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RESPONDING TO NEW SYNTHETIC OPIOIDS: A MULTICENTRE APPROACH

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FAN CONSTAR:

Que la memòria presentada per María Alías i Ferri amb el títol: "Responding to New Synthetic Opioids: a multicentre approach" ha estat realitzada sota la seus direcció i que reuneix totes les condicions per ser presentada per a la obtenció del Grau de Doctora per la Universitat Autònoma de Barcelona

I perquè consti als efectes oportuns signen el present



Dr. Magí Farre Albaladejo Dra. Marta Torrens Mèlich María Alías I Ferri

Setembre 2022

A mis padres y hermana.

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ABBREVIATIONS

COVID-19	Corona Virus Disease 2019
DEA	Drug Enforcement Administration
DSM	Diagnostic and Statistical Manual of Mental Disorders
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
EWA	Early Warning Advisory
EWS	Early Warning System
NPS	New Psychoactive Substance
NSO	New Synthetic Opioid
OEDA	Observatorio Español de la Drogas y las Adicciones
OUD	Opioids Use Disorder
SUD	Substance Use Disorder
UNODC	United Nations Office on Drugs and Crime
WHO	World Health Organization

ABSTRACT

New Synthetic Opioids (NSO), a class of New Psychoactive Substance (NPS), have emerged rapidly in the drug market in recent years posing a public health problem. These substances show similar effects to morphine but with a higher addictive potential and toxicity increasing the risk of overdose. Often they have been detected mixed with other substances or sold as counterfeits, placing even more at risk the consumer who is unaware of what substances he is consuming. The standard tests used in clinical practice cannot detect NSOs and their study is usually focused on recreational populations such as people attending music festivals or raves. So, we hypothesize that there is a growing phenomenon of consumption of new synthetic opioids in Europe.

The aim of the present thesis is to provide updated data to know the current situation with respect to these substances in our region in a population of individuals with Opioid Use Disorder (OUD). For this purpose, the main objective of this work is to determine the prevalence of NSO use among OUD people in addiction care centers from Barcelona and Badalona. Thus, a characterization of the NSO user and its consume was made including the reasons for its use, detect other NPS used in subjects OUD and, assess the polydrug-use among this population taking into account a gender perspective.

In the first work presented in this thesis, OUD patients were interviewed and asked to provide a urine sample to find out if they had consumed any NSOs. In the second study of this thesis, a urine sample was collected anonymously from OUD patients. All samples were analyzed by two methods, one screening and one confirmatory. Broadly our results showed that, in our environment, there is both voluntary and involuntary consumption of NSO.

RESUMEN

En los últimos años, la rápida aparición y crecida en el mercado negro de los Nuevos Opiáceos Sintéticos (NSO), una clase de Nueva Sustancia Psicoactiva (NPS), ha provocado un problema de salud pública a nivel global. Estas sustancias tienen efectos parecidos a los de la morfina pero con un potencial adictivo y una toxicidad muy superior, por lo que aumenta el riesgo de sobredosis. A menudo, son detectadas en mezclas con otras sustancias o son vendidas como falsificaciones, aumentando así los riesgos asociados a su consume ya que muchos usuarios no saben que están consumiendo. Estas sustancias no son detectadas por los test que se suelen utilizar en la práctica clínica y su estudio ha estado más centrado en consumidores esporádicos y en contextos recreativos como raves o festivales de música. Así pues, creemos que el consumo de NSO en Europa está incrementando.

Con esta tesis se pretende aportar información actualizada del consumo de NSO en nuestra región en individuos con un Trastorno por Uso de Opiáceos (OUD). El objetivo principal de este trabajo es determinar la prevalencia de uso de los NSO en una población OUD de los centros de adicciones de Barcelona y Badalona. Además se ha descrito el uso que se hace de estas sustancias, así como de su consumidor, incluyendo las razones para su uso, la detección de otras NPS y el policonsumo desde una perspectiva de género.

En el primer trabajo de esta tesis, pacientes con OUD fueron entrevistados y se adjuntó una muestra de orina para detectar las sustancias consumidas. En el segundo trabajo, una colección de muestras anónimas de orina de pacientes OUD fue analizada con el mismo fin. En ambos trabajos, se hizo un análisis de detección de sustancias y un segundo análisis confirmatorio. A grandes rasgos, nuestros resultados, mostraron que existe consumo de NSO tanto voluntario como involuntario.

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1. INTRODUCTION

1. INTRODUCTION

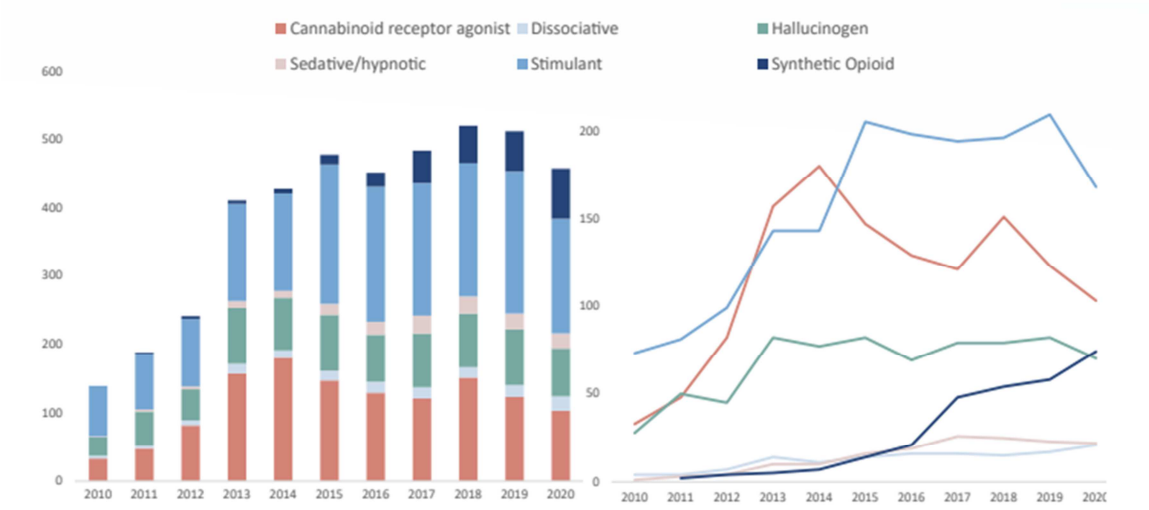
1.1. New Psychoactive Substances

New Psychoactive Substances (NPS) are a wide group of substances that have been emerge in the drug market in last years as a substitute of the classic drugs of abuse. These substances are defined as “a new narcotic or psychotropic drug, in pure form or in preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat comparable to that posed by substances listed in these conventions” (United Nations Office on Drugs and Crime (UNODC), 2013). Several of these compounds already existed and were synthesized years ago only recently emerging on the drug market (Papaseit, Farré, Schifano, & Torrens, 2014). So it is the recent use of them what the term "new" refers to and not by the fact that they are new synthetized compounds (United Nations Office on Drugs and Crime (UNODC), 2013). These substances have several presentations, such as tablets or powder, which are the most common, but they are also found in crystals, herbal mixtures or liquids. The large number and different compositions of NPS make difficult their detection and law control, posing a public health challenge (Helander, Bäckberg, Hultén, Al-Saffar, & Beck, 2014; Shafi, Berry, Sumnall, Wood, & Tracy, 2020).

In recent years, the great emerge and availability of these substances made the drug market expanded exponentially (United Nations Office on Drugs and Crime (UNODC), 2021b). Despite the fact that worldwide 1,124 unique NPS have been reported to the UNODC Early Warning Advisory (EWA) from 2009 up to January 2022 (United Nations Office on Drugs and Crime, 2022a), hundreds of them has been synthetized in the last few years (United Nations Office on Drugs and Crime, 2021c) (see Figure 1). This new drug market is characterized as changing and dynamic, so some of these substances disappear quickly, while others become established over the years.

Despite the great chemical variety of the NPS, there are 6 main groups attending to their effect: stimulants (e.g., cathinones, phenethylamines; tryptamines, etc.), synthetic cannabinoids, benzodiazepines, opioids (e.g., fentanyl, fentanyl analogues and non-fentanyl compounds), hallucinogens (e.g., 1P-LSD and 4-AcO-DMT), and dissociatives (Papaseit et al., 2014; United Nations Office on Drugs and Crime, 2018; Varì et al., 2020).

FIGURE 1. Number of NPS identified in Member States, by effect group, 2010-2020 (United Nations Office on Drugs and Crime, 2021a).



From mid-2017 to the end of 2020, Internet drug sales increased 4 times compared to previous years and NPS and their chemicals precursors are often found easily on Internet (United Nations Office on Drugs and Crime, 2021c). Several advantages make online platforms a suitable channel for their sale. First of all, online platforms makes it possible to reach a wider audience, including younger users, making the acquisition simple and fast (Miliano, Margiani, Fattore, & De Luca, 2018). They also save extra costs for intermediaries by allowing a direct connection between the seller and the buyer(United Nations Office on Drugs and Crime, 2021b). Finally, using conventional mail for shipment reduces the possibility of detection substances, which makes it safer than traditional drug trafficking (United Nations Office on Drugs and Crime, 2021b).

With the aim of monitoring these substances and provide a global response to the rapid worldwide emerge of these substances, the UNODC EWA was established in June 2013. The objectives are to monitoring, analyzing and identifying trends in NPS in order to provide technical assistance to Member States in the implementation of evidence-based policy measures (United Nations Office on Drugs and Crime, 2022d)

Similarly, the EMCDDA in cooperation with Europol operates the NPS Early Warning System (EWS) since 1997 under Joint Action 97/396/JHA. The EWS is a network composed of 27 EU member states, plus Turkey and Norway, Europol and its law enforcement networks, the European Medicines Agency (EMA), the European Commission and other partners. The EMCCDA and Europol are responsible to collect, collate, evaluate and provide the information compiled to the EWS network to enable them to have the necessary information for early warning and to the EMCDDA to be able to raise health and social issues arising from the NPS (European Monitoring Centre for Drugs and Drug Addiction, 2019). It is necessary to take into account that, since March 29th, 2017, the United Kingdom ceased his condition of member state of the European Union (Brexit) determined in accordance with Article 50 of the Treaty on European Union (TFUE).

The group of NPS users is very diverse but most of them are young adult, male who use other substances in recreational contexts without other concomitant substance use disorders (SUD) (Graddy, Buresh, & Rastegar, 2018). Although there are also NPS users from stigmatized populations like people who injected drugs (United Nations Office on Drugs and Crime, 2021b).

In Europe, the prevalence of NPS use during last year among adults between 15-64 years has been estimated in a 0.6%, but if we focus on young adults, between 15 and 34 years of age, the prevalence rises to 1.1% (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)., 2021b). The highest prevalence of use is observed in adolescents between 15 and 16 years of age, with an estimated prevalence of between 0.6% and 4.9% (European Monitoring

Centre for Drugs and Drug Addiction (EMCDDA), 2021b). Despite this, according to the latest European Web Survey on Drugs 2021, which enrolled 50,852 people over 18 years of age, shows higher prevalence of use for NPS in the last year of 16% (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)., 2021d). When specific populations like people who go to raves or clubs, or psychoestimulants users, a higher frequency of use is found (Benschop et al., 2020).

Since 2009, 381 unique substances have been detected for the first time and reported to the EWS, of which 48 were reported during 2021 (United Nations Office on Drugs and Crime, 2022c). Recently, in March 2022, three more substances have been added to the list of controlled NPS: brorphine, metonitazene and eutilone (United Nations Office on Drugs and Crime, 2022b). The European drug market is one of the most active, offering a large number of these substances (Lovrecic et al., 2019). Every year, 400 different substances have been detected since 2015, and to the date a total of 880 NPS were monitored (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)., 2022b).

Most commonly detected types of NPS, both seizures and intoxications, are NPS stimulant-type and NPS cannabinoid-type, but, in recent years there has been a rapid and constant emergence of New Synthetic Opioids (NSO) (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)., 2020a). The NSO are characterized by their high potency, increasing the risk of abuse, addiction and overdose for those who use them. This along with the scarce information of these substances short and long term harms and even sometimes, depending on the substance, unknown, point them as a risk for NPS and NSO users (United Nations Office on Drugs and Crime, 2018).

To date, NPS have been extensively studied in recreational contexts such as raves or music festivals, either through drug checkpoints or through the wastewater from these events (Bade et al., 2021; Bijlsma et al., 2020). However, these substances are rarely studied or

detected in clinical practice among SUD patients (Shafi et al., 2020). Some of the reasons for not widely studying them are: the lack of detection of these substances in standard procedures (Pichini, Pacifici, Marinelli, & Busardò, 2017a) and the lack of knowledge of their use (Volkow & Blanco, 2021)

The few studies conducted in clinical practice have been mostly in emergency rooms when NPS related intoxication or overdose is suspected (Elliott, Sedefov, & Evans-Brown, 2018; Helander et al., 2014) or in detoxification treatment (Specka et al., 2020a), although these studies are more scarce.

1.2. New Synthetic Opioids

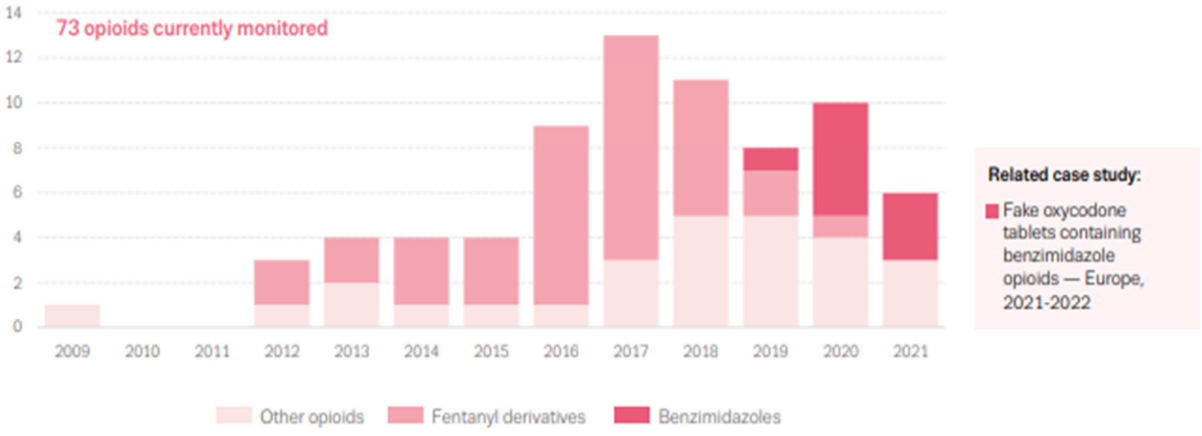
1.2.1. Description

New synthetic opioids are a class of NPS that have recently emerged and pose a new global public health challenge (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)., 2017; Lovrecic et al., 2019). Two main reasons explain the growing concern about NSO in recent years. The first one is related to the increased number of detections of these substances (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA, 2019), and the second one is due to the high toxicity of these compounds elevating the risk of overdose (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)., 2020a, 2020b).

In Europe, synthetic opioids first report has increased from 2007 with no detections, to being the third group of NPS with the most detections in 2018 (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)., 2019a). This makes us focus on this group of substances and that is why their monitoring is important in order to be able to know the situation and take the necessary health, social, prevention and legal measures.

A total of 73 different NSOs have been detected since 2009 (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)., 2022b). Since 2016, an increase in NSO detections has been observed and gradually non-fentanyl related opioids have started to gain prominence. In 2019 and 2020, among NSOs first reports, non- fentanyl opioids were the most common (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)., 2020a, 2021b) (Figure 2). Therefore, NSO compounds have increased their presence in the drug market (Prekupec, Mansky, & Baumann, 2017). The proportion of both types of opioids is maintained, but a sudden spread of synthetic benzimidazole opioids has been observed in the last 3 years, accounting for 60% of the drug seizures (Di Trana, Pichini, Pacifici, Giorgetti, & Busardò, 2022; European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)., 2022a)..

Figure 2. Number and types of new synthetic opioids notified to the EU Early Warning System for the first time, 2009-2021 (EU+2) (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)., 2022a).



Regarding drug seizures, in 2019 fentanyl doubled the amount seized, with 15 kilograms, compared to the previous year, with 6 seized kilograms (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)., 2021b).

Fentanyl and fentanyl derivatives reach the European drug market through two main sources: one is the illegal manufacture of these substances and the other is the re-routing of

pharmacological fentanyl from the regulated market to illegal drug market (Mounteney, Giraudon, Denissov, & Griffiths, 2015). Most of the illegal fentanyl production arriving in Europe is done in frontier countries such as Russia, Belarus and Ukraine (Mounteney et al., 2015). During the COVID-19 pandemia, the main routes for opioid trafficking has been from Afghanistan and Balkans (United Nations Office on Drugs and Crime (UNODC), 2021a). Also, precursors of fentanyl and non-controlled fentanyl precursor were seized in Estonia and Belgium and Germany, respectively (EMCDDA)., 2021a) suggesting the existence of illegal laboratories in Europe.

These substances have been found in different forms: powder, tablets or capsules, patches and liquid and can be consumed by different routes such as oral, ingested, transdermal, injected, sublingual and others (Solimini, Pichini, Pacifici, Busardò, & Giorgetti, 2018). In the United States (US), it is very common to find these substances in the form of pills or tablets, simulating pharmacological opioids (Drug Enforcement Administration (DEA), 2020b). While in Europe, according to seizures reported to the EWS, the most common form is powder (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)., 2018).

Despite the monitoring of fentanyl analogues, fentanyl is a molecule that can be modified in multiple ways to produce a multitude of derivatives, making it difficult to control and leaving regulatory systems to lag behind illegal manufacture (Higashikawa & Suzuki, 2008).

1.2.2. Pharmacology of NSOs.

NSO can be classified in three groups: fentanyl, fentanyl analogs and other non-fentanyl-derived synthetic opioids (Armenian, Vo, Barr-Walker, & Lynch, 2018; Pérez-Mañá et al., 2018; Suzuki & El-Haddad, 2017; Tabarra et al., 2019).

Paul Janssen first synthesized fentanyl in 1960. It was later, in 1963, when it was used for the first time as an intravenous analgesic and to this day is one of the most widely used

(Stanley, 2014). Fentanyl is a piperidine widely used for pain and anesthesia which is available in several formulations: injection, transdermal patch, tablets, etc. (Pérez-Mañá et al., 2018). The first reported misuse of fentanyl was in the 1970s-80s by health professionals, mostly anesthesiologists, who had easy access to the substance (Jungerman, Palhares Alves, Carmona, Conti, & Malbergier, 2012).

After the synthesis of fentanyl, several analogs were synthesized, including alfentanyl, remifentanyl and subfentanyl, which were approved for pharmaceutical use, and carfentanyl, only approved for veterinarian use (United Nations Office on Drugs and Crime, 2017). Other high-potency fentanyl derivatives have also appeared and are not approved for human medical use such as 4-fluorofentanyl or acetylfentanyl among others (Armenian et al., 2018).

These compounds and their metabolites have frequently been found together with some of the classical drugs, especially heroin, in biological samples of individuals with intoxications or overdoses (Pichini, Solimini, Berretta, Pacifici, & Busardò, 2018), but this mixture has also been found in seized substances (United Nations Office on Drugs and Crime (UNODC), 2017). Adding more potent and addictive compounds, such as fentanyl and its analogues, to classic drugs of abuse is a strategy used by drug dealers to achieve a higher market share (Schueler, 2017).

Most of non-fentanyl related opioids were synthetized in the 1970's (Tabarra et al., 2019). These compounds are a large group that can be classified in the benzamide (e.g. U-47700), actamide (e.g. U-50488) and piperazine (e.g. MT-45) family (Solimini et al., 2018).

Figure 3. Novel Synthetic Opioids (NSO) notified to the UNODC Early Warning Advisory for the first time in the period 2013-2019.

NSO have a different chemical structure than morphine, opioid referend compound (Concheiro, Chesser, Pardi, & Cooper, 2018). Similar to classic opiates (e.g. morphine) in general, the NSO are agonists of the μ-, δ-, and κ-opioid receptors in the endogenous opioid system (Frisoni et al., 2018). Despite this, we found some differences between the different substances belonging to the NSO group. Fentanyl presents a full affinity for the μ-receptor (Stanley, 2014). Fentanyl analogs produce the same effects as fentanyl but with structural differences (Drug Policy Alliance, 2021).

Although non-fentanyl opioids show particular pharmacodynamics and pharmacokinetics characteristics (Salle et al., 2019) most of them , primarily have affinity for μ-opioid receptors, but also have affinity for δ- and κ-opioid receptors (Solimini et al., 2018).

Furthermore, some of these compounds, such as U-47700, show a higher affinity for the and κ-opioid receptors (Sharma et al., 2019).

Each one of these compounds show different potencies to bind the receptors, but in general, they are more potent than morphine (Armenian et al., 2018) reaching their peak effects very quickly (Scholz, Steinfath, & Schulz, 1996). In the case of fentanyl, its potency is between 50 and 100 times higher compared to morphine (Suzuki & El-Haddad, 2017) but other compounds such as carfentanil are up to 10,000 times more potent than morphine (Salle et al., 2019) (Table 1).

The effects produces by NSO are pretty similar to those produced by morphine, including analgesia, sedation, anxiolysis, euphoria, somnolence, and feelings of relaxation (Kuczyńska, Grzonkowski, Kacprzak, & Zawilska, 2018; Suzuki & El-Haddad, 2017). In addition, some side effects can occur like nausea/vomiting, dizziness, delusions, tachycardia and constipation among others (Zawilska, 2017). Along with this, immunosuppression may also appear (Han et al., 2019)

Regarding overdoses, the symptomatology associated is very similar with both type of opioids (Pourmand, Mazer-Amirshahi, Chistov, Li, & Park, 2018). Among the most common symptoms of overdose are unconsciousness, myosis, bradycardia, hypotension, hypoxia and hypothermia and respiratory depression, being this last one which can result in a fatal overdose (Kraemer, Boehmer, Madea, & Maas, 2019; Tamama & Lynch, 2020).

Table1. Different groups of NSO and their potency compared to morphine in times more potent.

Type	Name	IUPAC* name	Potency compared to morphine (times)
Fentanyl	Fentanyl	*N*-phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]propanamide	50-100 [1]
Fentanyl analogs	3-methylfentanyl	N-[3-methyl-1-(2-phenylethyl)piperidin-4-yl]-N-phenylpropanamide	400-600 [2]
	Acetylfentanyl	N-phenyl-N-[1-(2-phenylethyl)piperidin-4-yl]acetamide	15 [2]
	Acetyl-alpha methylfentanyl	N-phenyl-N-[1-(1-phenylpropan-2-yl)piperidin-4-yl]acetamide	3.1 [5]
	Acryloylfentanyl	N-phenyl-N-[1-(2-phenylethyl)piperidin-4-yl]prop-2-enamide	100 [2]
	Alfentanil	N-[1-[2-(4-ethyl-5-oxotetrazol-1-yl)ethyl]-4-(methoxymethyl)piperidin-4-yl]-N-phenylpropanamide	UNKNOWN [7]
	Alpha-methyl fentanyl	N-phenyl-N-[1-(1-phenylpropan-2-yl)piperidin-4-yl]propanamide	±57 [5]
	Alpha-methylthiofentanyl	N-phenyl-N-[1-(1-thiophen-2-ylpropan-2-yl)piperidin-4-yl]propanamide	UNKNOWN [9]
	Beta-hydroxyfentanyl	N-[1-(2-hydroxy-2-phenylethyl)piperidin-4-yl]-N-phenylpropanamide	UNKNOWN [9]
	Beta-hydroxy-3-metyl fentanyl	N-[1-(β- hydroxyphenethy)-3-methyl-4-piperidyl]propionanilide	6300 [8]
	Beta-hydroxythiofentanyl	N-[1-(2-hydroxy-2-thiophen-2-ylethyl)piperidin-4-yl]-N-phenylpropanamide	UNKNOWN [9]
	Butyrylfentanyl (or butyrfentanyl)	N-phenyl-N-[1-(2-phenylethyl)piperidin-4-yl]butanamide	20-25 [2]
	Carfentanil	methyl 1-(2-phenylethyl)-4-(N-propanoylanilino)piperidine-4-carboxylate	10,000-100,000 [2]
	Furanylfentanyl	N-phenyl-N-[1-(2-phenylethyl)piperidin-4-yl]furan-2-carboxamide	20 [2]
	Ocfentanil	N-(2-fluorophenyl)-2-methoxy-N-[1-(2-phenylethyl)piperidin-4-yl]acetamide	100 [3]
	Valerylfentanyl	N-phenyl-N-[1-(2-phenylethyl)piperidin-4-yl]pentanamide	20 [2]
Non fentanyl analogs	AH-7921	3,4-dichloro-N-[[1-(dimethylamino)cyclohexyl]methyl]benzamide	Equal [6]
	U-47700	3,4-dichloro-N-[(1R,2R)-2-(dimethylamino)cyclohexyl]-N-methylbenzamide	±7.5[4]

* International Union of Pure and Applied Chemistry

1. (Prekupec et al., 2017)
2. (Schueler, 2017)
3. (Karila, Marillier, Chaumette, Nicolas, & Amine, 2019)
4. (Moody, Diaz, Shah, Papsun, & Logan, 2018)
5. (Higashikawa & Suzuki, 2008)
6. (Zawilska, 2017)
7. (Halliburton, 1988)
8. (Jin et al., 1981)
9. No data available at the moment.

1.2.3. Epidemiology

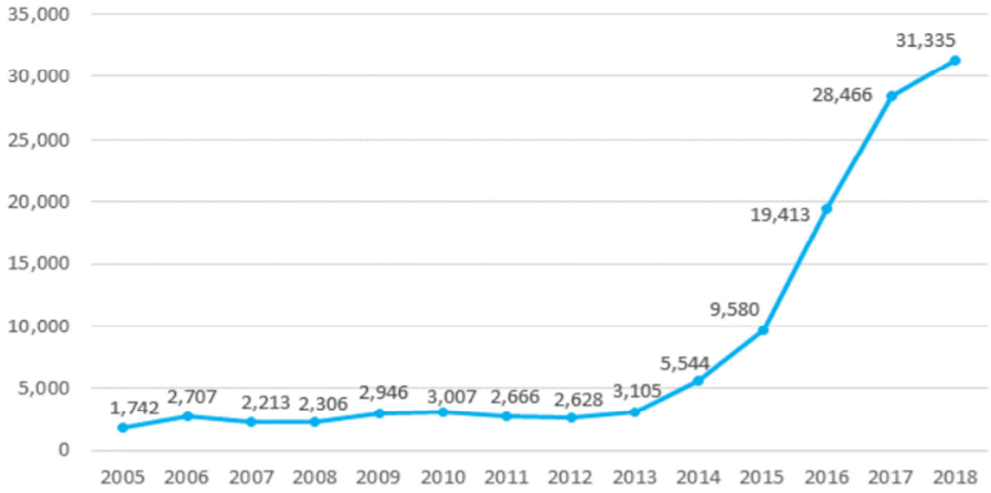
For two decades, the US has faced a worsening opioid crisis, resulting from the misuse prescription opioids and the abuse of illegal opioids, which represents one of the greatest health and political challenges for the country (Skolnick, 2018; Vadivelu, Kai, Kodumudi, Sramcik, & Kaye, 2018). Three different waves can be distinguished in this crisis. The first one and the beginning of the epidemic, starts in the 1990, is characterized by the increasing prescriptions of opiates , until 1999 at least, resulting in abuse of them for non-medical purposes. The second wave, in 2010, was characterized by the increased use of heroin. Finally, the third wave, in 2013, is characterized by the predominant use of new synthetic opioids (Centre for Disease Control and Prevention (CDC), 2017). It was during this last wave when detections of fentanyl only and fentanyl in other substances increased exponentially and since then it has not ceased (Drug Enforcement Administration (DEA), 2019).

There has also an increase in the number of intoxications or fatal overdoses related to NSO. In US overdose deaths in which synthetic opioids were involved raised from 3007 deaths in 2010 to 19.413 in 2016 representing early the 50% of opioids overdose (Jones, Einstein, & Compton, 2018). According to DEA synthetic opioid related deaths increase to 28,466 in 2017, being the most present illicit drug in fatal overdoses for two years consecutive (Drug Enforcement Administration (DEA), 2019) (see figure 4).

In fact, over the past 5 years, the number of fatal overdoses involving fentanyl or its derivatives exceeded those involving heroin and this trend continues until the latest population

data in January 2021 multiplying by 4 the deaths of 2016 (Centre for Disease Control and Prevention (CDC), 2021).

Figure 4. Fatal overdoses involving Synthetics Opioids other than methadone, 2005-2018 (Drug Enforcement Administration (DEA), 2020a).



Based on drug seizures, reported substances and forensic laboratory detections in US during 2019, fentanyl becomes widely available compared to previous years (Drug Enforcement Administration (DEA), 2019; United Nations Office on Drugs and Crime (UNODC), 2017). However, the detection of these substances is not always easy because their high potency allows small amounts and therefore makes them difficult to detect. (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)., 2019a).

Although fentanyl is usually detected in itself, it has also been found on several occasions mixed with other substances, mainly heroin, but also cocaine and other synthetic opioids (Drug Enforcement Administration (DEA), 2019, 2020a). Hence a problem is posed in differentiating those users who really want to consume NSO from those who consume them accidentally (Amlani et al., 2015; Volkow & Blanco, 2021). This involuntary consumption points to heroin users in particular, as a population vulnerable to intoxication or overdose due to lack of knowledge of the substance consumed.

There are several reasons that make it difficult to estimate the prevalence of NSO use. The first one is the nature of the drug market, which is constantly changing, making very difficult to monitor all the substances being synthesized (Lovrecic et al., 2019). Another reason is the lack of knowledge of its use, as these substances are often used as adulterants or sold as counterfeits of other illicit or pharmaceutical opioids (Drug Enforcement Administration (DEA), 2020a; European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)., 2020b; Rinaldi, Negro, & Minutillo, 2020). Finally, we found a 12-year delay from the start of opioid use until treatment is sought, making it difficult to estimate the prevalence of use in real time (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)., 2019a). In addition, the NSOs are not tested in standard toxicology tests, being under-reported (Pichini et al., 2017a)

In the case of Europe, although heroin remains the most widely used opiate, the inappropriate use of opioids used in the treatment of opioid use disorder (OUD), such as methadone or buprenorphine, or other synthetic opioids such as fentanyl, tramadol, codeine or oxycodone, is becoming increasingly common (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)., 2020a). Among new admissions to treatment for opioid use disorder, those referring opioids other than heroin as the primary drug increased from 8% in 2015 to 22% in 2019 (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)., 2015, 2019b).

In Europe, there is also a year-over-year increase since 2012 in the drug seizures of these substances (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)., 2021a). Since 2009, 73 unique NSO have been detected in the European drug market, 10 of which were reported for the first time in 2020 (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)., 2021c, 2022b).

Regarding fentanyl related fatal overdose, did not vary much from 2016 to 2017, which were 326 and 333 respectively (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)., 2021b). However, over the same period, there was a notable increase in those

deaths involving a fentanyl analogue, from 141 in 2016 to 242 in 2017. Despite these data, the toxicity caused by the consumption of NSO is underestimated because it is not sufficiently explored, and in many cases of intoxication there is a lack of specific analysis to help determine the presence or absence of these substances (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)., 2019b; Pichini, Pacifici, Marinelli, & Busardò, 2017b). Nevertheless, each country in Europe shows its particularities regarding synthetic opioids drug seizures, intoxications, overdoses and treatment admissions (Pierce, Amsterdam, Kalkman, Schellekens, & Brink, 2021). Along with that, the characteristics of the market are also different in each country, but in general terms, fentanyl and fentanyl derivatives reach the European drug market through two main sources. 1) The re-routing of pharmacological fentanyl from the regulated market to illegal drug market (Mounteney et al., 2015). 2) The illegal fentanyl production arriving in Europe is done in frontier countries such as Russia, Belarus and Ukraine (Mounteney et al., 2015). During the COVID-19 pandemia, the main routes for opioid trafficking has been from Afghanistan and Balkans (United Nations Office on Drugs and Crime (UNODC), 2021a). Also, precursors of fentanyl and non-controlled fentanyl precursor were seized in Estonia and Belgium and Germany respectively (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)., 2021b) suggesting the existence of illegal laboratories in Europe

In Spain, the prevalence of opioid analgesic misuse is relatively low, even so, the *Observatorio Español de las Drogas y las Adicciones (OEDA)* included it in its latest survey, thus pointing out the importance of this health problem that affects us. The prevalence of consumption for the last 12 months was 0.6% of the total population, and 0.2% for the last 30 days (Observatorio Español de las Drogas y Addicciones, 2021).

1.2.4. Biological samples analysis for detecting NSO

The screening of NSOs is not widely extended and considering the rapid emergence of NSOs, fentanyl, fentanyl derivatives and other synthetic opioids other than fentanyl, in our environment, we need effective methods for their detection in biological samples (Moody et al., 2018). Drug-checking, a service that offers users to check their drugs, is a very useful strategy for this purpose and also to identify substances available on the market (Maghsoudi et al., 2021). While this procedure is quite popular among recreational users (raves, music festivals…) it is not among people with SUD (Shafi et al., 2020). Along with these traditional methods used in clinical practice for the detection of substances normally do not include these compounds (Pichini et al., 2017a). So, it require to find standard methods that allow the qualitative and quantitative detection of these compounds (Zhang et al., 2020).

The objective of bioanalytical methods is to be able to determine the amount of drug or its metabolite in a different biological samples such as urine or blood among others (Nováková, Svoboda, & Pavlík, 2017). In this procedure, the analysis is performed after consumption, which makes it possible to know all the substances to which the subject has been exposed.

Therefore, several studies have validated different analytical methodologies to detect and quantified various NSO and their metabolites in different biological samples as hair, blood or urine (Gerace, Salomone, & Vincenti, 2018; Marchei et al., 2018). Some of these techniques are gas chromatography (GC-MS) and ultra-high-performance liquid chromatography-high-resolution mass spectrometry (UHPLC-HRMS) which are described below.

Gas chromatography, and concretely gas chromatography-mass spectromy (GC-MS) (Roda, Faggiani, Bolchi, Pallavicini, & Dei Cas, 2019) is the most widely used analytical technic for biological samples for both clinical and forensic toxicological applications (Zhang et al., 2020). GC-MS has been used and validated by several studies for the screening and

confirmation of fentanyls, fentanyl analogs and other NSOs (Jannetto et al., 2019) in different types of biological samples such as urine, blood or hair (Finkelstein, Chronister, Stanley, Ogilvie, & Goldberger, 2019; Goldberger, Chronister, & Merves, 2010; Misailidi et al., 2019; Mochizuki, Nakazawa, Adachi, Takekawa, & Shojo, 2018). Also has been used in *in vitro* studies (Blanckaert et al., 2020).

Ultra-high-performance liquid chromatography-high-resolution mass spectrometry (UHPLC-HRMS) is one of the most widely used in drug metabolism studies because of its greater capacity to detect metabolites, expected and unexpected, also, allows a quick analysis, around 5 minutes (Nováková et al., 2017). Studies, such as Zhang et al., 2020, have been able to demonstrate the usefulness of this analysis tool, being able to detect up to 32 different fentanyl-related substances. Other studies have pointed out its utility in post mortem cases in different samples such as whole blood, femoral blood, urine or even brain tissue (Mardal et al., 2018; Sofalvi et al., 2019).

Due to the complexity of these substances, it is necessary to use various analytical methodologies in the analysis of biological samples for the detection of NSO (Ameline et al., 2019). Both techniques described above have proven to be useful in the detection of these substances. Finally, the non-invasive nature of urine sampling and the availability of reliable procedures for its analysis, it is often the biological sample of choice for drug testing (Amante et al., 2021)

1.2.5. Treatment

Naloxone is the first-line treatment to reverse opioids overdoses (United Nations Office on Drugs and Crime & World Healt Organitation, 2013) as it is efficient in reverting the respiratory depression. It is very effective in managing heroin overdoses and also for other

opioids, including NSO, but it is important to administered at the correct time and the correct dose (Kuczyńska et al., 2018).

Despite this, overdoses with fentanyl or in combination with heroin or other substances appear to be more difficult to reverse with naloxone (Suzuki & El-Haddad, 2017). It has been shown that the management of overdoses with fentanyl requires a more rapid and dose-escalating administration of naloxone in order to reverse the overdose (Schumann, Erickson, Thompson, Zautcke, & Denton, 2009). Several reasons could explain the reduced effectiveness of naloxone in the management of fentanyl overdose, these include: the high potency at the μ-opioid receptor, the very rapid pharmacokinetics, and the longer duration of its respiratory depressant effects (Suzuki & El-Haddad, 2017). Together, different names for the same substance on the street market put consumers at risk and make it difficult to identify and study (Richards, Sitkowski, Heneghan, & Aronson, 2021).

Taking account these characteristics some implications in reducing the likelihood of death in NSO overdose include naloxone kits with higher dose and availability of easy-to-use formulations, like intranasal and auto-injector (Volkow & Blanco, 2021). These actions should be implemented in harm reduction rooms, emergency services, as well as provided directly to the users.

As important as it is to have tools to treat NSO overdoses is to be able to prevent them. Hence the importance of having the ability to detect these substances effectively and quickly, in the substance itself to provide information to the consumer of its composition, and also in biological fluids in the case of overdose, intoxication or even if the person reports the possibility of non-intentional consumption.

2. JUSTIFICATION

2. JUSTIFICATION

In Europe, the last decade has been characterized by an increase in the of first-time detections of NSO well as a wide variety of these substances on the market (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)., 2022b) together with an increase in the chemical variety of them (Pichini et al., 2017b).

The large number of heroin adulteration with NSO entails a risk for opioids consumers who are unaware of the drug they are using, increasing the chance of intoxication, overdose and/or fatal overdose (Morales et al., 2019; Rinaldi et al., 2020). This fact makes it difficult to know which people are consciously consuming NSO and which are doing it unknowingly (Volkow & Blanco, 2021). Hence, opioid users represent a very vulnerable population with regard to the challenge of the NSO.

Fewer studies have studied a population with subjects with OUD. This population presents a high prevalence of poly-consumption, which includes NPS (Heikman, Sundström, Pelander, & Ojanperä, 2016). These substances are widely investigated through recreational users (e.g. raves, musical festivals, etc.) (Bijlsma et al., 2020), by the detection in the wastewater (Bade et al., 2021) or in emergency rooms when intoxication, overdose or death is related to substance use (Elliott et al., 2018). But, in contrast NPS screening is not common in clinical practice (Shafi et al., 2020). Drug-checking is a very useful strategy for harm reduction as well as a way to identify substances available on the market (Maghsoudi et al., 2021) and post-consumption drug checking allows to know all the substances to which the subject has been exposed (Palamar, Salomone, & Barratt, 2020).

There are still some regions in Europe, and in particular in our country, where there is no data or insufficient information available on the consumption of NSO. Along with that, since 2019, the OEDA has included in its consumption survey opioids analgesics without prescription, pointing out a possible problem in our country. There is a need to know the situation in our

region regarding the NSO challenge in order to detect any emerging trends of individuals with OUD.

3. HYPOTHESIS AND OBJECTIVES

3. HYPOTHESIS AND OBJECTIVES

3.1. Hypothesis

There is a growing phenomenon of consumption of new synthetic opioids in Europe, so there is use in our area of these substances among opioid use disorder population.

3.2. Objectives

3.2.1. General objective

The main objective of this study is to determine the prevalence use of NPS, including NSO, among individuals with OUD diagnosis in two different addiction care centers from Barcelona and Badalona.

3.2.2. Specific objectives.

- To establish the prevalence of NSO use in individuals with OUD and characterize their use taking into account a gender perspective.
- To establish the prevalence use of NPS and other illegal drugs among OUD population.

4. METHODS AND RESULTS

4. METHODS AND RESULTS

4.1. Manuscript 1: New Synthetic Opioids Use among Patients in Treatment for an Opioid Use Disorder in Barcelona.

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New Synthetic Opioids Use among Patients in Treatment for an Opioid Use Disorder in Barcelona

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Keywords
New drugs · Opiate addiction · Psychoactive substances · New synthetic opioids

Abstract
Introduction: New synthetic opioids (NSO), a class of new psychoactive substances (NPS), have recently emerged and pose an upcoming global public health challenge. The effects produced by NSO are similar to those from morphine, but they present greater pharmacological potency and abuse potential. Due to the increasing number of fatal overdoses and seizures in which NSO have been detected as heroin substitutes or adulterants, individuals with Opioid Use Disorder (OUD) represent a vulnerable population. The aim of our study was to describe and characterize from a gender perspective a Spanish cohort of potential conscious or unconscious NSO users. ***Methods:*** A cross-sectional study was conducted in a cohort of OUD participants under treatment in addiction care services in Barcelona and Badalona, Spain. Clinical evaluation was performed through an ad hoc survey, a scale to evaluate reasons to use an opioid without prescription (range 0–4) and the Wellbeing Index (WHO-5) (range 0–100). Objective consumption of NSO was assessed by urinalysis carried out by two validated methods: high-sensitivity gas chromatography-mass spectrometry (MS) and ultra-high-performance liquid chromatography-high-resolution MS. ***Results:*** A total of 154 participants with OUD were enrolled. They were mainly men (72.7%), mean age 47.8 years. Methadone was the predominant medication for opioid agonist treatment (mean dose 61.25 mg/day). A total of 32 (20.8%) participants reported having consumed some opioid to become "high" in the previous 3 months. The principal reasons for consuming illicit opioids were Replacing other

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drugs (mean 2.03) and Availability (mean 1.62), although Low price, was more highly valued by men ($p = 0.045$) and Shorter effect duration, most highly rated by women ($p = <0.001$). In the WHO-5, the mean score was 55 (SD = 30.1) without differences by gender. Fentanyl and derivatives or/and metabolites were detected in 7 (6.1%) participants, but illicit/non-prescribed NSOs were found in 5 out of 114 patients (4.4%), and other non-fentanyl opioids in 36 participants (26 men and 10 women). ***Conclusion:*** A non-negligible consumption of NSO-fentanyl's (positive detection in 6.1% of biological samples) was detected. The reasons for using these substances and also the well-being differed between the genders. There is therefore both voluntary and involuntary NSO consumption in our country which highlights the importance of approaching this potential public health problem.

Introduction

The most recent emerging class of New Psychoactive Substances (NPS) has been the one of new synthetic opioids (NSO) [1, 2]. For two decades, the USA has faced a worsening opioid crisis, resulting from the misuse prescription opioids and the abuse of illegal opioids, which represents one of the greatest health and political challenges for the country [3, 4]. Different waves in this epidemic can be differentiated according to the predominant opioid and it is since 2013 approximately that synthetic opioids are the predominant ones [5], with an increase in the detection of fentanyls and derivatives [6]. This opioid crisis extended to other Commonwealth Countries (e.g., Australia and New Zealand) finally reaching Europe with case data are fragmented and quite different between countries [7].

In fact, since 2009, 67 NSO have been detected on the European drug market, 10 of which were reported for the first time in 2020 [1]. NSO selectively bind to the μ-, δ-, and/or κ-opioid receptors in the endogenous opioid system [8–10]. In a similar manner to morphine and other mu-agonist active compounds, NSO effects include analgesia, sedation, anxiolysis, euphoria, somnolence, and feelings of relaxation [11]. NSO potency, ranging from 100- to 1,000-fold that of morphine/heroin, results in an elevated risk of overdose [12–14] especially when administered as a heroin substitute, heroin adulterants, or counterfeit products.

NSO can be classified into three groups: fentanyl, fentanyl analogues, and nonfentanyl-derived synthetic opioids [11, 15, 16]. Of these, fentanyl analogues have been the compounds most involved in non-fatal and fatal intoxications particularly in the USA and in recent years in some European countries, [1, 17, 18]. It is believed that the real extent of NSO toxicity is probably underestimated. This is probably due to the fact that NSO overdoses are underdisclosed; in the majority of cases, they lack specific investigations and objective evidence of the intoxicating agent (parent drugs and/or metabolites) in the biological fluids of the affected individuals [12, 19].

Actual figures for the fentanyl epidemic are unknown given that NSO are used in place of heroin, as a cheaper alternative, or as an adulterant [12, 20]. There is therefore a greater likelihood of substitution, adulteration, and miss-selling of these substances which could lead to a higher number of intoxications [21].

To date, there is scarce information regarding NSO use by individuals with heroin or other opioid addictions who represent a predominantly male population (around 80% men). Moreover, specific data on some European regions and Mediterranean areas are missing. The aim of the study was to establish the prevalence of NSO consumption in individuals with Opioid Use Disorder (OUD) attending two different addiction care facilities in the greater Barcelona area (Barcelona city and Badalona) and to characterize the feature of their use and related motivation.

Materials and Methods

Participants

This is a cross-sectional study with one group of treatment seeking patients with an OUD. The study sample was made up of patients with an OUD diagnosis according to DSM-5 criteria [22] in treatment at the addiction care services from Hospital del Mar, Barcelona, Spain and Hospital Universitari Germans Trias i Pujol, Badalona, Spain. Recruitment was carried out from February 2019 to March 2020 and from July to October 2020.

Inclusion criteria were subjects with an OUD (DSM-5), older than 18 years, attending any of the two addiction care facilities and speaking/understanding Spanish. The exclusion criteria included linguistic and cognitive barriers that impaired the subject's correct evaluation. All participants gave their written consent, and the study was approved by the Ethics Committee in Clinical Research Parc de Salut MAR (CEIC-PSMAR, number 2018/8138/I) and Hospital Universitari Germans Trias i Pujol (CEIC-HUGTiP number (PI-18-126).

Clinical Assessments

Clinical assessment was performed through a structured face-to-face interview of approximately 20–30 min. Instruments used in the interview were: (a) An "*ad-hoc questionnaire*" including: sociodemographic data, history of opioid use (lifetime history of overdoses, age of first treatment, current treatment), any substance

Table 1. Sociodemographic and clinical characteristics of the 154 study participants

	Total *N* = 154	Men *N* = 112 (72.72%)	Women *N* = 41 (26.62%)	Transgender *N* = 1 (0.66%)	*p* value
Age (mean SD)	47.80 (8.72)	48.20 (8.49)	46.76 (9.46)	46 (–)	0.653
Birthplace, *N* (%)					
Spain	121 (78.57)	90 (80.35)	30 (73.17)	1 (100)	0.550
Other	33 (21.43)	22 (19.65)	11 (26.83)	–	
Current opioid agonist treatment, *N* (%)					
Total	142 (92.20)	102 (91.07)	39 (95.12)	1 (100)	0.768
Buprenorphine	20 (12.99)	12 (10.71)	8 (19.51)	–	
Methadone	116 (75.32)	87 (77.67)	28 (68.29)	1 (100)	0.444
Morphine slow release	5 (3.25)	2 (1.79)	3 (7.32)	–	
Other*	1 (0.65)	1 (0.89)	–	–	
Methadone doses, mg/day (mean SD)	61.25 (44.92) Min: 2 Max: 235	61.03 (42.00) Min: 5 Max: 235	62.15 (54.32) Min: 2 Max: 220	55 (–)	0.137
Age of first drug treatment (mean SD)	28.53 (13.82)	29.42 (14.76)	26.07 (10.82)	30 (–)	0.415
Lifetime overdose, *N* (%)					
Heroin	59 (38.31)	41 (36.60)	18 (43.90)	–	0.522
Other opioid	2 (1.30)	0 (–)	2 (4.88)	–	0.684
Other substances	25 (16.23)	7 (6.25)	17 (11.04)	1 (100)	0.072
Substances used in the last 3 months, *N* (%)					
Heroin	52 (33.77)	43 (38.39)	9 (21.95)	–	0.126
Speedball	16 (10.39)	13 (11.60)	3 (7.32)	–	0.701
Cocaine	59 (38.31)	45 (40.18)	14 (34.15)	–	0.581
Amphetamine**	11 (7.14)	9 (8.04)	2 (4.88)	–	0.768
Cannabis	63 (40.91)	48 (42.86)	14 (34.15)	–	0.302
Alcohol	24 (15.58)	21 (18.75)	3 (7.32)	–	0.205
Gabapentin**	1 (0.65)	1 (0.89)	–	–	0.828
Benzodiazepines**	18 (11.69)	13 (11.61)	5 (12.19)	–	0.931
Ketamine	2 (1.30)	–	2 (4.88)	–	–
MDMA	1 (0.65)	1 (0.89)	–		

* Fentanyl. ** Without prescription.

consumed in the previous 3 months, and being "high" without prescription; (b) in the case participants reported the use of any without prescription opioid resulting a sensation of being "high," they were asked to evaluate 14 potential reasons why they chose it on a five-point Likert scale (0 = not true at all; 1 = rather not true; 2 = partly true; 3 = rather true; 4 = "completely true") [23]; and (c) *The Wellbeing Index* (WHO-5, 1998) was used to assess the subject's subjective and psychological well-being [24]. This self-administered index consists of five questions that refer to the patient's physical-emotional state over the previous two weeks. The total score obtained ranges from 0 to 100 points; the higher the score, the greater the well-being.

Sample Collection and Biological Analysis

At recruitment, a clinical assessment was performed and a urine sample obtained. Urine samples were analysed by two different set-ups and validated methodologies: ultra-high-performance liquid chromatography-high-resolution mass spectrometry for extensive of screening for NSOs and general NPS and high-sensitivity gas chromatography-mass spectrometry for confirmation of identified compounds [25].

Data Analysis

The statistical analysis was carried out with the SPSS version 22.0 (SPSS Inc., Chicago, IL, USA) software. Descriptive analyses were used to characterize the samples. The estimations of the rates for each categorical variable were described in frequencies and percentages, and for the continuous ones in mean and standard deviations. Comparisons of sociodemographic and clinical data, reasons for NSO use, and the WHO-5 score according to gender were performed using Student's *t* test for continuous variables and the χ^2 test for categorical ones. Statistical significance level was set at $p < 0.05$.

Results

Sociodemographic and Clinical Assessment

A total of 154 participants were enrolled in the study. Men (*N* = 112; 72.7%) outnumbered women (*N* = 41; 26.6%) and there was one transgender individual (0.7%). The mean age for all participants was 47.80 years and 121

Table 2. Participants who reported use of any opioid (except heroin) in the previous 3 months to get "high" and reasons for selecting them (*N* = 32)

	Total *N* = 32	Men *N* = 24 (21.04%)	Women *N* = 8 (19.51%)	*p* value
Opioids (except heroin), *n* (%)				
Oxycodone	1 (0.65)	1 (0.89)	–	0.828
Fentanyl	7 (4.55)	3 (2.68)	4 (9.76)	0.173
Other fentanyl derivatives*	1 (0.65)	–	1 (2.44)	0.250
Tramadol	11 (7.14)	8 (7.14)	3 (7.32)	0.961
Morphine	4 (2.60)	3 (2.68)	1 (2.44)	0.983
Tapentadol	1 (0.65)	1 (0.89)	–	0.828
Methadone	16 (10.39)	14 (12.50)	2 (4.88)	0.370
Buprenorphine	1 (0.65)	1 (0.89)	–	0.828
Codeine	2 (1.30)	1 (0.89)	1 (2.44)	0.918
Hydrocodone	1 (0.65)	1 (0.89)	–	0.828
Reasons for consuming opioids (mean scale score [0–4**]), *n* (%)				
Curiosity	1.09 (1.67)	1.17 (1.76)	0.88 (1.46)	0.161
Replacing other drugs	2.03 (1.84)	1.88 (1.90)	2.5 (1.69)	0.094
Availability	1.62 (1.69)	1.63 (1.69)	1.63 (1.85)	0.742
My friends also used it	0.91 (1.44)	0.88 (1.33)	1 (1.85)	0.241
More intense subjective effects	1.06 (1.66)	0.92 (1.67)	1.5 (1.69)	0.713
I did not know why I used	0.53 (1.19)	0.46 (1.10)	0.75 (1.49)	0.279
Low price	1.56 (1.70)	1.92 (1.67)	0.5 (1.41)	0.045
Legality	0.56 (1.24)	0.58 (1.21)	0.5 (1.41)	0.888
Shorter effect duration	0.31 (0.93)	0.13 (0.45)	0.88 (1.64)	<0.001
It cannot be detected in biological samples	–	–	–	–
Exotic brand name	–	–	–	–
I thought it was safer	0.13 (0.55)	0.17	–	0.134
Attractive packaging	0.09 (0.53)	0.13	–	0.242
I thought it was more natural	0.03 (0.17)	–	0.13	–

* Carfentanyl. ** Scale scores (0–4): 0 = not true at all, 1 = rather not true, 2 = partly true, 3 = rather true, 4 = completely true.

(78.6%) were Spanish, without differences between genders. Main clinical characteristics are described in Table 1.

Of the 154 participants, 142 (92.2%) were included in a current opioid agonists treatment program, and 12 (7.8%) were not in an opioid agonist treatment (first day of contact to the addiction facility). Among opioid agonist treatment, methadone was the most frequent prescribed medication (*N* = 116, 75.3%), with a mean dose of 61.25 mg/day, although near half of the participants (*N* = 59, 51%) received a dose below 60 mg/day (mean = 31.8; SD = 13.6). The mean age for the first treatment for OUD was 28.53 years, and heroin was the main drug consumed (*N* = 123, 79.9%). Interestingly, 59 (38.3%) participants reported at least one lifetime overdose episode with heroin. In relation to the use of other substances in the previous 3 months, the most commonly consumed ones were cannabis (*N* = 63; 40.9%), cocaine (*N* = 59; 38.3%), and benzodiazepines without prescription (*N* = 18; 11.7%).

A total of 32 (20.8%) participants, 24 (21.4%) men and 8 (19.5%) women, reported the use of non-prescribed opioids other than heroin to get "high" in the previous 3 months. The mean number of non-prescribed reported opioids was 1.28 (SD = 0.57). Methadone without prescription was the most commonly reported (16 participants), followed by tramadol (11) and fentanyl (7). The reasons for use were principally *Replacing other drugs* (mean 2.03, SD = 1.84) and *Availability* (mean 1.62, SD = 1.69). Differences between gender were found in two reasons: *Low price*, which was more highly valued by men than women (*p* = 0.045), and *Shorter effect duration,* most highly rated by women (*p* = <0.001) (Table 2).

The mean score for all the participants in the WHO-5 questionnaire was 55 (SD = 30.1) without significant gender differences [men: mean = 57.1 (SD = 29.7) versus women mean = 49.9 (SD = 30.8), *p* = 0.860]. In addition, no differences between male and female participants were

Table 3. Opioids and metabolites detected in biological samples (*N* = 114)

	Detected in biological sample (*N* = 114), *N* (%)	Non-prescribed, *N* (%)
Substitution opioids	**93**	**7**
Methadone	90 (79)	7 (6.1)
Morphine	4 (3.5)	–
Buprenorphine	1 (0.9)	–
Fentanyl and derivatives	**7**	**5**
Fentanyl/norfentanyl	4 (3.5)	2 (1.8)
Beta-hydroxyfentanyl	1 (0.9)	1 (0.9)
Fluorofentanyl	2 (1.8)	2 (1.8)
Fluoro acetyl fentanyl	1 (0.9)	1 (0.9)
Fluoro valeril fentanyl	1 (0.9)	1 (0.9)
Norfentanyl	1 (0.9)	1 (0.9)
Heroin and other opioids	**36**	**34**
Heroin	14 (12.3)	14 (12.3)
Anileridine	3 (2.6)	3 (2.6)
Codeine	2 (1.8)	2 (1.8)
Dextromethorphan	20 (17.5)	20 (17.6)
Difenoxin	1 (0.9)	1 (0.9)
Hydromorphone	3 (2.6)	3 (2.6)
Hydrocodone	1 (0.9)	1 (0.9)
Levorphanol/Dextrorphan	5 (4.4)	5 (4.4)
N-Desmethyltramadol	1 (0.9)	1 (0.9)
Norpropoxifene	5 (4.4)	5 (4.4)
Tapentadol	1 (0.9)	–
Tramadol	3 (2.6)	2 (1.8)

found for age, current opioids agonist treatment, methadone dose, age of first drug treatment, lifetime overdose, and substances used in last 3 months (Table 1).

Sample Collection and Biological Sample Analysis

Of the 154 participants in the study, we obtained single samples from 114 of the subjects (74.0%). All the types of opioids detected in the samples are shown in Table 3. In some of them more than one opioid was found (164 opioids in total). In addition, information provided by the participants regarding the prescription or not of any opioid is depicted in the same table.

Opioids other than those prescribed were detected in the urine samples of 41 subjects (35.9%). Of these 41 subjects, 27 were on methadone treatment (mean mg/day 74.3; SD = 52.3), five on buprenorphine treatment (mean buprenorphine mg/day 6.8; SD = 5.6) and four on morphine slow sustained-release preparation (mean 390 mg/day; SD = 128.1).

In case of fentanyl and derivatives/analogues, fentanyl itself was detected in 7 participants (6.1%), but the presence of illicit/non-prescribed NSOs was found in only 5 out of 114 patients (4.4%). Other opioids (non-fentanyl derivatives) were found in 36 (31.6%) participants, and in only 2 (1.8%) cases, those were under prescription, one case for tapentadol and one case for tramadol. The remaining 34 (29.8%) cases were illegal/non-prescribed opioids. Dextromethorphan, without prescription, was the most frequent opioid detected in the urine samples (*N* = 20; 17.5%), followed by heroin (*N* = 14; 12.3%), and methadone without prescription (*N* = 7; 6.1%).

Regarding awareness of having taken NSO, none of the 5 participants in which illegal fentanyl/derivatives was detected, reported having used it in the previous 3 months to "get high." With respect to other opioids (non-fentanyl derivatives), among the 36 subjects, 19 (52.8%) participants reported their use in the previous 3 months to "get high."

Discussion

The results show that synthetic opioids are consumed within our social context. Only 30% of the participants in the identified cases reported using an opioid to "get high." When distinguishing in between nonprescribed non-fentanyl opioids and fentanyls and analogues, we observed that the prevalence of detection in urine for the former was higher (25.4%) than that of the latter (4.4%). Such findings are consistent with recent data provided by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) in which the proportion of non-fentanyl opioids reported in seizures was higher than that of fentanyl's and analogues [21, 26]. It is also important to mention that in case non-prescribed fentanyls, this 4.4% is a very limited figure that is significantly smaller than similar numbers in the USA, Canada, and some other European countries [7, 27]. Considering opioids detected, most frequent was dextromethorphan, it is a weak opioid that is present as an ingredient in coughing syrup and probably available as a licit over the counter opioid, and this may explain its relatively frequent use in this population. It is also important to mention that oxycodone was not detected (and hydrocodone only once) in this group of patients although oxycodone/naloxone a relative used analgesic in our country (13% opioid prescriptions) [27, 28].

When participants were asked why they consumed opioids other than heroin, the two principal responses were *Replacing other drugs* and *Availability* of the substance. These two reasons are also among those most highly rated in the study by Kapitány-Fövény et al. [23].

Other authors have indicated pleasure/enjoyment and coping with some kind of problem such as pain, anxiety, and insomnia as the main causes for NSO consumption [29]. Relatively low dose of methadone might suggest that higher doses in this population may be having resulted in lower levels of illicit opioid use. Understanding the reasons behind NSO use is crucial in order to design better harm reduction and treatment strategies, as increase the doses of the opioid agonist treatment, provide information of different NSO in addiction facilities (including self-injection rooms), easy availability of naloxone kits.

A key issue in the World Health Organization health definition is well-being. In this respect, our participants obtained a WHO-5 score lower than the average reported for the general Spanish population (65.4 points) [30]. When classifying the scores for the general Spanish population by gender, it can be observed that the women's was lower (mean = 64.9) than the men's (mean = 70.5) [31]. Several studies have pointed to the usefulness of the WHO-5 as an instrument to detect depression, reaching a consensus that a score equal to or less than 50 indicates that there is a risk of depressive disorder [32]. In the particular case of OUD women, their mean score was lower than 50 points for the WHO-5 so a comprehensive examination would be recommended in order to determine whether or not a depressive disorder exists. In our results, among 154 participants included in this study, 19 (46.3%) women and 45 (40.2%) men, showed a score <50 for the WHO-5 scale, suggesting the presence of comorbid depression that emphasizes the need of study the presence of other psychiatric disorders in patients with substance use disorder, mainly in women [33].

According to the EMCDDA 2020 [26], opioids, often together with other substances, have been detected in the majority of fatal overdoses, and the age at which this occurs is increasing year by year. Several acute intoxications and fatal overdoses involving fentanyls, fentanyl analogues, and non-fentanyl opioids have been reported in recent years [34–37]. In addition, the number of NSO poisonings has been rising [38] and these substances are being sold as heroin or added as adulterants [39]. Taking into account such circumstances, individuals with an OUD are a population particularly at risk. Among our participants, approximately 5 of 7 (70%) of those with urinalysis positive for fentanyl and/or metabolites/analogues and synthetic opioids were unaware they were taking that type of substance. Although the small sample size in our study, such a finding highlights the need to develop effective strategies for the detection in biological samples of these compounds in order to provide effective treatment response and prevent NSO overdose [40, 41]. In addition, as a high percentage of NSOs are adulterants or counterfeit substances, consumers need to be informed about the products contained in the substances they intend to use.

In a similar manner to other studies, a tendency for patients in opiate agonist treatment to use other illicit drugs, particularly cocaine, was observed [42, 43]. Regarding the detection of classical illicit drugs, we confirmed that cocaine was the most frequent substance. Some studies have suggested that cocaine consumption may have a negative impact on treatment retention for opioid substitution [43], facilitating the use of opioids, including NSO.

Among the limitations of the study is the relatively small sample size. The mean methadone dose was relatively low, which may be a risk factor for illicit opioid use in patients receiving opioid agonist treatment. A higher maintenance dose or greater control of the treatment could be effective in reducing the consumption of illicit opioids.

Nevertheless, NSO represent a minor percentage of NPS, the consumption prevalence of which in Spain is 1.7% among individuals aged 15–64 years [44]. In addition, gender differences require further research due to the limited number of women in our sample. With respect to design, our study was transversal, a sole one-point sample was collected from each participant, and thus the possibility of substance detection depended on the time of consumption, dose, and elimination half-life in urine. Although further research is warranted for a comprehensive understanding of the situation, our study provides an overview of NSO use and highlights the importance of remaining vigilant to this potential public health problem.

Conclusion

This study provides a preliminary description of the situation regarding NSO consumption among opiate users in the greater Barcelona area. We can conclude there is no an opioid crisis in our area as reported by the USA and some European countries. Despite this, a starting phenomenon has been observed which, according to the experience of the countries mentioned above, requires attention even in a limited number of individuals. Our preliminary results demonstrate that in individuals with OUD there is both voluntary and involuntary NSO consumption with the risks such behaviour entails, particu-

larly when such substances are used unknowingly. Such findings emphasize the importance of appropriately adapting the public health system so as to reduce any consequences arising from NSO consumption in individuals with OUD.

Statement of Ethics

The study was conducted in accordance to the national and international guidelines (Declaration of Helsinki) and the legislation regarding the treatment, communication, and transfer of personal data (protection data regulation of the EU 2016/679 of the European Parliament and of the Council April 27th 2016, Protection data [RGPD]). The study was approved by the Ethics Committee in Clinical Research Parc de Salut MAR (CEIC-PSMAR, number 2018/8138/I) and Hospital Universitari Germans Trias I Pujol (CEIC-HUGTiP number (PI-18-126). Written informed consent was obtained from each participant after a complete description of the study and any question/issue being fully answered.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Conception and design of the work: Marta Torrens, Simona Pichini, Roberta Pacifici, and Magí Farré. Validation and investigation: Marta Torrens, Maria Alías-Ferri, Emilia Marchei, Roberta Pacifici, Simona Pichini, Manuela Pellegrini, Clara Pérez-Maña, Esther Papaseit, Roberto Muga, Francina Fonseca, and Magí Farré. Analysis and interpretation of the data: Marta Torrens, Maria Alías-Ferri, Emilia Marchei, Simona Pichini, Manuela Pellegrini, and Magí Farré. Draft preparation: Maria Alías-Ferri, Simona Pichini, Francina Fonseca, Marta Torrens, and Magí Farré. Critical review: Marta Torrens, Maria Alías-Ferri, Roberta Pacifici, Simona Pichini, and Magí Farré. All the authors: Marta Torrens, Maria Alías-Ferri, Emilia Marchei, Roberta Pacifici, Simona Pichini, Manuela Pellegrini, Clara Pérez-Maña, Esther Papaseit, Roberto Muga, Francina Fonseca, and Magí Farré approved the final version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Data Availability Statement

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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4.2. Manuscript 2: New Psychoactive Substances Consumption in Opioid-Use Disorder Patients.

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biology

MDPI

Article

New Psychoactive Substances Consumption in Opioid-Use Disorder Patients

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Simple Summary: We applied a toxicological screening on 187 urine samples collected from patients with opioid-use disorder treated with opioid agonists in Barcelona and Badalona addiction care services, Spain. We found that 27.3% of urine samples were positive for any type of new psychoactive substance and 8.6% of samples were positive for a new synthetic opioid (NSO). These results show a new trend of consumption in patients with opioid-use disorder that requires social and political actions to stem associated health threats.

Abstract: (1) Background: Since the beginning of the 21st century, the large number and wide chemical variety of new psychoactive substances (NPS) that enter the market every year has become a public health problem. Given the rapidity with which the drug market is changing, many NPS are not clinically investigated and their effects and health risks are unknown. Drug testing is a very useful tool for this purpose, but, unfortunately, it is not very widespread in individuals with opioid-use disorder under detoxification treatment. The aim of this study is to investigate the use of illicit drugs and NPS in opioid-use disorder (OUD) patients on opioid agonist treatment. (2) Methods: A multicenter, descriptive, cross-sectional study was conducted at two addiction care services in Barcelona and Badalona, Spain. Urine samples were collected from OUD individuals attending these two centers, who anonymously donated a urine sample at the time of a periodical visit. Samples were analyzed by high-sensitivity gas chromatography-mass spectrometry (GC-MS) and ultra-high-performance liquid chromatography-high –resolution mass spectrometry (UHPLC-HRMS). (3) Results: Out of the 187 collected and analyzed urine samples, 27.3% were positive for any type of NPS and 8.6% were positive for new synthetic opioids, including fentanyl and its derivatives (NSO). Other frequently detected substances were benzodiazepines in 46.0% of samples, antipsychotics in 27.8% of samples, or cocaine and cannabis in 23.5% of samples. (4) Conclusion: A wide number of NPS, including NSO, have been detected in urine samples from an OUD population. A lack of NPS detection in standard drug screening among drug users can hide the identification of a potential public health problem.

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1. Introduction

In the 20th century, the illicit market of drugs of abuse was limited to a few classes of psychotropic substances such as cannabis, opiates, cocaine, amphetamines, hallucinogens, and benzodiazepines [1]. In the 21st century, that market expanded exponentially with the availability of new psychoactive substances (NPS), a very heterogeneous group of substances with a wide range of mechanisms and chemical variety [2–4].

Worldwide, 1124 NPS have been reported to the UNODC Early Warning Advisory from 2009 up to January 2022 [5], and in Europe, in these first twenty years of the 21st century, more than 1000 NPS [6] were made available on the street and internet dealing. Some of these entered and left the illicit market very quickly, while others persisted over the years and some showed a sharp demand increase, especially during the COVID-19 pandemic [7,8]. Although in Europe, the NPS most commonly detected in both drug seizures and intoxications are synthetic cannabinoids and synthetic cathinones, in recent years, a rapid and constant barrage of New Synthetic Opioids (NSO) has been observed [9].

Polysubstance use typically includes the simultaneous consumption of three or four psychotropic substances from opiates, cocaine, cannabinoids, and amphetamines classes [10]. More recently, polyconsumption can also involve the addition of NPS [11,12]. However, it is yet unclear how many users of "classical" illicit psychotropic substances are attracted by NPS. Along with this, some NPS have been used as street opiates adulterants, being fentanyl and their analogues as the most common ones [13]. This latter occurrence entails a risk for users, either because of a lack of knowledge of the consumed product and/or because of the high potency of the above-reported adulterants, which can result in a fatal overdose [14]. In general, NPS users are young individuals who also use other substances in a recreational setting, usually did not have a concomitant substance use disorder [15], and are the most frequently intoxicated by the use of these substances [16]. One exception can be the use of NSO, such as fentanyl and its derivatives, that are most commonly abused by subjects with an opioid-use disorder (OUD) [17].

The screening of NPS in clinical practice is not widespread, as well as in recreational consumers [18]. Drug checking is a very useful strategy for harm reduction, as well as a way to identify substances available on the market [3]. This service usually offers consumers the possibility to analyze illicit drugs before consumption, whereas post-consumption drug checking, which allows one to know the substances consumed, is less common [19].

Several studies have investigated the use and/or detection of classical psychoactive drugs and NPS in mainly recreational users (e.g., raves, musical festivals, etc.) [20], through wastewater analysis [21], in emergency rooms when intoxication, overdose, or death was attributed to the use of these substances [22], or in patients in detoxification treatment [23]. Fewer studies have focused on individuals with OUD on opioid maintenance treatment. This population presents with a high prevalence of polysubstance use, including NPS among the abused compounds [23]. Apart from these studies, the consumption of NPS in populations with OUD is scarcely studied in some European regions and specifically in Mediterranean areas.

In this regard, we investigated the consumption of common drugs of abuse and NPS in individuals with OUD attending outpatient addiction care services in the greater Barcelona area (Barcelona city and Badalona, Spain) by urinalysis.

2. Materials and Methods

2.1. Study Design and Participants

A multicenter, descriptive, cross-sectional study was conducted at the addiction care services of the Hospital del Mar, Barcelona, Spain and the Hospital Universitari Germans

Trias i Pujol, Badalona, Spain, from February 2019 to March 2020 and from July to October 2020. Due to the COVID-19 pandemic and the impact it has caused on the functioning of addiction care services, sample collection was cancelled from 13 March until 6 July 2020.

The subjects enrolled in the study donated an anonymous urine sample during their regular urine test at the addiction care service and, since the participation was voluntary and anonymous, their personal data or any other medical information were not recorded. Subjects had to meet the following inclusion criteria: being over 18 years of age, having an opioid-use disorder according to DSM-5 criteria [24], and being on opioid agonist treatment. No exclusion criteria were applied.

The study was approved by the Ethics Committee in Clinical Research Parc de Salut MAR (CEIC-PSMAR, number 2018/8138/I) and Hospital Universitari Germans Trias i Pujol (CEIC-HUGTiP number PI-18-126).

2.2. Urinalysis

Urine samples from recruited individuals were collected without any preservative and stored at −20 °C until analysis. Urinalysis was performed by two different set-ups and validated methodologies. An ultra-high-performance liquid chromatography-high-resolution mass spectrometry (UHPLC-HRMS) assay was used for extensive screening of more than 1000 pharmacologically active substances, including prescription psychoactive drugs, classic drugs of abuse (e.g., opiates, cocainics, amphetamine-type substances, cannabinoids, hallucinogens, etc.), NPS (parent drugs and metabolites), prescription opioids (e.g., oxycodone, hydromorphone, hydrocodone, etc.), NSO such as fentanyl and analogs, and benzoimidaloles (e.g., etonitazene, isotonitazene and metonitazene) [25]. A last generation gas chromatography-mass spectrometry method was used for the confirmation of identified compounds [25].

2.3. Data Analysis

The rates for each detected substance and metabolites were described as frequencies and percentages using the SPSS version 22.0 (SPSS Inc., Chicago, IL, USA) software.

3. Results

One hundred eighty-seven participants were recruited for the study and donated a urine sample. Although the samples were collected anonymously, they are part of an opioid agonist treatment program (main characteristics: 68% men, mean age 52 years old, range: 28–77). The NPS detections are shown in Table 1 and the detection of other substances in Table 2.

Table 1. Detected NPS and metabolites in biological samples (N = 187).

NPS	Detected in Urine, *n* = 187
NSO AND FENTANYL	16 (8.6)
2F-ortho-fluorofentanyl	1 (0.5)
2-Fluorofentanyl	1 (0.5)
Acetyl-methyl fentanyl	1 (0.5)
Beta-hydroxyfentanil	2 (1.1)
Fentanyl	7 (3.7)
Fluorofentanyl	2 (1.1)
Fluoro valeril fentanyl	1 (0.5)
Meta fluoro valeril fentanyl	3 (1.6)
Norfentanyl	7 (3.7)
Thiofentanyl	2 (1.1)
NPS STIMULANT TYPE	35 (18.7)
1-3-chlorophenyl piperazine	3 (1.6)
1-(4-chlorophenyl) piperazine	4 (2.1)
2,fluorophenyl piperazine	1 (0.5)

Table 1. *Cont.*

NPS	Detected in Urine, *n* = 187
2,4 Dimethoxyamphetamine	1 (0.5)
25N BoMe	1 (0.5)
3.4 methylendioxypyrovalerone	2 (1.1)
3.4 methylendioxy PV8	1 (0.5)
4-cloro N butyl cathione	1 (0.5)
4-Fluoro-PV8	1 (0.5)
4-Fluoroamphetamine	1 (0.5)
4-Methoxy-PV8	1 (0.5)
4-Methyl-PV8	7 (3.7)
5-AEDB	1 (0.5)
B-Methylphenethylamine (BMPEA)	1 (0.5)
BK-MPA	1 (0.5)
Buphedrone	2 (1.1)
Dimethylcathione	1 (0.5)
Ephinidine	1 (0.5)
Fenethylline	2 (1.1)
Lefetamine	1 (0.5)
m-CPP (1-(3-chlorophenyl)piperazine)	7 (3.7)
Methoxyamphetamine	2 (1.1)
Methoxyphenedine	1 (0.5)
MD-Benzyl MDMA	1 (0.5)
Methylendioxypyrovalerone (MDPV)	1 (0.5)
Ortho-chlorophenylpiperazine	3 (1.6)
NPS CANNABINOID TYPE	6 (3.2)
JWH-018	1 (0.5)
JWH-032	1 (0.5)
JWH-122	4 (2.1)
JWH-122 N-4-hydroxypentyl / JWH-122 N-5-hydroxypentyl	3 (1.6)
JWH-200	1 (0.5)
JWH-210	2 (1.1)
JWH-210 N-4-hydroxypentyl / JWH-210 N-5-hydroxypentyl	2 (1.1)
UR-144	1 (0.5)
UR-144 N-5-hydroxypentyl	1 (0.5)

Table 2. Other detected substances and metabolites in biological samples (N = 187).

Other Substances	Detected in Urine, *n* = 187
OPIOID SUBSTITUTION DRUGS	
Methadone	174 (93)
Morphine	2 (1.1)
Buprenorphine	1 (0.5)
PSYCHIATRIC TREATMENT DRUGS	
Antidepressants	50 (26.7)
Antipsychotics	52 (27.8)
Anticonvulsant	43 (23.0)
Benzodiazepines	86 (46.0)
OTHER THERAPEUTIC DRUGS	
Non-steroidal anti-inflammatory	6 (3.2)
Non-opioid analgesic	27 (14.4)
Anesthetic	2 (1.1)
Non-opioid alkaloid	2 (1.1)
Anesthetic (Lidocaine)	2 (1.1)
Other drugs *	43 (23)

Table 2. *Cont.*

Other Substances	Detected in Urine, *n* = 187
STIMULANTS	49 (26.2)
Cocaine	44 (23.5)
Amphetamine	2 (1.1)
Ephedrine	2 (1.1)
Ethylamphetamine	3 (1.6)
Feprosidnine	1 (0.5)
Methamphetamine	2 (1.1)
Norephedrine	1 (0.5)
OPIOIDS	30 (16)
Heroin	11 (5.9)
Alfa-propoxyphene	1 (0.5)
Codeine	7 (3.7)
Desmethyltramadol	2 (1.1)
Dextromethorphan	11 (5.9)
Hydromorphone	3 (1.6)
Levophanol/Dextrorphan	6 (3.2)
Norcodeine	1 (0.5)
Norpropoxyphene	2 (1.1)
Oxymorphone ether TMS	1 (0.5)
Tramadol	1 (0.5)
OTHER DRUGS	
Alcohol	17 (9.1)
11-Nor-9-carboxy-Δ9-tetrahydrocannabinol (11-COOH-THC)	44 (23.5)
LSD/LAMPA	3 (1.6)

* Levamisol, azapetine, etidronate, bisopropol, domperidone, furosemide, enalapril, etidronate.

Some type of NPS (opioid, stimulant, or cannabinoid) was detected in 51 (27.3%) of the 187 urine samples and a total of 45 different NPS were detected (Table 1). In addition, more than one substance was detected in 124 (66.3%) urine samples.

Fentanyl and derivatives were present in the urine of 16 (8.6%) participants and only one of these samples was positive for heroin too. Stimulant-type NPS were detected in 35 (18.7%) participants, being 4-Methyl-PV8 (*n* = 7, 3.7%) and m-CPP (*n* = 7, 3.7%) as the most detected substances, followed by 1-(4-chlorophenyl) piperazine (*n* = 4, 2.1%). In addition, seven of these subjects were also positive for cocaine (25%). Cannabinoid-type NPS were detected in six (3.2%) participants, with the most detected being was JWH-122 (*n* = 4, 2.1%).

In agreement with the administered treatment, an opioid agonist was detected in the majority of samples (*n* = 177, 94.6%): methadone in 174 (93.0%) participants, morphine in two (1.1%) participants, and buprenorphine in one (0.5%) participant (Table 2). Benzodiazepines (*n* = 86, 46.0%) were the most frequently detected psychiatric treatment drugs, followed by antipsychotics (*n* = 52, 27.8%) and antidepressants (*n* = 50, 26.7%). In 43 (23.0%) participants, an anticonvulsant was found and the main one was gabapentin (*n* = 20, 10.7%), followed by pregabalin (*n* = 7, 3.7%). Non-opioid analgesics were detected in 27 (14.4%) participants. Stimulants were present in 49 (26.2%) samples and the majority were positive for cocaine (*n* = 44, 23.5%). Less commonly detected was amphetamine or methamphetamine, being positive in two (1.1%) samples each. Opioids were detected in a total of 30 (16%) participants. Among these, most detected opioids were dextromethorphan and heroine, being positive in 11 (5.9%) samples each. Finally, other detected drugs were alcohol in 17 (9.1%) samples, metabolites of THC (11-COOH-THC) in 44 (23.5%), and LSD/LAMPA in three (1.6%) samples.

4. Discussion

Different types of NPS, other substances of abuse, and psychiatric and other treatment drugs have been detected in our study. We detected the presence of any type of NPS in

27.3% of urine samples from patients with an OUD diagnosis attending a treatment centre. Differentiating by type of NPS, we detected NSO and/or fentanyl in 8.6% of the samples, NPS stimulant type in 18.7%, NPS cannabinoid type in 3.2%, and other NPS in 1.6% of samples. Additionally, opioids other than NSO were found in 16% of our samples.

Specifically, the presence of fentanyl in our samples agree with what was previously highlighted in one of our previous studies (6.1% versus 8.6%) [17]. Another study conducted with opioid maintenance treatment shows a 13.0% prevalence of NPS use, although, unlike our results, no fentanyl and analogs or NSO was found [26]. Similar to these data, NSOs were also not found in the substance-use-disorder population despite subjects having reported their use [23]. These findings may explain the differences in the prevalence of NPS and NSO use between studies.

According to worldwide NPS identifications [2], most detected in our samples was stimulant-type NPS. However, our results showed a higher use of NSO in this population than expected based on drug seizure data.

The prevalence for NPS use in Europe is 1.1% among young adults (15–34 years old) [6], although its use is normally associated with another substance such as alcohol, cocaine, or heroin [27]. In our sample, 22.9% of those individuals consuming an NPS stimulant-type also used cocaine, the use of which is widespread among people receiving opiate substitution treatment [28].

Other combinations of substances detected were with heroin, which was present in 6.3% of the samples positive for fentanyl and in 20.8% of the samples positive for other opioids. This is in agreement with the reported heroin adulteration with NSO and other opioids with increased addictive potency and risk of unintended intoxication for heroin users [29]. Moreover, there is a high proportion of polysubstance use consumption among people with an OUD [26] and is often addressed to the classic prescription opioids and NSO [19,30].

We found an elevated consumption of psychiatric treatment drugs such as benzodiazepines, antipsychotics, antidepressants, and anticonvulsants in our sample of OUD patients, as described commonly in other similar studies. The possible biological role of this high prevalence of psychiatric drug use is probably related to a dual diagnosis including psychosis, affective disorders, anxiety, and personality disorders [31–33]. Estimating the prevalence of use of NPS and NSO is complicated for several reasons: the non-detection of these substances in standard toxicological tests [4], the unawareness of their use by consumers [34], and the continuous change in the drug market [35]. In addition, as in our case, many of the NPS detected have not previously been described in the scientific literature, so their mechanism of action, effects, and health risks are unknown.

One relevant difference between young recreational NPS users and our population is that people with an OUD are not always aware of drug-checking services or are not sufficiently motivated to bring their substances for testing. These circumstances point to the post-consume drug checking as a suitable tool in the OUD population. This technique allows us to get an overview of all the substances being consumed, voluntary or involuntary, and detect substances missed by ordinary controls. The importance of drug-checking as a harm reduction tool in the clinical setting should be emphasized, not only in recreational contexts [19].

In the last 10 years, the use of NPS, as well as NSO, has been consolidated as a global health problem. Hence, new public health and social measures are needed, including the development of detection methods for these substances, early detection strategies, as well as specific prevention and treatment strategies [36].

5. Limitations

This study has some limitations: (I) the most relevant is the sample under investigation, which selected between participants who want to collaborate in the research and was not a random sample; (II) the anonymous collection cannot permit one to know the origin of the opioids (prescription and/or illegal market); (III) the design of our study is cross-sectional

and subjects were not followed for a period; (IV) differences between gender or ages cannot be evaluated due to study design. The possibility of substance detection will depend on the time of consumption, dose, and elimination half-life in urine in relation to the sample collection.

6. Conclusions

We detected a wide variety of NPS of different types in a sample of patients with an OUD. The detection of NSO and other opioids in our sample suggests a non-therapeutic use of these substances. Difficulties in analyzing NSO and NPS in urine samples makes it difficult to extend the knowledge of the use of these substances in opioid treatment centers. It is necessary to follow up the NPS phenomenon in different populations of drug users through its detection in urine and other biological matrices.

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Informed Consent Statement: Participants were informed about the study and participation was voluntary and anonymous.

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

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5. GENERAL DISCUSION

5. GENERAL DISCUSION

The NPS phenomenon, and specifically the NSO, has experienced growing interest in recent years as these substances have increased their presence in the drug market and have been gaining popularity. With this study, we have contributed to a better knowledge of this phenomenon in our region among people with OUD. Although we are not facing an epidemic of synthetic opioid use, we believe it is important to keep monitoring the use of these in order to take action sooner rather than later, given the relevant health problems associated with the disease.

Our study reveals, through the urinalysis and the self-reported, the use of NSO and synthetic opioids in our region among individuals with OUD. The study showed a very high amount of different substances including NPS opioid-type, stimulant-type and cannabinoid-type, classical illegal drugs (cocaine, heroin, cannabis, hallucinogens…) and psychiatric and other treatment drugs.

The methodology used for the detection of substances in urine showed good reliability and robustness (Jannetto et al., 2019; Marchei et al., 2021; Nováková et al., 2017). With an ultra-high-performance liquid chromatography-high-resolution mass spectrometry (UHPLC-HRMS) assay can be performed an exhaustive screening of more than 1000 active subtances and with gas chromatography-mass spectrometry method the confirmation of the detected compounds can be obtained (Marchei et al., 2021). It demonstrates that it could be a suitable method to identify substances in case of suspecting of been involved in intoxication, overdose or even in addiction services.

There is use of synthetic opioids including NSO among OUD patients in our region. Among detections, we found consumption previously reported by the participants as well as consumption that was not reported, suggesting it was involuntary, with the risk that this entails.

Contrary to our findings, very similar previous studies did not detect the use of NSO in the biological samples even though the subjects did report its use (Heikman et al., 2016; Specka et al., 2020a). Considering the NSO detected, in our sample the most detected is fentanyl, while other studies detect more other NSO such as Kratom, AH-7921 and o-desmethyltramadol (Soussan & Kjellgren, 2016).

Regarding self-reported use of synthetic opioids, compared with similar studies, we found that our prevalence (20.8%) is in the middle of what other studies have described, since we found prevalence ranging from 1% to 40% (Amlani et al., 2015; Heikman et al., 2016; Scherbaum et al., 2021; Specka et al., 2020a). Distinguishing among synthetic opiates detected into non-fentanyl opioids and fentanyl and analogues, we find a greater detection of the first ones which is consistent with the synthetic opioids detections reported by the EMCDDA (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)., 2020b, 2021c). Estimating the prevalence of use for these substances is therefore a difficult task, as it varies greatly depending on the characteristics of the population studied, the type of substance, and as well as the resource or social context in which the recruitment is made.

To characterize the use of synthetic opioids, we asked the reasons for their use to those participants reported their use. The two most highly rated reasons were *Replacing other drugs* and *Availability,* the same as those reported in a previous study (Kapitány-Fövény et al., 2017). Differences were found in the reason *Shorter effect duration,* which is a highly rated reason by women for the use of these substances. Other studies have identified pleasure/enjoyment or coping with problems such as anxiety, pain or insomnia as the main reason for NSO use (Soussan & Kjellgren, 2016). In order to determine and design the most accurate prevention, harm reduction and treatment measures, it is important to understand consumers' reasons.

We include the assessment of well-being given its importance in health according to the World Health Organization. Our participants obtained a WHO-5 score lower than the average

reported for the general Spanish population (65.4 points) (Regueras-Escudero & Lopez-Guzman, 2021). Several studies have pointed to the usefulness of the WHO-5 as an instrument to detect depression, been agree that a score equal to or less than 50 indicates a risk of depressive disorder (Topp, Østergaard, Søndergaard, & Bech, 2015). In our study, the 46.3% women and 40.2% men had score lower than 50 points being advisable an exhaustive assessment to determine whether a depressive disorder exists. This finding supports the need to screen out other psychiatric disorders in SUD population, especially in women (Fonseca et al., 2021).

A large number of different NPS were detected among our samples of individuals with OUD and the prevalence of detection reaches early the 30% which is similar than those found in a similar studies (Larabi et al., 2019) while other studies did not found any NPS among their participants (Specka et al., 2020b). Stimulant-type NPS were the most frequently detected in our sample, which is consistent with NPS identifications worldwide (United Nations Office on Drugs and Crime, 2021b). NPS use is usually associated with the use of other substances such as alcohol, cocaine or heroin (Elliott et al., 2018). One of the substances we found to be most associated with the use of stimulant-type NPS was cocaine, which is frequently used by patients in opioid substitution treatment (Hser et al., 2014; Roux et al., 2016; Tzilos, Rhodes, Ledgerwood, & Greenwald, 2009). Another finding supports this fact is that the self-reported cocaine use prevalence is early 40% among our OUD population. Although this is a high prevalence, other studies have found higher prevalence of 84% (Scherbaum et al., 2021).

Polyconsumption in people with OUD has been described several times in the scientific literature (Hassan & Le Foll, 2019; Heikman et al., 2016; Mahoney et al., 2021) and often the mixtures includes prescription opioids and NSO (Palamar et al., 2020; Schulte et al., 2016). In congruence, in our sample we found several samples with mixtures of heroin with fentanyl or other opioids.

A high consumption of psychiatric medications was found, mainly benzodiazepines, antipsychotics, antidepressants and anticonvulsants. This consumption could be explained by the high incidence of dual diagnosis in these patients, the most common being psychosis, affective disorders, anxiety or personality disorders (Astals et al., 2009; Roncero et al., 2016; Torrens, Mestre-Pintó, Domingo-Salvany, Montanari, & Vicente, 2015).

The detection of these substances is not a common practice in clinical contexts, and their use may go unnoticed by the professionals of these resources. This type of analysis, where a high number of substances can be detected, is more common in recreational contexts such as raves and music festivals where the consumption of NPS is apparently more common (Bade et al., 2021; Bijlsma et al., 2020; Shafi et al., 2020). Despite this, there are a few studies in the clinical setting, although they are more likely in emergencies rooms in cases where the presence of these substances is suspected in overdoses or intoxications (Elliott et al., 2018). Conducting this type of study, in which we can detect more types of substances than with standardized tests in clinical settings, is of great interest for several reasons. First of all, we can detect consumption of these substances, voluntarily or involuntarily, in patients who come to treatment resources. In the case of involuntary use, we can check if the person has used any of these substances in case he reports having suspicions of having experienced any unwanted or expected effects when consuming. Related to this, we can detect these substances which are sometimes used by individuals in order to falsify test results because they are not detected (Kapitány-Fövény et al., 2017; Smith & Staton, 2019). Another reason for carrying out these studies is the possibility of knowing all the substances consumed by the patients in the last few days. In this way, it allows us to know which substances are moving in the drug market.

Drug cheking is a valuable tool in harm reduction that should also be implemented in clinical practice and not only be used in recreational contexts (Palamar et al., 2020). Despite the existence of drug-checkin services, our population is not normally users of these services

because many of them do not know of their existence, are not always accessible to people with an addictive disorder (due to distance, analysis time, etc.) or they are not sufficiently motivated to carry their drugs to test. This makes the post-consume test a very suitable tool for these population.

There are some limitations to our study; the first one has to do with the sample we have investigated. The participants selected for the study agreed to participate voluntarily, so it is not a random sample. In the case of the anonymous collection of urine samples, we cannot determine the origin of the opioids detected, so we do not know whether they could have been prescribed or come from the illegal market. In addition, differences in age or sex could not assess. Regarding substance detection it depends on the time of consumption, dose and elimination half-life in urine in relation to the collection of the sample. Finally, the study is a cross-sectional design study, so the subjects have not been followed up.

The results of this study help us to have a general and updated overview of the situation with respect to NSO and other types of NPS in our area. This study is relevant for several reasons. The first is because the drug field is always in constant change due to the dynamism of the illegal market. Another reason is the scarcity of studies we have to date in this field and therefore we do not have enough data to be able to design strategies. Finally, mention should be made of the population with which the study has been carried out, patients with OUD, which has not been practically studied and in which patterns of consumption may also change over the years.

This study opens the door to future research in the field of NSO and NPS in populations with SUD. We believe it is important to study this phenomenon in this population as well, and not only in recreational users, since addiction is a chronic and periodic disorder in which the patient is not always abstinent. This way we can know which substances are moving in the

illegal market and therefore are consuming these patients in order to be able to design good strategies for prevention, harm reduction and treatment.

6. CONCLUSIONS

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1. There is consumption of new synthetic opioids (NSO) among patients with opioid use disorder (OUD) in our region. A prevalence of NSO use was detected in 7.6% (23 of 301 participants) through urinalysis. All NSO detected were fentanyl or fentanyl derivatives.
2. The use of synthetic opioids and/or NSO was reported by 20.8% of the participants with no gender differences.
3. Most common reasons self-reported by the participants for the consumption of synthetic opioids and/or NSO were “Replace other drugs” and “Availability”. No gender differences were observed.
4. “Shorter effect duration” self-reported reason for use were highly rated by women and gender differences were found.
5. Other new psychoactive substances (NPS) types than NSO were detected in urine among OUD patients.
6. The most detected NPS types were synthetic stimulants with a prevalence of detection of 18.7% and synthetic cannabinoids with a prevalence of detection of 3.2% among OUD population.
7. Among all non-NPS/NSO types of substances detected in urine, opioid substitution treatments, psychiatric treatment drugs, classical stimulants and natural cannabis were the most frequent detected.
8. Polisubstance use, including classical drugs of abuse and NPS, is common among OUD population.

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8. ANNEX

8. ANNEX.

8.1. Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) Criteria for Diagnosis of Opioid Use Disorder Diagnostic Criteria.

Opioid Use Disorder

Diagnostic Criteria

A. A problematic pattern of opioid use leading to clinically significant impairment or distress, as manifested by at least two of the following, occurring within a 12-month period:

1. Opioids are often taken in larger amounts or over a longer period than was intended.
2. There is a persistent desire or unsuccessful efforts to cut down or control opioid use.
3. A great deal of time is spent in activities necessary to obtain the opioid, use the opioid, or recover from its effects.
4. Craving, or a strong desire or urge to use opioids.
5. Recurrent opioid use resulting in a failure to fulfill major role obligations at work, school, or home.
6. Continued opioid use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of opioids.
7. Important social, occupational, or recreational activities are given up or reduced because of opioid use.
8. Recurrent opioid use in situations in which it is physically hazardous.
9. Continued opioid use despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by the substance.
10. Tolerance, as defined by either of the following:
    a. A need for markedly increased amounts of opioids to achieve intoxication or desired effect.
    b. A markedly diminished effect with continued use of the same amount of an opioid.

    Note: This criterion is not considered to be met for those taking opioids solely under appropriate medical supervision.
11. Withdrawal, as manifested by either of the following:
    a. The characteristic opioid withdrawal syndrome (refer to Criteria A and B of the criteria set for opioid withdrawal, pp. 547–548).
    b. Opioids (or a closely related substance) are taken to relieve or avoid withdrawal symptoms.

    Note: This criterion is not considered to be met for those individuals taking opioids solely under appropriate medical supervision.

Specify if:

In early remission: After full criteria for opioid use disorder were previously met, none of the criteria for opioid use disorder have been met for at least 3 months but for less than 12 months (with the exception that Criterion A4, "Craving, or a strong desire or urge to use opioids," may be met).

In sustained remission: After full criteria for opioid use disorder were previously met, none of the criteria for opioid use disorder have been met at any time during a period of 12 months or longer (with the exception that Criterion A4, "Craving, or a strong desire or urge to use opioids," may be met).

Specify if:

On maintenance therapy: This additional specifier is used if the individual is taking a prescribed agonist medication such as methadone or buprenorphine and none of the criteria for opioid use disorder have been met for that class of medication (except tolerance to, or withdrawal from, the agonist). This category also applies to those individ-

uals being maintained on a partial agonist, an agonist/antagonist, or a full antagonist such as oral naltrexone or depot naltrexone.

In a controlled environment: This additional specifier is used if the individual is in an environment where access to opioids is restricted.

Coding based on current severity: Note for ICD-10-CM codes: If an opioid intoxication, opioid withdrawal, or another opioid-induced mental disorder is also present, do not use the codes below for opioid use disorder. Instead, the comorbid opioid use disorder is indicated in the 4th character of the opioid-induced disorder code (see the coding note for opioid intoxication, opioid withdrawal, or a specific opioid-induced mental disorder). For example, if there is comorbid opioid-induced depressive disorder and opioid use disorder, only the opioid-induced depressive disorder code is given, with the 4th character indicating whether the comorbid opioid use disorder is mild, moderate, or severe: F11.14 for mild opioid use disorder with opioid-induced depressive disorder or F11.24 for a moderate or severe opioid use disorder with opioid-induced depressive disorder.

Specify current severity:

305.50 (F11.10) Mild: Presence of 2–3 symptoms.

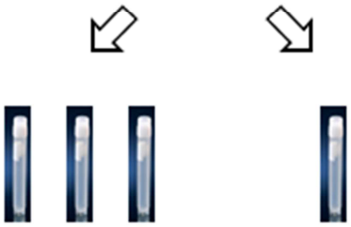
304.00 (F11.20) Moderate: Presence of 4–5 symptoms.

304.00 (F11.20) Severe: Presence of 6 or more symptoms.

8.2. Procedure for aliquoting and storage of collected samples.

URINE

Urine 25 mL divided in 4 cryotubes: 3 for ANALYTICAL TEAM and 1 for the center (BACK-UP)



3 tubes (NUNC cryotubes) 3.6 mL each (to be send) + 1 tube (NUNC cryotube) 3.6 mL (back-up)
All tubes freeze at -20ºC

BLOOD

Blood collected in EDTA tubes (1 tube 10 mL to obtain plasma, 1 tube 3mL (complete blood))

1 tube EDTA 10 mL

1 tube EDTA 2 mL

Centrifugation 10 min x 3000 rpm

No centrifugation

2 tubes of 1,8 mL (NUNC cryotubes) with plasma (to send) + 1 tube of 1,8 mL (NUNC cryotube) with plasma (back-up) 1 tube 2 mL

All tubes freeze at -20ºC

8.3. Ad-hoc survey for collect sociodemographic and clinical data.

ID: _____
FECHA: ____/____/_____

Por favor, responda a las siguientes preguntas. Todas las respuestas serán anónimas.

1. **Sexo**
   ☐ Hombre
   ☐ Mujer **Si es usted mujer, ¿está embarazada?** ☐ Sí ☐ No

2. **Fecha de nacimiento:** ____/____/_____
   Edad: _____ años.

3. **País de origen:**

4. **País de residencia:**

5. **¿Es usted profesional de la salud?** ☐ Sí ☐ No
   ¿Cuál?

6. **Actualmente, ¿Está en tratamiento por un trastorno de uso de opiáceos?** ☐ Sí ☐ No

7. **¿Qué tipo de tratamiento está recibiendo en este momento? (marcar todas las opciones aplicables)**

☐ Tratamiento con buprenorfina	☐ Tratamiento con metadona	☐ Tratamiento con morfina (MST)	☐ Counselling
☐ Tratamiento supervisado en centro (dispensario)	☐ Tratamiento supervisado en centro (dispensario)	☐ Tratamiento supervisado en centro (dispensario)	
☐ Tratamiento en casa	☐ Tratamiento en casa	☐ Tratamiento en casa	☐ Otros
☐ Ambos (supervisado y en casa)	☐ Ambos (supervisado y en casa)	☐ Ambos (supervisado y en casa)	
☐ Farmacia	☐ Farmacia	☐ Farmacia	

8. **¿Qué edad tenía la primera vez que inició un tratamiento por drogas?** _____ años

9. ¿Cuál era la sustancia que utilizaba preferentemente (droga principal) para colocarse antes de iniciar tratamiento? (Marcar UNA).

- ☐ Buprenorfina
- ☐ Codeína
- ☐ Fentanilo
- ☐ Heroína
- ☐ Hidrocodona
- ☐ Hidromorfona
- ☐ Metadona
- ☐ Morfina
- ☐ Oxicodona
- ☐ Oximorfona
- ☐ Sufentanil
- ☐ Tramadol
- ☐ Tapentadol
- ☐ Otros, especificar:

10. ¿De qué manera tomaba la droga principal por la que ahora recibe tratamiento?

- ☐ Oral, tragada entera
- ☐ Oral, masticada y tragadala
- ☐ Oral, disuelta en la boca
- ☐ Fumada
- ☐ Esnifada
- ☐ A través de la piel
- ☐ Inyectada
- ☐ Otra, especifique:

11. ¿Dónde conseguía la sustancia? (Marque todas las opciones aplicables)

- ☐ "Camello" (mercado ilegal)
- ☐ Robado
- ☐ Prescripción médica
  - ☐ Médico
  - ☐ Dentista
  - ☐ Otra
- ☐ Receta falsificada
- ☐ Amigo / Familiar
- ☐ Internet
- ☐ Urgencias
- ☐ Otro, especificar

12. Alguna vez ha solicitado tratamiento médico por:

	Nunca	Últimos 90 días	Hace más tiempo
Sobredosis por heroína			
Sobredosis por opiáceos con prescripción			
Sobredosis por otras sustancias (no opiáceos). ¿Cuál?			

13. A continuación hay una lista con diferentes sustancias, marque cual de ella ha consumido con el fin de colocase y de qué manera la ha consumido.

OXICODONA	Consumida en los últimos 90 días para colocarse	Masticada y luego tragada en los últimos 90 días	Fumada en los últimos 90 días	Esnifada en los últimos 90 días	Inyectada en los últimos 90 días
Formulación desconocida					
Oxicodona, formulación desconocida					
Comprimidos/Capsulas de liberación inmediata (IR)					
OxyNorm® cápsulas					

Oxicodina genérica comprimidos IR					
Otros comprimidos IR oxicodona					
Comprimidos/capsulas de liberación retardada (ER)					
Dolanor® comprimidos					
OxyContin® comprimidos					
Targin® comprimidos					
Oxicodona genérica comprimidos ER					
Otros comprimidos ER oxicodona					

FENTANILO	**Consumida en los últimos 90 días para colocarse**	**Masticada y luego tragada en los últimos 90 días**	**Fumada en los últimos 90 días**	**Esnifada en los últimos 90 días**	**Inyectada en los últimos 90 días**
Formulación desconocida					
Fentanilo, formulación desconocida					
Comprimidos/Capsulas de liberación inmediata (IR)					
Abstral® comprimidos					
Effentora® comprimidos					
Comprimidos de fentanilo genérico					
Otros comprimidos de fentanilo					
Película/caramelo/ piruleta/pulverizador nasal					
Actiq® caramelo/película					
Breakyl® película					
Instanyl® pulverizador nasal					
PecFent® pulverizador nasal					
Película, caramelo, pulverizador nasal de fentanilo genéricos					
Otros película, caramelo, pulverizador.					
Parches					
Durogesic® parches					
Fendivia® parches					
Matrifen® parches					
Nylafent® parches					
Parches de fentanilo genéricos					
Otros parches de fentanilo					

OTROS FENTANILOS, ESPECIFICAR	**Consumida en los últimos 90 días para colocarse**	**Masticada y luego tragada en los últimos 90 días**	**Fumada en los últimos 90 días**	**Esnifada en los últimos 90 días**	**Inyectada en los últimos 90 días**

SUFENTANIL	Consumida en los últimos 90 días para colocarse	Masticada y luego tragada en los últimos 90 días	Fumada en los últimos 90 días	Esnifada en los últimos 90 días	Inyectada en los últimos 90 días
Sufentanil (i.e. Sufenta®, Zalviso)					

HIDROMORFONA	Consumida en los últimos 90 días para colocarse	Masticada y luego tragada en los últimos 90 días	Fumada en los últimos 90 días	Esnifada en los últimos 90 días	Inyectada en los últimos 90 días
Formulación desconocida					
Hidromorfona formulación desconocida					
Comprimidos/Capsulas de liberación inmediata (IR)					
Hidromorfona genérica comprimidos IR					
Otros comprimidos IR de hidromorfona					
Comprimidos/capsulas de liberación retardada (ER)					
Jurnista® comprimidos					
Palladone SR® cápsulas					
Hidromorfona genérica, comprimidos ER					
Otros comprimidos ER hidromorfona					

TRAMADOL	Consumida en los últimos 90 días para colocarse	Masticada y luego tragada en los últimos 90 días	Fumada en los últimos 90 días	Esnifada en los últimos 90 días	Inyectada en los últimos 90 días
Formulación desconocida					
Tramadol formulación desconocida					
Comprimidos/Capsulas/ Comp.bucodispersables de liberación inmediata (IR)					
Adolonta capsulas					
Ceparidin cápsulas					
Tioner cápsulas					
Zaldiar® comprimidos					
Tramadol genérico comprimidos IR					
Otros comprimidos IR tramadol					
Comprimidos/capsulas/ Comp.bucodispersables de liberación retardada (ER)					
Adolonta Retard comprimidos					
Dolodol ER cápsulas					
Tioner ER comprimidos					
Zytram® comprimidos					
Tramadol genérico comprimidos ER					
Otros comprimidos ER tramadol					
Solución inyectable					
Tramadol Normon					

inyectable					
Tramadol Stada inyectable					
Tramadol genérico inyectable					
Otra solución tramadol inyectable					

MORFINA	**Consumida en los últimos 90 días para colocarse**	**Masticada y luego tragada en los últimos 90 días**	**Fumada en los últimos 90 días**	**Esnifada en los últimos 90 días**	**Inyectada en los últimos 90 días**
Formulación desconocida					
Morfina, formulación desconocida					
Comprimidos/Capsulas de liberación inmediata (IR)					
Sevredol® comprimidos					
Morfina genérica, comprimidos IR					
Otros comprimidos IR morfina					
Comprimidos/capsulas de liberación retardada (ER)					
Morfina Lannacher comprimidos					
MST Continus® comprimidos					
Zomorph® cápsulas					
Morfina genérica, comprimidos ER					
Otros comprimidos ER morfina					
Solución inyectable					
Morfina B. Braun inyectable					
Morfina Serra inyectable					
Morfina genérico inyectable					
Otra solución de morfina inyectable					
Solución oral					
Oramorph® solución oral					
Solución oral de morfina genérica					
Otra solución de morfina					

TAPENTADOL	**Consumida en los últimos 90 días para colocarse**	**Masticada y luego tragada en los últimos 90 días**	**Fumada en los últimos 90 días**	**Esnifada en los últimos 90 días**	**Inyectada en los últimos 90 días**
Formulación desconocida					
Tapentadol, formulación desconocida					
Comprimidos/Capsulas de liberación inmediata (IR)					
Palexla® comprimidos					
Tapentadol comprimidor IR genérico					
Otros comprimidos IR tapentadol					
Comprimidos/capsulas de liberación retardada (ER)					
Palexla SR® comprimidos					

Yantil®comprimidos					
Tapentadol comprimidos ER genérico					
Otros comprimidos ER tapentadol					

METADONA	**Consumida en los últimos 90 días para colocarse**	**Masticada y luego tragada en los últimos 90 días**	**Fumada en los últimos 90 días**	**Esnifada en los últimos 90 días**	**Inyectada en los últimos 90 días**
Formulación desconocida					
Metadona, formulación desconocida					
Comprimidos/Capsulas					
Metasedin comprimidos					
Comprimidos de metadona					
Otros comprimidos de metadona					
Solución inyectable					
Metasedin inyectable					
Metadona genérica inyectable					
Otra solución de metadona inyectable					
Solución oral					
Eptadone® solución oral					
Metadona fórmula magistral					
Solución oral de metadona genéricos					
Otra solución de metadona					

BUPRENORFINA	**Consumida en los últimos 90 días para colocarse**	**Masticada y luego tragada en los últimos 90 días**	**Fumada en los últimos 90 días**	**Esnifada en los últimos 90 días**	**Inyectada en los últimos 90 días**
Formulación desconocida					
Buprenorfina, formulación desconocida					
Comprimidos/Capsulas – Un solo ingrediente					
Buprenorfin comprimidos					
Buprex comprimidos					
Subutex®comprimidos					
Comprimidos de buprenorfina genérica un solo ingrediente					
Otros comprimidos de buprenorfina un solo ingrediente					
Comprimidos/capsulas - Combinación					
Suboxone® comprimidos					
Tabletas de buprenorfina genérica, combinación					
Otras tabletas de buprenorfina combinación					
Solución inyectable					
Buprex inyectable					
Buprenorfina genérico inyectable					

Otra solución buprenorfina inyectable					
Parches					
Felben parches					
Transtec® parches					
Parches buprenorfina genéricos					
Otros parches de buprenorfina					

CODEINA	**Consumida en los últimos 90 días para colocarse**	**Masticada y luego tragada en los últimos 90 días**	**Fumada en los últimos 90 días**	**Esnifada en los últimos 90 días**	**Inyectada en los últimos 90 días**
Formulación desconocida					
Codeina formulación desconocida					
Comprimidos/Cápsulas					
Codeisan comprimidos					
Dolomedil comprimidos					
Migraleve™ comprimidos/capsulas					
Perduretas comprimidos					
Comprimidos codeína genéricos					
Otros comprimidos de codeína					
Supositorios de codeína					
Dolviran supositorios					
Supositorios de codeína genérico					
Otros supositorios de codeina					

HIDROCODONA	**Consumida en los últimos 90 días para colocarse**	**Masticada y luego tragada en los últimos 90 días**	**Fumada en los últimos 90 días**	**Esnifada en los últimos 90 días**	**Inyectada en los últimos 90 días**
Hidrocodona, cualquier formulación (i.e. Hycodan®, Lortab™, Tussionex®, Vicodin®)					

OXIMORFONA	**Consumida en los últimos 90 días para colocarse**	**Masticada y luego tragada en los últimos 90 días**	**Fumada en los últimos 90 días**	**Esnifada en los últimos 90 días**	**Inyectada en los últimos 90 días**
Oximorfona, cualquier formulación (i.e. Opana®)					

HEROÍNA	**Consumida en los últimos 90 días para colocarse**	**Masticada y luego tragada en los últimos 90 días**	**Fumada en los últimos 90 días**	**Esnifada en los últimos 90 días**	**Inyectada en los últimos 90 días**
Heroína, cualquier formulación					
Heroína combinada con cocaína "speedball"					

COCAÍNA	Consumida en los últimos 90 días para colocarse	Masticada y luego tragada en los últimos 90 días	Fumada en los últimos 90 días	Esnifada en los últimos 90 días	Inyectada en los últimos 90 días
Cocaína, cualquier formulación					

METILFENIDATO	Consumida en los últimos 90 días para colocarse	Masticada y luego tragada en los últimos 90 días	Fumada en los últimos 90 días	Esnifada en los últimos 90 días	Inyectada en los últimos 90 días
Formulación desconocida					
Metilfenidato, formulación desconocida					
Comprimidos/Capsulas de liberación inmediata (IR)					
Medicebran® comprimidos					
Rubifen®comprimidos					
Metilfenidato comprimidos IR					
Otros comprimidos IR					
Comprimidos/capsulas de liberación retardada (ER)					
Concerta®comprimidos					
Equasym®comprimidos					
Medikinet XL®cápsulas					
Metilfenidato Tecnigen ER comprimidos					
Metilfenidato comprimidos ER genérico					
Otros comprimidos de metifenidato ER					

ANFETAMINA FABRICADA POR COMPAÑÍA FARMACEUTICA	Consumida en los últimos 90 días para colocarse	Masticada y luego tragada en los últimos 90 días	Fumada en los últimos 90 días	Esnifada en los últimos 90 días	Inyectada en los últimos 90 días
Formulación desconocida					
Anfetamina formulación desconocida					
Comprimidos/Capsulas de liberación inmediata (IR)					
Anfetamina comprimidos IR genérico					
Otros comprimidos IR de anfetamina					
Comprimidos/capsulas de liberación retardada					
Anfetamina comprimidos ER genérico					
Otros comprimidos ER de anfetamina					
Sales/Polvos					
Anfetamina sales/polvos genérico					
Otros sales/polvos anfetamina					

ANFETAMINA NO FABRICADA POR COMPAÑÍA FARMACEUTICA	Consumida en los últimos 90 días para colocarse	Masticada y luego tragada en los últimos 90 días	Fumada en los últimos 90 días	Esnifada en los últimos 90 días	Inyectada en los últimos 90 días
Anfetamina, formulación desconocida					
Comprimidos/Cápsulas					
Sales/Polvos					

ALCOHOL	Consumida en los últimos 90 días para colocarse	Cantidad (UBE's)
Alcohol fermentado (cerveza, vino…)		
Licores (<20º)		
Bebidas de alta graduación >20º		

THC/CANNABINOIDES/MARIHUNA FABRICADA POR COMPAÑÍA FARMACEUTICA NO resina, aceite, comestible, "chocolate"	Consumida en los últimos 90 días para colocarse	Masticada y luego tragada en los últimos 90 días	Fumada en los últimos 90 días	Esnifada en los últimos 90 días	Inyectada en los últimos 90 días
Formulación desconocida					
THC, cannabinoides, marihuana formulación desconocida					
Comprimidos/Capsulas					
Cesamet®comprimidos					
Marinol®comprimidos					
Pulverizador nasal					
Sativex®pulverizador nasal					

THC/CANNABINOIDES/MARIHUNA NO FABRICADA POR COMPAÑÍA FARMACEUTICA Resina, aceite, comestible, "chocolate"	Consumida en los últimos 90 días para colocarse	Masticada y luego tragada en los últimos 90 días	Fumada en los últimos 90 días	Esnifada en los últimos 90 días	Inyectada en los últimos 90 días
THC, cannabinoides, marihuana, cualquier formulación					

PREGABALINA	Consumida en los últimos 90 días para colocarse	Masticada y luego tragada en los últimos 90 días	Fumada en los últimos 90 días	Esnifada en los últimos 90 días	Inyectada en los últimos 90 días
Pregabalina cualquier formulación (ie. Lyrica®)					

GABAPENTINA	Consumida en los últimos 90 días para colocarse	Masticada y luego tragada en los últimos 90 días	Fumada en los últimos 90 días	Esnifada en los últimos 90 días	Inyectada en los últimos 90 días
Gabapentina, cualquier formulación (i.e. Neurotin®)					

BENZODIAZEPINA	Consumida en los últimos 90 días para colocarse	Masticada y luego tragada en los últimos 90 días	Fumada en los últimos 90 días	Esnifada en los últimos 90 días	Inyectada en los últimos 90 días
Benzodiazepina, cualquier formulación (i.e. lorazepam, midazolam, temazepam, diazepam, alprazolam)					

OTRO FÁRMACO DE PRESCRIPCIÓN NO CITADO PREVIAMENTE	Consumida en los últimos 90 días para colocarse	Masticada y luego tragada en los últimos 90 días	Fumada en los últimos 90 días	Esnifada en los últimos 90 días	Inyectada en los últimos 90 días
Anfetamina, formulación desconocida					
Comprimidos/Cápsulas					
Sales/Polvos					

14. ¿Alguna vez ha presentado un dolor crónico (dolor que ocurre durante al menos 3 meses, de manera constante o que aparece frecuentemente)?

- ☐ **Sí**
- ☐ **No – Usted ha acabado la encuesta**

15. Para las siguientes preguntas, dolor crónico se refiere a aquel dolor que dura al menos 3 meses; este dolor puede ser constante o intermitente pero frecuente (marcar todo lo que se aplique).

	NO	SÍ, en los últimos 7 días	SÍ, en los últimos 30 días	SÍ, en los últimos 12 meses	SÍ, durante toda mi vida
¿Ha consultado alguna vez con un profesional de la salute para este dolor crónico?					
¿Alguna vez ha recibido una receta de opioides (analgésicos) para tratar su dolor? ¿Cuál? - - -					

8.4. Instrument to collect reasons to use New Synthetic Opioids

RAZONES PARA ELEGIR NUEVOS OPIOIDES SINTÉTICOS (FENTANILOS).

Señala en qué medida estás de acuerdo, siendo 0 “Muy en desacuerdo” y 4 “Totalmente de acuerdo”, con las siguientes afirmaciones hacen referencia a tus motivaciones para cada uno de los opiáceos que consume.

OPIÁCEO:

	0. Muy en desacuerdo	1. Algo en desacuerdo	2. Ni en desacuerdo ni en acuerdo	3. De acuerdo	4. Totalmente de acuerdo
Curiosidad					
Remplazar otras drogas					
Disponibilidad					
Mis amigos también las consumen					
Efectos subjetivos más intensos					
No sé por qué las utilizo					
Bajo precio					
Estado legal					
Menor duración de los efectos					
No se pueden detectar en muestras de orina y/o sangre					
Nombre exótico					
Pienso que son más seguras					
Presentación atractiva					
Pienso que son más naturales					

8.5. Five Well-Being Index (WHO-5)

Psychiatric Research Unit
WHO Collaborating Centre in Mental Health

WHO (Five) Well Being Index (1998 version)
OMS (cinco) Indice de Bienestar (versión 1998)

Por favor, indique para cada una de las cinco afirmaciones cual define mejor como se ha sentido usted durante la últimas dos semanos. Observe que cifras mayores significan mayor bienestar. **Ejemplo**: Si se ha sentido **alegre y de buen humor más de la mitad del tiempo durante las últimas dos semanas,** marque una cruz en el recuadro con el número 3 en la esquina superior derecha.

Durante las últimas dos semanas:	Todo el tiempo	La mayor parte del tiempo	Más de la mitad del tiempo	Menos de la mitad del tiempo	De vez en cuando	Nunca
1. Me he sentido alegre y de buen humor	5	4	3	2	1	0
2. Me he sentido tranquilo y relajado	5	4	3	2	1	0
3. Me he sentido activo y enérgico	5	4	3	2	1	0
4. Me he despertado fresco y descandado	5	4	3	2	1	0
5. Mi vida cotidiana ha estado llena de cosas que me interesan	5	4	3	2	1	0

Instrucciones de puntuación.
Para calcular la punctuación, sume las cifras en la esquina superior derecha de los recuadros que usted marcó y multiplique la suma por quatro.
Usted habrá obtenido una punctuación entre 0 y 100. Mayor punctuatión significa mayor bienestar.

Interpretación:
Se recomienda administrar "The Major Depression (ICD-10) Inventory" si la primera punctuación calculada es menor que 13 o si las respuestas del paciente oscilan entre 0 y 1 en cualquiera de las afirmaciones citadas. Una punctuación menor que 13 indica bajo bienestar y es un indicador para la aplicación del test de depresión (ICD-10).

Cambios en el monitoreo:
Para poder monitorear posibles cambios en el bienestar se usa la punctuación porcentual. Una diferencia de 10% indica un cambio significativo (ref. John Ware, 1996).

8.6. Other publications

8.6.1. Manuscript 3: Ultra-high performance liquid chromatography-high resolution mass spectrometry and high-sensitivity gas chromatography-mass spectrometry screening of classic drugs and new psychoactive substances and metabolites in urine of consumers.

Marchei, E., Alías-Ferri, M., Torrens, M., Farré, M., Pacifici, R., Pichini, S., & Pellegrini, M. (2021). Ultra-High Performance Liquid Chromatography-High Resolution Mass Spectrometry and High-Sensitivity Gas Chromatography-Mass Spectrometry Screening of Classic Drugs and New Psychoactive Substances and Metabolites in Urine of Consumers. *International journal of molecular sciences*, *22*(8), 4000. https://doi.org/10.3390/ijms22084000

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Article

Ultra-High Performance Liquid Chromatography-High Resolution Mass Spectrometry and High-Sensitivity Gas Chromatography-Mass Spectrometry Screening of Classic Drugs and New Psychoactive Substances and Metabolites in Urine of Consumers

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Abstract: The use of the new psychoactive substances is continuously growing and the implementation of accurate and sensible analysis in biological matrices of users is relevant and fundamental for clinical and forensic purposes. Two different analytical technologies, high-sensitivity gas chromatography-mass spectrometry (GC-MS) and ultra-high-performance liquid chromatography-high-resolution mass spectrometry (UHPLC-HRMS) were used for a screening analysis of classic drugs and new psychoactive substances and their metabolites in urine of formed heroin addicts under methadone maintenance therapy. Sample preparation involved a liquid-liquid extraction. The UHPLC-HRMS method included Accucore™ phenyl Hexyl (100 × 2.1 mm, 2.6 µm, Thermo, USA) column with a gradient mobile phase consisting of mobile phase A (ammonium formate 2 mM in water, 0.1% formic acid) and mobile phase B (ammonium formate 2 mM in methanol/acetonitrile 50:50 (*v*/*v*), 0.1% formic acid) and a full-scan data-dependent MS2 (ddMS2) mode for substances identification (mass range 100–1000 *m*/*z*). The GC-MS method employed an ultra-Inert Intuvo GC column (HP-5MS UI, 30 m, 250 µm i.d, film thickness 0.25 µm; Agilent Technologies, Santa Clara, CA, USA) and electron-impact (EI) mass spectra were recorded in total ion monitoring mode (scan range 40–550 *m*/*z*). Urine samples from 296 patients with a history of opioid use disorder were examined. Around 80 different psychoactive substances and/or metabolites were identified, being methadone and metabolites the most prevalent ones. The possibility to screen for a huge number of psychotropic substances can be useful in suspected drug related fatalities or acute intoxication/exposure occurring in emergency departments and drug addiction services.

Keywords: classic drugs of abuse; new psychoactive substances (NPS); novel synthetic opioids (NSO); urine; liquid chromatography; high-resolution mass spectrometry; gas chromatography-mass spectrometry

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1. Introduction

A new psychoactive substance (NPS) is defined as "a new narcotic or psychotropic drug, in pure form or in preparation, that is not controlled by the United Nations drug

conventions, but which may pose a public health threat comparable to that posed by substances listed in these conventions" [1].

In Europe, seizures of NPS mainly concern synthetic cannabinoids which together with synthetic cathinones account for more than 70% of NPS seizures [2]. Nevertheless, the more recent and most toxic NPS showed to be the novel synthetic opioids (NSOs). Since 2009, 57 new NSOs have been detected on Europe's drug market [2]. Several NSOs were originally synthesized by pharmaceutical companies in their research for analgesic drugs as compounds with a similar chemical structure to natural opiates without addictive properties, but their toxicity or abuse potential posed a very high risk of poisoning to consumers. Whereas some of them were then marketed as prescription drugs, some others were eliminated from the licit market and some others were chemically modified to exclusively enter illicit market [3–5].

The chemical variety of NSOs, ranging from several illicit analogs of fentanyl and derivatives to newly synthesized molecules, make their identification extremely difficult and need the investigation of qualified analysts/toxicologists [6].

Since NSOs and particularly fentanyl-related compounds are active in very low doses, due to their potency and many users are unknowingly consuming these as adulterants in products sold as heroin, or as pain killers [7,8], parent drugs and metabolites are present in biological material at extremely low concentrations. One consequence of this is that they may escape detection because routine testing of these drugs is rarely performed and requires dedicated analytical methods with sufficiently high sensitivity and specificity [9].

The 2020 COVID-19 pandemic has transformed daily life and the different intensity of the lockdown across countries showed important consequences on drug users. The legal restrictions modified their ability to access classic illicit drugs (e.g., heroin, cocaine, cannabinoids) and shifted consumptions towards prescription psychoactive drugs, frequently available at home or from the use of psychoactive recreational NPS (e.g., synthetic cathinones, synthetic cannabinoids, phenethylamines to narcotic analgesics such as NSOs or to anxiolytics such as new benzodiazepines [10,11]. Nine new uncontrolled NSOs have been reported during 2020 [12]and the global shortage of heroin due to pandemic may have forced regular users to take other substances with similar effects, such as fentanyl analogs and NSOs [13].

In 2018 the JUSTSO project (analysis, dissemination of knowledge, implementation of Justice and special tests of new synthetic opioids), funded by European Commission, intended to evaluate, test profile and feedback into education and prevention, knowledge related to the NSO currently used in Europe, their nature, effects and associated harm [14].

Our main involvement in the project was to develop and validate analytical methodologies for the screening analysis of NSO and their metabolites, together with all other possible psychoactive drugs in urine samples of drug users collected in different settings (detoxification units, methadone maintenance clinics, drug addiction services, etc.).

Targeted/untargeted screening workflows based on gas or liquid chromatography coupled with mass spectrometry or tandem mass spectrometry (GC-MS, LC-MS and LC-MS/MS, respectively) play a central role in the daily activities of analytical laboratories operating in clinical and forensic toxicology. Specifically, urinalysis with multiple analytical technologies can increase the number of licit and illicit drugs band metabolites with different physicochemical properties that can be determined [15–23]. New pharmacologically active substances, both licit and illicit, are constantly being introduced and this occurrence has increased demand for new MS solutions that go beyond conventional GC-MS and LC-MS/MS. High-resolution mass spectrometry (HRMS) enables determination of the exact molecular formula (<5 ppm mass error) that can be useful for presumptive assignment of unknowns in general toxicology screenings [18].

Few previous studies performed in this field used one or more than one analytical tool for identification of a high number of unreported psychotropic substances in biological matrices of users.

Ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) methodology has been applied not only to detect, but also to quantify 87 NPS and 32 classic illicit drugs and their metabolites in hair and nails [16] and 77 among the most abused NPS in blood, urine and oral fluid [17]. These two assays used only one type of instrument, but required the availability of all the pure standards of analytes under investigation for their quantification. Others screening methods coupled LC or GC with detection methods as time-of-flight mass spectrometry for analytical determination of NPS in seized samples [19] or in serum of consumers [20]. Moreover, to solve a complex toxicological fatal case due to NPS, several different analytical methodologies, including 1H nuclear magnetic resonance (NMR), GC–MS and UPLC–MS/MS to examine unambiguously seized material and biological fluids [21].

Finally, a combination of last generation GC-MS and UHPLC-HRMS has been recently employed by our investigation group to determine a selection of synthetic cannabinoids in oral fluid of consumers. Specifically, GC-MS has proven useful to identify and quantify parent compounds whereas UHPLC-HRMS also confirmed the presence of their metabolites in oral fluid [22].

Using the same combination of analytical methodologies, we hereby propose a screening method for urinalysis of principal NSOs, classical drugs of abuse and other NPS with main metabolites using a fast sample extraction.

2. Results

2.1. GC-MS and UHPLC-HRMS Methods

A simple and selective screening analysis with simultaneous use of high-sensitivity GC-MS and UHPLC-HRMS was applied for the identification of classic drugs of abuse, new psychoactive substances and metabolites in urine of drug addicts. The extraction procedure was tested with above reported fortified urine samples using different solvents. The mixture of chloroform and isopropanol has been found as the best compromise for the extraction of drugs and with acceptable signal-to noise ratio in an analytical screening, optimizing the extraction times and costs. Furthermore, even if the total analysis time was not short (each chromatographic run was completed in 32 min in GC/MS and 15 min for UHPLC-HRMS) the combined use of two instruments allowed to screen with a high percentage of compounds matched several different substances.

The characteristic retention times and monitored m/z ions used for the identification of mostly found substances monitored in urine samples are reported in Table 1.

Table 1. List of different target compounds, retention times (Rt) and monitored ions (m/z) using for the screening gas chromatography-mass spectrometry (GC/MS) and ultra-high-performance liquid chromatography-high-resolution mass spectrometry (UHPLC-HRMS) analysis.

Compound	Formula	GC/MS			UHPLC-HRMS		
		Rt (min)	Target *m/z* ion (Q)	Fragment *m/z* ions (Q/q) [a]	Rt (min)	Target *m/z* ion $[M+H]^+$ (Δ-error, ppm) [b]	Fragment *m/z* ions
Anticonvulsants							
Carbamazepine	$C_{15}H_{12}N^2O$	15.9	236	193(0.21) 165(1.4)	5.83	237.1022(−2.53)	194.0963 192.0805
Gabapentin	$C_9H_{17}NO_2$	7.74	171	153(0.06) 110(0.13) 81(0.05)	2.79	172.1332(−3.48)	154.1227 137.0961 95.0860
Levetiracetam	$C_8H_{14}N_2O_2$	7.75	170	126(0.05) 98 (0.33) 69(0.13)	2.85	171.1128(−3.51)	154.0863 126.0914
Pregabalin	$C_8H_{17}NO_2$	6.54	159	141(0.05) 103(0.03) 84(0.04)	2.61	160.1332(−3.75)	142.1227 97.1016 83.0861

Table 1. *Cont.*

Compound	Formula	GC/MS			UHPLC-HRMS		
		Rt (min)	Target *m/z* ion (Q)	Fragment *m/z* ions (Q/q) [a]	Rt (min)	Target *m/z* ion $[M+H]^+$ (Δ-error, ppm) [b]	Fragment *m/z* ions
Topiramate	$C_{12}H_{21}NO_8S$	5.12	324	206(2.61) 189 (2.61) 127(1.62)	5.19	357.1326 * (2.20)	264.0532 184.0970 127.0391
Antidepressants							
Amitriptyline	$C_{20}H_{23}N$	9.59	277	215 (0.33) 202 (0.17) 58 (0.02)	5.98	278.19033 (−1.94)	233.1332 191.0861 105.0700
Bupropion	$C_{13}H_{18}ClNO$	17.53	239	139 (0.14) 100 (0.02) 44 (0.01)	4.52	240.1150 (−2.08)	184.0521 166.0419 131.0731
Citalopram	$C_{20}H_{21}FN_2O$	17.27	324	238 (0.33) 208 (0.37) 58 (0.02)	5.44	325.1711(−1.59)	262.1026 234.0712 109.0452
Clomipramine	$C_{19}H_{23}ClN_2$	17.22	314	268 (0.34) 85 (0.23) 58 (0.11)	6.23	315.1623(−1.59)	270.1044 86.0964 58.0651
Desmethylcitalopram	$C_{19}H_{19}FN_2O$	17.42	310	238 (0.23) 138 (0.56) 44 (0.05)	5.40	311.1554(−1.60)	293.1446 262.1025 109.0451
Desmethylmirtazapine	$C_{16}H_{17}N_3$	19.71	251	208 (2.50) 195 (0.08)	4.08	252.1495(−2.38)	235.1229 209.1073 195.0918
Mirtazapine	$C_{17}H_{19}N_3$	19.51	265	208(0.39) 195(0.05) 167(0.5)	4.24	266.1652(−1.88)	209.1076 195.0917 72.0816
Trazodone	$C_{19}H_{22}ClN_5O$	30.50	371	278(0.26) 205(0.05) 176(0.16)	4.95	372.1586(−1.34)	176.0819 148.0505 96.0446
Antipsychotics							
Levomepromazine	$C_{19}H_{24}N_2OS$	19.32	328	282(6.34) 100(6.01) 58(0.79)	6.12	329.1682(−1.82)	242.0633 100.1126 58.0660
Norquetiapine	$C_{17}H_{17}N_3S$	20.01	295	239(0.46) 227(0.09) 210(0.16)	5.26	296.1216(−1.69)	221.1080 210.0373 139.2405
Olanzapine	$C_{17}H_{20}N_4S$	19.01	312	242(0.20) 229(0.25) 213(0.33)	3.09	313.1481(−1.92)	256.0901 213.0480 84.0814
Quetiapine	$C_{21}H_{25}N_3O_2S$	19.38	383	239(0.09) 210(0.04) 144(0.06)	5.61	384.1740(−1.56)	279.0949 253.0792 221.1071
Risperidone	$C_{23}H_{27}FN_4O_2$	8.1	410	233(0.09) 191(2.04) 177(1.30)	4.78	411.2191(−1.22)	191.1179 110.0600 69.0334
Amphetamines							
Amphetamine	$C_9H_{13}N$	5.40	135	91(0.04) 44(0.005)	2.84	136.1121(−3.67)	119.0857 91.0547
Ethylamphetamine	$C_{11}H_{17}N$	6.98	163	148 (0.11) 91 (0.02) 72 (0.005)	3.38	164.1434 (−3.05)	119.0858 91.0547
MDA	$C_{10}H_{13}NO_2$	6.67	179	136(0.03) 77(0.08) 44(0.02)	3.24	180.1019(−3.33)	163.0753 135.0439 105.0699

Table 1. *Cont.*

Compound	Formula	GC/MS			UHPLC-HRMS		
		Rt (min)	Target *m*/*z* ion (Q)	Fragment *m*/*z* ions (Q/q) [a]	Rt (min)	Target *m*/*z* ion $[M+H]^+$ (Δ-error, ppm) [b]	Fragment *m*/*z* ions
MDMA	$C_{11}H_{15}NO_2$	6.88	193	135(0.10) 77(0.83) 58(0.01)	3.31	194.1176(−2.58)	163.0753 135.04393 105.06986
Methamphetamine	$C_{10}H_{15}N$	5.80	149	134(0.25) 91(0.04) 58(0.01)	3.20	150.1277(−3.99)	119.0855 91.0541
Benzodiazepines							
7-Aminoclonazepam	$C_{15}H_{12}ClN_3O$	12.60	285	256(1.15) 222(6.82) 194(6.82)	4.06	286.0742(−1.75)	250.0974 222.1025 194.0831
7-Aminoflunitrazepam	$C_{16}H_{14}FN_3O$	11.33	283	264(5.00) 255(1.53) 240(5.55)	4.65	284.1194(−1.76)	227.0978 256.1243 148.0631
7-Aminonitrazepam	$C_{15}H_{13}N_3O$	15.12	251	222(1.64) 195(5.55) 110 (5.55)	3.20	252.1131(−2.38)	224.1182 146.0714 121.0762
Alprazolam	$C_{17}H_{13}ClN_4$	13.54	308	279(0.64) 245(2.29) 204(0.83)	6.38	309.0902(−1.62)	274.1208 241.0528 205.0747
Clonazepam	$C_{15}H_{10}ClN_3O_3$	12.34	315	288(1.14) 280(0.73) 234(1.14)	6.18	316.0484(−1.58)	302.0448 241.0521 214.0415
Clonazolam	$C_{17}H_{12}ClN_5O_2$	17.99	353	324 (0.60) 249 (1.00) 203 (0.82)	5.65	354.0752 (−1.69)	326.0563 319.1064
Diazepam	$C_{16}H_{13}ClN_2O$	17.66	284	283(0.77) 256(0.59) 221(1.43)	6.83	285.0789(−2.10)	222.1150 193.0885 154.0417
Etizolam	$C_{17}H_{15}ClN_4S$	18.01	342	313 (2.64) 266 (3.22) 137 (4.83)	6.54	343.0779 (−1.46)	314.0388 259.0216
Flubromazolam	$C_{17}H_{12}BrFN_4$		370	341 (0.60) 222 (0.45) 195 (2.25)	6.22	371.0302 (−1.62)	343.0096 292.1105 237.0951
Flunitrazepam	$C_{16}H_{12}FN_3O_3$	22.31	313	312(0.71) 285(0.65) 266(0.95)	6.25	314.0936(−1.59)	300.0902 268.1003 239.0976
Flualprazolam	$C_{17}H_{12}ClFN_4$		326	297 (0.55) 257 (2.75) 222 (0.61)		327.0806 (−2.14)	299.0625 292.1124 223.0662
Nitrazepam	$C_{15}H_{11}N_3O_3$	24.08	281	280 (0.44) 253 (0.64) 206(0.78)	5.96	282.0873(−2.12)	268.0842 236.0944 207.0918
Nordiazepam	$C_{15}H_{11}ClN_2O$	18.66	270	242(1.04) 235(3.61) 207(4.87)	6.41	271.0633(−1.84)	208.0994 165.0214 140.0261
Oxazepam	$C_{15}H_{11}N_2O_2Cl$	16.70	286	268(0.06) 239(0.07) 205(0.06)	6.11	287.0581(−2.09)	241.0525 269.0475 104.0498
Temazepam	$C_{16}H_{13}ClN_2O_2$	19.93	300	273(0.35) 271(0.12) 256(0.86)	6.51	301.0738(−1.99)	283.0630 256.0715 255.0681

Table 1. *Cont.*

Compound	Formula	GC/MS			UHPLC-HRMS		
		Rt (min)	Target *m*/*z* ion (Q)	Fragment *m*/*z* ions (Q/q) [a]	Rt (min)	Target *m*/*z* ion $[M+H]^+$ (Δ-error, ppm) [b]	Fragment *m*/*z* ions
Cocaine							
Benzoylecgonine	$C_{16}H_{19}NO_4$	15.15	289	168(0.27) 124(0.07) 105(0.22)	3.84	290.1387(−1.72)	168.1019 105.0335 82.0650
Cocaethylene	$C_{18}H_{23}NO_4$	15.03	317	196(0.23) 82(0.11) 105(0.35)	4.72	318.1704(−0.31)	196.1330 82.0657 105.0341
Cocaine	$C_{17}H_{21}NO_4$	14.27	303	272(2.00) 182(0.24) 82(0.17)	4.25	304.1543(−1.97)	182.1175 82.0657 105.0337
Ecgonine methyl ester	$C_{10}H_{17}NO_3$	7.12	199	182(1.63) 94(0.39) 82(0.31)	0.6	200.1281(−2.99)	182.1177 150.0911 82.0658
Cannabinoids							
11-OH-THC	$C_{21}H_{30}O_3$	15.91	330	300(0.74) 299(0.16) 41(1.86)	8.15	331.2267(−1.81)	313.2161 193.1224 105.0703
Cannabidiol	$C_{21}H_{30}O_2$	16.42	314	246(0.53) 231(0.06) 193(0.75)	8.64	315.2319(−1.59)	193.1225 135.1169 93.0704
Cannabinol	$C_{21}H_{26}O_2$	17.30	310	295(0.11) 238(0.79) 165(2.36)	8.88	311.2006(−1.61)	293.1901 241.1224 223.1118
Delta-9-tetrahydrocannabinol	$C_{21}H_{30}O_2$	16.90	314	299(0.79) 271(1.66) 231(1.01)	9.02	315.2319(−1.59)	193.1223 123.0441 93.0701
THC-COOH	$C_{21}H_{28}O_4$	17.20	344	329 (0.70) 299(0.41) 41(0.40)	8.26	345.2060(−1.74)	327.1953 299.2004 193.1223
Fentanyls and NSOs							
4-ANPP	$C_{19}H_{24}N_2$	18.16	280	189(0.08) 146(0.07) 91(0.24)	5.04	281.2012(−2.13)	188.1435 134.0965 105.0703
Acetyl fentanyl	$C_{21}H_{26}N_2O$	18.01	322	231(0.03) 188(0.08) 146(0.05)	4.89	323.2118 (−1.54)	188.1434 105.0703 132.0809
AH-7921	$C_{16}H_{22}Cl_2N_2O$	11.22	329	172(0.20) 144(0.20) 126(0.05)	3.73	329.1182(−1.52)	284.0610 189.9555 172.0610
Alfentanil	$C_{21}H_{32}N_6O_3$	18.47	416	289(0.01) 268(0.03) 140(0.04)	5.35	417.2609(−1.20)	268.17651 197.1284 165.10223
Alpha-methylfentanyl	$C_{23}H_{30}N_2O$	18.30	350	259(0.05) 146(0.20) 91(0.25)	5.50	351.2431(−1.42)	202.1588 119.0856 91.0546
Beta-Hydroxyfenatnyl	$C_{22}H_{28}N_2O_2$	17.52	352	245 (0.02) 189 (0.05) 146 (0.03)	4.90	353.2224 (−1.42)	204.1384 186.1276 132.0809
Carfentanil	$C_{24}H_{30}N_2O_3$	18.72	394	303(0.01) 187(0.05) 105(0.08)	5.60	395.2329(−1.52)	134.0965 105.0702 113.0600

Table 1. *Cont.*

Compound	Formula	GC/MS			UHPLC-HRMS		
		Rt (min)	Target *m/z* ion (Q)	Fragment *m/z* ions (Q/q) [a]	Rt (min)	Target *m/z* ion $[M+H]^+$ (Δ-error, ppm) [b]	Fragment *m/z* ions
Despropionyl *para*-fluorofentanyl	$C_{19}H_{23}FN_2$	18.45	298	207(0.08) 164(0.08) 136 (0.40)	5.33	299.1918(−2.01)	188.1435 134.0966 105.0703
Fentanyl	$C_{22}H_{28}N_2O$	18.89	245	189(2.77) 146(1.57) 105(4.27)	5.38	337.2279 (−0.30)	188.1436 105.0703 132.08010
Fluorofentanyl	$C_{22}H_{27}FN_2O$	17.05	354	263(0.01) 207(0.04) 164(0.02)	3.55	355.2180(−1.69)	234.1289 188.1433 105.0699
Isotonitazene	$C_{23}H_{30}N_4O_3$	17.76	410	236 (0.40) 107 (0.12) 86 (0.01)	7.02	411.2391(−1.21)	250.1077 100.1109 72.0809
MT-45	$C_{24}H_{32}N_2$	12.01	348	257(0.01) 165(0.17) 91(0.05)	4.03	349.2638(−1.72)	181.1011 169.1699 87.0916
N-methyl Norfentanyl	$C_{15}H_{22}N_2O$	17.32	246	189(0.12) 96(0.08) 82(0.22)	4.20	247.1805(−2.02)	150.0915 98.0969 69.0707
Norfentanyl	$C_{14}H_{20}N_2O$	17.90	232	175(0.09) 159(0.12) 83(0.05)	3.77	233.1649 (−2.14)	204.1038 150.0914 84.0814
Ocfentanil	$C_{22}H_{27}FN_2O_2$	17.34	370	279(0.01) 176(0.05) 105(0.05)	4.83	371.2129(−1.62)	188.1434 134.0966 105.0702
Remifentanil	$C_{20}H_{28}N_2O_5$	16.81	376	227(0.02) 212(0.02) 168(0.01)	4.48	377.2071(−1.33)	228.1230 146.0964 113.0600
Sufentanil	$C_{22}H_{30}N_2O_2S$	18.50	386	289(0.01) 140(0.03) 93(0.03)	5.97	387.2101(−1.29)	355.1838 238.1257 111.0266
Thienyl fentanyl	$C_{19}H_{24}N_2OS$	17.99	328	179(0.20) 97(0.03) 82(0.04)	4.87	329.1682(−1.82)	97.0111 82.0657
U-47700	$C_{16}H_{22}C_{l2}N_2O$	10.80	329	172(0.05) 125(0.02) 84(0.01)	3.52	329.1182(−1.52)	284.0596 172.9579 81.0699
Opioids and SOs							
6-Monoacetylmorphine	$C_{19}H_{21}NO_4$	18.83	327	268(0.92) 214(2.44) 162(4.40)	3.37	328.1543(−1.82)	268.1327 211.0753 165.0698
Buprenorphine	$C_{29}H_{41}NO_4$	32.0	467	434 (0.33) 410(0.17) 378 (0.04)	5.72	468.3108(−1.28)	396.2165 84.0808 55.0544
Codeine	$C_{18}H_{21}NO_3$	16.94	299	229(3.33) 214(5.00) 162(3.00)	2.88	300.1594(−1.28)	243.1012 215.1065 58.0659
EDDP	$C_{20}H_{23}N$	11.96	277	262(2.17) 220(3.09) 165(3.82)	5.61	278.1903(−1.43)	249.1509 234.1275 186.1275
EMDP	$C_{19}H_{21}N$	11.60	263	208(0.08) 130(0.17) 115(0.20)	5.95	264.1747(−1.89)	235.1355 234.1275 220.1121
Hydrocodone	$C_{18}H_{21}NO_3$	16.01	299	284(7.80) 242(1.50) 185(2.44)	3.35	300.1594(−1.99)	283.175 133.0860 89.0602

Table 1. *Cont.*

Compound	Formula	GC/MS			UHPLC-HRMS		
		Rt (min)	Target *m*/*z* ion (Q)	Fragment *m*/*z* ions (Q/q) [a]	Rt (min)	Target *m*/*z* ion $[M+H]^+$ (Δ-error, ppm) [b]	Fragment *m*/*z* ions
Hydromorphone	$C_{17}H_{19}NO_3$	16.35	285	229(3.12) 214(4.08) 200(5.30)	2.49	286.1438(−1.75)	185.0597 227.0699 199.0753
Methadone	$C_{21}H_{27}NO$	13.41	309	178(0.33) 165(0.25) 72(0.03)	6.15	310.2165(−1.93)	105.0338 265.1584 223.1116
Morphine	$C_{17}H_{19}NO_3$	17.18	285	268(6.67) 215(2.50) 162(2.13)	1.91	286.1438(−1.75)	201.0912 229.0857 183.0807
Norcodeine	$C_{17}H_{19}NO_3$	16.84	285	242(6.67) 215 (2.00) 148 (2.50)	2.91	286.1438(−1.74)	268.13263 215.10689 225.09088
Normorphine	$C_{16}H_{17}NO_3$	16.12	271	201(0.02) 150(1.05) 148(1.33)	1.23	272.1281(−2.20)	254.1173 201.0916 121.0649
Noroxycodone	$C_{17}H_{19}NO_4$	15.32	301	216(1.76) 201(4.14) 188(3.63)	3.17	302.1387(−1.65)	284.1281 227.0941 187.0754
Noroxymorphone	$C_{16}H_{17}NO_4$	15.30	287	253(5.93) 202(1.63) 174(4.15)	1.78	288.1230 (−2.08)	270.1122 213.0783 173.0597
Oxycodone	$C_{18}H_{21}NO_4$	15.83	315	258(4.42) 230(1.91) 187(7.64)	3.21	316.1543(−1.90)	298.1438 256.1330 241.1093
Oxymorphone	$C_{17}H_{19}NO_4$	16.25	301	244(9.07) 216(2.62) 203(6.18)	2.24	302.1387(−1.65)	284.1278 242.1173 227.0934
Tramadol	$C_{16}H_{25}NO_2$	14.41	263	188 (2.00) 135 (2.00) 58(0.13)	4.13	264.1958(−2.27)	58.0659
Synthetic Cannabinoids							
JWH 018	$C_{24}H_{23}NO$	8.55	341	284(1.50) 214(1.31) 127(0.82)	8.74	342.1852(−1.75)	214.1224 155.0605 144.0444
JWH 073	$C_{23}H_{21}NO$	6.98	327	284(1.62) 200(0.98) 127(0.84)	8.58	328.1696(−1.52)	230.1172 155.0489 125.0962
JWH 073 N-4-Hydroxybutyl	$C_{23}H_{21}NO_2$	11.10	343	270(0.95) 144(1.11) 127(0.77)	7.32	344.1645(−1.74)	155.0490 127.1062
JWH 081	$C_{25}H_{25}NO_2$	11.57	371	314(2.00) 214(1.43) 185(1.43)	8.92	372.1958(−1.61)	214.1223 185.0596 144.0443
JWH 081 4-Hydroxynaphtyl	$C_{24}H_{23}NO_2$	12.44	357	300(1.32) 214(1.31) 171(1.48)	8.36	358.1802(−1.39)	214.1222 171.0438 144.0443
JWH 081 N-5-Hydroxypentyl	$C_{25}H_{25}NO_3$	19.93	387	314(1.45) 230(1.50) 185(0.90)	7.70	388.1907(−1.55)	230.1172 185.0596 144.0443
JWH 122	$C_{25}H_{25}NO$	9.33	355	338(1.82) 298(1.38) 214(1.45)	8.91	356.2009(−1.40)	214.1223 169.0646 141.0697

Table 1. *Cont.*

Compound	Formula	GC/MS			UHPLC-HRMS		
		Rt (min)	Target *m*/*z* ion (Q)	Fragment *m*/*z* ions (Q/q) [a]	Rt (min)	Target *m*/*z* ion $[M+H]^+$ (Δ-error, ppm) [b]	Fragment *m*/*z* ions
JWH 122 N-4-Hydroxypentyl	$C_{25}H_{25}NO_2$	13.26	371	284(0.66) 169(0.92) 144(0.96)	7.81	372.1958(−1.61)	169.0647 141.0698
JWH 122 N-5-Hydroxypentyl	$C_{25}H_{25}NO_2$	15.87	371	284(1.57) 141(1.29) 115(1.56)	7.80	372.1958(−1.61)	169.0646 141.0697
JWH 210	$C_{26}H_{27}NO$	10.77	369	352(1.71) 312(1.64) 214(0.90)	9.21	370.2165(−1.62)	214.1223 183.0804 144.0443
JWH 210 N-4-Hydroxypentyl	$C_{26}H_{27}NO_2$	14.56	385	298(0.64) 183(0.86) 144(0.90)	8.08	386.2115(−1.29)	183.0804 155.0854 144.0443
JWH 210 N-5-Hydroxypentyl	$C_{26}H_{27}NO_2$	17.79	385	368(2.75) 230(3.24) 144(2.20)	8.06	386.2115(−1.29)	230.1172 183.0803 155.0853
UR 144	$C_{21}H_{29}NO$	9.94	311	296(0.98) 214(0.13) 144(0.40)	9.07	312.2322(−1.60)	214.1223 125.0962 97.1016
UR 144 N-5-Hydroxypentyl	$C_{21}H_{29}NO_2$	10.70	327	231 (0.33) 230(0.001) 144(0.10)	7.85	328.2271(−1.83)	230.1172 125.0962 97.1016
XLR 11	$C_{21}H_{28}FNO$	10.73	329	314(0.90) 232 (0.09) 144(0.36)	8.64	330.2228(−1.51)	232.1129 125.0962 97.1016
XLR 11 N-4-Hydroxypentyl	$C_{21}H_{28}FNO_2$	11.73	345	330(0.83) 248(0.11) 144(0.29)	7.57	346.2177(−1.44)	248.1077 144.0443 67.0550
AM-2201	$C_{24}H_{22}FNO$	10.35	359	342 (0.20) 284 (1.25) 232 (1.30)		360.1764	
Synthetic Cathinones							
MDPV	$C_{16}H_{21}NO_3$	8.23	275	149(0.25) 126(0.01) 119(0.50)	4.35	276.1594 (−2.17)	126.1278 149.0232
4-MEC	$C_{12}H_{17}NO$	6.43	191	119(0.33) 91(0.17) 72(0.03)	3.66	192.1383 (−2.60)	174.1277 159.1040 119.0857
Butylone	$C_{12}H_{15}NO_3$	8.72	221	149(0.10) 121(0.20) 72(0.02)	3.52	222.1125(−2.25)	204.1018 174.0913 72.0815
Mephedrone	$C_{11}H_{15}NO$	6.45	177	119(0.20) 91(0.10) 58(0.02)	3.37	178.1226(−3.36)	160.1121 145.0886 119.0857
Methcathinone	$C_{10}H_{13}NO$	5.98	163	105(0.20) 77(0.07) 58(0.02)	2.67	164.107(−1.83)	146.0965 131.0731 105.0703
Pentylone	$C_{13}H_{17}NO_3$	8.13	235	149(0.17) 121(0.25) 86(0.01)	4.16	236.1281(−2.54)	218.1174 188.1069 86.0969

Table 1. *Cont.*

Compound	Formula	GC/MS			UHPLC-HRMS		
		Rt (min)	Target *m*/*z* ion (Q)	Fragment *m*/*z* ions (Q/q) [a]	Rt (min)	Target *m*/*z* ion $[M+H]^+$ (Δ-error, ppm) [b]	Fragment *m*/*z* ions
Miscellaneous							
4-FA	$C_9H_{12}FN$	4.81	153	109(0.06) 83(0.10) 44(0.01)	2.95	154.1027(−3.24)	109.0451 137.0761 114.0917
4-MA or PMA	$C_{10}H_{15}NO$	9.50	165	122(0.03) 78(0.08) 44(0.01)	3.27	166.1226(−3.61)	150.0499 137.0419 117.0701
PMMA	$C_{11}H_{17}NO$	10.33	179	121(0.10) 78(0.13) 58(0.01)	3.43	180.1383(−2.78)	149.0961 121.0649
m-CPP	$C_{10}H_{13}ClN_2$	7.39	196	154 (0.25) 138 (2.00)	3.97	197.0840(−3.04)	154.0416 119.0730
Ketamine	$C_{13}H_{16}ClNO$	8.28	237	209(0.07) 179(0.02) 125(0.08)	3.77	238.0993(−2.51)	207.0574 179.0622 125.0154

a, Q/q ion abundance ratio; b, delta error (ppm); * Sodium adduct; MDA: 3,4-Methylenedioxyamphetamine; MDMA: 3,4-Methylenedioxymethylamphetamine;11-OH-THC: 11-Hydroxy-delta-9- tetrahydrocannabinol; THC-COOH: 11-nor-9-carboxy-delta-9- tetrahydrocannabinol carboxilc acid; NSOs: novel Synthetic Opioids;4-ANPP: 4-Aminophenyl-1-phenethylpiperidine or Despropionyl fentanyl; AH 7921: 3,4-dichloro-N-[[1-(dimethylamino)cyclohexyl]methyl]-benzamide;U-47700: *trans*-3,4-dichloro-N-[2-(dimethylamino)cyclohexyl]-N-methyl-benzamide; MT-45: 1-cyclohexyl-4-(1,2-diphenylethyl)-piperazine, dihydrochloride; SO: Synthetic Opioids; EDDP: 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; EMDP: 2-ethyl-5-methyl-3,3-diphenylpyrroline; MDPV: 3,4-Methylendioxy Pyrovalerone; 4-MEC: 4-Methylethcathinone;4-FA: 4-Fluoroamphetamine; 4-MA or PMA: 4-Methoxyamphetamine or *para*-methoxymethylamphetamine; PMMA: *para*-methoxymethamphetamine; *m*-CPP: 1-(3-Chlorophenyl)piperazine, SO: synthetic opioids.

The results obtained by screening proficiency urine testing samples from UNODC International Quality Assurance Program and those from in "NPS-LABVEQ" project showed an excellent agreement (98% agreement as screened substances) between substances declared and those found in the samples. Since these latter substances were analyzed at a concentration of 1 ng/mL urine with a signal to noise ratio, calculated at the baseline, always higher than 10, we could assume that our methodologies could screen substances present in concentrations equal or above 1 ng/mL. Moreover, from the analysis of blank urine no additional peaks due to endogenous substances, which could have interfered with the detection, were observed.

2.2. Methods Application

Drug screening applied to 296 former heroin addicts under methadone maintenance therapy urine disclosed the presence of different psychoactive prescription drugs, classical drugs of abuse, NSO, NPS and their metabolites. The presence of a certain drug and/or metabolites was confirmed only if both methodologies identified the molecules, which occurred in 95% cases.

Pharmaceuticals like benzodiazepines, antidepressants, antipsychotics, anticonvulsants and opioids were detected. Drugs of abuse (opioids, amphetamines, cocaine and cannabinoids), NPS (synthetic cannabinoids, synthetic cathinones), fentanyls, NSO and other drug classes were also found. The frequency of different drug classes found in urine samples using the developed GC-MS and LC–HRMS screening methods is reported in Figure 1.

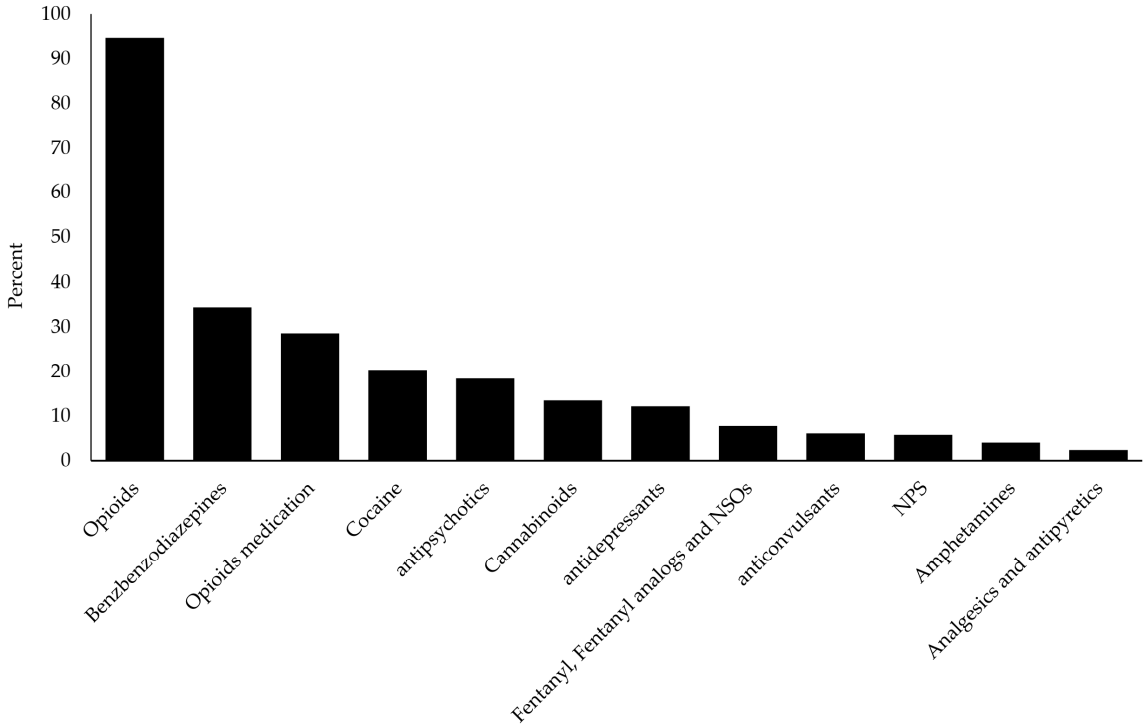

Figure 1. Percentage plot of classic drugs and new psychoactive substances found in 296 urine samples from former heroin users at methadone maintenance clinics and drug addiction services.

The most frequent found substances (about 90%) were methadone and its metabolites, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP). Urine samples resulted positive also to benzodiazepines (mainly Clonazepam, Diazepam and their metabolites), antipsychotics (principally Risperidone, Quetiapine and their metabolites), antidepressants (Citalopram, Mirtazapine and their metabolites, Trazadone and its psychoactive metabolite meta-Chlorophenylpiperazine) and Gabapentin. Additional findings included samples positives for cocaine and its metabolites BZE and EME, cannabinoids, amphetamine and synthetic cathinone methylenedioxypyrovalerone and synthetic cannabinoids from JWH family.

In urine samples in which methadone was not found, screening analysis revealed the presence of the opiates (buprenorphine, 6-MAM, morphine, codeine, dextromethorphan), cocaine, cannabinoids and fentanyl and analogs.

2.3. Fentanyl, Fentanyl Analogs and Novel Synthetic Opioids

Toxicological screening analysis revealed the presence of fentanyl and analogs and/or metabolites in 23 (7.8%) out of 296 screened urine samples. No other NSOs were found.

In 4 out of 23 samples, the substances matched while in other cases, parent drug was identified by one method and metabolite by the other, or similar compounds were determined.

Chromatogram sin GC-MS and UHPLC-HRMS of 2 positive fentanyl samples are shown in Figures 2 and 3, respectively, and screening results on fentanyl positive samples were reported in Table 2.

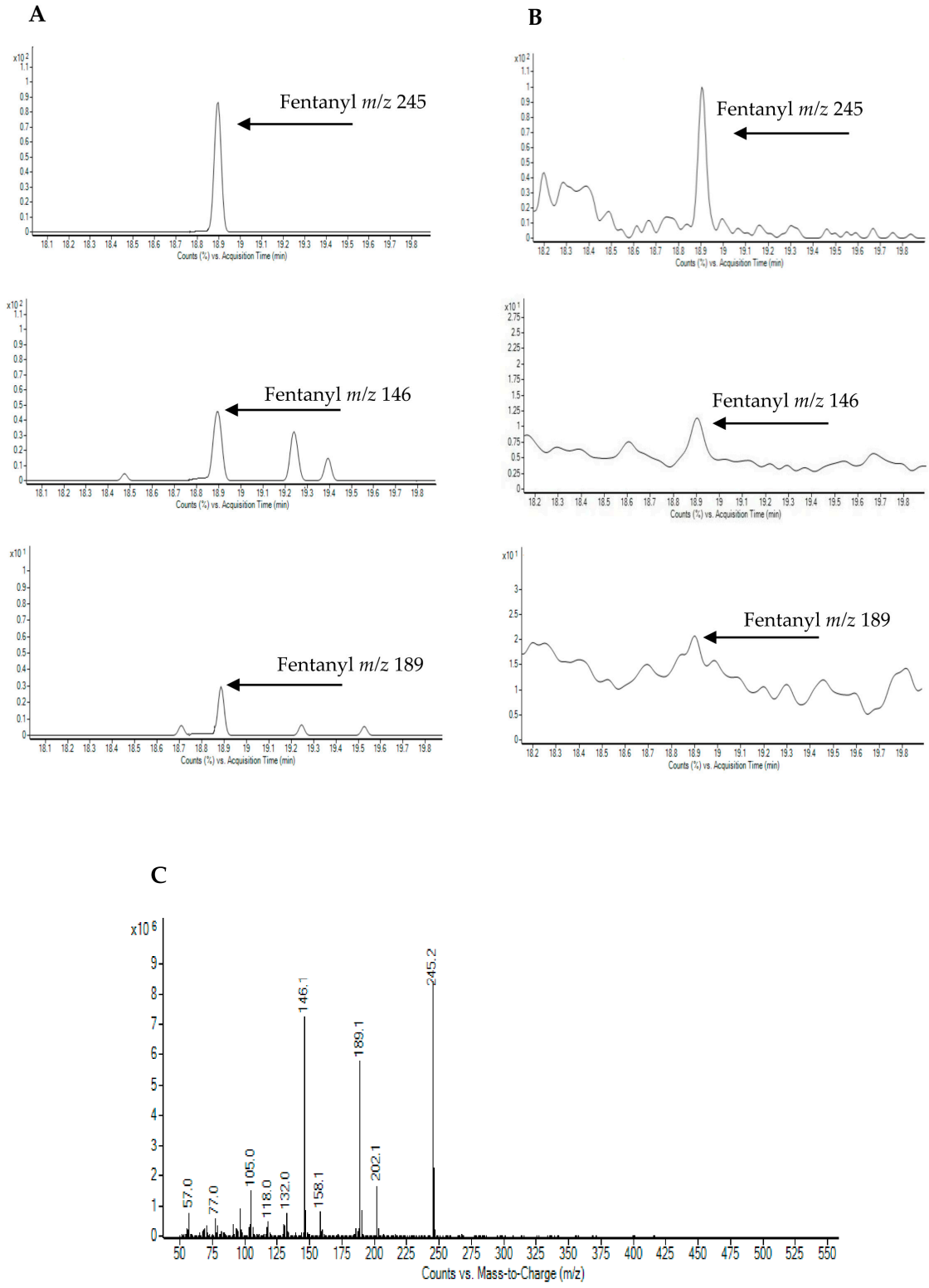

Figure 2. Representative selected ion monitoring GC-MS chromatograms of: urine samples positive to Fentanyl (**A**,**B**) and mass spectrometry or tandem mass spectrometry (MS/MS) full scan mass spectrum used for substance identification (**C**).

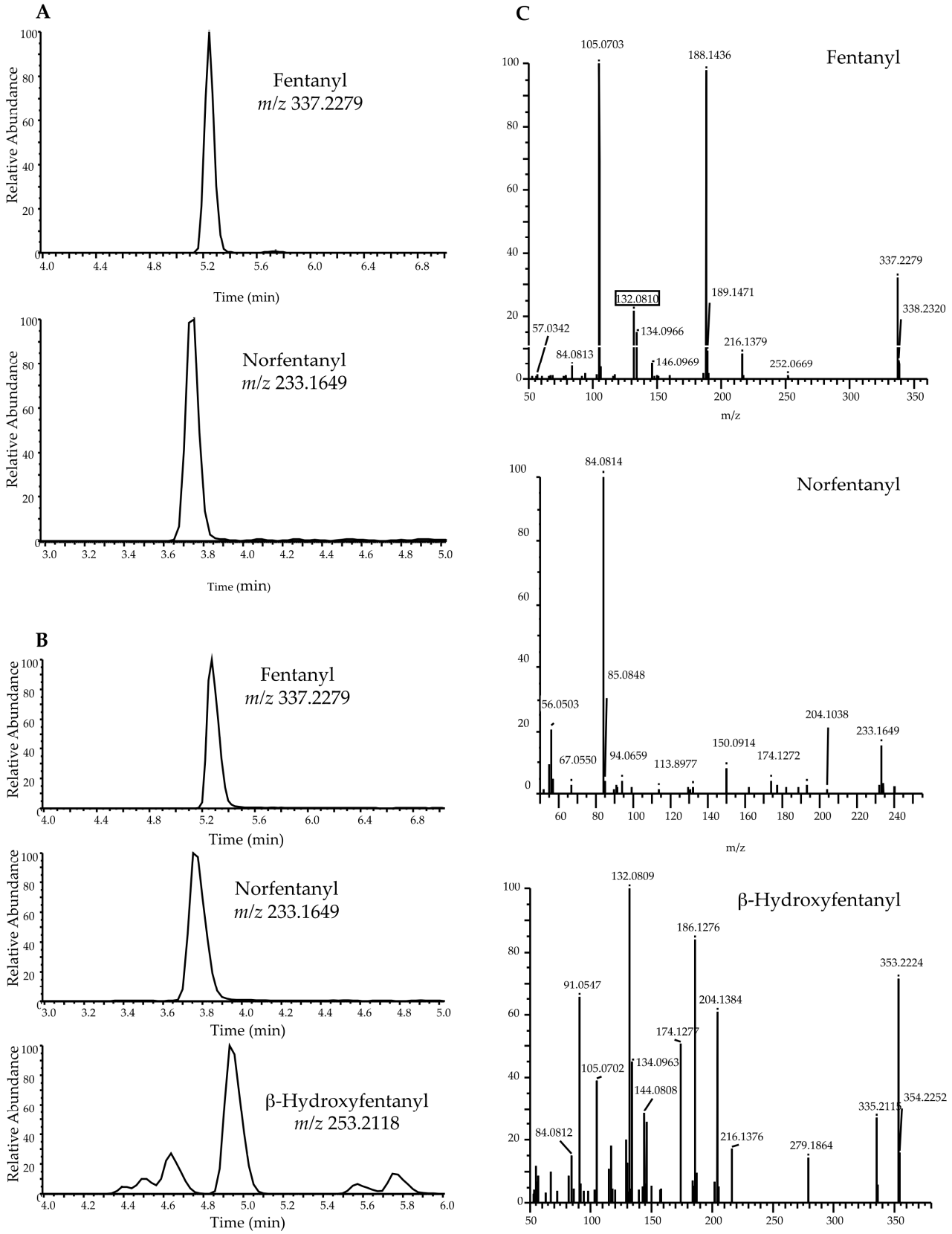

Figure 3. Representative extracted-ion UHPLC-HRMS chromatograms of: (**A**) urine sample positive to Fentanyl and Norfentanyl (**B**) urine sample positive to Fentanyl, Norfentanyl and β-hydroxyfentanyl and MS/MS full scan mass spectrum used for substances identification (**C**).

Table 2. Comparison of GC/MS and UHPLC-HRMS fentanyl and/or its metabolites and analogs urine sample screening and confirmation results.

Sample Code	Detected Compound (GC/MS)	Detected Compound UHPLC-HRMS
MI-1029	ND	Fentanyl Norfentanyl
MI-1077	Fluorfentanyl	ND
MI-1078	N-(3-ethylindole) Norfentanyl	ND
MI-1079	Fluorfentanyl	Fentanyl Beta-Hydroxyfentanyl Norfentanyl
BS-2003	Fentanyl	Fentanyl
MI-3009	Fluoro acetyl Fentanyl	ND
MI-5016	Fluoro Valeryl fentanyl	ND
US-010	Fentanyl	Fentanyl Norfentanyl
US-017	Fentanyl	Norfentanyl
US-039	Fentanyl	Beta-hydroxyfentanyl Fentanyl Norfentanyl
US-059	Fluorfentanyl	ND
US-060	ND	Norfentanyl
US-065	Fluorfentanyl	ND
US-077	Fluoro Valeryl fentanyl	ND
US-083	Fluoro Valeryl fentanyl	ND
US-095	Fluoro Valeryl fentanyl	ND
US-109	Fluoro Valeryl fentanyl	ND
US-139	Acetyl-methylfentanyl 2′-fluoro ortho-Fluorofentanyl	ND
US-142	Thiofentanyl	ND
US-144	Fentanyl	Fentanyl Norfentanyl Beta-Hydroxyfentanyl
US-145	Fluorfentanyl	ND
US-148	Fentanyl	Norfentanyl
US-155	Thiofentanyl	Norfentanyl

ND: not detected.

2.4. Other NPS

The NPS, other than NSOs, detected in the 296 analyzed samples by both methodologies belonged to the class of synthetic cathinones (4.4%) and to that of synthetic cannabinoids (1.3%) (Table 3).

Table 3. New psychoactive substances(NPS) found in urine samples under investigation.

NPS Classes	Substances (*n*)
synthetic cathinones	MDPV (2) 4-cloro N butylcathinone (1) 4-Methyl-PV8 (6) Fenethylline (4)
synthetic cannabinoids	JWH-122 (1) JWH-032 (1) JWH-200 (1) UR-144 (1)

n = number of positive samples; MDPV: 3,4-Methylendioxy Pyrovalerone; 4-Methyl-PV8: 2-(pyrrolidin-1-yl)-1-(p-tolyl)heptan-1-one.

The analysis by UHPLC-HRMS method of real sample obtained from the subject that results positive to UR-144 showed two peaks with different retention time but similar mass spectrum (Figure 4).

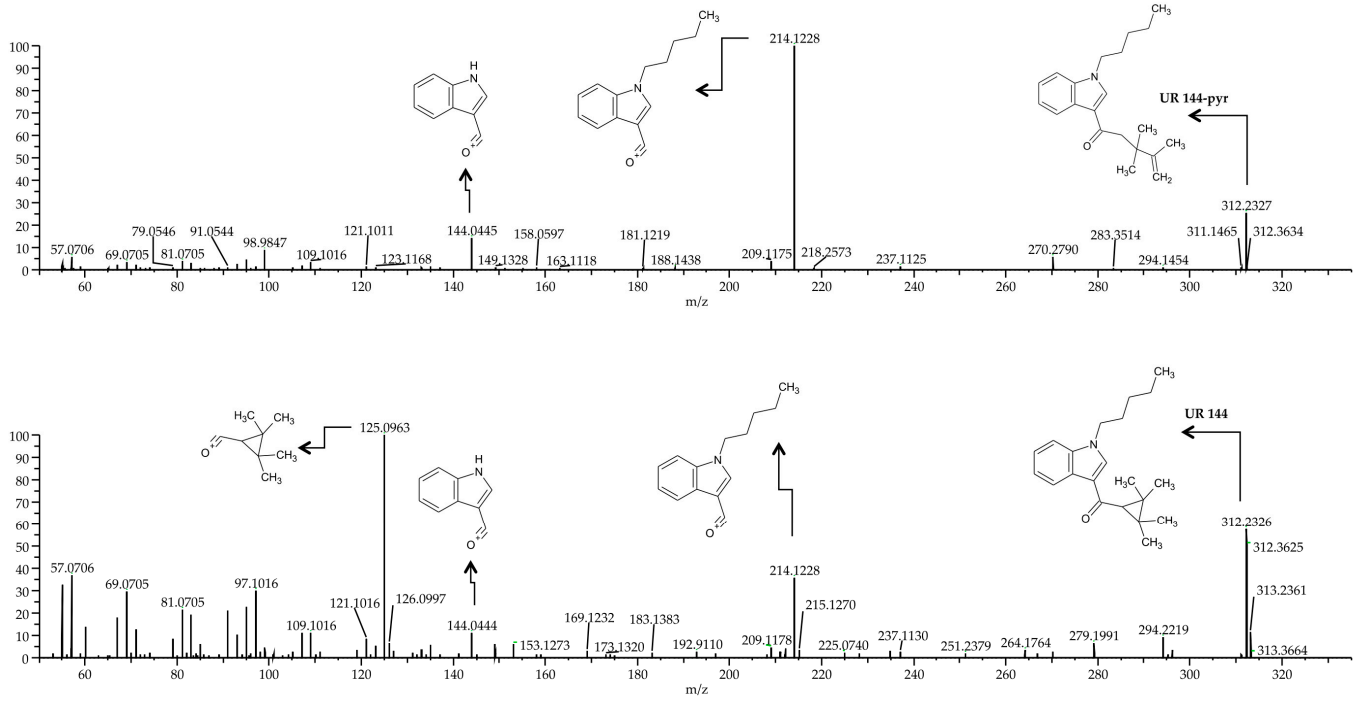

Figure 4. Full mass spectra of UR-144-pyr and UR-144.

3. Discussion

Methadone is frequently prescribed for the maintenance therapy of opioid addiction detoxification. Patients needing treatment with this and other medications often have co-occurring medical and mental illnesses that require medication treatment [24].

Untargeted mass spectrometry techniques have become essential tools for toxicological analysis [25].

The poor availability of reference standards for many NPS and metabolites presents a large challenge to forensic toxicology laboratories when trying to detect and identify both known and unknown NPS and other xenobiotics. What toxicologists expect both in clinical and forensic analysis from a general unknown screening procedure is the unequivocal identification of the xenobiotics involved in intoxication cases, even when they have no evidences to guide the search.

In general, the combination of different complementary methods (immunoassays, liquid chromatography and gas chromatography) was shown to be a good approach for

screening samples in forensic and clinical toxicology [26]. Currently, the most competent approach for compound identification involves mass spectral library search [27].

We here presented two complementary analytical methods for screening of classic drugs of abuse and new psychoactive substances and metabolites in urine samples. Low resolution GC-MS and high-resolution instruments (UHPLC-HRMS) can both be used to develop efficient screening workflows. It was possible to obtain an identification, based on the obtained mass spectrometry information, of different xenobiotics.

The main purpose of this initial screening technique has been to identify samples positive to classical drugs of abuse, NPS and NSOs while simultaneously eliminating negative specimens from any subsequent analytical examination. Once a NPS or a NSO are detected, quantification could be further performed to provide information regarding concentrations found in urine of users and in cases of fatal and non-fatal intoxications.

The principal limitation of the presented methodology was the difficulty associated with data processing to get the information from single sample analysis that required qualified expertise. Moreover, in some cases it can be extremely difficult to chromatographically separate certain NPS to facilitate identification via mass spectrometry, such as in the case of isomers and isobaric compounds which display the same or significantly related chemical formulae [22,28–32].

In the current method and in agreement with a previous study [29] the isomers JWH-019 and JWH-122 as well the two metabolites of JWH-122 (JWH 122 N-4-Hydroxypentyl and N-5-Hydroxypentyl) and JWH-210 (JWH 210 N-4-Hydroxypentyl and N-5-Hydroxypentyl) were not distinguishable, since their masses and retention times matched. Otherwise, the isomers UR-144 and UR-144-pyr could be distinguished (Figure 4). Moreover, the opiate family contains a number of isobaric couples that can complicate the correct identification of e.g., morphine versus hydromorphone, or codeine versus hydrocodone. Other potential isobaric/isomeric interfering compounds that we found in our run were amitriptyline versus EDDP, and Tramadol versus O-desmethylvenlafaxine. Nevertheless, in our developed methodology, the above reported substances exhibited different retention times.

Isomeric and isobaric substances require gas or liquid chromatographic conditions that enable adequate separation of the compounds prior to MS analysis or include other mass spectrometry data such as m/z, isotope pattern, retention time and fragmentation information [22,30–32].

However, even if the total analysis time was not short, this method could screen several psychoactive substances of different chemical structures in epidemiological studies aimed to disclose the use of compounds with a high risk of toxicity, leading to severe acute intoxications and overdoses. Moreover, High resolution full scan data also provides retrospective analysis for identifying previously unknown drugs of abuse [31].

Indeed, for this particular study, no reference standards were used, but only mass spectrometric libraries and the coupling of both methodologies. As above reported, positivity to a certain substance was only provided when both methodologies, independently run by different operators, matched with the identification of a specific molecule.

In agreement with previous studies [15,19,21], the HRMS procedure was shown to be superior to screening by GC-MS, the costs still limit the widespread distribution in routine laboratories.

On the other hand, a last generation GC-MS assay highlighted the similar specificity of UHPLC-HRMS and therefore the simultaneous use of the two instruments allowed to demonstrate that a simple and traditional methodology can be used to screen unknown samples this also due to the presence of the latest generation of libraries present in support to toxicologist whose experience allows to identify unknown substances or to exclude false positives.

In this concern, analytical methodologies used for the identification of NPS continuously emerging in illicit markets should be developed, validated, updated and analytical data should always be shared across different communication platforms to help health professionals involved in clinical and forensic toxicology issue [6,33].

In addition, once substances identification has been accomplished, it can be of interest to confirm and quantify identified substances to expand information on concentration found in biological fluids of consumers and eventually associate obtained data with clinical evidence. In this concern, pure standards of parent compounds and/or metabolites are needed an extensive method validation whatever is the applied methodology (e.g., LC-MS/MS, GC-MS, GC-MS/MS or HRMS) considering the maximum cost-benefit ration for a high throughput laboratory facing with this kind of analyses.

4. Materials and Methods

4.1. Chemicals and Reagents

Water, methanol (MeOH) and acetonitrile (ACN) MS grade, chloroform, isopropanol and formic acid analytical grade were purchased by Carlo Erba (Milan, Italy). Ammonium formate, phosphate buffer and N,O-bis-trimethylsilyl-trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) was obtained from Sigma–Aldrich (Milan, Italy).

4.2. Study Design

Urine samples collection took place at Consorcio Mar Parc De Salut De Barcelona, Spain and Hospital Universitari Germans Trias i Pujol from March, 2019 through October, 2020. Here, 296 patients with a history of opioid use disorder were enrolled in this study. All individuals were under methadone maintenance therapy (MTT). In this case, 109 patients provided identified urine samples after obtaining a signed informed consent, while 187 accepted to provide an anonymous sample, but no personal information was collected.

In order to secure the participants' privacy, the survey data and collected urine were coded and the local Human Research Ethics Committee of both centers (ref. 2018/2138/I and PI-18-126) approved the study protocol. Prior to analysis aliquots of urine were stored at −20 °C.

4.3. Sample Preparation for Screening Analysis by GC-MS and UHPLC-HRMS

A liquid-liquid extraction was performed after diluting 0.5 mL of urine in 1 mL 0.1 M phosphate buffer pH 3.0 and 0.5 mL of the same sample in 0.1 M phosphate buffer pH 10 (the desired pH was eventually adjusted using drops of 1 N HCl or 1N KOH, respectively). The samples were vortex mixed and then the solutions were extracted twice with 1.5 mL chloroform/isopropanol (9:1, v:v). After centrifugation, the organic layer from each buffered sample was divided into two 1.5 mL aliquots and evaporated to dryness at 40 °C under a nitrogen stream.

The first dry aliquot was derivatized with a mixture of 25 µL of acetonitrile and 25 µL of N,O-bis-trimethylsilyl-trifluoroacetamide (BSTFA) with 1%trimethylchlorosilane (TMCS) at 70 °C for 30 min. The second dry aliquot was dissolved in 50 µL ethyl acetate. A 1 µL amount of underivatized and derivatized acid and alkaline extracts were injected into the GC-MS system.

After the analysis in GC-MS, the underivatized samples were evaporated to dryness under a nitrogen stream and then dissolved in 150 µL of a mixture of mobile phase A (Ammonium formate 2 mM, 0.1% HCOOH) and B (Ammonium formate 2 mM in MeOH/ACN 50/50, 0.1% HCOOH, 1% H2O) (50:50, v/v). 5 µL were injected into UHPLC-HRMS.

4.4. Gas Chromatography-Mass Spectrometry (GC-MS) Instrumentation

The GC-MS instrument consisted of an Agilent 7890 A gas chromatograph coupled with 5975 C mass spectrometry detector (Agilent Technologies, PaloAlto, CA, USA). Ultra-Inert GC column Zebron (ZB-Drug-1, 15m × 250 µm i.d, film thickness 0.25 µm; Phenomenex, Milan, Italy) was installed.

The GC-MS condition for the screening procedure was as follows: splitless injection mode; helium (purity 99%) carrier gas flow 1.2 mL/min; the injection port, ion source, quadrupole and transfer line temperatures were 260, 230, 150 and 320 °C, respectively;

column temperature was programmed at 70 °C for 2 min and increased to 190 °C at 30 °C/min and then increased to 290 °C at 5 °C/min for 10 min. Subsequently the programed temperature was increased to 340 °C at 40 °C/min to eliminate impurities from the column.

The electron-impact (EI) mass spectra were recorded in total ion monitoring mode (scan range 40–550 m/z).

The full scan data files were processed by an Agilent Workstation (Agilent Technologies). The mass spectra international libraries used for peaks identification were NIST Research Library (National Institute of Standards and Technology)

4.5. Ultra-High-Performance Liquid Chromatography-High-Resolution Accurate Masses Spectrometry (UHPLC-HRMS) Instrumentation

The UHPLC/ESI Q-Orbitrap system consisted of an Ultimate3000 LC pump and an Ultimate 3000 autosampler coupled with a QExactive Focus mass spectrometer equipped with a heated electrospray ionization (HESI) probe operating in positive ionization mode and the system was controlled by Trace finder 4.0 software (Thermo Fisher Scientific, Bremen, Germany).

Separation was performed on an Accucore™ phenyl Hexyl (100 × 2.1 mm, 2.6 µm, Thermo, USA). They were maintained at 40 °C. The flow rate was set at 500 µL/min. Elution was achieved as follow: 99% A for 1 min, linear gradient to 99% B in 10 min, held for 1.5 min. The column re-equilibration was performed with a linear gradient to 99% A in 0.01 min, held for 4.0 min. A heated electrospray ionization (HESI) source in positive/negative ion mode was used for the ionization of compounds.

The mass parameters were as follows: ionization voltage was 3.0 kV; sheath gas and auxiliary gas were 35 and 15 arbitrary units, respectively; S-lens RF level 60; vaporizer temperature and capillary temperature were setting both at 320 °C. Nitrogen was used for spray stabilization, for collision induced dissociation experiments in the HCD cell and as the damping gas in the C-trap. The instrument was calibrated in the positive and negative modes every week.

Data were acquired in full-scan in data-dependent MS2 (ddMS2) mode. In this mode, both positive and negative high-resolution, full-scan data at resolution of 70 k were collected with a scan range of 100–1000 m/z, then MS2 spectra at a resolution of 17.5 k with an isolation window of 2 m/z were triggered for compounds entered in the inclusion list and expected retention times of the target analytes, with a 1 min time window.

The MS and fragmentation data acquired in full scan is processed by Thermo Scientific TraceFinder™ software. This specific software performs a thorough interrogation of the database by making use of the built-in database and mass spectral library of over 1400 compounds, retention times, isotope pattern matching, elemental composition determinations to identify and confirm drugs and metabolites in the analyzed samples. Moreover, mzCloud Mass Spectral Library was used as mass spectra international library for unknown peak identification (Advanced Mass Spectral Database; www.mzcloud.org, accessed on 1 April 2021).

4.6. Analytical Performance

To check the robustness and the reliability of the developed analytical methods, 10 different proficiency urine testing samples from UNODC International Quality Assurance Program (some with no analytes, some with one and some with more substances), whose previous qualitative and quantitative GC–MS results were available, were re-analyzed using the present methods.

Moreover, we also tested 10 urine samples fortified with 1 ng/mL 40 different most popular NPS and main metabolites prepared within the framework of an Italian Project ("NPS-LABVEQ" project) founded by Italian antidrug policy department aimed to allow pharmacotoxicological laboratories along the Italian peninsula to identify these substances in biological and non-biological matrices with different NPS [34]. Finally, 20 blank urine

samples from laboratory personnel were also tested to check for false positives during the different batches.

5. Conclusions

This study presents a comprehensive gas chromatography-mass spectrometry (GC-MS) and liquid chromatography (UHPLC)–high-resolution mass spectrometry (HRMS) general screening procedure for classic drugs and new psychoactive substances in urine of consumers involving an easy, quick and low-cost sample preparation. This screening method based on two different chromatographic and mass spectrometry methodologies can be applied to disclose suspected drugs of abuse related fatalities or acute intoxications occurring in emergency departments and drug addiction services.

Author Contributions: "Conceptualization, S.P., M.F., M.T. and R.P.; methodology, E.M., M.P. and M.A.F.; validation, E.M., M.P. and M.A.F.; investigation, S.P., E.M. and M.P. writing—original draft preparation, E.M., M.P.; writing—review and editing, all the authors. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Human Research Ethics Committees of Parc de Salut MAR (protocol code 2018/8138/I and date of approval 7 February 2019) and the Hospital Universitary Germans Trias i Pujol (protocol code PI-18-126 and date of approval 20 June 2018).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All data generated or analyzed during this study are included in this published article.

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Conflicts of Interest: The authors declare no conflict of interest

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8.6.2. Manuscript 4: Synthetic cannabinoids use in a sample of Opioid-Use Disorder Patients.

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Synthetic cannabinoids use in a sample of opioid-use disorder patients

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Cannabis is the most widely consumed illegal drug in the world and synthetic cannabinoids are increasingly gaining popularity and replacing traditional cannabis. These substances are a type of new psychoactive substance that mimics the cannabis effects but often are more severe. Since, people with opioids use disorder use widely cannabis, they are a population vulnerable to use synthetic cannabinoids. In addition, these substances are not detected by the standard test used in the clinical practice and drug-checking is more common in recreational settings. A cross-sectional study with samples of 301 opioid use disorder individuals was carried out at the addiction care services from Barcelona and Badalona. Urinalysis was performed by high-sensitivity gas chromatography-mass spectrometry (GC-MS) and ultra-high-performance liquid chromatography-high –resolution mass spectrometry (UHPLC-HRMS). Any synthetic cannabinoid was detected in 4.3% of the individuals and in 23% of these samples two or more synthetic cannabinoids were detected. Among the 8 different synthetic cannabinoids detected, most common were JWH-032 and JWH-122. Natural cannabis was detected in the 18.6% of the samples and only in the 0.7% of them THC was identified. Several different synthetic cannabinoids were detected and a non-negligible percentage of natural cannabis was detected among our sample. Our results suggest that the use of synthetic cannabinoids may be related to the avoidance of detection. In the absence of methods for the detection of these substances in clinical practice, there are insufficient data and knowledge making difficult to understand about this phenomenon among opioid use disorder population.

KEYWORDS

cannabis, synthetic cannabinoid, opioid use disorder, new psychoactive substances, urine sample analysis

Introduction

Cannabis is the most used illicit drug worldwide, with an estimated 200 million users (approximately 4% of the world's population) between the ages of 15 and 64 in 2019 (1). In Europe, it is also the most widely used drug, with a prevalence of daily use of 1.8% in the general population and 10.3% in individuals between 15 and 24 years of age, respectively (2).

In recent years, synthetic cannabinoids (SCs) have emerged as substitutes for natural cannabis as they are cannabinoid types 1 and 2 receptor agonists similar to tetrahydrocannabinol (THC), the main psychoactive component of cannabis, which is a partial agonist (3). The most common products containing SCs are smoking mixtures, e-liquids, and infused paper (4). Some effects of SCs intoxication are loss of consciousness, respiratory depression, and behavioral alterations such as aggression or self-injury (5). Over the years, different generations of SCs have appeared, each time showing a higher potency than THC, making them attractive to some consumers (3, 6). Recently, a fourth generation of SCs has been described, which could cause serious damage to health based on its pharmacological and toxicological activity (7). The effects experienced may differ between SC users, as in the case of cannabis use, including feelings of euphoria, relaxation, or even paranoia (5); however, they are usually more intense than those experienced with natural cannabis (8).

SCs were first synthesized in 1970 in an attempt to find new analgesics for pain treatment, but it was not until the 2000s that they appeared in the market (9). The popularity of these compounds was boosted in 2004 by the emergence of a new product called "Spice" (4). A few years later, in 2008, the first cases of poisoning related to SCs use were reported (4), some of which resulted in fatal overdoses (10). Since 2008 to date, the European Union Early Warning System controls 209 different SCs; thus, SCs are the largest group of new psychoactive substances (NPS) monitored (2, 4).

Cannabis is commonly used by patients with opioid use disorder (OUD) (11), which points to this population as a potential consumer of SCs. Other SC users include regular cannabis users, people who experiment with new drugs (e-psychonauts), increasingly vulnerable groups, such as high-risk users (4, 12), and men aged between 13 and 59 with a history of polydrug use who consider SCs a good alternative to cannabis (13). Since SCs are not detected in the standard toxicological urine tests used in clinical practice, they can be easily used by individuals for pleasure and enjoyment (13).

Notably, the prevalence of SC use is <1% in the European general population, but this is higher if we focus on subpopulations such as young adults or psychiatric patients, especially those with psychosis (4). The national survey among the Spanish general population shows a prevalence of SC use of the 0.6% and increases to 1.2% in the group aged between 25 and 34 years (14).

OUD individuals have not been the focus population for studying these substances, which are more likely to be present in recreational settings, such as music festivals or raves, or among e-psychonauts. In the present study, we investigated the use of natural and synthetic cannabis in an OUD population from Barcelona through urinalysis.

Abbreviations: NPS, new psychoactive substance; OUD, opioid use disorder; SC, synthetic cannabinoid; THC, tetrahydrocannabinol.

Materials and methods

Study design and participants

A secondary analysis from a cross-sectional study of 301 OUD individuals was conducted. The samples belong to two collections: one from identified patients from whom sociodemographic and clinical data were collected, and the other from anonymous patients with no data collected. Patients were recruited at addiction care facilities at Hospital del Mar (Barcelona) and Hospital Universitari Germans Trias i Pujol (Badalona) in Spain, from February 2019 to March 2020 and from July to October 2020, respectively. Due to the impact and changes in the functioning of addiction care services during the COVID-19 pandemic, no samples were collected from March 13th until July 6th, 2020.

Participation in the study was voluntary, and urine samples were collected from each participant. All participants, as an inclusion criterion, have been diagnosed with OUD by a psychiatrist/psychologist according to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (15), participation in an opioid agonist treatment program, and >18 years of age. None of the eligible participants were excluded from the study.

The study was approved by the local Ethical Committee of Clinical Research of the Parc de Salut MAR (CEIC-PSMAR number: 2018/8138/I) and the Hospital Universitari Germans Trias i Pujol (CEIC-HUGTiP number: PI-18-126).

Other details of the participants and methods can be found in previous publications that did not focus on SCs (16, 17).

Urine analysis

Urine samples (9 ml) from recruited individuals were collected and stored at −20 °C (in Nunc CryoTubesTM) until analysis. Sample preparation involved a liquid-liquid extraction and urinalysis was performed using two different validated methodologies. Ultra-high-performance liquid chromatography high-resolution mass spectrometry (the full scan MS and fragmentation data-dependent MS/MS)

was processed by Thermo Scientific TraceFinder™ software. The built-in database, mass spectral library of over 1,000 compounds of which more than fifty SCs (naphthoylindoles, phenylacetylindoles, indazole carboxamides, tetramethylcyclopropylindoles), retention times, isotope pattern matching, elemental composition determinations are used to identify and confirm drugs and metabolites in the analyzed samples. The matching threshold to establish LOIs (limit of identification) was set at 80%. Moreover, mzCloud Mass Spectral Library was also used as mass spectra international library for peak identification (Advanced Mass Spectral Database; www.mzcloud.org). In gas chromatography, the full scan data files were processed by an AgilentWorkstation (Agilent Technologies). The mass spectra international library (NIST, National Institute of Standards and Technology research library) was used for peaks identification (18).

Data analysis

Frequency-based descriptive analysis was carried out using SPSS (version 22.0; SPSS Inc., Chicago, IL, USA).

Results

A total of 301 urine samples were collected and analyzed. Although more than 50% of the samples were collected anonymously, subjects were part of an opioid agonist treatment program, the sociodemographic characteristics of patients in this program indicate that 68% of the patients were men and the mean age was 52 years (range: 22–77 years old).

The SCs and cannabis derivatives detected in these samples are shown in Table 1.

Some SCs were detected in 13 (4.3%) urine samples, and 8 different substances of this type were identified. Among these, two or more SCs were found in three (23.07%) samples. The most detected NPS cannabinoid types were JWH-032 and JWH-122 in four (1.3%) cases each. JWH-018, RCS-8, and UR-144 were less common, present in only one (0.3) case each.

Natural cannabis was detected in 56 (18.6%) samples. In all cases, carboxy-THC, cannabidiol, or cannabinol were identified. THC was identified in only two (0.7%) of the samples.

TABLE 1 Cannabis-related substances and metabolites detected among opioid use disorder urine samples ($N = 301$).

Substances and metabolites detected	N (%)
Samples positive to SCs*	**13 (4.3)**
JWH-018	1 (0.3)
JWH-032	4 (1.3)
JWH-122 or JWH-122 N-4-hydroxypentyl / JWH-122 N-5- hydroxypentyl	4 (1.3)
JWH-200	2 (0.7)
JWH-209	2 (0.7)
JWH-210 or JWH-210 N-4-hydroxypentyl / JWH-210 N-5- hydroxypentyl	2 (0.7)
RCS-8	1 (0.3)
UR-144 or UR-144 N-5-hydroxypentyl	1 (0.3)
Samples positive to natural cannabinoids*	**56 (18.6)**
Cannabidiol	-
Cannabinol	-
THC	2 (0.7)
11-Nor-9-carboxy-THC (THC-COOH), cannabidiol, or cannabinol	56 (18.6)

*Samples can contain more than one SCs o natural cannabinoid.

Discussion

We detected the use of SCs in individuals with OUD who were attending addiction care facilities in Barcelona and Badalona. Notably, cannabis use was widespread among the study participants with a Contrary to our results, previous studies in Finland and Germany did not find the presence of SCs in a similar population (19, 20). Furthermore, previous studies have investigated the prevalence of natural cannabis use in OUD populations. While some show a similar, although somewhat lower prevalence of 15% in positive urine samples (21), other studies show a higher prevalence of e.g., 58% (20) and 63% (22). Differences in the prevalence of cannabis use in this population could be explained by characteristics of the sample such as the country where the studies were conducted.

This study highlights the importance of investigating the consumption of SCs in this population, as there are limited studies on this topic. Along with this lack of knowledge, we found that polydrug use in the OUD population is widespread and often includes cannabis and cannabinoids among other substances (17, 23). Polydrug use is associated with an increased risk of relapse, fatal overdose, and suicidal ideation and attempts (23). Of note, SCs are one of the groups of NPS with the highest number of reported intoxications (24).

Human studies for the investigation of the clinical aspects of SCs are currently limited and usually focused on cases of intoxication or fatalities (25). Although, these are some reports of observational studies focused on pharmacological effects and fewer on the detection of these substances in addicted populations, such as OUD individuals, as is the case of the present study (26–28).

We hypothesized that there are several factors that could explain the increased use of cannabis and SCs, in recent years. First, the legalization of this substance in several countries has contributed to a lower perception of the risk of consumption in the population than the risk perception pre-legalization

(1, 29). Another reason is the increase in the consumption of cannabinoids for therapeutic or medical purposes, which can lead to misuse and even abuse of these substances (5). Finally, the view that SCs are safer than other drugs and are a good alternative to natural cannabis indicates that these substances are potentially abused (30).

Conclusion

We detected several types of SCs in patients with OUD in Barcelona and Badalona. Additionally, a non-negligible percentage of cannabis use was detected in our sample. These findings suggest that cannabis use is prevalent among patients with OUD and may be substituted by cannabinoid-like NPS to avoid detection in clinical tests. Since we do not have the instruments and protocols for NPS detection in clinical practice, knowledge about this phenomenon is very limited in this population. It would be interesting to continue this line of research to have more updated knowledge about the use of SCs. Importantly, this study had limitations: first, the samples analyzed were provided by voluntary participants; therefore, random sampling was not exercised. Second, the possibility of detecting substances is linked to the time of use, dose, and elimination half-life in urine before elimination; these factors were not analyzed in this study.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by Ethical Committee of Clinical Research of the Parc de Salut MAR (CEIC-PSMAR Number: 2018/8138/I) and Hospital Universitari Germans Trias i Pujol (CEIC-HUGTiP Number: PI-18-126). The patients/participants provided their written informed consent to participate in this study.

Author contributions

MA-F, MT, and MF: formal analysis. MT, MF, RP, and SP: conceptualization, study design, funding acquisition, and supervision. MA-F, MP, EM, MR, RP, SP, CP-M, RM, EP, FF, MT, and MF: investigation. MA-F, MP, RP, SP, MT, and MF: methodology. RP, MT, and MF: project administration. MA-F, SP, MT, and MF: writing–original draft. All authors writing–review and editing and read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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