2.4 SÍNTESI D'UN POLÍMER AMB EMPREMTA MOLECULAR PREPARAT AMB OXITETRACICLINA COM A MOLÈCULA *TEMPLATE* I APLICACIÓ A L'EXTRACCIÓ EN FASE SÒLIDA DE TETRACICLINES

Degut als bons resultats obtinguts en l'estudi anterior corresponent al MIP empremtat amb naproxen, es va decidir ampliar el camp d'aplicació i portar a terme l'extracció de compostos que es troben en mostres de matriu més complexa com poden ser les de teixit animal. Amb aquest objectiu, es va preparar un MIP amb oxtetraciclina (OTC) com a *template* i es va aplicar a l'extracció d'un grup d'antibiòtics (tetraciclines) en ronyó de porc. La presència d'aquests antibiòtics en mostres de teixit està regulada per la Unió Europea (UE), ja que si l'animal ha estat sacrificat abans de poder metabolitzar-los podrien passar fàcilment a la persona durant la ingesta d'aquest teixit i produir al·lèrgies en individus sensibles a aquests compostos. Per tal d'assegurar que els consumidors no estan exposats a concentracions de residus potencialment perilloses per la seva salut, la UE estableix el que es coneix com límits màxims de residu (MRL) [1]. La determinació d'aquests compostos és important i degut a la complexitat que aquest tipus de mostres presenten, l'aplicació dels MIPs com a sorbents pot resultar interessant.

La majoria de treballs on la MISPE ha estat aplicada a l'extracció de compostos de mostres biològiques tracten principalment amb biofluids [2-5], sent minoritaris els estudis realitzats en mostres de teixit [6-8]. Degut a la complexitat d'aquest tipus de mostra, normalment es treballa amb un sistema d'extracció en dues etapes on la mostra (l'extracte obtingut del tractament del teixit) es fa passar primer a través d'un sorbent comercial, per exemple C_{18}, Extrelut 20, etc. de manera que permet fer una primera neteja de la matriu de la mostra. A més a més, aquest mètode també presenta l'avantatge de poder preconcentrar els analits a través del MIP en absència d'aigua, ja que l'elució dels compostos retinguts al sorbent comercial es pot realitzar amb un solvent orgànic. No obstant, Muldoon *et al*. [6] és un dels pocs autors que va aplicar directament l'extracte de la mostra al MIP. Tot i no haver realitzat una etapa de neteja prèvia amb un solvent comercial, l'analit d'interès (atrazina) podia ser quantificat sense problemes d'interferents.
En el present estudi es presenta l'aplicació d'un MIP empremtat amb OTC tot i que també s'havia preparat un altre MIP sintetitzat amb la tetraciclina (TC) com a *template*. La síntesi dels dos MIPs amb aquestes molècules no va ser senzilla

degut al gran nombre de grups funcionals que presenten a la seva estructura. Per tal de solubilitzar els *templates* i d'assegurar-nos que hi havia suficient monòmer funcional present al medi de síntesi per a que tots els grups funcionals de la TC i OTC poguessin establir interaccions específiques, la relació entre T:M:X emprada va ser 1:8:40. La diferent solubilitat entre els dos compostos va donar lloc a l'ús de l'acetonitril com a porogen en el cas de la TC i del dimetilsulfòxid (DMSO) en el cas de l'OTC.

D'igual manera que en els estudis anteriors, els MIPs sintetitzats es van aplicar com a sorbents en processos d'SPE. Primerament, es va intentar optimitzar el procés de MISPE en línia amb la cromatografia de líquids, però es van obtenir recuperacions molt baixes després de preconcentrar diversos patrons en aigua Milli-Q. Les baixes recuperacions podien ser degudes per una banda a que la quantitat de sorbent emprada no fos suficient i per una altra banda a que les condicions d'elució no fossin les adients. Per aquests dos motius els MIPs van ser aplicats finalment fora de línia.

El procés d'optimització es va dur a terme tant pel MIP empremtat amb la TC com amb l'OTC i es comprovar com en emprar una major quantitat de sorbent, els quatre antibiòtics estudiats (TC, OTC, 4-epiclorotetraciclina i doxiciclina) quedaven retinguts en ambdós MIPs. A més a més, també es van provar altres solvents d'elució en lloc de l'ACN/àcid acètic (99:1) corresponent al solvent orgànic de la fase mòbil i es va veure que en metanol amb un 10% de KOH 1M l'elució dels compostos era completa. Després d'optimitzar l'etapa de neteja, es va observar com tots dos MIPs presentaven reactivitat creuada. No obstant, degut a que el MIP empremtat amb l'OTC donava millors recuperacions per a tots quatre compostos, va ser aquest polímer el que es va emprar per a l'extracció d'aquests antibiòtics en extractes de teixit d'animal.
L'objectiu de l'estudi que s'adjunta a continuació va ser avaluar l'efecte que tenia sobre la selectivitat del MIP l'aplicació d'un extracte de teixit animal. Per aquest motiu i per tal d'evitar possibles pèrdues durant l'etapa de pretractament de mostra, es va fortificar amb la mescla de tetraciclines l'extracte final.

D'aquesta manera va ser possible avaluar les pèrdues degudes al procés de MISPE pròpiament.

L'extracte del teixit, que es va obtenir després d'un procés d'homogenització en un tampó EDTA-McIlvine [9] i de centrifugació, es va aplicar directament sobre el MIP, ja que durant l'etapa d'optimització de la MISPE es va comprovar com el MIP presentava bona afinitat pels compostos a extreure tot i la presència d'aigua a la mostra.

La recuperació per la TC i OTC va ser lleugerament inferior a l'obtinguda en aigua Milli-Q degut possiblement a que, tot i fer el tractament adient al teixit, restes de proteïnes i greixos són encara presents en l'extracte final, i per tant, poden donar lloc a l'obstrucció de les cavitats de reconeixement molecular, fent disminuir doncs la recuperació dels analits a extreure. Com a conseqüència de les restes de proteïnes a l'extracte final del teixit es va comprovar com un mateix cartutx no podia ser emprat per a més de dues extraccions.

Els resultats obtinguts en aquest estudi s'inclouen en el treball que s'adjunta a continuació i que ha estat enviat a la revista *Analytica Chimica Acta* per a la seva publicació.

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2.4.1 *Synthesis and application of an oxytetracycline imprinted polymer for the solid-phase extraction of tetracycline antibiotics*

SYNTHESIS AND APPLICATION OF AN OXYTETRACYCLINE IMPRINTED POLYMER FOR THE SOLID-PHASE EXTRACTION OF TETRACYCLINE ANTIBIOTICS

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Abstract

Two molecularly imprinted polymers (MIPs) were synthesised using tetracycline and oxytetracycline antibiotics as template molecules in non-covalent molecular imprinting procedures. After a chromatographic evaluation, the performance of the MIPs as selective SPE sorbents was evaluated. In this study, it was demonstrated that after a clean-up step to disrupt the non-specific interactions between the MIPs and the compounds retained on them, the polymers showed cross-reactivity for certain other tetracycline analytes. The feasibility of the MIP to selectively extract tetracycline antibiotics in pig kidney tissue extract was demonstrated when the oxytetracycline MIP, which gave rise to the best MISPE results, was then applied.

Keywords: Column liquid chromatography; Solid-phase extraction; Molecularly Imprinted Polymer; Tetracycline antibiotics; Tissue samples

INTRODUCTION

Molecularly imprinted polymers (MIPs) are tailor-made materials which can be applied in several different applications [1-3]. However, in the last few years they have been increasingly exploited as selective sorbents in molecularly imprinted solid-phase extraction (MISPE) [4]. To date, the main application of MISPE has been to the extraction of analytes from biological samples [5-14]. Biofluids such as plasma, serum and urine have been the most analysed biomatrices [5-10] and only few studies

have been performed to the extraction of compounds from tissue samples [11-14].

Tissue samples are very complex matrices, for this reason, a pre-treatment step is always required. Then, the extract obtained is percolated through the MIP. Nevertheless, some proteins and lipids may still be present in the extract and subsequently they can hinder the access to the imprinted sites, which represents a decrease in the recognition properties of the MIP. Consequently, in most of the studies in which MISPE has been used for the extraction of analytes from tissue samples a two-step MISPE procedure has been applied [12-14]. Therefore, the extract coming from the pre-treatment sample is first percolated through a commercial sorbent ($C_{18,}$ OASIS HLB, Extrelut 20) and the retained analytes are then eluted and passed through the MIP.

Thus far, there is only a study in which the tissue extract has been applied directly through the MIP [11]. In this work, Muldoon *et al*. prepared a MIP with atrazine as template molecule and the polymer was then applied to selectively extract this analyte from beef liver extract which was obtained after a pre-treatment step. In this study the beef liver extract was directly applied to the MISPE cartridge and good results were obtained. Therefore, it was not necessary to use a commercial SPE sorbent before the MISPE cartridge.

The interest in detecting and quantifying drugs in animal tissues is increasing since it has been shown that these substances can leave residues in such tissues which can be directly toxic or cause allergic reactions in hypersensitive individuals. For this reason, in the present manuscript, the determination of tetracycline antibiotics has been studied. Two papers have been recently published regarding the imprinting with these compounds [15,16]. Nevertheless, in the study performed by Cai *et al* [15], the MIP prepared with TC was not used in a real sample application and, on the other hand, Suedee *et al.* [16] describes the application of molecularly imprinted membrane to the extraction of TC and its degradation products from water.

The present study describes for the first time the application of a MIP imprinted with tetracycline antibiotics to the direct extraction of tetracycline (TC) and oxytetracycline (OTC) from tissue sample extracts. TC and OTC were completely bound to their respective MIPs, which allow the tissue extract to be directly applied through the MIP.

EXPERIMENTAL

Reagents and standards

The chemicals used for the polymer syntheses were TC, methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), and dimethylsulfoxide (DMSO), all from Aldrich (Steinheim, Germany), OTC from Sigma (Steinheim, Germany), 2,2'-azobisisobutyronitrile (AIBN) from Acros Organics (Geel, Belgium), and HPLC grade acetonitrile (ACN) from Rathburn Chemicals (Walkerburn, UK). The monomers were purified prior to use *via* standard procedures in order to remove stabilisers, and the solvents dried over molecular

sieves. The AIBN was recrystallised from acetone.

The HPLC-grade solvents (acetonitrile and methanol) were provided either by Rathburn Chemicals or SDS (Peypin, France) and the water collected from a Millipore water purification system (Milli-Q water). The acetic and hydrochloric acids were from Probus (Badalona, Spain) and dichloromethane (DCM) from SDS (Peypin, France).

The structurally related tetracycline compounds used to investigate the selectivity of the imprinted polymers were TC, OTC, 4-epichlorotetracycline hydrochloride (4-EPI), and doxycycline hydrochloride (DC). TC and OTC were kindly purchased by Cenavisa (Tarragona, Spain) and 4-EPI and DC were provided from Across Organics (New Jersey, USA). The structure of these compounds is shown in Figure 1.

Preparation of the Imprinted Polymers.

Polymers P1 and P2 were prepared by the non-covalent approach using TC and OTC as the template molecules, respectively. The same functional monomer (MAA) was used in both polymerisations but the porogenic solvents were different (for solubility reasons).

The pre-polymerisation mixture for P1 comprised TC (1.16 mmol), MAA (9.28 mmol), the cross-linking monomer EGDMA (46.41 mmol) and the initiator AIBN (1.02 mmol) dissolved in13.33 ml of the porogen acetonitrile, in a 25 ml thick-walled glass tube. This solution was cooled on an ice bath, sparged with oxygen-free nitrogen for five minutes, sealed under nitrogen and then left to polymerise in a water bath at 60 ºC for 24 h. The polymer monolith obtained was crushed, ground and wet-sieved using acetone to obtain polymer particles with diameters between 25 and 38 µm suitable for the chromatographic and MISPE evalua-tions.

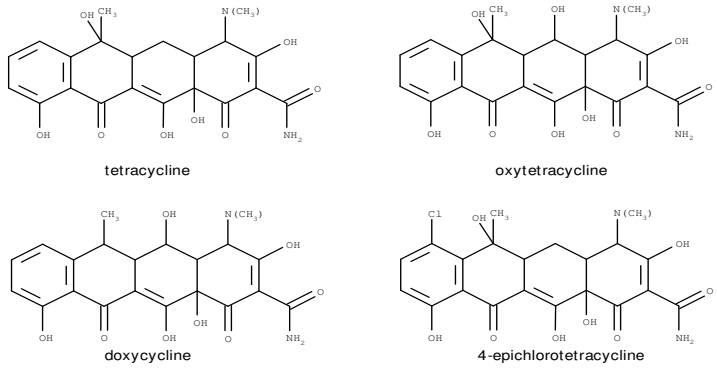

Figure 1. Chemical structures of the tetracycline antibiotics used to study the selectivity of the MIPs.

The P2 pre-polymerisation mixture comprised OTC (1.16 mmol), MAA (9.28 mmol), EGDMA (46.42 mmol) and AIBN (1.02 mmol) dissolved in the porogen, DMSO, (13.33 ml) in a 25 ml thick-walled glass tube. The synthetic procedure

followed during the polymerisation step was the same as for P1 polymer.

A reference, non-imprinted control polymer (NIP) based upon MAA (9.28 mmol) and EGDMA (46.41 mmol) with acetonitrile (13.33 ml) as the porogenic solvent, was prepared in absence of template.

Instrumentation

The polymers were initially evaluated in analytical columns to check the imprinting effects. 15 x 0,46 cm i.d. stainless steel HPLC columns were slurry packed with the ground polymer particles (25-38 μm) using an air-driven fluid pump (Haskel) with acetone as the slurrying and packing solvent at 2500 psi. An SP 8800 ternary HPLC pump and an SP 8450 UV detector (Spectra-Physics, Mountain View, CA, USA) were used in this pre-screening work.

The MISPE study was developed in an off-line mode using a solid-phase extraction manifold supplied by Teknokroma (Barcelona, Spain) connected to a vacuum pump. 200 mg or 500 mg of each polymer suspended in MeOH was packed into an empty 6 ml polyethylene cartridge. The liquid chromatographic system consisted of two LC-10AD pumps, a DGU-14A degasser, a CTO-10A oven and an SPD-10A UV spectrophotometric detector from Shimadzu (Tokyo, Japan). The loop for direct injection was 20 μl and the analytical column was a end-capped 25 x 0.4 cm i.d. Tracer Extrasil ODS2, 5 μm, supplied by Teknokroma.

Chromatographic Conditions

After packing of the polymers into analytical columns, the polymers were washed on-line with a mixture of acetonitrile/water/acetic acid (92.5/2.5/5 (*v/v/v*)) to eliminate interfering compounds arising from the synthesis (template and unreacted monomers).

For the chromatographic evaluation, 10 μl of each analyte and the void marker (acetone) were injected. P1 was evaluated using acetonitrile as the mobile phase at 0.21 ml min^{-1} in isocratic mode and P2 was evaluated using methanol as the mobile phase at 0.12 ml min^{-1} due to solubility issues and back-pressure problems. The NIP was evaluated under identical chromatographic conditions to P1 and to P2 in each case. The UV detector wavelength was set at 254 nm to detect acetone and 350 nm to detect the TCs. The analysis were performed at room temperature.

The parameters in the HPLC system for the MISPE experiments were as follows. The mobile phase was a mixture of two solvents: Milli-Q quality water containing 3% of MeOH and 1% of acetic acid (solvent A), and acetonitrile (solvent B). The flow-rate of the mobile phase was 1 ml min^{-1}, the gradient profile was 10-15% B from 0-15 min, 100% B at 35 min and then isocratic elution for 2 min. The column temperature was 35 ºC.

Molecularly Imprinted Solid-Phase Extraction Conditions

When tetracycline antibiotics were extracted from tissue sample extract, a

standard protocol was followed in treating the matrix prior to the SPE [17]. Thus, 5 g of tissue was placed in a tube and 20 ml of EDTA-McIlvaine's buffer added. The mixture was homogenised for 1 min using an Ultra-Turrax T-25 (Jankle & Kunkel, IKA-Labortechnik, Staufen, Germany) and the mixture then centrifuged at 9,000 r.p.m. for 10 min. After this period, the pellet was blended and centrifuged twice more with 20 ml and 10 ml EDTA-McIlvaine's buffer, respectively. The supernatants obtained were mixed and centrifuged for 25 min and, after filtration of the supernatant, 25 ml of the filtrate was passed through the MISPE cartridge.

For the MISPE extractions, the cartridges were packed with 500 mg of polymer. 25 ml of tissue extracts (adjusted to pH 2 with HCl 0.1M) were spiked with the mixture of tetracycline antibiotics and applied to the cartridges which had already been conditioned with 10 ml Milli-Q water (pH 11 with NaOH 0.1M). The polymers were then washed with 3 ml of ACN and the retained analytes desorbed with 20 ml of MeOH containing 10% of 1 M aqueous KOH. 20 µl of each sample was injected on to the analytical column.

RESULTS AND DISCUSSION

Chromatographic Evaluation of the Polymers

In this study, the chromatographic evaluation of the MIPs was performed to have an idea about the imprinting effect of the polymers. For this reason, 10 µl of 10 mM of TC in ACN was injected onto the chromatographic columns containing P1 and the NIP and the capacity factors calculated (k'^{TC}_{P1}= 1.23, k'^{TC}_{NIP}= 0.31). From these results, it was concluded that P1 showed higher affinity for TC than the control polymer. Furthermore, there was extensive tailing of the TC peak on the P1 column, a characteristic of an imprinted stationary phase. The imprinting factor (IF= k'^{TC}_{P1}/ k'^{TC}_{NIP}) was also calculated (IF =4) and this was indicative of a good imprinted effect in P1.

Similar behaviour was observed when 10 µl of 10 mM of OTC in MeOH was injected onto the column packed with P2. The capacity factors were not calculated because the NIP was not prepared in the same conditions; nevertheless, the extensive tailing of OTC observed on P2 but not on the NIP, indicated a possible good imprinted effect.

The next step was to develop a SPE procedure to extract TC and OTC from animal tissue extracts. Both MIPs were applied to this end and in this way, it would be also possible to confirm the imprinting effect.

MISPE

The SPE process was optimised for the TC and the OTC MIPs and the performance of these imprinted polymers for the extraction of tetracycline antibiotics was compared with the non-imprinted polymer. First of all, the conditioning and the loading steps were optimised. 10 ml of a standard solution, spiked with 5 mg l^{-1} of each compounds (TC, OTC, 4-EPI and DC), was passed through the cartridges packed, initially, with 200 mg of polymer. The sample prepared was in Milli-Q water at pH 2 and 9 and the cartridge conditioned with Milli-Q water at

several pH values (2, 6 and 11). The analytes were only strongly retained on the MIPs when the conditioning was using Milli-Q water at pH 11 and the sample was loaded in Milli-Q water at pH 2 because in this way electrostatic interactions were established. When any other conditions were applied for either of these two steps, it was seen that the aqueous solution collected during the loading step contained approximately 70%-80% of the compounds indicating that minimum retention of the analytes had been achieved.

Using these efficient loading conditions, the elution step was then optimised. When ACN or MeOH were used, the recoveries of the analytes were lower than 50%. However, when 3% of 1 M aqueous KOH was added to the MeOH, the recoveries increased significantly. This is likely to be because the interactions between the tetracyclines and the MIPs are disrupted at this pH. When volume of the elution solvent was studied, it was demonstrated that any volume lower than 10 ml was not sufficient to elute the TCs completely from the polymers. For this reason, 10 ml of this solvent was finally chosen as the optimum volume.

It is well known that compounds can be retained on MIPs due to both specific and non-specific interactions. Thus, it was thought that a suitable clean-up process would remove the non-specifically bound compounds from the MIPs, while the analytes used as templates would remain bound. Several organic solvents such as DCM, ethyl acetate and ACN, were investigated as washing solvents. A drying step which consisted of applying a vacuum to the SPE cartridges for 15 min prior to the clean-up was included to remove small amounts of water remaining on the cartridge [18-20]. When DCM or ethyl acetate were used, the non-specific interactions established between the analytes and P1, P2 and NIP were not removed, thus all the compounds present in the mixture previously described (TC, OTC, 4-EPI and DC) were still retained on the polymers. Nevertheless, a selective clean-up step was possible with ACN. Different volumes of this solvent were tested and the imprinting effect was revealed when 3 ml was applied, as can be seen in Figure 2. In this situation, tetracycline antibiotics were retained on both MIPs, but not in the NIP where the analytes were nearly washed off during this clean-up step. Figure 2 also shows that in these clean-up conditions, the analytes are stronger retained on OTC MIP than in TC MIP which represents higher recoveries for tetracycline

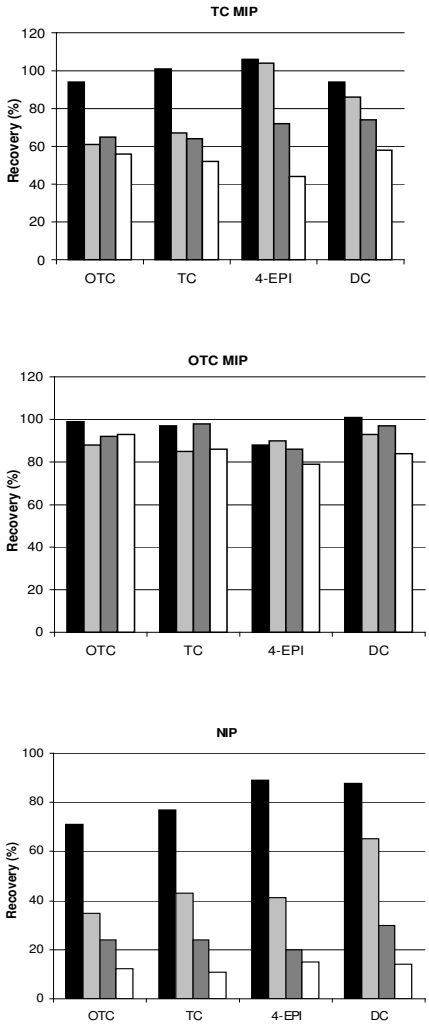

Figure 2. Recovery of the molecularly imprinted polymers when 10 ml of a standard solution spiked at 5 mg l^{-1} with the mixture of compounds was percolated through each polymer. a) TC MIP (P1), b) OTC MIP (P2), and c) NIP, without clean-up (■) and when a clean-up step was applied using 1 (■), 2 (■) and 3 (□) ml of ACN, respectively, as the washing solvent. (RSDs= 11% (n= 3))

antibiotics in OTC MIP. This allowed the conclusion to be drawn that OTC MIP shows more cross-reactivity [21] for other structurally close analogues than TC MIP.

In order to decrease the cross-reactivity shown by the MIPs, acid acetic was added to the ACN washing solvent (ACN-acetic acid (99:1)). This mixture was then evaluated as a potential solvent for the clean-up step, but there was no improvement in terms of selectivity, neither for TC MIP nor OTC MIP, because all the analytes in the sample were still retained (recoveries slightly lower than those using ACN alone). This allows to confirm that the interactions established between the analytes and the MIP during the loading step are based on selective ionic interactions since they are not disrupted by the presence of acetic acid in this clean-up step. ACN was finally chosen as the selective washing solvent for further experiments.

The recovery of the compounds at different sample volumes (10, 25, 50 and 100 ml) was studied. When the sample volume was increased to 25 ml it was observed that the recoveries for the tetracyclines decreased slighlty; consequently, the cartridges were packed with 500 mg of polymer, which allowed the retention of the analytes to be improved. The volume of MeOH and the percentage of KOH used in the elution step had to be increased to 20 ml and 10%, respectively, to elute the retained analytes. At these conditions, after performing the clean-up step the recoveries were similar than those obtained using the 200 mg cartridge.
It has been shown that after the clean-up step, the OTC MIP gives not only the best recovery values, but also shows a higher imprinting effect than the TC MIP in the MISPE experiments. For this reason, the OTC MIP was selected for further applications using tissue samples extracts.

MISPE of Tissue Sample Extracts

Tissue samples are complex biological matrices and for this reason an exhaustive pretreatment of the sample is always required. The use of a selective sorbent such a MIP can be very useful to obtain cleaner chromatograms. However, if proteins or lipids remain in the resultant tissue extract they can hinder the access to the imprinted sites and the recoveries can decrease. To evaluate the effect of a tissue extract in the procedure of molecular recognition, the extract was spiked with a mixture of the tetracycline

antibiotics. In this way, it was possible to check the recovery and selectivity of only MISPE process avoiding possible looses of recoveries arising from the tissue pre-treatment step.

5 g of a pig kidney tissue was selected and the standard sample treatment [22] followed prior to the MISPE experiments. After performing the standard sample treatment, 25 ml of the extract obtained was spiked with 0.12 mg l^{-1} of TC and OTC (600 µg of TC and OTC per kg of tissue) to evaluate the performance of the MIP in this kind of matrix. The tissue extract was directly applied to the MIP since it has been demonstrated that TC and OTC are completely retained on OTC MIP when they are applied in water samples. After the loading step, the cartridge was dried and the polymer was then washed with 3 ml of ACN and the compounds eluted with 20 ml of MeOH containing 10% of 1 M aqueous KOH. The eluate was evaporated to dryness and the residue reconstituted in 1 ml of HCl (0.01 M). 20 µl of this extract were injected onto the analytical HPLC column.

No peaks were observed at the retention time of the analytes when the non-spiked blank kidney tissue was analysed. When the tissue extract was spiked with TCs, the recoveries in the absence of the clean-up step were 66% and 69% for OTC and TC respectively, which were lower than those obtained with the acidified water standard solution. The lower recoveries obtained for the tetracycline antibiotics studied could be explained as a result of the matrix content still present in the solution extract after the standard sample treatment. When the clean-up step was included, the recoveries obtained were the same as those without washing the cartridge. Figure 3a shows the chromatogram obtained when the clean-up step was not performed and it should be pointed out that the impurity which coelutes with the TC analyte is nearly completely removed after the clean-up step as can be observed in Figure 3b, which represents a better quantification for the TC analyte. The repeatability of the method for 25 ml of spiked (0.12 mg ml^{-1}) tissue sample extract, expressed as RSD (n=3), was lower than 8%. Thus, the selectivity and the recovery of MISPE when biological extracts are percolated through P2 have been checked and the applicability of the MIP demonstrated.

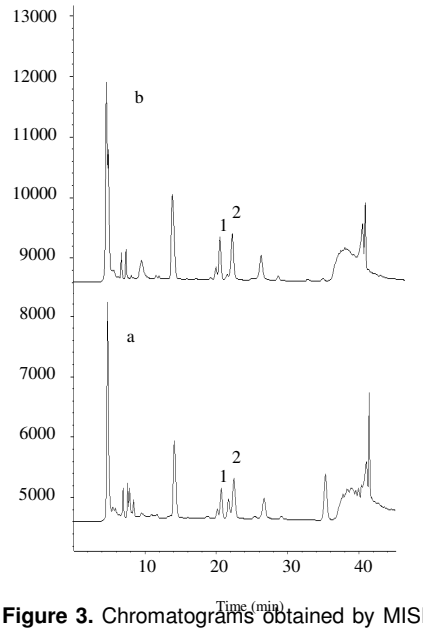

Figure 3. Chromatograms obtained by MISPE with the OTC imprinted polymer (P2) using 25 ml of extract from the standard tissue sample treatment (pH 2) spiked at 0.12 mg ml^{-1} with the mixture of compounds. a) Without clean-up step, and b) With a

clean-up step involving 3 ml of ACN. Peak designation: **1**= OTC, **2**= TC.

CONCLUSIONS

In this paper, two imprinted polymers (TC MIP and OTC MIP) prepared by a non-covalent molecular imprinting protocol, have been synthesised using TC as template molecule for TC MIP and OTC for OTC MIP. Both MIPs were applied in SPE protocols as selective sorbents. The imprinting effect and selectivity of the MIPs was confirmed and it was seen that OTC MIP was deemed to be the most promising polymer for further study because it showed the greatest imprinting effect and the highest recoveries for all the compounds pre-concentrated. Thus, only the OTC MIP was applied to the direct extraction of TC and OTC from tissue extracts. It has been demonstrated the applicability of the MIP when complex biological samples are passed through it.

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