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Preparation and evaluation of molecularly imprinted polymers as selective SPE sorbents for the determination of cathinones in river water

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ABSTRACT

In this study, a series of molecularly imprinted polymers (MIPs) were synthesised using (1R,2S)-(−)-ephedrine or (1S,2S)-(+)-pseudoephedrine as template, methacrylic acid as functional monomer and ethylene glycol dimethacrylate as cross-linking agent. A highly selective and sensitive method using the best performing MIP as sorbent in the solid-phase extraction (SPE) followed by liquid chromatography-high resolution mass spectrometry was established for the enantiomeric determination of five cathinones in river water samples. The SPE parameters (sample loading volume and pH, washing and elution solutions) were optimised so that recoveries and selectivities for the analytes were high. The enantioselectivity of MIPs towards the enantiomers of the analytes during the SPE process was also investigated. Under optimal conditions, the method developed provided satisfactory recoveries ranging from 67.6 to 83.2 % with a negligible matrix effect ranging from −5.5 to 1.8 %. The detection limits ranged from 0.3 to 0.8 ng/L and quantification limits ranged from 1.0 to 2.0 ng/L. The application of the method to the analysis of the river water samples indicated that methedrone and butylone were present at low concentration levels in river water samples.

1. Introduction

Nowadays the determination of drug residues in environmental waters is still a challenge that requires highly sensitive and selective analytical methods, due to the very low drug concentrations and extremely complex matrices. Efficient sample preparation can remove interference components and concentrate target analytes prior to instrumental analyses and so plays an important role in improving method sensitivity and selectivity. Solid-phase extraction (SPE) with various sorbents is the most commonly used sample preparation technique [1]. However, classical SPE sorbents, such as silica-based sorbents modified with C_8 or C_{18} chains and hydrophilic-hydrophobic balance (HLB), lack selectivity since analytes are retained via non-specific interactions. Thus, other compounds in the sample are co-extracted with target analytes, leading to significant matrix effects (ME), particularly when liquid chromatography-mass spectrometry (LC-MS) is used [2]. Mixed-mode ion-exchange sorbents may show selectivity towards ionic analytes when a clean-up step is included. However, molecularly imprinted polymers (MIPs) are the sorbents which have attracted considerable attention as promising SPE sorbents that have high selectivity during the preparation of environmental samples [3,4].

MIPs are highly cross-linked and porous synthetic polymers with high selectivity to the target analytes. They are obtained by polymerising functional and cross-linking monomers around a template molecule, which is usually the target analyte. Afterwards, the template molecules are removed to leave cavities complementary to the target molecule in size, shape and functional groups. Thus, MIPs are able to selectively bind target molecules from the complex matrix, with fewer interferences than classical sorbents. In addition, MIPs have the advantage that they have a high loading capacity, are easy to prepare and are remarkably robust under a wide range of operating conditions, which increases their applicability [3]. However, the main obstacle in the application of the traditional MIPs is the template bleeding during the analysis resulting from incomplete template elution from polymeric matrix, which could cause overestimated results [5]. To overcome this, the use of structural analogue as the template, called a "dummy template", has been proposed to prepare MIPs. Besides, the introduction of dummy templates is a useful solution when the target molecule is

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expensive or difficult to synthesise [6]. To date, the application of dummy MIPs in the SPE (MISPE) of various drugs has been reported in other studies [7–9]].

An emerging class of new psychoactive substances (NPSs), cathinones have gained great popularity in illicit drug markets all over the world [10]. The ease of online purchase makes them difficult to regulate. After consumption, cathinones are released into the environment via the sewage system. Determining their residues in environmental samples is a fast and cheap method for estimating their community consumption [11]. However, it should be noticed that all cathinones are chiral and are usually marketed as racemates. Their enantiomeric composition is often different from the racemic form after excretion as a consequence of the stereoselective metabolism [12]. Monitoring the enantiomeric composition of chiral drugs in the environment can distinguish between the residues of consumed drugs and unused drugs that have been directly disposed, which would make the estimation of consumption more accurate [13,14]. It would also help to identify the synthesis pathway of chiral drugs [15].

Methods based on SPE followed by LC-MS have been applied to determine cathinones at environmental level [11,16–19]. An important consideration during the extraction of chiral drugs is that stereoselectivity can occur since the enantiomers might show different affinities for the sorbents, which would result in a deviation from its initial enantiomeric fraction (EF). In particular, MIPs have already been shown to have a great potential for the *enantio*-separations of various chiral drugs [20–22]. Therefore, the stereoselectivity of MIPs towards the enantiomers should be assessed during the sample extraction.

The aim of the present study is to establish a highly sensitive and selective method for the enantiomeric determination of a group of cathinones in river water applying MISPE followed by LC–(Orbitrap) high resolution mass spectrometry (HRMS). For this purpose, novel MIPs using (1R,2S)-(−)-ephedrine or (1S,2S)-(+)- pseudoephedrine as dummy template were synthesised and compared for the extraction of cathinones. Ephedrine was chosen as a dummy template molecule because its structure is similar to that of cathinones and it is easy to obtain. Although MIPs employing ephedrine as template have been

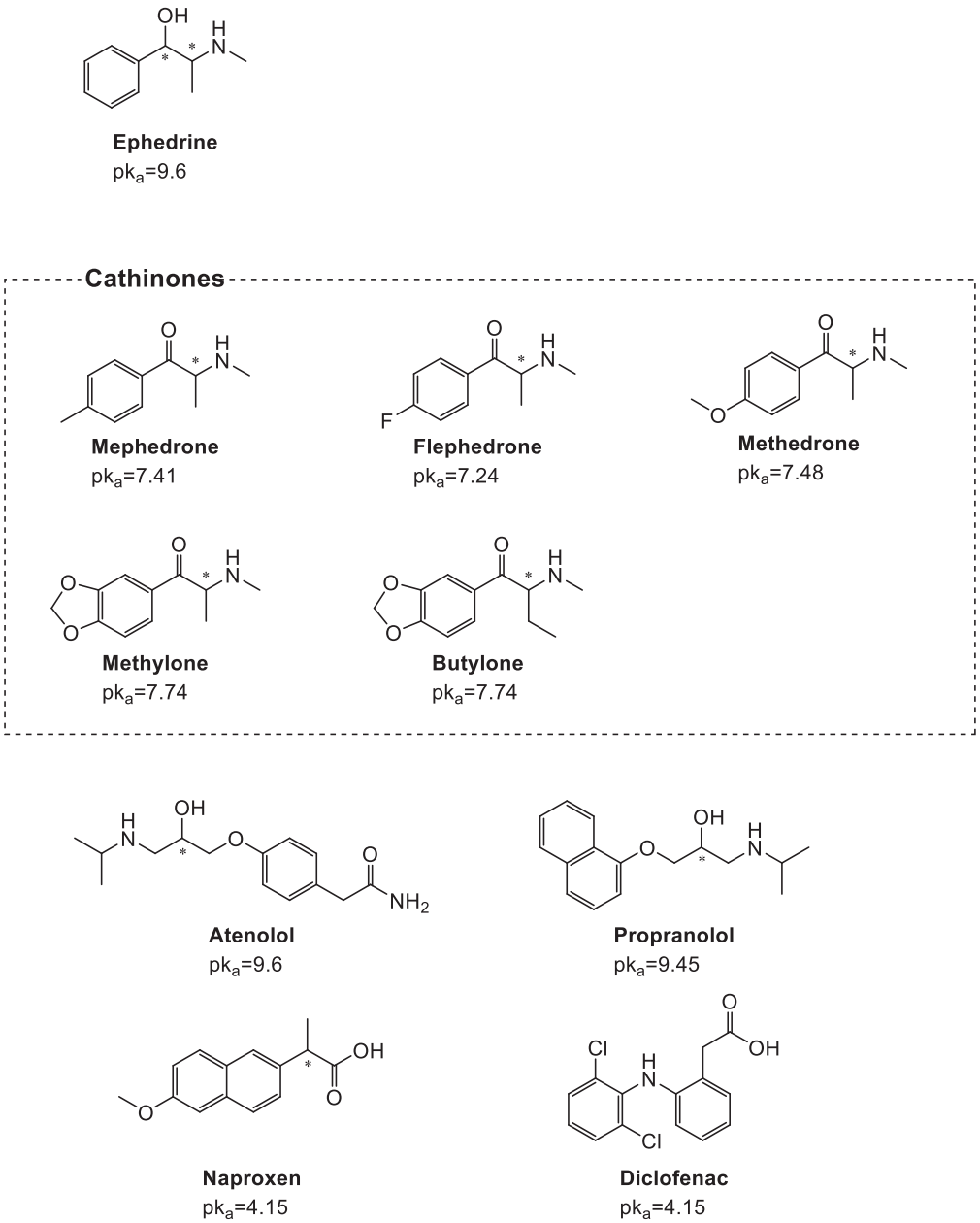

Fig. 1. Structures and pk_a values of ephedrine (template) and the analytes. Chiral centers are indicated by asterisks.

wildly reported in fundamental molecular imprinting studies [23–25] and as LC stationary phases [22,26–28], their application as SPE sorbent has been rarely reported [29,30], and to the best of our knowledge, this is the first time applying it in the extraction of cathinones.

2. Experimental

2.1. Chemicals and solvents

For the preparation of the MIP and corresponding non-imprinted polymer (NIP) based SPE cartridges, methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), (1R,2S)-(–)-ephedrine, (1S,2S)-(+)-pseudoephedrine, chloroform, 1-hydroxycyclohexyl phenyl ketone (IRG184), acetic acid (HAc), empty polypropylene SPE cartridges (3 mL) and 20 μm porous polyethylene frits were purchased from Sigma Aldrich (Gillingham, UK). The cathinone standards – (±)-flephedrone, (±)-3,4-methylenedioxymethcathinone (methylone), (±)-4-methylmethcathinone (mephedrone), (±)-butylone and (±)-4-methoxymethcathinone (methedrone) – were purchased from LGC Standards (Luckenwalde, Germany), while the standards of (±)-atenolol, (±)-propranolol, (±)-naproxen and diclofenac, which were used to prove the specific selectivity of the MIPs towards cathinones, were purchased from Sigma–Aldrich (St. Louis, MO, USA). The structures and pK_a values of these analytes and ephedrine are shown in Fig. 1. Individual stock standard solutions were prepared in methanol (MeOH) at 1 or 2 mg mL^{-1}, and kept at −20 °C. Working solutions were prepared by diluting the stock standard solutions with mobile phase, and kept at 4 °C.

MS grade acetonitrile (ACN), MeOH and water for the LC-HRMS mobile phase were purchased from Scharlab (Barcelona, Spain), and HPLC grade ACN and MeOH were purchased from J. T. Baker (Deventer, The Netherlands). Analytical-grade ammonium hydroxide (NH_4OH) and ammonium acetate (NH_4Ac) were obtained from Sigma–Aldrich. Formic acid (FA) and HAc were purchased from Honeywell (Augsburg, Germany). Ultrapure water was produced through a water purification system (Merk-Millipore, Billerica, MA, USA).

2.2. Synthesis of imprinted polymers

Imprinted and non-imprinted polymers were prepared by photochemically initiated free radical polymerisation. The compositions of all the polymers prepared are presented in Table 1. Briefly, the template and the selected functional monomer were transferred into a glass vial and mixed with the porogen (chloroform). Upon complete dissolution, the cross-linker was added followed by the initiator. The resulting pre-polymerisation solutions were ultrasonicated for 5 min, purged with argon and then hermetically sealed. The vials were then placed in the chamber of a UVP CX-2000 UV curing reactor (UVP, Jena, Germany) and irradiated at 365 nm for 3 h at room temperature. The resulting rigid monoliths were coarsely ground and washed with MeOH in a Soxhlet apparatus for 24 h to remove the template and any unreacted monomers. The coarse polymer particles were further ground using a mortar and pestle, wet-sieved with acetone (to further ensure template removal), and the 25–50 μm fraction was collected, dried and stored at room temperature for further experiments. The corresponding NIPs were prepared in a similar fashion, although the template was not added to the pre-polymerisation mixture.

2.3. MISPE procedure

100 mg of each polymer (MIP/NIP (R,S) 1:4, MIP/NIP (R,S) 4:4, MIP/NIP (S,S) 1:4 and MIP/NIP (S,S) 4:4) was packed into a 3 mL empty polypropylene SPE cartridge. Parameters such as the volume and pH of the loading sample, and the type and volume of the washing and elution solution were systematically investigated for the effect they might have on the extraction recovery and selectivity.

The optimised MISPE protocol using the best MIP is as follows: the MIP cartridge is conditioned with 3 mL of ultrapure water (pH = 7); 100 mL of sample (pH = 7) is loaded onto the cartridges; the cartridges are then washed with 1 mL of 0.02 % HAc in MeOH; subsequently, 1 mL of 5 % NH_4OH in MeOH is used as elution solution; the eluates are evaporated to dryness using a MiVac vacuum concentrator (Genevac, Ipswich, UK), reconstituted with 1 mL of mobile phase and filtered through a 0.45 μm PTFE syringe filter prior to analysis.

Before SPE, the river water samples were consecutively filtered through a 1.2 μm glass-fiber membrane filter and a 0.45 μm nylon membrane filter (both from Whatman, Maidstone, UK).

2.4. Instruments and chromatographic conditions

SPE development experiments were performed by analysing the extracts using an Agilent 1100 series LC system (Waldbronn, Germany) equipped with a diode array detector (DAD). To identify and quantify analytes, a non-chiral analysis method was used with a Brisa LC2 C_{18} column (150 mm × 4.6 mm i.d., 5 μm) (Teknokroma, Sant Cugat del Vallès, Spain). The mobile phase consisted of 0.1 % FA in water (solvent A) and 0.1 % FA in ACN (solvent B). The gradient was as follows: 10 % to 15 % B in 10 min, to 30 % B in 5 min, to 100 % B in 3 min, hold for 2 min, and then back to initial conditions in 2 min and hold for 5 min. The flow rate was 0.4 mL min^{-1} and the injection volume was 20 μL. The column was maintained at 35 °C, and UV detection was operated at 230 or 254 nm.

A chiral analysis method was used with a Chiralpak CBH column (150 mm × 2 mm i.d., 5 μm) and a Chiralpak CBH guard column (10 mm × 2 mm i.d., 5 μm), both purchased from Daicel (Illkirch, France) to verify the stereoselectivity of MIP cartridges towards the analytes and determine the enantiomeric composition of analytes in river water samples. The enantioseparation conditions were selected using the criteria established in a previous study by our group [17]. Briefly, the enantioseparations were carried out using a mobile phase of 1 mM NH_4Ac aqueous solution/MeOH (98/2, v/v) at 0.4 mL min^{-1} in isocratic mode. The column was maintained at 30 °C, and the analysis time was 10 min.

For method validation and analyses of river water samples, an Accela 1250 LC system coupled to an Exactive Orbitrap™ mass spectrometer (Thermo Scientific, Bremen, Germany) was used. The LC system consisted of an automatic injector, a quaternary pump and a column oven. The separations were carried out under the same chromatographic conditions used in the LC-DAD. The HRMS system was equipped with a heated electrospray ionisation (HESI) source operating in the positive ionisation mode. For the non-chiral analysis method, the optimal ion-source parameters were: sheath gas flow rate, 40 AU (adimensional units); auxiliary gas flow rate, 2 AU; spray voltage, 3.5 kV; skimmer voltage, 20 V; tube lens voltage, 75 V; capillary voltage, 30 V; capillary temperature, 350 °C; heater temperature, 350 °C. For the chiral analysis

Table 1
Amounts of all reagents used for the synthesis of MIPs.

Polymer	(1R,2S)-(–)-ephedrine (mmol)	(1S,2S)-(+)-pseudoephedrine (mmol)	MAA (mmol)	EDGMA (mmol)	Chloroform (mL)	IRG184 (g)
MIP (R,S) 1:4	1	–	4	20	5.60	0.0380
MIP (R,S) 4:4	4	–	4	20	5.60	0.0380
MIP (S,S) 1:4	1	–	4	20	5.60	0.0380
MIP (S,S) 4:4	–	4	4	20	5.60	0.0380

method, the HRMS parameter values were the same as above except the sheath gas flow rate and auxiliary gas flow rate, which were 50 and 5 AU, respectively.

The data were acquired in a single time window by alternating two scan events: (1) a full scan at 50,000 FWHM with 250 ms of injection time; and (2) a fragmentation scan at 10,000 FWHM with 50 ms of injection time using a HCD cell voltage of 15 eV. The analytes were identified based on the retention time (with a tolerance of 0.1 min) and the accurate mass of diagnostic and two fragment ions (shown in Table 1S) (with a mass tolerance of 5 ppm). Ion ratios between the selected fragment ions and diagnostic ions were monitored for purposes of confirmation.

3. Results and discussion

3.1. Preparation and characterisation of the imprinted polymers

Ephedrine was used as dummy template as ephedrine and cathinone have similar structures since ephedrine contains amine and alcohol, whereas cathinone contains amine and carbonyl (see Fig. 1). In addition, in the formation of the template-monomer complex, as reported earlier [28] there is hydrogen bonding and electrostatic interactions between the carboxylic groups in the MAA and amine groups of either ephedrine or cathinones. There can be secondary interactions to the carbonyl or hydroxyl groups but these are a much weaker.

The MIPs were prepared using photochemical bulk polymerisation approach in which many different template, monomers, crosslinkers as well as solvents are feasible to be polymerised. Thus, all the tested compositions outcame with final monolithic polymers. Moreover, FT-IR spectra (shown in Fig. 1S) proved the incorporation of all the monomers during the polymerisation, and thus, the functional groups in the MIPs available for interaction during the MISPE. After this, the monolith was properly crushed to provide particulate material in form of irregular shape but with narrow size distribution (see scanning electronic microscope (SEM) image in Fig. 2S) able to be packed for MISPE.

3.2. Optimisation of chromatographic conditions and MS detection

For the non-chiral analysis of cathinones, a Brisa LC2 C_{18} column (150 × 4.6 mm, 5 μm) was tested with a mobile phase of 0.1 % FA in water (solvent A) and 0.1 % FA in ACN (solvent B) under various gradient profiles. The best separation in a reasonable analysis time (less than 16 min) was achieved with the gradient described in Section 2.4 (Fig. 3S).

After the chromatographic conditions had been optimised, Orbitrap-based HRMS parameters were also investigated to maximise the response of the analytes, and the optimal values are described in Section 2.5. For all the analytes, the protonated ion $[M + H]^+$ was measured for quantification.

With the optimal LC–(Orbitrap) HRMS conditions, the instrumental limits of detection (IDLs), corresponding to the concentrations that gave a signal-to-noise ratio (S/N) of 3 and a signal intensity higher than 1×10^3, ranged from 0.05 to 0.1 μg L^{-1}. The instrumental quantification limits (IQLs), defined as the concentrations that produce a S/N of 10, ranged from 0.1 to 0.25 μg L^{-1}. Good linearity ($r^2 > 0.996$) between IQLs and 100 μg L^{-1} was obtained for all the analytes.

3.3. MISPE optimisation

A preliminary test was conducted to compare the performance of four MIP cartridges – MIP (R,S) 1:4, MIP (R,S) 4:4 , MIP (S,S) 1:4 and MIP (S,S) 4:4 – with the initial protocol. That is, conditioning with 3 mL of 10 % HAc in MeOH and 3 mL of ultrapure water (pH = 7), loading with 100 mL of ultrapure water spiked with 5 cathinones at 0.1 mg L^{-1}, washing with 1 mL of 0.02 % HAc in MeOH and eluting with 1 mL of 10 % HAc in MeOH. The eluates were evaporated to dryness, then reconstituted with 1 mL of mobile phase and injected to LC-DAD. The results indicated that MIP (R,S) 1:4 and MIP (S,S) 4:4 performed better than MIP (R,S) 4:4 and MIP (S,S) 1:4 in terms of both recovery and selectivity. Therefore, MIP (R,S) 1:4 and MIP (S,S) 4:4 cartridges were selected to optimise such SPE conditions as the volume and pH value of the loading sample, and the type and volume of the washing and elution solutions.

3.3.1. MISPE optimisation with ultrapure water

Initially, ultrapure water spiked with 5 cathinones at 0.1 mg L^{-1} was used to evaluate the extraction recoveries. First, the loading volume was set to 100 mL, and the effect of the pH of the loading sample was evaluated with 1 mL of 0.02 % HAc in MeOH as washing solution and 1 mL of 10 % HAc in MeOH as elution solution. The pH of the loading sample is an important parameter since the retention mechanism of the analytes on the MIPs is based on ionic interactions between the amine moieties of cathinones and the carboxylic acid groups in the MIPs. The effect of the sample pH on the recovery was investigated at pH 3 and pH 7. With both MIP cartridges, the recoveries for all the analytes at pH 3 (3.1–6.4 % in the case of MIP (R,S) 1:4 and 2.0–4.9 % in the case of MIP (S,S) 4:4) were found to be much lower than those at pH 7 (62.2–73.4 % in the case of MIP (R,S) 1:4 and 68.4–86.6 % in the case of MIP (S,S) 4:4). The carboxylic acid groups in MIPs have a pK_a of about 5, and the cathinone analytes have a pK_a between 7.2 and 7.8. At pH 3, although the amino group of the cathinones are fully protonated, the carboxylic acid groups of MIPs are primarily in their non-ionised form. In contrast, at pH 7 both the carboxylic acid in the polymer and the amine moieties of cathinones are ionised, which promotes ionic interactions. Thus, the retention of cathinones on the MIPs are more effective at pH 7 than at pH 3. Therefore, the pH of the loading samples was set at 7 in the successive experiments.

Then different washing solutions were tested: 1 or 2 mL of MeOH, and 1 mL of 0.02 % HAc in MeOH. The similar trends were observed for both MIP (R,S) 1:4 and MIP (S,S) 4:4 sorbents. Recoveries were good in all the tests. When pure MeOH was used as the washing solution, there was no obvious difference in recoveries between MIP and NIP cartridges, which indicated that pure MeOH was not strong enough to disrupt the non-specific interactions between the MIPs and cathinones. By comparison, when 1 mL of 0.02 % HAc in MeOH was used as the washing solution, MIP cartridges showed much higher recoveries than NIP cartridges. By way of example, the results of MIP (R,S) 1:4 are shown in Fig. 2. On the basis of the results, 1 mL of MeOH with 0.02 % HAc was selected as the washing solution.

The elution solution was optimised by applying three fractions of 1 mL of 10 % HAc in MeOH. The results indicated that 1 mL of 10 % HAc in MeOH was sufficient to elute all the analytes both for MIP (R,S) 1:4 and MIP (S,S) 4:4 cartridges.

Subsequently, we increased the sample loading volume to 200 mL which significantly decreased the recoveries (from 50 % to 75 %) of all the analytes on both cartridges (the results are detailed in Fig. 4S). Thus, the loading volume was set at 100 mL.

Besides cathinones, four other pharmaceuticals (atenolol, propranolol, naproxen and diclofenac) with very different structures (shown in Fig. 1) from cathinones were analysed to confirm the selectivity of the MIP cartridges. With the optimised protocol, the recoveries of these four compounds were lower than 30 %. The poor retention of the four pharmaceuticals further demonstrated the selectivity of the MISPE protocol towards cathinones.

In the next step, we used an Orbitrap analyser to evaluate the recovery at lower concentration levels and ME. Firstly, 100 mL of ultrapure water spiked with the analytes at 0.4 μg L^{-1} was percolated following the above protocol. Recoveries were good, ranging from 61.7 to 78.5 % for MIP (R,S) 1:4 and 67.6 to 83.7 % for MIP (S,S) 4:4, which were comparable with those obtained at higher concentrations in LC-DAD.

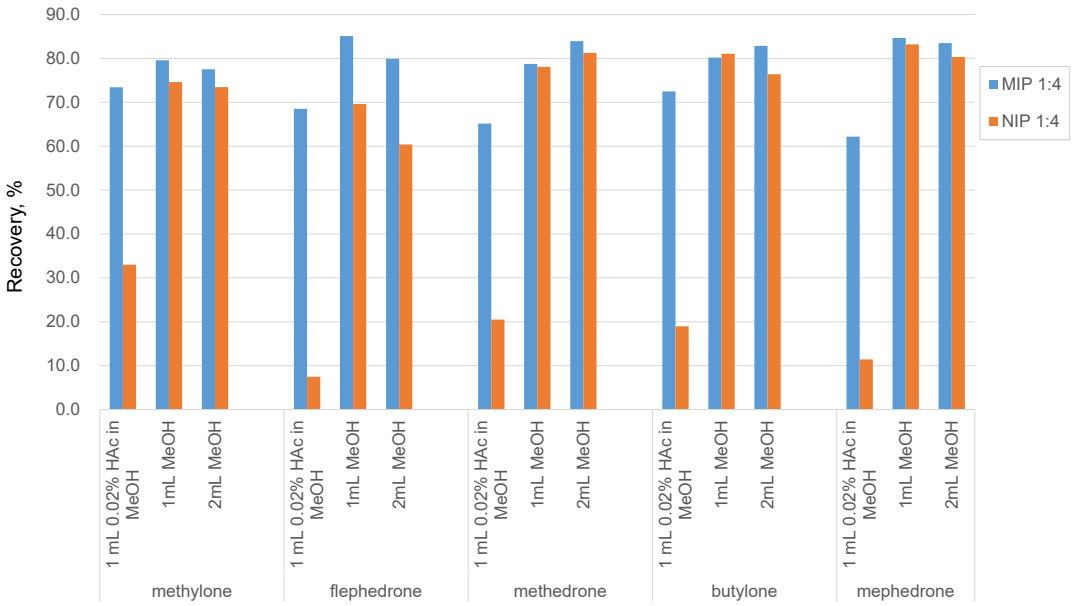

Fig. 2. The effect of washing solutions on recovery for all the analytes with MIP/NIP (R,S) 1:4 sorbents.

3.3.2. *Evaluation of the stereoselectivity of MIP sorbents*

To evaluate the stereoselectivity of MIP sorbents during the SPE process, the above extracts were also analysed using the chiral chromatographic method. Under the optimised conditions described in Section 2.4, baseline enantioseparations ($R_s \geq 1.2$) were achieved for mephedrone, flephedrone, methylone and methedrone, while partial enantioseparation was achieved for butylone ($R_s = 0.8$). One example of the enantioselective chromatograms of the analytes obtained with a MIP (R,S) 1:4 cartridge is shown in Fig. 3. The chiral analysis showed that the EF values of all the analytes in the eluates were around 0.5 except for butylone (EF = 0.45), which indicates that there was no stereoselectivity during the MISPE procedure. In the case of butylone EF, the slight deviation from 0.5 is due to the poor enantioseparation of butylone. Although the MIP was synthesised with one stereoisomer, the MIP did not show any stereoselectivity towards the analytes.

Subsequently, 100 mL of river water spiked at 0.4 μg L^{-1} was also analysed following the same protocol as for ultrapure water. When the chromatographic method with the C_{18} column was used for non-chiral analysis of analytes in the extracts, good recoveries (57.2–75.9 % in the case of MIP (R,S) 1:4 and 63.4–84.9 % in the case of MIP (S,S) 4:4) were attained for all the analytes. In addition, lower ME ranging from −8.1 % to 3.4 % in the case of MIP (R,S) 1:4 and from −6.6 to 4.1 % in the case of MIP (S,S) 4:4 were obtained. However, when the CBH column was used for the determination of enantiomeric composition of analytes in the extracts, the analyte peaks shifted forward significantly and enantioseparations were lost with both MIP sorbents. This phenomenon was also observed in one of our previous studies [17] where the same cathinone analytes were extracted using an Oasis WCX cartridge with 5 mL of 5 % FA in MeOH as elution solution injected with the same CBH column. Considering that just such a problem was solved by changing the elution solution to 5 mL of 5 % NH_4OH in MeOH in the previous study [17], we tried 1 mL of the same elution solvent (5 % NH_4OH in MeOH) in the MISPE. As expected, the retention time and enantioseparation were not interfered. Moreover, the recovery values attained using this elution solution were similar and the difference in the recoveries between MIP and its respective NIP were notable (Table 2). Moreover, the ME encountered (-9.0–1.1 % in the case of MIP (R,S) 1:4 and −5.5–1.8 % in the case of MIP (S,S) 4:4) were similar to those obtained when 1 mL of 10 % HAc in MeOH was used as elution solution. This effect on the CBH column could be explained by interferences in the river water which can be eluted with 10 % HAc in MeOH, and might compete with analytes to bind to active sites of the CBH column, thus leading to the lack of retention of the cathinones. Consequently, 1 mL of 5 % NH_4OH in MeOH was selected as the elution solution.

3.3.3. *Selection of the MIP sorbent*

Comparing the performance of MIP (R,S) 1:4 and MIP (S,S) 4:4 sorbents, MIP (R,S) 1:4 sorbent showed higher selectivity in comparison to that of corresponding NIP. However, MIP (S,S) 4:4 provided improved recoveries (67.6–83.2 %) with good selectivity. For this reason, subsequent experiments were conducted using MIP (S,S) 4:4 as the SPE sorbent. The recovery values obtained with MIP (S,S) 4:4 are comparable to those of other studies [16,17,31] reporting the extraction of cathinones with commercial SPE cartridges. However, much lower ME values between −5.5 and 1.8 % were achieved in the present study. For example, in a previous study [16] in which the Oasis WCX sorbent was used to extract a group of synthetic cathinones from river water samples, signal suppression (from −16 % to − 28 %) was observed for flephedrone, methylone, methedrone and butylone in spite of washing with 5 mL of MeOH. In another study [31], signal suppression ranging from −13 % to −17 % was observed for flephedrone, methylone, methedrone, mephedrone and butylone in river water samples, when the Oasis MCX sorbent was applied. The lower ME values in the present study indicated high selectivity of the MIP towards cathinones since the river water samples were cleaned-up efficiently.

The EF values of each analyte in river water sample extracts obtained using the optimal protocol were also determined and compared to those measured in the standard solution. The EFs of each analyte spiked in river water samples were in the range 0.45–0.49. For all the analytes no apparent change in the EF was observed, so the MIP (S,S) 4:4 cartridges showed the same retention capacity towards both enantiomers.

3.4. *Validation*

The method that used MIP (S,S) 4:4 as SPE sorbent followed by LC-HRMS with a C_{18} column was validated with river water samples by evaluating the method detection limits (MDLs), method quantification limits (MQLs), accuracy, repeatability (intra-day precision) and reproducibility (inter-day precision). All validation data are presented in Table 3.

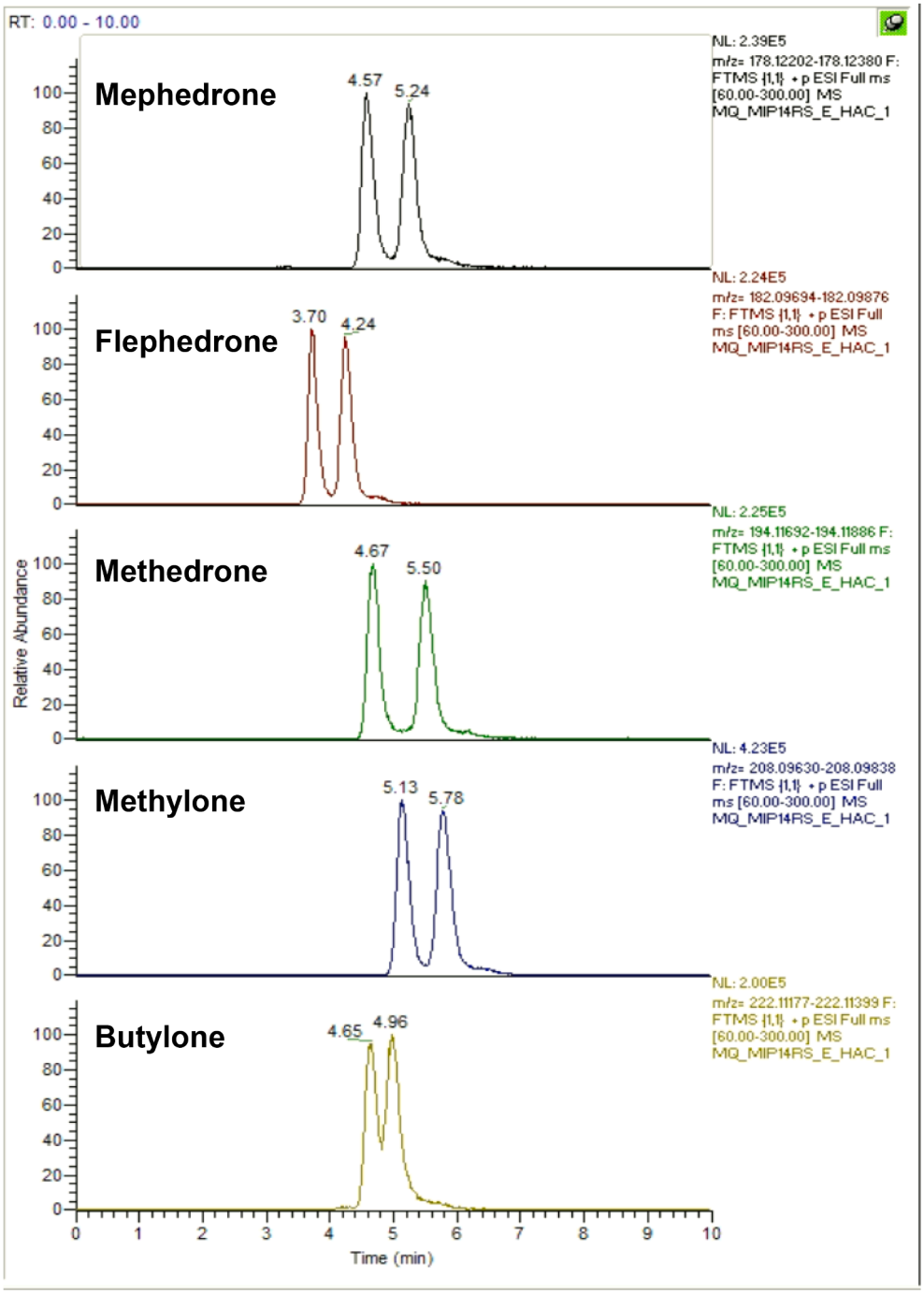

Fig. 3. Enantioselective chromatograms of the analytes spiked in ultrapure water at 0.4 μg L^{-1} extracted by SPE using MIP (R,S) 1:4 followed by LC-HRMS with CBH column. See the text for experimental conditions.

MDLs and MQLs, defined as the minimum concentration with a S/N of 3 and 10 respectively, were determined by analysing a series of spiked water samples. The MDLs in river water were in the range 0.3–0.8 ng/L and the MQLs were in the range 1.0–2.0 ng/L. The values were somewhat higher than those reported in the previous studies due to the lower loading volume. For instance, in previous studies [16] the MDLs and MQLs reported were 0.08–0.15 ng/L and 0.25–1.5 ng/L for river water, when 250 mL of river water was analysed using an SPE (Oasis WCX)-LC-(Orbitrap)HRMS technique. Nevertheless, MDLs and MQLs obtained in our study were still considered to be suitable for environmental analysis.

Accuracy, repeatability and reproducibility were assessed by determining five replicates of river water samples spiked with cathinones at 0.4 μg L^{-1}. As a measure of accuracy, relative recoveries were calculated as the percentage of the mean experimental concentration and the theoretical concentration. They were between 87 and 116 % for all the analytes. Repeatability and reproducibility, expressed as the relative standard deviation (%RSD) of these replicate analyses in one day and five successive days, respectively, ranged from 8.8 to 13.5 % and 7.4 to 14.7 %, respectively.

In summary, the method developed showed satisfactory MDL and MQL values, accuracy, repeatability and reproducibility, and therefore can be used to detect target cathinones in river water.

Table 2
Comparison of recovery (R_{app}) obtained when 100 mL of river water spiked with the analytes at 0.4 μg L^{-1} were extracted with MIP (R,S) 1:4, MIP (S,S) 4:4 and their NIP cartridges using the optimal protocol that includes elution with 1 mL 5 % NH_4OH in MeOH.

Compounds	MIP (R,S) 1:4	NIP (R,S) 1:4	MIP (S,S) 4:4	NIP (S,S) 4:4
	R_{app} (%)	R_{app} (%)	R_{app} (%)	R_{app} (%)
Methylone	75.8	19.6	79.5	47.3
Flephedrone	58.6	6.0	67.6	15.7
Methedrone	72.4	12.8	79.0	42.3
Butylone	81.5	21.7	83.2	55.0
Mephedrone	65.2	12.9	78.3	33.3

4. Application of the analytical method in river water analysis

The validated method was used to analyse river water samples collected from the Ebre River. Methedrone and butylone were detected in the river water samples after meeting all the confirmation criteria (mass error, tolerance and ion ratio). Fig. 4 displays one example of the extracted ion chromatograms of the protonated ion (A) and the two fragments (B, C) for one of the river water samples analysed.

Methedrone and butylone were detected in all the river water samples at concentrations between < MQL − 3.5 ng/L and < MQL − 2.4 ng/L respectively. These values are in agreement with a previous study [16] with samples from the same river, in which butylone was detected below MQL and methedrone was detected at concentrations of up to 1.8 ng/L in river water samples.

Mephedrone, flephedrone and methylone were not found in any river water samples. Indeed, after passing through a waste water treatment plant (WWTPs), these drugs are rarely present in river water samples. For example, Senta et al. [31] reported that flephedrone, mephedrone and methylone were detected at concentrations of up to 4.2, 3.2 and 1.6 ng/L in raw wastewater, while mephedrone and flephedrone were found in neither effluent wastewater nor in river water and only methylone was detected at 1.8 ng/L in one of the river water samples.

The enantiomeric composition of the analytes detected in river water samples was checked by injecting the eluates into CBH column. The extracted ion chromatograms of the enantiomers of methadone and butylone are shown in Fig. 5. The EF values for methedrone and butylone found in river water samples were 0.48 ± 0.01 and 0.44 ± 0.01, respectively. They were not significantly different from those recorded in the validation, which means that methedrone and butylone were present in racemic form in the river water samples. The EF value determined for methedrone was consistent with our previous study [17], which suggests that little stereoselective degradation occurred during various processes or that the cathinones present in the river water came from the direct disposal of unused drugs [15].

Table 3
Validation parameters of the non-chiral analysis method based on SPE with MIP (S,S) 4:4 followed by LC-(Orbitrap) HRMS for the determination of the target cathinones in river water samples.

Analytes	MDL (ng/L)	MQL (ng/L)	Accuracy[a](%)	Repeatability[a]RSD (%)	Reproducibility[a]RSD (%)
Mephedrone	0.5	2.0	91	9.3	11.1
Butylone	0.5	1.5	87	11.9	9.1
Flephedrone	0.3	1.0	116	8.8	7.4
Methylone	0.8	1.5	95	13.5	14.7
Methedrone	0.8	2.0	104	10.4	12.4

[a] spiked at 0.4 μg L^{-1}.

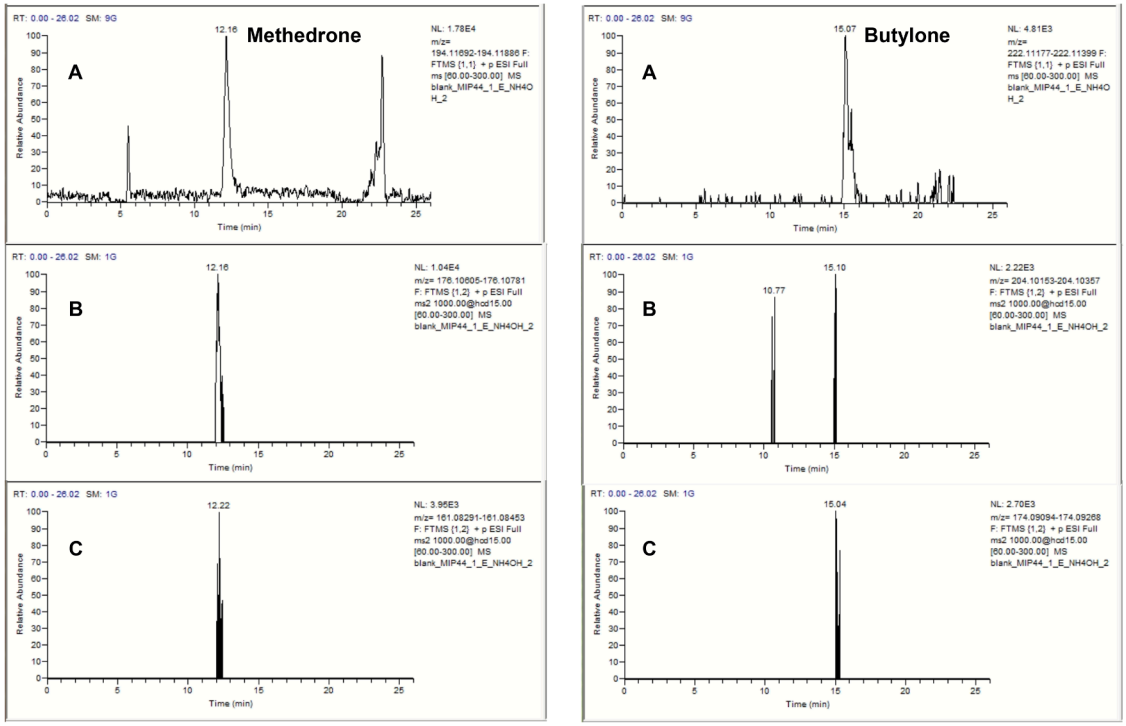

Fig. 4. Extracted ion chromatograms of the protonated ion (A) and the two fragments (B, C) for a river water sample extracted by SPE with MIP (S,S) 4:4 and analysed with LC-HRMS with a C_{18} column.

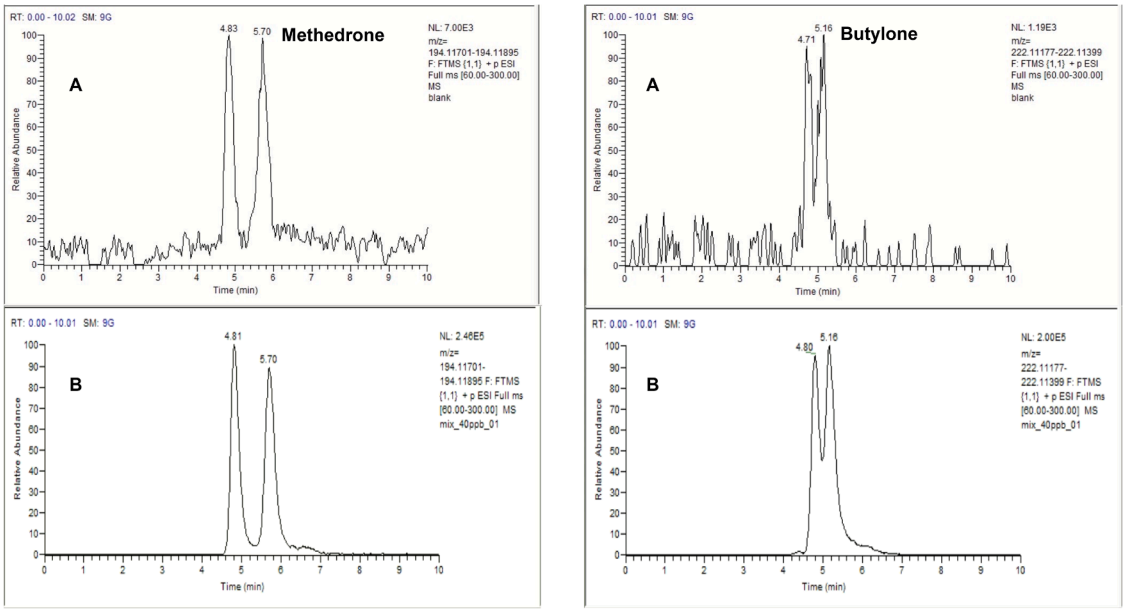

Fig. 5. Extracted ion chromatogram of the enantiomers of methedrone and butylone obtained when 100 mL of (A) Ebre River water sample or (B) ultrapure water spiked at 0.4 μg L^{-1} were extracted by SPE with MIP (S,S) 4:4 and analysed by LC-HRMS using a CBH column.

5. Conclusions

A series of MIPs were synthesised and evaluated as sorbent for the extraction of five cathinones. The MIP (S,S) 4:4 with (1S,2S)-(+)-pseudoephedrine as template and a template/monomer molar ratio of 4:4 has been shown to provide best recoveries. Then, a highly selective and sensitive method was developed using MIP (S,S) 4:4 as sorbent in SPE followed by LC-(Orbitrap)HRMS. It was successfully applied to determine five cathinones from river water samples. Racemic methedrone and butylone were detected in Ebre River water samples at concentrations of up to 3.5 and 2.4 ng/L, respectively.

The good recoveries achieved with high selectivity in the study indicate that using MIPs as the selective sorbent in SPE followed by LC-HRMS analysis can be a promising method for determining these chiral drugs in the environment.

CRediT authorship contribution statement

Yandi Fu: Investigation, Validation, Writing – original draft. **Federica Pessagno:** Investigation. **Panagiotis Manesiotis:** Conceptualization, Methodology, Supervision. **Francesc Borrull:** Conceptualization, Methodology. **Núria Fontanals:** Conceptualization, Methodology, Writing – review & editing, Supervision. **Rosa Maria Marcé:** Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2021.107100.

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