data were interrogated by a custom-made database that included more than 60 veterinary drugs (anthelmintics, antibiotics, coccidiostats, hormones, etc.) commonly used to produce medicated feedstuffs. The custom-made database included the ionisation mode and the expected ions (protonated and deprotonated molecules, adduct ions, in-source fragments, etc.) that can be generated in the DESI source. Veterinary drugs at dose levels between 37 and 107 mg/kg in medicated feed were easily detected and confirmed by the proposed DESI-HRMS screening method, and in one of these samples an unexpected cross-contamination of monensin (3.5 mg/kg) was also detected (see DESI-HRMS full-scan spectrum in Fig. 7). Additionally, the authors reported that the results obtained for non-medicated feed indicated that cross-contamination occurs quite

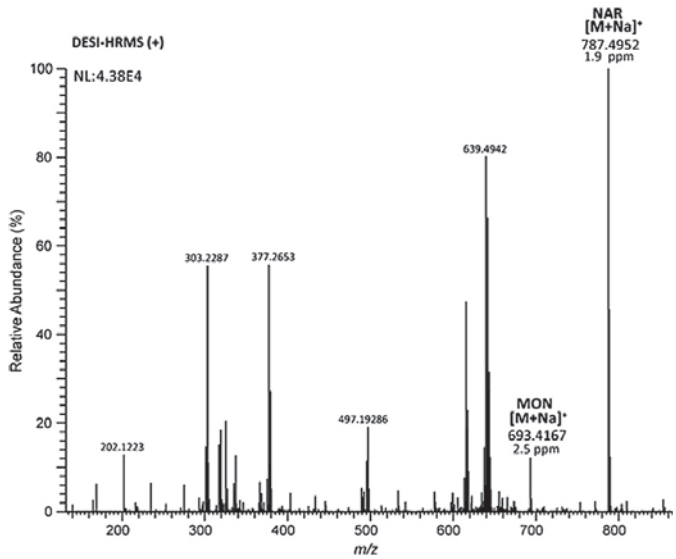

FIGURE 7 DESI-HRMS full-scan spectrum obtained from a narasin (NAR) medicated feed (37 μg/g) cross-contaminated with monensin (MON) (3.5 μg/g). *Reproduced with permission from reference R. Seró, O. Núñez, J. Bosch, J.M. Grases, P. Rodríguez, E. Moyano, M.T. Galceran, Anal Bioanal. Chem. (2015), http://dx.doi.org/10.1007/s00216-015-8899-4. Copyright (2015) Springer.*

frequently and values above the legislated levels were detected in 28% of the samples analysed by DESI-HRMS.

Paper spray in combination with HRMS has been described for the analysis of food contaminants generated during food processing procedures such as heating. For example, Li et al. [86] reported the rapid qualitative and quantitative analysis of 4-methylimidazole (4-MEI), a carcinogenic by-product generated through the Maillard reaction when heating caramel-based food products, in beverage and caramel samples using the paper spray form of ambient ionisation MS. For the caramel samples and typical beverages examined by the authors, ionisation competition/suppression was observed but was not significant. However, these matrices all have three to six isobaric peaks at m/z 83, being matrix interferences for the detection of 4-MEI. This scenario made HRMS necessary for the reliable identification and quantification of 4-MEI. By performing paper spray analysis on an Orbitrap-HRMS instrument, rapid screening of the analyte of interest was achieved as an alternative to tandem MS. As an example, Fig. 8 shows a paper spray high-resolution full-scan spectrum of a typical cola beverage. The accurate masses of the peaks were compared to those of compounds known to be present in the sample and several matching peaks are labelled in the figure. Among them, the peak that matches 4-MEI was subjected to tandem MS to additionally confirm the assignment. The proposed paper spray-Orbitrap-MS method provided a limit of detection of 100 pg/μL in beverage samples.

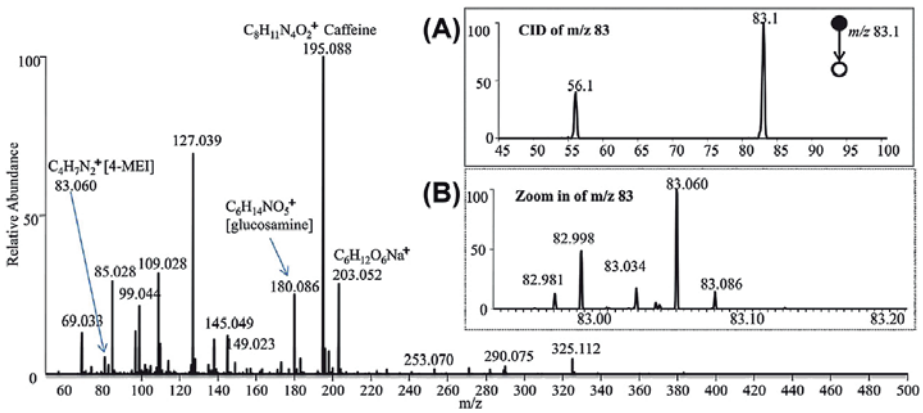

FIGURE 8 Paper spray HRMS analysis of a cola beverage; the accurate masses observed match with chemical compounds labelled in the figure. As shown in inset (B), the high-resolution mass spectrometer successfully resolves protonated 4-MEI (m/z 83.060) from isobaric interferences. Its 13C isotope is also recorded in the spectrum, although not shown in the insets. Additional structural confirmation was carried out by MS2 for the analyte (4-MEI) of interest, as shown in the inset (A). *Reproduced with permission from reference A. Li, P. Wei, H.-C. Hsu, R.G. Cooks, Analyst 138 (2013) 4624–4630. Copyright (2013) Royal Society of Chemistry.*

Ambient MS techniques in combination with HRMS have also been proposed in migration studies of potentially harmful contaminants from materials intended to be in contact with food (food-contact materials). For example, Mattarozzi et al. [87] studied the migration of melamine into food from melamine tableware using DESI-Orbitrap-MS. The migration test was performed using acetic acid 3% (v/v) as food simulant. A two-tailed migration test (*t*-test) allowed the authors to assess the good agreement between the qualitative results obtained applying the proposed DESI-HRMS method with data provided by conventional LC-ESI-MS, thus demonstrating the reliability of DESI-HRMS as a rapid technique for the study of melamine release from plastic materials into food. Bentayeb et al. [88] proposed the use of DART-TOF-MS for the detection and identification of compounds from print set-off not visible to the human eye. The set-off is the unintentional transfer of substances used in printing form the external printed surface of food packaging to the inner, food-contact surface. The authors compared the DART mass spectra from inner and outer surfaces of printed and non-printed food packaging to detect and identify non-visible set-off components, and developed a protocol to identify unknowns by using a custom open-source database of printing inks and food packaging compounds. The protocol matched print-related food-contact surface ions with the molecular formulae of common ions, isotope patterns, and fragments of compounds from the database. The authors were able to detect print set-off and identify seven different compounds by DART-TOF-MS.

3.3 Forensics

An interesting and broad area of applications of ambient ionisation techniques is forensic science, in which on-location analysis, speed, specificity and wide applicability are all important [89–91]. Furthermore, ambient MS methods are intrinsically very efficient tools for high-throughput analysis, which plays an important role in drug counterfeit and forensic identifications.

The ability to rapidly detect explosive compounds and chemical warfare agents in the ambient environment with minimal sample manipulation using ambient techniques such as DART [92–97], DESI [98], LTP [99], APLD [100], APTDI [101], DEFFI [102] and EASI [103] has been widely described. In these applications, ambient MS techniques have been coupled to high-resolution mass analysers to take advantage of the high sensitivity and selectivity they can offer. In addition to the detection of the explosive source, one key goal in this field is detection of trace amounts of explosive residues, since they can be indicative of close contact with a concrete one. Saha et al. [99] developed an LTP-Orbitrap method for the analysis of explosive residues in soil and pond water at parts per billion levels with minor sample pre-treatment and with the advantage of the unequivocal identification by accurate mass measurements. Furthermore, Sisco et al. [94] studied the viability of DART-TOF as screening tool for the analysis of trace explosive. They used acetone as dopant in combination with thermal assistance (225°C) to improve the response of some explosives, while for EGDN and diethylene-glycol dinitrate the temperature had to be decreased for a better sensitivity. In this study, authors used searching lists to identify explosives in samples and categorised the explosive *m/z* signals (peaks) into three groups. The first one included the unique peaks, which could be assigned to only one explosive. Thus, these compounds could be easily confirmed. The second group was composed of the shared peaks, which could be assigned to more than one different explosive belonging to the same compound class, thus hindering the mass spectra interpretation. Finally, the third group classified the mass overlap peaks, which were peaks from two or more ions with exact masses that shifted only ± 0.005 Da and which were difficult to interpret. Thus, even without the benefits of the second dimension provided by a separation technique, authors demonstrated that ambient HRMS can be used to screen a high number of trace-levels explosives, although confirmatory identification of specific explosives can be sometimes compromised due to the similarity between fragmentation patterns of some of them.

The manufacture and trafficking of illicit drugs and counterfeiting of legitimate pharmaceuticals remains a priority concern for law enforcement. Furthermore, the constant appearance of new psychoactive substances requires currently rapid preliminary confirmatory testing methods. Ambient MS techniques coupled to HRMS offer advantages over other classical methods (GC-MS, LC-MS) due to their high-throughput, retrospective analysis capabilities

and their ability to analyse the specimens in different forms and surfaces (eg, tablets, capsules, gels, skin, hair or blood). Regarding forensic investigations of falsified pharmaceuticals, different qualitative and quantitative methods using ambient MS techniques have been described [32,104—109]. For instance, DESI-MS in both conventional and imaging modes, DART-TOF and 2D diffusion-ordered ^{1}H nuclear magnetic resonance (2D DOSY ^{1}H NMR) were complementarily used to characterise the chemical composition of counterfeit antimalarial drugs [110]. Both types of methods enabled the detection of active pharmaceutical ingredients and tablet excipients. For a total of 16 samples, the correct active pharmaceutical ingredient was observed in only six formulations, while the remaining formulations contained either wrong active pharmaceutical ingredients such as acetaminophen or dipyrone, or only pharmaceutical excipients. Stearate and polymeric excipients were not detected neither by DESI nor by DART, while disaccharides (lactose, sucrose) were detected only by DESI, probably due to difficulties in TD of these class of compounds in the DART source. Valuable structural information were obtained in DESI when coupled to an ion trap to perform multi-stage mass spectrometry experiments (MS^n) and the accurate mass information offered by DART-TOF complemented and validated the DESI results. Additionally, DESI imaging was also performed in this study to provide information about sample homogeneity and impurities distribution in malarial tablets that may not be detectable with other approaches. Fig. 9 shows the spectra obtained in the analysis of a counterfeit formulation when employing the ambient MS techniques in parallel with the 2D DOSY ^{1}H NMR method. As can be seen, all the assayed methods detected the active principal ingredient artemisinin, which is less effective in antimalarial treatments than artesunate because of its poor water solubility. Furthermore, two excipients were found in this sample, dextrin and a stearate-based lubricant, but they were only detected by 2D DOSY ^{1}H NMR, maybe due to the higher ionisation efficiency of artemisinin that suppresses the ionisation of other compounds in the sample when analysed by DESI and DART.

Ambient MS has also been used for quick and easy determination of illicit drugs allowing the simultaneous identification of active ingredients, additives, adulterants, etc. in complex samples such as hair, blood, breath or skin [111—114]. For example, Su et al. [113] proposed paper spray MS for the rapid monitoring and quantitation of drugs of abuse at ng/mL level in dried blood spots. Because a very small amount of blood is necessary to perform the analysis, less invasive methods such as collected pricks can be used to take the sample. For screening analysis, quadrupole-Orbitrap mass analyser was used, while for the quantification analysis the high signal-to-noise ratio obtained by tandem MS with a triple quadrupole mass analyser provide the required sensitivity and specificity to detect trace levels of drugs in blood. In addition, solvent spray composition was optimised because of the role it plays in both analyte extraction and ESI at the tip of the paper substrate. Thus, the highest

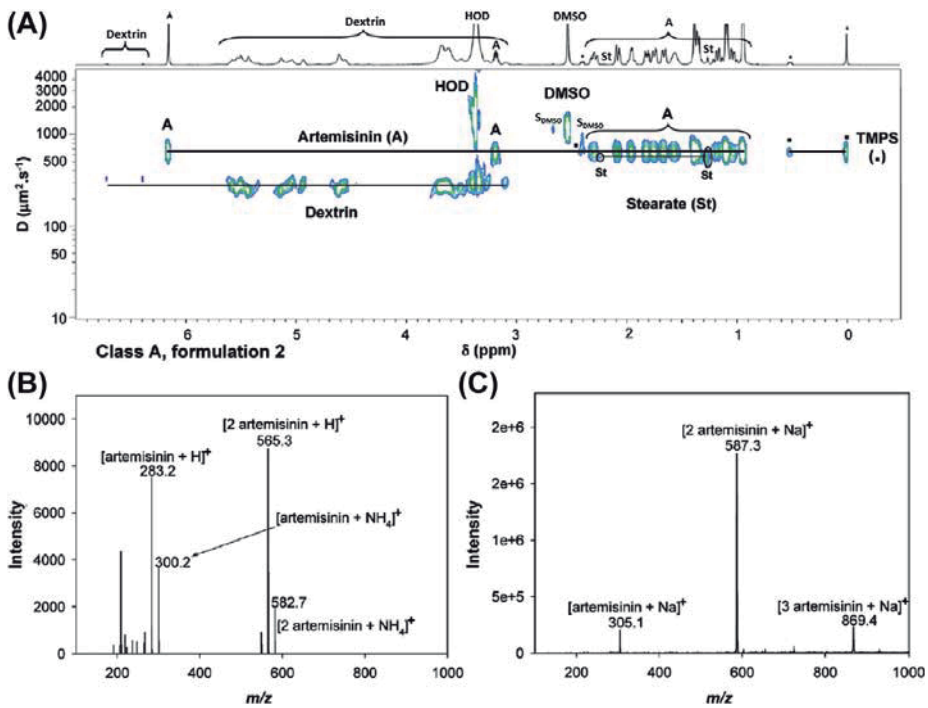

FIGURE 9 Results from the analysis of formulation 2 by (A) 2D DOSY 1H NMR in dimethyl sulfoxide-d6 (DMSO-d6), with trimethyl(phenyl)silane (TMPS) as internal reference standard (where SDMSO represents DMSO satellite signals), (B) DART-MS in positive-ion mode, and (C) DESI-MS in positive-ion mode. *Reproduced with permission from reference L. Nyadong, G.A Harris, S. Balayssac, A.S. Galhena, M. Malet-Martino, R. Martino, R.M. Parry, M.D. Wang, F.M. Fernández, V. Gilard, Anal Chem. 81 (2009) 4803−4812. Copyright (2009) American Chemical Society.*

sensitivity was obtained with 9:1 acetonitrile-water solution and decreased rapidly when increasing the water percentage, perhaps because more water-soluble chemicals are extracted, which negatively affected the final ionisation of the analytes.

Another interesting field where ambient MS has been applied is the authentication of documents, which is critical for establishing facts in court. This often requires the examination of inks to identify any document amendments. Typically, analytical proof of a document's authenticity is determined by taking sections and analytical conventional methods usually require the ink to be removed from the support. However, the use of ambient MS techniques quickly provides the chemical mapping of the surface, without damaging the original document and providing precise details about the entire document. One of the most frequently employed ambient MS technique in documents authentication [115,116] and identification of counterfeit bills [117,118] has been EASI. One interesting example is the application of EASI-HRMS to the investigation and molecular information acquisition of second-generation Brazilian Real (R$) banknotes and seized suspect banknotes [116].

With the aim to implement this rapid and non-destructive method in forensic laboratories and routine analysis, a single quadrupole mass analyser was used to establish the chemical signature or chemical fingerprint for authentication of banknotes. Nevertheless, to confirm the counterfeiting, diagnostic markers have to be unequivocally identified; hence, HRMS was required for the correct identification and characterisation of each diagnostic ion. Fig. 10 shows the EASI-FT-ICR MS chemical profiles of genuine R$100 banknote and seized suspect counterfeited R$100 banknote. Few minor ions were detected in the authentic banknotes (Fig. 10A,B) and two plasticisers, bis(2-ethylhexyl) phthalate and dibutyl phthalate, mostly related to the official offset printing process were used as diagnostic markers for genuine banknotes. In contrast, high-resolution chemical profiles of the seized suspect counterfeit banknotes displayed abundant diagnostic ions within the *m/z* 400−800 range due to the presence of oligomers. As can be seen in Fig. 10c, the mass spectrum shows a distribution of ions separated by 44 *m/z* units, which enabled their characterisation as Surfynol 4XX, wherein increasing XX (XX = 40, 65, and 85) values indicate increasing amounts of ethoxylation on a backbone of Surfynol 104. Furthermore, sodiated triethylene glycol monobutyl ether, another ink constituent of ink-jet printers was also identified in the counterfeit banknotes.

Finally, fingerprints at the crime scene have been historically used by forensic experts to identify a suspect or to determine the movements of an individual [119]. The surface chemical analysis of the fingerprints by ambient MS techniques can provide information about the person's ethnic origin, the detection of trace amounts of drugs or explosives deposited with the fingerprint, as well as other social indicators such as people lifestyles [120−122]. As an example, Bailey et al. have successfully demonstrated the use of MALDI and DESI-linear ion trap-Orbitrap working at a mass resolution of 100,000 FWHM for the analysis of cocaine and its metabolites in latent fingerprints [122]. Samples were obtained from five individuals who were attending a drug and alcohol treatment service to receive treatment for drug dependence. Using the Orbitrap mass analyser in full-scan mode, cocaine and its metabolites benzoylecgonine and methylecgonine were detected in four donors. Furthermore, high-resolution tandem MS was used to confirm the correct identification of the analytes and the analysis of latent fingerprints showed good correlation compared with the results obtained from oral fluid testing for the same donor. Although the fingerprints were not spatially uniform, the ability to detect excreted substances in latent fingerprints allowed the differentiation between drug consumption and contact solely based on the presence of metabolites in the residue.

3.4 Doping

For doping control testing, reliable, robust, cost-reduced and easy-to-perform sampling is of utmost importance [123]. The need for fast multi-residue

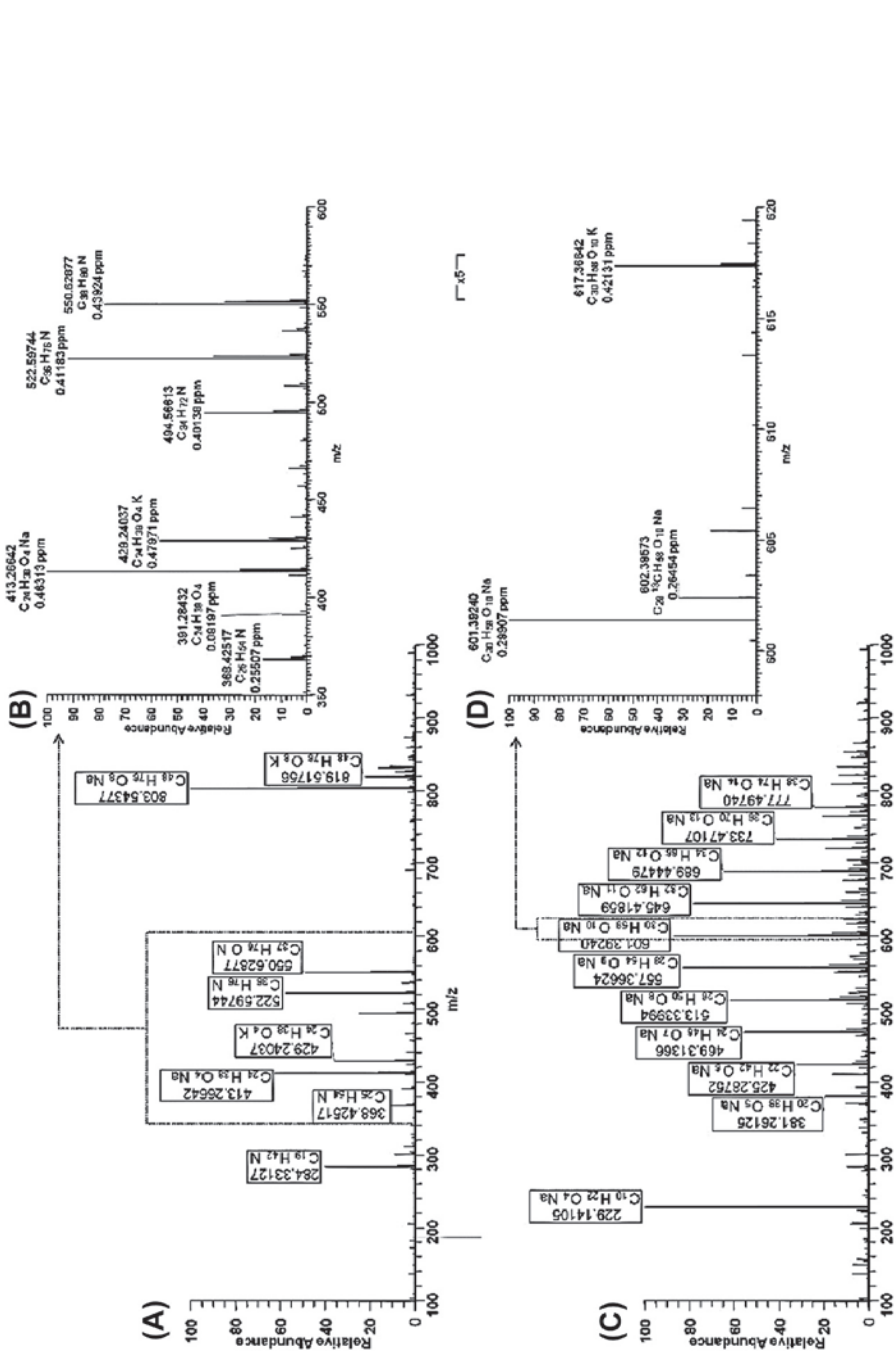

FIGURE 10 EASI(+)-FT-ICR MS high-resolution chemical profiles of (A) genuine R$100 banknote in the range 100−1000 m/z and (B) in the range 350−600 m/z, (C) seized suspect counterfeited second-generation R$100 banknote in the range 100−1000 m/z and (D) the range 560−620 m/z. *Reproduced with permission from reference E.M. Schmidt, M.F. Franco, K.G. Regino, E.L. Lehmann, M.A.Z. Arruda, W.F. de Carvalho Rocha, R. Borges, W. de Souza, M.N. Eberlin, D.N. Correa, Sci. Justice 54 (2014) 459–464. Copyright (2014) Elsevier.*

approaches is crucial today, since the quicker the analysis, the more efficient is the control. In particular, it is required the availability of methods able to rapidly characterise seized preparations and to detect the presence of prohibited substances at trace level in complex biological samples. When using conventional analytical methods (LC-MS and GC-MS) long, tedious and time-consuming extraction and purification procedures are often required to obtain clean extracts and to enhance sensitivity. In this context, ambient MS techniques can be used as wide screening methods due to their low discrimination in the analytical procedure, high throughput and fast and simple specimen preparation requirements [124]. Many applications have been published demonstrating the applicability of ambient MS for the detection of drugs and metabolites in biological matrices, but most of them use low-resolution mass analysers [34,125,126]. However, when analysing complex biological samples without previous sample treatment, ion suppression effects were observed, hindering to obtain suitable detection limits. Lin et al. developed a DESI-MS/MS method for the analysis of clenbuterol in urine [126], but a SPE step was required in order to minimise ion suppression effects caused by the urine matrix components, with the consequent increase in sample analysis time. As an alternative, other authors are proposing the use of reactive DESI-MS to enhance selectivity and sensitivity by in situ transformation of analytes into more easily ionisable species thus improving method selectivity and the signal-to-noise ratio when analysing urine samples. Nevertheless, only low-resolution MS applications of reactive DESI-MS to doping control have been found in the literature [34]. Alternatively, high-resolution mass analysers, such as Orbitrap, TOF, or hybrid analysers like quadrupole-Orbitrap, are a key of benefit when coupled to ambient MS techniques in this application field. Modern high-resolution mass spectrometers are more sensitive and can compensate in part the suppression signal produced. Additionally, they provide more detailed chemical information and selectivity to achieve the unequivocal identification of banned substances at detection limits for doping testing [127].

Among the ambient MS techniques, DART in combination with HRMS either using TOF [128,129] or Orbitrap [130] mass analysers has been most frequently used to analyse prohibited substances by the World Anti-Doping Agency (WADA) [131]. As an example, Lesiak et al. [129] developed a DART-TOF (6000 FWHM) method to rapidly identify the presence of dimethylamylamine (DMAA), a stimulant present in some athletic training or pre—workout supplements, but banned by WADA. A number of DMAA-containing supplements were tested directly by DART-MS in their solid form by just holding the sample between the ionisation source and the mass spectrometer inlet. Under the soft ionisation conditions of DART, DMAA only provided the $[M+H]^+$ ion and the accurate mass (m/z of 116.1439; ±5 mDa) and the collision induced dissociation (CID) fragmentation allowed the identification/confirmation of this compound in the suspected samples (Fig. 11A). Additionally, in this work identification and/or differentiation of DMAA from

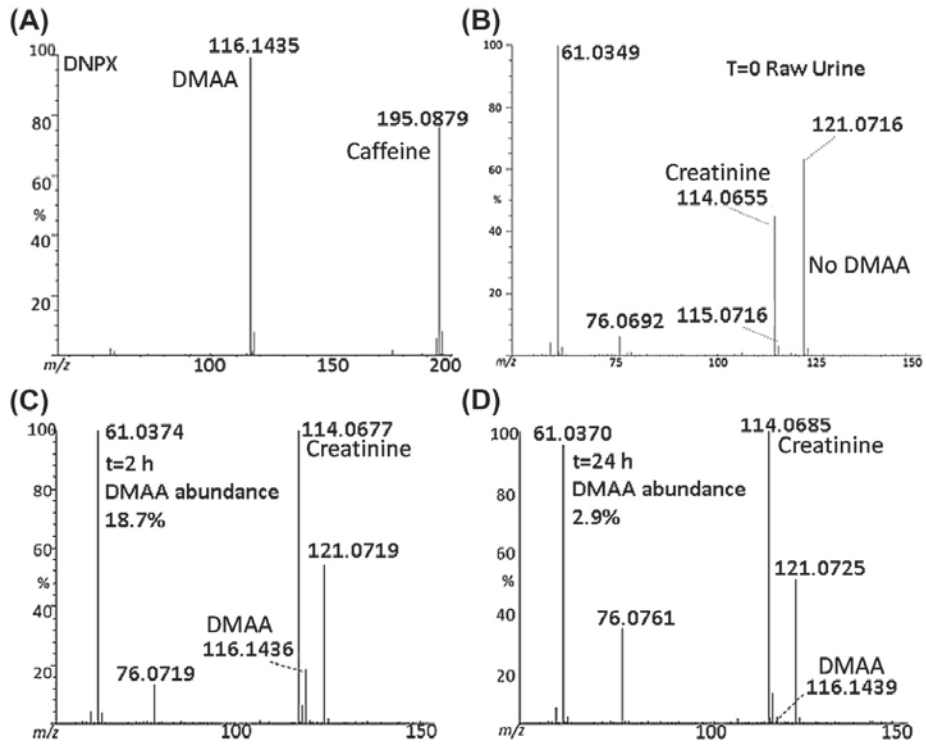

FIGURE 11 DART-HRMS spectra obtained from nutritional supplement samples in positive-ion mode. (A) DART-MS spectra for the supplement DNPX in pill form. (B) DART-MS control data from a urine sample prior to ingestion of a DMAA-containing supplement. (C) DART-MS spectrum of unprocessed urine containing DMAA at 2 h after the supplement ingestion, representing a maximum relative abundance of DMAA in urine. (D) DART-MS spectrum of unprocessed urine 24 h after the supplement ingestion. *Adapted with permission from reference A.D. Lesiak, K.J. Adams, M.A. Domin, C. Henck, J.R.E. Shepard, Drug Test. Anal. 6 (2013) 788−796. Copyright (2013) John Wiley & Sons, Ltd.*

the isomer tuaminoheptane was successfully performed based on the CID fragmentation pattern differences observed between the two compounds. The relative abundance of $[M-H]^+$ (m/z 114) at 60 V for DMAA was relatively smaller than that observed for tuaminoheptane under the same experimental conditions. Regarding sports doping and urine testing, the urine from a volunteer who ingested one of the DMAA-containing supplements was monitored over 48 h. Urine was directly analysed by DART-MS by holding a glass capillary tube dipped into the urine between the source and the MS inlet. The DMAA diagnostic ion was detected/confirmed within 2 hours of ingestion of the supplement; even after 48 hours the relative signal was $\geq$1% (Fig. 11C,D). Furthermore, other major components in the urine sample, specifically urea and creatinine, were also identified by DART-MS. Regardless, matrix effects did not preclude the detection of DMAA under the conditions and time periods studied.

In addition to the DART, other ambient HRMS techniques have also been used for doping control testing. Thus, an Leidenfrost phenomenon-assisted thermal desorption (LPTD) quadrupole-Orbitrap method was used for the identification of trace anabolic steroids in urine. Limits of detection (LODs) ranged from 2 to 50 ng/mL but they were improved one order of magnitude by using a simple and rapid (1 min to analyse a single sample) dichloromethane extraction before the LTPD-MS analysis [132]. Furthermore, Kennedy et al. demonstrated the feasibility of the use of C18 solid phase microextraction (SPME) fibres in combination with DESI-MS for the rapid detection of drugs in raw urine samples [133]. The extraction procedure was performed by inserting the SPME fibre into raw urine and under a gentle agitation. The SPME fibre was directly analysed by DESI-MS after being rinsed, thus combining sampling and sample preparation in a single step. The screening of samples was performed using a TOF mass analyser, while quantitative analysis was performed by a triple quadrupole mass analyser operating in selected reaction monitoring acquisition mode to provide the maximum sensitivity and selectivity.

4. CONCLUDING REMARKS

The simplicity of ambient MS techniques combined with the selectivity and sensitivity of HRMS offer powerful and throughput analytical tools for modern analytical laboratories. Considering the pressures on these laboratories in terms of workload, turnaround time, and cost per sample, it is not surprising that ambient MS would rise so quickly to the forefront of analytical science.

Many new ambient MS variants, combinations, hybridization, and applications are continuously appearing, which make difficult to predict the directions where we will see important developments in the near future. Nevertheless, only the truly simple-to-operate yet effective and rugged interfaces will survive over the years.

Although little or no sample pre-separation, preparation, or derivatisation is required before the mass analysis, the combination of ambient techniques with well-established simple and fast sample pre-treatment procedures may be necessary to reduce matrix effects and to improve limits of detection. The development of robust hybrid multimode ion sources will make possible to obtain additional chemical information in a single experiment and to expand the applications to a broader spectrum of chemical species. The automation of the effective and rugged interfaces will be necessary to enable their integration into platforms that can respond to the demands of the modern routine analytical laboratories. Quantitative measurements are one of the main challenges, but reproducible and robust strategies to quantify samples by ambient MS will be also necessary to exploit their applicability in routine analytical laboratories. Nevertheless, the better understanding of the differential enrichment of analytes during ablation/desorption, the extent of ion suppression due to charge competition, and further insights into the ionisation

mechanisms for a more rational development of quantitative applications are still needed. Finally, machine learning of ambient MS fingerprints is required to allow expert-independent decision based on highly complex HRMS data. Combination of high-throughput ambient MS approaches with advanced chemometrics will be useful to develop automated systems for environmental, food, forensic and doping analysis applications.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support received from Spanish Ministry of Economy and Competitiveness under the project CTQ2012-30836 and from the Agency for Administration of University and Research Grants (Generalitat de Catalunya, Spain) under the project 2014SGR-539. Raquel Seró is gratefully to the University of Barcelona for an APIF grant.

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1.2. ACTUALITZACIÓ BIBLIOGRÀFICA

Avui dia, el nombre de treballs publicats a la literatura que demostren el potencial de les tècniques *Ambient MS* segueix creixent, tot i que l'aplicació en laboratoris de control és encara minsa i hi ha poques revisions bibliogràfiques centrades en les aplicacions d'aquestes tècniques. A mode d'exemple, es poden citar algunes de les publicacions que se centren en demostrar l'aplicabilitat d'aquestes tècniques per determinar la distribució fraudulenta de fàrmacs de mala qualitat (Culzoni et al., 2014), per a la detecció de pesticides en tot tipus d'aliments (Garcia-Reyes et al., 2012; Kauppila et al., 2014; Moyano et al., 2015), per al diagnòstic de càncer en diferents òrgans (Brown et al., 2018; Ifa et al., 2016) o per demostrar el potencial d'aquestes tècniques per a l'anàlisi d'evidències involucrades en investigacions forenses (Correa et al., 2016a; Forbes et al., 2018). En les *Publicacions I i II* (apartat 1.1.1 i 1.1.2), incloses en aquesta introducció, s'han comentat les tècniques *Ambient MS* més utilitzades durant els últims anys per a la resolució de problemes analítics associats als camps de l'alimentació i del medi ambient (referències fins l'any 2018). En la *Publicació II* s'han inclòs també aplicacions de les tècniques *Ambient MS* en els camps de l'anàlisi forense i del control antidopatge fins l'any 2016 (any de publicació) que no han estat considerades en la *Publicació I*. Per aquesta raó, en aquest últim apartat de la introducció es presenta, en primer lloc, una actualització de la bibliografia relacionada amb aquestes dues darreres temàtiques. A més, s'ha considerat d'interès incloure una revisió de les metodologies analítiques que empren tècniques *Ambient MS* per a la diagnosi clínica, un dels camps d'aplicació en què aquestes tècniques presenten importants perspectives de futur, tema que està relacionat amb la *Publicació V* que s'inclou a l'apartat 3.2.1 d'aquesta tesi doctoral.

1.2.1. Anàlisi forense i control antidopatge

A la Taula 1.1 es resumeixen els articles més rellevants publicats els darrers 3 anys que fan referència a l'ús de les tècniques *Ambient MS* en anàlisi forense i en el control antidopatge. La major part dels treballs publicats estan enfocats a desenvolupar mètodes per a la detecció i la identificació d'un elevat nombre de substàncies en mostres de diferent naturalesa. Encara que moltes de les substàncies analitzades són conegudes per la seva aplicació en pràctiques il·legals (compostos diana i sospitosos), les tècniques d'escombratge que es proposen s'orienten a fer possible la detecció i la caracterització de nous compostos (compostos desconeguts) que van apareixent en les diferents pràctiques delictives.

Pel que fa als tipus de tècniques *Ambient MS* utilitzades durant aquests darrers 3 anys, les fonts DESI, DART i EASI són les que es continuen emprant amb més freqüència, possiblement perquè els seus mecanismes d'ionització són ben coneguts i els paràmetres més crítics per a la optimització de les condicions de treball estan ben establerts. A més, actualment es troben disponibles comercialment les fonts de DART i DESI i, fins i tot, és possible aprofitar la font comercial DESI per dur a terme anàlisis emprant la tècnica EASI si no s'aplica un potencial d'electroesprai. Aquestes tècniques s'utilitzen especialment (Taula 1.1) per a la detecció de traces d'explosius en diferents tipus de mostres (roba, bales, bitllets bancaris), per a l'anàlisi de drogues d'abús, així com d'estimulants i d'esteroides anabolitzants que estan regulats per l'agència mundial antidopatge (WADA) en matrius biològiques o en suplements dietètics/nutricionals (Taula 1.1)(Bailey et al., 2015; Black et al., 2017; Correa et al., 2017, 2016b; de Morais et al., 2017; Doué et al., 2015; Forbes et al., 2017; Habala et al., 2016; Hall et al., 2017; Kern et al., 2018b; Kerpel dos Santos et al., 2018; Khatami et al., 2017; Lian et al., 2017; Mirabelli et al., 2015; Schmidt et al., 2015; Zhou et al., 2017). Altres fonts *Ambient MS*, com ara el *paper spray* (PS-MS) i la més recent *touch spray* (TS-MS) comencen a ser emprades en aquests darrers 3 anys, probablement per la seva simplicitat, que possibilita el mostreig i l'anàlisi directa de la mostra sobre el mateix substrat mostrejador (Bain et al., 2018; Damon et al., 2016; Domingos et al., 2017a; Jeong et al., 2016; Lawton et al., 2017; Mckenna et al., 2017; Teunissen et al., 2017). Com a suport s'utilitza un paper triangular (PS) o una petita sonda (agulles metàl·liques) (TS) on s'incorpora un dissolvent per tal de dur a terme l'extracció i s'aplica un potencial per a la ionització dels compostos d'interès mitjançant la generació d'un electroesprai a la punta del suport. Ara bé, encara que les tècniques PS i TS permeten l'anàlisi directa de substàncies psicoactives o estimulants en matrius biològiques (orina o saliva), en la majoria dels casos, els compostos analitzats s'han de trobar a concentracions relativament altes, de l'ordre dels $\mu g\ mL^{-1}$. Si els valors de concentració permesos per la legislació són menors, cal dissenyar estratègies que permetin assolir-los millorant la selectivitat del procés d'extracció que té lloc *in situ* durant l'anàlisi a fi d'evitar els efectes de supressió iònica causats per l'elevada concentració de sals i/o de proteïnes que contenen les mostres biològiques. Amb aquest objectiu s'han proposat procediments ràpids de tractament de la mostra. Per exemple, Damon *et al.* proposa sililar el paper convencional utilitzat en *paper spray* per transformar-lo en hidrofòbic i ho aplica a l'anàlisi de drogues d'abús en mostres d'orina, de sang i de sèrum (Damon et al., 2016). En aquest paper hidrofòbic la mostra queda dipositada en forma de gota esfèrica de manera que disminueix la difusió de la mostra líquida en aplicar el

dissolvent orgànic (acetat d'etil) durant el procés d'extracció i de transport que té lloc *in situ* durant l'anàlisi. El dissolvent orgànic permet l'extracció selectiva de les substàncies orgàniques de menor pes molecular, deixant enrere les proteïnes i les sals presents a la matriu, la qual cosa redueix l'efecte matriu i possibilita la detecció de les drogues a concentracions per sota dels ng mL^{-1}.

La simplicitat dels sistemes de les fons *Ambient MS*, que permet que siguin controlades per usuaris que no són experts en espectrometria de masses, fa que siguin idònies per a l'anàlisi de camp emprant analitzadors miniaturitzats. Poder realitzar l'anàlisi en el mateix lloc on sorgeix el problema resulta de gran utilitat en l'anàlisi forense, com per exemple, per a la detecció de substàncies il·legals en punts de control de seguretat (de Araujo et al., 2018; Lawton et al., 2017). Ara bé, encara avui, molts dels treballs publicats relacionats amb l'anàlisi de camp són estudis conceptuals (el que es coneix en anglès com *proof of concept*) que pretenen demostrar el potencial d'aquestes tècniques, per exemple per a la detecció de traces d'explosius, de drogues d'abús o de tintes en bitllets bancaris (Fedick et al., 2017; Hall et al., 2017). Tot i que la miniaturització de la majoria de les tècniques *Ambient MS* per al seu ús en espectròmetres de masses portàtils és relativament senzilla, algunes tècniques com la DESI o la DART presenten alguns desavantatges derivats, principalment, de la necessitat de bombejar dissolvent (DESI), de l'elevat volum de gas a alta pressió (He, N_2) o de la font d'alimentació d'alt voltatge (de Araujo et al., 2018). Per contra, la font LTP, que permet analitzar qualsevol tipus de superfície sense restriccions en la mida i en la forma (la torxa del plasma es dirigeix directament a la superfície de la mostra), presenta menys limitacions, la qual cosa ha permès un disseny portàtil utilitzant bateries recarregables i un petit tanc d'heli per a l'anàlisi de camp (de Araujo et al., 2018). Altres fonts *Ambient MS*, com ara les ja esmentades tècniques PS-MS i TS-MS, també s'han emprat per a l'anàlisi forense de camp per les seves capacitats per mostrejar *in situ* gairebé qualssevol tipus de mostra. A mode d'exemple, es pot comentar el treball de Hall *et al.,* en el què es compara l'efectivitat de les tècniques DESI-MS que utilitzen hisops per al mostreig i el *paper spray* per a la detecció de traces de compostos relacionats amb la producció de desomorfina, un opiaci més econòmic que l'heroïna responsable dels efectes psicoactius d'una droga clandestina injectable anomenada *krokodil*, utilitzant un espectròmetre de masses portàtil amb un analitzador de trampa d'ions (Hall et al., 2017). Tot i que els resultats del treball posen de manifest l'aplicabilitat d'ambdues tècniques per a la detecció dels anàlits a nivells de traces (ng mL^{-1}), el PS-MS va permetre obtenir uns límits de detecció dos ordres de

magnitud per sota. Els mateixos autors indiquen que els pitjors resultats obtinguts amb el DESI-MS podrien estar directament relacionats amb el tipus de substrat utilitzat per a l'anàlisi (l'hisop utilitzat per al mostreig), la composició i la textura del qual afecten directament al procés d'extracció i d'ionització dels anàlits (Takáts et al., 2005).

Com s'ha comentat en la *Publicació I* (apartat 1.1.1), algunes fonts *Ambient MS* es poden adaptar per generar imatges en dos dimensions (2D) per tal d'obtenir informació de la distribució espacial de la composició química a la superfície de les mostres, el que resulta de gran interès en aplicacions forenses. Alguns articles de la literatura descriuen les capacitats de les tècniques DESI, EASI i LESA per generar imatges moleculars que permeten obtenir informació sobre l'ètnia o el gènere d'una persona sospitosa, o bé per detectar la presència de substàncies que evidenciïn la pràctica d'una activitat il·legal, com ara traces de drogues d'abús, d'explosius o d'armes de foc (Bailey et al., 2016; de Morais et al., 2017; Perez et al., 2018; Zhou et al., 2017). Els treballs més recents se centren, sobretot, en la generació d'imatges emprant la font DESI per a l'anàlisi de tintes en diferents tipus de documents bancaris susceptibles d'ésser robats o falsificats, com ara les tintes termocròmiques, la visibilitat de les quals canvia de forma reversible amb la temperatura (Correa et al., 2016b; Khatami et al., 2017). Per exemple, a través de la generació d'imatges amb DESI-MS, Khatami *et al.* van identificar ions específics dels estats visibles, invisibles i de reaparició de la tinta termocròmica en estudis d'identificació de documents falsificats. Ara bé, una de les principals preocupacions en aquest tipus d'aplicacions és la possibilitat de danyar les mostres analitzades quan s'estudien documents valuosos. Per aquest motiu, alguns autors posen de manifest la importància del control del cabal i la selecció del tipus de dissolvent emprat per a la generació de l'electroesprai, ja que són els paràmetres més crítics per evitar que la tinta s'estengui sobre la superfície i malmeti el document en estudi.

Pel que fa al tipus d'analitzadors de masses emprats aquests darrers anys amb les tècniques *Ambient MS* en el camp forense i pel control d'antidopatge, en molts estudis s'han seguit utilitzant les trampes d'ions lineals (LIT), principalment en el mode de *full scan* i d'escombratge d'ions producte amb fragmentacions en etapes successives (MS^2 i MS^3). La relativa elevada sensibilitat que presenten les LIT en el mode de *full scan* facilita la detecció de compostos diana a baixes concentracions, a la vegada que possibiliten l'anàlisi retrospectiva de la mostra per a la detecció de compostos sospitosos (Amador et al., 2017; Damon et al., 2016; Khatami et al., 2017; Mirabelli et al., 2015; Pirro et al., 2015). Tot i que alguns estudis empren analitzadors de QqQ en el mode d'adquisició de MRM (Crevelin et

al., 2016; Kerpel dos Santos et al., 2018; Teunissen et al., 2017), aquests treballs se centren en la monitorització selectiva de determinats compostos diana (anàlisi *target*). No obstant això, i atès que els analitzadors de masses de baixa resolució presenten limitacions a l'hora d'identificar i de caracteritzar substàncies, especialment els compostos sospitosos i els nous compostos desconeguts presents en matrius molt complexes, els espectròmetres de masses d'alta resolució actuals comencen a ser utilitzats amb força freqüència. Com es pot observar a la Taula 1.1, els analitzadors que s'utilitzen majoritàriament són l'Orbitrap i el TOF en la seva configuració híbrida, combinant-los amb un quadrupol o amb una trampa d'ions lineal per tal de poder dur a terme experiments d'espectrometria de masses en tàndem (Bailey et al., 2015, 2016; Bain et al., 2018; de Morais et al., 2017; Doué et al., 2015; Fedick et al., 2017; Habala et al., 2016; Jeong et al., 2016; Schmidt et al., 2014; Zhou et al., 2017). La ja mencionada capacitat d'aquests espectròmetres de masses de combinar informació indiscriminada (*full scan*) amb informació molt més selectiva, treballant en el mode d'escombratge d'ions producte o en altres modes d'adquisició menys dirigits, com ara els DDA o els DIA, proporciona informació estructural que possibilita la caracterització de nous compostos utilitzats en pràctiques delictives. Així, aquestes metodologies permeten augmentar i actualitzar les bases de dades dels compostos diana que es van incorporant a les llistes de compostos regulats o dels quals se'n coneix una relació directa amb activitats il·legals i possibiliten l'anàlisi retrospectiva de les mostres per a la identificació de nous compostos d'interès.

Els treballs publicats els últims 3 anys referents a la possibilitat de desenvolupar mètodes d'anàlisi quantitativa emprant tècniques *Ambient MS* per a l'anàlisi forense i, sobretot, per al control antidopatge, segueixen essent gairebé inexistents. Alguns autors, com ara Bailey *et al.*, posen de manifest la dificultat que existeix per conèixer la quantitat de mostra que es transfereix a l'espectròmetre de masses i com es distribueix en els diferents dispositius comercials utilitzats per suportar-la (portaobjectes, paper de filtre o hisops) (Bailey et al., 2015). Aquests factors augmenten la variabilitat dels resultats obtinguts i, per tant, limiten les possibilitats de desenvolupar mètodes *Ambient MS* quantitatius. Pel que fa referència a l'anàlisi de matrius líquides, el volum de mostra que s'analitza és perfectament conegut i, per tant, l'anàlisi quantitativa es pot realitzar emprant el calibratge per addició de patró intern, utilitzant patrons isotòpicament marcats que permeten corregir els efectes de la matriu. Tot i així, en alguns treballs de la literatura es comenta la necessitat de realitzar manipulacions ràpides de la mostra, com ara la dilució d'un oli o l'alcalinització de l'orina,

per tal de disminuir els ja esmentats efectes de supressió iònica observats en l'anàlisi de matrius complexes, que afecten considerablement a la sensibilitat, a la selectivitat i a la reproductibilitat dels mètodes *Ambient MS*, sobretot quan la ionització per electroesprai hi està involucrada (Crevelin et al., 2016; Doué et al., 2015; Habib et al., 2018).

La velocitat, l'especificitat i l'ampli interval d'aplicabilitat de les tècniques *Ambient MS* obren grans perspectives de futur en el desenvolupament de metodologies analítiques per a l'anàlisi forense. Ara bé, en aquest camp d'aplicació és de gran importància la selectivitat i sensibilitat de la tècnica, així com la robustesa dels resultats quantitatius. Per aquests motius, és indispensable continuar desenvolupant estratègies en les metodologies *Ambient MS* que permetin millorar els actuals límits de detecció dels mètodes desenvolupats fins avui. Indiscutiblement, s'hauran d'utilitzar espectròmetres de masses d'alta resolució capaços de proporcionar una gran capacitat d'identificació i de caracterització. Encara que part de la variabilitat dels resultats és inherent a l'anàlisi de superfícies i a la manca d'homogeneïtat en la distribució dels anàlits en la superfície de la mostra, per millorar la reproductibilitat dels mètodes quantitatius és necessari seguir explorant en el disseny de noves estratègies que permetin augmentar la robustesa dels mètodes *Ambient MS*.

Taula 1.1. Articles més rellevants publicats els últims tres anys sobre l'ús de les tècniques Ambient MS per a l'anàlisi forense i el control antidopatge.

Tècnica *Ambient MS*	Analitzador de masses	Mostra	Mostreig	Àmbit de l'anàlisi	Ref.
			Anàlisi forense		
DART	TOF	Bales, cartutxos i roba amb possibles residus d'armes de foc	Bales i cartutxos: capil·lar de vidre per raspar Mostres tèxtils : extracció amb MeOH	Identificació de possibles marcadors associats a armes de foc impreses en 3D	(Black et al., 2017)
DART	LTQ-Orbitrap	Plantes i cannabinoides en pols	Plantes: extracció amb MeOH Mostres en pols: dissolució amb MeOH	Identificació de 6 cannabinoides sintètics	(Habala et al., 2016)
DART	Orbitrap	Taques de gelat en pantalons i peces ceràmiques	Frotis de les superfícies amb un hisop	Identificació de teobromina i cafeïna per aportar evidències en un cas forense real relacionat amb un crim violent	(Kern et al., 2018b)
DART	IM-TOF	Drogues d'abús (plantes i pastilles)	Dissolució en MeOH	Identificació de 53 drogues d'abús	(Lian et al., 2017)
TD-DART	TOF	Sals oxidants i patrons comercials d'explosius	Dissolució amb aigua i dilució amb MeOH	Anàlisi d'explosius inorgànics volàtils	(Forbes et al., 2017)
DBDI	Orbitrap	Cru de síntesi d'explodius	Dilució en acetonitril i cloroform. Dipòsit en superfícies de vidre, paper i tela de cotó	Determinació de 2 peròxids (TATP i DADP)	(Hagenhoff et al., 2017)
DESI DESI-MSI	LTQ	Tinta termocromàtica	Paper de filtre pintat amb la tinta	Identificació de marcadors per a la detecció de documents falsificats	(Khatami et al., 2017)
DESI	LTQ-Orbitrap	Empremtes dactilars i saliva	Empremtes marcades en un portaobjectes. Mostres de saliva recollides amb un kit Quantisal™	Identificació de cocaïna i dels seus metabòlits per a la detecció del consum il·legal de drogues	(Bailey et al., 2015)
DESI i EASI	LTQ	Condons	Cap	Identificació d'abús sexual basat en perfils polimèrics (PEGs)	(Mirabelli et al., 2015)
DESI i PS	IT cilíndrica (MS portàtil)	Estris de cuina	Frotis de la superfície amb un hisop (DESI) i amb un paper de filtre (PS)	Detecció de desomorfina i codeïna en la producció clandestina de *Krokodile*	(Hall et al., 2017)

Tècnica *Ambient MS*	Analitzador de masses	Mostra	Mostreig	Àmbit de l'anàlisi	Ref.
DESI-MSI	Q-Orbitrap	Bitllets bancaris	Cap	Detecció de residus de tintes antirobatori no visibles	(Correa et al., 2016b)
DESI-MSI	LTQ-Orbitrap	Teixit dèrmic	Frotis de la superfície amb un portaobjectes	Identificació de patrons espacials per obtenir informació sobre el gènere, l'ètnia i l'edat	(Zhou et al., 2017)
EASI	Q and FT-ICR	Bitllets bancaris	Cap	Detecció de residus de triperòxid de triacetona (TATP) per a la identificació de robatoris en caixers automàtics per explosió	(Correa et al., 2017)
EASI	Q	Bitllets bancaris	Cap	Identificació del colorant de tinció Rodamina B contra robatori	(Schmidt et al., 2015)
EASI-MSI	Q-Orbitrap	Paper secant	Cap	Anàlisis d'una nova substancia psicoactiva (25I-NBOH)	(de Morais et al., 2017)
LESA-MSI	LTQ orbitrap	Empremtes dactilars, saliva i orina	Empremtes dactilars: en un portaobjectes. Saliva i orina: recollides amb un kit Quantisal™	Detecció d'explosius i de drogues d'abús	(Bailey et al., 2016)
PS	LCQ	Tintes de color negre	Aplicació de la tinta en papers A4 ordinaris	Classificació de bolígrafs comercials utilitzant els perfils químics	(Amador et al., 2017)
PS	FT-ICR	Paper assecant, fulles de cànnabis, cannabinoides sintètics, tintes de color blau, bitllets bancaris falsificats	Paper assecant: extracció amb MeOH Fulles de cànnabis: extracció amb ACN	Identificació i quantificació de substàncies psicoactives i determinació dels perfils químics de diferents bolígrafs comercials	(Domingos et al., 2017a)
PS	LTQ	Sang, sèrum i orina	Dipositat en paper sililat	Identificació de drogues d'abús	(Damon et al., 2016)
PS	Orbitrap	Sang i orina	Cap	Determinació de 3 agents químics bèl·lics i els seus productes d'hidròlisi	(Mckenna et al., 2017)
PS	QqQ	Sang	Cap	Determinació de 8 amfetamines	(Teunissen et al., 2017)

Tècnica *Ambient MS*	Analitzador de masses	Mostra	Mostreig	Àmbit de l'anàlisi	Ref.
TS	LTQ-Orbitrap	Superfícies de vidre, metall, tefló, plàstic, guants i pell humana	Hisops prèviament mullats amb ACN	Anàlisi de 6 residu d'explosius	(Bain et al., 2018)
TS	LTQ-Orbitrap and Mini 12 rectilinear IT	Pell, guants, roba i cartutxos	Hisops	Escombratge de residus orgànics d'armes de foc	(Fedick et al., 2017)
TS-MS	LTQ	saliva	Cap	Identificació de 14 drogues d'abús	(Pirro et al., 2015)
			Control antidopatge		
ASAP	QqQ	Orina	Capil·lars de vidre	Identificació d'estimulants de tipus amfetamínic	(Crevelin et al., 2016)
DART	LTQ-Orbitrap	Olis comercials	Dilució amb MeOH	Identificació i quantificació de 21 esteroides anabolitzants	(Doué et al., 2015)
DART	QqQ	Suplements dietètics	Dilució amb MeOH	Identificació i quantificació de DMAA i d'altres estimulants no declarats	(Kerpel dos Santos et al., 2018)
HS-DBDI	LTQ	Orina	Alcalinització de l'orina	Identificació d'estimulants de tipus amfetamínic	(Habib et al., 2018)
PS	LTQ-Orbitrap	Orina	Cap	Quantificació d'efedrines	(Jeong et al., 2016)

1.2.2. Anàlisi clínica

Un dels camps d'aplicació importants de les tècniques *Ambient MS* és l'anàlisi clínica. A la Taula 1.2 es resumeixen els treballs més rellevants en aquest camp d'aplicació publicats a la literatura els darrers 6 anys on s'indiquen l'objectiu de l'anàlisi, el tipus de mostres i el tipus d'analitzadors, a més de les corresponents referències. Com es pot observar, són nombroses les tècniques *Ambient MS* emprades i les aplicacions analítiques desenvolupades que se centren, sobretot, en demostrar les possibilitats d'aquestes tècniques per al diagnòstic i el control ràpid de malalties.

La facilitat operacional així com la capacitat d'obtenir informació molecular gairebé en temps real que ofereixen les tècniques *Ambient MS* són característiques molt atractives en el camp de la salut, ja que poden permetre disposar de metodologies analítiques útils en els serveis de diagnosi ràpida de malalties (*point of care*: POC). De manera semblant al que ja s'ha vist en l'apartat anterior per a la detecció del consum de drogues d'abús i per al control antidopatge, moltes de les aplicacions clíniques de les tècniques *Ambient MS* descrites a la bibliografia se centren en l'anàlisi de mostres de sang, d'orina i de sèrum on es monitoritzen un nombre relativament limitat de compostos diana. L'objectiu d'aquestes aplicacions és poder detectar, de la forma més ràpida i menys invasiva possible, la presència de substàncies a concentracions clínicament rellevants i que permetin donar respostes en tests POC. Per aquestes aplicacions s'han utilitzat les tècniques DART, LTP i EESI (Jones et al., 2013; Li et al., 2013; Song et al., 2015; Wang et al., 2013a), tot i que destaca el nombre de treballs que proposen emprar la PS-MS, sobretot en la seva vesant d'anàlisi quantitativa. Alguns articles de revisió posen de manifest que els protocols poc invasius per a la recollida de les mostres i la possibilitat de dur a terme l'anàlisi amb una quantitat mínima de mostra (<10 µL) són característiques que compleixen amb les necessitats de rapidesa i rendiment dels assajos POC (Chiang et al., 2018; Ferreira et al., 2016; Manicke et al., 2016; Pu et al., 2019). Entre les aplicacions més estudiades emprant la tècnica PS-MS, en destaquen la monitorització d'una gran varietat de fàrmacs (drogues oncològiques, dextrorfan, amitriptilina, imipramina, citalopram o imitinib, entre d'altres) i la identificació de substàncies que puguin ser emprades com a biomarcadors per al pronòstic de nombrosos desordres metabòlics en mostres de sang, orina i saliva (Taula 1.2).

Pel que fa a l'anàlisi quantitativa, la tècnica que ha permès obtenir molt bons resultats en termes de sensibilitat, d'exactitud, de precisió i d'interval de linealitat és la PS-MS emprant

el patró intern per a la calibració i obtenint uns límits de quantificació (LOQs), en la majoria dels casos, inferiors als ng mL^{-1} (Espy et al., 2012; Kennedy et al., 2018; Naccarato et al., 2013; Shi et al., 2015; Wang et al., 2013b). Tot i que en moltes de les publicacions s'utilitza la PS-MS de forma manual, s'ha dissenyat i comercialitzat un sistema automatitzat que utilitza cartutxos de plàstic per depositar la mostra i que permet augmentar la reproductibilitat dels mètodes PS-MS per a l'anàlisi clínica. Aquests cartutxos contenen el paper suport, que s'ha tallat amb gran precisió i reproductibilitat emprant tècniques làser. A més, els cartutxos disposen d'una zona metàl·lica i d'un reservori amb un sistema de bombeig que permeten que el potencial i el dissolvent necessaris per a la generació de l'ESI siguin aplicats sempre en la mateixa posició i de la mateixa manera. L'ús d'aquests dispositius fa possible millorar la robustesa dels mètodes PS-MS ja que evita la manipulació del paper que podria fer malbé la punta on té lloc la ionització, proporciona una certa protecció de la mostra fins que s'introdueix en el sistema i minimitza la possibilitat de que es puguin produir casos de contaminació creuada (Kennedy et al., 2018; Shi et al., 2015). La senzillesa i l'automatització d'aquest disseny comercial possibilita que pugui ser emprada en espectròmetres de masses portàtils i per usuaris del camp clínic que no són experts en MS (Zhang et al., 2017b). Alguns treballs de la literatura també descriuen l'ús d'altres tècniques *Ambient MS* per al desenvolupament de mètodes per assajos POC, com ara el TS-MS, que introdueix directament en el sistema d'ionització el substrat (agulla metàl·lica) emprat pel mostreig dels fluids biològics (Kerian et al., 2014).

Una altra de les àrees de l'anàlisi clínica en què s'ha estudiat l'aplicabilitat de les tècniques *Ambient MS* és l'anàlisi de teixits per a la diagnosi de malalties, sobretot de càncer, que provoca anualment a l'entorn de 6 milions de morts a nivell mundial (Ifa et al., 2016). Els procediments més freqüents per al diagnòstic del càncer es basen en estudis de mostres del teixit obtingut en una biòpsia per part de patòlegs especialitzats. En aquests estudis s'avalua la morfologia del teixit cel·lular, l'estructura i l'organització de les cèl·lules del teixit per poder determinar si el pacient sofreix o no la malaltia. Els resultats es transmeten al cirurgià per tal que prengui decisions en plena intervenció d'extirpació del tumor. Tanmateix, aquests procediments tenen una durada que va dels 20 als 60 minuts, cosa que implica que, en molts casos, sigui inevitable haver de perllongar la cirurgia per poder prendre una decisió sobre la necessitat, o no, de seguir extirpant teixit. En aquest context, alguns dels articles de revisió posen de manifest els avantatges que ofereix l'anàlisi directa emprant les tècniques *Ambient MS* a l'hora de proporcionar informació molecular rellevant en el mateix quiròfan dels teixits

extirpats de manera molt ràpida (en qüestió de pocs minuts) (Ifa et al., 2016; Woolman et al., 2018). Aquesta informació, a més, pot complementar l'avaluació histològica, sobretot quan existeixen desacords en les lectures patològiques.

Els estudis de diagnosi de càncer amb *Ambient MS* que es troben a la literatura es basen, principalment, en obtenir perfils específics discriminants per al diagnòstic, aprofitant les diferències en les abundàncies relatives d'algunes espècies lipídiques en mostres de teixit sa i en mostres de teixit tumoral. Com es pot observar a la Taula 1.2, la DESI és una de les tècniques *Ambient MS* que més s'ha emprat per a l'exploració de perfils lipídics en teixits (Taula 1.2). Molts dels treballs publicats mostren la capacitat d'aquesta tècnica en la seva versió de generació d'imatges (DESI-*imaging MS)* per discriminar, en una mateixa mostra de teixit, la zona de teixit tumoral i la de teixit sa mitjançant l'escombratge de la superfície del teixit. La tècnica DESI s'ha utilitzat per interrogar la distribució lipídica espacial en diferents tipus de teixits obtinguts en biòpsies per al diagnòstic de càncer de mama, de pàncrees o d'ovari (Eberlin et al., 2016, 2012; Porcari et al., 2018; Sans et al., 2017; Zhang et al., 2017a). A més, la DESI també s'ha emprat per a la generació d'imatges de diferents seccions de teixit cerebral extirpat, obtenint diferents perfils de fosfolípids per a la matèria grisa, la matèria blanca, gliomes, meningiomes i tumors de la hipòfisi, mostrant la capacitat d'aquesta tècnica no només per la detecció de teixit tumoral, sinó també per diferenciar entre diferents tipus de tumors (Eberlin et al., 2016, 2012; Pirro et al., 2017a; c). L'anàlisi és molt poc invasiva, la qual cosa facilita que la mateixa mostra de teixit també pugui ser utilitzada per realitzar l'avaluació patològica. Ara bé, el temps necessari per generar una imatge és relativament llarg, ja que cal adquirir l'espectre de masses d'un gran nombre de punts de la superfície, molt propers entre si, per tal de determinar la distribució de les espècies estudiades amb suficient resolució espacial. Si el que es vol és obtenir informació representativa i reproduïble de manera ràpida del teixit extirpat, algun autors proposen realitzar un frotis del teixit amb un suport, com ara un portaobjectes o una superfície de PTFE, i analitzar-ne la superfície emprant DESI-MS mono-dimensional (Jarmusch et al., 2016; Pirro et al., 2017a; c; Woolman et al., 2017). Aquesta estratègia s'està avaluant com una eina per poder guiar als cirurgians durant les intervencions de resecció de tumors, especialment d'òrgans on és important extirpar la mínima quantitat de teixit possible, facilitant informació gairebé de forma immediata. Ara bé, aquests procediments impossibiliten que les mostres de teixit puguin utilitzar-se també en els estudis patològics, i només proporcionen informació sobre el teixit que s'ha extirpat, la qual cosa implica que,

per assegurar que s'ha eliminat qualssevol residu del tumor, sigui necessari seguir seccionant teixit fins obtenir un perfil lipídic de teixit sa.

En aquest context, la utilització de la tècnica REIMS, que integra una modalitat quirúrgica rutinària amb l'anàlisi MS utilitzant una sola sonda per realitzar l'anàlisi de teixits *in vivo*, pot representar un avenç important. Aquesta tècnica permet obtenir perfils MS de forma gairebé instantània mentre el cirurgià està extirpant teixit emprant mètodes quirúrgics d'ablació tèrmica. La tecnologia anomenada *iKnife* utilitza un bisturí elèctric en línia amb la tècnica REIMS i en combinar els procediments quirúrgics amb la informació molecular que proporciona l'espectrometria de masses i les eines estadístiques multivariants, que permeten l'anàlisi ràpida de les dades, permet obtenir un diagnòstic durant les operacions de resecció tumoral (Balog et al., 2013). La REIMS s'ha aplicat en la identificació de perfils lipídics discriminants, resultant una eina molt útil per al diagnòstic de càncer en diferents òrgans (estómac, còlon, pit i fetge, entre d'altres) (Balog et al., 2015, 2013; Golf et al., 2015; St John et al., 2017). D'altra banda, alguns autors també han investigat l'ús de la tècnica REIMS per estudiar les característiques de l'absorció de lípids nutricionals i el seu procés de digestió, tot i que els estudis realitzats fins ara s'han dut a terme *in-vitro*, simulant el procés gastrointestinal dels humans (Lin et al., 2018). Emprant la mateixa filosofia que l'*iKnife*, també s´ha explorat l'acoblament d'altres tècniques quirúrgiques, com ara la cirurgia làser o l'aspirador quirúrgic CUSA (C*avitron Ultrasonic Surgical Aspirator*), amb les fonts DESI o EASI per al diagnòstic en línia, però fins ara no s'han realitzat proves *in-vivo* (Ifa et al., 2016). Tot i les possibilitats que presenten aquestes tècniques, és important mencionar que es requereix una formació específica dels cirurgians i del personal mèdic per aplicar-les en les intervencions quirúrgiques, cosa que pot dificultar la seva acceptació per arribar, en un futur, a ser considerades una alternativa o inclús un substitut de les eines quirúrgiques actuals. En aquest context, les perspectives de futur per a l'anàlisi clínica *in-vivo* se centren en l'ús i l'adaptació de dispositius comercials amb els quals els cirurgians ja estan familiaritzats (agulles, hisops, etc.) per al mostreig *in-vivo* i la posterior la ionització.

Com ja s'ha comentat en aquesta introducció, les matrius biològiques són especialment complexes i solen donar efectes matriu significatius en la seva anàlisi per espectrometria de masses. Els efectes de supressió iònica, sobretot en les tècniques *Ambient MS* basades en ionització per ESI, així com les interferències isobàriques causades pels components presents en la matriu, poden afectar negativament a la sensibilitat i la selectivitat del mètode. Per tal de millorar l'especificitat en els mètodes que se centren en l'anàlisi de compostos

diana, la majoria d'autors proposen treballar en tàndem en mode MRM emprant analitzadors de QqQ, que permeten discriminar entre compostos isobàrics o inclús entre isòmers estructurals, sempre que presentin ions producte específics diferents (Espy et al., 2012; Lee et al., 2016a; b; Naccarato et al., 2013; Ren et al., 2013; Song et al., 2015; Wang et al., 2013b; a; Yan et al., 2017; Yang et al., 2012a). Una estratègia que utilitzen alguns autors per millorar la selectivitat i la sensibilitat obviant els processos d'extracció, de neteja o de preconcentració per a l'anàlisi de mostres biològiques, és la derivatització *in situ* dels anàlits (Bag et al., 2015; Ferreira et al., 2016; Lostun et al., 2015; Manicke et al., 2016; Zhou et al., 2014). L'ús del terme "*in situ*" implica que l'agent derivatitzant s'incorpora durant l'anàlisi de la mostra mitjançant el dissolvent d'extracció/ionització sense realitzar cap etapa que impliqui una preparació prèvia. Alguns treballs publicats posen de manifest l'aplicabilitat de la derivatització en línia emprant les tècniques DESI i PS per a l'anàlisi de compostos que presenten grups funcionals difícilment ionitzables amb aquestes tècniques (aldehids, carbonils, alcohols, tiols i alquens). En la majoria dels casos, la principal motivació és millorar l'eficàcia de la ionització dels compostos d'interès, sobretot per a la detecció de substàncies que presenten baixa afinitat protònica, com ara el colesterol, tot i que també millora l'especificitat del mètode, possibilitant la discriminació entre isòmers estructurals o la caracterització de pèptids i de proteïnes (Ferreira et al., 2016; Manicke et al., 2016).

Pel que fa a les metodologies que se centren en la detecció de perfils lipídics per al diagnòstic de càncer, la recerca de compostos desconeguts o la detecció de biomarcadors requereix la utilització de modes d'adquisició no dirigits i molt poc discriminants. Així, els analitzadors de masses de trampes d'ions han estat sovint emprats, possiblement per l'avantatge que ja s'ha comentat anteriorment d'obtenir els espectres de masses en *full scan* amb bona sensibilitat, tot i que avui dia comencen a ser substituïts per instruments d'alta resolució, especialment l'Orbitrap (Taula 1.2). En aquestes metodologies s'obté una gran quantitat d'informació espectral i, per aquest motiu, sovint és necessària la utilització de mètodes estadístics (supervisats i no supervisats) que permetin la seva avaluació, com ara per identificar els compostos lipídics més rellevants i discriminants en mostres de teixits que, posteriorment, poden ser emprats com a possibles biomarcadors.

La implementació fora del laboratori de mètodes *Ambient MS* per donar una resposta diagnòstica ràpida de malalties és un camp d'indubtable interès. La simplicitat, rapidesa i baix cost de l'anàlisi emprant les tècniques *Ambient MS* possibilita que puguin ser utilitzades en un gran ventall de proves, com ara per a l'anàlisi toxicològica, la monitorització de

fàrmacs, l'escombratge de metabòlits o l'obtenció de perfils lipídics discriminants de teixits. Tanmateix, encara existeixen limitacions i reptes que cal afrontar. D'una banda, la baixa selectivitat, en part solucionada emprant l'HRMS, i els elevats límits de detecció dificulten la presa de decisions sobretot quan s'analitzen fluids biològics (sang, orina o saliva). D'altra banda, fins avui, la majoria de les investigacions s'han centrat en la instrumentació i rarament s'han dut a terme estudis que demostrin les possibilitats de les metodologies *Ambient MS* per donar un servei mèdic real. Així doncs, és necessari realitzar assajos de validació i desenvolupar protocols regulats que permetin assegurar tant la viabilitat real d'aquestes metodologies com la veracitat del resultat de la prova diagnòstica.

Taula 1.2. Articles més rellevants publicats els últims sis anys sobre l'ús de les tècniques Ambient MS per a l'anàlisi clínica.

Tècnica *Ambient MS*	Analitzador de masses	Mostra	Mostreig	Àmbit de l'anàlisi	Ref.
DART	QqQ	Sang humana	Extracció amb MeOH:àcid fòrmic (0.1%)	Diagnòstic de fenilcetonúria en nadons	(Wang et al., 2013a)
DART	QqQ	Plasma humà	Dilució ACN/H2O (80/20, *v:v*)	Quantificació de tirosina	(Song et al., 2015)
DART	Q-TOF	Sèrum humà	Precipitació de proteïnes	Escombratge del perfil metabòlic	(Jones et al., 2013)
DESI	IT	Teixit mamari	Cap	Obtenció de perfils lipídics per al diagnòstic de càncer de mama	(Calligaris et al., 2014)
DESI	LTQ	Teixit prostàtic	Cap	Obtenció de perfils lipídics per al diagnòstic de càncer de pròstata	(Kerian et al., 2015)
DESI	LTQ, Orbitrap	Teixit cerebral humà i animal	Frotis del teixit en un portaobjectes de vidre	Obtenció de perfils lipídics per al diagnòstic de tumors cerebrals	(Jarmusch et al., 2016; Pirro et al., 2017a; c)
DESI i DESI-MSI	Q-TOF	Teixit animal	Frotis del teixit en un portaobjectes de vidre i PTFE	Obtenció de perfils lipídics per al diagnòstic de càncer	(Woolman et al., 2017)
DESI-MSI	Orbitrap i LTQ-Orbitrap	Teixit mamari	Cap	Obtenció de perfils lipídics per al diagnòstic del càncer de mama	(Porcari et al., 2018)
DESI-MSI	LTQ-Orbitrap	Teixit mamari	Cap	Obtenció de perfils lipídics i de metabòlits per al diagnòstic de càncer de mama	(Zhang et al., 2017a)
DESI-MSI	LTQ-Orbitrap	Teixit ovàric	Cap	Detecció de marcadors metabòlics per a la predicció de l'agressivitat del càncer d'ovari	(Sans et al., 2017)
DESI-MSI	LTQ	Teixit cerebral i pancreàtic	Cap	Obtenció de perfils lipídics per al diagnòstic de càncer cerebral i de pàncrees	(Eberlin et al., 2016, 2012)
Reactive DESI-MSI	LTQ	Teixit cerebral	Cap	Obtenció de perfils lipídics per al diagnòstic de càncer	(Lostun et al., 2015)
EESI	LTQ	Alè humà	Cap	Determinació d'acetonitril per a la detecció d'usuaris fumadors	(Li et al., 2013)
LMJ	Orbitrap	Sang	Cap	Determinació d'acetaminofèn	(Gaissmaier et al., 2016)
NanoDESI	LTQ-Orbitrap	Teixit de la pell	Cap	Identificació de proteïnes i pèptids	(Hsu et al., 2015, 2013)

Tècnica *Ambient MS*	Analitzador de masses	Mostra	Mostreig	Àmbit de l'anàlisi	Ref.
Probe-ESI	IT-TOF i QqQ	Plasma i teixit aòrtic	Dilució del plasma (20 vegades) amb EtOH/H_2O (50:50, *v/v*) Extracció del teixit aòrtic amb 50 µL EtOH/H_2O (50:50, *v/v*), centrifugació i dilució (10 vegades) del sobrenedant (EtOH/H_2O 50:50, *v/v*)	Detecció de biomarcadors per al diagnòstic d'arteriosclerosi	(Johno et al., 2018)
PS	QqQ	Sèrum humà	Mescla del sèrum amb una solució que conté el substrat i el coenzim en HCl per a la reacció enzimàtica (37 °C). L'alíquota es mescla amb el dissolvent de PS (MeOH/H_2O:àcid fòrmic, 90:10:0.2%) abans de l'anàlisi	Activitat de l'aspartat aminotransferasa com a biomarcador per al diagnòstic clínic	(Yan et al., 2017)
PS	QqQ	Sang	Dipositada en paper amb coagulant	Anàlisi de drogues oncològiques	(Espy et al., 2012)
PS	QqQ	Sèrum i sang	Cap	Anàlisi quantitativa d'acilcarnitines	(Yang et al., 2012a)
PS	QqQ	Sang, saliva i orina	Cap	Anàlisi quantitativa de 4 alcaloides de nicotina	(Wang et al., 2013b)
PS	QqQ	Orina	Cap	Anàlisi quantitativa d'acilcarnitines (C_2-C_{18})	(Naccarato et al., 2013)
PS	QqQ	Sang	Cap	Anàlisi quantitativa de tracolimus per al diagnòstic clínic	(Shi et al., 2015)
PS	QqQ, Orbitrap	Orina	Cap	Anàlisi quantitativa del fentanil i els seus anàlegs i identificació de drogues d'abús	(Kennedy et al., 2018)
PS	Mini12, QqQ	Sang	Cap	Identificació de proteïnes i monitorització de drogues terapèutiques	(Ren et al., 2016, 2013; Yang et al., 2012b)
Reactive PS	LTQ-Orbitrap, Mini 12	Solucions patró en ACN/H_2O (80:20, *v:v*)	Derivatització in-situ amb 4-aminofenol	Determinació d'aldehids relacionats amb el diagnòstic de càncer de pulmó	(Bag et al., 2015)
Reactive PS	LTQ	Sèrum i orina	Derivatització *in situ* amb cisteamina	Determinació de 4 quinones per al diagnòstic de mutacions	(Zhou et al., 2014)
REIMS	Q-TOF	Diversos teixits humans	Cap	Escombratge de perfils lipídics per al diagnòstic del càncer	(Balog et al., 2015, 2013; Golf et al., 2015; St John et al., 2017)

Tècnica *Ambient MS*	Analitzador de masses	Mostra	Mostreig	Àmbit de l'anàlisi	Ref.
REIMS	Q-TOF	Teixit gastrointestinal	Digestió *in vitro*	Obtenció de perfils lipídics per observar l'absorció de lípids durant la digestió gastrointestinal	(Lin et al., 2018)
TD-ESI	QqQ	Suc gàstric	Cap	Detecció de fàrmacs d'administració oral en sucs gàstric	(Lee et al., 2016b)
TD-ESI	QqQ	Saliva	Hisops i extracció amb MeOH	Identificació de pesticides per al diagnòstic d'autoenverinament	(Lee et al., 2016a)
TS-MS	LTQ	Mostres clíniques simulades	Cap	Detecció de bactèries per al diagnòstic de faringitis estreptocòccia	(Jarmusch et al., 2014)
TS-MS	LTQ	Sang	Cap	Detecció de imatinib per a la monitorització de drogues terapèutiques	(Kerian et al., 2014)

CAPÍTOL 2

Ionització per Desorció per Electroesprai (DESI): identificació de compostos sospitosos i desconeguts

En aquest capítol s'estudien dos problemes analítics de naturalesa diferent per avaluar l'aplicabilitat de la font d'ionització de desorció per electroesprai (DESI) per a l'establiment de metodologies analítiques ràpides d'anàlisi de compostos en matrius d'elevada complexitat. El primer cas tracta de l'anàlisi de compostos sospitosos d'estar presents a les mostres i dels quals es disposa de patrons comercials, a més de conèixer el tipus de matriu. El segon problema es basa en avaluar l'aplicabilitat de la tècnica DESI per a la identificació de compostos desconeguts en mostres de les quals se'n desconeix la seva naturalesa química. En ambdós casos, s'ha combinat la DESI amb l'espectrometria de masses d'alta resolució (HRMS) per tal de facilitar la identificació dels compostos i d'eliminar interferències. A més, s'han dut a terme experiments de tàndem amb alta resolució (MS/HRMS) dels ions generats a la DESI per tal d'obtenir informació estructural dels compostos desconeguts.

Aquest capítol consta d'una breu introducció, un apartat experimental on s'inclouen dues publicacions en les que es proposa l'ús de la tècnica DESI combinada amb l'espectrometria de masses d'alta resolució per a la identificació de compostos sospitosos (*Publicació III*) i per a la caracterització de compostos desconeguts (*Publicació IV*) i, per últim, una discussió conjunta dels resultats obtinguts en el treball experimental realitzat.

2.1. INTRODUCCIÓ

Com s'ha comentat a la Introducció d'aquesta memòria (Capítol 1, *Publicació I*), en la font DESI el processament de la mostra (extracció sòlid-líquid dels compostos en la superfície sòlida) i la seva posterior ionització pel mecanisme d'ESI, té lloc *in situ* de manera que, ambdós processos, l'extracció i la ionització, es realitzen en un sol pas. En DESI, les microgotes carregades generades en l'electroesprai primari que impacten en la superfície de la mostra són les responsables tant de l'extracció/desorció dels compostos com de la seva ionització en la fase líquida. Ara bé, aquesta tècnica originàriament dissenyada per a l'anàlisi directa de la superfície de matrius sòlides, no sempre es pot aplicar directament a causa, sovint, de la naturalesa de la matriu. En l'anàlisi de mostres sòlides amb superfícies irregulars o de matrius semi-sòlides o pulverulentes és necessari dissenyar estratègies ràpides i senzilles que impliquen una manipulació mínima de la mostra abans de dur a terme l'anàlisi per DESI, com ara una etapa de dissolució o una d'extracció ràpida dels compostos d'interès o inclús la impregnació d'una superfície amb la mostra. Per altra banda, cal esmentar que els límits de detecció en DESI són, en general, relativament elevats, cosa que limita la seva aplicabilitat per a la detecció directa de compostos presents a concentracions baixes, que són a les que es troben habitualment els contaminants en mostres alimentàries o mediambientals. En aquest context, convé emprar estratègies de manipulació de la mostra prèvies a l'anàlisi que permetin preconcentrar els anàlits, a la vegada que ajudin a disminuir els efectes derivats de la matriu per millorar els límits de detecció dels mètodes DESI.

Tot i que la tècnica DESI es caracteritza per la seva simplicitat operacional, és necessària l'optimització prèvia de determinades condicions de treball. En concret, s'han d'optimitzar els paràmetres geomètrics com són l'angle d'incidència de l'esprai primari, la distància de l'agulla de l'electroesprai a la superfície de la mostra i la d'aquesta al capil·lar d'entrada a l'espectròmetre de masses. A més, es requereix l'optimització de la composició i del cabal del dissolvent per l'electoesprai DESI, així com del cabal del gas de nebulització. Tots aquest paràmetres permeten controlar la mida i la velocitat de les microgotes de l'electroesprai, magnitud que repercuteix en la formació de la pel·lícula de dissolvent a la superfície de la mostra, que és on es produeix l'extracció dels compostos i la posterior formació de l'electroesprai secundari. Posteriorment, els anàlits ionitzats en la fase líquida (pel·lícula de dissolvent) es transfereixen a la fase gas pel mecanisme d'evaporació iònica a través d'un electroesprai secundari que es produeix en el si de la pel·lícula de líquid i, finalment, es

dirigeixen cap a l'entrada de l'espectròmetre de masses mitjançant la diferència de potencial aplicat (Figura 2.1). Convé tenir present que qualsevol canvi en la composició del dissolvent de l'electroesprai DESI fa variar la tensió superficial de les microgotes carregades, cosa que fa necessari modificar tots aquests paràmetres (geomètrics i de cabal del dissolvent i del gas de nebulització) per tal d'aconseguir una mida i una velocitat de les microgotes carregades adients per a la desorció/ionització eficient dels compostos a la superfície.

Un altre dels paràmetres que poden fer variar les condicions de treball de la font DESI és la pròpia naturalesa de la superfície de la mostra. Les propietats d'aquesta superfície com ara la seva porositat, la capacitat d'interacció amb els anàlits i la seva naturalesa química poden afectar al procés de desorció/ionització i, per tant, a l'abundància dels ions generats. De forma similar, en l'anàlisi de matrius líquides, en què es diposita un volum de la mostra sobre una superfície (substrat) i es deixa assecar per a l'anàlisi per DESI, les propietats del substrat emprat (naturalesa química, textura i conductivitat del substrat) també poden afectar de manera significativa al procés d'extracció/ionització així com al temps de l'anàlisi, considerant que també pot influir al temps d'assecat. Les superfícies que presenten una baixa afinitat amb els anàlits d'interès i/o una certa rugositat faciliten l'extracció dels compostos en la fase líquida (pel·lícula de solvent DESI) però també afavoreixen una major concentració de la mostra en una àrea de superfície més reduïda, ja que es disminueix la dispersió del volum de mostra dipositat. Pel que fa a la composició del dissolvent de l'electroesprai primari, aquest ha de ser elèctricament conductor i ha de presentar una tensió superficial relativament baixa per tal de facilitar la ionització dels compostos pel mecanismes d'ESI. D'altra banda, l'elecció del dissolvent de l'electroesprai DESI també té un efecte significatiu tant en l'eficàcia com en la selectivitat de l'extracció i de la ionització dels anàlits. Aquest aspecte s'ha de tenir present sobretot quan l'objectiu del mètode és la identificació de compostos sospitosos o desconeguts, atès que un augment de la selectivitat en l'extracció pot comportar la pèrdua de compostos durant l'anàlisi. En aquests casos, és més adient l'ús de dissolvents menys selectius per tal d'extraure compostos d'un ampli rang de polaritats. Ara bé, una extracció poc selectiva també implica l'obtenció d'espectres de masses més complexos i un augment dels problemes d'interferències derivats de la matriu.

Els treballs experimentals que s'inclouen en aquest Capítol s'han dut a terme emprant la font comercial DESI 1D (Prosolia Inc.). Aquesta font d'ionització (Figura 2.1) permet l'anàlisi automatitzada en una dimensió, al llarg de l'eix X. Hi ha altres models més moderns, com el DESI 2D, que permeten la generació d'imatges, doncs és possible l'anàlisi automatitzada

en dues dimensions. El *software* del DESI 1D permet programar una seqüència per a l'anàlisi automatitzada d'un nombre important de mostres així com també l'anàlisi de la superfície de la mostra emprant diferents modes de mostreig. Així, és possible programar el temps i la posició de l'esprai sobre la mostra per dur a terme l'anàlisi en el mode estàtic però també permet establir la velocitat i la longitud d'escombratge de la superfície de la mostra al llarg de l'eix horitzontal per realitzar l'anàlisi en el mode dinàmic.

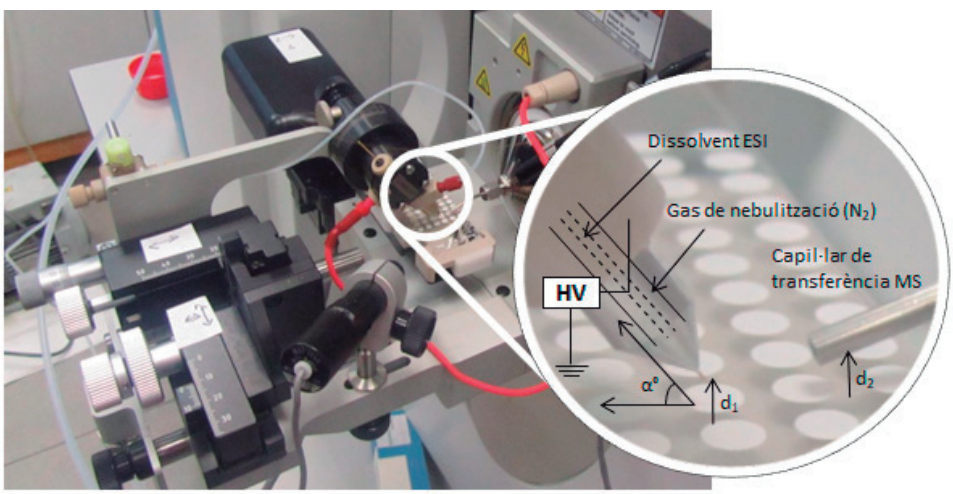

Figura 2.1. Font comercial DESI 1D (Prosolia Inc.) emprada en aquesta tesi

La rapidesa de l'anàlisi amb els mètodes DESI-MS suposa un avantatge per al desenvolupament de mètodes de cribratge que permetin augmentar el rendiment dels laboratoris de control. Tanmateix, cal tenir present que, en general, s'obté una gran quantitat d'informació espectral, ja que tot compost ionitzable de la superfície donarà un senyal en l'espectre de masses. En aquest context, la ionització suau per DESI combinada amb l'espectrometria de masses d'alta resolució permet evitar els possibles problemes d'interferències isobàriques en l'anàlisi de matrius complexes alhora que les mesures de massa exacta i les distribucions isotòpiques faciliten l'assignació de les fórmules moleculars dels compostos en el procés d'identificació/confirmació. L'analitzador de masses híbrid d'alta resolució amb què s'ha realitzat la major part del treball experimental d'aquesta tesi, un quadrupol-Orbitrap (Q-Exactive, ThermoFisher Scientific) (Figura 2.2), ha permès treballar a resolucions de fins a 140,000 FWHM (a *m/z* 200) i adquirir les dades en el mode d'escombratge d'ions totals (*full* scan) amb una elevada sensibilitat/selectivitat i proporcionant una molt bona exactitud en la mesura de la massa (<2-3 ppm). Aquest mode

d'adquisició ha possibilitat l'anàlisi de compostos diana (*target)* i de compostos sospitosos (*non-target*) i, a més, ha fet possible l'anàlisi retrospectiva de les mostres. Així mateix, amb aquest analitzador híbrid ha estat possible combinar la informació obtinguda dels espectres de masses de *full scan* amb l'obtinguda amb els experiments d'espectrometria de masses en tàndem, facilitant l'elucidació estructural dels ions detectats en l'anàlisi de compostos desconeguts (*unknowns*). Els instruments actuals com el Q-Orbitrap, permeten la fragmentació dels ions generats a la font de dues maneres diferents: per una banda, una fragmentació selectiva a una cel·la HCD *(high-energy collision dissociation)* dels ions precursors prèviament seleccionats al quadrupol (*target* MS^2) i, per altra banda, una fragmentació a la cel·la HCD sense selecció de precursors (*all-ion fragmentation*), és a dir, utilitzant el quadrupol en mode de transmissió per a la fragmentació simultània de tots els ions generats a la font d'ionització que arriben a la vegada a la cel·la HCD. Ara bé, en aquest cas l'espectre de masses de tàndem que s'obté és una barreja de tots els ions fragments generats a la cel·la HCD. Així doncs, aquest mode té una utilitat limitada en els mètodes *Ambient MS*, atesa la gran quantitat d'ions que es generen de forma simultània amb aquestes tècniques.

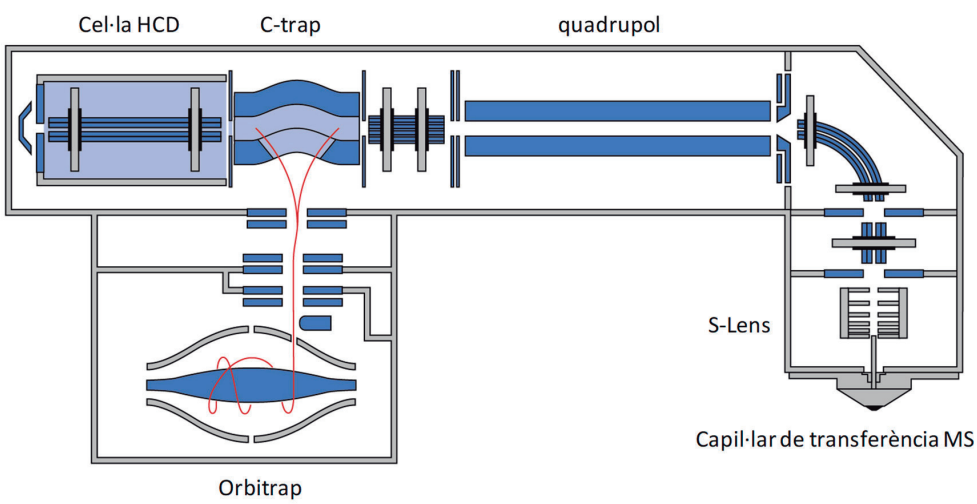

Figura 2.2. Esquema de l'espectròmetre de masses amb analitzador híbrid Q-Orbitrap (Q-Exactive) emprat en aquesta tesi (imatge de ThermoFisher Scientific)

La gran quantitat d'informació espectral que s'obté en els mètodes DESI-HRMS(/MS) implica que sigui necessari utilitzar estratègies que permetin simplificar el processament de les dades ja que, a diferència dels mètodes LC-MS o GC-MS en què també es disposa dels

temps de retenció cromatogràfics per al cribratge de les dades, tota la informació per a la identificació dels compostos s'ha d'extreure dels espectres de masses de *full scan* i de tàndem. En el cas de disposar d'informació prèvia sobre els compostos que poden estar presents a la mostra (sospitosos), es pot fer ús de diferents programaris que permeten creuar, de forma automatitzada, els valors de *m/z* dels ions observats en l'espectre de masses de *full scan* amb la informació continguda en una base de dades, per tal de confirmar la presència dels compostos d'interès en les mostres. En canvi, si no es disposa d'informació sobre la possible composició química de la mostra és més complex establir una estratègia per tal de dur a terme l'anàlisi de compostos desconeguts. Una característica dels ions que ajuda en la seva assignació és el defecte de massa, que es defineix com la diferència entre la massa exacta i la massa nominal d'un determinat ió. L'ús del defecte de massa pot ser d'utilitat en l'estudi d'espectres de masses molt complexos ja que la representació gràfica del defecte de massa envers la massa nominal dels ions detectats permet visualitzar, de forma senzilla, agrupacions d'ions que tenen característiques comunes. Per exemple, aquesta estratègia s'ha proposat per a la caracterització d'hidrocarburs en mostres de petroli o per a la identificació de metabòlits en mostres biològiques amb l'objectiu d'identificar els compostos relacionats que presenten una mateixa estructura química base. A més, en el cas de compostos que presenten unitats repetides com ara els hidrocarburs o els polímers, se solen utilitzar escales modificades en les quals l'escala de la IUPAC (Da) es normalitza en base a la unitat de repetició. Per exemple, en l'anàlisi de les dades emprant el defecte de massa de Kendrick (KMD) (Kendrick, 1963) es proposa la transformació de les masses de l'escala de la IUPAC (basada en la massa exacta del ^{12}C=12.0000) a una nova escala denominada escala de Kendrick en què s'estableix, de forma arbitrària, que la massa del metilè (CH_2) en l'escala de Kendrick és KM_{CH_2}=14.0000, enlloc de 14.0157 en l'escala de la IUPAC. Per a l'anàlisi de les dades emprant el KMD, tots els valors de *m/z* del ions observats en l'espectre de masses d'alta resolució es converteixen a l'escala de Kendrick simplement multiplicant el valor experimental de *m/z* pel factor que relaciona les dues escales, la de Kendrick i de la IUPAC (14.0000/14.01561). En l'escala de Kendrick, les sèries homòlogues d'ions que només difereixen en *n* unitats de CH_2 presenten el mateix valor de KMD. Així doncs, quan es representen gràficament els valors del defecte de massa de Kendrick (KMD) –diferència entre la massa exacta de Kendrick (KM) i la massa nominal de Kendrick (NKM)– envers la massa nominal de Kendrick (NKM) és possible identificar ràpidament les sèries d'ions que presenten la mateixa composició elemental amb *n* unitats de CH_2, ja que els ions que

pertanyen a la mateixa sèrie es troben alineats horitzontalment en el gràfic. Tot i que, com s'acaba d'esmentar, l'anàlisi usant el KMD s'ha emprat originàriament per a la identificació d'hidrocarburs, alguns autors han proposat modificar l'escala de Kendrick per a la caracterització de polímers amb unitats de repetició diferents a la del metilè (Fouquet et al., 2017a; b; Sato et al., 2014). En aquests casos, s'utilitza la unitat de repetició observada en la distribució d'ions en l'espectre de masses per convertir els valors de *m/z* a l'escala de Kendrick modificada. En el gràfic KMD *vs* NKM, tots els ions que pertanyen a la mateixa distribució i presenten la mateixa unitat de repetició seleccionada es trobaran alineats horitzontalment, mentre que els ions amb una unitat de repetició diferent quedaran alineats obliquament i els ions que no són característics de compostos polimèrics tindran valors diferents de KMD i quedaran aïllats en altres punts del gràfic.

2.2 TREBALL EXPERIMENTAL

En el primer dels treballs inclosos en aquest Capítol es proposa utilitzar la DESI combinada amb l'HRMS per al cribratge ràpid de mostres en els laboratoris de control. En concret, s'ha aplicat a l'anàlisi de drogues veterinàries en pinsos medicats i no medicats, amb l'objectiu de detectar contaminacions creuades. El treball experimental es troba recollit a la *Publicació III*, "*Desorption electrospray ionization-high resolution mass spectrometry for the screening of veterinary drugs in cross-contaminated feedstuffs"* (apartat 2.2.1). En aquest estudi s'estableixen les condicions de treball, s'avalua la viabilitat del mètode DESI-HRMS proposat per a la detecció de contaminacions creuades mitjançant l'anàlisi de mostres de pinso medicats i no medicats i es comparen els resultats amb els obtinguts emprant un mètode d'UHPLC-MS/MS, prèviament establert.

El segon dels treballs experimentals inclosos en aquest capítol, que es recull a la *Publicació IV* intitulada "*Desorption electrospray ionization-high resolution mass spectrometry for the analysis of unknown materials: The phytosanitary product case"* (apartat 2.2.2), pretén posar de manifest l'aplicabilitat de la tècnica DESI i de l'espectrometria de masses d'alta resolució per a l'anàlisi de mostres desconegudes, tant pel que fa a la seva naturalesa com als compostos a estudiar. En aquest cas, es tracta d'una mostra sospitosa d'haver estat adulterada de forma fraudulenta amb finalitats fitosanitàries on tant la matriu com l'adulterant són totalment desconeguts. Es tracta d'un cas real en què el producte fitosanitari era una de les proves principals per incriminar el responsable d'haver comès el delicte d'espargir un fungicida il·legal en un parc natural. En aquest treball es proposa l'ús de l'espectrometria de masses d'alta resolució i de l'espectrometria de masses en tàndem, així com l'anàlisi de les dades emprant el defecte de massa de Kendrick per a la identificació i la caracterització dels components de la mostra.

2.2.1 PUBLICACIÓ III

Desorption electrospray ionization-high resolution mass spectrometry for the screening of veterinary drugs in cross-contaminated feedstuffs

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Analytical and Bioanalytical Chemistry, 407 (**2015**) 7369-7378

Anal Bioanal Chem (2015) 407:7369–7378
DOI 10.1007/s00216-015-8899-4

RESEARCH PAPER

Desorption electrospray ionization-high resolution mass spectrometry for the screening of veterinary drugs in cross-contaminated feedstuffs

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Received: 22 May 2015 / Revised: 29 June 2015 / Accepted: 1 July 2015 / Published online: 14 July 2015

Abstract In this study, a desorption electrospray ionization-high resolution mass spectrometry (DESI-HRMS) screening method was developed for fast identification of veterinary drugs in cross-contaminated feedstuffs. The reliable detection was performed working at high resolution (70,000 full width half maximum, FWHM) using an orbitrap mass analyzer. Among the optimized DESI parameters, the solvent (acetonitrile/water, 80:20, *v*/*v*) and the sample substrate (polytetrafluoroethylene, PTFE) were critical to obtain the best sensitivity. To analyze the solid feed samples, different approaches were tested and a simple solid-liquid extraction and the direct analysis of an aliquot (2 μL) of the extract after letting it dry on the PTFE printed spot provided the best results. The identification of the veterinary drugs (target and non-target) in the cross-contaminated feedstuffs based on the accurate mass measurement and the isotopic pattern fit was performed automatically using a custom-made database. The positive cross-contaminated feed samples were quantified by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). The results obtained demonstrate that DESI-HRMS can be proposed as a fast and suitable screening method to identify positive cross-contaminated feedstuffs reducing the number of samples to be subsequently quantified by UHPLC-MS/MS, thus improving the productivity in quality control laboratories.

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Keywords Veterinary drugs · Ambient mass spectrometry · Desorption electrospray ionization · High resolution mass spectrometry · Cross-contamination

Introduction

One of the most effective ways for farmers to administer medicines to the livestock after veterinary prescription is by medicated feed. The production and marketing of medicated feed are regulated by the European Commission [1], and many European countries have implemented residue monitoring plans to control the illegal use of these substances in feed and the misuse of authorized veterinary medicines, and to minimize drug residual occurrence [2]. The European Parliament and the Council of the European Union have established, under the Regulation 183/2005/EC, the general rules to control feed production and their manufacturing conditions, thus ensuring the traceability of feed [3]. Despite the requirements set for feed business, multi-product plants manufacture both medicated and non-medicated feed in the same production line [4, 5], and, under practical conditions, during the production, a certain percentage of the previous batch remains in the production circuit contaminating the subsequent feed batch. This *carry-over* or *cross-contamination* is recognized by the Current Good Manufacturing Practice Regulations (CGMPR) which requires adequate clean-out procedures to prevent the "unsafe" contamination. This cross-contamination may result in the exposure of non-target animals and, as a consequence, potential health risks for these animals as well as the presence of residue contamination in food products might occur. Several studies have shown that production of premixes and composed feed free of contamination is, in practice, very difficult in the existing multi-product plants [5]. If the drug carry-over results in the unsafe contamination of other medicated or non-

medicated feed, it constitutes a violation of the maximum limits established by Directive 574/2011/EC [6], resulting in adulterated feed.

To increase the productivity in agricultural and food laboratories, the rapid screening of (il)legal preparations to identify veterinary drugs in feedstuffs is widely demanded [7–13]. Today, liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) is the technique most currently used for the determination of drug contamination in feed samples. However, the complexity of feed samples requires extensive and time-consuming sample treatment protocols to provide clean extracts to be analyzed by the selective target LC-MS/MS methods [9, 10, 12–16]. In the last decade, the introduction of high resolution mass spectrometry (HRMS) has improved selectivity and specificity of LC-MS methods. However, only few methods have been published until now regarding the analysis of feed samples by LC-HRMS [17–19].

The recent introduction of ambient ionization techniques in mass spectrometry such as desorption electrospray ionization (DESI) [20] and direct analysis in real time (DART) [21] open the possibility for the direct analysis of compounds from the sample acquiring the mass spectra from bulk samples in their native state and without sample treatment or chromatographic separation [22, 23]. The analysis is performed in a few seconds, which is a significant advantage when compared to conventional analytical methods. Particularly, in DESI, a spray of charged liquid droplets is directed to the sample creating a solvent film on the surface. Further droplets hit this film splashing secondary droplets containing the analytes into the mass spectrometer [24]. Since their introduction, ambient techniques have been applied to multitude of fields, such as environmental [24–26], food [27–29], clinical diagnosis [30], and forensic analysis [31]. Nevertheless, only few papers described the use of ambient techniques for the analysis of veterinary drugs [32, 33]. DESI-MS has been applied for a rapid screening of hormones and veterinary drugs in samples from forensic investigations using an ion trap (IT) mass analyzer, although authors indicated the difficulty to detect tetracyclines under the DESI-MS conditions used [34]. Moreover, DART-HRMS has been applied for the target analysis of coccidiostats in feed samples using an orbitrap mass analyzer demonstrating the feasibility of this ambient technique to quantify these analytes at the levels established by the EU legislation [32].

The aim of this work is to study the applicability of DESI coupled to HRMS (orbitrap) for the fast screening of veterinary drugs in cross-contaminated feed samples in order to improve throughput analysis and productivity of feed control laboratories. For this purpose, the most critical DESI-HRMS working parameters are evaluated and discussed. A home custom-made database with mass spectral information of veterinary drugs is used for the fast identification of target compounds and suspect cross-contaminants.

Experimental

Chemicals and materials

Nine veterinary drugs were used as model standards for the optimization of DESI parameters. Diclazuril (DIC), narasin (NAR), monensin (MON), oxibendazole (OXI), amoxicillin (AMO), lincomycin (LIN), tiamulin (TIA), and spiramycin (SPI) were purchased from Sigma-Aldrich (Steinheim, Germany) while tylosin (TYL) was purchased from Rikilt (Wageningen, Netherlands). All the standards were of the highest purity available. LC-MS-grade methanol (MeOH), acetonitrile (ACN), and water were supplied by Sigma-Aldrich (Steinheim, Germany) as well as formic acid (≥99 %). Nitrogen (99.9995 % purity) used for nebulization gas was supplied by Linde Group (Barcelona, Spain). Individual stock solutions (1 mg mL^{-1}) were prepared in MeOH and stored at 4 °C, while the working standard mixtures were prepared weekly by appropriate dilution in ACN.

Desorption electrospray ionization-high resolution mass spectrometry

A desorption electrospray ionization (DESI) source (Omnispray Ion Source; Prosolia Inc., Indianapolis, IN) equipped with a 1D moving stage and coupled to a quadrupole-orbitrap mass spectrometer (Q-Exactive; Thermo Fisher Scientific, San Jose, CA, USA) was used in this study. DESI solvent (acetonitrile/water, 80:20 *v*/*v*) was infused by a syringe pump at 2.5 μL min^{-1} and N_2 gas was used as nebulizer gas at a pressure of 9 bar. DESI solvent was directed onto the sample surface at a nebulization capillary angle of 55° and a distance of ~9.2 mm between the mass spectrometer inlet and the spray tip. The electrospray voltage was ±4.8 kV (positive/negative). The transfer capillary temperature was set at 250 °C. Samples were deposited onto microscope glass slides of 7.1 mm^2 polytetrafluoroethylene (PTFE) (Teflon; McMaster-Carr, Santa Fe, CA, USA) printed spots. The Q-Exactive mass spectrometer was operated in positive and negative ion mode within an *m*/*z* scan range of 100–1000 *m*/*z*. Omni Spray ion source software v2.0 (Omnispray Ion Source; Prosolia Inc., Indianapolis, IN) was used to control the DESI source, while data acquisition and data processing were performed with Xcalibur software v2.2 and Exact Finder software v2.0 (Thermo Fisher Scientific, San Jose, CA, USA), respectively.

To control the reproducibility and to determine the initial DESI conditions, a red permanent marker (containing rhodamine-6G dye) purchased from Fine Sharpie (Stanford Corp., Oak Brook, IL) was used. Accurate mass calibration was performed in the Q-Exactive mass spectrometer every 48 h in both positive and negative ion modes. For positive ion mode, a calibration solution consisting of caffeine, MRFA

peptide, Ultramark 1621, and *n*-butylamine in acetonitrile/methanol/water containing 1 % formic was used, while for negative ion mode calibration, a mixture solution containing dodecyl sulfate, sodium taurocholate, and Ultramark 1621 in acetonitrile/methanol/water with 1 % of formic acid was used.

Samples and sample preparation

Feed samples, collected from farms and feed mills, that were received by the *Laboratori Agroalimentari* of the *Generalitat de Catalunya* (LAC) for their analysis by UHPLC-MS/MS [10] were used to demonstrate the applicability of the DESI-HRMS in this study.

Feed samples were extracted using a simple and fast solid-liquid extraction procedure. Briefly, 2 g of the sample was placed in a 15-mL polypropylene centrifuge tube and were extracted for 15 min in an ultrasonic bath (Bransonic B-5510, Soest, Germany) using 5 mL of a mixture of acetonitrile/water (80:20, *v*/*v*) acidified with 1 % formic acid. Finally, the extract was centrifuged (Selecta-Macrotronic; J.P. SELECTA S.A., Abrera, Spain) for 1 min at 3500 rpm and 2 μL of the supernatant was deposited onto the PTFE printed spot and allowed to dry for 5 min at ambient temperature before the DESI-HRMS analysis.

Results and discussion

DESI-HRMS

Nine veterinary drugs (macrolides, coccidiostats, and benzimidazoles) were used as model compounds to evaluate and to set up the DESI-HRMS working conditions. Standard solutions in pure acetonitrile (10 μg mL^{-1}) were deposited on PTFE surfaces and DESI full mass spectra were recorded using both positive and negative ion modes. Figure 1 shows the mass spectra obtained for a standard mixture where MON, NAR, TIA, TYL, ESP, LIN, and OXI were detected in positive ion mode mainly as protonated molecules $[M+H]^+$, except MON and NAR for which sodium adducts $[M+Na]^+$ were observed. Regarding DIC and AMOX, they were only detected in negative ion mode as deprotonated molecules $[M-H]^-$. Additionally, the DESI-HRMS analysis of individual standard solutions indicated that no significant in-source CID fragmentation and other adducts formation were expected for these compounds, allowing us to assign one ion (isotope cluster) to each veterinary drug during the screening.

The DESI-HRMS screening of veterinary drugs in feed samples was based on the accurate mass measurement and the isotope pattern distribution of the detected ions. Orbitrap can operate at a mass resolution high enough to prevent possible endogenous matrix interferences without sacrificing sensitivity. However, a compromise between acquisition duty cycle and mass resolution was necessary to provide both accurate mass measurements with mass errors below 5 ppm and enough sensitivity to detect the analytes in the complex mass spectrum. To select the working mass resolution, a blank sample extract spiked with the nine veterinary drugs (10 μg mL^{-1}) was analyzed at values between 17,500 and 140,000 FWHM (full width half maximum). All target compounds showed a drop in sensitivity when working above 70,000 without any significant improvement in mass accuracy. Thus, this mass resolution was used for further screening analysis.

Moreover, the sensitivity of the DESI-HRMS method also depended on the number of ions accumulated inside the orbitrap and also on the accumulation time applied. Since the automatic gain control (AGC) algorithm controls the number of ions inside the orbitrap to prevent space charge effects, the injection time (accumulation time) had to be optimized. Thus, the AGC was kept constant at 1×10^6 and the injection time was varied between 50 and 500 ms. The best signal was obtained for 300 ms as injection time. This relatively high injection time compared to conventional ESI is due to the low ion intensity generated in the DESI process that required longer injection times to accumulate a number of ions high enough to obtain a reasonable spectrum.

Optimization of DESI working conditions

To maximize the DESI signal, two main groups of working conditions must be optimized. The first group comprises those conditions related to the electrospray process such as nebulizing gas pressure, electrospray solvent composition, electrospray solvent flow rate, and the substrate/surface. The second group is related to the geometrical DESI parameters that include the nebulization capillary angle, the tip distance to the sample surface, and the distance to the mass spectrometer inlet. Initial DESI conditions were established using rhodamine-containing marker and the most critical DESI ion source parameters (nebulization capillary angle, tip distance to the sample surface, distance to mass spectrometer inlet, nebulizing capillary gas, solvent flow rate, and capillary voltage) were individually optimized using blank feed extracts spiked with a set of veterinary drugs (10 μg g^{-1}).

It has been demonstrated that the sample surface (substrate) plays a crucial role in DESI performance. Since the DESI process involves the landing and release of charged particles on a surface, the fundamental features of the solid surface, including its chemical composition and texture, severely affect the energy and charge transfer processes and consequently the ionization efficiency in DESI. Thus, several important parameters such as limit of detection, signal stability, carry-over, and reproducibility of the DESI method can be influenced by the surface [33]. In this work, three different surfaces were tested as substrates to analyze spiked acetonitrile feed extracts: glass, filter paper, and PTFE. The highest and most stable signal was

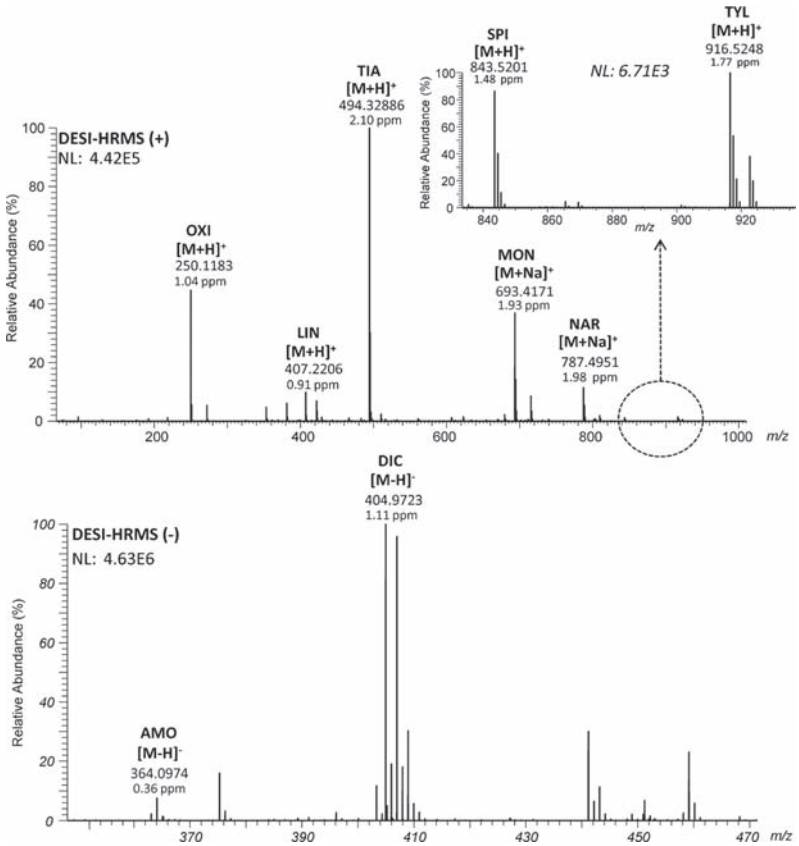

Fig. 1 DESI-HRMS (+/−) full-scan mass spectrum of a standard mixture with nine representative veterinary drugs. DESI solvent: acetonitrile/water (80:20, *v*/*v*); sample volume: 2 μL; sample substrate: PTFE

observed when using the PTFE surface. In filter paper, worse reproducibility than in PTFE was obtained, which can be due to uneven distribution of the analytes on the surface caused by chromatographic effects that occur in the course of the solution deposition [35].

DESI solvent composition and analyte solubility in the DESI solvent have an important effect in both desorption and transfer of analytes from the surface to the mass spectrometer. DESI solvent composition strongly affects electrospray droplet formation influencing the primary droplet size and the droplet charge, as well as the focus of the spray. Additionally, DESI solvent composition could favor the extraction and electrospray ionization of the analyte. To select the most adequate DESI solvent, different solvent mixtures of methanol/water and acetonitrile/water and the addition of formic acid to promote the protonation of target compounds in positive ion mode were evaluated. As an example, the effect of the DESI solvent composition on the ion signal intensity of MON, NAR, and TIA in positive ion mode and DIC in negative ion mode is depicted in Fig. 2a. As can be seen, the composition of the DESI solvent dramatically affects the compound's signal. The highest signal intensity, in both positive and negative ion modes, was achieved when using acetonitrile/water. The increase in the compound response may be due to the higher solubility of the analytes in the acetonitrile/water solvent that improves the transfer efficiency of the analytes into the secondary ESI droplets. It should be noted that an important decrease on the relative abundance of the ions generated from the veterinary drugs was observed when adding formic acid to the DESI solvent (Fig. 2a). These results were expected for veterinary drugs such as MON and NAR because the ion abundance of $[M+Na]^+$, the base peak in the non-acidic DESI solvent, can decrease due to the competition with $[M+H]^+$ ion generated in acidic medium. For acidic compounds that ionized in negative ion mode generating deprotonate molecules $[M-H]^-$, the ion signal also decreased when using acid in the DESI solvent because the neutral species are favored in the liquid phase. However, unexpected results were observed for basic compounds such as TIA, for which the acidic media should facilitate the protonation of

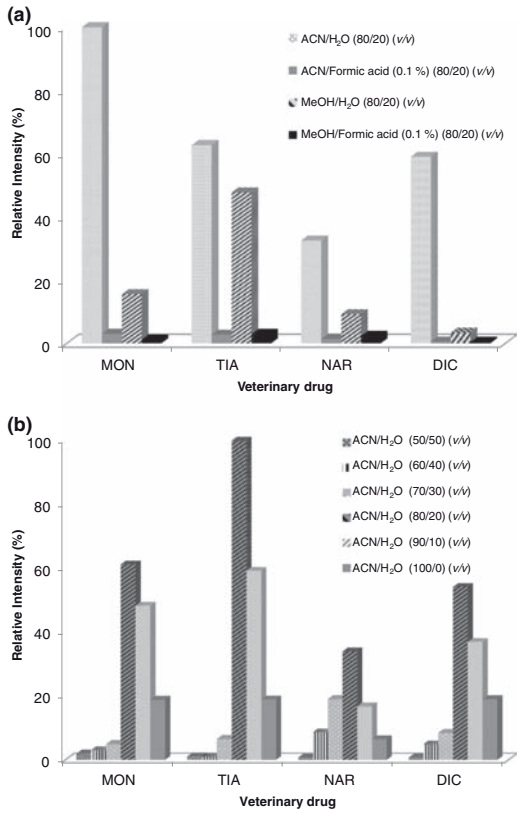

Fig. 2 Effect of the DESI solvent nature (**a**) and the percentage of acetonitrile in the DESI-HRMS signal for some representative veterinary drugs (**b**)

analytes in positive-ion mode. This might be due to an increase in the DESI droplet size caused by the enhancement of the surface tension produced by the higher ionic strength of the acidic DESI solvent (formic acid), in agreement with the results obtained by Green et al. [36]. Moreover, the effect of the organic solvent percentage of the DESI solvent on the ion signal intensity was also studied. The ion abundances observed for each compound using different acetonitrile/water mixtures are shown in Fig. 2b. All compounds studied showed a similar behavior. The ion signal intensity increased when increasing the organic solvent content from 50 to 80 %. This could be explained by the highest solubility of the analytes in the enriched acetonitrile solvent mixture. Nevertheless, the ion signal intensity dropped when using 90–100 % acetonitrile probably due to a worse wettability of the surface when using a solvent with lower hydrophilicity (>90 % acetonitrile). The optimal conditions, acetonitrile/water 80:20 (*v*/*v*), were supposed to be satisfactory for the other veterinary drugs with similar physicochemical properties to the chemicals studied.

The DESI solvent flow rate and nebulizing gas pressure affect the wetting and the flow dynamics on the surface as well as the size and velocity of the electrospray droplets, thus playing an important role in both ionization and desorption of analytes from the surface [37]. In this study, these parameters were optimized using the previously selected DESI solvent (acetonitrile/water 80:20, *v*/*v*). The gas pressure was tested within the range of 7–10 bar, and it was observed that when working at gas pressure values below 9 bar, the intensity dropped. This might be due to the formation of electrospray droplets of slow velocity and to the generation of secondary droplets with less kinetic energy to escape from the surface. In contrast, when applying a gas pressure of 10 bar, the signal also dropped probably because the high gas flow rate pushed the secondary droplets back to the surface leading to enhance droplet splashing. Regarding DESI solvent flow rate, it was varied from 1 to 5 μL min^{-1}, and it was observed that when increasing flow rate the signal improved probably due to the better surface wetting. Nevertheless, a wider surface area was eroded, thus worsening the spatial resolution [38]. As a compromise between sensitivity and spatial resolution, a gas pressure of 9 bar and a DESI solvent flow rate of 2.5 μL min^{-1} were chosen as optimal working conditions.

To optimize the geometrical parameters, we used acetonitrile/water (80:20, *v*/*v*) as DESI solvent. The position of the spray tip (both within the spray head and relative to the surface area) is critical for a successful DESI signal. Thus, the nebulizing capillary angle (α) and the tip distance to the sample surface (d_1) have direct effects on the ionization process, while the distance to the mass spectrometer inlet (d_2) have important effects on the ion collection efficiency and, hence, on the sensitivity of the method. The effect of α on the DESI signal was evaluated by modifying the incident angle (45–75°) of the electrospray tip relative to the surface that changes the impact angle of the droplets on the surface. The highest intensity was observed for an α value of 55°, which is generally used as optimum value in other DESI applications [39]. The d_1 and d_2 values were varied from 1.5 to 4 mm and from 4 to 10 mm, respectively. For a DESI solvent flow rate of 2.5 μL min^{-1}, the closer the sprayer was to the surface (d_1), the highest was the signal, being 1.7 mm the optimal value for all the analytes. Moreover, for d_2, the best response was observed at 5 mm when analyzing the spiked feed extract.

DESI-HRMS analytical performance

The complexity of the matrix and the wide polarity range among the different chemical groups of the veterinary drugs make the analysis of feedstuffs a challenge. Different sample manipulation strategies were evaluated to screen veterinary drugs in feed. Because of the powder nature of the feed samples studied, the direct analysis by DESI-HRMS was not possible. As a first attempt, we prepared pressed feed pellets of

Table 1 Screening results

Sample	Detected antibiotics	LOD[b] (μg g^{-1})	DESI-HRMS screening						UHPLC-MS/MS quantification (MRL)[a] (μg g^{-1})
			Exact mass (*m*/*z*)	Accurate mass (*m*/*z*)	Ion assignment	Elemental composition	Mass accuracy (ppm)	Isotopic cluster fit (%)	
Medicated feed									
MF1	Lincomycin		407.2210	407.2205	$[M+H]^+$	($C_{18}H_{35}N_2O_6S$)	1.3	95	107
MF2	Monensin		693.4184	693.4169	$[M+Na]^+$	($C_{36}H_{62}O_{11}Na$)	2.2	89	100
MF3	Monensin		693.4184	693.4184	$[M+Na]^+$	($C_{36}H_{62}O_{11}Na$)	0.1	92	87
MF4	Narasin		787.4967	787.4947	$[M+Na]^+$	($C_{43}H_{72}O_{11}Na$)	2.5	88	44
MF5	Narasin		787.4967	787.4952	$[M+Na]^+$	($C_{43}H_{72}O_{11}Na$)	1.9	84	37
	Monensin	0.5 [c]	693.4184	693.4167	$[M+Na]^+$	($C_{36}H_{62}O_{11}Na$)	2.5	80	3.5* (1.25)
Non-medicated feed									
BF1	Florfenicol	0.7[d]	379.9897	379.9891	$[M+Na]^+$	($C_{12}H_{14}Cl_2FNO_4SNa$)	1.5	80	7.0
BF2	Salinomycin	0.7[d]	773.4810	773.4794	$[M+Na]^+$	($C_{42}H_{70}O_{11}Na$)	2.1	86	20* (0.7)
	Amoxicillin	15[c]	364.0973	n.d.	$[M-H]^-$	($C_{16}H_{19}O_5N_3S$)	–	–	0.13
	Tiamulin	0.5[c]	494.3299	n.d.	$[M+H]^+$	($C_{28}H_{48}NO_4S$)	–	–	0.11
BF3	Oxytetracycline	0.5[c]	461.1555	461.1546	$[M+H]^+$	($C_{22}H_{25}N_2O_9$)	1.9	93	6.3
BF4	Decoquinate	0.4[d]	440.2407	440.2412	$[M+Na]^+$	($C_{24}H_{35}NO_5Na$)	1.1	91	5.0* (0.4)
BF5	Decoquinate	0.4[d]	440.2407	440.2410	$[M+Na]^+$	($C_{24}H_{35}NO_5Na$)	0.5	89	3.3* (0.4)
BF6	Lasalocid	0.4[d]	613.3711	613.3705	$[M+Na]^+$	($C_{34}H_{54}O_8Na$)	1.0	80	0.45 (1.25)
	Decoquinate		440.2407	n.d.	$[M+Na]^+$	($C_{24}H_{35}NO_5Na$)	–	–	0.21 (0.4)
BF7	Narasin	0.5[c]	787.4967	787.4957	$[M+Na]^+$	($C_{43}H_{72}O_{11}Na$)	1.2	84	1.3* (0.7)
BF8	Tiamulin	0.5[c]	494.3299	494.3288	$[M+H]^+$	($C_{28}H_{48}NO_4S$)	2.1	86	1.6
	Amoxicillin	15[c]	364.0973	n.d.	$[M-H]^-$	($C_{16}H_{19}O_5N_3S$)	–	–	0.80
BF9	Narasin	0.5 [c]	787.4969	787.4955	$[M+Na]^+$	($C_{43}H_{72}O_{11}Na$)	1.5	89	2.1* (0.7)
	Nicarbazin		301.0573	n.d.	$[M-H]^-$	($C_{19}H_{18}O_6N_6$)	–	–	0.42 (1.25)
BF10	Narasin	0.5[c]	787.4969	787.4967	$[M+Na]^+$	($C_{43}H_{72}O_{11}Na$)	1.3	91	29* (0.7)
BF11	Tiamulin	0.5[c]	494.3299	494.3297	$[M+H]^+$	($C_{28}H_{48}NO_4S$)	0.4	83	1.7
	Doxycycline	1.2[c]	463.1711	463.1714	$[M+H]^+$	($C_{22}H_{27}N_2O_9$)	0.6	94	7.2
	Amoxicillin	15 [c]	364.0973	n.d.	$[M-H]^-$	($C_{16}H_{19}O_5N_3S$)	–	–	2.0
BF12	Decoquinate	0.4[d]	440.2407	440.2413	$[M+Na]^+$	($C_{24}H_{35}NO_5Na$)	1.2	88	5.0* (0.4)
BF13	Narasin	0.5[c]	787.4967	787.4964	$[M+Na]^+$	($C_{43}H_{72}O_{11}Na$)	0.3	83	1.7* (0.7)
	Monensin	0.5[c]	693.4184	693.4187	$[M+Na]^+$	($C_{36}H_{62}O_{11}Na$)	0.3	84	1.6* (1.25)
	Robenidine		334.0621	n.d.	$[M+H]^+$	($C_{15}H_{13}Cl_2N_5$)	–	–	0.32 (0.7)
	Diclazuril	1[c]	404.9718	n.d.	$[M-H]^-$	($C_{17}H_9Cl_3O_2N_4$)	–	–	0.01* (0.01)
BF14	Amoxicillin	15[c]	364.0973	n.d.	$[M-H]^-$	($C_{16}H_{19}O_5N_3S$)	–	–	0.17
	Tiamulin	0.5[c]	494.3299	n.d.	$[M+H]^+$	($C_{28}H_{48}NO_4S$)	–	–	0.50
BF15	Oxibendazole	0.5[c]	250.1186	250.1188	$[M+H]^+$	($C_{12}H_{16}N_3O_3$)	–	–	0.13

Table 1 (continued)

Sample	Detected antibiotics	DESI-HRMS screening							UHPLC-MS/MS quantification (MRL)[a] (μg g^{-1})
		LOD[b] (μg g^{-1})	Exact mass (*m/z*)	Accurate mass (*m/z*)	Ion assignment	Elemental composition	Mass accuracy (ppm)	Isotopic cluster fit (%)	
BF16	Amoxicillin	15[c]	364.0973	n.d.	$[M-H]^-$	$(C_{16}H_{19}O_5N_3S)$	–	–	0.17
	Lincomycin	0.5[c]	407.2210	n.d.	$[M+H]^+$	$(C_{18}H_{35}N_2O_6S)$	–	–	0.25
	Oxibendazole	0.5[c]	250.1186	n.d.	$[M+H]^+$	$(C_{12}H_{16}N_3O_3)$	–	–	0.20
	Tiamulin	0.5[c]	494.3299	n.d.	$[M+H]^+$	$(C_{28}H_{48}NO_4S)$	–	–	0.18
BF17	Amoxicillin	15[c]	364.0973	n.d.	$[M-H]^-$	$(C_{16}H_{19}O_5N_3S)$	–	–	0.15
	Lincomycin	0.5[c]	407.2210	n.d.	$[M+H]^+$	$(C_{18}H_{35}N_2O_6S)$	–	–	0.39
BF18	Nicarbazin		301.0573	n.d.	$[M-H]^-$	$(C_{19}H_{18}O_6N_6)$	–	–	0.16 (1.25)

*MRL

[a] Maximum residue levels legislated in Directive 574/2011/EC

[b] Limits of detection (LOD) calculated by DESI-HRMS

[c] LODs estimated by spiking blank feed extracts with standards

[d] LODs calculated taking into account the concentration quantified by HPLC-MS/MS

1.5 cm in diameter using a manual hydraulic press to get a smooth surface to be screened by DESI-HRMS. However, the dusty texture of the feed samples made it difficult to obtain good results because of the damaging of the feed pellet surface by the nebulizing gas and the contamination of the mass spectrometer transfer line by the powdery sample. To enhance the pellets' compactness, different pressures (from 10 to 15 tons) were tested as well as the addition of boric acid to increase pellet agglutination, although no significant differences were observed.

As an alternative to the direct analysis of the sample surface, a simple solid-liquid extraction procedure was considered. Several sample extraction multi-analyte methods based on organic solvent mixtures have been developed for the detection of a wide range of veterinary drugs in animal feed by LC-MS [12, 13, 17, 40] mainly using acetonitrile and methanol. Hence, the behavior of both solvents for the analysis by DESI of feed samples was tested. For this purpose, blank feed extracts extracted individually with these solvents and spiked with the nine representative veterinary drugs (10 μg g^{-1}) were deposited onto a PTFE surface after letting it dry and were analyzed by DESI-HRMS. The results showed that higher ion intensities were obtained when using acetonitrile as extraction solvent since methanol may extract too many matrix compounds that can cause ion suppression. In contrast, acetonitrile allows protein precipitation and enzyme denaturation resulting in cleaner extracts. However, it has been described that the use of only organic solvents (acetonitrile, methanol, or a combination of both) at different percentages led to low intensities for non-ionophore coccidiostats (clopidol, ethopabate, amprolium), macrolides, and tetracyclines [41]. Moreover, some authors recommend the addition of a small amount of water, up to 20 %, to the organic solvent to favor the extraction of polar compounds [40]. So, acetonitrile/water (80:20, *v/v*) with 1 % of formic acid recommended to increase the extraction of basic compounds was chosen as extraction solvent for the DESI-HRMS multi-residue method.

The effect of the feed matrix in the ionization efficiency was tested for the nine representative veterinary drugs. A blank feed extract was spiked at 10 μg g^{-1} level and then extracted with acetonitrile/water (80:20, *v/v*) with 1 % formic acid. The mass spectra of this spiked blank feed extract and that obtained for a standard mixture at the same concentration level prepared in acetonitrile/water (80:20, *v/v*) with 1 % formic acid were compared. For all the studied compounds, the ion signal in the spiked feed blank extracts were one order of magnitude lower than in the standard mixture indicating that ion suppression occurs. Even though the limits of detection (LODs) estimated for the tested compounds were lower than 1 μg g^{-1} (Table 1), except for amoxicillin, a higher estimated LOD value (15 μg g^{-1}) was obtained, probably because of a partial degradation in acidic solutions, especially at low concentrations [2]. LODs, based on a signal-to-noise ratio of

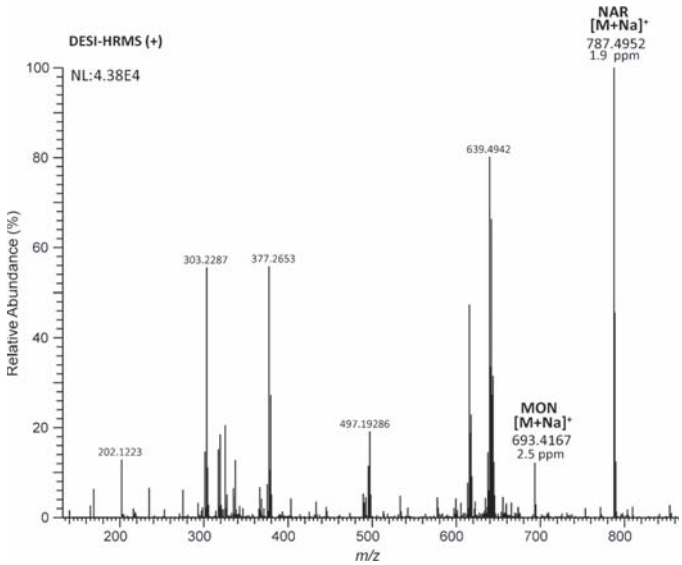

Fig. 3 DESI-HRMS full-scan spectrum obtained from a narasin (NAR) medicated feed (37 μg g^{-1}) cross-contaminated with monensin (MON) (3.5 μg g^{-1})

3:1, were estimated by analyzing blank feed samples spiked with standards at low concentrations. For those compounds that the standard was not available, LODs from sample were calculated taking into account a signal-to-noise ratio of 3:1 and the concentrations of veterinary drugs quantified by UHPLC-MS/MS. These values are below the legal limits legislated for most of the veterinary drugs due to the unavoidable carry-over in the line production (μg g^{-1} levels) except for diclazuril, which the maximum residue level is legislated at 0.01 μg g^{-1} [6].

DESI-HRMS screening of feed samples

To evaluate the applicability of the developed DESI-HRMS method, 50 feed samples (medicated and non-medicated feed) received from LAC were analyzed using the DESI-HRMS method in order to detect those samples suspected of being cross-contaminated by veterinary drugs.

Feed samples were screened and the acquired mass spectral raw data were interrogated by a custom-made database that included more than 60 veterinary drugs (anthelmintics, antibiotics, coccidiostats, hormones, etc.) commonly used to produce medicated feedstuffs. For each substance, the compound name, the CAS number, the elemental composition, and the chemical structure were included. The ionization mode and the expected ions (protonated and deprotonated molecules, adduct ions, in-source fragments, etc.) that can be generated in the DESI source were also added to the custom-made database.

Feed samples (three replicates) were submitted to the simple sample treatment detailed in the experimental section and analyzed by the DESI-HRMS multi-residue method. The sample raw data files were processed using the Exact Finder software and interrogated by the custom-made database to automatically identify the veterinary drugs in the feedstuffs. The criteria applied to confirm the presence of the suspected compounds were the following: a mass accuracy of less than 5 ppm on the exact mass, a minimum signal-to-noise ratio of 3:1, and an isotope cluster fit higher than 80 % (both mass relative deviation and relative intensity differences, for each isotope peak within the cluster ion, were taken into account). Feed samples were also analyzed by a well-established UHPLC-MS/MS method for the quantification of the identified compounds [10].

Table 1 lists the positive samples and the veterinary drugs identified along with the DESI-HRMS identification criteria and the quantitative results obtained by target UHPLC-MS/MS method. The veterinary drugs at dose levels between 37 and 107 μg g^{-1} in the medicated feed were easily detected by the DESI-HRMS screening method and only in one of these samples (MF5) an unexpected cross-contamination of monensin (3.5 μg g^{-1}) was detected. Figure 3 shows the DESI-HRMS spectrum of a narasin medicated feed where both narasin and monensin were identified. Additionally, results obtained for non-medicated feed indicated that cross-contamination occurs quite frequently and values above the legislated levels were detected in 28 % of the samples analyzed by DESI-HRMS. Coccidiostats (monensin, narasin, decoquinate, nicarbazin, salinomycin, and lasalocid),

benzimidazoles (oxibendazole), amphenicols (florfenicol), tetracyclines (doxycycline and tetracycline), lincosamides (lincomycin), and pleuromutilins (tiamulin) were identified in the non-medicated feed samples at concentrations ranging from 29 to 1.3 μg g^{-1}. For most of these samples, the cross-contamination was at concentrations close to the maximum residue levels, except for sample BF2, where salinomycin was detected at 20 μg g^{-1}, a third of the minimum dose recommended for a medicated feed (60 μg g^{-1}) [42]. Furthermore, in most of the non-medicated feeds, several veterinary drugs were detected in the same sample. For instance, sample BF11 was cross-contaminated with tiamulin (1.7 μg g^{-1}) and doxycycline (7.2 μg g^{-1}) and in sample BF13 monensin and narasin (at< μg g^{-1} level) were positively identified. The UHPLC-MS/MS analysis of the whole set of samples confirmed the DESI-HRMS results and also allowed the identification of additional veterinary drugs at sub-microgram-per-gram level. However, these low concentrations are much lower than the maximum residue levels and they are considered unavoidable carry-over.

Regarding the obtained results, the developed DESI-HRMS method could be suitable to detect cross-contamination of veterinary drugs in feed samples in quality control laboratories since it is simple, with minimum sample manipulation, less time consuming, and able to detect cross-contamination at the maximum residue levels legislated.

Conclusions

DESI-HRMS has been shown to be an effective approach for the screening of veterinary drugs in cross-contaminated feedstuffs. A minimal sample manipulation based on a simple extraction procedure (acetonitrile/water 80:20 *v/v* acidified with 1 % formic acid) is proposed to analyze dusty homogenized feed samples. Among the DESI working parameters optimized using nine representative veterinary drugs, the most critical ones for the feed extract analysis were the substrate and the DESI solvent. PTFE substrate and acetonitrile/water (80:20 *v/v*) as DESI solvent provided the highest signal intensity. Although ion suppression due to matrix effects was observed, the sensitivity achieved by DESI-HRMS was enough to identify veterinary drugs as cross-contamination above the legislated levels. Data acquired in high resolution mass spectrometry (70,000 FWHM), processed and interrogated with the custom-made database, provided the identification of cross-contamination of non-target veterinary drugs based on accurate mass measurements and isotope cluster fit from HRMS full-scan spectra. The results obtained in the feed sample analysis correlated well with those found by UHPLC-MS/MS and demonstrate the potential of the DESI-HRMS as screening method to identify cross-contaminated feedstuffs reducing the number of samples to be quantified by UHPLC-MS/MS in quality control laboratories.

Acknowledgments The authors gratefully acknowledge the financial support received from the Spanish Ministry of Economy and Competitiveness under the project CTQ2012-30836 and from the Agency for Administration of University and Research Grants (Generalitat de Catalunya, Spain) under the project 2014SGR-539. Raquel Seró gratefully acknowledges the University of Barcelona for an APIF grant.

Conflict of interest The authors declare that they have no conflict of interest.

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2.2.2 PUBLICACIÓ IV

Desorption electrospray ionization-high resolution mass spectrometry for the analysis of unknown materials: The phytosanitary product case

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Analyst, 194 (**2019**) 350-356

Talanta 194 (2019) 350–356

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Desorption electrospray ionization-high resolution mass spectrometry for the analysis of unknown materials: The phytosanitary product case

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ARTICLE INFO

Keywords:
Ambient mass spectrometry
Desorption electrospray ionization
High-resolution mass spectrometry
Unknown materials
Unknown compounds
Triphenyltin compounds
Kendrick mass defect

ABSTRACT

In this work, we tested the potential of desorption electrospray ionization-high resolution mass spectrometry (DESI-HRMS) for the analysis of unknown materials. To this end, our study focused on analyzing an unknown complex sample suspected of being an adulterated phytosanitary product or a fraud. A simple and fast sample manipulation procedure (filter paper impregnated with the sample) was used for the chemical characterization of the phytosanitary product by DESI-HRMS(/MS). Kendrick Mass Defect (KMD) analysis was used to process the DESI high-resolution mass spectral data, allowing the identification of a low molecular weight polymer (polyethylene glycol) and the detection of other ions, which did not follow polymer distributions. The characteristic isotope patterns of these ions suggested the presence of organometallic compounds. Accurate mass measurements, isotope pattern fits and the structural information obtained by DESI-MS/HRMS (wide isolation window) allowed identifying the presence of triphenyltin, a biocide extensively used for agricultural purposes and restricted by the European Commission, in the phytosanitary product. The concentration of triphenyltin in the sample was 35%, which corresponds to ~12% expressed as weight of tin, 120 times higher than the maximum legislated level.

1. Introduction

The analysis of unknown materials is a complex process, which become more challenging when the nature of the concerned compounds is also unknown. In this context, the selection of the appropriate analytical technique, sample treatment, isolation, identification/quantitation, etc., is a difficult task. Among the myriad of analytical techniques available today, mass spectrometry (MS) can provide valuable chemical information, even if the identity of the analytes is not known, which can help in the identification of the chemical composition of unknown materials. However, classical mass spectrometry-based analytical methods generally require extensive sample treatments to dissolve the sample, isolate analytes, minimize sample matrix and avoid interferences that could bias the results, since they may be compound discriminant. Nowadays, a new group of techniques named "ambient ionization mass spectrometry" (ambient MS) gathers a series of characteristics that can help to solve these real-life analytical problems.

Ambient MS, firstly defined by Cooks and co-workers [1], includes those desorption/ionization techniques that operate in the open air and are able to analyze samples in their native environment. Ambient MS techniques offer advantageous characteristics for the analysis of unknown samples, such as in-situ and fast analysis, soft ionization, minimal or no sample preparation, and relatively low matrix effects. These features allow global non-targeted analysis, far from applications focused on target analytes, which are generally performed using liquid chromatography or gas chromatography coupled to mass spectrometry (LC-MS and GC-MS) [2,3]. Among ambient MS, desorption electrospray ionization (DESI) [4] and direct analysis in real time (DART) [5] are the most popular, although a wide range of new techniques have been developed, which differ in how the sample processing takes place and the ionization mechanism employed [6,7]. In ambient MS, the modification of the working conditions to fit to the sample characteristics is easy. Another advantage is the use of soft ionization that yields low internal energy ions, which suffer no or little fragmentation in the atmospheric pressure region, allowing assigning one ion (isotope cluster) to each compound. However, since all ionisable compounds on the sample surface will contribute to the sample mass spectrum, the complexity of mass spectral data obtained when analyzing complex samples requires the use of MS instruments capable of acquiring data at high-resolution and/or performing tandem MS experiments. Today, highly sensitive and selective instruments such as time-of-flight or Orbitrap and hybrid instruments such as quadrupole-Obritrap (Q-Obitrap),

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https://doi.org/10.1016/j.talanta.2018.10.038
Received 27 July 2018; Received in revised form 8 October 2018; Accepted 9 October 2018
Available online 15 October 2018

quadrupole-TOF (Q-TOF) or LIT-Orbitrap, allow overcoming interference problems, avoid overlapping isotope clusters and provide high-accuracy mass measurements and high-quality mass spectral information for the identification of both, chemical formulas and chemical structures of compounds [8,9].

The major application of ambient HRMS relies on the development of screening methods for the analysis of a wide range of compounds for food, environmental and forensic applications [10]. These methods are usually developed for the rapid qualitative identification of a family or families of compounds of interest in samples where their chemical composition is usually known or suspected. In this context, a set of compounds is used as models to optimize ambient MS working conditions and online libraries are used to narrow candidate lists for further confirmatory analyses. In cases generally related to frauds and crimes, where sample information provided is minimal or ignored and/or it is not known what has to be looked for, the use of ambient MS techniques could be a good option. Ambient MS could be considered a better strategy than LC-MS or GC-MS due to their ability to perform the analysis of samples in its native environment and without applying prior sample preparation procedures, including extraction and clean-up steps, which may be discriminatory, causing the loss of some compounds during the sample process.

In this paper, a real case is presented for the chemical characterization of an unknown sample. A phytosanitary product suspected of being adulterated was the main evidence of an environmental crime for its unexpected activity. A sample was received in the laboratory for the identification of the adulterant in order to assign responsibility for applying the concerned product in a protected natural park. However, no information about the nature of the sample or about its chemical composition was available. In this context, the aim of this work was to evaluate the applicability of DESI-HRMS(/MS) for the chemical characterization of the sample in order to identify the compounds responsible of causing the very high activity of the phytosanitary product.

2. Experimental

2.1. Chemicals, materials and sample

LC-MS grade acetonitrile (ACN), methanol (MeOH), water, formic acid (98%) and Whatman® Grade 41 filter paper were supplied by Sigma-Aldrich (Steinheim, Germany). Triphenyltin chloride (≥ 98.8% purity) and triphenyltin hydroxide (≥ 99.9% purity) were purchased from Sigma-Aldrich (Steinheim, Germany) and bis(tryphenyltin) oxide (≥ 98.0% purity) was provided by Alfa Aesar (Karlsruhe, Germany). Individual stock solutions (1 mg mL^{-1}) were prepared using ACN as solvent and stored at 4 °C, while the working standard solutions were prepared weekly by appropriate dilution in ACN from stock solution.

A phytosanitary product suspected of being adulterated was collected by official inspectors in a Spanish National Park and sent to our laboratory for its analysis. The sample was a white dense liquid product packed in an opaque bottle with no information about the nature of the sample or its composition.

2.2. Desorption electrospray ionization-high resolution mass spectrometry (DESI-HRMS)

To perform the sample analysis by DESI-HRMS, a Whatman filter paper impregnated with the phytosanitary product was placed onto a microscope glass slide (McMaster-Carr, Santa Fe, CA, USA) with double side tape and let it dry at room temperature. A desorption electrospray ionization (DESI) source (Omnispray Ion Source, Prosolia Inc., Indianapolis, IN) with a 1D moving stage and coupled to a quadrupole-Orbitrap mass spectrometer (Q-Exactive, Thermo Fisher Scientific, San José, CA, USA) was used. A red permanent marker that contains rhodamine 6G (ion at *m/z* 443.2329, positive ion mode), purchased form Fine Sharpie (Stanford Corp., Oak Brook, IL), was used to establish the DESI geometric source conditions. DESI solvent (ACN:water, 80:20, *v:v*) was infused by a syringe pump at 3.5 µL min^{-1} and directed onto the sample surface at a nebulization capillary angle of 55° and a distance of ~ 9.2 mm between the mass spectrometer inlet and the spray tip. Nitrogen (99.95% pure, Air Liquide, Madrid, Spain) was used as nebulizer gas (8 bar) and as collision gas. Electrospray (ESI) voltage was set at 4.5 kV in positive ionization mode and 5 kV in negative ion mode, while transfer capillary temperature was set at 250 °C.

DESI data was acquired by both, scanning mode moving the DESI source through the filter paper at a stage scan speed of 250 µm s^{-1}, and fixed sampling mode, acquiring the MS data keeping the DESI in a single position. For the analysis of the standards, 1 µL of the standard solution in ACN (100 µg mL^{-1}) were deposited onto microscope glass slides of 7.1 mm polytetrafluoroethylene (PTFE) (Teflon; McMaster-Carr, Santa Fe, CA, USA) printed spots. The Q-Exactive mass spectrometer was operated in positive and negative ionization modes in full-scan and target MS^2 acquisition modes. For both, full-scan and product ion scan, a mass resolution of 70,000 FWHM (full width at half maximum) at *m/z* 200, a maximum injection time of 300 ms and a scan range from *m/z* 120–1200 were used. Automatic gain control (AGC) values were set at 1×10^6 and 5×10^5 for full-scan and target MS^2 experiments, respectively. For tandem mass spectrometry an isolation width 5–12 *m/z* was used.

Omni Spray ion source software *v*2.0 (Omnispray Ion Source, Prosolia Inc., Indianapolis, IN) was used to control the DESI source, while Xcalibur software *v*2.2 (Thermo Fisher Scientific, San José, CA, USA) was used for data acquisition and data processing.

Accurate mass calibration was performed in the Q-Exactive mass spectrometer every 72 h by ESI source using a calibration solution of caffeine, MRFA peptide, Ultramark 1621 and butylamine in ACN/MeOH/water (50:25:25, *v:v*) containing 1% formic acid.

2.3. Kendrick mass defect analysis

Modified Kendrick mass defect (KMD) analysis using the repeat unit of the polymer backbone as base unit (monomer) for the calculation of Kendrick mass (KM) was used [11,12]. Modified KM values were calculated according to:

$$\mathrm{KM(ion)} = m/z\,\mathrm{(ion)} \times \frac{\mathrm{nominal(IUPAC\ mass\ of\ monomer)}}{\mathrm{IUPAC\ mass\ of\ monomer}} \quad (1)$$

being *m/z* (ion) the accurate mass of the ion and the IUPAC mass of monomer the exact mass in the IUPAC mass-scale. The nominal mass is the rounded value of the mass of the monomer to the nearest integer. The Kendrick mass-scaling factor was calculated using -CH_2CH_2O- as repeating unit, giving a factor value of 44/44.0262 = 0.99940.

All the *m/z* values in the mass spectral data with relative intensities above 1% were converted to the modified Kendrick mass-scale and used to obtain the corresponding KMD-NKM plots, where nominal Kendrick mass (NKM) and KMD values were calculated according to:

$$\mathrm{NKM} = \mathrm{round\,(KM)} \quad (2)$$

$$\mathrm{KMD} = \mathrm{KM–NKM} \quad (3)$$

being NKM the nearest integer of KM. These plots are usually represented using a “bubble chart”, where each disk expresses a data triplet (NKM, KMD, abundance) and the size of the disk is proportional to peak intensities.

Additionally, the “remainder of NKM” (RNKM) was calculated to obtain information about the composition of the end groups of polymeric series following the equation proposed by Fouquet *et al.* [12]:

$$\mathrm{RNKM_R} = \mathrm{round(R)}\left\{\frac{\mathrm{NKMR}}{\mathrm{round(R)}}\right\} \quad (4)$$

being {*x*} the fractional part of *x* defined as *x* – ⌊x⌋.

3. Results and discussion

3.1. Sample analysis by DESI-HRMS

As mentioned above, a dense white liquid sample arrived to our laboratory with no information neither on the nature of the sample nor on its composition. Based on the declaration of the user to the official inspectors, we were informed that the phytosanitary product was saponins-based used to treat rice crops against apple snail pest. It was suspected that this sample was adulterated since the phytosanitary effect was found to be higher than that expected for a product based only on saponins. Because of the lack of information, direct analysis of the sample by DESI was performed in order to identify the potential adulterant/s avoiding both sample treatment and chromatographic separation, thus preventing discriminatory treatments and reducing analysis time. High-resolution mass spectrometry was used to prevent possible interferences and to obtain information about accurate mass, high-resolution isotope pattern and chemical structural information.

Several parameters, potentially important to maximize DESI signal, such as those related to the geometry of the ion source, the DESI solvent composition and flow-rate and the nebulizing gas pressure were optimized. Regarding DESI solvent composition, a mixture of ACN:water was selected because of its capability to extract both polar and non-polar compounds and for its conductivity and relative volatility that help to improve the electrospray formation. Although DESI typically operates with a solvent mixture of 50% organic solvent:water, the organic solvent was increased up to 80% to improve ionization efficiency [13]. DESI solvent flow-rate and the nebulizing gas pressure were optimized directly on the sample. DESI solvent flow-rate was raised to 3.5 $\mu L\,min^{-1}$ to increase both primary ion current and average droplet size, thus improving both the wetting of the sample surface and the extraction efficiency [14]. Nebulizing gas pressure was reduced to 116 psi to prevent contamination and carryover due to the dusty texture of the dried phytosanitary product.

DESI-HRMS full scan mass spectrum of the sample obtained under scanning mode for 1 min after background subtraction is shown in Fig. 1A. To identify background noise ions and to subtract them from the sample mass spectrum, a blank filter paper was analyzed under the same working conditions. The most abundant signals observed in the mass spectrum correspond to a series of peaks (singly charged) separated by 44 nominal mass units. The accurate mass data showed that the mass difference for the 13 most abundant peaks varies from 44.0251 to 44.0268, with an average accurate mass of 44.0262. The distribution of these ions as well as the accurate mass measurements are consistent with characteristic pattern distributions of low molecular weight polymers with n ethylene oxide (EO) repeat units, which might indicate the presence of poly(ethylene glycol) (PEG_n) in the sample [15].

Since the analysis of complex samples by high-resolution mass spectrometry without any previous chromatographic separation deals with an enormous collection of peak data, Kendrick mass defect analysis, which does not rely on peak assignments, was used to characterize components in this phytosanitary product. The Kendrick mass-scale, based on CH_2 was developed by Kendrick to better separate homologous series of hydrocarbons [16]. Modified KMD combined with high-resolution mass spectral data has been used to obtain information in the field of polymer chemistry [11]. To create a graphical distribution of the data obtained by DESI-HRMS on a two-dimensional KMD plot, a modified Kendrick mass-scale was used, with the ethylene oxide as the repeating unit [11,17]. All *m/z* values corresponding to ions with a relative intensity higher than 1.0% were transformed from IUPAC mass-scale into Kendrick one. The Kendrick mass (KM), nominal Kendrick mass (NKM) and KMD were calculated as described in the Experimental section. Fig. 2A shows the two-dimensional plot of KMD versus NKM using a "bubble chart". The KMD plots show ions separated in three horizontal lines with KMD values of -0.095, -0.047 and -0.012.

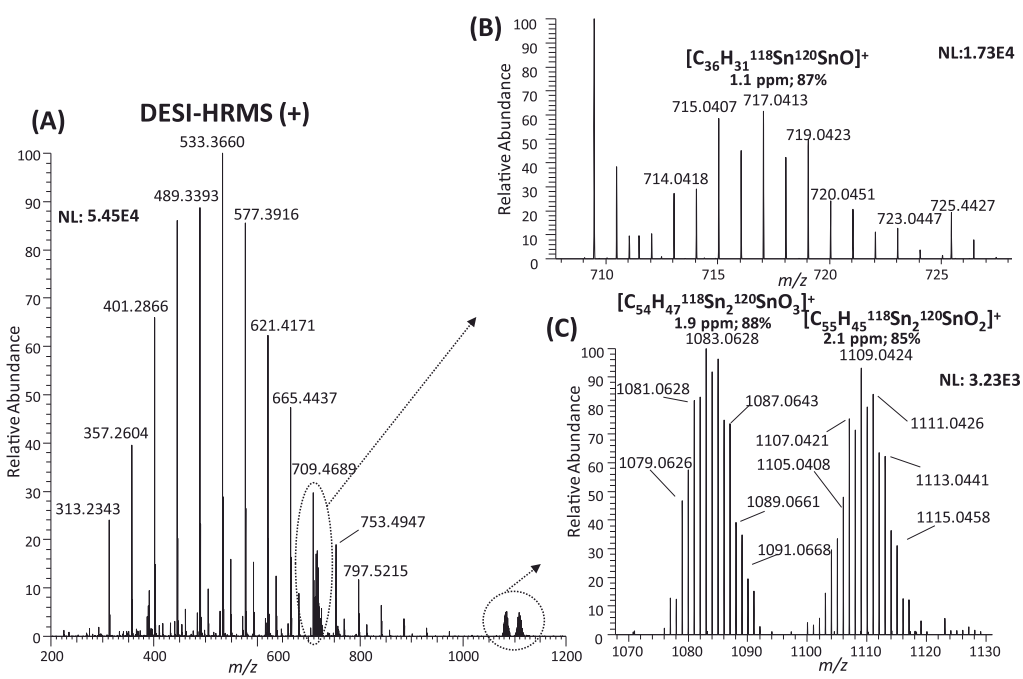

Fig. 1. Full scan mass spectrum obtained for the analysis of the phytosanitary product by DESI-HRMS (+) under scanning mode at 250 $\mu m\,s^{-1}$.

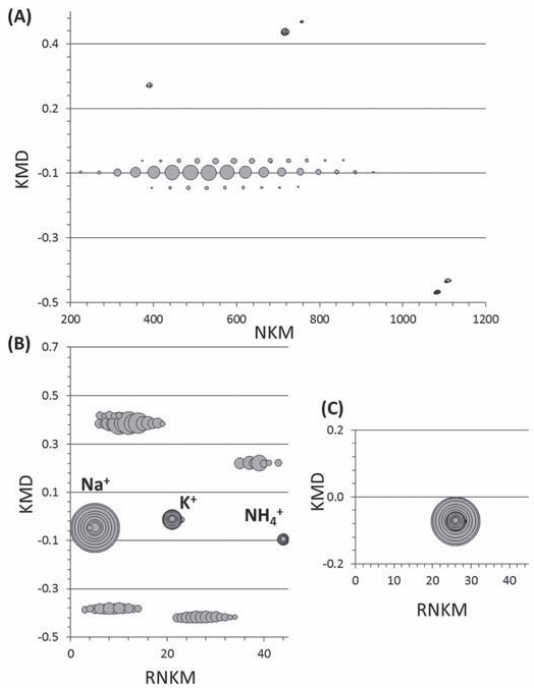

Fig. 2. Two-dimensional KM plots obtained from the DESI-HRMS mass spectrum of the sample: (A) KMD *vs* NKM using a mass scale-based on EO units, (B) KMD *vs* RNKM (C) KMD *vs* RNKM calculated using the $M_{(obs\text{-}adduct)}$ of the peaks associated to the characteristic distributions of the polymer.

Considering the general concept when applying the Kendrick mass scale, those ions with the same KMD value have the same chemical backbone but differ by one or more EO units. By contrast, the difference between the three aligned ions with different KMD values is due to the end-groups and/or the possible adducted cation. After the identification of the homologous series by using KMD, the determination of the end-group is needed for polymer characterization. The mass of end-groups (M_{end}) can be given by:

$$M_{end} = M_{obs} - M_{monomer} \times n - M_{cation} \quad (5)$$

where M_{obs}, $M_{monomer}$ and M_{cation} are the *m/z* values of the observed ion, the monomer unit and the mass of the cation, respectively, and n is the degree of polymerization. When applying this strategy to polymer analysis, M_{cation} is usually known (e.g. Na^+ or H^+) since it depends on the solution added during sample preparation [11,12]. The difference between the accurate mass of the ions of the first line (KMD values of −0.012) and its nearest ion on the second line (KMD values of -0.047) was 15.9741 Da (as an average of all calculated mass differences). This accurate mass difference corresponds to that between sodium and potassium adducts, with a mass tolerance of ± 2 ppm. Applying the same calculating procedure to the second (KMD values of −0.047) and the third horizontal ion distributions (KMD values of −0.095), the accurate mass difference obtained was 4.9550 Da, which corresponds to that between sodium and ammonium adducts. Thus, potassium, sodium and ammonium adducts are present in the sample, being the sodium adducts the most abundant ones. After identifying the adduct of each KMD-NKM horizontal distribution, the *m/z* of the cation (M_{cation}) was subtracted to each aligned group of ions (M_{obs}) to simplify Eq. (5):

$$M_{(end)} = M_{(obs-cation)} - M_{monomer} \times n \quad (6)$$

The use of the "remainded NKM" (RNKM) (described in the Experimental section), combined with accurate mass measurements can help to obtain information of the composition of the end-groups of polymer series [12]. The RNKM does not depend on the EO unit, ergo all the homologs of a distribution with the same end-groups and same adducted ion, have the same RNKM. Since different adducts were found for each polymer distribution, the KMD-RNKM plots were calculated using the $M_{(obs\text{-}cation)}$. In the KMD-RNKM bubble chart (Fig. 2C), the three horizontal series observed in the KMD-NKM plot (Fig. 2A) are condensed in a single disk with KMD and RNKM values of -0.0718 and 26, respectively. Therefore, the three different distributions observed in the mass spectrum have the same end-group but differ on the cation adduct, thus confirming the presence of a single homopolymer in the sample. The elemental composition of the end-group was calculated within the elemental composition range of [$C_{0–100}$ $H_{0–200}$ $N_{0–5}$ $O_{0–20}$] with a mass tolerance of ± 2 ppm. Since members of homologous series differ only by integer multiples of EO, assignment of a single member of the series was enough for all mass members. The ion with lowest *m/z* was used to perform the putative identification, being the molecular formula of the polymer [$(C_{10}H_{22}O(EO)_n)$] (Table S1, Supporting information).

The presence of this polymer in the sample fits with the high density of the phytosanitary product (quite similar to white paint or glue) and could be related to their high water solubility ensuring its effectiveness as transport agent. Considering its low toxicity and chemical and microbiological inertness, the polymer was discarded as the agent of causing the high phytosanitary activity of the product.

In the KMD-NKM plot (Fig. 2A), some other ions that are not following any singular polymer distribution were also present. Better separation of these ions was observed in the KMD-RNKM plot (Fig. 2B), where five ion distributions that not merge in a single disk can be distinguished at KMD values of −0.4183, −0.3830, 0.2210, 0.3858 and 0.4183. The DESI-HRMS mass spectrum of Fig. 1 helps to explain the presence of these ions. Three wide isotope pattern distributions can be distinguished that might be due to the presence of metal elements in the chemical structure of these ions. By comparing the experimental isotope distributions with the theoretical ones, the ions detected in the DESI-HRMS spectrum are consistent with the presence of tin atoms. Therefore, the different ions separated horizontally by one RNKM unit in the KMD-RNKM plot (Fig. 2B) could correspond to the contribution of different tin isotopes to the isotope cluster. To simplify the discussion of the mass spectra, only the most abundant ion of the isotope cluster will be mentioned in this paper, being the *m/z* values assigned to ions with one, two and three tin atoms, respectively. The signals at *m/z* 717.0396 (Fig. 1B) might include two tin atoms ($^{120}Sn^{118}Sn$), while the signals at *m/z* 1083.0628 and *m/z* 1109.0424 (Fig. 1C) could be assigned to ions with three tin atoms ($^{120}Sn^{118}Sn_2$) in their structure (Fig. S1). Based on the accurate mass measurements and the isotope pattern, the chemical formulas were proposed for the suspected organotin (OT) ions (Fig. 1B and C) taking into account the following criteria: elemental compositions limited to $^{12}C_{0–100}{}^{1}H_{0–100}{}^{14}N_{0–10}{}^{16}O_{0–10}{}^{23}Na_{0–1}{}^{39}K_{0–1}{}^{120}Sn_{0–3}{}^{118}Sn_{0–3}$ mass accuracy lower than ± 2 ppm and isotope cluster fit higher than 80% (Fig. S2: isotope distribution simulations).

In order to obtain complementary information, MS data were acquired by DESI-HRMS on static mode (sampling for 1 min) instead of scanning mode. During the first 12 s of the analysis in positive ion mode, only ions from the polymer were observed whereas the presence of ions from the OT compounds was not detected until 42 s (Fig. S3). The wash away of the sample matrix at the beginning of the analysis by the DESI solvent reduced the concentration of the polymer in the sample surface, which consequently decreased the ion suppression effect on the OT ions [18]. Performing the DESI-HRMS analysis at this conditions (Fig. 3A) improves the desorption/ionization efficiency of OTs (ion signal intensities are one order of magnitude higher than those obtained in scanning mode). Moreover, the interpretation of OT ions in

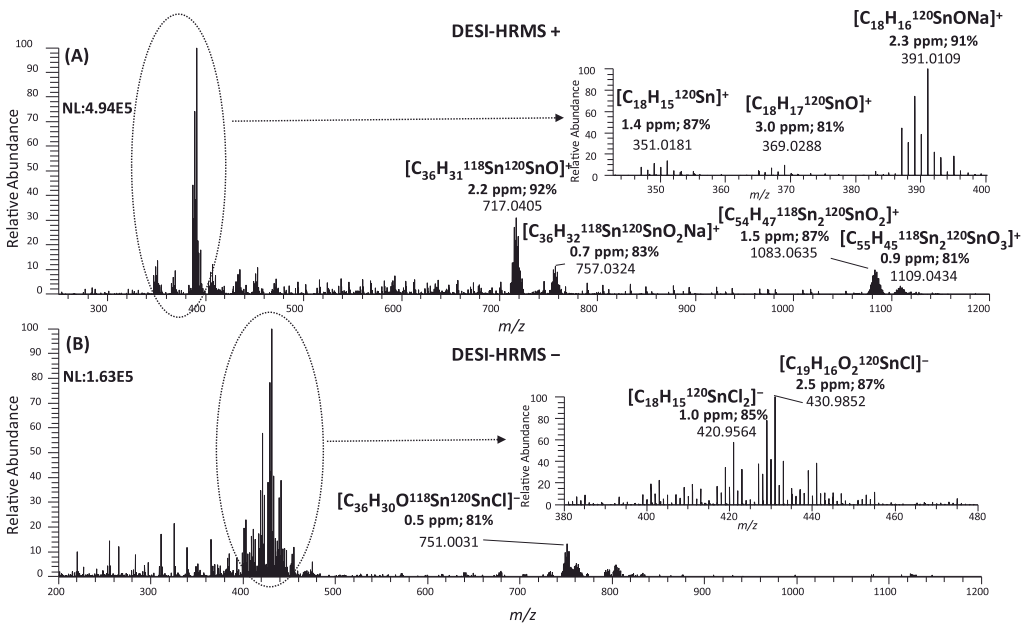

Fig. 3. Average DESI-HRMS mass spectrum of the phytosanitary product under static sampling mode: (A) positive ion mode mass spectrum from 0.7 to 1 min with the first 12 s of the analysis background-subtracted. (B) Negative ion mode mass spectrum from 0.5 to 0.7 min with the first 10 s of the analysis background subtracted.

the mass spectrum was also facilitated by subtracting the polymer ions and other matrix interference signals. The highest desorption ionization efficiency obtained using the static mode allowed detecting other OT ions with one tin atom (Fig. 3A).

Negative DESI-HRMS experiments were also performed in fixed scanning mode. As can be seen in Fig. 3B, a cleaner mass spectrum than the one in positive ion mode was obtained. Two different OT pattern profiles (corresponding to *m/z* 420.9564 and *m/z* 430.9852 ions) were observed in this ionization mode and their proposed molecular formulas are given in Fig. 3B.

3.2. Characterization of organotin ions

At this point, it is suspected that OT ions could be related with the high activity of the phytosanitary product. In order to obtain structural information, DESI-MS/HRMS experiments in a quadrupole-Orbitrap were performed. To keep relevant isotope pattern information of product ions, DESI-MS/HRMS experiments were performed by isolating the OT precursor ions in the quadrupole using an isolation width of 12 *m/z* for those ions containing two or three tin atoms and 5 *m/z* for OT ions with one tin atom in their chemical structure.

The obtained DESI-MS/HRMS spectra in positive ion mode of the most abundant OT ions detected in the sample are shown in Fig. 4. In all tandem mass spectra, a common product ion at *m/z* 351.0187 (also detected in the DESI-HRMS spectrum) was observed. The base peak at *m/z* 119.9017 in the product ion spectrum of *m/z* 351 (Fig. 4A) matches with the exact mass and isotope pattern of the tin radical ion $[Sn]^{+\bullet}$. Additionally, accurate mass differences of 77.0391 Da between ions at *m/z* 196.9408 and *m/z* 119.9017 and 154.0779 Da between *m/z* 315.0187 and *m/z* 196.9408 can be assigned to the losses of one and two phenyl molecules, respectively. This fragmentation pattern suggested that the ion at *m/z* 351.0187 could correspond to $[Ph_3Sn]^+$, which is in agreement with reported data for triphenyltin compounds (Ph_3SnX, X: halide, oxide or hydroxide) [19–21]. The ions at *m/z* 369.0295 and *m/z* 214.9512 (Fig. 4A) can be assigned to water adducts, $[Ph_3Sn+H_2O]^+$ and $[PhSn+H_2O]^+$, respectively, generated via ion-molecule reactions in the collision cell. The formation of water adducts in quadrupole-Orbitraps has been previously reported as a consequence of the moisture content (99.995% purity) of the nitrogen used in the collision cell [22]. The high reactivity of OT compounds [21] suggests that adduct formation (water, ACN, sodium) can also occur in the DESI process, which could explain the ion at *m/z* 391.0109 observed in the DESI-HRMS spectrum, assigned to a sodium adduct. The tandem mass spectrum of the ion at *m/z* 391(Fig. 4B) yielded the product ions at *m/z* 351.0187 and *m/z* 369.0295 assigned to $[Ph_3Sn]^+$ and its water adduct ion, respectively. Considering both, the tandem mass spectrum and the accurate mass measurements, the ion at *m/z* 391.0109 detected in the DESI-HRMS spectrum might be assigned to $[Ph_3SnOH+Na]^+$. These results may allow hypothesizing that triphenyltin hydroxide could be a possible candidate for the adulterant. This hypothesis can be also supported by the presence of the ion at *m/z* 369.0288 in the DESI-HRMS, that can also be assigned as $[Ph_3SnOH+H]^+$. However, Ph_3SnOH could also be generated by the hydrolysis of triphenyltin halide, another potential candidate.

The same product ions were observed in tandem mass spectra of *m/z* 717, *m/z* 1083 and *m/z* 1109 (Fig. 4C, D and E). A low normalized collision energy (NCE) (10–17 V) was required for the fragmentation of these precursor ions, which may indicate that they could be aggregates, assumption supported by the high reactivity of triphenyltin compounds. The generation of aggregated ions has been previously reported for triphenyltin halides and triphenyltin hydroxide using soft ionization sources [23–25]. These three ions (DESI-HRMS spectrum) were attributed to complexes of Ph_3Sn^+ and OH^-, in agreement with previously reported results for triphenyltin halides [23]. Hence, ions at *m/z* 717.0405 and *m/z* 1083.0635 were assigned to $[(Ph_3Sn)_2OH]^+$ and $[(Ph_3Sn)_3(OH)_2]^+$, respectively. The presence of the ion at *m/z* 717 has also been reported as the result of the reaction of two Ph_3SnOH molecules with the subsequent loss of water to yield bis(triphenyltin) oxide

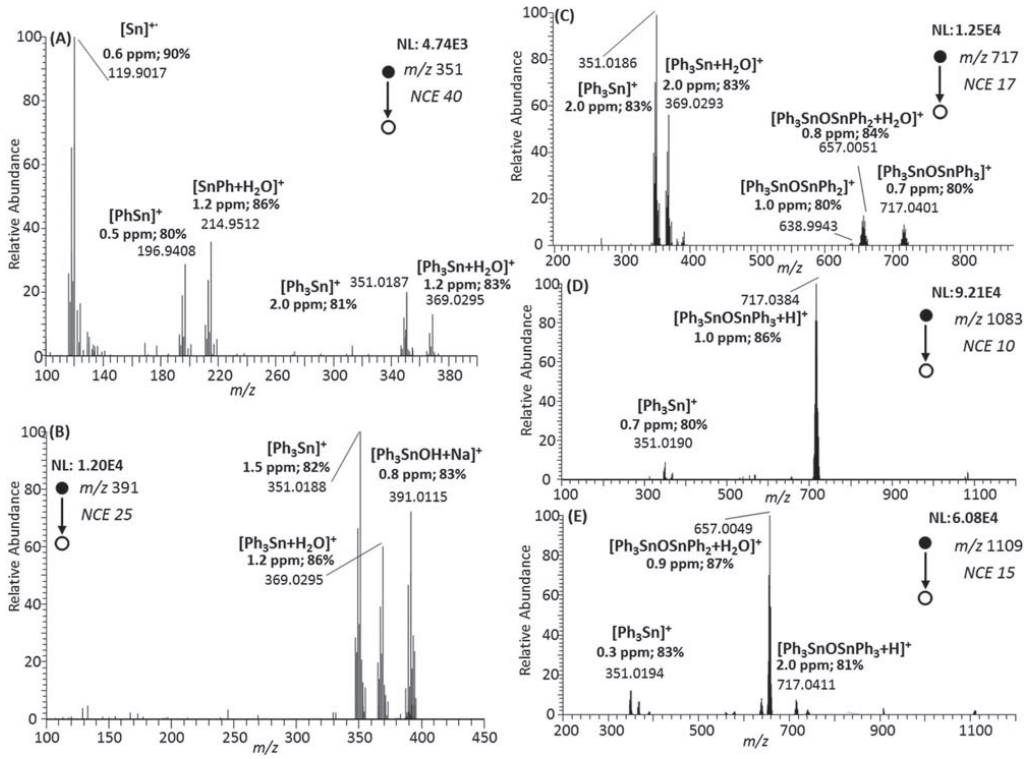

Fig. 4. DESI-MS/HRMS product ion scan of the OT ions: (A) *m/z* 351 (B) *m/z* 391 (C) *m/z* 717 (D) *m/z* 1083 (E) *m/z* 1109. Isolation window: 5 *m/z* for precursor ions at *m/z* 351 and 391; 12 *m/z* for precursor ions at *m/z* 717, 1083 and 1109. Mass relative errors (ppm) calculated for the base peak of each isotope cluster ion. Elemental compositions correspond to the cluster ion.

($Ph_3SnOSnPh_3$) as reaction product [25,26]. Taking into account this assumption, the ion at *m/z* 1083.0635 in the DESI-HRMS spectrum (Fig. 3A) could be explained as an aggregate between Ph_3SnOH and $[Ph_3SnOSnPh_3 + H]^+$ (*m/z* 717.0405). This last ion was also observed as product ion in the tandem mass spectrum of *m/z* 1083 (Fig. 4D).

Regarding tandem mass spectrum of *m/z* 1109 (Fig. 4E), in addition to the ion at *m/z* 717.0412 $[Ph_3SnOSnPh_3 + H]^+$, two other product ions were observed. The accurate mass difference of 78.0482 Da between *m/z* 717.0412 and *m/z* 638.9936 corresponds to C_6H_6, allowing to assign the product ions at *m/z* 638.9936 and *m/z* 657.0048 to $[Ph_3SnOSnPh_2]^+$ and $[Ph_3SnOSnPh_2 + H_2O]^+$, respectively. Considering the accurate mass measurements, the isotope cluster fit as well as the product ions, the ion at *m/z* 1109.0434 could be tentatively assigned to an aggregate $[(Ph_3Sn)_3OCO_2]^+$. This ion was not observed in ESI experiments (data not shown) and it may be explained because ambient mass spectrometry techniques operate in a more open environment, which could favor the interaction between $[Ph_3Sn]^+$ and the atmospheric CO_2.

DESI-MS/HRMS experiments were also performed for those OT ions detected in the negative DESI-HRMS sample spectrum. Although no information was obtained from the tandem mass spectrum of *m/z* 421, previously reported results suggested that this ion could be attributable to the complex ion $[Ph_3SnCl_2]^-$ [23]. The main product ion from the fragmentation of this ion would be $[Cl]^-$, out of the mass range of Orbitrap. Regarding the tandem mass spectrum of *m/z* 431, only the presence of $[Ph_3Sn]^-$ (*m/z* 351.0199) was observed (data not shown). Taking into account the elemental composition, the ion at *m/z* 430.9852 could be assigned to the complex ion $[Ph_3SnClCO_2H]^-$. The presence of chlorine and the absence of any other halide may suggest that triphenyltin chloride could be present in the sample.

At this point, three different compounds, triphenyltin chloride, triphenyltin hydroxide and bis(triphenyltin) oxide, were suspected of being possible adulterant/s responsible/s of causing the unexpected activity of the phytosanitary product. In the past decades, triphenyltin compounds have been used as major components in antifouling products and as biocides, which is consistent with the sample of interest [27]. These compounds have been reported for being highly toxic for organisms into aquatic systems as well as for their high bioaccumulation potential and persistence in sediments [28–30]. In light of the extensive effects of triphenyltin compounds, the European Commission, under the Directive 2009/425/EC, has restricted their marketing and use stablishing a maximum concentration level in any product equivalent of 0.1% by weight of tin [31]. In order to get more information about the identity of triphenyltin compound/s detected in the sample, the standards of suspect compounds were purchased and analyzed by DESI-HRMS(/MS).

The DESI-HRMS (positive ion mode) spectra of the standards (data not shown) were quite similar, matching with the ions observed in the sample and confirming the presence of triphenyltin compounds in the adulterated phytosanitary product, but not allowing distinguishing between the three candidates. By contrast, the tandem mass spectra provided some significant information. Among the ions studied, noticeable differences in the product ion scan of *m/z* 1109 were observed between triphenyltin chloride and that of the sample. The relative abundances of all product ions for triphenyltin chloride were higher

than 40% (Fig. S4A). By contrast, the ion at m/z 657.0049 was the base peak in the mass spectra of the sample (Fig. 4E), triphenyltin hydroxide and bis(triphenyltin) oxide standards (Fig. S4B and S4C), and other product ions were not found at relative abundances higher than 10%. Additionally, the sample DESI-HRMS mass spectrum in negative ion mode was more similar to those obtained for triphenyltin hydroxide and bis(triphenyltin) oxide standards than for triphenyltin chloride where, as expected, the ion at m/z 420.95675 $[Ph_3SnCl_2]^-$ prevailed over the other ions (Fig. S5). Taking into account all the data obtained, only triphenyltin hydroxide and bis(triphenyltin) oxide may be the suspected adulterants. Since the legislated restrictions (0.1% of the weight of tin) affect to any type of triphenyltin compound [31], it is not significant if these two triphenyltin compounds or a mixture of both is the responsible of the unexpected phytosanitary activity of the sample.

In order to confirm the presence of triphenyltin compounds, the sample was analyzed by ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS) (experimental conditions in Supporting information). The UHPLC-HRMS chromatogram showed two peaks (Fig. S6). The mass spectrum of the first eluting peak showed the characteristic ions of organotin compounds also observed in the DESI-HRMS spectrum, while the mass spectrum of the second peak showed the series of ions characteristics of the PEG_n polymer previously identified. In addition, the analysis of triphenyltin hydroxide and bis(triphenyltin) oxide standards provided the same retention time as well as the same mass spectrum, which may indicate an interconversion of one compound into the other (Fig. S7).

Quantitation was performed by UHPLC-HRMS using standard addition method and the concentration of triphenyltin in the phytosanitary product was 35.0 ± 0.8%, which corresponds to 11.8 ± 0.3% of tin. This result is in agreement with that obtained (12.1 ± 0.1%) by inductively-coupled plasma-optical emission spectroscopy (ICP-OES) (experimental conditions in Supporting information), which is 120 times higher than the maximum permitted [31].

4. Conclusions

In this work, the potential of DESI-HRMS(/MS) for the analysis of unknown materials has been demonstrated, revealing that ambient MS techniques could be proposed as a valuable analytical tool to solve these real life analytical problems. KMD analysis was successfully used to extract information from the complex mass spectrum, allowing the identification of a polyethylene glycol polymer in the sample. The use of HRMS in both, full scan and tandem experiments revealed the presence of triphenyltin compounds at a concentration of 35%. The unexpected high activity of the phytosanitary product was attributed to the presence of these compounds with a tin concentration highly above (120 times) the legislated levels, thus confirming the use of an illegal product.

Acknowledgments

The authors gratefully acknowledge the financial support received from Spanish Ministry of Economy and Competitiveness (CTQ2015-63968-C2-1-P) and from the Agency for Administration of University and Research Grants (Generalitat de Catalunya, Spain) (2017SGR-310). Raquel Seró is also grateful to the University of Barcelona for an APIF grant.

Conflict of interest

The authors declare that they have no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2018.10.038.

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INFORMACIÓ SUPLEMENTÀRIA DE LA PUBLICACIÓ IV

Desorption electrospray ionization-high resolution mass spectrometry for the analysis of unknown materials: The phytosanitary product case

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Analyst, 194 (**2019**) 350-356

Supporting Information

Ultra-high performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS) experimental conditions:

Ultra-high performance liquid chromatography was used for the chromatographic separation of the sample under study. The chromatographic separation was carried out on an Accucore C_{18} column (150x2.1 mm, 2.6 µm superficially porous particles) (Thermo Fisher Scientific, San Jose, CA, USA) using an Accela UHPLC system equipped with an autosampler (Accela AS) and a quaternary pump (Accela 1250 Pump) (Thermo Fisher Scientific). The UHPLC system was coupled to a quadrupole-Orbitrap Q-Exactive (Thermo Fisher Scientific) mass spectrometer equipped with an electrospray (ESI) ion source. For the analysis, a methanol/water gradient elution program (total runtime of 15 min) was as follows: 1 min isocratic step at 50% methanol; from 50 to 100% methanol in 9 min and a final 1 min isocratic step at 100% methanol before returning to the initial conditions (4 min of conditioning time). The injection volume and the flow rate were 10 µL and 300 µL min^{-1}, respectively. The Q-Exactive mass spectrometer operated in positive ionization mode at a mass resolution of 70,000 FWHM (at *m/z* 200), the maximum injection time (IT) was 200 ms, automatic gain control (AGC) was set at 1×10^6 and the scan range was *m/z* 100-1,500. The ionization source parameters were as follows: nebulizer gas and auxiliary gas (N_2, 99.95% pure, Air Liquide, Madrid, Spain) were 50 and 30 arbitrary units (a.u.), respectively; capillary temperature, spray voltage and S-lens were set at 320 °C, 3.2 kV and 50 a.u., respectively.

Triphenyltin hydroxide and bis(triphenyltin) oxide working standard solutions were prepared separately by appropriate dilution in MeOH/H_2O (1:1, *v*:v) from stock solutions described in the manuscript. The standard addition method was used to quantify the triphenyltin in the sample correcting potential matrix effects on the electrospray ionization. The sample was properly diluted in methanol/water (1:1, *v*:*v*) by triplicate and triphenyltin hydroxide and bis(triphenyltin) oxide were added to the diluted sample aliquots (1 mL) separately. The standard addition calibration curves were stablished using 5 calibration levels S_0-S_5, 0/0.3/0.6/0.9/1.2 µg g^{-1}. Triphenyltin concentration was calculated based on the calibration curves obtained by plotting the area of the chromatographic peak vs the fortified concentration as $[Ph_3Sn]^+$.

Inductively-coupled plasma optical emission spectroscopy (ICP-OES) experimental conditions

The studied sample was analyzed by ICP-OES using 0.1 g (by triplicate) which was placed in a Teflon Vessel with a mixture of 4 mL HNO_3, 1 mL HF, 1 mL of HCl and 2 mL of H_2O_2 for the subsequent microwave digestion at a final temperature of 220 °C. After let it cool, 45 mL of H_2O were added and the solution was diluted with H_2O 20 times before the analysis. Tin was determined in the aqueous solution using a Perkin-Elmer OPTIMA 8300 ICP-OES spectrometer under standard conditions. This equipment consists of a radiofrequency source (working at 1150 W and 40 MHz), a cross-flow nebulizer and a segmented-array charge-coupled device (SCD) detector. The emission line used for the determination of tin was 189.927 nm. For the quantification of the amount of tin in the sample, a four-point external calibration curves ranging from 2 to 20 $\mu g\ g^{-1}$ was used.

Table S1. Tentative identification of polymer adduct ions observed in the sample studied using Kendrick Mass Defect analysis, based on a scaling factor of ethylene oxide (EO) (44.0000/44.0262)

Accurate mass (*m/z*)	Kendrick mass	Kendrick mass defect	Tentative ion assignment	Exact mass (*m/z*)	Error (ppm)
313.2343	313.0479	0.05	$[C_{10}H_{22}O(EO)_3+Na]^+$	313.2349	2.0
357.2604	357.0478	0.05	$[C_{10}H_{22}O(EO)_4+Na]^+$	357.2611	2.1
401.2866	401.0478	0.05	$[C_{10}H_{22}O(EO)_5+Na]^+$	401.2874	1.9
445.3128	445.0478	0.05	$[C_{10}H_{22}O(EO)_6+Na]^+$	445.3136	1.7
489.3393	489.0481	0.05	$[C_{10}H_{22}O(EO)_7+Na]^+$	489.3398	1.0
533.3660	533.0486	0.05	$[C_{10}H_{22}O(EO)_8+Na]^+$	533.3660	0.0
577.3916	577.0480	0.05	$[C_{10}H_{22}O(EO)_9+Na]^+$	577.3922	1.1
621.4171	621.0473	0.05	$[C_{10}H_{22}O(EO)_{10}+Na]^+$	621.4184	2.1
665.4437	665.0477	0.05	$[C_{10}H_{22}O(EO)_{11}+Na]^+$	665.4446	1.4
709.4689	709.0467	0.05	$[C_{10}H_{22}O(EO)_{12}+Na]^+$	709.4709	2.8
753.4947	753.0463	0.05	$[C_{10}H_{22}O(EO)_{13}+Na]^+$	753.4971	3.2
797.5215	797.0469	0.05	$[C_{10}H_{22}O(EO)_{14}+Na]^+$	797.5233	2.2
841.5468	841.0460	0.05	$[C_{10}H_{22}O(EO)_{15}+Na]^+$	841.5495	3.2
417.2607	417.0124	0.01	$[C_{10}H_{22}O(EO)_5+K]^+$	417.2613	1.4
461.2869	461.0124	0.01	$[C_{10}H_{22}O(EO)_6+K]^+$	461.2875	1.3
505.3135	505.0128	0.01	$[C_{10}H_{22}O(EO)_7+K]^+$	505.3137	0.4
549.3401	549.0132	0.01	$[C_{10}H_{22}O(EO)_8+K]^+$	549.3399	-0.3
593.3657	593.0126	0.01	$[C_{10}H_{22}O(EO)_9+K]^+$	593.3662	0.8
637.3914	637.0121	0.01	$[C_{10}H_{22}O(EO)_{10}+K]^+$	637.3924	1.5
681.4178	681.0123	0.01	$[C_{10}H_{22}O(EO)_{11}+K]^+$	681.4186	1.0
725.4432	725.0115	0.01	$[C_{10}H_{22}O(EO)_{12}+K]^+$	725.4448	2.2
769.4686	769.0107	0.01	$[C_{10}H_{22}O(EO)_{13}+K]^+$	769.4710	3.1
813.4956	813.0115	0.01	$[C_{10}H_{22}O(EO)_{14}+K]^+$	813.4972	2.0
440.3575	440.0954	0.10	$[C_{10}H_{22}O(EO)_5+NH_4]^+$	440.3582	1.5
484.3840	484.0957	0.10	$[C_{10}H_{22}O(EO)_6+NH_4]^+$	484.3844	0.8
528.4109	528.0964	0.10	$[C_{10}H_{22}O(EO)_7+NH_4]^+$	528.4106	-0.6
572.4360	573.0953	0.10	$[C_{10}H_{22}O(EO)_8+NH_4]^+$	572.4368	1.4
616.4620	616.0951	0.10	$[C_{10}H_{22}O(EO)_9+NH_4]^+$	616.4630	1.7
660.4885	660.0954	0.10	$[C_{10}H_{22}O(EO)_{10}+NH_4]^+$	660.4893	1.1
704.5143	704.0950	0.10	$[C_{10}H_{22}O(EO)_{11}+NH_4]^+$	704.5155	1.7
748.5405	748.0950	0.10	$[C_{10}H_{22}O(EO)_{12}+NH_4]^+$	748.5417	1.6
792.5667	792.0950	0.10	$[C_{10}H_{22}O(EO)_{13}+NH_4]^+$	792.5679	1.5

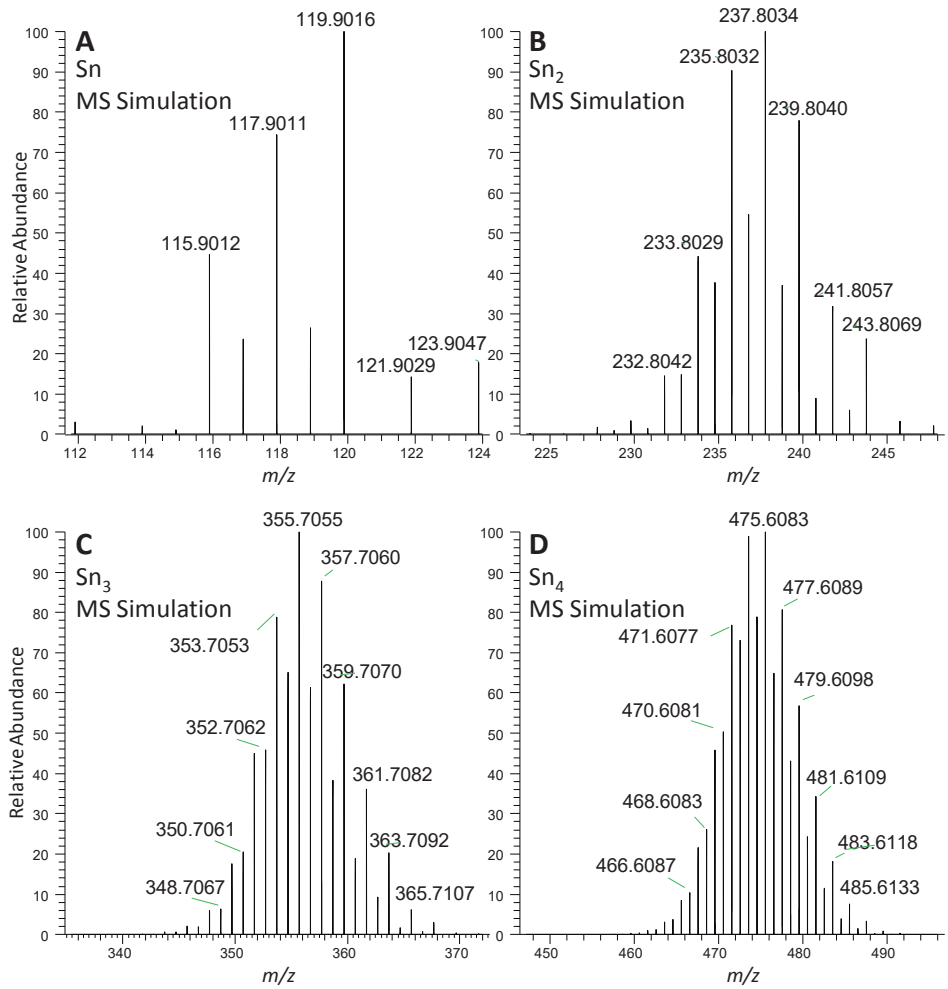

Fig. S1. Simulation of isotope pattern distributions of (A) one, (B) two, (C) three and (D) four tin atoms.

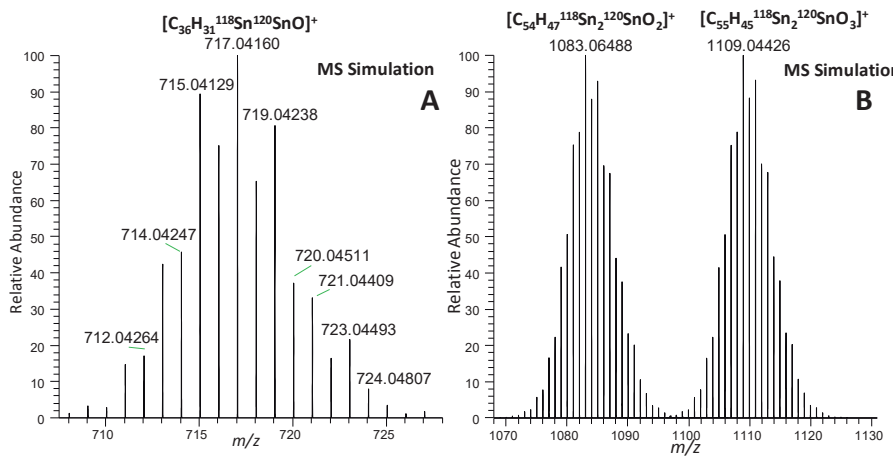

Fig. S2. Simulation of isotope pattern distributions of (A) $[C_{36}H_{31}Sn_2O]^+$, (B) $[C_{54}H_{47}Sn_3O_2]^+$ and $[C_{55}H_{45}Sn_3O_3]^+$

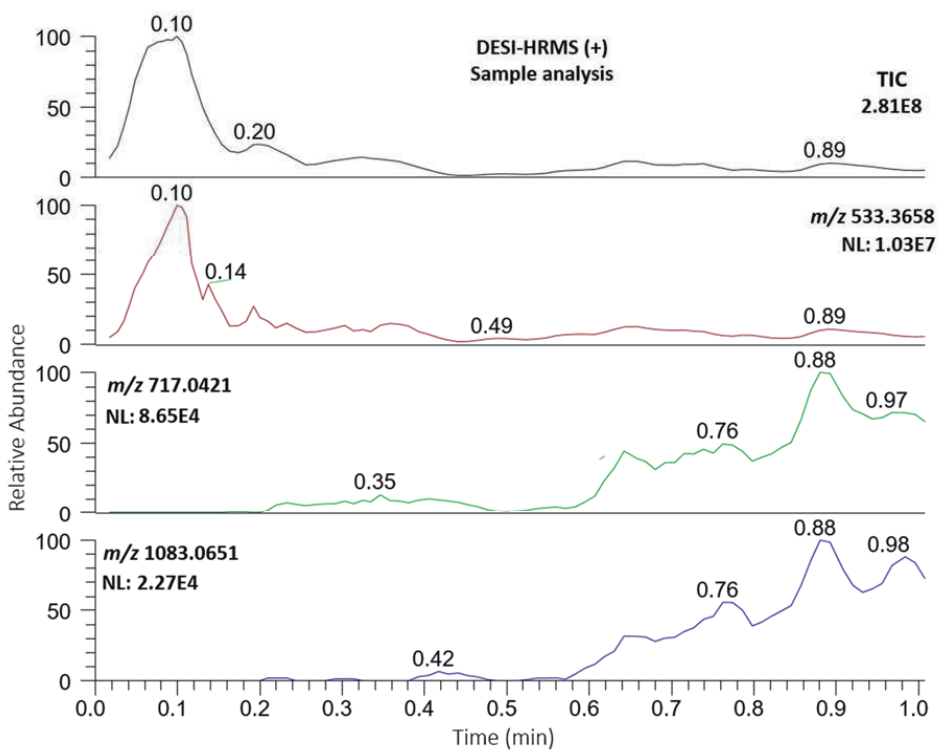

Fig. S3. Static mode DESI-HRMS (+) total ion current and selected ion current profiles for *m/z* 533.3658 (base peak of the polymer sodium adduct distribution), *m/z* 717.0421 (the

most intense peak of the isotope cluster of $[C_{36}H_{31}{}^{120}Sn^{118}SnO]^+$) and *m/z* 1083.0651 (the most intense peak of the isotope cluster of $[C_{54}H_{47}{}^{120}Sn^{118}Sn_2O_2]^+$).

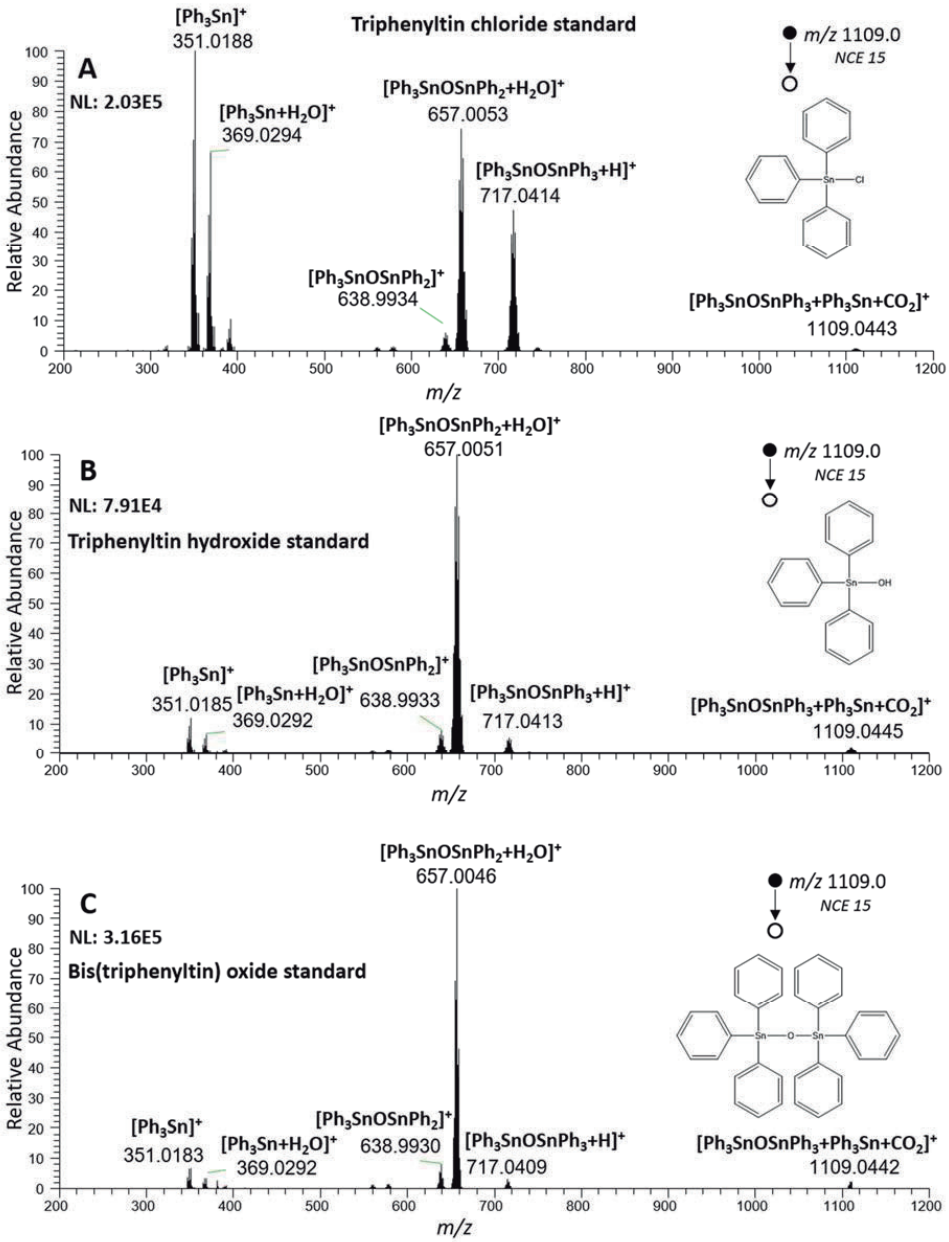

Fig. S4. DESI-MS/HRMS product ion scan of precursor ion at *m/z* 1109 obtained from (A) triphenyltin chloride, (B) triphenyltin hydroxide and (C) bis(triphenyltin) oxide standards.

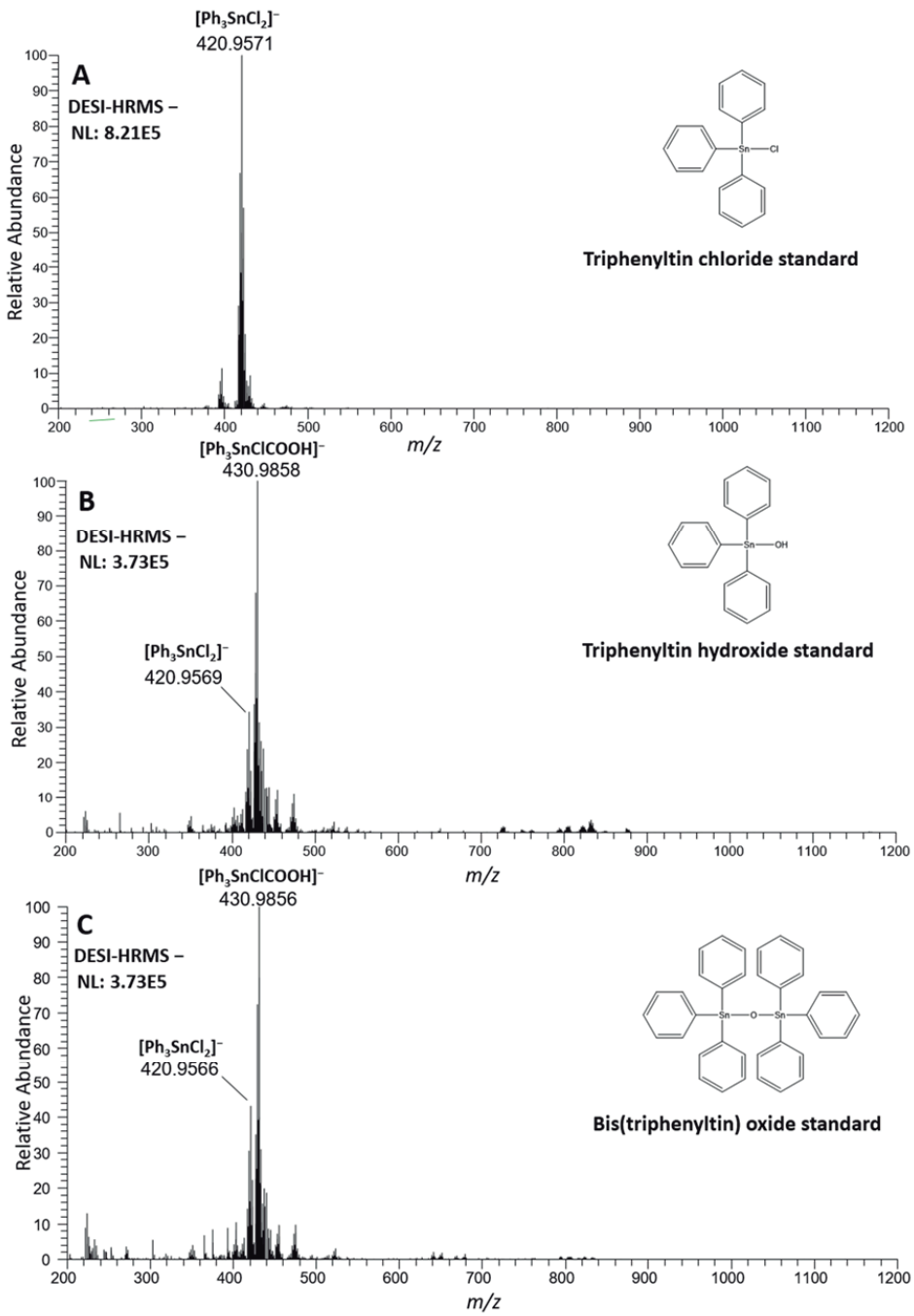

Fig. S5. DESI-HRMS (-) full scan mass spectra of (A) triphenyltin chloride, (B) triphenyltin hydroxide and (C) bis(triphenyltin) oxide standards.

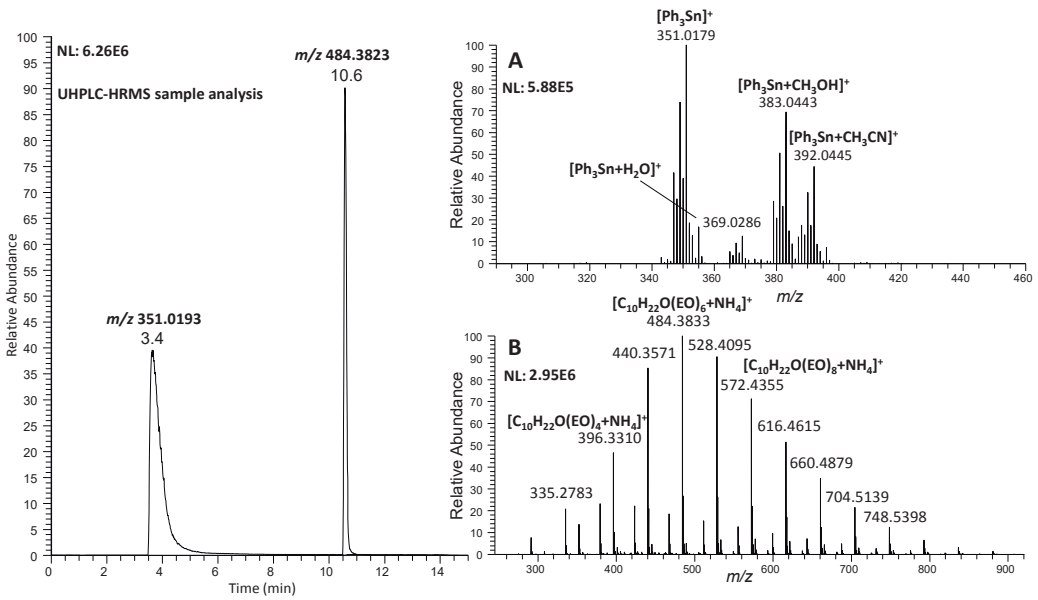

Fig. S6. UHPLC-HRMS sample analysis. (A) sample mass spectrum at the retention time 3.4 min and (B) sample mass spectrum at the retention time 10.6 min.

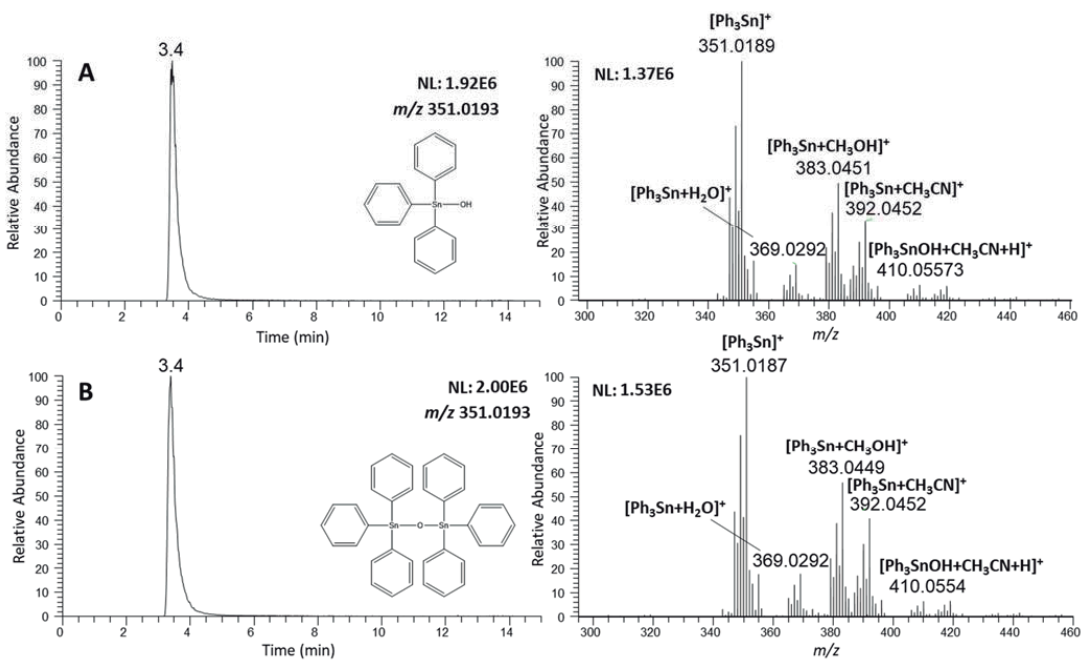

Fig. S7. UHPLC-HRMS standard analysis of (A) triphenyltin hydroxide and (B) bis(triphenyltin) oxide at a concentration of 0.2 μg g^{-1}.

2.3. DISCUSSIÓ DE RESULTATS

En aquest apartat es discuteixen els resultats dels treballs experimentals *(Publicacions III i IV)* inclosos en aquest capítol referits a la utilització de la tècnica DESI-HRMS per a la identificació, per una banda, de drogues veterinàries i la detecció de contaminacions creuades en mostres de pinso i, per l'altra, a la possibilitat de caracteritzar compostos desconeguts en un producte fitosanitari sospitós d'haver estat adulterat de forma fraudulenta i del qual no es disposa d'informació prèvia. La discussió s'ha dividit en tres apartats: estratègies de mostreig i manipulació de les mostres, optimització dels paràmetres de la font DESI i utilització de l'espectrometria de masses d'alta resolució.

2.3.1. Mostreig i manipulació de la mostra

Com s'ha comentat prèviament, la utilització de tècniques *Ambient MS* possibilita dur a terme l'anàlisi directa de mostres sense cap tipus de processament previ, més enllà del que té lloc *in situ* durant l'anàlisi. També és cert que, en alguns casos, és necessari realitzar una mínima manipulació de la mostra per adequar-la a la tècnica *Ambient MS* emprada. En aquesta tesi s'ha afrontat l'anàlisi de dues mostres de característiques molt diferents, un pinso i una mostra líquida molt densa, que han obligat a utilitzar estratègies diferents.

En el cas de les mostres de pinso (*Publicació III*, apartat 2.2.1), a causa de la coneguda variabilitat en la composició entre els pèl·lets del mateix lot, es va considerar necessari triturar i homogeneïtzar les mostres per tal d'analitzar una mostra composta que donés resultats més representatius. Aquesta transformació de les mostres en una polsina (pols fina) va dificultar l'anàlisi directa per DESI, ja que el gas de nebulització que assisteix la generació de l'esprai de gotes carregades provocava la dispersió del material, allunyant-lo del punt de la superfície on incideix l'esprai. Això va provocar que resultés impossible el procés d'extracció i d'ionització dels anàlits en l'anàlisi per DESI. Per tal de solucionar aquesta problemàtica, en una primera fase es va optar per preparar pastilles compactant la mostra (pols) mitjançant una premsa hidràulica (Figura 2.3A). Malauradament, malgrat que es van fer proves amb diferents quantitats de mostra (entre 0.5 i 1.5 g) i emprant diferents pressions de càrrega (entre 10 i 15 T), les pastilles s'esquerdaven amb facilitat durant l'anàlisi per DESI a causa de l'efecte del cabal del gas de nebulització i acabaven provocant la dispersió del material polsós i contaminant la font DESI. Tot i que l'ús d'aglutinants com la cera permetia millorar la resistència de les pastilles, la resposta dels anàlits disminuïa

considerablement, probablement a causa que la cera emprada com a aglutinant en la pastilla dificultava que l'esprai mullés la superfície i, per tant, feia més difícil el procés d'extracció i d'ionització dels compostos presents a la superfície. De fet, altres autors han descrit un efecte similar en l'anàlisi directa de fulles (Muller et al., 2011; Thunig et al., 2011), on la pel·lícula de cera que recobreix la superfície dificultava tant la formació adient de la pel·lícula líquida de dissolvent com l'extracció dels compostos. Per evitar aquests problemes, en aquesta tesi s'ha optat per dur a terme una extracció sòlid-líquid de les mostres de pinso en pols (Figura 2.3B) prèvia a l'anàlisi DESI-HRMS. Es tracta d'una extracció simple i ràpida que, a més, presenta l'avantatge addicional de permetre extreure selectivament els anàlits en detriment de la matriu. Entre els solvents assajats, metanol i acetonitril, aquest últim va facilitar la disminució dels efectes de supressió iònica en la ionització per DESI gràcies a la seva major selectivitat en aquest problema analític. A fi i efecte d'afavorir l'extracció de les diferents famílies de drogues veterinàries que poden contenir compostos amb diferents característiques fisicoquímiques (polaritat i àcid-base), en aquesta tesi es proposa emprar com a dissolvent extractant una mescla ACN/H_2O (80/20, *v/v*) amb un 1% d'àcid fòrmic.

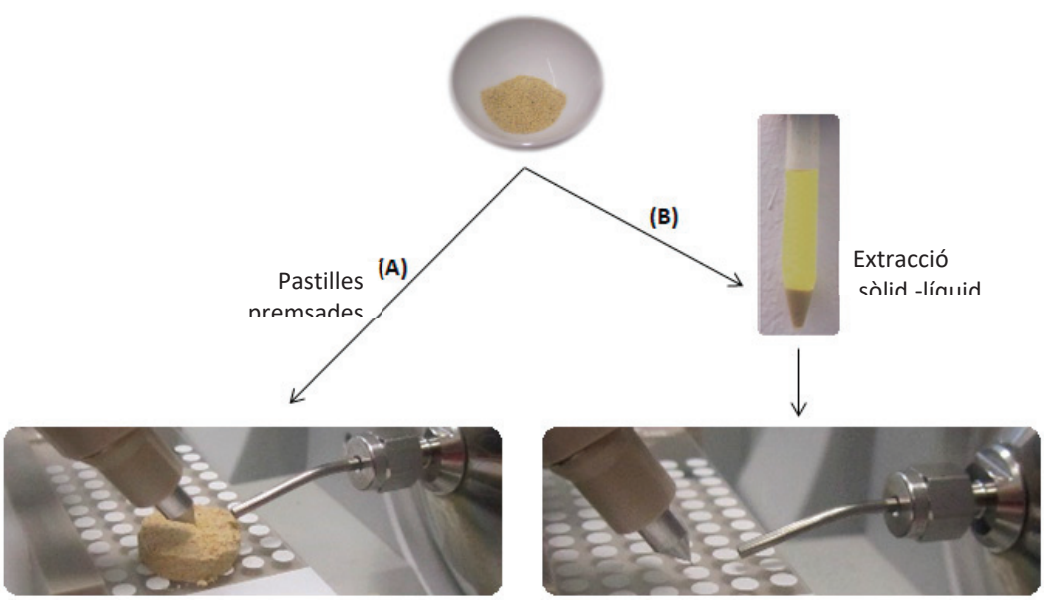

Figura 2.3. Estratègies de manipulació de les mostres de pinso per a la seva anàlisi amb DESI-HRMS: (A) pastilles premsades, (B) extracció sòlid-líquid amb ACN/H_2O (80/20, *v:v*) amb un 1% d'àcid fòrmic.

Com s'ha mencionat a la introducció d'aquest capítol, per a l'anàlisi de matrius líquides, com l'extracte de les mostres de pinso o el producte fitosanitari, la naturalesa del substrat on es diposita la mostra afecta a l'eficàcia d'extracció i d'ionització dels compostos. En aquesta tesi s'han avaluat tres superfícies (substrats) que s'empren habitualment per a l'anàlisi de matrius líquides amb DESI: vidre, politetrafluoroetilè (PTFE) imprès sobre vidre i paper de filtre. Les millors respostes en l'anàlisi dels extractes de les mostres de pinso es van obtenir emprant el PTFE com a substrat (Figura 2.3B), ja que la baixa afinitat que presenta aquesta superfície amb els anàlits facilita la transferència dels compostos des de la superfície a la fase líquida. D'altra banda, la rugositat i baixa porositat que presenta aquesta superfície de PTFE impresa sobre vidre permet reduir l'àrea que ocupa el volum d'extracte líquid dipositat, de manera que augmenta la quantitat de matèria per unitat d'àrea i disminueix la difusió del dissolvent de l'esprai primari que impacta sobre la superfície. Per a l'anàlisi de drogues veterinàries en les mostres de pinso, l'elevat percentatge de dissolvent orgànic (acetonitril) de l'extracte a analitzar permet agilitzar el procés d'evaporació previ a l'anàlisi per DESI (<5 min en les tres superfícies avaluades). Per contra, per a l'assecatge del producte fitosanitari dipositat sobre les superfícies de vidre i de PTFE es van necessitar més de 24 h, probablement a causa de l'elevada viscositat d'aquest producte. Per aquest motiu, es aquest cas es va emprar un paper de filtre com a substrat (*Publicació III*, apartat 2.2.1). L'elevada capacitat d'absorció del paper de filtre va facilitar la fixació de la mostra en el suport i l'evaporació del dissolvent de la mostra, fent possible una disminució significativa del temps d'assecatge (<5 min) i, per tant, també de l'anàlisi. Atesos els bons resultats emprant el paper com a substrat per a l'anàlisi d'aquesta mostra per DESI, amb posterioritat a la *Publicació IV* (apartat 2.2.2) s'han realitzat algunes proves emprant la tècnica *paper spray* (PS), tal com es pot apreciar a la Figura 2.4, on es mostra el dispositiu utilitzat al laboratori per dur a terme aquests experiments amb PS. En aquest muntatge es fa servir un clip de coure tant per

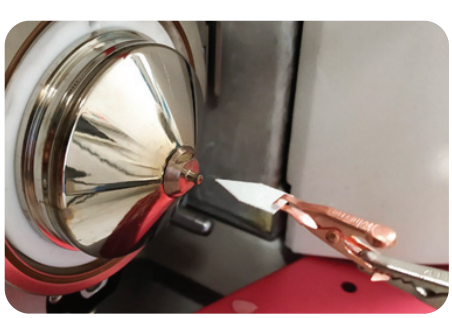

Figura 2.4. Muntatge de la font PS emprat per a l'anàlisi del producte fitosanitari. Dimensions del paper cromatogràfic Whatman 31ET: 10 mm d'alçada i 8 mm de base.

subjectar el paper de forma triangular on s'ha dipositat la mostra (~ 0.1 g), com per subministrar el potencial necessari per a la generació de l'electroesprai a la punta del paper.

2.3.2. Optimització de les condicions de treball en DESI

En DESI, l'optimització dels paràmetres operacionals de la font juga un paper important pel que fa a la sensibilitat de la metodologia desenvolupada. La velocitat, la composició química, la mida, la càrrega i la distribució de les microgotes de l'esprai primari que impacten en la superfície tenen una relació de causa i efecte directa amb l'extracció, la desorció i la transferència dels anàlits cap a l'interior de l'espectròmetre de masses. Per a l'optimització dels paràmetres operacionals de la font DESI, en aquesta tesi s'han seguit diferents estratègies en funció del problema concret plantejat. Així, en el mètode establert per a l'anàlisi de drogues veterinàries (*Publicació III*, apartat 2.2.1), pel qual es disposa de patrons, es va emprar un extracte de pinso blanc fortificat amb alguns dels compostos en estudi. En canvi, per a l'establiment de les condicions de treball per a l'anàlisi del producte fitosanitari (*Publicació IV*, apartat 2.2.2), atesa la manca d'informació sobre la composició i la naturalesa química de la mostra per establir els paràmetres de la font DESI (procediment que s'especifica en l'apartat 2.2.1), s'han emprat com a punt de partida els paràmetres operacionals prèviament optimitzats per a la detecció de contaminacions creuades en les mostres de pinso. Amb l'objectiu d'aconseguir realitzar una optimització individualitzada dels paràmetres operacionals de la font DESI, en ambdós casos es va establir una configuració inicial dels paràmetres geomètrics utilitzant un retolador vermell que conté Rodamina 6G, un colorant fluorescent que s'ionitza fàcilment en mode positiu generant un ió a *m/z* 443.2329 que correspon a la molècula protonada de Rodamina 6G $[M+H]^+$. La coloració del retolador així com la resposta estable d'aquest compost faciliten el posicionament de l'esprai sobre la zona del substrat en què es pretén realitzar l'anàlisi. Com es descriu en la discussió de resultats de la *Publicació III* (apartat 2.2.1), entre els paràmetres de la font DESI que afecten d'una forma més crítica a la sensibilitat dels mètodes DESI en destaquen: la composició química del dissolvent, l'angle i la distància de l'esprai en relació amb la superfície (α, d_1), el cabal del dissolvent i del gas de nebulització, el potencial d'ionització i, com ja s'ha esmentat prèviament, el tipus de substrat. A la Taula 2.1 es resumeixen les condicions de treball DESI emprades en ambdues metodologies (*Publicacions III i IV*) així com les condicions òptimes de treball que es troben descrites en

alguns treballs de la literatura que se centren en l'estudi del fonament i en les característiques de la tècnica DESI.

Taula 2.1. Paràmetres operacionals emprats en l'anàlisi dels extractes de les mostres de pinso i del producte fitosanitari i valors òptims dels paràmetres DESI recomanats a la literatura (Green et al., 2010, 2009; Takáts et al., 2005; Venter et al., 2006)

Paràmetre operacional	Pinso en pols	Producte fitosanitari	Dades de la literatura
Matriu que s'analitza	Extracte de la mostra en ACN/H_2O (80/20, *v:v*) amb 1% àcid fòrmic	Mostra	—
Anàlits	Drogues veterinàries	Compostos desconeguts	Compostos de polaritat mitjana/alta
Substrat	PTFE	Paper de filtre	PTFE, vidre
Dissolvent de l'ESI primari	ACN/H_2O (80/20, *v:v*)	ACN/H_2O (80/20, *v:v*)	ACN, MeOH/H_2O (50/50 a 90/10, *v:v*)
Cabal del dissolvent	2.5 μL min^{-1}	3.5 μL min^{-1}	0.1-3 μL min^{-1}
Pressió del gas de nebulització	9 bar	8 bar	7-10 bar
Potencial d'ionització	4.8 kV (pos/neg)	4.5/5 kV (pos/neg)	1-5 kV
Angle del capil·lar de l'ESI (α)	55°	55°	40-70°
Distància del capil·lar ESI/superfície (d_1)	1.7 mm	2.0 mm	1.0-3.0 mm
Distància de la zona d'impacte/entrada MS (d_2)	5.0 mm	3.5 mm	3.0-5.0 mm
Distància superfície/entrada MS (d_3)	1.0 mm	1.0 mm	1.0-2.0 mm
Temperatura del capil·lar	250 °C	250 °C	200-350 °C
Tipus d'escaneig DESI	Punt fix	Punt fix/mòbil (250 μm s^{-1})	—

L'eficàcia d'extracció i d'ionització en DESI depèn de la solubilitat dels anàlits en el dissolvent de l'electroesprai primari. Per tant, és coherent que la màxima intensitat del senyal de les drogues veterinàries s'hagi obtingut emprant ACN/H_2O (80:20, *v:*v) com a dissolvent. Aquesta mescla de dissolvents és la mateixa que la utilitzada en l'etapa d'extracció, tot i que s'ha eliminat l'àcid que, com es mostra en la Figura 2A de la *Publicació III* (apartat 2.2.1), fa disminuir dràsticament la resposta de tots els compostos model, fins i tot la dels compostos bàsics que s'ionitzen en mode positiu donant l'ió $[M+H]^+$. Un augment de la mida de les microgotes carregades a causa de la major força iònica del dissolvent acidificat, que n'augmenta la tensió superficial, pot ser la causa de la disminució del senyal. D'altra banda, la composició química del dissolvent té una relació directa amb la dinàmica de les

microgotes carregades i amb l'eficàcia d'ionització de la font DESI, ja que un elevat percentatge de dissolvent orgànic fa disminuir la mida de les microgotes i facilita la desorció dels anàlits en l'electroesprai secundari. Tenint en compte les millores d'eficàcia en la ionització quan s'augmenta la proporció del component orgànic en el dissolvent de l'esprai i, atesa la capacitat de la mescla ACN/H_2O (80/20, *v:v*) d'extraure compostos d'un ampli interval de polaritats, aquesta també es va utilitzar en l'anàlisi del producte fitosanitari (*Publicació IV*, apartat 2.2.2).

Com es pot observar a la Taula 2.1, els valors dels paràmetres operacionals de l'esprai primari emprats en les dues aplicacions estudiades (cabal del dissolvent, pressió del gas de nebulització i potencial d'ionització) són diferents. Per a l'anàlisi dels extractes de les mostres de pinso dipositats en PTFE, el valor òptim del cabal del dissolvent és inferior a l'emprat en l'anàlisi del producte fitosanitari viscós impregnat en el paper de filtre, mentre que en l'anàlisi de les mostres de pinso la pressió del gas de nebulització és superior. Aquestes diferències es deuen essencialment a les propietats del substrat; atès que el paper de filtre presenta una major capacitat d'absorció que el PTFE, cal augmentar el cabal del dissolvent i disminuir la pressió del gas de nebulització per tal d'incrementar la mida de les microgotes i, per tant, l'acumulació de líquid a la superfície, que és imprescindible per a que es pugui generar la pel·lícula de dissolvent on tenen lloc els processos d'extracció i d'ionització dels anàlits.

Pel que fa als paràmetres geomètrics, els més crítics són l'angle (α) i la distància (d_1) de l'electroesprai primari respecte a la superfície. A la Figura 2.5 es mostra, a mode d'exemple, l'efecte de α i d_1 en la detecció de les drogues veterinàries en l'extracte de la mostra blanca de pinso fortificada. Les millors respostes es van obtenir per angles d'incidència entre 55 i 60°, valors en què la trajectòria de l'esprai primari també afavoreix la generació de les microgotes secundàries i la transferència dels ions cap a l'entrada de l'espectròmetre de masses (Figura 2.5B). D'altra banda, la resposta de les drogues veterinàries es va veure afavorida en augmentar la distància d_1 fins a 1.7 mm. A distàncies curtes, la baixa resposta dels anàlits pot explicar-se per l'alta velocitat de les microgotes i l'efecte del gas, que dificulta la formació de la pel·lícula de líquid en la superfície on té lloc l'extracció i la ionització dels anàlits. A distàncies per sobre del valor òptim, la baixa energia cinètica de l'esprai primari així com la possible evaporació de les microgotes, que pot provocar la re-deposició del material extret durant l'etapa d'extracció, poden explicar la disminució de la resposta de les drogues veterinàries. Pel que fa a l'anàlisi del producte fitosanitari, es van

utilitzar uns paràmetres geomètrics similars als anteriors excepte en la distància d_1, que es va augmentar fins a 2.0 mm per tal d'afavorir l'acumulació de líquid en la superfície del paper impregnat amb la mostra.

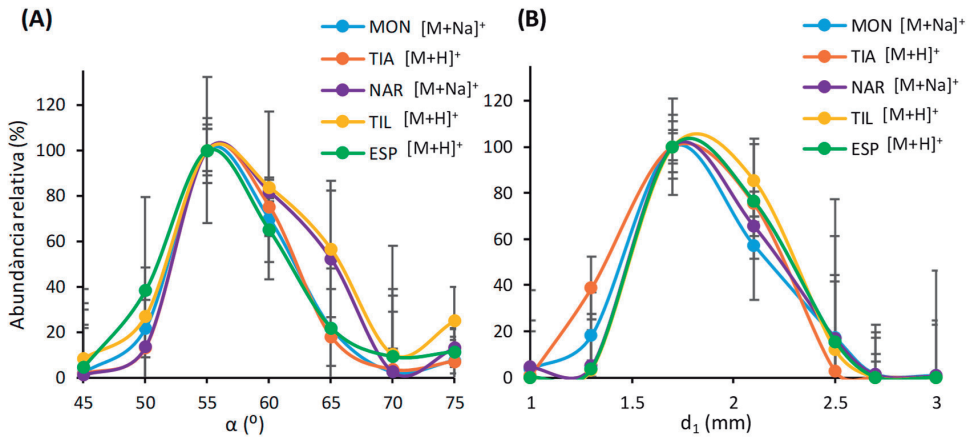

Figura 2.5. Abundàncies relatives de 5 drogues veterinàries a diferents paràmetres geomètrics del mètode DESI-HRMS en mode positiu. (A) l'angle d'incidència (α) i (B) distància de l'esprai primari a la superfície.

Com s'ha comentat a la introducció, la font comercial DESI 1D (Prosolia Inc.) emprada en aquesta tesi permet utilitzar diferents modes d'escombratge de la superfície. En aquesta tesi, s'ha treballat emprant ambdós modes, l'estàtic i el dinàmic. En l'anàlisi de drogues veterinàries per a la detecció de contaminacions creuades (*Publicació III*, apartat 2.2.1), la millor resposta dels ions es va obtenir treballant en mode estàtic, analitzant durant 30 segons la zona central de la superfície de PTFE on s'ha dipositat l'extracte, punt de la superfície en què la quantitat de mostra és màxima. En canvi, en l'anàlisi del paper de filtre impregnat amb el producte fitosanitari es van observar diferències significatives en els espectres de masses obtinguts en utilitzar ambdós modes. Com es pot observar a la Figura 2.6B, en analitzar la mostra en el mode dinàmic es va detectar la presència dels tres ions seleccionats amb masses nominals a *m/z* 533, *m/z* 717 i *m/z* 1083 durant tot el temps de mesura (1 min). En canvi, en analitzar la mostra en mode estàtic (Figura 2.6A), no es va detectar la presència dels ions a *m/z* 717 i *m/z* 1083 fins passats els primers 42 segons d'anàlisi. Com es comenta a la *Publicació IV* (apartat 2.2.2), el procediment estàtic permet millorar la resposta dels ions a *m/z* 717 i *m/z* 1083 (compostos organoestànnics identificats a la mostra) quan s'augmenta el temps de lectura en el mateix punt de mostreig, fet que pot tenir una relació directa amb una certa neteja de la superfície de la mostra durant el primer període de l'anàlisi. En aquesta primera etapa, el dissolvent de l'esprai DESI sembla que podria estar provocant la

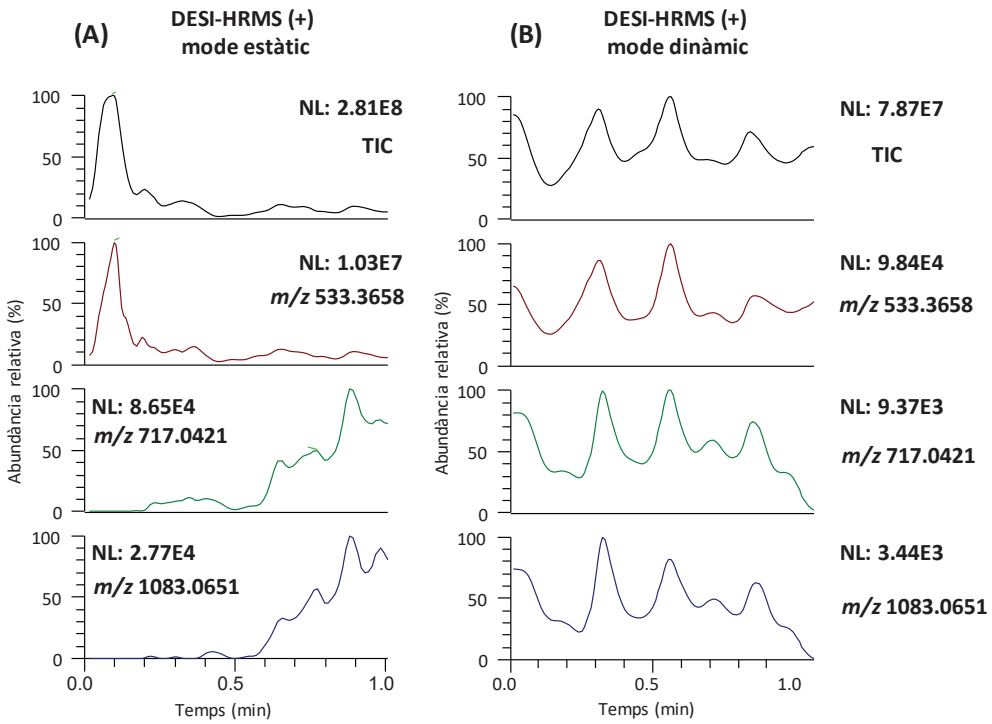

Figura 2.6. Perfils corresponents a la corrent total d'ions i a les corrents dels ions a diferents *m/z* (5 ppm d'error) obtinguts en analitzar la mostra fitosanitària emprant DESI-HRMS: (A) mode estàtic i (B) mode dinàmic.

disminució continuada del compost polimèric (*m/z* 533) a la superfície, fet que podria reduir l'efecte de supressió de la ionització del compost organoestànnic (ions *m/z* 717 i *m/z* 1083) per part del polímer present a una concentració molt més elevada. Aquesta disminució del compost polimèric també podria disminuir la tensió superficial de les microgotes de l'esprai secundari, la qual cosa facilitaria el procés d'evaporació iònica dels ions organoestànnics generats a la fase líquida. A més, l'anàlisi en el mode estàtic ha permès detectar la presència d'ions orgànoestànnics amb un únic àtom d'estany (Figura 3 de la *Publicació IV*, apartat 2.2.2), els quals no s'havien observat treballant en el mode dinàmic.

Aquestes diferències en l'eficàcia d'extracció i d'ionització dels compostos al llarg de l'anàlisi per DESI del producte fitosanitari també s'han observat en les proves realitzades amb la tècnica *paper spray* (PS). Així, a la Figura 2.7A es mostra el cronograma de l'anàlisi per PS-HRMS en mode positiu on es poden diferenciar dues zones: la Zona 1, on la presencia del dissolvent que s'ha aplicat és màxima i la Zona 2, on s'ha esgotat la major part del dissolvent. Com es pot observar a la Figura 2.7B, en la Zona 1 es detecten majoritàriament els ions característics del polímer (distribució centrada en l'ió a *m/z* 577.3922), mentre que

l'abundància relativa dels ions a *m/z* 717.0421 i *m/z* 1109.0445 (ions organoestànnics) augmenta considerablement en l'espectre de masses adquirit en la Zona 2 (figura 2.7C). Aquest increment en la resposta podria associar-se a un canvi en el mecanisme d'ionització en comptes d'una disminució de l'efecte de supressió de la ionització, aspecte que es discuteix amb més detall en el Capítol 3.

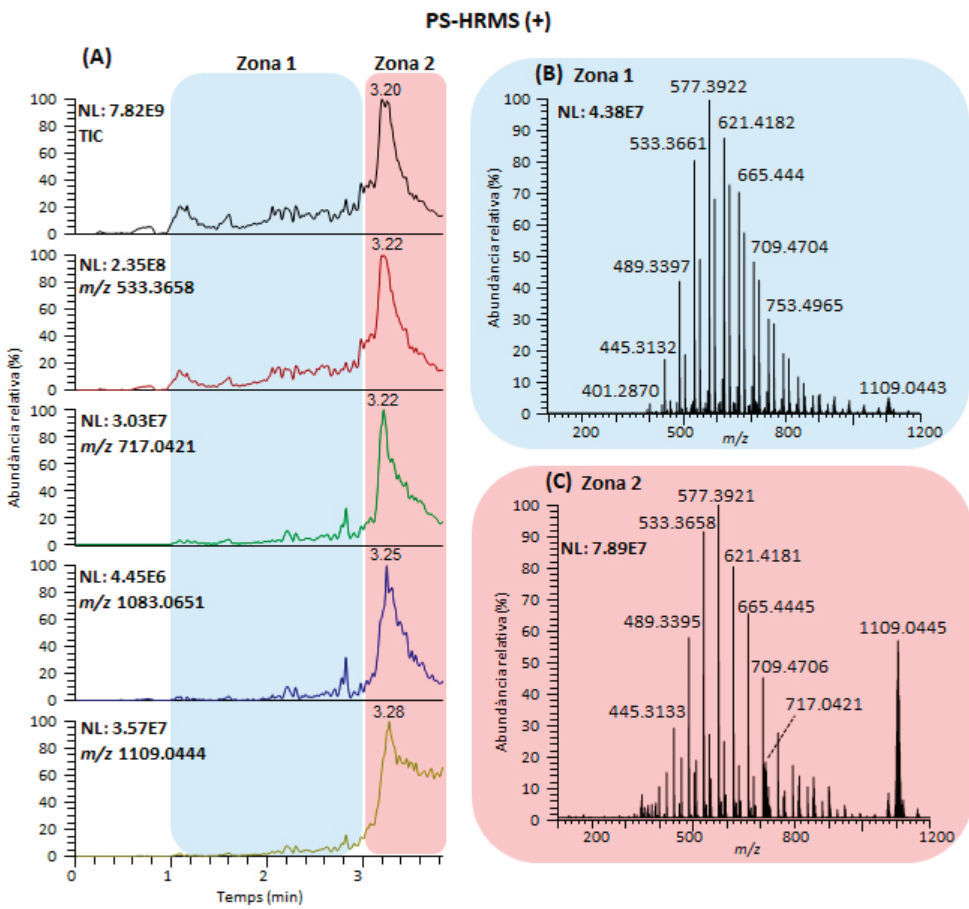

Figura 2.7. Anàlisi del producte fitosanitari emprant la tècnica PS-HRMS: (A) Perfils corresponents a la corrent total d'ions i a les corrents d'ions seleccionats (5 ppm d'error), (B) espectre de masses mitjà obtingut entre els minuts 2 i 3, (C) espectre de masses mitjà obtingut entre els minuts 3 i 4. Condicions de l'anàlisi: 10 µL de mostra dipositats al paper, distància de 4 mm entre la punta del paper i el capil·lar d'entrada a l'espectròmetre de masses, 150 µL de dissolvent ACN-H_2O (80/20, *v:v*) i un potencial de 4 kV.

2.3.3. Espectrometria de Masses d'alta resolució

La complexitat de les matrius de pinso i del producte fitosanitari així com la mínima o nul·la manipulació realitzada prèviament a l'anàlisi d'ambdues mostres suposa que els espectres de masses obtinguts siguin més complexos. En aquest marc, la utilització de l'espectrometria de masses d'alta resolució resulta molt adequada. Com ja s'ha comentat, en aquesta tesi s'ha emprat la tècnica DESI en un espectròmetre de masses híbrid Q-Exactive (quadrupol-Orbitrap). En ambdues metodologies DESI-HRMS desenvolupades, els espectres de masses s'han adquirit en el mode de *full scan* a resolucions superiors a 70,000 FWHM (*m/z* 200). Aquesta elevada resolució ha permès obtenir una molt bona exactitud en la mesura de la massa (< 2-3 ppm) i una òptima separació dels ions i dels clústers isotòpics, cosa que ha proporcionat una elevada selectivitat per a la detecció/identificació de les drogues veterinàries i per a la caracterització dels compostos desconeguts en el producte fitosanitari, a més d'una sensibilitat suficient per detectar les contaminacions creuades en les mostres de pinso. Cal esmentar que en aquesta tesi s'ha utilitzat un temps d'acumulació (*injection* time) dels ions en el C-Trap de 300 ms, valor que es troba molt per sobre al que s'utilitza habitualment en les anàlisis emprant la font convencional ESI (50 ms). Això és degut al fet que en DESI la producció d'ions en el procés de desorció/ionització és molt menor que en ESI. El temps d'acumulació afecta directament a la sensitivitat del mètode quan s'utilitzen espectròmetres de masses que es basen en l'emmagatzematge de paquets d'ions, com ho és el Q-Exactive, de manera que el temps d'acumulació estableix el temps màxim en què els ions són acumulats a la C-Trap. Com es comenta a la *Publicació III* (apartat 2.2.1), cal augmentar el valor d'aquest paràmetre per garantir una acumulació suficient d'ions a la C-Trap, els quals són enviats en forma de paquet d'ions a l'Orbitrap per obtenir un espectre de masses de qualitat.

Considerant la ja esmentada gran quantitat d'informació (ions) que s'obté en els mètodes d'escombratge en DESI, és necessari emprar estratègies que permetin tractar les dades d'una manera fàcil i eficaç per tal d'identificar els compostos presents en les mostres. Per a l'anàlisi de les drogues veterinàries en les mostres de pinso, en aquesta tesi s'ha emprat el programari ExactFinder (ThermoFisher Scientific) per interrogar les mostres analitzades. Així, els espectres de masses de *full scan* obtinguts per a cadascuna de les mostres s'han comparat amb la informació continguda en una base de dades que s'ha construït en aquesta tesi a partir de la informació més rellevant d'espectrometria de masses obtinguda per les més de 60 drogues veterinàries que s'utilitzen habitualment en la producció de pinsos medicats.

Aquesta informació és producte tant de les dades experimentals dels compostos dels quals se'n disposen patrons comercials, com de les dades extretes de la literatura. En la base de dades s'ha inclòs per a cada compost el número CAS, la composició elemental, l'estructura química, el mode d'ionització (positiu/negatiu) així com els ions esperats (molècula protonada/desprotonada, tipus d'adductes i la possible presència d'ions producte com a conseqüència d'una fragmentació a la font d'ionització). Els criteris emprats per a la identificació de les drogues veterinàries s'han basat en: un error en la mesura de la massa exacta inferior a 5 ppm, una relació senyal-soroll mínima 3:1 i un coeficient de semblança del clúster isotòpic superior al 80%. En base a aquests criteris, es va detectar la presència de contaminacions creuades per sobre dels nivells legislats en un 28% de les 50 mostres de pinsos (medicats i no medicats) analitzades amb el mètode DESI-HRMS (resultats inclosos a la Taula 1 de la *Publicació III*, apartat 2.2.1). A més, la posterior anàlisi de totes les mostres de pinso amb un mètode d'UHPLC-MS/MS prèviament establert va posar de manifest l'absència de falsos positius/negatius en els resultats obtinguts per DESI-HRMS, la qual cosa permet proposar-lo com un mètode ràpid de cribratge per augmentar el rendiment dels laboratoris de control.

Com ja s'ha esmentat a la introducció d'aquest capítol, l'estratègia proposada per a l'anàlisi de compostos desconeguts pot variar en funció del problema analític. En el cas concret de l'anàlisi del producte fitosanitari emprant la tècnica DESI-HRMS (*Publicació IV*, apartat 2.2.2) es va detectar la presència d'una sèrie d'ions amb un perfil de distribució característic de polímers de baix pes molecular equidistants 44.0262 unitats de *m/z,* cosa que indicava una possible unitat de repetició d'òxid d'etilè (44.0262 Da). En aquesta tesi s'han analitzat les dades de l'espectre de masses obtingut (Figura 1, *Publicació IV*, apartat 2.2.2) utilitzant l'estratègia del defecte de massa de Kendrick (KMD). Per a tal propòsit, s'han convertit els senyals de *m/z* detectats en l'espectre de masses DESI-HRMS a l'escala de Kendrick modificada en base a la unitat de repetició d'òxid d'etilè (les equacions s'indiquen a l'apartat experimental de la *Publicació IV*). La representació gràfica de les dades del KMD *vs* la massa nominal de Kendrick (NKM) ha permès visualitzar tres sèries horitzontals d'ions que contenen l'òxid d'etilè com a unitat de repetició (Figura 2A, apartat 2.2.2). Amb l'objectiu d'identificar la composició elemental de cada una d'aquestes sèrie d'ions, a més s'han calculat les masses nominals de Kendrick residuals (RNKM), les quals no depenen de la unitat de repetició. En la representació gràfica que es mostra a la Figura 2.8A es pot observar que els ions de les sèries observades convergeixen en diferents valors de RNKM (5, 21 i 44),

cosa que indica diferències entre les seves estructures químiques troncals i/o en el tipus d'adducte generat. La diferència en la massa exacta mitjana mesurada entre els diferents ions homòlegs ha permès concloure que es tractaven d'adductes de diferent naturalesa. Així, la diferència entre les masses exactes experimentals de la primera i la segona sèrie d'ions concorden amb la teòrica entre adductes de sodi i potassi (15.9772 Da), mentre que la comparació entre les masses dels ions de la segona i tercera sèrie ha permès assignar a aquests últims com adductes d'amoni. Les identificacions dels adductes ha permès concloure que a la mostra només hi era present un sol polímer de composició elemental ($C_{10}H_{22}O(EO)_n$). Com es pot observar a la Figura 2.8B, en sostraure la contribució en la massa de l'ió que s'ha adductat (Na^+, K^+, NH_4^+) tots els ions de les diferents sèries coincideixen amb els mateixos valors de KMD i RNKM.

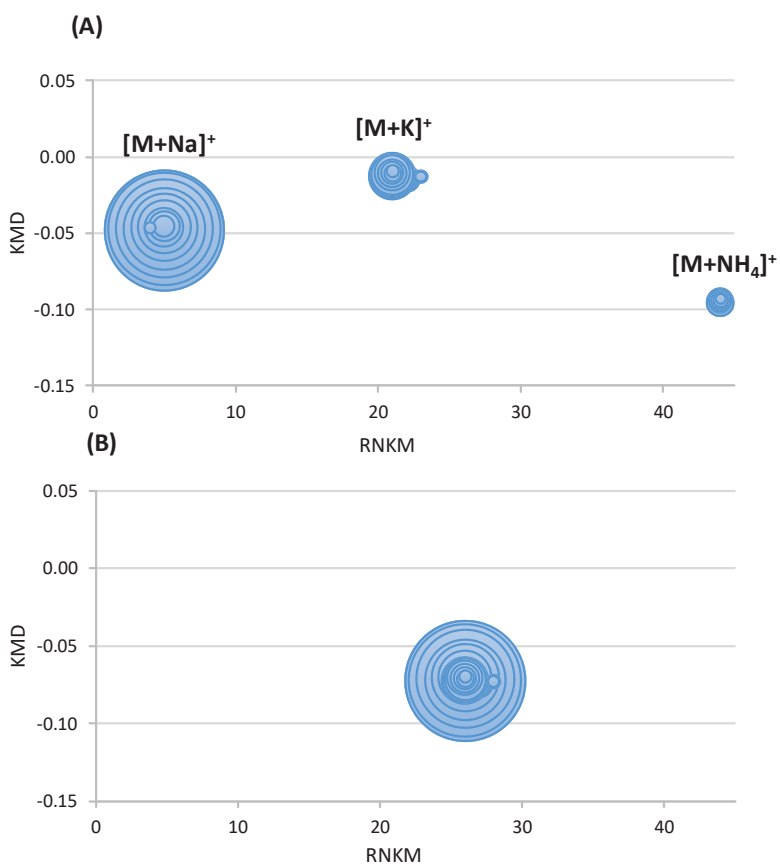

Figura 2.8. Representació gràfica basada en l'anàlisi del defecte de massa de Kendrick dels ions corresponents a les sèries polimèriques detectades en l'espectre de masses obtingut en l'anàlisi del producte fitosanitari per DESI-HRMS: (A) gràfic KMD *vs* RNKM, (B) gràfic KMD *vs* RNKM (calculats sense considerar la massa de l'ió que s'ha adductat).

D'altra banda, l'anàlisi de les dades emprant el defecte de massa de Kendrick també ha posat de manifest la presència d'ions que no presenten cap distribució polimèrica (Figura 2 A i B, *Publicació IV*, apartat 2.2.2), identificats com distribucions isotòpiques característiques de compostos organometàl·lics en l'espectre de masses DESI-HRMS. Comparant els perfils isotòpics detectats amb els teòrics s'han pogut confirmar que es tractaven d'ions organoestànnics (Figura 3, apartat 2.2.2). A més, l'estudi dels clústers isotòpics d'aquests ions organoestànnics ha permès determinar el nombre d'àtoms d'estany en la composició elemental d'aquests ions. A mode d'exemple, la Figura 2.9 mostra les distribucions isotòpiques dels ions detectats en el mode positiu a *m/z* 351.0188 i *m/z* 717.0411, que coincideixen amb els perfil teòrics d'ions organoestànnics característics d'un i dos àtoms d'estany, respectivament, mentre que la distribució isotòpica de l'ió a *m/z* 1083.0639 coincideix amb la simulada per a 3 àtoms d'estany.

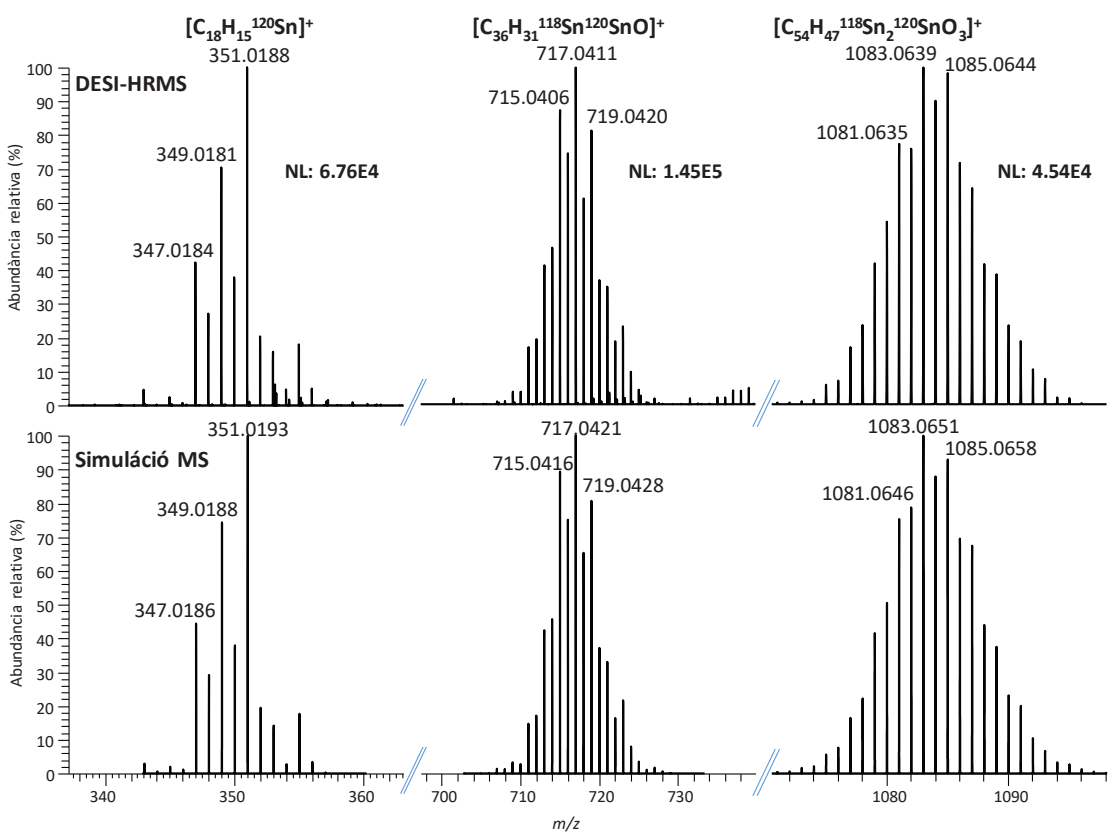

Figura 2.9. Distribucions isotòpiques dels ions organoestànnics a *m/z* 351.0188, *m/z* 717.0411 i *m/z* 1083.0639 observats en l'espectre de masses DESI-HRMS en mode positiu (a dalt) i els seus respectius clústers isotòpics simulats (a baix).

La informació obtinguda dels perfils isotòpics, combinada amb les mesures de massa exacta, ha facilitat l'assignació de les composicions elementals dels diferents ions organoestànics disminuint, així, el nombre de possibles candidats. Tanmateix, per a la caracterització d'aquests ions s'ha obtingut informació estructural realitzant experiments de tàndem DESI-MS/HRMS en el mode positiu. Amb l'objectiu de mantenir la informació de les distribucions isotòpiques en els espectres d'ions producte, en aquesta tesi s'han seleccionat en el quadrupol finestres d'aïllament pels ions precursors suficientment grans com per permetre abraçar tots els isòtops (de *m/z* 12 per als ions precursors amb tres i dos àtoms d'estany i de *m/z* 5 per als ions precursors amb un àtom de Sn) del clúster isotòpic. En tots els espectres de masses d'ions producte de tots els ions que contenen estany (Figura 4A, *Publicació IV*, apartat 2.2.2) es va observar la presència d'un ió producte comú amb massa nominal 351. En l'espectre de masses DESI-MS/HRMS obtingut en fragmentar aquest ió es van observar els ions producte a *m/z* 196.9408 i *m/z* 119.9017. Les diferències entre l'ió precursor (*m/z* 351.0187) i l'ió producte (*m/z* 196.9408) (*Δm/z* 154.0779) així com la diferència entre ambdós ions producte (*Δm/z* 77.0391) es van poder assignar, respectivament, a les pèrdues de dos i d'un grup fenil, cosa que ens va permetre suggerir la presència d'un compost de trifenilestany (Ph_3SnR) en el producte fitosanitari. De fet, els compostos de trifenilestany encara que van ser extensament utilitzats com a fungicides agrícoles per les seves propietats biocides, actualment el seu ús es troba restringit per la Comissió Europea donada la seva elevada toxicitat en el medi aquàtic i l'alt potencial de bioacumulació i persistència en els sediments (EU directive, 2009). Un dels possibles compostos a considerar és l'hidròxid de trifenilestany, atès que l'espectre de masses de tàndem de l'ió precursor a *m/z* 391.0115 ha permès caracteritzar aquest ió com $[Ph_3SnOH+Na]^+$. A més, la baixa energia de col·lisió necessària per fragmentar els ions a *m/z* 717.0405 i *m/z* 1083.0635 observat a l'espectre de *full scan,* així com la detecció d'ions producte comuns en ambdós espectres de masses de tàndem, suggereix que aquests ions podrien correspondre a la formació d'agregats de l'hidròxid, $[(Ph_3Sn)_2OH]^+$ i $[(Ph_3Sn)_3(OH)_2]^+$, respectivament. Tanmateix, l'òxid de bis(trifenilestany) també podria considerar-se un dels possibles candidats, ja que l'ió a *m/z* 717.0405 també podria correspondre a la molècula protonada d'aquest compost $[Ph_3SnOSnPh_3+H]^+$ i l'ió a *m/z* 1083.0635 podria atribuir-se al complex entre l'ió $[Ph_3SnOSnPh_3+H]^+$ i una molècula de Ph_3SnOH. D'altra banda, és important comentar la presència de l'ió a *m/z* 1109.0434 en l'espectre de *full-scan* del producte finosanitari (Figura 3A, *Publicació* IV, apartat 2.2.2). Aquest ió s'ha assignat

temptativament a un agregat $[(Ph_3Sn)_3OCO_2]^+$, que pot provenir de la interacció entre els ions $[Ph_3Sn]^+$ i una molècula de CO_2 de l'atmosfera. Aquest ió no s'ha observat emprant la font ESI convencional, en la qual l'ionització te lloc en un ambient més tancat. En canvi, aquest ió sí que s'ha detectat en analitzar la mostra amb la tècnica *paper spray* que, com la font DESI, opera en un entorn molt més obert. Per últim, atès que en l'espectre de masses DESI-HRMS en mode negatiu es detecten ions organoestànnics que contenen clorurs en la seva composició elemental, com ara l'ió a *m/z* 430.9852 $[Ph_3SnCl_2]^-$, també s'ha considerat la possible presència de clorur de trifenilestany a la mostra com un altre potencial candidat. L'anàlisi per DESI-HRMS(/MS) dels patrons dels compostos sospitosos (clorur de trifenilestany, hidròxid de trifenilestany i òxid de bis(trifenilestany) va permetre descartar el clorur de trifenilestany com a candidat, donat que es van observar diferències significatives en els espectres de masses d'ions producte del patró i de la mostra en fragmentar l'ió a *m/z* 1109.0445 (Figura S4, *Publicació IV*, Informació suplementària). Per tant, el producte fitosanitari podria ser una mescla de l'hidròxid de trifenilestany i l'òxid de bis(trifenilestany), que es poden trobar en equilibri en la mostra atès que el primer es pot obtenir per hidratació del segon.

La identificació i caracterització dels compostos del producte fitosanitari duta a terme en aquesta tesi per DESI-HRMS ha possibilitat la posterior quantificació de trifenilestany en la mostra per UHPLC-HRMS i ICP-OES emprant un tractament de la mostra adequat. Finalment, cal esmentar que la concentració a la que es va detectar la presència d'aquest compost en la mostra va ser de 120 vegades superior al nivell màxim legislat (<0.1% Sn) (EU directive, 2009).

CAPÍTOL 3

Utilització de tècniques *Ambient MS* basades en substrats mostrejadors com a sondes generadores de l'esprai

3.1. INTRODUCCIÓ

En els darrers anys, el nombre de noves tècniques *Ambient MS* ha anat augmentat exponencialment i, avui dia, ja se'n descriuen més de 80. Algunes de les raons d'aquest increment són la simplicitat en el disseny i el baix cost que suposa la implementació d'aquestes tècniques en els laboratoris analítics. A més, una característica interesant és que, en molts casos, és fàcil adequar/modificar el mètode *Ambient MS* a l'estratègia de mostreig, a les propietats dels compostos que es pretenen detectar o al tipus de matriu que s'analitza.

Com es comenta en el Capítol 1 d'introducció d'aquesta tesi, en les estratègies de mostreig per a l'anàlisi emprant metodologies *Ambient MS* sovint s'utilitzen estris com ara agulles, hisops, escuradents, capil·lars de vidre o papers cromatogràfics. Aquests estris permeten, per exemple: analitzar mostres que tenen una mida tant gran que excedeix l'espai disponible del sistema instrumental per a dur a terme l'anàlisi directa de la superfície, assegurar la representativitat del resultat mitjançant el mostreig de diferents punts d'una superfície emprant el mateix estri o permetre la presa de mostres biològiques, com ara teixit humà o saliva, entre d'altres. El mostreig amb aquests estris s'ha emprat en el desenvolupament de metodologies basades en les tècniques DESI, DART, DAPCI o DBDI o LTP, mitjançant les quals es realitza l'anàlisi de superfície de la mostra dipositada/adsorbida sobre l'estri (Hagenhoff et al., 2017; Hall et al., 2017; Kern et al., 2018a; Khatami et al., 2017). Tanmateix, aquest tipus d'estris també s'han utilitzat en el desenvolupament de noves tècniques *Ambient MS* on el propi estri s'utilitza com a sonda d'electroesprai. La *probe electrospray ionizaton* (PESI), la *touch spray* (TS) o el *paper spray* (PS) són algunes de les tècniques *Ambient MS* basades en la ionització per electroesprai emprant sondes, normalment amb forma punxeguda, que s'utilitzen tant en l'etapa de mostreig com en la d'ionització, ja que la ploma de l'electroesprai es genera directament a la punta de la sonda en aplicar-hi un volum determinat de dissolvent i un elevat potencial. Aquestes tècniques presenten un disseny significativament més senzill que la DESI o la DART, ja que no és necessari l'ús de gasos durant el procés d'ionització i, fins i tot en alguns casos, no es requereix l'ús de dissolvents, com és el cas de la tècnica PESI, on únicament cal introduir la punta d'una agulla metàl·lica on s'ha dipositat la mostra humida o líquida en la font d'ionització. En l'anàlisi de les mostres líquides per PESI, el mostreig es realitza de forma automatitzada, introduint l'agulla en el recipient que conté la mostra. En canvi, en la tècnica TS la presa de mostra (matrius sòlides o líquides) es realitza *offline,* fregant o punxant la mostra amb l'agulla per tal que hi quedi adherida, cosa que permet realitzar el mostreig en el

mateix lloc on es troba originalment la mostra. Aquesta agulla pot ser emmagatzemada per a la seva posterior anàlisi al laboratori, on simplement se li aplica un volum de dissolvent petit (< 100 µL) i un elevat potencial per tal de generar la ploma de l'electroesprai a la punta (Alfaro et al., 2016; Kerian et al., 2014). Aquestes agulles també poden substituir-se per altres tipus de sondes, com ara els hisops mèdics, que són els més adequats per a l'anàlisi de matrius biològiques. Els hisops mèdics s'utilitzen habitualment en el camp de l'anàlisi clínica, sobretot per dur a terme proves de cultius per a la detecció d'infeccions bacterianes. A més, es troben disponibles comercialment com a dispositius mèdics de la classe IIA (poden estar en contacte amb teixit humà durant un màxim de 60 minuts) i la presa de mostra és mínimament invasiva. Tot i els avantatges que presenten aquestes tècniques *Ambient MS* basades en sondes, cal apuntar que és difícil conèixer exactament la quantitat de mostra que queda dipositada (adsorbida) en el dispositiu. En canvi, en la tècnica PS, on la punta del paper emprat com a substrat actua com a sonda de l'electroepsrai, el volum de mostra dipositat en el paper és perfectament conegut i, a més, és possible l'addició d'una quantitat coneguda de patró intern a la mostra líquida o a l'extracte d'una mostra sòlida, característiques que fan que aquesta tècnica s'hagi emprat per al desenvolupament de mètodes quantitatius (Domingos et al., 2017b; Jeong et al., 2016; Kennedy et al., 2018; Teunissen et al., 2017).

Com s'ha posat de manifest en els comentaris anteriors, les tècniques *Ambient MS* que empren sondes/substrats normalment utilitzen l'electroesprai per a la ionització. En aquests casos, la formació d'ions es produeix en la fase líquida, normalment emprant dissolvents hidro-orgànics, la qual cosa fa que resultin poc adequats per a la ionització de compostos de baixa polaritat o que no presentin característiques àcid-base que afavoreixin l'intercanvi protònic. Tanmateix, en algunes d'aquestes tècniques és factible l'anàlisi de compostos de baixa polaritat. Així, per exemple, en el cas concret de la tècnica PS, si bé el mecanisme d'ionització més habitual és l'electroesprai, alguns autors han demostrat que és possible la ionització de determinats compostos que presenten dificultats per ionitzar-se mitjançant aquest mecanisme si s'utilitzen dissolvents apolars, com ara el toluè, el diclorometà o l'hexà, entre d'altres (Li et al., 2011). L'aplicació d'un potencial elevat en presència de dissolvents apròtics, que impossibiliten l'intercanvi protònic, permet la generació d'ions en fase gas, possiblement per un mecanisme de desorció/ionització per camp o d'ionització química mitjançant la formació d'una descàrrega de corona a la punta del paper emprat com a substrat. En aquest context, la simplicitat en el disseny del sistema de PS permet que la seva

configuració original es pugui modificar fàcilment al laboratori per tal d'adequar el mecanisme d'ionització als compostos d'interès o, fins i tot, combinar el mecanisme d'electroesprai amb altres mecanismes d'ionització que tenen lloc en la fase gas, per tal de detectar compostos d'un rang de polaritats més ampli. Per exemple, alguns autors proposen incorporar una agulla connectada a un alt potencial sobre el paper triangular per tal d'augmentar i focalitzar el camp elèctric entre la punta del paper i l'entrada a l'espectròmetre de masses i afavorir, així, la ionització/desorció per camp, en comptes del mecanisme d'electroesprai (Aronco et al., 2016).

Com s'ha posat de manifest a la Introducció d'aquesta tesi, ja des de l'inici de les tècniques *Ambient MS* s'han utilitzat tant l'electroesprai (ESI) com la ionització química a pressió atmosfèrica (APCI) per a la ionització. La fotoionització a pressió atmosfèrica (APPI), tècnica alternativa a l'APCI que resulta molt eficient per a la detecció de compostos de molt baixa polaritat, també s'ha utilitzat en les tècniques *Ambient MS* (Kauppila et al., 2017). De forma similar a la font APPI convencional emprada en les metodologies LC-MS, en les tècniques *Ambient MS* basades en APPI, com ara la *desorption atmospheric pressure photoionization* (DAPPI) o la *laser-ablation atmospheric pressure photionization* (LAAPPI), s'utilitza una làmpada d'ultraviolat (UV) de Criptó que emet fotons de 10-10.6 eV d'energia, els quals desencadenen una sèrie de reaccions en la fase gas que possibiliten la ionització dels compostos d'interès. Encara que l'etapa d'ionització és similar, aquestes dues tècniques es diferencien en el procés pel qual les molècules neutres de la superfície de la mostra són transferides a la fase gas. En la tècnica DAPPI, el dissolvent escalfat i nebulitzat amb assistència pneumàtica (gas) es fa impactar sobre la superfície de la mostra per desorbir tèrmicament els compostos, mentre que en la tècnica LAAPPI és un làser infraroig, situat ortogonalment a la làmpada UV, el responsable de la desorció dels compostos de la superfície per ablació. Ara bé, la fotoionització directa dels compostos a la fase gas és poc eficient a causa, en part, de la pèrdua d'energia dels fotons, que resulten atenuats per interacció amb els gasos presents a pressió atmosfèrica. Per tal d'augmentar l'eficàcia d'ionització s'utilitzen dissolvents (dopant, D) que presenten un potencial d'ionització inferior a l'energia dels fotons emesos per la làmpada de Criptó i, per tant, són fàcilment fotoionitzables. Aquests dissolvents-dopants, introduïts en forma d'esprai de gotes neutres, generen cations radicals ($D^{+\bullet}$) en interaccionar amb els fotons i alliberen electrons, desencadenant una cascada de reaccions en fase gas que fan possible la ionització dels anàlits d'interès a través de reaccions de transferència protònica ($[M+H]^+$, $[M-H]^-$) o de càrrega

($[M]^{+\bullet}$, $[M]^{-\bullet}$). Tot i els avantatges que presenta l'APPI, cal fer esment que no s'han trobat treballs que facin referència a tècniques *Ambient MS* que utilitzen sondes/substrats combinades amb l'APPI, possibilitat que podria ampliar l'àmbit d'aplicació d'aquestes tècniques.

En aquest capítol de la tesi s'avalua l'aplicabilitat de dues tècniques *Ambient MS* basades en sondes/substrats per resoldre dos problemes analítics concrets: d'una banda l'adequació de la presa de mostra al tipus de matriu i al problema analític i, de l'altra, implementar un mecanisme d'ionització adequat als compostos que es pretenen detectar. En primer lloc, s'estudia l'ús d'hisops comercials per al desenvolupament d'un mètode TS-MS que possibiliti la presa de mostra *in vivo* de teixit cerebral i la seva posterior anàlisi *Ambient MS offline* emprant el mateix hisop. La familiarització del personal mèdic amb l'ús dels hisops comercials així com la possibilitat que ofereixen aquests estris per dur a terme mostrejos mínimament invasius de les mostres, podrien facilitar la seva implementació en els protocols mèdics per al mostreig *in vivo* de teixit humà en les intervencions quirúrgiques. A més, el fet que el protocol de mostreig sigui independent del procés de desorció/ionització/detecció evita que l'espectròmetre de masses hagi d'estar instal·lat en el mateix quiròfan o molt a prop, com succeeix en els mètodes en què s'empren tècniques de mostreig/ionització/detecció en línia, com ara la REIMS (*Rapid Evaporative Ionization Mass Spectrometry)*. Aquest estudi forma part del treball experimental dut a terme al grup *Aston Labs,* sota la supervisió del Dr. Robert Graham Cooks, en el període d'una estada formativa de 5 mesos a la Universitat de Purdue (Indiana, EUA). El treball s'inclou en una de les línies de recerca que se segueixen en aquest grup d'investigació i en la qual ja s'havien obtingut resultats satisfactoris al laboratori emprant la tècnica DESI-MS per al diagnòstic de gliomes en teixits cerebrals. Durant el període de l'estada s'estava valorant la possibilitat d'implementar un mètode DESI-MS, en una sala contigua al quiròfan, per a l'anàlisi *offline* de teixit humà extirpat durant les intervencions quirúrgiques de resecció de tumors, a l'hospital universitari d'Indianapolis Health Methodist (Pirro et al., 2017b). Atès que mitjançant el mètode DESI-MS només era possible obtenir informació del teixit extirpat, en aquest capítol s'avalua la possibilitat d'utilitzar hisops per dur a terme el mostreig del teixit viu i la generació d'ions per TS-MS.

En segon lloc, en aquesta tesi s'ha considerat d'interès estudiar la possibilitat d'emprar tècniques *Ambient MS* per a l'anàlisi de compostos de baixa polaritat, camp d'interès en el nostre grup de treball. Atès que l'objectiu era desenvolupar un mètode ràpid per a la

determinació d'aquests tipus de compostos en matrius líquides, s'ha optat per emprar la tècnica PS, que en permet la quantificació. A més, a fi i efecte de potenciar la sensibilitat del mètode, s'ha combinat, per primera vegada, l'APPI per a la ionització amb el PS. Amb aquest objectiu s'ha dissenyat i efectuat el muntatge de la font i s'han optimitzat les condicions de treball. En concret, la combinació del PS amb l'APPI s'ha avaluat emprant com a model de compostos poc polars els fluorotelòmer alcohols (FTOHs), les fluorooctanosulfonamides (FOSAs) i els fluorooctanosulfonamido-etanols (FOSEs). Aquests compostos, pertanyents a la família de les substàncies per- i poli-fluoroalquilades (PFAS), presenten dificultats per ionitzar-se mitjançant el mecanisme d'electroesprai. Com sigui que avui dia hi ha molt poca informació sobre l'ús de PFASs en productes de consum, el desenvolupament de mètodes ràpids que permetin la seva monitorització i determinació pot resultar de gran interès pels laboratoris de control. Així, en aquest treball s'ha desenvolupat un mètode basat en PS-APPI utilitzant un analitzador de masses d'alta resolució Q-Orbitrap per aconseguir la determinació ràpida de FTOHs, FOSEs i FOSAs.

3.2. TREBALL EXPERIMENTAL

En aquest apartat s'inclou la *Publicació V* intitulada *"Analysis of human gliomes by swab touch spray-mass spectrometry: application to intraoperative assessment of surgical margins and presence of oncometabolites"*, en què s'avalua l'ús d'hisops mèdics per al desenvolupament d'un mètode que permet el mostreig *in vivo* de teixit cerebral i el seu anàlisi *offline* per TS-MS. En aquest estudi s'estableixen les condicions òptimes de treball i s'avalua la viabilitat de la tècnica TS-MS per a la detecció de perfils fosfolipídics discriminants i d'oncometabòlits en teixits cerebrals humans amb l'objectiu de proposar un mètode que, en un futur, pugui ser considerat una alternativa a les proves patològiques que es realitzen actualment en les intervencions quirúrgiques d'extirpació de tumors cancerígens.

D'altra banda, en aquest apartat també s'inclou el treball experimental en què es combina el PS amb l'APPI per tal d'ampliar el rang de compostos a analitzar amb aquesta tècnica. En el treball experimental, que es recull en la *Publicació VI* "*Paper Spray-Atmospheric Pressure Photoionization-High Resolution Mass Spectrometry for the Direct Analysis of Neutral Fluorinated Compounds in Waterproof Impregnation Sprays"*, s'incorpora una làmpada de Criptó en el disseny original del PS a fi i efecte de fer possible l'anàlisi de compostos de baixa polaritat. En concret, es pretén proposar un mètode que utilitzi el PS-APPI per a la determinació de compostos neutres per- i polifluoroalquilats (PFAS). En aquest treball s'estudien els paràmetres més crítics de la incorporació de l'APPI a la tècnica PS, es compara l'eficàcia d'ionització d'ambdues tècniques, PS i PS-APPI, per a la detecció dels anàlits d'interès i s'avalua l'aplicabilitat del mètode PS-APPI-HRMS proposat per a la determinació de PFASs en productes d'impregnació resistents a l'aigua.

3.2.1 PUBLICACIÓ V

Analysis of human gliomas by swab touch spray-mass spectrometry: application to intraoperative assessment of surgical margins and presence of oncometabolites

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Analyst, 142 (**2017**) 4058-4066

Analyst

PAPER

View Article Online
View Journal | View Issue

Cite this: *Analyst*, 2017, **142**, 4058

Analysis of human gliomas by swab touch spray-mass spectrometry: applications to intraoperative assessment of surgical margins and presence of oncometabolites†

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Touch spray mass spectrometry using medical swabs is an ambient ionization technique (ionization of unprocessed sample in the open air) that has potential intraoperative application in quickly identifying the disease state of tissue and in better characterizing the resection margin. To explore this potential, we studied 29 human brain tumor specimens and obtained evidence that this technique can provide diagnostic molecular information that is relevant to brain cancer. Touch spray using medical swabs involves the physical sampling of tissue using a medical swab on a spatial scale of a few mm^2 with subsequent ionization occurring directly from the swab tip upon addition of solvent and application of a high voltage. Using a tertiary mixture of acetonitrile, *N*,*N*-dimethylformamide, and ethanol, membrane-derived phospholipids and oncometabolites are extracted from the tissue, incorporated into the sprayed microdroplets, vacuumed into the mass spectrometer, and characterized in the resulting mass spectra. The tumor cell load was assessed from the complex phospholipid pattern in the mass spectra and also separately by measurement of *N*-acetylaspartate. Mutation status of the isocitrate dehydrogenase gene was determined *via* detection of the oncometabolite 2-hydroxyglutarate. The lack of sample pretreatment makes touch spray mass spectrometry using medical swabs a feasible intraoperative strategy for rapid surgical assessment.

Received 10th August 2017,
Accepted 27th September 2017

DOI: 10.1039/c7an01334e

rsc.li/analyst

Introduction

We describe the rapid analysis of neurological tissue by touch spray mass spectrometry with medical swabs (*i.e.*, swab TS-MS). Swab TS-MS is envisioned as a tool for molecular diagnosis of gliomas in which tissue is sampled *in vivo* along the surface of the resection cavity, and then analyzed intraoperatively but *ex vivo* to provide rapid feedback on the pathological state of the tissue and to guide surgical maneuvers for maximal tumor excision. Added diagnostic information for surgical margin assessment is provided by these chemical measurements to complement standard intraoperative histopathology. Intraoperative histopathology, as currently performed during neurosurgical tumor resection, identifies tumor type and grade from tumor core tissue, but it is not used for assessment of surgical margins. Surgical margins are defined based on the surgeon's experience, visual and tactile observation of the tissue during surgery, and neuronavigation according to preoperative MRI.[1–4] No intraoperative molecular measurement indicative of tissue pathology is currently made to assist in surgical decision-making.

Here we present the swab TS-MS methodology for analysis of neurological tissue and provide proof-of-concept data of its diagnostic utility from 29 human brain specimens selected for their known histopathology. Swab TS-MS is an ambient ionization method in which a minute amount of sample (*e.g.*, tissue) is transferred to a swab tip by a gentle touch, and subsequently ionized with the application of solvent to the swab tip and a high voltage directly to the swab shaft.[5,6] Sampling is minimally invasive, analysis is straightforward and requires no other sample handling or pretreatment, making it highly appropriate for clinical testing in hospitals.[7] Swabs are used both as sampling devices and as electrospray emitters for MS

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†Electronic supplementary information (ESI) available: Tables S1 and S2. Fig. S1–S7. Video S1. (PDF). See DOI: 10.1039/c7an01334e

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analysis. Rapid evaporative ionization mass spectrometry (REIMS) combines tissue sampling and ionization in an online approach to provide quasi real-time feedback.[8] In REIMS, a modified monopolar cutting electrode is used for both tissue removal and for collection of the surgical smoke produced from electrocauterization.[8] We envision swabs as a tool to maximize analysis throughput while removing the need for positioning the MS instrument close to the surgical field or requiring a transfer line to interface the ionization source to the mass spectrometer. We consider an offline approach, where tissue sampling is performed remotely from the mass spectrometer, to best suit neurosurgery needs while limiting deviations from current surgical procedures.

Swab touch spray is based on the ambient ionization method of paper spray mass spectrometry[9] which has proven successful in biofluids analysis. Applications of swab TS-MS that have been presented so far include the detection of microbial lipids from culture[5] and from human skin, and illicit drug detection in oral fluid.[6] The development of swab TS-MS follows that of TS and probe electrospray ionization (PESI), two methods that use metallic teasing probes for tissue sampling. TS in this previous form has been investigated for prostate and kidney cancer detection[10–12] while PESI has been tested for detection of renal cell carcinoma and metabolite profiling in mouse brain.[13,14] Differently from metal probe TS and PESI, swab TS utilizes swabs that are already commercialized as probes for *in vivo* sampling of deep surgical wounds, body orifices and surfaces, facilitating the translation of this tool into surgical application. Electrospray ionization occurs readily from the porous material of the swab tip, similarly to paper spray[15,16] and biocompatible solid phase micro-extraction fibers.[17,18]

Three items of information were sought from the mass spectra obtained using swab TS-MS: (i) tissue type was assessed by monitoring the expression of complex phospholipids; (ii) tumor infiltration was measured as percentage of tumor cells (TCP; relative percentage of tumor cells compared with parenchyma) by monitoring the abundance of *N*-acetylaspartate (NAA); and (iii) isocitrate dehydrogenase (*IDH*) mutation status was assessed by monitoring the presence of the oncometabolite 2-hydroxyglutarate (2HG). Previous studies using desorption electrospray ionization-mass spectrometry (DESI-MS) demonstrated that all three items of diagnostic information are achievable by electrospray-based ambient ionization MS[19–22] and that this technology can be used for intraoperative molecular pathology.[23–25] DESI-MS is emerging as a molecular diagnostic intraoperative tool for analysis *ex vivo* of biopsied tissue smears. Phospholipid profiles, as detected by DESI-MS, change dynamically with the density of the tumor and with the composition of the infiltrated brain parenchyma (*i.e.*, grey matter, white matter, or a mixture of both). NAA signal intensities in DESI mass spectra decrease proportionally with the degree of tumor infiltration, measured as TCP.[20,21,23] The oncometabolite 2HG accumulates in glioma tissue carrying *IDH* mutations.[26] Its presence has been determined using DESI-MS and the data compared well with clinical genetic tests carried out post-operatively.[22] The development of swab TS-MS, also an electrospray-based method, represents an additional step towards the use of intraoperative MS and it provides the neurosurgeon with a simple manual tool for *in vivo* tissue sampling and direct chemical evaluation of tissue pathology.

Experimental

Study protocol

Cryopreserved human neurological specimens were obtained from 29 patients through the Biorepository of the Methodist Research Institute (Purdue IRB #1410015344). The list of specimens analyzed in this study is reported in Table S1.† Tissue specimens were stored at −80 °C before analysis. For each specimen, a few tissue sections (15 μm thickness) were cut using a cryotome (Cryotome™ FSE Cryostats, Thermo Fisher Scientific, San Jose, CA) in order to obtain a flat open surface of the tissue. The last tissue section cut was H&E stained for blind pathological examination. The tissue sample was then allowed to thaw at room temperature, and the flat surface of the tissue, adjacent to the section that was stained, was touched with the swab (approximately an area of 6 mm^2) to perform the TS-MS experiments. Multiple touches were performed for most of the samples that showed macroscopically-heterogeneous areas, as detailed in Table S1.† For all the specimens used in this study, another tissue biopsy had been previously used for preparation of tissue sections and smears and analyzed by DESI-MS imaging. Results are reported elsewhere.[19,20] Mouse brain (Purdue IRB #1704001561) was used for the initial stages of method development.

Chemicals

Electrospray was generated using a mixture of acetonitrile–dimethylformamide–ethanol (ACN–DMF–EtOH) in a ratio of 45 : 5 : 50% v/v, doped with octyl β-D-glucopyranoside (non-ionic surfactant, ≥98% pure) and the internal standard NAA-d_3 (10 μg mL^{-1}). All solvents and standards were purchased from Sigma Aldrich (Minneapolis, MN).

Medical swabs

Sterile medical swabs were purchased from Copan Diagnostics Inc. (Murietta, CA). The swabs have an aluminum handle and rayon mini tip of fused shape and largest diameter of ~2.4 mm (Fig. S1†). The swabs are packaged in individual tubes for easy transport and storage. They are mounted in a plastic cap that serves as a convenient holder. Each tube and cap assembly is sealed with a tamperproof label for assurance of sterility and chain of custody. These swabs are commercialized for purposes other than ESI probes for MS analysis. They have been used with no modification from their commercial form.

Swab touch spray mass spectrometry

Swab touch spray experiments were performed by touching gently a region of interest of a sample and rotating the swab

on its shaft to transfer minute quantities of tissue on the swab tip. Each swab was weighed before and after the touch to measure tissue quantity transferred to the swab tip (tissue weight, Table S1†), and then submitted to MS analysis with no further treatment.

MS experiments were performed using a linear ion trap mass spectrometer (Finnigan LTQ, Thermo Fisher Scientific). The ion source was custom-built to allow positioning the swab vertically with respect to the mass spectrometer; a configuration similar to that used in commercial electrospray sources. The use of an extended MS inlet capillary, bent 90° upwards and held directly underneath the swab tip, improved stability of the swab electrospray despite ion transmission losses (Fig. S2†). The absence of carry-over using a bent inlet capillary was tested by spraying concentrated mouse brain extracts (1 mg mL^{-1} in ACN–DMF–EtOH 45–5–50% v/v) using a clean swab as probe substrate, in alternation with blank solvent. The swab tip was positioned 5–8 mm above the inlet. A precision motion control system was used to adjust the position of the swab whenever necessary. A silica capillary was used to deliver the solvent to the swab tip using an external syringe pump. The silica capillary was held in a fixed position in such a way that the end of the capillary directly touched the surface of the swab tip once positioned. Electrospray was generated using ACN–DMF–EtOH 45 : 5 : 50% v/v as solvent system. The solvent was doped with 250 ng mL^{-1} of octyl β-D-glucopyranoside to facilitate solvent flow on the probe, and 10 μg mL^{-1} of the internal standard NAA-d_3. Electrospray was initiated after addition of solvent directly on the swab tip *via* a fused silica capillary and external syringe pump. The syringe pump flow rate was set at 50 μL min^{-1} for about 30 s, accounting for dead volume and wetting the swab tip. When the swab tip was visibly wet, high voltage (−6.5 kV) was applied directly to the metallic handle. Solvent flow rate was changed to 25 μL min^{-1}. It was possible to generate an electrospray from all the swabs analyzed. In only a couple of instances fibers distend from the body of the swab tip during data acquisition compromising the stability of the electrospray; these swabs had to be disregarded and the experiments repeated.

Full scan mass spectra over the range *m*/*z* 700–1000 were acquired in negative ion mode first; a second acquisition over the mass range *m*/*z* 80–200 was performed in negative ion mode. After this, collision-induced dissociation MS/MS product ion scans were acquired to measure NAA (precursor ion *m*/*z* 174 $[M - H]^-$) and NAA-d_3 (precursor ion *m*/*z* 177 $[M - H]^-$), followed by MS^3 sequential product ion scans of 2HG (precursor ion *m*/*z* 147 $[M - H]^-$). Total acquisition time was 1.2 min; approximately 15 seconds were acquired for each mode so that spray and signal stability could be evaluated. MS^n spectra other than for NAA and 2HG were acquired separately when additional structural information was needed for compound identification. The automatic gain control was always activated to adjust for variable ion flux. The MS instrumental settings are reported in Table S2.† Signal-to-noise ratios were calculated using ion intensity at maximum peak height and signal intensity at peak onset.

Microscopic videography

For each swab tested, videos of the spray plume were recorded to observe the electrospray behavior. A Watec WAT-704R camera was used to acquire the videos; the software Cyberlink PowerDirector v.14 (http://www.cyberlink.com) was used to record them; Adobe Premier Pro CC (http://www.adobe.com) was used for video editing. The spray plume was illuminated with a red laser pointer as shown in Fig. S2.†

Data analysis and chemical diagnosis

Data were exported from XCalibur 2.0 (Thermo Fisher Scientific) and imported into MATLAB (The Matworks Inc., Natick MA) for elaboration. Full scan mass spectra were used to provide molecular diagnosis of the tissue *via* comparison with a reference spectral library acquired by DESI-MS. Spectral profiles were used comprehensively to classify the tissue as either glioma, white matter, or grey matter. The methodology is based on multivariate pattern recognition and is described extensively elsewhere.[20,21] The abundance of NAA, detected using tandem MS (*m*/*z* 174 → 114) and normalized to the intensity of the internal standard NAA-d_3 (*m*/*z* 177 → 116), was plotted in relation to tumor infiltration (low, medium, and high as evaluated by histopathology). Box-plots were created using MATLAB. The Kruskal–Wallis non-parametric test was used to compare population medians. *P* values <0.05 were considered significant. The signal intensity of 2HG, detected using MS^3 (*m*/*z* 147 → 129 → 101) and normalized to the intensity of the internal standard NAA-d_3 (*m*/*z* 177 → 116), was plotted against the *IDH* mutation status (wild-type *vs.* *IDH* mutant) to monitor differential distribution. The receiver operating characteristic (ROC) curve analysis was used to determine the ability of 2HG measurement to assess *IDH* mutation of gliomas. The predicting ability was considered strong when the area under the curve (AUC) exceeded 0.8 (80%). Receiver operating characteristic (ROC) curve analysis and Kruskal–Wallis test were performed using SPSS v.22 (SPSS Inc. IBM Corp, Chicago, IL).

Adobe Photoshop (Adobe Systems Inc., San Jose, CA) was used to produce publication-quality figures.

H&E staining

Tissue staining was performed as described elsewhere.[20]

Histopathology

An expert neuropathologist (E.M.H.) identified regions of interest as glioma (G) or infiltrated tissue (IT), further specifying grey matter (GM), white matter (WM), a mixture of both, or infiltrated tissue not otherwise specified (NOS). Estimation of tumor cell percentage was roughly provided in the categories of low (<33%), medium (34–67%), or high (>67%) simply by visual observation. Immunohistochemistry for assessment of *IDH* mutation was performed on separate tissue biopsies than those analyzed by swab TS-MS. Analysis was conducted from an independent pathology laboratory. Results were provided from the Biorepository of the Methodist Research Institute as dichotomous answer (immunoreactive *vs.* non-immunoreactive, Purdue IRB #1410015344).

Results

Instrumental set-up and analytical considerations

Swab TS-MS was performed in such a way as to mimic one foreseeable implementation: collection of tissue *in vivo* along the surface of the resection cavity, followed by placement of the swab probe in front of a mass spectrometer located in the operating room and direct MS analysis (Fig. 1). Analysis required a few minutes with the current methodology; this time was equally divided between swab positioning onto a custom-built source and MS data acquisition, while tissue sampling was immediate. MS data acquisition was prolonged for over a minute to test electrospray and signal stability but could be shortened to a few seconds for each mode of data acquisition, since the entire tissue sampled on the swab is interrogated by the constantly-flowing solvent, leading to a chemical profile that is stable over time and represents the average signature arising from the heterogeneous morphological features sampled. The analysis can be multiplexed easily, as done in this study, to specifically monitor particular biomarkers and oncometabolites. In this study, cryopreserved tissue biopsies served as a proxy for *in vivo* sampling. Swab TS-MS incorporates a manual user-guided method of collecting minute amounts of tissue with direct MS analysis from the sampling device. A current drawback of this approach is the lack of control over the quantity of tissue transferred on the swab tip by touch. The average quantity for the specimens analyzed in this study was 3.1 mg but acceptable signal-to-noise ratios (>3) for the diagnostic peaks in the mass spectra were obtained for as little as 1.0 mg. Tissue quantities are minimal and smaller than those of a typical tissue biopsy resected for pathological examination. The minute amount of sample collected emphasizes the value of MS methods in which ionization is generated directly from the sampling device; *viz.*, no sample loss and higher signal intensity resulting from minimal solvent consumption (flow rate ~25 μL per minute). Table S1† gives the signal-to-noise ratios calculated on the most intense peak of the lipid profile mass spectra. As already observed using DESI-MS,[21,28] the absolute signal intensity changed with the quantity of tissue sampled, as well as with its composition and cellularity. High-grade and high-density tumor tissue provided lower absolute signal compared to low-infiltrated tissue, partly due to the presence of calcified, hemorrhagic, and necrotic tissue. Note that an internal standard added to the extraction solvent normalizes variations of absolute ion counts due to swab positioning and instability of the electrospray, but cannot correct for matrix differences. Importantly, the relative spectral profiles (*i.e.*, relative intensity of ions within a scan) used as a diagnostic fingerprint of the tissue were grossly uninfluenced by global intensity variations which allowed us to obtain accurate diagnostic information from the mass spectra despite the lack of control over the absolute quantity of tissue analyzed. We monitored the spectral profiles repeatedly over a total period of 10 minutes in both low- and high-glioma infiltrated samples and changes in their respective spectral profiles were minimal (relative standard deviations <15% for diagnostic ions, Fig. S3†). The absolute signal decreased monotonically over time, which is typical of a continuous extraction process (Fig. S4†).

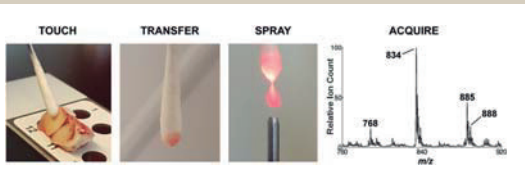

Fig. 1 Swab TS-MS experiment. (Left to right) Tissue is touched with the swab tip and transferred by rotating the swab on its shaft. The swab is positioned in front of a mass spectrometer and an electrospray is generated directly from the swab tip upon application of solvent and a high voltage, which allows mass spectra to be recorded.

Different extraction solvents (methanol, ACN, DMF, EtOH, dichloromethane, and acetone) were tested during method development based on previous experience with DESI-MS.[27] The system empirically chosen for these analyses (ACN–DMF–EtOH, 45 : 5 : 50 v/v%) is the result of an optimization process meant to select a mixture of organic solvents that would provide a chemical fingerprint of the tissue similar to that obtained using DESI-MS (which uses ACN–DMF 50 : 50 v/v% as solvent system) but one that would also generate an electrospray from the swab tip. Video S1† shows the generation of the electrospray plume from the swab and its remarkable stability. The caption describes briefly the swab electrospray process (ESI, Video S1†). The use of the original ACN–DMF DESI solvent was not acceptable because of poor spray behavior observed in the negative ionization mode for swab TS-MS. The addition of ethanol and a non-ionic surfactant to the solvent system significantly decreases the surface tension of the solvent mixture, thereby facilitating electrospray plume formation from the swab tip and improving signal stability.

Assessment of glioma presence and estimation of tumor infiltration

The lipid profiles detected by swab TS-MS resembled those detected by DESI-MS.[20] They indicated the presence of tumor when present and could distinguish the type of normal tissue into which the tumor infiltrated (*i.e.*, grey matter or white matter); an information not always assessable by histopathology as tumors efface the morphology of normal brain parenchyma. The main MS feature characterizing grey matter is *m*/*z* 834.5, the deprotonated ion of phosphatidylserine 40 : 6 (Fig. 2A). The main MS features characteristic of white matter are *m*/*z* 888.6 and 904.5, deprotonated ions for (3′-sulfo)GalCer 24 : 1 and (3′-sulfo)GalCer 24 : 1(OH) (Fig. 2B). For gliomas, the characteristic ions are *m*/*z* 768.4, the chlorinated adduct of phosphatidylcholine 32 : 0, *m*/*z* 794.5, chlorinated adduct of phosphatidylcholine 34 : 1, and *m*/*z* 885.5, the deprotonated phosphatidylinositol 38 : 4 (Fig. 2C). Fig. S5† shows spectra for these categories of samples acquired by DESI-MS for comparison. The similarity between the lipid profiles acquired by DESI-MS and swab TS-MS for the same subjects (from

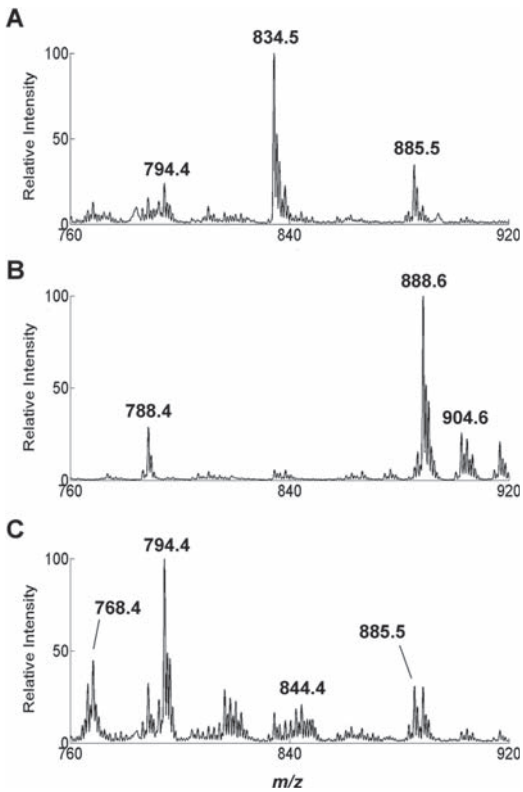

Fig. 2 Full-scan mass spectra in negative ion mode over m/z 760–920. (A) Sample #24; pathological assignment: grey matter with low TCP. (B) Sample #1; pathological assignment: white matter with low TCP. (C) Sample #20; pathological assignment: glioma with high TCP. *Y*-Axes are normalized to the base peak (m/z 834, 888, and 794, respectively) in the mass range m/z 760–920. TIC values are 7.98×10^6, 6.71×10^6, and 2.22×10^5 respectively for A, B, and C.

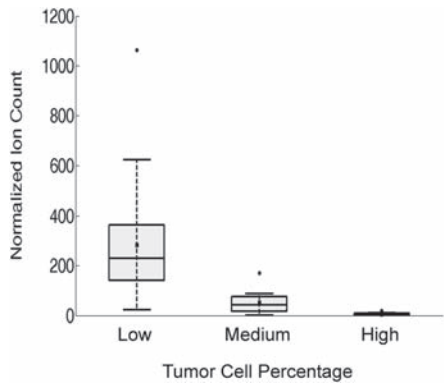

Fig. 3 Box and whisker plot. NAA abundance in tissue was categorized as low (<34%, n = 24), medium (34–67%, n = 12) or high (>67%, n = 11) TCP as visually assessed by histopathology. Note, multiple touches from the same specimens were considered independent measurements as the neuropathologist annotated the presence of heterogeneous areas in adjacent tissue sections (Table S1†). The ion counts correspond to the signal intensity of the transition m/z 174 → 114 normalized to the ion counts of the transition m/z 177 → 116 (NAA-d_3, the internal standard). The box represents the interquartile range with a median line and whiskers at ±1.5 SD. Squares represent the mean value. Circles represent outliers. Zero intensity was assigned to Sample #13 in which no NAA signal was detected (Table S1†).

different portions of tissue biopsies) was measured in terms of spectral correlation using canonical correlation analysis[12,20] and the correlation coefficient was equal to 0.9.

High-resolution MS and MS^2 experiments have been previously recorded using DESI-MS for structural identification.[20] These experiments were repeated using swab TS-MS and confirmed the structural assignments (Fig. S6†). Increased intensity of m/z 810.4, corresponding to phosphatidylserine 38 : 4 was observed in a few specimens. We attribute it to blood absorption on the swab tip as this lipid is a major membrane constituent of erythrocytes. This interference did not compromise our ability to determine the presence of tumor in the tissue but further evaluation is needed from specimens collected *in vivo*.

The neurometabolite NAA is one of the most abundant in healthy human brain tissue. However, its abundance (*i.e.* relative signal) observed in swab TS mass spectra decreased as the TCP (*i.e.*, tumor cells relative to normal cells) increased (Fig. 3). Population medians are statistically different (p values = 0.0046 using Kruskal–Wallis non-parametric test). NAA measurement provides an estimate of tumor infiltration within the tissue, and corroborates prior DESI-MS observations made on tissue sections and smears,[20,21,23] as well as literature reports regarding the inhibited expression of the biosynthetic enzyme L-aspartate *N*-acetyltransferase in glioma cells.[29] For validation, we confirmed that the decreased signal abundance of NAA in the swab TS-MS spectra corresponded to decreased concentrations of NAA in the specimens. We quantified NAA in the same specimens using an independent protocol, which is described elsewhere.[30] Briefly, an adjacent portion of the tissue to that sampled by swab TS-MS was removed and extracted using methanol–water (3 : 2 v/v). The solution was analyzed by traditional electrospray ionization triple quadrupole MS operated in the multiple reaction monitoring (MRM) mode. NAA concentrations decreased proportionally with increasing fraction of tumor cells (averages for low, medium, and high TCP were equal to 797, 406, and 42 ng mg^{-1}, respectively), hence validating the trend we observed qualitatively in swab TS mass spectra (see results in Table S3†). A more accurate regression analysis to estimate tumor infiltration is yet to be developed, as is assessment of a detection limit for the minimal and maximum amounts of infiltrating cells discernable *via* NAA measurements. The use of controlled cell cultures and mixtures is foreseen as a possible strategy to address such issues.

Overall, the changes in the lipid and NAA features in swab TS-MS spectra reflect the known complexity and heterogeneity

of gliomas that diffusely infiltrate into the surrounding brain parenchyma, as corroborated by pathological examination of the tissue (Table S1†). Fig. 4 depicts an example of such dynamic yet diagnostic changes. Three regions of interest were sampled from Case #19. The touch spray at the first spot (Sample #31) showed a low abundance of NAA (indicative of high TCP) and a lipid profile indicative of glioma tissue infiltrating grey matter (Fig. 4A and B). The second spot touched (Sample #32) showed low abundance of NAA as well but the lipid profile was indicative of glioma invading a mixture of white and grey matter (Fig. 4D and E). The third touch (Sample #33) showed higher intensity of NAA (lower tumor infiltration) into prevalently grey matter, as indicated by the lipid profile dominated by *m*/*z* 834.5 (Fig. 4G and H). Pathological examinations matched with the swab TS-MS results (Table S1†).

Assessment of *IDH* mutation *via* 2HG measurement

IDH mutations result in accumulation of 2HG in glioma cells by conversion of alpha ketoglutarate *via* NADPH oxidation. We detected a 50-fold increase in the average 2HG normalized signal intensity between wild-type gliomas and *IDH*-mutant gliomas (Fig. 5A). A wide range in 2HG signal intensity was detected for the *IDH*-mutant gliomas and can be attributed to differences in tissue cellularity and the known heterogeneity of tumor density in the samples. We averaged the 2HG measurements of multiple touches from the same specimens to avoid bias due to sample size (N = 29). The possibility that other compounds give ions that interfere with the signal for 2HG in the full scan mass spectrum led us to increase specificity by using the MS^3 collision-induced dissociation sequence *m*/*z* 147 → 129 → 101. A sequential product ion scan spectrum from an *IDH*-mutant glioma is shown in Fig. S7.† A relative signal intensity of 2HG (signal intensity of the sequential product ion *m*/*z* 147 → 129 → 101 normalized to the signal intensity of the internal standard NAA-d_3 *m*/*z* 174 → 114) equal to 1.02 is a cut-off that discriminates this set of *IDH*-mutant gliomas and wild-type gliomas with 100% accuracy (using ROC curve analysis). This observation was confirmed by quantitation of 2HG in adjacent tissue of the same specimens, performed as described above for NAA (N = 28; Case #29 was excluded because insufficient tissue was available to perform both experiments). A cut-off of 45 ng mg^{-1} for the 2HG concentrations was found to discriminate *IDH*-mutant and wild-type tumors with 100% accuracy.[30] Concentrations of 2-HG are reported in Table S3† for validation. Fig. 5B shows complete agreement between the qualitative swab TS-MS measurements and the concentrations determined by ESI-MS (*i.e.* all IDH mutant samples show swab TS-MS intensity and ESI-MS concentrations of 2HG above the set cut-offs, while wild-type tumors show values below such cut-offs). There is also agreement with intraoperative DESI-MS measurements reported recently.[23]

Discussion

In this study, we demonstrated the feasibility of obtaining accurate diagnostic information (Table S4†) by touching tissue using a medical swab followed by direct MS analysis from the sampling device. Rapid analysis and minimally invasive sampling are major advantages of swab TS-MS. A larger cohort

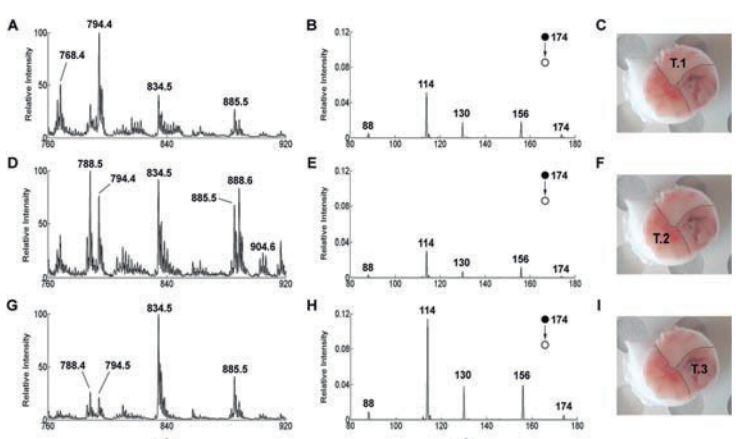

Fig. 4 Case #19. (On the left) Negative ion mode full-scan mass spectra over *m*/*z* 760–920 of (A) Sample #31, pathological assignment: glioma 70% TCP. (D) Sample #32, pathological assignment: glioma 70% TCP (G) Sample #33, pathological assignment, grey matter with 40% TCP. Lipid profile spectra are normalized to the base peak (*m*/*z* 794, 788, and 834, respectively) over the mass range *m*/*z* 760–920. (Middle column) Negative ion mode product ion scan for NAA of (B) Sample #31, (E) Sample #32, (H) Sample #33. MS/MS product ion spectra are normalized to the signal of the signal intensity of the internal standard NAA-d_3 (transition *m*/*z* 177 → 116). (On the right) Picture of Specimen #19 with superimposed annotation of the touch number for swab TS-MS analysis (C) Touch #1. (F) Touch #2. (I) Touch #3. The tissue was allowed to thaw at room temperature before performing the swab TS-MS analysis.

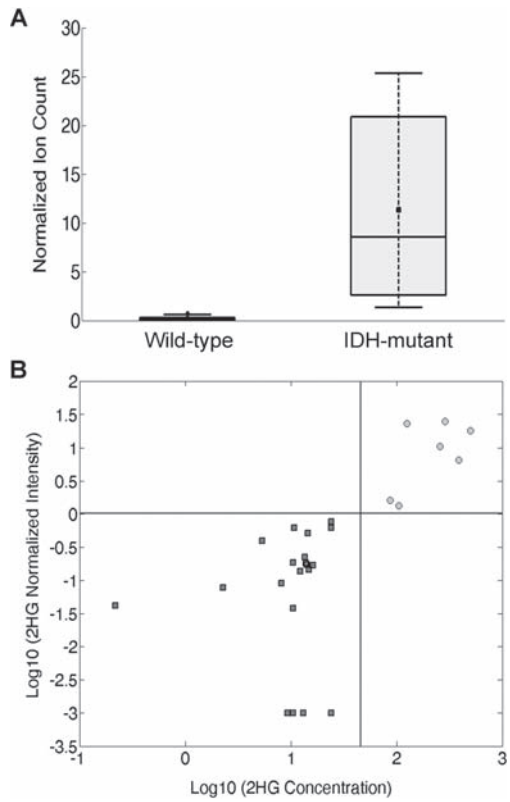

Fig. 5 2HG expression. (A) Box and whisker plot for 2HG in tissue analyzed by swab TS-MS. Wild-type, n = 21; *IDH*-mutant gliomas, n = 8. Normalized ion counts correspond to the signal intensity of the transition m/z 147 → 129 → 101 normalized to the signal intensity of the internal standard NAA-d_3 (transition m/z 177 → 116). The box represents the interquartile range with a median line and whiskers at ±1.5 SD. Squares represent the mean value. (B) 2HG concentration (ng mg^{-1} tissue) from tissue extracts by ESI-MS *versus* 2HG normalized signal from swab TS-MS for 28 cases (Case #29 was of insufficient quantity to perform the quantitative measurement). Dark grey squares, wild-type gliomas; light grey circles, *IDH*-mutant gliomas; black lines represent the cut-offs for the Logarithmic 2HG normalized signal intensity (horizontal line) and for the Logarithmic 2HG concentration (45 ng mg^{-1}, vertical line). The value of 0.001 was assigned to samples in which no 2HG signal was detected in order to compute the logarithm (−3). 2HG concentrations are reported in Table S3† for validation.

of samples is certainly needed to validate the findings described herein and to evaluate its use in routine clinical practice. Nonetheless, we consider this technique to be worth investigating as a strategy to implement chemical pathology into the standard intraoperative diagnostic consultation. The collection of tissue remotely to the mass spectrometer using a medical swab is a simple process. Changes to the swab design and the source interface could improve automation and reduce analysis time. MS analysis of neurological tissue directly from the sampling device can be performed inside the operating room with no interference with surgical practice. Commercial MS instruments and ESI sources can easily be adapted to allocate the swab as probe rather than the conventional ESI needle. Rapid analysis of tissue should allow assessment of the surgical margin status in multiple locations selected by the neurosurgeon as tumor resection is executed. The diagnostic feedback can provide guidance on further surgical maneuvers to maximize safe tumor resection, especially in proximity of critical anatomical structures. Neurosurgeons are already familiar with the use of swabs and absorbent pads and the device we are describing to touch neurological tissue would be used no differently. A fit-for-purpose swab design is wanted, however, for optimal MS performance. The medical swab we used in this study is commercialized as a class IIA device for surgical invasive transient use (*i.e.*, contact with tissue for less than 60 min). The swab has a sterile mini rayon tip with a fused shape. It is sufficiently small to sample minute amounts of tissue by gentle touch and minimize the invasiveness of the sampling procedure but tip dimensions could be reduced further and the shape of the tip changed to a conical geometry to improve upon electrospray formation. The stiffness and the crevasses in the tip hold the tissue during sample transfer and MS analysis, but different biocompatible functionalized surfaces could be designed to improve tissue transfer, reduce chemical noise, and enhance extraction and recovery of target compounds. The aluminum handle is conductive and allows the generation of the electrospray directly from the swab upon application of a high voltage; a different design of the swab with hollowed handles could facilitate the delivery of solvent and allow for the development of a swab probe that is easier to interface with commercial ESI sources.

The spectra obtained from swab TS-MS recapitulate previously reported DESI-MS spectra and pathology. The lipid signature provides information as to the disease state of the tissue. The oncometabolite NAA provides estimate of tumor infiltration which is of utmost importance when attempting to maximize glioma resection, an outcome that is favorably prognostic for glioma patients. Our results support the hypothesis that tumor infiltration can be monitored through neuronal cell damage in its path by measuring NAA depletion.[31] Estimation of tumor infiltration directly from points of interest along the resection margins can guide further tumor excision, assisting and validating neurosurgeons' decisions; keeping in mind that complete tumor resection is unachievable, and safe removal of tissue with high tumor infiltration is a primary goal of neurosurgery, whereas areas of low infiltration are more likely to be the target of coadjuvant postsurgical therapies. In the current surgical practice, the amount of residual tumor near the resection margins is not pathologically assessed during surgery. Neuronavigation with preoperative MRI images is typically used to judge extent of resection but several studies have highlighted the limitations and the subjectivity of such a practice as tumor infiltration can extend beyond MRI contrast-enhanced areas, and the enhancement itself poorly correlates to tissue histopathology.[3,4] In our parallel work using DESI-MS, we were able

to detect variable and even large amounts of residual tumor *via* NAA measurements at the resection margins, even when they appeared clear and non-enhanced by postoperative MRI,[23] remarking the utility of such a measurement.

In addition to tumor presence and infiltration, we can monitor the oncometabolite 2HG to assess the *IDH* mutation status of the tumor which is clinically relevant and a strong prognostic marker.[26,32] *IDH* mutation status is normally assessed postoperatively as it relies on laborious immunohistochemistry or genetic assays on biopsied tissue; however, its intraoperative assessment *via* MS measurement of 2HG could influence surgical decisions. An increasing body of evidence shows that more aggressive resection of *IDH*-mutant gliomas improves overall and progression-free survival, while more aggressive resection of wild-type tumors does not.[33,34] Furthermore, the assessment of *IDH* mutation is required for tumor diagnosis following the 2016 WHO diagnostic criteria for central nervous system tumors.[35] Intraoperative testing could benefit neuropathologists by providing a more accurate diagnostic consultation, for example, achieving an unequivocal diagnosis of diffuse glioma intraoperatively, particularly at the edge of a tumor. This is very challenging by pathology and often results in a nonspecific diagnosis. Having concurrent access to supporting molecular information should bolster the diagnostic yield. In addition, it is conceivable that emerging intraoperative therapies (*e.g.*, BCNU wafers)[36] may require better classification of diffuse gliomas intraoperatively to guide better decision making.

Conflicts of interest

There are no conflict of interest.

Acknowledgements

We thank Dr Zane Baird for assembling the swab touch spray interface. The research was supported by the National Institute of Biomedical Imaging and Bioengineering, NIH grant R21EB015722, and the Purdue University Center for Cancer Research.

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INFORMACIÓ SUPLEMENTÀRIA A LA PUBLICACIÓ V

Analysis of human gliomas by swab touch spray-mass spectrometry: application to intraoperative assessment of surgical margins and presence of oncometabolites

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Analyst, 142 (**2017**) 4058-4066

Analysis of human gliomas by swab touch spray - mass spectrometry: applications to intraoperative assessment of surgical margins and presence of oncometabolites

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SUPPLEMENTARY INFORMATION

Table S1. Pathological Evaluation and Chemical Assessments

				Pathological Evaluation				Chemical Assessment				
Case	**# Touches**	**Tissue Weight (mg)**	**# Samples in Main Text**	**Diagnosis**	**Comments[a]**	**TCP**	**IDH[b]**	**Base Peak Lipid Profile (*m/z*)**	**S/N**	**Diagnosis**	**NAA[c]**	**2HG[d]**
1	1	1.9	1	IT	Mostly WM	Low	0	888.6	58	IT (WM)	623	0.18
	2	3.1	2	IT	Mixture of GM and WM	Low		834.4	63	IT (GM)	344	
2	1	4.5	3	G	Presence of necrosis	High	0	n.d.[e]	n.d.	-	2.4	n.d.
3	1	4.9	4	G	-	High	1	788.4	27	G	8.2	1.34
	2	n/a	5	G	-	High		794.4	17	G	6.9	
4	1	3.5	6	G	Mostly necrotic and hemorrhagic tissue	High	0	885.5	36	G	3.1	n.d.
5	1	5.4	7	IT	Infiltrated GM	Medium	1	834.4	62	IT (GM)	52.5	10.51
6	1	1.9	8	IT	Mixture of GM and WM	Low	0	888.6	71	IT (WM)	236	0.14
	2	1.5	9	IT	GM	Low		834.5	45	IT (GM)	179	
7	1	1.2	10	G	Presence of calcification	High	1	885.5	18	G	10.1	18.22
	2	1.1	11	G	Presence of calcification	High		885.5	42	G	19.0	
8	1	1.8	12	IT	Mostly GM	Low	0	834.4	50	IT (GM)	531	0.19

	2	1.8	13	IT	Mostly WM	Low		888.6	226	IT (WM)	232	
9	1	3.3	14	G	-	High	0	885.5	45	G	7.8	0.09
	2	3.7	15	G	-	High		885.5	52	G	1.6	
10	1	3.2	16	IT	Mixture of GM (90%) and WM (10%)	Low	0	834.5	36	IT (GM)	131	0.63
	2	1.0	17	IT (NOS)	-	Low		888.5	130	IT (WM)	1063	
11	1	1.7	18	IT	Mostly WM	Low	0	888.6	88	IT (WM)	81.0	0.40
	2	1.9	19	IT (NOS)	-	Low		888.6	157	IT (WM)	171	
12	1	2.5	20	G	-	High	0	794.4	10	G	1.9	0.08
13	1	1.6	21	G	Mostly necrotic tissue	High	0	885.5	11	G	n.d.	n.d.
14	1	1.8	22	IT	Mixture of WM (50%) and GM (50%)	Low	0	834.4	83	IT (GM)	150	0.17
	2	2.5	23	IT	Mixture of WM (50%) and GM (50%)	Low		888.6	38	IT (WM)	89.2	
15	1	1.8	24	IT	Mostly GM	Low	1	834.4	22	IT (GM)	99.6	1.63
16	1	8.6	25	IT (NOS)	-	Medium	1	794.4	31	G	10.1	25.33
	2	4.7	26	IT (NOS)	-	Medium		794.4	28	G	63.0	
17	1	3.1	27	IT	Mostly WM	Low	0	888.6	57	IT (WM)	23.6	0.14

	2	2.6	28	IT	Mostly GM	Low		834.4	58	IT (GM)	249	
18	1	3.9	29	IT	Mostly GM	Medium	0	834.4	113	IT (GM)	71.8	0.63
	2	4.3	30	IT	Mixture of GM and WM	Medium		794.4	28	G	88.4	
19	1	2.4	31	G	-	High	1	794.5	29	G	22.3	6.54
	2	2.7	32	G	-	high		788.4	15	G	7.91	
	3	3.5	33	G	Infiltrated GM	Medium		834.5	84	IT (GM)	29.6	
20	1	2.4	34	IT	Mostly GM	Low	0	834.5	30	IT (GM)	117	n.d.
21	1	3.5	35	IT	Mostly GM	Low	1	794.4	28	IT (GM)	101	23.51
	2	2.2	36	IT	Mixture of GM (65%) and WM (35%)	Low		788.4	3	IT (WM)	165	
22	1	4.0	37	IT	Mostly GM with small pockets of WM	Low	0	834.5	58	IT (GM)	277	0.17
	2	4.1	38	IT	Mostly GM	Low		834.5	136	IT (GM)	227	
23	1	4.7	39	IT	GM	Low	0	834.5	24	IT (GM)	419	0.78
24	1	2.4	40	IT	Mostly GM	Low	0	834.4	45	IT (GM)	312	0.04
	2	1.3	41	IT	Mostly GM	Low		834.4	113	IT (GM)	207	
25	1	8.6	42	IT (NOS)	Presence of edema	Medium	0	788.4	43	IT (WM)	1.67	0.04

26	1	2.4	43	IT	Mixture of GM and WM	Medium	0	888.6	55	IT (WM)	31.7	0.22
27	1	1.3	44	IT	Mostly GM	Low	0	834.5	77	IT (GM)	441	0.52
28	1	1.0	45	IT	Mostly GM	Low	0	834.5	42	IT (GM)	383	0.02
	2	1.1	46	IT	Mixture of GM and WM	Low		888.6	91	IT (WM)	191	
29	1	3.5	47	G	-	High	1	885.5	74	G	3.5	3.62

[a]GM, grey matter; WM, white matter; G, glioma; IT, infiltrated tissue; IT (NOS), infiltrated tissue not otherwise specified

[b]0 = non-immunoreactive; 1 = immunoreactive

[c]NAA signal intensity of the transition *m/z* 174→114 is normalized to the signal intensity of the internal standard NAA-d_3 using the transition *m/z* 177→116.

[d]2HG signal intensity of the sequential product ion *m/z* 147→129→101 is normalized to the signal intensity of the internal standard NAA-d_3 using the transition *m/z* 177→116.

[e]n.d. = not detected

Table S2. MS Instrumental Settings

Full Scan MS	**Lipid Profile**		**Metabolite Profile**
Mass range	*m/z* 700-1000		*m/z* 80-200
Tuned mass	*m/z* 786		*m/z* 174
Tube lenses potential	−80 V		−20 V
Capillary voltage	0 V		−8 V
Microscans	2		
Injection time	25 ms		
Capillary temperature	275 °C		
Capillary voltage	−6.5 kV		
MS^n CID fragmentation*	**NAA**	**NAA-d_3**	**2-HG**
MS^n transitions	*m/z* 174 → ○	*m/z* 177 → ○	*m/z* 147 → 129 → ○
Tube lenses potential	−20 V		
Capillary voltage	−8 V		
Microscans	2		
Injection time	25 ms		
Collision Energy (a.u.)	28		
Q value	0.3		

Table S3. Concentrations of NAA and 2HG determined by ESI-MS[30.] Data reproduced with permission, copyright from Clinical Chemistry 2017.

Case	NAA concentration (ng/mg)	2HG concentration (ng/mg)
1	15.9	13.8
2	33.0	13.0
3	81.0	104.6
4	12.1	10.4
5	223.3	254.5
6	1182.5	12.3
7	43.7	506.4
8	827.3	10.3
9	76.4	8.15
10	821.0	10.8
11	313.0	5.4
12	43.0	2.3
13	3.3	9.2
14	604.6	14.3
15	610.8	87.9
16	332.0	289.7
17	679.4	14.9
18	718.1	24.0
19	270.6	385.8
20	27.9	24.2
21	144.2	124.7
22	1113.2	16.1
23	1358.0	24.2
24	1160.7	10.4
25	0.4	0.2
26	984.9	13.4
27	1424.9	14.4
28	1186.7	12.2
29	n.d.	n.d.

Table S4. Association between chemical predictions of disease state vs. pathology assessment

	Chemical Evaluation of Disease State		
Pathological Evaluation	**Grey Matter**	**White Matter**	**Glioma**
Infiltrated tissue	18	12	3
Glioma	1	0	12

Figure S1. Photograph of medical swabs, model 160C, from Copan Diagnostics Inc. with and without the plastic sealing tube.

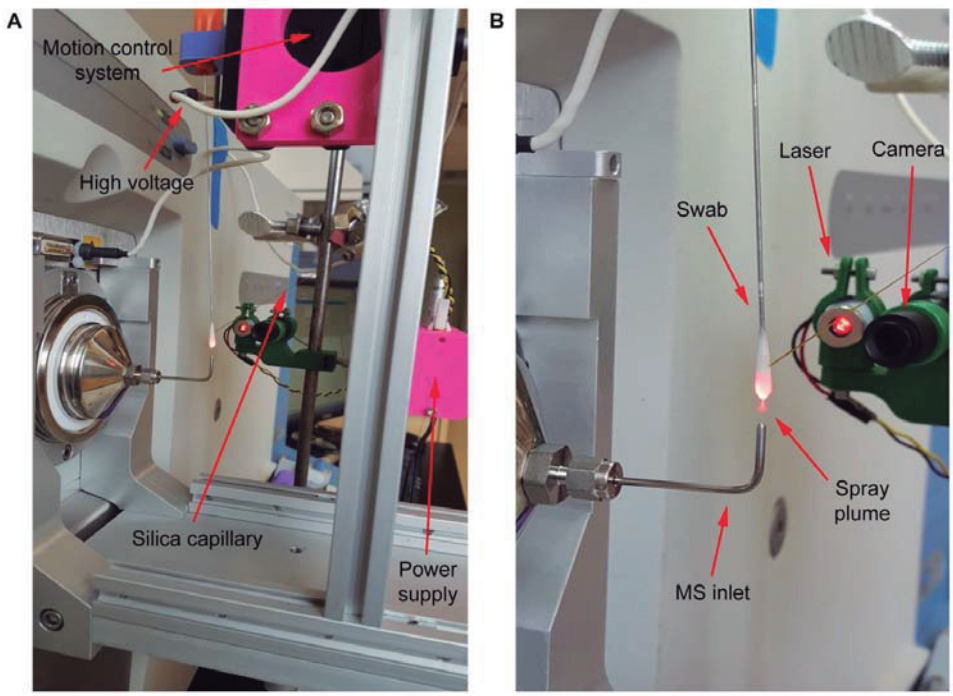

Figure S2. (A) Image of the custom-build ion source for TS-MS with medical swabs. **(B)** Photograph of electrospray generated from the swab, red laser pointer was used to illuminate the spray plume.

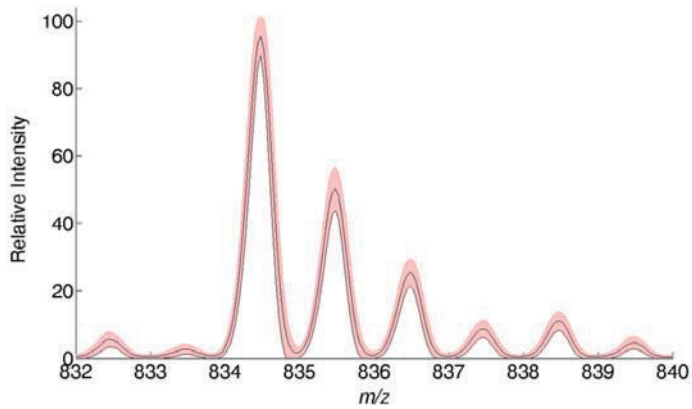

Figure S3. Isotope distribution of PS 18:0_22:6, *m/z* 834.5. Mean ion intensity denoted by solid line with ± standard deviation illustrated by the shaded area between the dotted lines. Number of scans averaged = 1026 over 10 min of data acquisition. Ion Intensities are normalized to the base peak (*m/z* 834.5) over the mass range *m/z* 760-920.

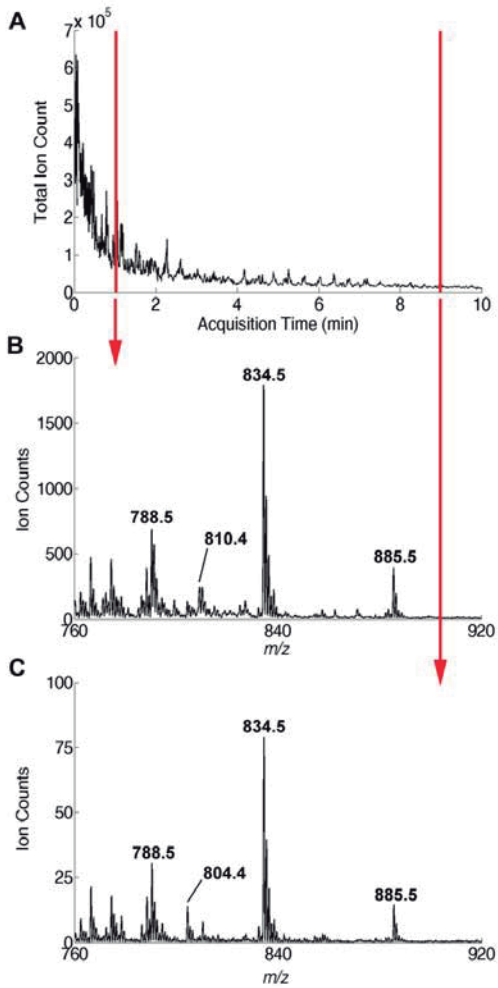

Figure S4. Case #17; pathological diagnosis: grey matter infiltrated with low TCP. **(A)** Total ion count (TIC) over a 10-minute window of data acquisition. Vertical red lines are drawn at minutes 1 and 9, respectively; the red arrows point at the full-scan mass spectrum acquired at those two-time points. **(B)** Full-scan mass spectrum in negative ion mode after 1 minutes of data acquisition; TIC = $1.67 \cdot 10^5$ **(C)** Full-scan mass spectrum in negative ion mode after 9 minutes of data acquisition; TIC = $0.26 \cdot 10^5$. Absolute ion counts are shown on the Y-axes.

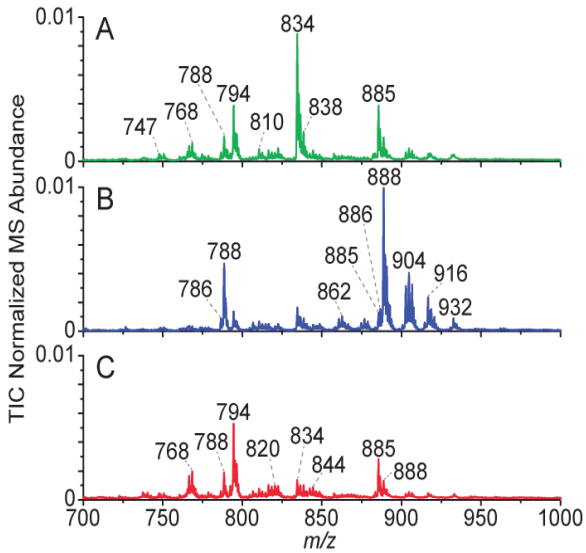

Figure S5. Average DESI-MS lipid (*m/z* 700–1000) MS profiles for **(A)** grey matter (N=223), **(B)** white matter (N=66), and **(C)** glioma (N=158). Ion intensities are normalized to the total ion count (TIC). Figure is reproduced with permission of the National Academy of Sciences (2017).

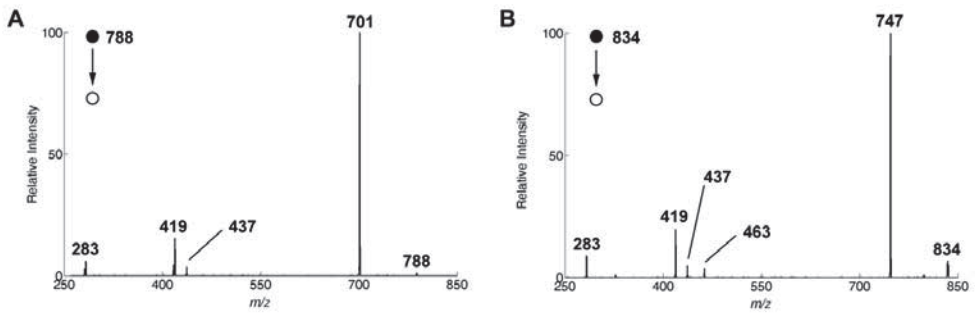

Figure S6. MS/MS product ion spectra for *m/z* 788 **(A)** and *m/z* 834 **(B)** detected in the negative ionization mode from Sample #8. Characteristic losses used in determining the lipid class; e.g., −87 (*m/z* 788→701, head group loss of phosphatidylserines). Furthermore, acyl chain could be determined based on fatty acid product ions; e.g., *m/z* 283, stearic acid. Ion intensities are normalized to the base peak over the product mass range *m/z* 250-850.

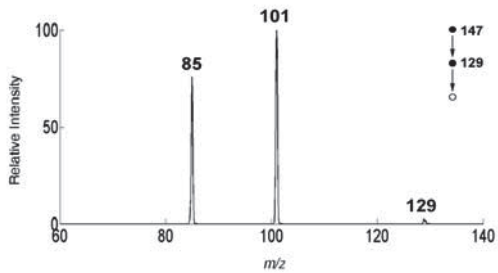

Fig. S7. Sequential product ion scan for 2HG from Case #16, IDH-mutant glioma. Fragmentation of 2HG matches previously reported pattern detected by DESI-MS (20, 22). The fragmentation pattern was matched also against a certified analytical standard. Ion intensities are normalized to the base peak over the product mass range *m/z* 60-140.

Video S1. Swab electrospray process. The video shows side-by-side the electrospray plume formation and the mass spectral data acquired simultaneously from Sample #12 (Case #8, Table S1). The swabs are unconventional probes for electrospray because of their fused shape and large tip (>1 mm). Swab TS differs from regular electrospray in that the solvent is pumped on a rough and porous surface; it is initially absorbed and then becomes suspended in a droplet at the apex of the swab tip once the porous material is completely saturated. When high voltage is applied to the handle of the swab, the voltage is transferred to the solvent at the swab tip. The suspended droplet becomes elongated. The elongation reduces droplet diameter, which in turn increases the electric field strength such that it exceeds the solvent surface tension resulting in the electrospray generation. The Taylor cone is formed at the tip of the swab. The plume of microdroplets generated by the electrospray process is visible through illumination with a laser. Microdroplets undergo cycles of solvent evaporation and Coulomb fission and are vacuumed into the mass spectrometer and mass analyzed.

3.2.2 PUBLICACIÓ VI

Paper Spray-Atmospheric Pressure Photoionization-High Resolution Mass Spectrometry for the Direct Analysis of Neutral Fluorinated Compounds in Waterproof Impregnation Sprays

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Analytical and Bioanalytical Chemistry, enviat

Paper Spray-Atmospheric Pressure Photoionization-High Resolution Mass Spectrometry for the Direct Analysis of Neutral Fluorinated Compounds in Waterproof Impregnation Sprays

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Keywords: Ambient Mass Spectrometry, Paper Spray, Atmospheric Pressure Photoionization, High Resolution Mass Spectrometry, Fluorotelomer alcohols, Fluorocotanesulfonamides, Fluorooctane sulfonamido-ethanols

Abstract

Paper spray-mass spectrometry (PS-MS) has been widely used as a fast ionization method for the direct analysis of polar compounds, since the main ionization mechanism that takes place is similar than that in electrospray ionization source. In this work, PS combined with atmospheric pressure photoionization (PS-APPI) is applied for the determination of non-polar and low polar compounds, such as the neutral per- and polyfluorinated alkyl substances (PFASs). The proposed PS-APPI method has been optimized for the analysis of fluorotelomer alcohols (FTOHs), fluoroctanesulfonamides (FOSAs) and fluorooctane sulfonamido-ethanols (FOSEs), using both negative ion mode and high-resolution mass spectrometry. The most critical working parameters (i.e. UV-krypton lamp position, sample drying time and spray dopant solvent) have been evaluated to study both the ionization behaviour and ionization efficiency of these compounds, achieving the best results using dopant-assisted PS-APPI with toluene as dopant solvent. The most intense ions observed in the mass spectra, $[M–H]^-$ for FOSAs and $[M+O_2]^{-\bullet}$ for FTOHs and FOSEs, were selected and proposed for the fast screening and quantitation of the target compounds in the samples. The developed dopant-assisted PS-APPI-HRMS method was applied for the quantification of neutral PFASs in waterproof impregnation sprays samples at μg L^{-1} levels using the internal standard calibration method, showing a good performance as demonstrate the satisfactory evaluation of the method quality parameters (repeatability, trueness and linearity). The analysis of raw impregnation sprays by the proposed method has allowed the identification and quantitation of several FTOHs (6:2 FTOH, 7-Me-6:2 FTOH, 8:2 FTOH, 10:2 FTOH) and *N*-MeFOSE at mg L^{-1} in several samples.

1. Introduction

In the last years, the use of ambient mass spectrometry techniques (Ambient MS) has shown an important increase, probably due to its attractive characteristics that allow the performance of direct analysis of samples in the open air with minimal or even no sample preparation [1]. Ambient MS techniques promote straightforward analysis with emphasis in simplicity, low cost and short analysis time. Among these techniques, paper spray ionization (PS-MS) is one of the easiest and simplest Ambient MS techniques. A liquid sample deposited onto a triangular shaped paper is analyzed by adding a solvent and the application of a high voltage, which produces a spray of charged droplets in the apex of the triangle paper [2]. Because PS-MS is based on electrospray ionization mechanism, its applications have been generally limited to the analysis of polar compounds (amines, amides, ketones or acids) that can be easily protonated or deprotonated in liquid-phase before being transferred into the gas-phase *via* ion evaporation [3]. Recently, some authors have proposed the use of some strategies to favour the ionization of non-polar compounds in PS-MS using alternative ionization mechanisms [4,5]. For instance, non-polar solvents have been used to favour field desorption ionization and/or chemical ionization by corona discharge. The monitoring of environmental and food contaminants and the evaluation of risk of human exposure to persistent pollutants frequently require the analysis of a large number of samples, although in many studies only few of them are positives. Under this scenario, the direct analysis of samples by Ambient MS methods could play an important role by detecting the positive samples and helping in the workload of control laboratories. PS-MS might would help to achieve these objectives, since the already described methods have been applied to several research areas, such as food, environmental, forensic and clinical analysis [6–9], and the feasibility of this technique have been shown for both qualitative and quantitative analysis of polar and moderate-polar compounds in raw samples.

Per- and polyfluorinated alkyl substances (PFASs), which comprise a huge group of chemicals that are characterized by a totally or partially fluorinated alkyl chain with a terminal functional group, have been used in many industrial and consumer products due to their special chemical properties such as hydrophobicity, oleophobicity, non-sticking and highly fire resistance, among others [10–14]. Within this family of compounds, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are of great concern because of their persistence, toxicity and potential bioaccumulation in organisms, as well as their biomagnification through the food chain [15–17]. From a regulatory point of view,

PFOS has been included by the Stockholm Convention as persistent organic pollutant in Annex B, while PFOA is still under evaluation [18, 19]. Over the last years, these PFASs have been substituted by other fluorinated compounds such as fluoroctanesulfonamides (FOSAs), fluoroctane sulfonamido-ethanols (FOSEs) or fluorotelomer alcohols (FTOHs), among others. Although these compounds show lower toxicity, they can degrade into the persistent PFASs, so their monitoring is of interest [13, 20]. Related to human exposure, there are few available data about PFAS content in consumer products, which are the responsible of the main emissions of these compounds into the environment. In addition, there is a lack of information about PFASs employed in consumer products such as impregnation products, cleansers, polishers and lubricants [21, 22]. Besides, this information is often hidden by the data owner, which makes it less accessible [23]. FTOHs, FOSAs and FOSEs have been analyzed by both gas chromatography (GC) [24–27] and liquid chromatography (LC) [28, 29] mainly coupled to mass spectrometry (MS). Regarding ionization sources, electron ionization (EI) and chemical ionization (CI) have been used for GC-MS analysis, although high fragmentation and poor ionization efficiency have been reported for some of these compounds. In LC-MS, electrospray ionization (ESI) is the source most currently used, although atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI) have also been recently proposed to overcome some ionization problems observed for FTOHs and FOSEs [10]. Concerning ambient ionization MS methods, so far none have been reported for these families of neutral PFASs, maybe due to the difficulties for their ionization under electrospray-based mechanisms. However, alternative ionization methods can be considered for the development of Ambient MS methods for these analytes to improve the high-throughput of quality control laboratories.

The present study explores the feasibility of a new Ambient MS approach that combines paper spray with APPI and high-resolution mass spectrometry (PS-APPI-HRMS) for the rapid analysis of FTOHs, FOSEs and FOSAs. The results obtained with the PS-APPI-HRMS method are compared with those achieved using the conventional PS-MS set-up in order to understand the procedures/ionization mechanisms involved in this new approach. Finally, the proposed PS-APPI-HRMS method has been applied for the determination of neutral PFASs in commercial waterproof impregnation sprays.

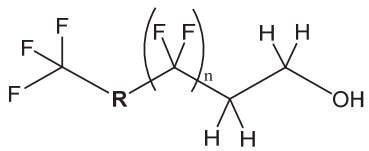
2. Experimental

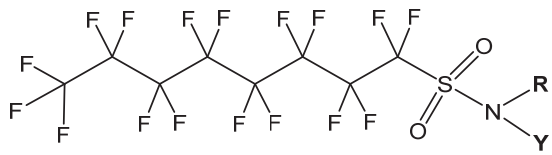
2.1 Materials, chemicals and standards

For paper spray experiments, Whatman 31ET chromatography paper (W31) obtained from Sigma-Aldrich (Steinhein, Germany) and copper clips purchased from Muller Electric (Akron, OH, USA) were used. Regarding solvents, acetonitrile (ACN), methanol (MeOH) (LC-MS Chromasolv® grade), ethanol (EtOH) (Chromasolv® for HPLC gradient grade) and dimethylformamide (DMF) (anhydrous grade) were purchased form Sigma-Aldrich (Steinheim, Germany). Solvents used as dopants, toluene and chlorobenzene (Chromasolv® Plus HPLC), were also acquired from Sigma-Aldrich, while anisole and acetone (pesticide residue analysis grade) were supplied by Fluka® Analytical (St. Louis, MO, USA) and tetrahydrofuran (PHOTREX^{TM}) was purchased from J.T. Baker (Deventer, Holland). The purity of all solvents used was higher than 99.8%.

FTOHs, FOSAs and FOSEs selected as target compounds for this study are shown in Fig. 1. Fluorotelomer alcohol standards (FTOHs), 1H, 1H, 2H, 2H-perfluorohexan-1-ol (4:2 FTOH), 1H, 1H, 2H, 2H-perfluorooctan-1-ol (6:2 FTOH) and 1H, 1H, 2H, 2H-perfluoro-7-trifluoromethyl-octan-1-ol (7-Me-6:2 FTOH) were acquired from Flurochem, Ltd. (Derbyshire, UK), whereas 1H, 1H, 2H, 2H-perfluorodecan-1-ol (8:2 FTOH) and 1H, 1H, 2H, 2H-perfluorododecan-1-ol (10:2 FTOH) were purchased from Alfa Aesar GmbH & Co KG (Karlsrube, Germany), at a purity higher than 96%. Regarding FOSEs and FOSAs standards, 2-(N-Methylperfluoro-1-octanesulfonamido)-ethanol (*N*-MeFOSE), 2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol (*N*-EtFOSE) and N-methylperfluoro-1-octanesulfonamide (*N*-MeFOSA) were supplied by Wellington Laboratories, Inc. (Guelph, Ontario, Canada) as individual standard solutions (50 mg L^{-1} in methanol, ≤ 98%), while N-ethylperfluoro-1-octanesulfonamide (*N*-EtFOSA) (99%) was obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Internal standards used for quantitation of FTOHs, 1H, 1H-pentadecafluoro-1-octanol (7:1 FA), 1H, 1H-perfluoro-1-nonanol (8:1 FA), 1H, 1H-perfluoro-1-decanol (9:1 FA) and 1H, 1H-perfluoro-1-dodecanol (11:1 FA) were obtained from Fluorochem LTd.. For the quantitative analysis of FOSA and FOSEs we used commercially available labelled standards, 2-(N-Ethyl-*d5*-perfluoro-1-octane-sulfonamido)-ethan-d_4-ol) (d_9-*N*-EtFOSE) and N-ethyl-*d5*-perfluoro-1-octanesulfonamide (d_5-*N*-EtFOSA) solutions (50 mg L^{-1} in methanol, supplied by Wellington Laboratories Inc.). Individual stock solutions (1 mg mL^{-1}) for FTOHs and *N*-EtFOSA were prepared in methanol and

stored at 4°C, while the working standard solutions were prepared weekly by appropriate dilution of the stock standard solutions in methanol and stored at 4°C until their analysis.





n	R	Compound
2	$-CF_2$	4:2 FTOH
4	$-CF_2$	6:2 FTOH
4	$-CF(CF_3)$	7-Me-6:2 FTOH
6	$-CF_2$	8:2 FTOH
8	$-CF_2$	10:2 FTOH

R	Y	Compound
$-CH_3$	–H	*N*-MeFOSA
$-C_2H_5$	–H	*N*-EtFOSA
$-CH_3$	$-C_2H_4OH$	*N*-MeFOSE
$-C_2H_5$	$-C_2H_4OH$	*N*-EtFOSE

Fig. 1 Chemical structures of the studied neutral PFASs

2.2 Instrumentation

The home-made PS-APPI set-up used in this work is shown in Fig. 2. A triangular piece of W31 paper, 10 mm (height) by 8 mm (base width), was held using a cooper clips with the apex in line with the inlet of the mass spectrometer at a distance of 4 ± 1 mm and the high-voltage was supplied through the cooper clips. The krypton lamp (Syagen, Santa Clara, CA, USA) used in APPI emitted photons of 10.6 eV energy and it was set at an angle of 30º and a distance of 1 cm from the triangle paper apex and the MS inlet. For the analysis of both, standards and liquid samples, 10 µL were loaded onto the W31 paper and let it dry for 2 min. Later, 70 µL of toluene (spray solvent) were deposited with a pipet onto the back of the paper. The high-voltage applied for PS-APPI was 2.5 kV to generate the spray of microdroplets and the mass spectra were recorded for 1-2 min.

Mass spectrometry analyses were performed using a quadrupole-Orbitrap mass spectrometer (Q-Exactive, Thermo Fisher Scientific, San Jose, CA, USA). High-resolution mass spectra were collected in negative-ion full-scan (HRMS) and target MS^2 acquisition modes (MS/HRMS). Mass resolution was set at 70,000 FWHM for both full-scan and product ion scan (full width at half maximum, *m/z* 200) while the mass range was 50-700 *m/z* in profile mode. All ions were assigned achieving accurate mass errors < 5 ppm. Automatic gain control (AGC) were set at 10^6 and 10^5 for full-scan and target MS/HRMS experiments, respectively, and maximum injection time values were set at 300 ms. The S-lens

radiofrequency and the capillary temperature were fixed at 50% and 300 °C, respectively. Nitrogen (99.95% pure, Air Liquide, Madrid, Spain) was used as collision gas and precursor ions were isolated using an isolation window of 1 *m/z*. Xcalibur™ software *v*3.1 (Thermo Fisher Scientific, San José, CA, USA) was used for data acquisition and data processing.

Accurate mass calibration was performed in the Q-Exactive mass spectrometer every 72 h using the ESI ion source and the calibration solution containing caffeine, MRFA peptide, Ultramark 1621 and butylamine in acetonitrile/methanol/water (2:1:1, *v/v*) with 1% (*v/v*) formic acid.

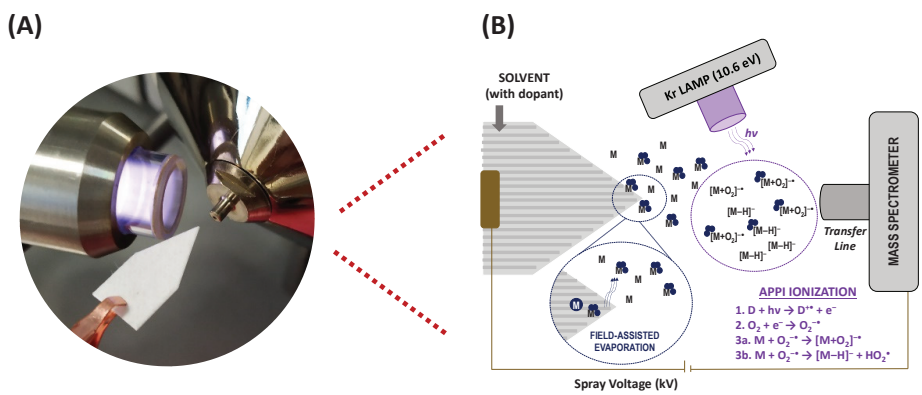


Fig. 2 (A) Home-made experimental set-up of voltage-assisted paper spray atmospheric pressure photoionization source (B) Scheme of PS-APPI ionization process. The figure is not drawn to scale.

2.3 Samples

Sixteen commercial waterproof impregnation sprays of different brands were collected from local supermarkets (Barcelona, Spain). Prior to PS-APPI-HRMS analysis, an adequate volume of a standard solution containing the internal standards were added to the samples, which were shaken in a vortex for few seconds before depositing 10 µL onto the W31 paper. No further sample manipulation was performed before the analysis and samples were quantified using the internal standard method using calibration standard solutions prepared in methanol at concentrations ranging from 0.05 to 110 mg L^{-1}.

3. Results and discussion

3.1 PS-APPI *vs* PS ionization behaviour of FTOHS, FOSAs and FOSEs

The ionization behaviour of FTOHs, FOSEs and FOSAs by paper spray using both approaches, standard PS and PS-APPI, have been explored in this work using the same home-made paper spray set-up. To perform the PS-APPI-HRMS experiments, a krypton lamp in the position described in the experimental section (Fig. 2) was included to the paper spray set-up and switched-on. For these studies, a methanol standard solution mixture (10 μL) containing the analytes (10 mg L^{-1} for FTOHs and 5 mg L^{-1} for FOSAs and FOSEs) was analyzed using several solvent mixtures (acetonitrile, toluene, methanol and acetone) and applying a high voltage within the range of 2-4 kV. After the first experiments, none of the target compounds were detected in positive ion mode neither in PS-HRMS nor in PS-APPI-HRMS. Thus, further PS studies of neutral PFASs were focused on the negative ion mode.

For the analysis of PFASs by PS in negative ion mode, the best results were obtained using both methanol/toluene (9:1, *v/v*) as spray solvent and 3.5 kV as spray voltage. At these conditions, FOSAs were ionized (Fig. 3A) via proton abstraction $[M–H]^–$, while any ions were observed from FTOHs and FOSEs. These results are in agreement with those obtained in previous studies performed by ESI, in which difficulties to generate deprotonated ions $[M–H]^–$ for FTOHs and FOSEs were reported and they only could be ionized via adduct formation with mobile phase components [10,11]. Although, the ionization mechanism occurring in paper spray under negative ion mode is still not well-understood, some authors [5,30] reported that other ionization mechanisms, in addition to electrospray, can also take place for non-acidic/basic, low polarity and high electron affinity compounds. These ionization mechanisms probably involve PS-based corona discharge ionization processes [30]. To evaluate this possibility, several non-polar organic solvents (acetone, toluene, cyclohexane and hexane) were tested to prevent electrospray-based mechanisms in PS. As occurred before, only FOSAs were ionized using non-polar solvents, obtaining the best responses using a spray solvent mixture of toluene/cyclohexane (3:1, *v/v*) and working at a 2.5 kV spray voltage (Fig. S1, Supporting information). At these conditions, the abundance of FOSAs ions was almost two orders of magnitude lower than that observed using polar solvent mixtures, which favour the ionization of FOSAs by electrospray-based mechanisms. However, the remained low intense ions $[M–H]^–$ when using non-polar solvents would

indicate that the ionization might be produced *via* corona discharge phenomena at the apex of the triangle shaped paper under these circumstances.

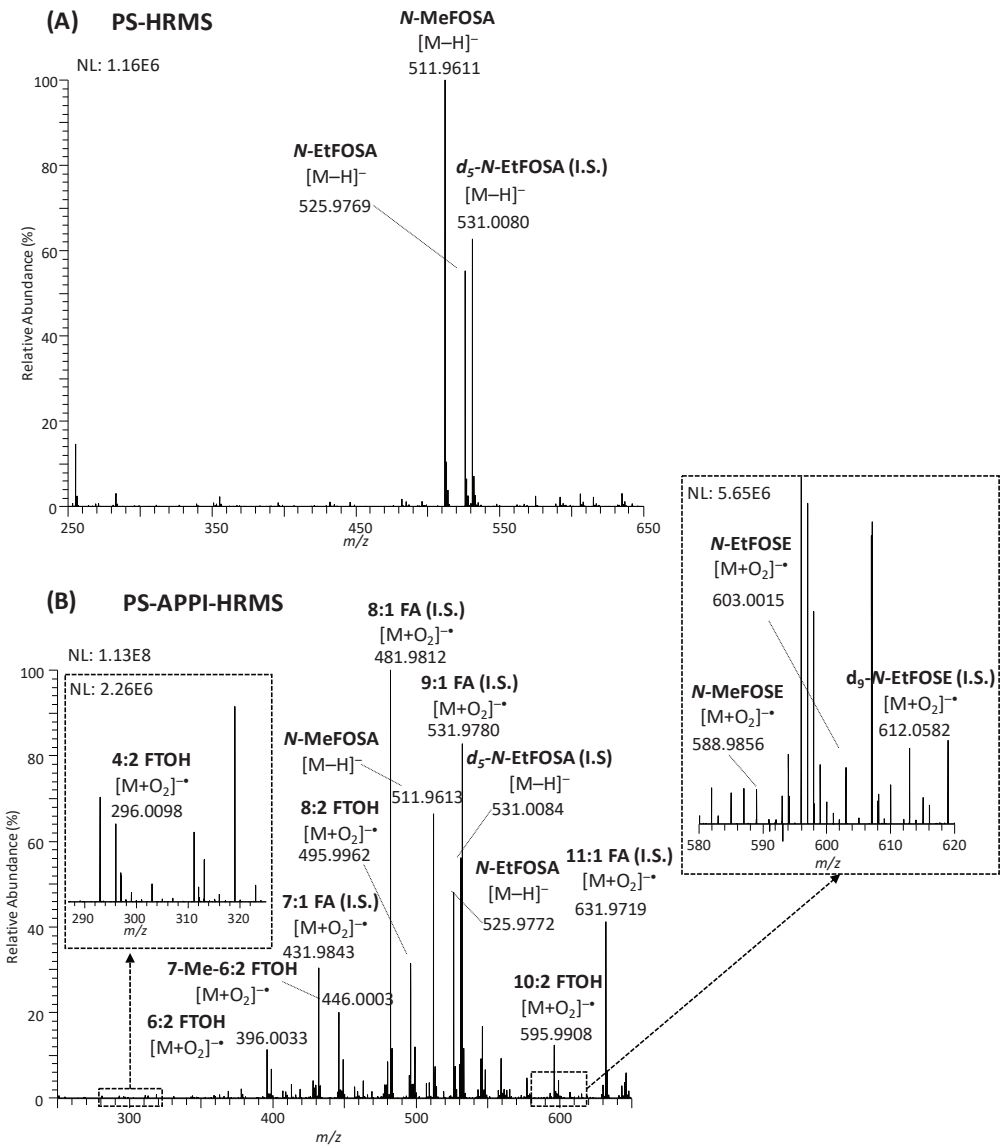


Fig. 3 Mass spectra of a standard mixture (10 µL) of the target compounds in pure methanol (10 mg L^{-1} for FTOHs and 5 mg L^{-1} for FOSAs and FOSEs) deposited onto the paper substrate by (A) PS-HRMS using methanol/toluene (9:1, *v/v*) as spray solvent and 3.5 kV as spray voltage and (B) PS-APPI-HRMS using toluene as spray solvent and a spray voltage of 2.5 kV.

Since previous studies performed in the research group showed that FTOHs and FOSEs can be ionized *via* LC-APPI-MS [10], a krypton lamp was incorporated into the original paper spray set-up to achieve the ionization of these compounds. In this new PS-APPI approach, the solvent used in PS plays two important roles, as solvent spray to extract and transport the analytes to the apex of the triangle shaped paper and as dopant solvent in the dopant-

assisted APPI process. Among the solvents and mixtures tested for PS-APPI, the best responses were obtained when using toluene as spray solvent at 2.5 kV spray voltage. As can be seen in Fig. 3B, FTOHs and FOSEs generated the characteristic superoxide adduct ions $[M+O_2]^{-\bullet}$ as it was previously observed in LC-APPI-MS [10]. The ionization mechanism of FTOHs and FOSEs would be initiated by the electrons released by the toluene photoionization process, which can be later captured by the atmospheric oxygen to form the superoxide radical ions $[O_2]^{-\bullet}$. Since no thermal-assisted evaporation was used in the PS-APPI, a field-assisted evaporation step might be the responsible to transfer neutral molecules from the paper substrate into the gas-phase, where would later interact with the superoxide radical ions $[O_2]^{-\bullet}$ to yield the superoxide adduct ion $[M+O_2]^{-\bullet}$ [31]. Regarding FOSAs, the ions observed in PS-APPI-HRMS mass spectra were also $[M–H]^-$, although their signal intensity was higher than that found using standard PS (Fig. 3B). As occurred for FTOHs and FOSEs, the field-assisted evaporation of FOSAs followed by their dopant-assisted photoionization in the gas-phase could be responsible of increasing the ions response in PS-APPI.

Tandem mass spectrometry MS/HRMS was also used to characterize the ions observed in both PS and PS-APPI and to confirm the presence of these compounds in real samples (Table S1, Supporting Information). All the product ions observed in the MS/HRMS mass spectra were consistent with those previously reported using LC-APPI-MS/HRMS for these families of compounds, being the fragmentation pathways similar to those described elsewhere [32].

3.2 PS-APPI-HRMS method optimization

To maximize the ion intensity in PS-APPI-HRMS, several operational parameters, such as the krypton lamp position, the spray solvent composition and the spray voltage, were optimized using 8:2 FTOH and *N*-EtFOSA as model compounds. To this end, 10 µL of a 10 mg L^{-1} methanol standard mixture solution were deposited onto the triangle shaped paper and analyzed by PS-APPI-HRMS.

The effect of the angle and the distance of the krypton lamp in the PS-HRMS response was studied. The ions generated from 8:2 FTOH and *N*-EtFOSA were only observed within an angle (α) between 20º and 45º and a distance from the paper tip (d) between 0.3 and 1.5 cm. As can be seen in Fig. 4A, differences in the ion signal intensity for 8:2 FTOH and *N*-EtFOSA were not critical at angles (α) between 30º and 45º and for distances (d) ranging

from 0.3 to 1 cm. Longer distances result in an important decrease in the ion abundances, which can be attributed to the spreading of the light beam. Thus, the krypton lamp optimal position was set at an angle (α) of 30º and a distance (d) of 1 cm for further studies. Furthermore, several spray solvents, such as toluene, acetone, chlorobenzene, tetrahydrofuran and anisole, which could also act as dopants, were tested to study their effect on the analytes response. As can be seen in Fig. 4B, toluene provided the highest signal intensity for both 8:2 FTOH and *N*-EtFOSA. Chlorobenzene and anisole also produced a relative intense ion signal for *N*-EtFOSA, because these two dopant-solvents might favour the proton abstraction of the analyte. In order to increase the analyte extraction and its transport to the paper tip, different solvent mixtures containing toluene as the main solvent were tested, but any of them provided a significant signal improvement. Thus, toluene (100%) was used in PS-APPI as both spray solvent and dopant solvent in the atmospheric pressure photoionization of target compounds.

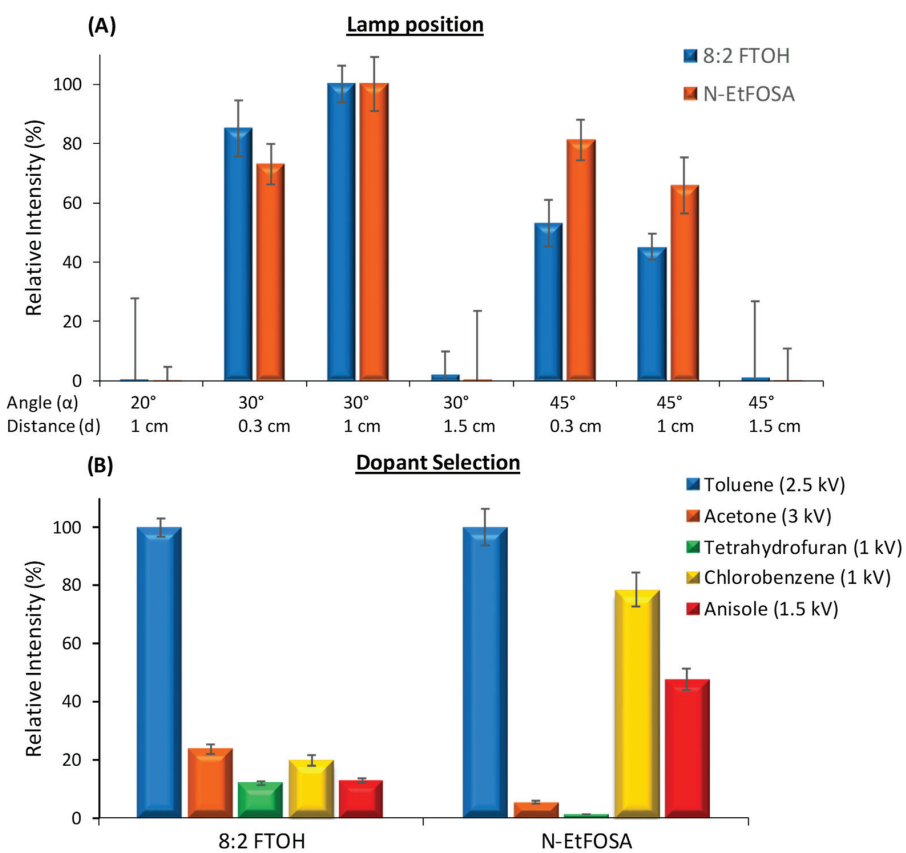


Fig. 4 Effect of the PS-APPI-HRMS working conditions using: (A) Lamp position, (B) Solvent selection.

Under the optimal PS-APPI-HRMS conditions, a standard mixture containing the nine target compounds at a concentration of 10 mg L^{-1} for FTOHs and 5 mg L^{-1} for FOSAs and FOSEs were analyzed. Interestingly, differences on the chronogram profiles of the studied compounds were observed within the acquisition time (2 min) (Fig. 5). For all FTOHs, the ion signal was intense within 0.5 and 1.5 min, although for 4:2 FTOH (*m/z* 296.0101) the ion response decreased over the time, while for 10:2 FTOH (*m/z* 595.9909) the signal increased over the same period. In contrast, the response of the less volatile FOSEs (*m/z* 588.9857 and *m/z* 603.0014) and FOSAs (*m/z* 511.9619 and *m/z* 525.9775) significantly raised at the end of the chronogram. This could be related with both the relative volatility of these compounds and the size of the droplets generated in the spray at the tip of the paper. The more volatile compounds are easily transferred to the gas phase, even when the presence of large amount of solvent generates relatively large droplets. However, when the solvent runs out faster over the time, the droplets generated are much smaller, which makes easier the desolvation process and the transfer of high molecular weight compounds to the gas phase. Furthermore, the absence of solvent at the end of the period recorded might increase

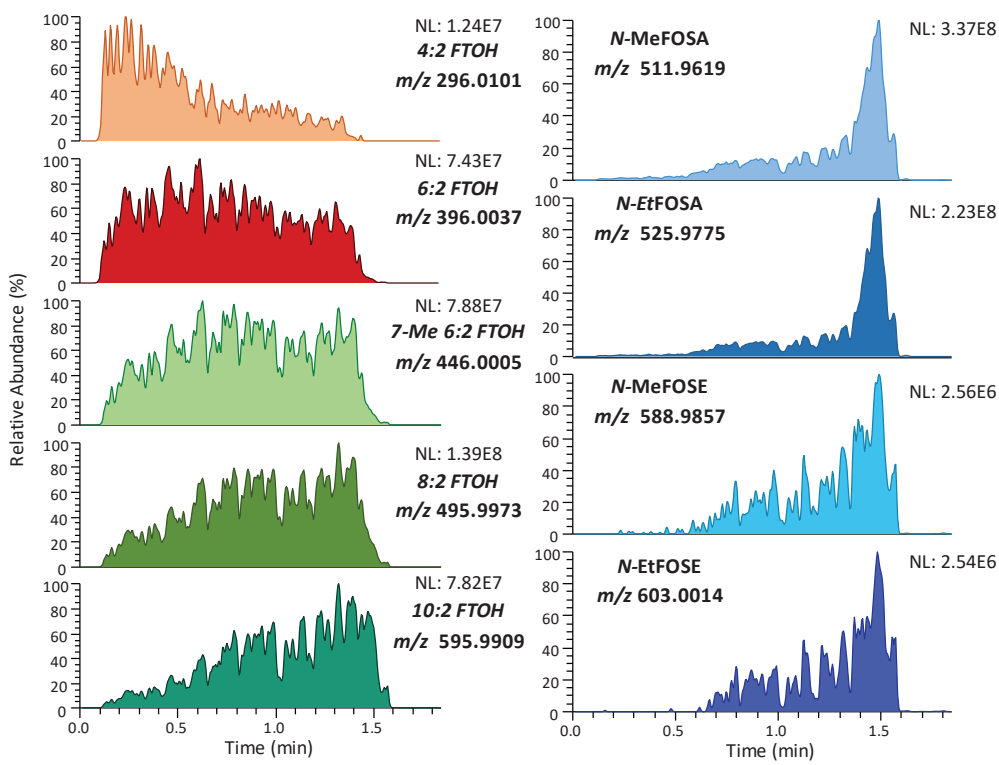


Fig. 5 PS-APPI-HRMS (-) selected ion current profiles of 4:2 FTOH, 6:2 FTOH, 7-Me-6:2 FTOH, 8:2 FTOH, 10:2 FTOH, *N*-MeFOSA, *N*-EtFOSA, *N*-MeFOSE and *N*-EtFOSE obtained analyzing a standard solution by PS-APPI-HRMS using toluene as spray solvent and a spray voltage of 2.5 kV.

the electrical field at the paper tip, which could also favour the field-assisted evaporation of the less volatile analytes [3, 33, 34].

3.3 Analysis of waterproof impregnation sprays by PS-APPI-HRMS.

Taking into account the volatility of target compounds, the drying time of the sample on the paper substrate before the PS-APPI-HRMS analysis was studied using a blank spiked sample. Different drying periods (0.5 - 10 min) were tested and both intensity and variability on the response of athe nalytes were evaluated. The highest analyte signals were achieved after letting the sample dry on the paper triangle at room temperature for 2 minutes (Fig. S2, Supporting Information). The use of longer drying times produced a significant decrease on the response of FTOHs, especially for 4:2 FTOH, probably due to the higher volatility of the short-fluoroalkyl chain FTOHs, which could cause the loss of analytes before its determination by PS-APPI-HRMS. Regarding ion signal variability (RSD %), it increased with the drying time and it was lower than 17% at drying times below 2 minutes, with the only exception of 4:2 FTOH (RSD 32%) because of its mentioned high volatility.

Internal standard method was used to quantify all neutral PFASs using d_5-*N*-EtFOSA and d_9-*N*-EtFOSE for FOSAs and FOSEs and 7:1 FA, 8:1 FA, 9:1 FA and 11:1 FA for FTOHs. Calibration curves obtained for all the target compounds showed good linearity (R^2 >0.998) within the studied concentration range and allowed the correction of both signal variability and possible matrix effects. Quality parameters such as limits of detection and quantitation as well as precision and bias have been estimated and the results obtained are summarized in Table 1. Method limits of detection (MLODs) and quantitation (MLOQs), based on the signal-to-noise (S/N) ratio of 3 and 10, respectively, were estimated by spiking a blank waterproof impregnation spray with standards at low concentration level. For most of the compounds, the estimated MLODs ranged from 3 to 27 µg L^{-1}, except for 4:2 FTOH (315 µg L^{-1}) because of its high volatility. These MLODs are at least 5 to 10 times lower than the concentration levels that have been reported for neutral PFAS in impregnating agents, which are currently above mg L^{-1} levels [21, 22]. To evaluate method precision (concentration intra-day repeatability as relative standard deviation, %RSD) and trueness (as relative concentration error, %), replicated analyses (n=5) at two concentration levels (low level: 0.08-0.6 mg L^{-1} and 2 mg L^{-1} for 4:2 FTOH, high level: 2-25 mg L^{-1} and 50 mg L^{-1} for 4:2 FTOH) were performed. For most of the compounds, RSD (%) values were lower than 18%

and bias were below 18%, except for 4:2 FTOH, for which the RSD was 32% and the bias was 39%, which is also attributed to its higher volatility.

Table 1. Quality parameters of the PS-APPI-HRMS method

Analyte	MLOD (μg L^{-1})	MLOQ (μg L^{-1})	Calibration range (mg L^{-1})	R^2	Precision (RSD, %)		Trueness (Re. Error, %)	
					Low Level	High Level	Low Level	High Level
4:2 FTOH	315	1000	1-110	0.9978	32	20	39	7
6:2 FTOH	25	85	0.15-60	0.9995	13	5	18	0.2
7-Me-6:2 FTOH	27	90	0.15-60	0.9998	9	4	12	0.3
8:2 FTOH	6	20	0.05-60	0.9984	9	9	9	9
10:2 FTOH	6	20	0.05-60	0.9995	5	2	5	2
N-MeFOSA	3	10	0.05-5	0.9998	6	4	11	0.3
N-EtFOSA	3	10	0.05-5	0.9993	12	1	17	0.7
N-MeFOSE	12	40	0.05-5	0.9999	10	9	10	0.2
N-EtFOSE	12	40	0.05-5	0.9999	18	4	16	0.2

Low level: 0.08-0.6 mg L^{-1} and 2 mg L^{-1} for 4:2 FTOH High level: 2-25 mg L^{-1} and 60 mg L^{-1} for 4:2 FTOH

FTOHs, FOSAs and FOSEs were determined by PS-APPI-HRMS in 16 waterproof impregnation spray samples purchased from local stores. Any label of the 16 samples analyzed indicated the use of neutral PFASs as an ingredient in the product composition. Internal standards (0.4 mg L^{-1} for d_5-*N*-EtFOSA and d_9-*N*-EtFOSE and 5 mg L^{-1} for FAs) were added to the samples after being transferred to an Eppendorf to perform the quantitative analysis. The sample raw data were acquired in both PS-APPI-HRMS for the quantitation of identified compounds (mass accuracy less than 5 ppm) and PS-APPI-MS/HRMS for confirmatory purposes (Table S1, Supporting Information). If necessary, positive samples with neutral PFASs at concentration levels above the calibration range were appropriately diluted in acetone to quantify the target compounds within the linear calibration range used. Table 2 lists the samples analyzed and the neutral PFASs identified along with the quantitative results obtained by PS-APPI-HRMS. Among the waterproof impregnating sprays analyzed, the presence of neutral PFASs was detected in seven samples. FTOHs were identified in 6 samples, being the 6:2 FTOH the most frequently detected at concentrations ranging from 0.27 to 167 mg L^{-1}. Fig. 6 shows the PS-APPI-HRMS and PS-APPI-MS/HRMS spectra obtained in the analysis of sample WP-06, where 6:2 FTOH was determined at a concentration of 167 ± 5 mg L^{-1}.

Table 2. PS-APPI-HRMS sample analysis

Sample	Detected compound	Ion assignment	Exact mass (*m/z*)	Accurate mass (*m/z*)	Mass accuracy (ppm)	Concentration ± SD (mg L^{-1})
WP-01	n.d.	–	–	–	–	n.d.
WP-02	n.d.	–	–	–	–	n.d.
WP-03	6:2 FTOH	$[M+O_2]^{-\bullet}$	396.0037	396.0032	1.3	0.27±0.01
	8:2 FTOH	$[M+O_2]^{-\bullet}$	495.9973	495.9956	3.4	0.057±0.003
WP-04	6:2 FTOH	$[M+O_2]^{-\bullet}$	396.0037	396.0024	3.3	25±1
	7-Me-6:2 FTOH	$[M+O_2]^{-\bullet}$	446.0005	445.9910	2.0	0.34±0.01
	8:2 FTOH	$[M+O_2]^{-\bullet}$	495.9973	495.9972	0.2	0.08±0.02
	10:2 FTOH	$[M+O_2]^{-\bullet}$	595.9909	595.9905	0.7	0.2±0.02
WP-05	6:2 FTOH	$[M+O_2]^{-\bullet}$	396.0037	396.0033	1.0	0.172±0.009
	N-MeFOSE	$[M+O_2]^{-\bullet}$	588.9857	588.9856	0.2	0.63±0.04
WP-06	6:2 FTOH	$[M+O_2]^{-\bullet}$	396.0037	396.0024	3.3	167±5[a]
WP-07	6:2 FTOH	$[M+O_2]^{-\bullet}$	396.0037	396.0024	3.2	102±1[a]
WP-08	n.d.	–	–	–	–	n.d.
WP-09	6:2 FTOH	$[M+O_2]^{-\bullet}$	396.0037	396.0020	4.3	n.q.
WP-10	n.d.	–	–	–	–	n.d.
WP-11	8:2 FTOH	$[M+O_2]^{-\bullet}$	495.9973	495.9949	4.8	n.q.
WP-12	n.d.	–	–	–	–	n.d.
WP-13	n.d.	–	–	–	–	n.d.
WP-14	n.d.	–	–	–	–	n.d.
WP-15	n.d.	–	–	–	–	n.d.
WP-16	n.d.	–	–	–	–	n.d.

n.d. not detected (<MLOD); n.q. not quantified (<MLOQ). [a] Sample diluted with acetone for analyte quantitation.

Tandem mass spectrum confirmed the presence of 6:2 FTOH in the sample since the perfluoroalkyl chain product ions observed are consistent with those observed for the standard (Table S1, Supporting Information). The concentration levels of FTOHs found in this study are in agreement with those reported for commercial impregnation products analyzed in Norway (0.5-330 mg L^{-1}) [21] and Switzerland (0.8-9400 mg L^{-1}) [22]. Moreover, *N*-MeFOSE was only detected (0.63 ± 0.04 mg L^{-1}) in one of the waterproof impregnation spray (WP-05), while FOSAs were not detected in any of the products tested.

4. Conclusions

In this work, the combination of APPI with the PS-based technique has demonstrated to be an effective approach to overcome the ionization problems observed for FOSEs and FTOHs using the standard PS set-up. Under PS-APPI conditions, FTOHs and FOSEs have shown a high tendency to generate superoxide adduct ions $[M+O_2]^{-\bullet}$ while the deprotonated molecule ion $[M–H]^-$ has been mainly detected for FOSAs. The krypton-lamp position (set horizontally at 30 ºC and at 1 cm from the sharp tip) and the spray solvent (toluene) have been the most critical working parameters to obtain the best signal intensity. Method quality

parameters, such as MLODs (at low µg L^{-1} levels), linearity (R^2 >0.998), intra-day precision (RSD% <18%) and trueness (relative errors <18%), have demonstrated the good performance of the PS-APPI-HRMS method proposed. Moreover, the analysis of waterproof impregnation sprays by PS-APPI-HRMS revealed the presence of several neutral PFASs up to mg L^{-1} levels, being the presence of 6:2 FTOH the most abundant one. These results demonstrated the potential of the proposed PS-APPI-HRMS method for the fast determination of these neutral fluorinated compounds in waterproof impregnation sprays, but the method could be further applied for fast monitoring of neutral PFASs in other samples to study the human exposure to these substances. Further studies should be performed to evaluate the general applicability of PS-APPI for the analysis of other low polar/non polar compounds.

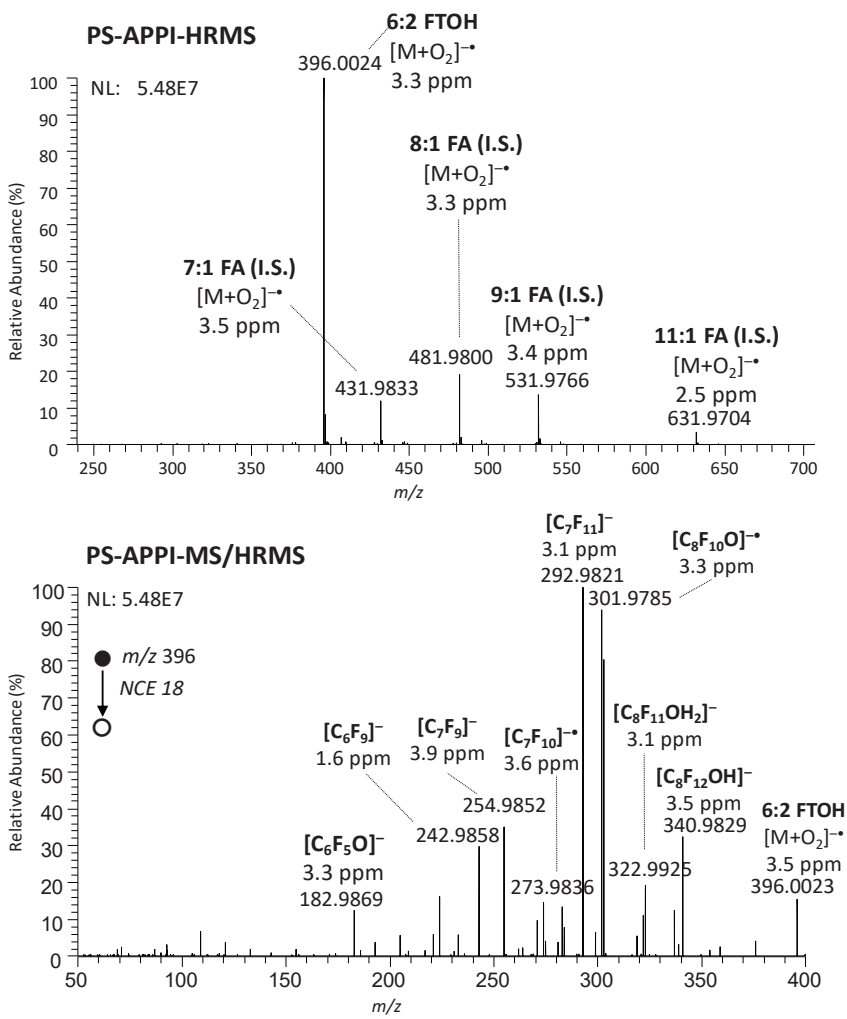


Fig. 6 PS-APPI-HRMS(/MS) spectra of a positive waterproof impregnation spray sample (WP-06) containing 6:2 FTOH (160 ± 4 mg L^{-1}).

Acknowledgments

The authors gratefully acknowledge the financial support received from Spanish Ministry of Economy and Competitiveness (CTQ2015-63968-C2-1-P), from the Spanish Ministry of Science, Innovation and Universities (PGC2018-095013-B-I00) and from the Agency for Administration of University and Research Grants (Generalitat de Catalunya, Spain) (2017SGR-310). Raquel Seró is grateful to the University of Barcelona for an APIF grant. Juan Francisco Ayala-Cabrera thanks the Spanish Ministry of Education, Culture and Sports for the FPU fellowship and the Water Research Institute (IdRA) from University of Barcelona for the PhD research financial assistance. The authors also acknowledge Professor M.T. Galceran for her willingness during the discussions in this paper.

Conflict of Interest:

The authors declare that they have no conflict of interest.

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INFORMACIÓ SUPLEMENTÀRIA A LA PUBLICACIÓ VI

Paper Spray-Atmospheric Pressure Photoionization-High Resolution Mass Spectrometry for the Direct Analysis of Neutral Fluorinated Compounds in Waterproof Impregnation Sprays

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Analytical and Bioanalytical Chemistry, enviat

Supporting Information

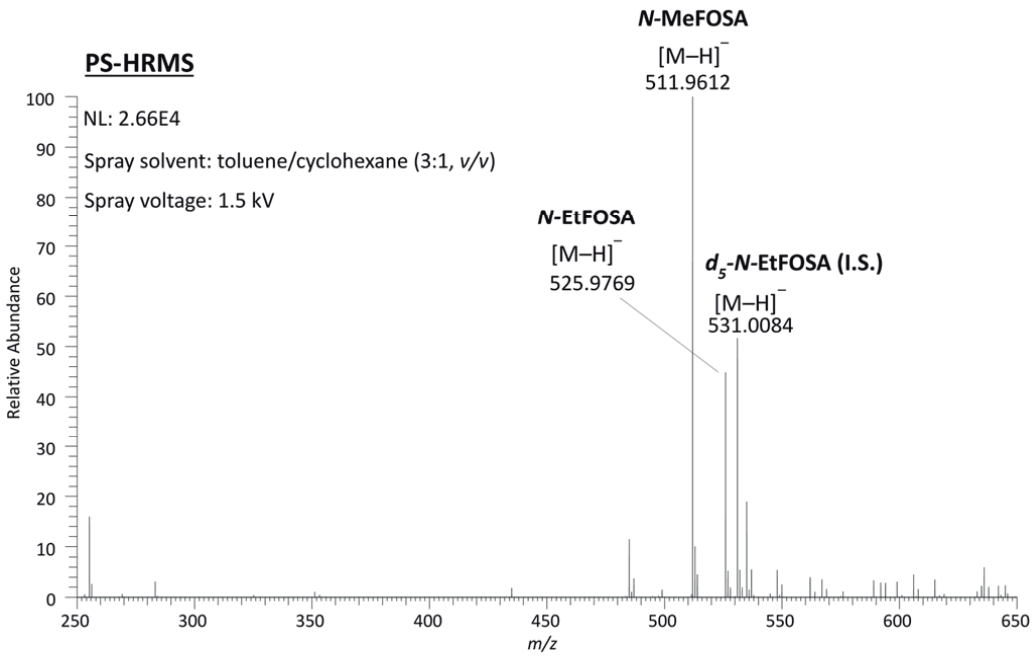

Figure S1 Negative-ion PS-HRMS mass spectrum of a standard solution mixture of the target compounds (10 mg L^{-1}) using toluene/cyclohexane (3:1, *v/v*) as spray solvent and the spray voltage set at 1.5 kV.

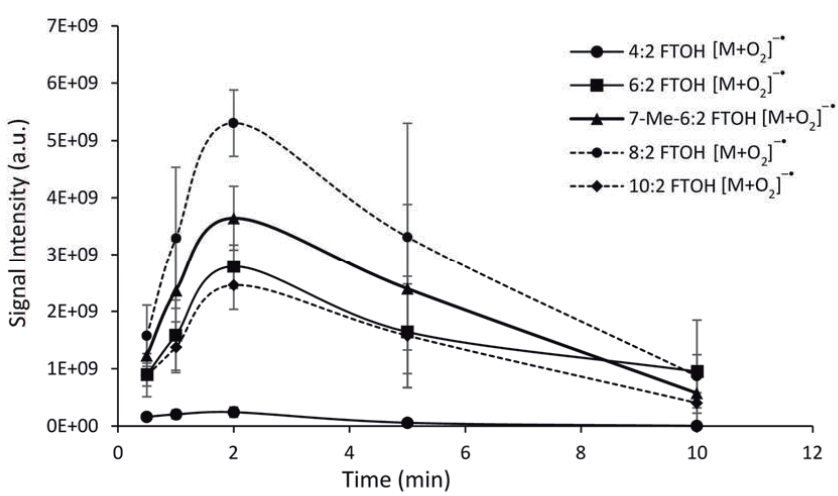

Figure S2 Effect of the drying time in the response of FTOHs ions.

Table S1. PS-APPI-MS/HRMS product ions, normalized collision energies (NCEs) and mass errors (ppm) for target FTOHs, FOSAs and FOSEs.

Compound	Precursor Ion			MS/HRMS Product ions	
	m/z	Ion Assignment	NCE (%)	*m/z* (Rel. Ab., %)	Ion Assignment (Mass Error, ppm)
4:2 FTOH	296.0103	$[C_6F_9H_4OH+O_2]^{-\bullet}$	22	296.0102 (6%)	$[C_6F_9H_5O+O_2]^{-\bullet}$ (0.3)
				240.9906 (30%)	$[C_6F_8OH]^-$ (0.4)
				222.9998 (16%)	$[C_6F_7HOH]^-$ (0.4)
				202.9940 (61%)	$[C_6F_6OH]^-$ (1.5)
				192.9896 (100%)	$[C_5F_7]^-$ (1.0)
				173.9912 (13%)	$[C_5F_6]^{-\bullet}$ (1.1)
				142.9925 (15%)	$[C_4F_5]^-$ (0.7)
				123.9941 (19%)	$[C_4F_4]^{-\bullet}$ (0.8)
6:2 FTOH	396.0038	$[C_8F_{13}H_4OH+O_2]^{-\bullet}$	18	396.0038 (15%)	$[C_8F_{13}H_4OH+O_2]^{-\bullet}$ (0.3)
				340.9842 (25%)	$[C_8F_{12}OH]^-$ (0.3)
				322.9938 (20%)	$[C_8F_{11}HOH]^-$ (0.9)
				301.9796 (100%)	$[C_8F_{10}O]^{-\bullet}$ (0.3)
				292.9833 (85%)	$[C_7F_{11}]^-$ (1.0)
				273.9847 (15%)	$[C_7F_{10}]^{-\bullet}$ (0.4)
				254.9861 (30%)	$[C_7F_9]^-$ (0.4)
				242.9860 (24%)	$[C_6F_9]^-$ (0.8)
				182.9873 (10%)	$[C_6F_5O]^-$ (1.1)
7-Me-6:2 FTOH	446.0007	$[C_9F_{15}H_4OH+O_2]^{-\bullet}$	17	446.0007 (7%)	$[C_9F_{15}H_4OH+O_2]^{-\bullet}$ (0.4)
				390.9812 (9%)	$[C_9F_{14}OH]^-$ (0.8)
				372.9908 (8%)	$[C_9F_{13}HOH]^-$ (1.1)
				342.9799 (44%)	$[C_8F_{13}]^-$ (0.3)
				323.9815 (100%)	$[C_8F_{12}]^{-\bullet}$ (0.3)
				304.9831 (39%)	$[C_8F_{11}]^-$ (0.3)
				274.9925 (37%)	$[C_7HF_{10}]^-$ (0.4)
				254.9861 (47%)	$[C_7F_9]^-$ (0.4)
8:2 FTOH	495.9977	$[C_{10}F_{17}H_4OH+O_2]^{-\bullet}$	16	495.9977 (10%)	$[C_{10}F_{17}H_4OH+O_2]^{-\bullet}$ (0.8)
				402.9812 (65%)	$[C_{10}F_{14}OH]^-$ (0.7)
				401.9734 (100%)	$[C_{10}F_{14}O]^{-\bullet}$ (0.7)
				392.9769 (60%)	$[C_9F_{15}]^-$ (0.8)
				373.9785 (10%)	$[C_9F_{14}]^{-\bullet}$ (0.8)
				354.9800 (30%)	$[C_9F_{13}]^-$ (0.6)
				342.9799 (20%)	$[C_8F_{13}]^-$ (0.3)
				323.9816 (8%)	$[C_8F_{12}]^{-\bullet}$ (0.6)
				232.9842 (5%)	$[C_7F_7O]^-$ (0.4)
10:2 FTOH	595.9916	$[C_{12}F_{21}H_4OH+O_2]^{-\bullet}$	15	595.9916 (12%)	$[C_{12}F_{21}H_4OH+O_2]^{-\bullet}$ (1.2)
				501.9672 (100%)	$[C_{12}F_{18}O]^{-\bullet}$ (1.0)
				492.9707 (55%)	$[C_{11}F_{19}]^-$ (1.0)
				454.9737 (40%)	$[C_{11}F_{17}]^-$ (0.7)
				442.9737 (15%)	$[C_{10}F_{17}]^-$ (0.7)
				292.9833 (10%)	$[C_7F_{11}]^-$ (1.0)
N-MeFOSA	511.9621	$[C_9F_{17}H_4NSO_2–H]^-$	37	511.9621 (7%)	$[C_9F_{17}H_4NSO_2–H]^-$ (0.4)
				268.9832 (5%)	$[C_5F_{11}]^-$ (0.7)
				218.9859 (16%)	$[C_4F_9]^-$ (1.4)
				168.9893 (100%)	$[C_3F_7]^-$ (0.6)
				118.9925 (29%)	$[C_2F_5]^-$ (0.8)
				64.9702 (58%)	$[HSO_2]^-$ (1.5)
N-EtFOSA	525.9773	$[C_{10}F_{17}H_6NSO_2–H]^-$	37	525.9773 (7%)	$[C_{10}F_{17}H_6NSO_2–H]^-$ (0.4)
				268.9834 (7%)	$[C_5F_{11}]^-$ (1.5)
				218.9859 (20%)	$[C_4F_9]^-$ (1.4)
				168.9893 (100%)	$[C_3F_7]^-$ (0.6)
				118.9924 (22%)	$[C_2F_5]^-$ (1.7)
				64.9702 (23%)	$[HSO_2]^-$ (1.5)
N-MeFOSE	588.9863	$[C_{11}F_{17}H_8NSO_3+O_2]^{-\bullet}$	10	588.9863 (6%)	$[C_{11}H_8F_{17}NSO_3+O_2]^{-\bullet}$ (1.0)
				541.9728 (10%)	$[C_{10}H_5F_{17}NSO_3]^-$ (0.7)
				511.9620 (100%)	$[C_9H_3F_{17}NSO_2]^-$ (0.2)
				138.0229 (6%)	$[C_3H_8O_3NS]^-$ (0.7)
N-EtFOSE	603.0018	$[C_{12}F_{17}H_{10}NSO_3+O_2]^{-\bullet}$	10	603.0018 (3%)	$[C_{12}F_{17}H_{10}NSO_3+O_2]^{-\bullet}$ (1.0)
				541.9729 (3%)	$[C_{10}F_{17}H_5NSO_3]^-$ (0.9)
				525.9776 (100%)	$[C_{10}F_{17}H_5NSO_2]^-$ (0.2)

3.3. DISCUSSIÓ DE RESULTATS

Com ja s'ha esmentat anteriorment, l'objectiu d'aquest capítol és l'avaluació de l'aplicabilitat de dues tècniques *Ambient MS*, la TS i la PS, per a la resolució de dos problemes analítics concrets. En aquest apartat es discuteixen conjuntament els resultats obtinguts en els treballs experimentals inclosos en aquest capítol (*Publicacions V i VI*). La discussió s'ha dividit en dos apartats: adaptació i optimització dels mètodes TS-MS i PS-HRMS i anàlisi de les mostres.

3.3.1 Muntatge i optimització dels mètodes TS-MS i PS-HRMS

En aquesta tesi, les dues tècniques TS i PS-APPI s'han implementat en el grup de recerca de manera molt senzilla i fent servir components de baix cost. Com es pot observar a la Figura 3.1, ambdues tècniques presenten un disseny molt simple amb algunes diferències en la seva configuració: la posició de la sonda/substrat, el tipus de línia de transferència (capil·lar d'entrada) utilitzat per enviar els ions cap a l'interior de l'espectròmetre de masses i la manera de subministrar el dissolvent necessari per a la generació de l'esprai. Tot i que és més habitual la fabricació d'hisops mèdics amb la vareta de plàstic, en aquesta tesi s'han utilitzat hisops amb la vareta metàl·lica per tal de poder connectar-la a la font de subministrament de l'alt potencial i conduir el corrent cap a la fibra on s'aplica el dissolvent durant l'anàlisi per TS-MS (*Publicació V*, apartat 3.2.1). En el TS (Figura 3.1, a dalt) l'hisop s'ha situat verticalment, a diferència del paper triangular en el PS, que s'ha posicionat horitzontalment en el mateix eix (*on-axis*) que el capil·lar d'entrada a l'espectròmetre de masses (Figura 3.1, a baix). Aquesta diferència en la posició es deu, principalment, a la major capacitat que té la fibra de raió de l'hisop per absorbir dissolvent, fet que comporta que sigui necessari emprar cabals més grans de dissolvent per aconseguir mantenir la fibra totalment mullada. En aquesta posició, la força de la gravetat facilita que el dissolvent flueixi cap a la punta de l'hisop on es forma una semiesfera de líquid de diàmetre relativament gran a partir de la qual es genera la ploma de l'electroesprai quan s'hi aplica el potencial. Tanmateix, la disposició vertical de l'hisop dificulta la formació d'una ploma estable de l'electroesprai en emprar una línia de transferència recta com la que normalment s'utilitza en els mètodes PS-MS i LC-MS. La substitució d'aquest capil·lar per un de més llarg i amb una configuració en forma de L (Figura 3.1, a dalt) va permetre millorar l'estabilitat de manera significativa. Cal indicar que aquesta configuració en L fa disminuir l'eficàcia en la transmissió d'ions cap a l'analitzador, tot i que no va suposar cap impediment per a l'aplicació que es volia

desenvolupar, ja que els anàlits a detectar es trobaven a concentracions suficientment elevades.

En el mètode PS-APPI, la incorporació d'una làmpada de Criptó al disseny original del PS ha requerit l'optimització de la seva posició (*Publicació VI*, apartat 3.2.2). Per obtenir la millor resposta dels anàlits, la làmpada s'ha posicionat perpendicularment a l'eix axial del paper triangular (Figura 3.1, a baix), però amb un angle d'inclinació respecte al pla del paper de 30° i a 1 cm de distància de la punta del paper (Figura 4A, *Publicació VI*). A angles superiors a 45º i inferiors a 30º no s'obté cap senyal i en augmentar la distància en més d'1 cm es produeix una disminució considerable de la resposta dels anàlits, probablement

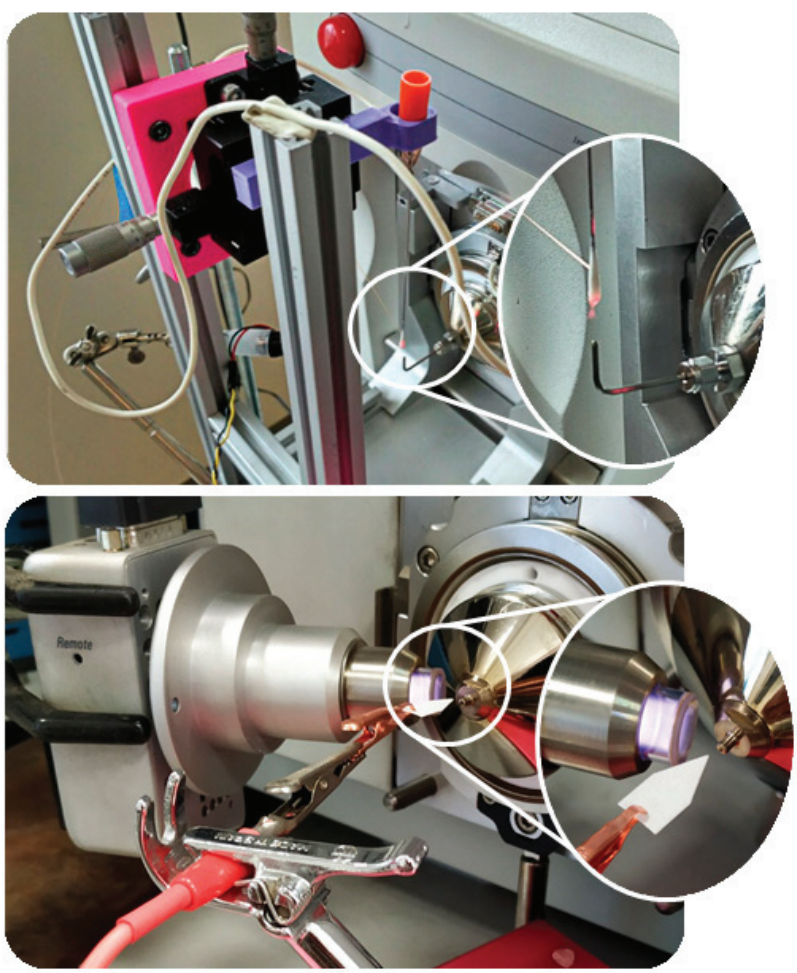

Figura 3.1. Muntatge de les fonts. TS emprant hisops (a dalt) i PS (a baix), emprant la làmpada de Criptó (PS-APPI).

causada per la dispersió del feix de llum que podria provocar l'atenuació parcial dels fotons emesos (Fig. 4A*, Publicació VI, apartat 3.2.2*).

D'altra banda, el mode de subministrament del dissolvent a l'hisop (TS) i al paper (PS) ha estat diferent per a cadascun dels mètodes. En el cas del TS, el dissolvent s'ha addicionat a l'hisop en continu a un cabal constant, mitjançant una bomba de xeringa i un capil·lar de sílice fosa en contacte amb la fibra. A l'inici de l'anàlisi, s'ha de saturar l'hisop amb el dissolvent bombejant-lo a un cabal alt (~50 μL min^{-1}) en absència de potencial fins a la formació d'una gota de líquid que quedi en suspensió a la punta. En aquest moment és quan s'ha d'aplicar l'alt potencial, a través de la connexió metàl·lica a la vareta de l'hisop, i reduir el cabal del dissolvent per tal d'evitar-ne el degoteig que desestabilitzaria l'electroesprai i, alhora, podria contaminar la línia de transferència. Cal indicar que el cabal i el valor del potencial necessari per a la formació de l'electroesprai depenen, majoritàriament, de la tensió superficial i de la volatilitat del dissolvent emprat, fet que fa necessària la seva optimització en cada cas. De forma similar, en el mètode de PS també cal dipositar un volum de dissolvent suficient que saturi (mulli completament) el paper triangular abans d'aplicar l'alt potencial però, a diferència del TS, l'addició no cal que sigui continuada sinó que es pot afegir un volum predeterminat a l'inici de l'anàlisi, després d'aplicar la mostra al paper. El volum de dissolvent varia en funció de la viscositat del dissolvent utilitzat, tot i que també depèn de la naturalesa i de la mida del paper triangular emprat. En aquesta tesi s'ha escollit el paper cromatogràfic Whatman 31ET (W31) (0.5 mm de gruix) per a l'anàlisi dels compostos PFAS emprant PS i PS-APPI (*Publicació VI*, apartat 3.2.2), ja que es va observar que la seva capacitat d'absorció i porositat permetia dipositar una major quantitat de mostra (10 μL) que en el paper Whatman de grau 1 (0.18 mm de gruix). La possibilitat de carregar un major volum de mostra ha permès assolir millors límits de detecció i mantenir el senyal durant un espai de temps més llarg. Tanmateix, la major capacitat d'absorció d'aquest paper també va implicar que fos necessari aplicar un volum més gran de dissolvent (70 μL).

La composició del dissolvent emprat en els mètodes TS i PS-APPI no només afecta a l'estabilitat de l'electroesprai sinó que, a més, pot influir en l'eficàcia d'extracció/transport dels anàlits en l'hisop/paper i en la naturalesa del mecanisme d'ionització que té lloc. En aquesta tesi, s'ha avaluat l'ús de diferents dissolvents per tal d'afavorir l'extracció dels fosfolípids en l'anàlisi de les mostres de teixit emprant TS-MS i per modificar la naturalesa del mecanisme d'ionització en PS i PS-APPI per tal d'ionitzar substàncies relativament no polars, com alguns PFASs neutres (FTOHs, FOSAs i FOSEs).

Respecte a l'anàlisi de teixits emprant TS-MS, s'ha avaluat l'ús de diversos dissolvents (*Publicació V*, apartat 3.2.1) per afavorir l'extracció del fosfolípids i dels oncometabòlits. Atesa la relativament elevada mida de la semiesfera líquida formada a la punta de la fibra de l'hisop així com les propietats físico-químiques dels dissolvents emprats, va ser necessari aplicar un potencial negatiu més elevat (entre 5.5-6.8 kV) que els utilitzats habitualment en TS amb agulles o en PS per aconseguir la generació de l'esprai a la punta. Com es pot observar a la Figura 3.2, la formació del tipus d'esprai que es genera varia en funció de la naturalesa del dissolvent, essent la tensió superficial i la volatilitat els paràmetres que hi afecten de manera més significativa. Per exemple, en utilitzar la *N,N*-dimetilformamida com a dissolvent (Figura 3.2A) no es va aconseguir formar cap ploma d'electroesprai estable a la punta de l'hisop, ja que l'elevada tensió superficial (37.9 Dines cm^{-1}) d'aquest dissolvent feia necessari aplicar potencials molt elevats que generaven descàrregues (corrents >10 μA). També ha estat impossible formar l'electroesprai emprant hexà, fet que podria estar relacionat amb l'alta volatilitat d'aquest dissolvent que dificultava l'acumulació d'un volum de líquid suficient a l'hisop abans d'aplicar-hi el potencial. En canvi, la utilització d'acetonitril, metanol, acetona i etanol (Figures 3.2 B-D) sí que va permetre la generació d'un electroesprai prou estable, tot i que la major tensió superficial del primer (30.2 Dines cm^{-1}) va requerir l'aplicació d'un potencial més elevat que per a la resta de dissolvents, que presenten tensions superficials inferiors (entre 22.9 i 24.9 Dines cm^{-1}). També és interessant assenyalar el fet que emprant acetona s'ha observat, en determinades ocasions, la formació simultània de dos plomes d'electroesprai que, aleatòriament, es fusionaven per generar una única ploma (Figura 3.2C). Aquest fenomen provocava una inestabilitat en l'electroesprai que causava una gran variabilitat en la intensitat dels ions detectats. Amb l'objectiu de millorar el comportament de l'esprai, en aquesta tesi s'ha addicionat un agent surfactant no iònic (+ β-D-glucopiranosida (0.25 mg mL^{-1}) al dissolvent, el qual va facilitar la formació d'una ploma de l'electroesprai més ampla i estable en reduir la tensió superficial del dissolvent (Figura 3.2E). D'altra banda, també s'ha avaluat l'efecte d'emprar mescles de dissolvents binàries i ternàries tant en l'estabilitat de l'electroesprai com en l'extracció dels fosfolípids i dels oncometabòlits. Les mescles que contenien acetona amb acetonitril, metanol i/o etanol generaven esprais polsats inestables, mentre que les mescles d'etanol amb acetonitril i/o metanol afavorien la formació de plomes més amples i estables. D'altra banda, tot i que la *N,N*-dimetilformamida és un molt bon agent extractant pels fosfolípids, únicament es van aconseguir la generació d'electroesprais estables amb mescles que

contenien una proporció d'aquest dissolvent inferior al 5%, atesa la seva ja mencionada elevada tensió superficial.

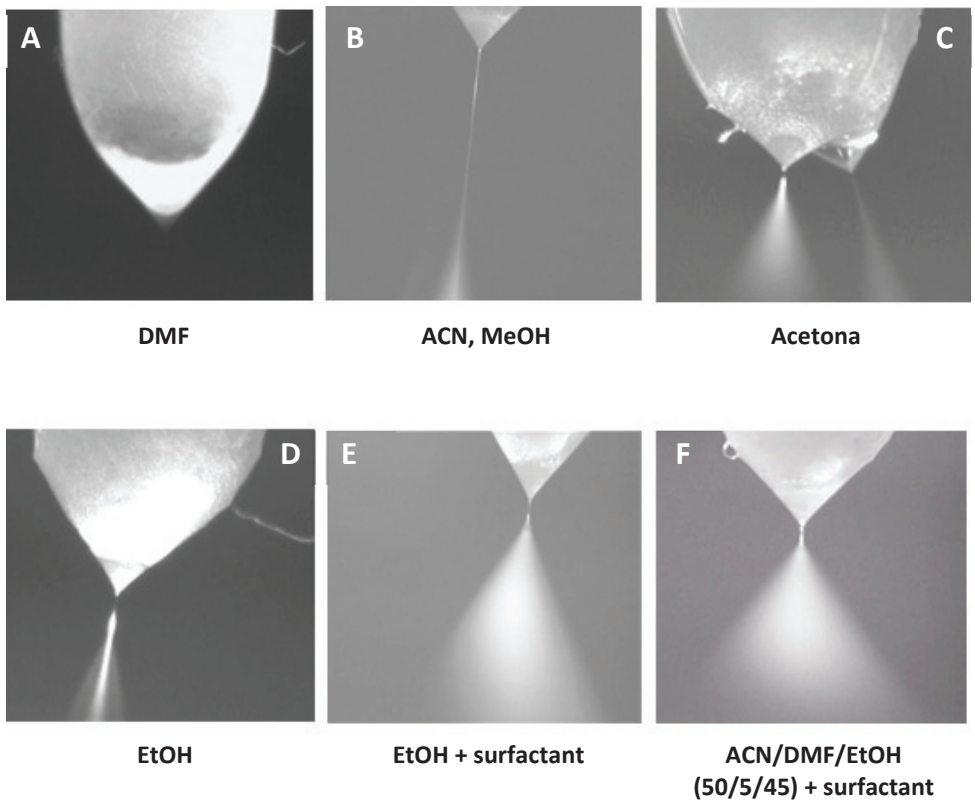

Figura 3.2. Fotografies dels electroesprais generats emprant diferents dissolvents: (A) *N,N*-dimetilformamida, -6.8 kV; (B) acetonitril, 6.5 kV i metanol, 6 kV (18 μL min^{-1}); (C) acetona (35 μL min^{-1}), 5.5 kV; (D) etanol (25 μL min^{-1}), 6 kV; (E) etanol (25 μL min^{-1})+ β-D-glucopiranosida (0.25 mg mL^{-1}), 6 kV; (F) acetonitril: *N,N*-dimetilformamida:etanol (50:5:45, *v/v*) (25 μL min^{-1}) + β-D-glucopiranosida (0.25 mg mL^{-1}), 6.5 kV.

Així, la mescla proposada per a l'anàlisi de teixit cerebral per TS-MS és acetonitril:etanol:*N,N*-dimetilformamida (50:45:5, *v/v*) (*Publicació V,* apartat 3.2.1). El subministrament d'aquesta mescla a un cabal de 25 μL min^{-1} i aplicant un potencial de 6.5 kV ha permès, per una banda, la formació d'un electroesprai estable a la punta de l'hisop durant tot el temps de l'anàlisi (Figura 3.2F) i, per l'altra, facilitar l'extracció i la ionització dels fosfolípids i oncometabòlits en les mostres de teixit. En aquestes condicions de treball, es va observar que aquests compostos s'ionitzaven en mode negatiu formant generalment l'ió $[M–H]^-$, encara que, com es discuteix més endavant, també es van detectar adductes amb l'ió clorur $[M+Cl]^-$ per alguns dels glicerofosfolípids característics (*Publicació V*, apartat 3.2.1).

Pel que fa al PS, ja s'ha apuntat que la composició del dissolvent també pot influir en el mecanisme d'ionització que té lloc durant l'anàlisi. En aquesta tesi, s'ha estudiat la naturalesa del mecanisme d'ionització dels FOSAs, FTOHs i FOSEs emprant diferents dissolvents en PS i en PS-APPI. Com es discuteix a la *Publicació VI* (*apartat 3.2.2*), es va analitzar una dissolució patró que contenia els FTOHs, FOSEs i FOSAs per PS utilitzant metanol:toluè (90:10, *v:*v) com a dissolvent i un potencial de 3.5 kV. En aquestes condicions experimentals, únicament es van detectar ions corresponents als compostos FOSAs, possiblement perquè són els més àcids i, per tant, els que s'ionitzen més fàcilment en fase líquida per desprotonació $[M–H]^-$ (Fig. 3A, *Publicació VI*), ja que aquesta mescla més polar afavoreix el mecanisme d'ionització per electroesprai. Per tal de forçar la ionització dels FTOHs i dels FOSEs pel mecanisme d'ionització química en la fase gas es va dur a terme l'anàlisi emprant dissolvents no polars. L'ús d'aquests dissolvents permet minimitzar la ionització via electroesprai i, a la vegada, produir un camp molt intens i un petit plasma (corona) a la punta del paper en aplicar l'alt potencial que desencadena una sèrie de reaccions ió-molècula en fase gas de forma similar al mecanisme d'APCI. Tanmateix, l'espectre de masses PS-HRMS obtingut en emprar toluè:ciclohexà (75:25, *v/v*) i un potencial de 2.5 kV (Figura S1, Informació suplementària a la *Publicació VI*) continuava mostrant únicament els ions corresponents als FOSAs $[M-H]^-$, però a una intensitat del senyal dos ordres de magnitud inferior a l'observada usant dissolvents polars. Això fa pensar que aquestes condicions experimentals no produïen especies d'afinitat protònica suficientment elevada per aconseguir ionitzar eficaçment els FOSAs via transferència protònica en la fase gas.

Atesos els bons resultats obtinguts prèviament al grup de recerca en la ionització de FOTHs, FOSAs i FOSEs en LC-MS emprant APPI (Ayala-Cabrera et al., 2018), en aquesta tesis s'ha considerat que la combinació del PS amb l'APPI podria ser una bona alternativa per a la ionització d'aquests compostos (*Publicació VI,* apartat 3.2.2). Els PFASs s'ionitzen en APPI en el mode negatiu i, per tant, és necessari l'ús d'un dopant per iniciar la cascada de reaccions ió-molècula en fase gas a partir de la fotoionització d'aquesta substància. Així, el dissolvent del PS-APPI, a més d'afavorir l'extracció i el transport dels anàlits cap a la punta del paper, ha de permetre la desorció de les molècules neutres cap a la fase gas i ha d'actuar com a dopant en el mecanisme d'APPI. Es van assajar diferents dissolvents potencialment compatibles amb aquestes funcions (acetona, tetrahidrofurà, clorobenzè, anisol i toluè) i es van detectar els mateixos ions en tots els casos. A mode d'exemple, a la Figura 3.3 es mostren els espectres de masses extrets a diferents temps (Figura 3.3B i C) durant l'anàlisi d'una

dissolució patró de PFASs per PS-APPI emprant toluè com a dissolvent. Com es pot observar, tant els FTOHs com els FOSEs formen principalment adductes amb l'ió radical superòxid $[M+O_2]^{-\bullet}$ (Figura 3.3B), mentre que els FOSAs donen l'ió de desprotonació $[M-H]^-$ (Figura 3.3C). El mecanisme d'ionització pels FTOHs i FOSEs s'inicia amb la fotoionització del dopant per generar el catió radical i alliberar un electró que pot ser capturat per l'oxigen atmosfèric per formar l'ió superòxid $O_2^{-\bullet}$, el qual pot interaccionar posteriorment amb l'anàlit per formar l'ió adducte $[M+O_2]^{-\bullet}$ (Esquema 3.1.). En canvi, l'ió $[M-H]^-$ dels FOSAs s'obté via transferència protònica en fase gas. Com es comenta a la *Publicació VI*, l'augment significatiu en la resposta d'aquest ió $[M–H]^-$ en PS-APPI davant

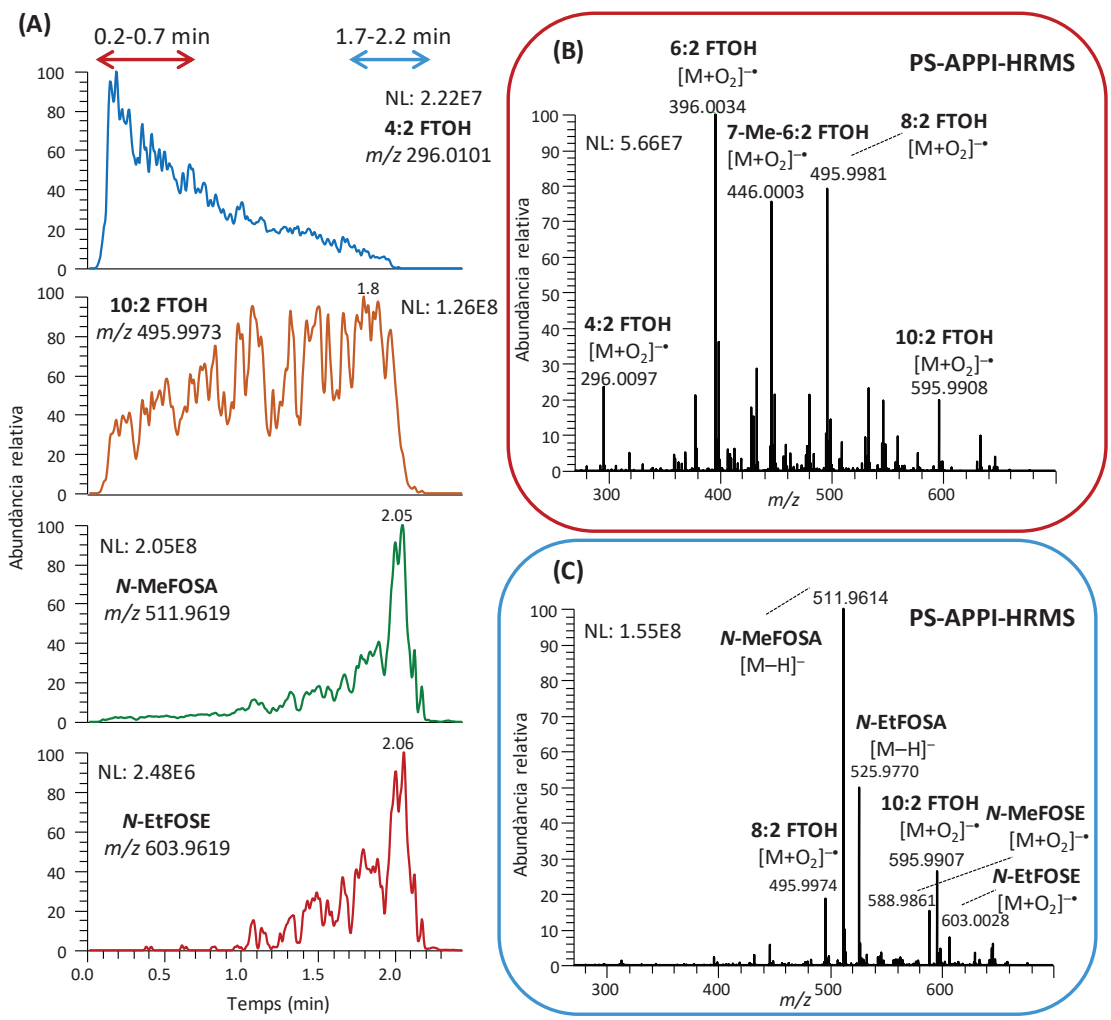

Figura 3.3. Anàlisi d'una mescla de patrons d'una concentració de 10 µg mL^{-1} pels compostos FTOHs i de 5 µg mL^{-1} pels FOSAs o FOSEs emprant el mètode PS-APPI-HRMS: (A) Perfils corresponents a la corrent total d'ions seleccionats (5 ppm d'error), (B) Espectre de masses mitjà obtingut entre els minuts 0.2 i 0.7, (C) Espectre de masses mitjà obtingut entre els minuts 1.7 i 2.2.

l'observada en PS convencional pot estar relacionat, en part, amb la generació en APPI d'espècies en fase gas amb afinitats protòniques més elevades que les dels FOSAs, la qual cosa podria afavorir la desprotonació d'aquests compostos.

$$D + h\nu \rightarrow D^{+\bullet} + e^{-}$$
$$O_2 + e^{-} \rightarrow [O_2]^{-\bullet}$$
$$M + O_2^{-\bullet} \rightarrow [M + O_2]^{-\bullet}$$
$$M + O_2^{-\bullet} \rightarrow [M-H]^{-} + HO_2^{\bullet}$$

Equacions 3.1. Reaccions d'ionització dels anàlits en el mode d'ionització negatiu per PS-APPI. (M = anàlit, D = dopant).

Entre els diferents paràmetres que afecten a la resposta dels anàlits per PS-APPI, s'ha observat que la composició del dissolvent i el valor del potencial aplicat en PS-APPI influeixen de forma important. A més, aquests paràmetres són interdependents entre si, cosa que fa que la major resposta dels anàlits per a cada un dels dissolvent assajats (acetona, tetrahidrofurà, clorobenzè, anisol i toluè) s'hagi obtingut a un potencial òptim diferent. Tenint en compte aquestes consideracions, les millors respostes dels anàlits s'han obtingut en utilitzar toluè com a dissolvent (Figura 4B de la *Publicació VI*), un dels dopants més freqüentment emprats en APPI i que també va proporcionar els millors resultats per aquesta família de compostos en LC-APPI-MS, tot i que també s'han observat bones respostes pel *N*-EtFOSA en utilitzar el clorobenzè i l'anisol com a dissolvents. Pel que fa a l'efecte del potencial aplicat en la resposta, a la Figura 3.4 es mostra, a tall d'exemple, l'abundància relativa dels ions generats per PS-APPI de tres FTOHs, un FOSA i un FOSE a diferents potencials d'ionització (entre 0.5 i 4 kV) usant toluè com a dissolvent. Com es pot observar, l'abundància relativa dels ions corresponents al 4:2 FTOH i 6:2 FTOH és màxima en aplicar un voltatge d'aproximadament 2.5 kV. Aquest valor augmenta pel 10:2 FTOH (2.5 kV) i encara augmenta més pels FOSAs i FOSEs, pels quals el màxim es troba a 3 kV. El valor del potencial on es troben els màxims sembla que es correlaciona amb la volatilitat d'aquests compostos i això fa pensar que la desorció de les molècules neutres cap a la fase gas en presència de dissolvents no polars podria estar relacionada amb la intensitat del camp elèctric que es genera a la punta del paper, que podria afavorir la seva desorció. Com a resultat

d'aquest estudi, en aquesta tesi s'ha seleccionat el toluè com a dissolvent i es proposa aplicar un potencial de 2.5 kV per a la determinació dels PFAS en el mètode PS-APPI.

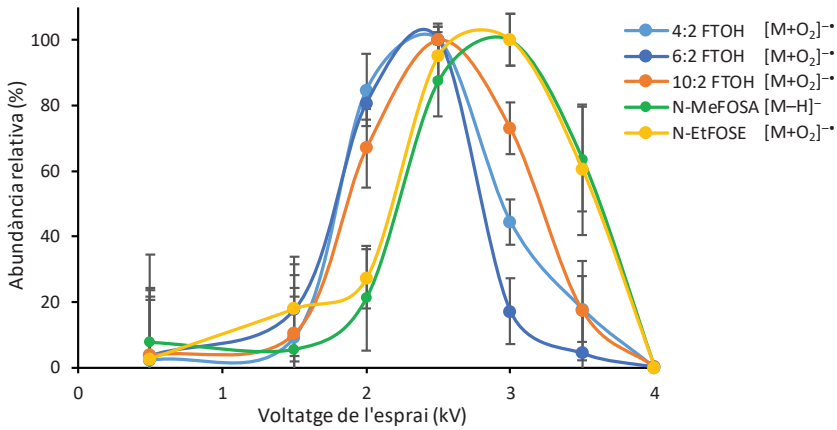

Figura 3.4. Abundàncies relatives dels ions corresponents als compostos 4:2FTOH, 6:2FTOH, 8:2FTOH, *N*-MeFOSA i *N*-EtFOSE en aplicar diferents valors de potencial (kV) emprant el mètode PS-APPI.

Un altre fet que es pot relacionar amb la volatilitat relativa dels PFASs és, com es discuteix a la *Publicació VI* (apartat 3.2.2), la variació dels espectres de masses obtinguts a diferents temps d'adquisició (Figura 3.3). Com es pot observar a la Figura 3.3B, a l'espectre de masses obtingut a l'inici del cronograma (0.2 - 0.7 min) només es detecta la presència dels FTOHs, mentre que els ions corresponents als FOSAs i FOSEs només s'observen en els espectres de masses adquirits cap al final del cronograma (Figura 3.3C). Aquest comportament concorda amb els perfils corresponents a la corrent dels ions seleccionats (Figura 3.3A). La intensitat del senyal del 4:2 FTOH és màxima a l'inici de l'anàlisi, moment a partir del qual la resposta de l'ió disminueix progressivament. En canvi, l'abundància relativa dels ions dels FOSEs i FOSAs augmenta significativament cap al final del cronograma, entre els minuts 1.7 i 2.2. Això sembla indicar que els compostos més volàtils (4:2 FTOH) es poden transferir cap a la fase gas a l'inici de l'anàlisi, encara que el cabal del dissolvent sigui més gran, condicions que impliquen la generació d'un esprai de gotes a la punta del paper d'una mida més gran. El consum del dissolvent al llarg de l'anàlisi, a més de produir una disminució en la mida de les gotes de l'esprai, també podria produir un augment del camp elèctric a la punta del paper quan es produeix l'esgotament del dissolvent, la qual cosa podria afavorir l'evaporació assistida per camp dels anàlits menys volàtils (FOSAs i FOSEs).

3.3.2 Anàlisi de mostres per TS-MS i PS-APPI-HRMS

Atès que els àmbits de l'anàlisi dels dos treballs inclosos en aquest Capítol de la tesi són molt diferents, les estratègies plantejades per tal de resoldre els corresponents problemes analítics també han estat força diferents. Així, en la *Publicació V* (apartat 3.2.1), on es pretén utilitzar la tècnica TS-MS per a l'anàlisi ràpida de teixits neurològics per al diagnòstic de càncer, s'ha utilitzat l'espectrometria de masses per a l'obtenció i l'anàlisi de perfils lipídics. En concret, per a l'anàlisi de mostres amb el mètode de TS-MS (hisops) es proposa emprar un analitzador de masses de trampa d'ions lineal (LIT), que permet enregistrar els espectres de masses en el mode d'escombratge d'ions totals (*full scan*) amb una sensibilitat suficient per obtenir informació molecular rellevant. A més, la utilització d'una trampa d'ions, que pot realitzar experiments de tàndem en el temps (MS^2 i MS^3), ha facilitat la identificació estructural dels ions discriminants i ha incrementat la selectivitat en la monitorització dels metabòlits més rellevants per al diagnòstic (*Publicació V*, apartat 3.2.1). En canvi, en la *Publicació VI* (apartat 3.2.2), que pretén la determinació de PFASs, s'ha combinat el PS-APPI amb un analitzador de masses d'alta resolució Q-Orbitrap amb el propòsit d'aconseguir la suficient selectivitat i sensibilitat per monitoritzar i determinar de manera ràpida els FTOHs, FOSEs i FOSAs escollits com a compostos model. L'adquisició dels espectres de masses en el mode de *full scan* a una resolució 70,000 FWHM (*m/z* 200) ha possibilitat la monitorització i la quantificació dels compostos diana amb una bona exactitud en la mesura de la massa (errors < 5 ppm) i, a més, ha permès l'anàlisi retrospectiva de les mostres per a la monitorització d'altres compostos sospitosos de ser-hi presents. Per tal de confirmar inequívocament la presència dels PFASs en les mostres, en aquesta tesi també s'ha dut a terme la caracterització, mitjançant l'adquisició dels espectres de masses d'ions producte (MS/HRMS) en alta resolució (Taula S1, Informació Suplementària de la *Publicació VI*). Els espectres de masses de PS-APPI-MS/HRMS adquirits són coherents amb els obtinguts prèviament en el grup de recerca utilitzant un mètode LC-APPI-MS/HRMS (Ayala-Cabrera et al., 2019).

El potencial del mètode TS-MS desenvolupat en aquesta tesi s'ha avaluat analitzant mostres de teixit humà cerebral (Taula S1, Informació suplementària de la *Publicació V*). L'anàlisi de les mostres es va dur a terme al laboratori de la Universitat de Purdue simulant l'estratègia que es podria seguir en els procediments de resecció de tumors per al mostreig *in vivo* de teixit. El procediment és senzill; simplement es toca la superfície del teixit amb l'hisop estèril per transferir una quantitat mínima de mostra a la fibra de l'hisop (Figura 1 de la *Publicació*

V, apartat 3.2.1) i s'analitza directament per espectrometria de masses. Per corregir les variacions en els senyals produïts per les petites diferències de mida dels hisops comercials així com les causades per la seva posició en el sistema TS i per l'electroesprai generat, es proposa afegir un patró intern (metabòlit NAA-d_3, 10 μg mL^{-1}) al dissolvent del TS. Amb aquest procediment s'ha aconseguit obtenir espectres de masses de qualitat i reproduïbles en analitzar hisops amb una quantitat de teixit d'entre 1.0 i 10.0 mg, molt menor que la que cal extirpar per a l'anàlisi de teixit *ex vivo* emprant la tècnica DESI-MS o pels estudis patològics. Els perfils lipídics que s'han obtingut en aquesta tesi amb el mètode TS-MS (mode d'ionització negatiu), en analitzar tant teixit corresponent a la parènquima cerebral normal (formada per substància blanca i substància grisa) com teixit tumoral (gliomes), són similars als obtinguts prèviament per DESI-MS (Jarmusch et al., 2016). L'espectre de masses TS-MS de la substància grisa, formada majoritàriament per neurones no mielinitzades i cèl·lules glials, es caracteritza per presentar com a pic base l'ió a *m/z* 834, el qual correspon a la molècula desprotonada de la fosfatidilserina (FS) 40:6 (Figura 2A de la *Publicació V*), mentre que l'espectre de masses de la substància blanca es caracteritza per la presència dels ions a *m/z* 788, 888 (pic base) i 904, identificats respectivament com els ions $[M–H]^–$ dels lípids FS 18:0-18:1, (3'-sulfo)GalCer 24:1 i (3'-sulfo)GalCer 24:1(OH), a abundàncies relatives significativament més elevades que en la substància grisa. Per tant, l'augment de la resposta d'aquests ions es pot atribuir a una major concentració de mielina en la substància blanca (Figura 2B de la *Publicació V*). Pel que fa a l'espectre de masses TS-MS dels gliomes, aquest és molt diferent de l'obtingut per la parènquima cerebral normal, ja que la intensitat relativa dels ions a *m/z* 834 i 888, característics de la substància blanca i grisa respectivament, disminueix significativament fins a valors inferiors al 10%, mentre que l'ió a *m/z* 794, corresponent a l'adducte clorat $[M+Cl]^–$ de la fosfatildilcolina (PC) 34:1, augmenta considerablement. A més, en l'espectre de masses apareix l'ió a *m/z* 768 (ió $[M+Cl]^–$ del lípid PC 32:0), el qual no s'observa en la parènquima cerebral normal (Fig. 2C, *Publicació V*). Aquestes diferències en els perfils lipídics són les que permeten detectar la presència del glioma. Ara bé, els perfils lipídics obtinguts en aquesta tesi en l'anàlisi de les mostres (Taula S1, informació suplementària de la *Publicació V*) ha posat de manifest la complexitat i la heterogeneïtat dels gliomes, els quals s'infiltren, en diferent grau, en la parènquima cerebral normal. A mode d'exemple, a la Figura 3.5 es mostren els espectres de masses obtinguts en analitzar per TS-MS dues regions (mostres 29 i 30) d'un teixit (cas 18) emprant dos hisops (t1 i t2). Com es pot observar a la Figura 3.5A, el perfil lipídic de la zona t1 és el propi de la substància grisa (pic base a *m/z* 834), resultat que concorda amb les

lectures patològiques. En canvi, en l'espectre de masses de la zona t2 (Figura 3.5A) es detecten els ions característics tant de la substància blanca (*m/z* 788, 888 i 904) com de la grisa (*m/z* 834) i del glioma (*m/z* 768, 794 i 885), cosa que indica la infiltració del tumor en la parènquima cerebral normal.

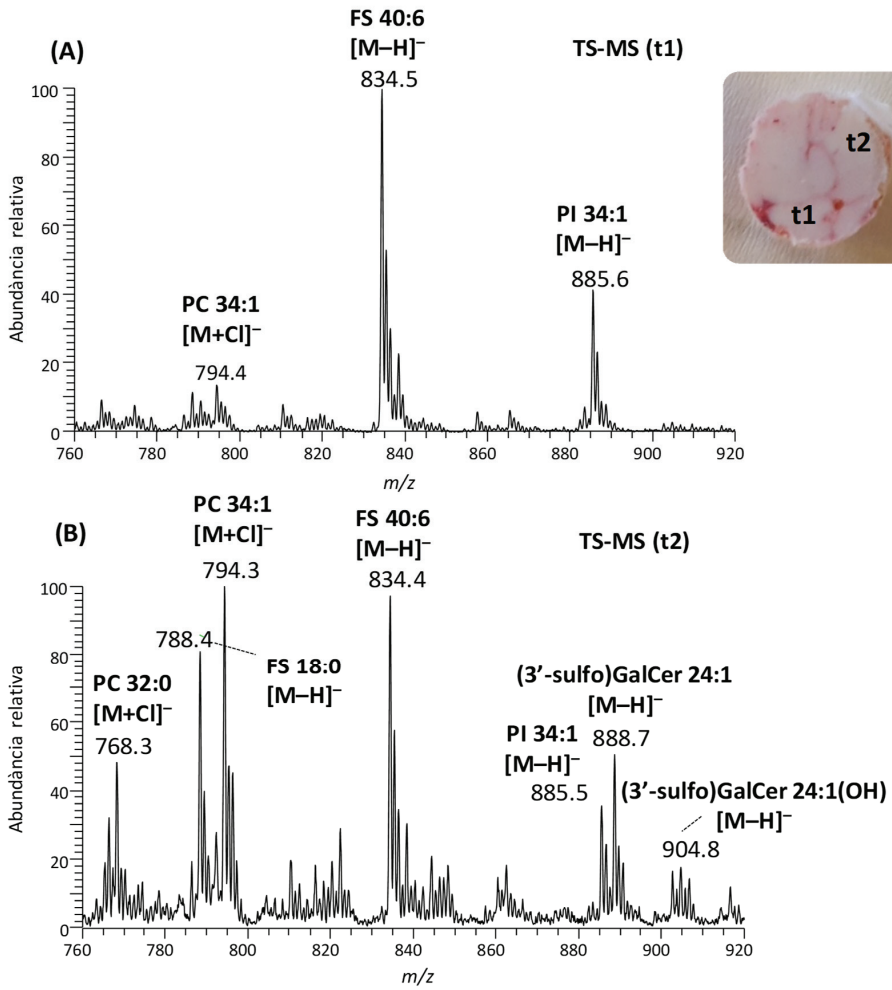

Figura 3.5. Espectres de masses de TS-MS (mode negatiu) en *full scan* obtinguts en analitzar els hisops utilitzats per mostrejar dues zones de la mostra d'un teixit (cas 18). (A) mostreig t1 (mostra 29), lectura patològica: substància grisa amb un TCP del 40%, i (B) mostreig t2 (mostra 30), lectura patològica: glioma amb un TCP del 40%. Llistat de casos i mostres en la Taula S1(*Publicació V*).

En aquesta tesi també s'ha avaluat la resposta en les mostres de dos metabòlits neuronals, el *N*-acetil aspartat (NAA, *m/z* 174) (un dels més abundants en la parènquima cerebral normal) i el 2-hidroxiglutarat (2HG, *m/z* 147) (més abundant en els gliomes que presenten una mutació en el domini d'unió del substrat de l'enzim isocitrat deshidrogenasa, IDH). A la Figura 3.6 A-C (a l'esquerra) es mostren els espectres d'ions producte (MS^2) del *N*-acetil

aspartat (NAA), normalitzats en base a l'espectre de masses MS^2 del patró intern (NAA-d_3, transició *m/z* 177 → 116). Com es pot observar, es produeix una disminució de la intensitat del senyal del pic base (*m/z* 114) en les mostres de teixit en què s'ha observat un perfil lipídic característic de gliomes (Figura 3.6 B i C). Aquesta disminució es pot correlacionar amb el percentatge relatiu de les cèl·lules tumorals en base a les normals (TCP, *tumor control probability*), percentatge que proporciona una estimació del grau d'infiltració del tumor en el teixit sa, ja que es va observar que una disminució en la resposta del NAA sempre venia acompanyada d'un augment del TCP. L'anàlisi estadística de les dades en base a la resposta normalitzada de l'ió producte a *m/z* 114 del NAA ha permès realitzar una estimació qualitativa del TCP en els teixits (nivell baix, mitjà o alt) (Figura 3 de la *Publicació V*), que resulta de gran importància quan s'intenta extirpar la major part del glioma.

Pel que fa al 2HG, en algunes de les mostres analitzades es va detectar la presència d'ions isobàrics que interferien amb l'ió $[M–H]^–$ corresponent a aquest metabòlit (*m/z* 147). Tot i així, l'espectrometria de masses en múltiples etapes (MS^n) ha proporcionat la selectivitat adequada per evitar aquestes interferències en la detecció del 2HG. Per aquest motiu, en aquesta tesi s'ha adquirit l'espectre de masses de MS^3 de l'ió producte a *m/z* 129. De la mateix manera que amb el NAA, aquest espectre MS^3 es va normalitzar en base al senyal de l'ió producte a *m/z* 116 del patró intern NAA-d_3. Com es pot observar a la Figura 3.6C (a la dreta), l'abundància relativa de l'ió producte a *m/z* 101 augmenta significativament en la mostra 47, en què s'ha identificat patològicament un glioma amb mutació IDH, en comparació amb l'obtinguda en l'anàlisi de les mostres en què no s'ha diagnosticat la mutació, com ara en la 39 i en la 30 (Figura 3.6A-B, a la dreta). L'anàlisi dels resultats obtinguts ha posat de manifest que una intensitat relativa i normalitzada del senyal del 2HG superior o igual a 1.02 permet diagnosticar amb un 100% de certesa la mutació IDH en els teixits (Figura 5A, *Publicació V*). Cal emfatitzar que la identificació d'aquesta mutació IDH, diagnòstic que pot tardar setmanes en obtenir-se mitjançant els estudis patològics, és de gran rellevància diagnòstica, doncs els pacients que pateixen la mutació presenten un millor pronòstic de supervivència amb un tractament mèdic adequat.

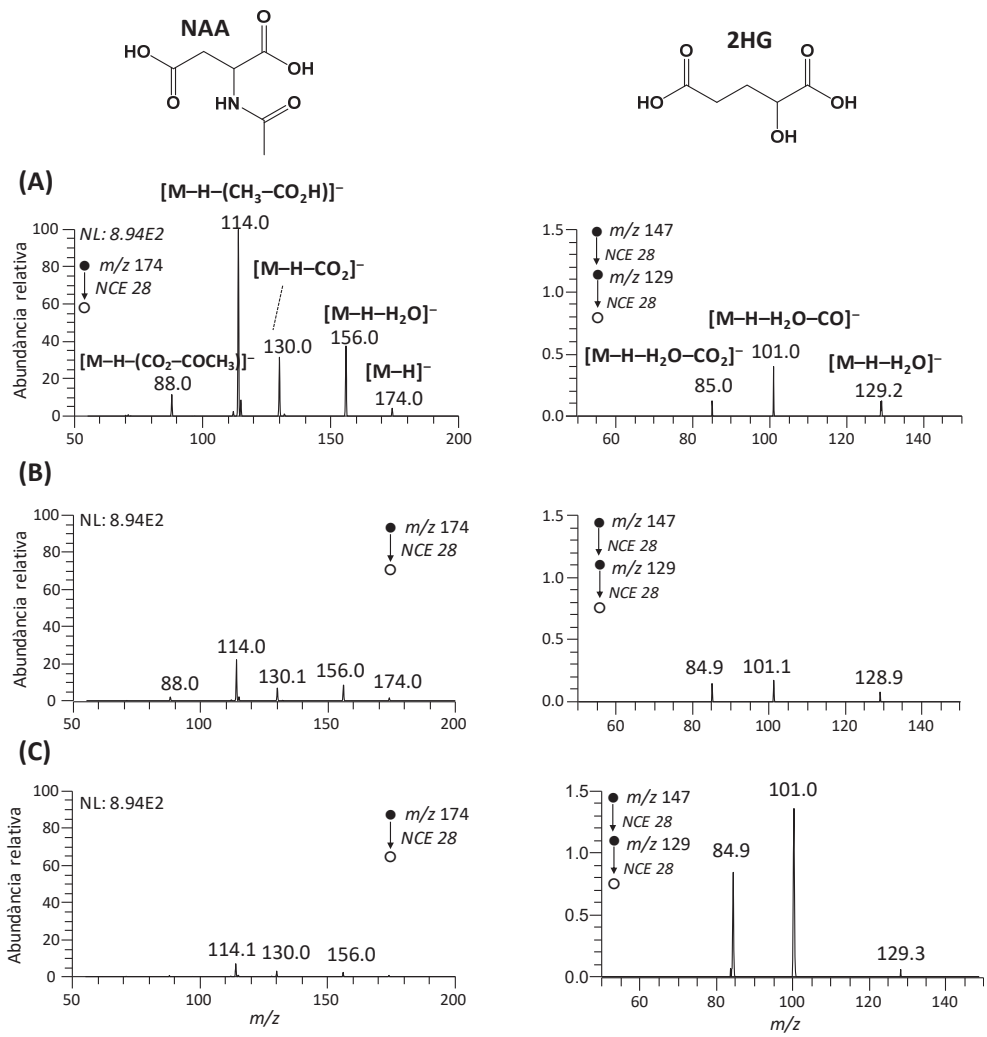

Figura 3.6. Espectres de masses d'ions producte MS^2 del metabòlit NAA (a l'esquerra) i MS^3 del metabòlit 2HG (a la dreta) obtinguts en el mode d'ionització negatiu en analitzar pel mètode TS-MS els teixits del (A) cas 23 (mostra 39), lectura patològica: glioma sense mutació IDH amb un TCP del 20% (baix), (B) cas 18 (mostra 30), lectura patològica: glioma sense mutació IDH amb un TCP del 40% (mitjà) i (C) cas 29 (mostra 47), lectura patològica: glioma amb mutació IDH amb un TCP del 90% (alt). Els espectres de masses s'han normalitzat en base a la intensitat del senyal obtingut pel patró intern NAA-d_3 (transició *m/z* 177 → 116).

En aquesta tesi, s'han estudiat 29 teixits (casos) dels quals se n'han realitzat un total de 47 mostrejos amb hisops (Taula S1, Informació Suplementària de la *Publicació V*) que s'han analitzat posteriorment per TS-MS. Del total de les mostres analitzades, en un 32% s'ha diagnosticat la presència de glioma en base als perfils lipídics i s'ha pronosticat en 8 dels casos la presència de gliomes amb mutació IDH, en base a l'abundància normalitzada del metabòlit 2HG. A més, en base a la intensitat del senyal normalitzada del NAA s'ha estimat un TCP alt en un 22% i mitjà en un 17% dels mostrejos realitzats. Els resultats obtinguts pel mètode TS-MS emprant hisops concorden amb les lectures patològiques, cosa que posa de manifest les possibilitats d'aquesta tècnica per proporcionar informació molecular rellevant per al diagnòstic de gliomes.

En el mètode PS-APPI-HRMS i atès que es pretén quantificar compostos neutres per- i polifluoroalquilats (PFASs) en productes d'impregnació, s'han utilitzat patrons interns marcats isotòpicament (*d*$_9$-*N*-EtFOSE, *d*$_5$-*N*-EtFOSA) pels FOSEs i FOSAs i els compostos 7:1 FA, 8:1 FA, 9:1 FA i 11:1 FA pels diferents FTOHs. Aquests patrons s'addicionen directament a les mostres i permeten corregir la variabilitat del senyal i els possibles efectes causats per la matriu en la ionització. Ara bé, atès que l'assecatge de la mostra afecta la reproductibilitat de la resposta, especialment dels FOTHs a causa de la major volatilitat d'aquests compostos, s'ha optimitzat el temps d'assecat (0.5-10 min) i els millors resultats pels FTOHs s'han obtingut en deixar assecar la mostra dipositada al paper durant un temps màxim de 2 min (Figura S2, Informació Suplementària a la *Publicació VI*).

Els paràmetres de qualitat del mètode PS-APPI-HRMS s'inclouen a la Taula 1 de la *Publicació VI* (apartat 3.2.2). La linealitat en el rang de concentració de treball estudiat és bona, amb coeficients de correlació (r^2) superiors a 0.995. Per a la majoria de compostos s'han obtingut uns límits de detecció de mètode (MLODs) entre 3 i 27 μg L^{-1}, els quals es troben entre 5 i 10 vegades per sota dels nivells de concentració trobats per aquests compostos en productes d'impregnació (Favreau et al., 2017; Herzke et al., 2012). Tanmateix, es va obtenir un MLOD més elevat pel compost 4:2 FTOH (315 μg L^{-1}) a causa de la seva major volatilitat. La precisió en un mateix dia (intra-dia, expressada com a %RSD) i la veracitat (expressada com a % d'error relatiu), calculades utilitzant 5 repeticions en un dia a dos nivells de concentració (0.08-0.6 mg L^{-1} i 2-25 mg L^{-1}, llevat del 4:2 FTOH que es va estudiar als nivells de concentració de 2 i 50 mg L^{-1}), van ser inferiors, en ambdós casos, al 18%, uns valors acceptables en les tècniques *Ambient MS*.

Per últim, i amb l'objectiu de demostrar que el mètode PS-APPI-HRMS desenvolupat pot ser útil per al control de la presència dels PFASs en productes d'impregnació resistents a l'aigua, en aquesta tesi s'han analitzat un total de 16 productes, que es van adquirir en diferents establiments de Catalunya i en l'etiqueta dels quals no s'hi indicava la presència de cap dels compostos PFASs. La identificació es basa en adquirir els espectres de masses en *full scan* (error en la mesura de la massa <5 ppm) per tal d'identificar els PFASs en les mostres i en confirmar les positives, per tal d'evitar falsos positius, adquirint l'espectre de masses d'ions producte (MS/HRMS). Els resultats obtinguts (Taula 2, *Publicació VI,* apartat 3.2.2), mostren la presència dels FTOHs en un 44% de les mostres analitzades en un interval de concentració entre 0.06 i 167 mg L^{-1}. El 6:2 FTOH ha estat el compost identificat amb més freqüència i a concentració més alta. En canvi, no es va detectar cap FOSA i només es va confirmar la presència del *N*-MeFOSE en una de les mostres, però a concentració baixa (0.63±0.04 mg L^{-1}). Cal indicar que en diverses mostres es va detectar més d'un compost PFAS. Per exemple, en la mostra WP-04 es van identificar el 6:2 FTOH, el 8:2 FTOH i el 10:2 FTOH, essent el 6:2 FTOH el que es troba a una concentració més elevada (25±1 mg L^{-1}) (Figura 3.7A). Els espectres de masses d'ions producte (MS/HRMS) han permès confirmar inequívocament la presència dels compostos en aquesta mostra. A mode d'exemple, a la Figura 3.7B i C es mostren els espectres de masses MS/HRMS adquirits pel 8:2 FTOH i 10:2 FTOH, els quals es correlacionen amb els obtinguts en l'anàlisi dels patrons (Taula S1, Informació Suplementària a la *Publicació VI*). Els nivells de concentració dels PFASs determinats en aquesta tesi emprant el mètode PS-APPI-HRMS són coherents amb els obtinguts en altres estudis realitzats a Noruega (Herzke et al., 2012) i Suïssa (Favreau et al., 2017), en què s'analitzaven mostres similars. En ambdós estudis també es van detectar majoritàriament la presència dels FTOHs en un interval de concentracions entre 0.5 i 330 mg L^{-1}. Els bons resultats obtinguts pel mètode PS-APPI-HRMS per a la determinació de PFASs posen de manifest que la combinació de l'APPI amb la tècnica PS podria proposar-se per a l'anàlisi quantitativa de compostos orgànics de baixa polaritat, ja que s'afavoreix la seva ionització.

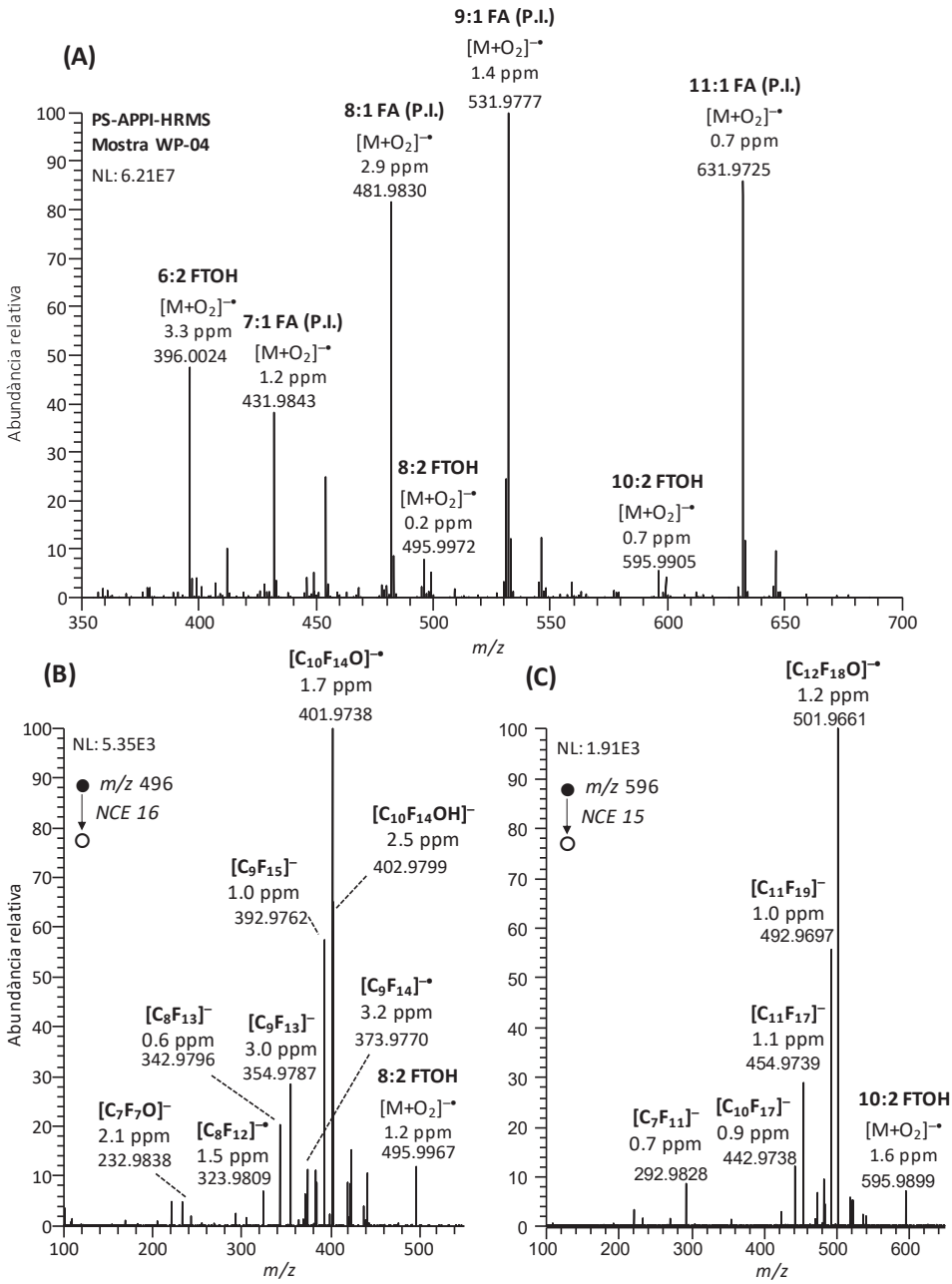

Figura 3.7. Espectres de masses PS-APPI-HRMS(/MS) obtingut d'una mostra positiva (WP-04) en què s'ha detectat la presència dels compostos 6:2 FTOH, 8:2 FTOH i 10:2 FTOH a una concentració de 25±1, 0.08±0.02 i 0.2±0.02 mg L^{-1}, respectivament. (A) Espectre de masses de *full scan* en alta resolució en el rang *m/z* 350-700, (B) Espectre de masses d'ions productes (MS/HRMS) obtingut en fragmentar l'ió $[M+O_2]^{-\bullet}$ del compost 8:2 FTOH i (C) Espectre de masses d'ions productes (MS/HRMS) obtingut en fragmentar l'ió $[M+O_2]^{-\bullet}$ del compost 10:2 FTOH.

CONCLUSIONS

CONCLUSIONS

En la present tesi doctoral s'han estudiat diferents tècniques *Ambient MS* per al desenvolupament de metodologies analítiques ràpides i selectives que poden millorar la productivitat dels laboratoris analítics i facilitar la presa de decisions en la diagnosi clínica. Per una banda, s'ha estudiat la tècnica d'ionització per desorció per electroesprai (DESI) per a la identificació de drogues veterinàries (compostos sospitosos) en mostres de pinso com a conseqüència d'una contaminació creuada i per a la caracterització de compostos desconeguts en un producte fitosanitari del que se'n desconeixia la seva naturalesa química. Per altra banda, s'han estudiat i modificat les tècniques *touch spray* (TS) i *paper spray* (PS) per adaptar-les a l'anàlisi directa de teixit viu en el diagnòstic de gliomes cerebrals i a la determinació de compostos per- i polifluoroalquilats neutres (PFASs) en l'anàlisi de productes d'impregnació.

De la realització del treball experimental dut a terme en aquesta tesi es poden extreure les següents conclusions:

Sobre els mètodes d'ionització per desorció per electroesprai (DESI),

Tot i que l'anàlisi per DESI no requereix un pretractament de la mostra, una mínima manipulació de les mostres de pinso i del producte fitosanitari ha permès resoldre problemes derivats de la textura d'ambdues matrius.

- En el mètode DESI-HRMS desenvolupat per a la detecció de drogues veterinàries en mostres de pinso com a conseqüència d'una contaminació creuada, es proposa: (*i*) la trituració i homogeneïtzació dels pèl·lets de pinso per tal d'obtenir una mostra composta que asseguri la representativitat dels resultats i (*ii*) una extracció sòlid-líquid simple i ràpida (< 5 min/mostra) emprant una mescla ACN/H_2O (80/20, *v:v*) amb un 1% d'àcid fòrmic per evitar la dispersió de les mostres pulverulentes a causa del gas de nebulització del sistema DESI. Aquesta estratègia ha permès l'extracció selectiva de la majoria de les drogues estudiades i reduir els efectes de la matriu en la resposta.
- En l'anàlisi del producte fitosanitari, un líquid d'elevada viscositat i de naturalesa química desconeguda, l'ús de la tècnica DESI ha permès la seva anàlisi directa. Es proposa la

impregnació d'un paper de filtre amb la mostra per fixar-la i reduir el temps d'assecatge (<5 minuts) previ a l'anàlisi per DESI-HRMS.

L'estudi i optimització dels paràmetres més crítics de la font DESI (tipus de substrat, composició i cabal de l'esprai DESI i del gas de nebulització i la configuració dels paràmetres geomètrics) ha permès obtenir la sensibilitat adient per a la resolució dels dos problemes analítics estudiats en el Capítol 2 d'aquesta tesi. Es recomana seguir estratègies d'optimització diferents segons la tipologia del problema (anàlisi de compostos sospitosos *vs* desconeguts).

- L'optimització dels paràmetres DESI emprant una mostra blanca fortificada amb una selecció de patrons comercials representativa de la família de les drogues veterinàries estudiades (compostos sospitosos), ha permès assolir una eficàcia d'extracció i d'ionització adequades per a la seva detecció per sota dels nivells establerts a la legislació. Es proposa utilitzar una superfície de PTFE com a substrat per dipositar els extractes líquids de les mostres, una mescla d'ACN/H_2O (80/20, *v:v*) com a dissolvent de l'esprai i un cabal de dissolvent de 2.5 μL min^{-1} amb el emissor DESI posicionat a un angle i una distància de la superfície de 55° i de 1.7 mm, respectivament.

- En l'anàlisi per DESI del producte fitosanitari es proposa emprar la mescla ACN/H_2O (80/20,*v:*v) com a dissolvent de l'esprai a un cabal de 3.5 μL min^{-1}, amb l'emissor DESI posicionat a un angle i una distància de la superfície de 55° i de 2.0 mm, respectivament. Aquestes condicions afavoreixen l'extracció i la ionització dels compostos d'un ampli rang de polaritats de la mostra desconeguda. A més, es recomana utilitzar el mode estàtic en l'anàlisi per DESI per reduir els efectes de supressió iònica detectats en l'anàlisi en el mode dinàmic.

Sobre els mètodes de *touch spray* (TS) i *paper spray* (PS),

S'han adaptat, de forma senzilla, els dissenys originals de les tècniques TS i PS per al mostreig *in vivo* de teixit biològic i per possibilitar la fotoionització a pressió atmosfèrica (APPI).

- S'ha desenvolupat un mètode ràpid de TS-MS (< 2 minuts) per a l'anàlisi *in vivo* de teixit humà utilitzant hisops comercials, habitualment emprats en el camp de l'anàlisi clínica, per al mostreig i, alhora, com una sonda per a la generació de l'electroesprai.

- La incorporació d'una làmpada de Criptó al disseny original del PS i l'ús de dissolvents no polars ha possibilitat l'aplicació d'aquesta tècnica per a la determinació ràpida (< 3 min) de compostos per- i polifluoroalquilats neutres (FOSAs, FTOHs i FOSEs) mitjançant l'APPI.

L'estudi de la configuració del disseny i de les condicions experimentals de la TS i del PS ha permès establir les condicions òptimes per a la resolució dels dos problemes analítics estudiats en el Capítol 3 d'aquesta tesi.

- En el mètode de TS-MS, la disposició vertical de l'hisop, el subministrament del dissolvent de forma contínua i l'ús d'una línia de transferència en forma de L han facilitat la generació d'un electroesprai estable a la punta de l'hisop.
- En el mètode de PS-APPI-HRMS es proposa l'addició del dissolvent (70 µL) a l'inici de l'anàlisi i la disposició de la làmpada vers al paper triangular amb un angle d'inclinació de 30° i a 1 cm de distància per a la ionització eficaç dels FTOHs, FOSAs i FOSEs estudiats.

L'avaluació dels diferents paràmetres operacionals ha fet possible l'extracció i la ionització dels compostos presents en la mostra de manera eficient.

- L'ús d'una mescla acetonitril:etanol:*N,N*-dimetilformamida (50:45:5, *v/v*), dopat amb un surfactant no iònic, i d'un potencial de 6.5 kV en el mètode TS-MS ha permès l'extracció eficaç dels fosfolípids i oncometabòlits del teixit biològic i la formació d'un electroesprai estable a la punta de l'hisop per a la ionització d'aquests compostos.
- En el mètode de PS-APPI-HRMS es proposa l'ús del toluè com a dissolvent i l'aplicació d'un potencial de 2.5 kV per potenciar tant la desorció dels anàlits neutres a la fase gas com la seva fotoionització assistida pel dopant (el toluè emprat com a dissolvent), generant l'ió adducte $[M+O_2]^{-\bullet}$ pels FTOHs i FOSES i la molècula desprotonada $[M–H]^-$ pels FOSAs.

Sobre l'anàlisi de les mostres emprant els mètodes *Ambient MS* desenvolupats,

Els resultats obtinguts en l'anàlisi de les mostres emprant els mètodes *Ambient MS* desenvolupats en aquesta tesi posen de manifest l'aplicabilitat d'aquestes tècniques en diversos camps d'anàlisi.

DESI-HRMS

- La selectivitat i sensibilitat assolides en el mètode DESI-HRMS emprant un analitzador d'alta resolució (Q-Orbitrap) han permès la detecció i confirmació de la majoria de drogues veterinàries en la producció de pinsos (medicats i no medicats) a nivells inferiors als establerts a la legislació.

- El mètode DESI-HRMS desenvolupat ha permès detectar la presència de contaminacions creuades en un 28% de les 50 mostres analitzades (pinsos medicats i no medicats). Per a la ràpida interrogació de les mostres es recomana l'ús d'una base de dades pròpia construïda amb informació experimental i de la literatura per a la identificació de les drogues veterinàries en base a criteris d'exactitud de massa (5 ppm d'error màxim) i d'adequació del clúster isotòpic (80% de semblança). A més, la determinació d'aquestes mostres emprant un mètode UHPLC-MS/MS prèviament establert ha posat de manifest l'absència de falsos positius/negatius en els resultats obtinguts per DESI-HRMS i ha demostrat l'aplicabilitat d'aquest nou mètode com una bona alternativa de cribratge de mostres per augmentar el rendiment dels laboratoris de control agroalimentari.

- En l'anàlisi del producte fitosanitari, l'estudi dels espectres de masses DESI-HRMS (resolució 70,000 FWHM, *m/z* 200) emprant el filtre del defecte de massa de Kendrick ha permès identificar la presència d'un polímer de polietilenglicol de composició elemental ($C_{10}H_{22}O(EO)_n$), així com la presència d'altres ions amb distribucions isotòpiques característiques de compostos organoestànnics.

- Les mesures de massa exacta combinades amb l'estudi dels perfils isotòpics dels ions organoestànnics i la interpretació dels espectres de tàndem han possibilitat la identificació i caracterització de trifenilestany en la mostra. La posterior quantificació de la mostra per UHPLC-HRMS i ICP-OES ha permès confirmar la presència fraudulenta d'aquest compost a una concentració 120 vegades per sobre del nivell màxim permès.

TS-MS

- Per a l'anàlisi de les mostres de teixit cerebral pel mètode TS-MS es proposa utilitzar un analitzador de baixa resolució de trampa d'ions lineal, ja que permet una velocitat d'escombratge elevada i l'adquisició dels espectres de masses en el mode de *full scan* amb una bona sensibilitat. L'estudi dels diferents perfils lipídics obtinguts en aquestes condicions en analitzar mostres de teixit sa i tumoral ha permès discriminar satisfactòriament entre la parènquima cerebral normal i la presència de gliomes.

- La possibilitat que ofereixen els analitzadors de trampes d'ions d'adquirir els espectres de tàndem en múltiples etapes permet augmentar la selectivitat del mètode desenvolupat. Aquest elevat grau d'especificitat s'ha emprat per estudiar la resposta dels oncometabòlits (NAA i 2HG) utilitzats com a biomarcadors, que ha permès estimar qualitativament el percentatge de cèl·lules tumorals (TCP) en el teixit analitzat i diagnosticar la presència de la mutació IDH en el glioma. En el cas del 2HG i per solucionar els problemes d'interferències isobàriques es proposa adquirir els espectres de masses de MS^3.

- L'anàlisi de 29 teixits pel mètode TS-MS ha posat de manifest la presència de gliomes en un 33% de les mostres i ha permès diagnosticar la mutació IDH en 8 casos. Aquests resultats han evidencien el potencial del mètode proposat per proporcionar, en un futur, informació molecular rellevant en el diagnòstic de càncer cerebral durant les intervencions de resecció tumoral.

PS-APPI-HRMS

- L'ús de l'analitzador Q-Orbitrap en el mètode PS-APPI-HRMS ha permès aconseguir la suficient sensibilitat i selectivitat per a la monitorització i la quantificació dels FTOHs, FOSAs i FOSEs en productes d'impregnació.

- Les bones característiques del mètode PS-APPI-HRMS, com ara els límits de detecció (per sota dels $\mu g\ L^{-1}$), la linealitat ($R^2 > 0.998$), la precisió intra-dia (RSD%< 18%) i la veracitat (error relatiu < 18%), han demostrat la viabilitat del mètode desenvolupat.

- Els bons resultats obtinguts en l'anàlisi quantitativa de 16 productes d'impregnació, en què s'ha determinat la presència de PFASs a uns nivells de concentració de $mg\ L^{-1}$, posen de manifest que el PS-APPI podria emprar-se com una alternativa a altres tècniques *Ambient MS* per a l'anàlisi de compostos de baixa polaritat.

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CONCLUSIONS

CONCLUSIONS

In the present thesis, Ambient MS techniques have been studied for the development of fast and selective analytical methods to improve the productivity of high-throughput laboratories and to provide clinical diagnosis information. Desorption electrospray ionization (DESI) has been studied for the identification of veterinary drugs (non-target analysis) in cross-contaminated feedstuffs. The applicability of DESI has also been explored for the characterization of unknown compounds in a phytosanitary product suspected of being adulterated and for which its chemical composition was also unknown. Moreover, modified touch spray (TS) and paper spray (PS) have also been evaluated as a tool for *in vivo* analysis of brain tissues for cancer diagnosis and for the determination of per- and polyfluorinated alkyl substances (PFASs) in waterproof impregnation sprays, respectively.

The following conclusions can be drawn from the experimental studies presented in this thesis:

In regard to the desorption electrospray ionization (DESI) methods,

Although DESI analysis does not require any additional sample preparation than the one that takes place during the analysis, a minimal manipulation of feed samples and the phytosanitary product has allowed to solve the problems related with the texture of both matrices.

- For the detection of veterinary drugs in cross-contaminated feedstuffs by DESI-HRMS, it is proposed the following manipulation strategy: (*i*) trituration and homogenization of feed pellets in order to guarantee the representativeness of the analyzed sample and (*ii*) a simple and fast solid-liquid extraction procedure using acetonitrile/water (80:20, *v/v*) acidified with 1% formic acid, to prevent the dispersion of the powdered homogenized samples by the nebulizing gas of the DESI system. This manipulation strategy has also allowed the selective extraction of most of the studied veterinary drugs, thus reducing severe matrix effects.
- For the characterization of unknown compounds in the phytosanitary product, a white dense liquid of unknown chemical nature, the use of DESI technique has allowed to

perform the direct analysis of the sample. The analysis of a filter paper impregnated with the sample is proposed to fix the sample and to reduce the drying time (<5 minutes) prior to DESI-HRMS analysis.

The study and optimization of the most critical DESI working parameters (sample substrate, DESI solvent composition and flow rate, nebulizing gas pressure and the configuration of the geometrical parameters) has provided suitable sensitivity for both DESI-HRMS developed methods. Different optimization strategies are recommended depending on the typology of the problem studied (non-target analysis *vs.* analysis of unknowns).

- For the analysis of veterinary drugs in feed samples (non-target analysis), DESI-HRMS optimization using a blank sample extract spiked with a representative selection of veterinary drugs has allowed to achieve satisfactory DESI extraction and ionization efficiencies for the detection of these compounds under the legislated levels. The analysis of sample extracts deposited in PTFE surface, using acetonitrile/water (80:20, *v/v*) as DESI solvent and a solvent flow rate of 2.5 μL min^{-1} provided the highest signal intensity. Among the DESI geometrical parameters, the best responses of veterinary drugs have been obtained by directing the DESI solvent onto the sample surface at a nebulization capillary angle of 55°, placed at a distance of 1.7 mm.

- For the analysis of the phytosanitary product, the use of a DESI solvent mixture of ACN/H_2O (80/20,*v:*v), infused at 3.5 μL min^{-1} and placed at a distance of 2.0 mm from the sample surface with a nebulization capillary angle of 55°, is proposed. These DESI working conditions favored the extraction and ionization of compounds with wide range of polarities from the unknown sample. In addition, it is recommended to perform the sample DESI-HRMS analysis under static mode to decrease the ion suppression effects observed when acquiring the MS data under scanning mode.

In regard to touch spray (TS) and paper spray (PS) methods,

TS and PS original set-ups have been easily adjusted for *in vivo* analysis of biological tissues and to enable atmospheric pressure photoionization (APPI), respectively.

- A fast TS-MS method (< 2 minutes) using commercial medical swabs, which are commonly used in the field of clinical analysis, has been developed for *in vivo* analysis

of human tissue. Swabs have been used for sampling and, at the same time, as electrospray probe.

- The incorporation of a Crypton lamp into the original PS set-up and the use of non-polar solvents has allowed the application of this technique for the rapid (< 3 minutes) determination of neutral per- and polyfluorinated alkyl substances (FTOHs, FOSAs and FOSEs) by APPI.

The evaluation of both the configuration of the design and the experimental conditions of TS and PS have allowed to establish the optimal set-up of the developed TS-MS and PS-APPI-HRMS methods included in the Chapter 3 of the present thesis.

- In regard to TS-MS method, the vertical arrangement of the swab, the supply of the solvent at a constant flow rate and the use of a L-shaped transfer line have promoted the generation of a stable electrospray at the swab tip.

- For the determination of the studied PFASs by PS-APPI-HRMS, the addition of the solvent (70 μL) at the beginning of the analysis and the disposal of the UV lamp at an angle of 30° and at a distance of 1 cm from the triangle paper apex are proposed for the efficient ionization of the studied FTOHs, FOSAs and FOSEs.

The optimization of the most critical TS and PS-APPI working parameters have allowed the suitable extraction and ionization of the studied compounds.

- The use of a solvent mixture acetonitrile:ethanol:*N,N*-dimethylformamide (50:45:5, *v/v*) doped with a non-ionic surfactant and the high voltage applied (6.5 kV) have allowed the efficient extraction and ionization of phospholipids and oncometabolites from the biological tissue. Under these conditions, the formation of a stable cone-jet electrospray plume at the swab tip has been favored, thus improving the ionization efficiency of the developed TS-MS method.

- For the developed PS-APPI-HRMS method, the use of toluene as spray solvent and a spray voltage of 2.5 kV is proposed to promote both the neutral analytes desorption and the dopant-assisted photoionization. Under these conditions, a high-tendency to generate $[M+O_2]^{-\bullet}$ adduct ions has been observed for FTOHs and FOSEs, while deprotonated molecules $[M–H]^-$ ions have been detected for FOSAs.

In regard to the sample analysis performed with the developed Ambient MS methods,

The sample analysis results obtained in the present thesis by the developed Ambient MS methods have demonstrated the applicability of these techniques in several fields of analysis.

DESI-HRMS

- The good sensitivity and selectivity achieved in the DESI-HRMS method using a high-resolution mass analyzer (Q-Orbitrap) has allowed the detection and confirmation of most of the veterinary drugs below the maximum residue levels legislated due to the unavoidable carry-over in the feed line production.
- The developed DESI-HRMS method has allowed the detection of veterinary drugs cross-contaminations in 28% of the 50 samples analyzed (medicated and non-medicated feeds). For the fast interrogation of the DESI-HRMS data acquired, the use of a custom-made database built with experimental and literature-based information of these compounds is recommended to perform high-throughput screening based on mass accuracy (<5 ppm) and isotope cluster fit (>80%) criteria for positive identification. Furthermore, the sample analysis performed with a previously established UHPLC-MS/MS method has revealed the absence of false positive/negative results by DESI-HRMS sample analysis, thus demonstrating the potential of this method to improve the productivity in quality control laboratories.
- For the analysis of the phytosanitary product, Kendrick mass defect analysis has been successfully used to extract information from the DESI-HRMS (resolution 70,000 FWHM, *m/z* 200) mass spectral data. A low molecular weight polyethylene glycol polymer ($C_{10}H_{22}O(EO)_n$) and the presence of other ions with characteristic isotope pattern profiles of organotin compounds have been detected in the sample.
- Accurate mass measurements, the isotope pattern fits and the structural information obtained from high-resolution tandem mass spectra (MS/HRMS) have allowed attributing the unexpected activity of the phytosanitary product to the fraudulent presence of triphenyltin compounds in the sample. The quantification results obtained by UHPLC-HRMS and ICP-OES have satisfactorily confirmed the presence of triphenyltin in the sample at a concentration 120 higher than the maximum legislated level.

TS-MS

- Considering the good performance capabilities of ion trap mass analyzers working in full scan acquisition mode in terms of scanning speed and sensitivity, the use of this low resolution mass analyzer (LIT) is proposed for the analysis of human brain tissue by TS-MS. The study of the spectral phospholipid profiles obtained under these conditions has allowed the successfully discrimination of normal parenchyma and tumor tissue.

- The possibility to perform multiple-stage tandem mass spectrometry using ion trap mass analyzers allows to increase the selectivity of the developed method. MS^n spectral data have been used to study the response of NAA and 2HG oncometabolites, thus providing enough specificity to qualitatively assess tumor infiltration grade (TCP) and mutation status of the IDH gene. In order to avoid isobaric interference problems, the acquisition of MS^3 product ion spectra is proposed to evaluate the response of 2HG.

- The developed TS-MS method has allowed the diagnosis of gliomas in 33% of the analyzed samples and identify IDH mutation in 8 of the 29 human brain specimens studied. These results demonstrate the potential of the proposed TS-MS method to provide, in a future, diagnostic molecular information for rapid intraoperative surgical assessment.

PS-APPI-HRMS

- The sensitivity and selectivity achieved using a Q-Orbitrap mass analyzer in the PS-APPI-HRMS method has allowed the reliable identification and quantification of FTOHs, FOSAs and FOSEs in waterproof impregnation sprays.

- Method quality parameters, such as method limits of detection (below $\mu g\ L^{-1}$), linearity ($r^2 > 0.998$), intra-day precision (RSD% < 18%) and trueness (relative errors < 18%) have demonstrated the good performance of the developed PS-APPI-HRMS method.

- The good results obtained in the analysis of 16 raw waterproof impregnation sprays, in which the presence of several neutral PFASs have been detected at $mg\ L^{-1}$ levels, indicate that PS-APPI could be considered as an alternative to other Ambient MS techniques for the analysis of low polar compounds.

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