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Synthesis and characterization of molecularly imprinted polymers for the selective extraction of cocaine and its metabolite benzoylecgonine from hair extract before LC–MS analysis

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ABSTRACT

A molecularly imprinted polymer (MIP) was synthesized and evaluated for the selective extraction of cocaine (COC) and its main metabolite benzoylecgonine (BZE) in hair extracts. To this end, a screening of different conditions of synthesis was performed by changing the nature of the crosslinker, and the functional monomer and also by changing polymerization's initiation mode. The selectivity of the different MIPs was evaluated by comparing the retention of COC and BZE between the MIP supports and also compared to a non-imprinted polymer for each. All the supports were selective for one or both molecules, but, the best results in terms of selectivity and retention were obtained for a MIP using methacrylic acid as functional monomer, ethyleneglycol dimethacrylate as crosslinker, and a photochemical initiation. An optimized procedure in acetonitrile media was developed for the selective extraction of COC and BZE with a recovery close to 80% for both molecules from the MIP. The capacity of the MIP for COC retention was also evaluated, and MIP showed a specific capacity of 8.96 μmol g^{-1}. Finally, the potential of this material for sample clean-up was demonstrated by the selective extraction of both COC and BZE from acetonitrile hair extracts spiked at the cutoff value for COC in hair analysis. By the selective purification with the MIP, a limit of quantification inferior to 0.07 ng mg^{-1} of hair was reached for both molecules.

1. Introduction

Cocaine is one of the most widely used illicit substances in the world. This is due to its ability to stimulate the central nervous system causing a very pleasant feeling of well-being, hyperactivity, restlessness, increased blood pressure, increased heart rate and euphoria; it also suppresses fatigue, hunger and thirst stimuli [1,2]. Cocaine is rapidly metabolized by hydrolysis in the human body to benzoylecgonine and ecgonine methyl ester, which are the main metabolites in blood and urine [3]. Typical doses are around 20–100 mg, and the detection of cocaine in blood lasts around 4–6 h while benzoylecgonine and ecgonine methyl ester may be detected up to 48 h [4]. For urine analysis, low concentration levels of cocaine are found since it only represents 1%–9% of the original dose. This is the reason why benzoylecgonine is generally analyzed since it represents between 35% and 54% of the initial dose. Ecgonine methyl ester can also be studied since it represents between 32% and 49% of the initial dose. Positive signal for both metabolites only remains until one or two days after intravenous injection [5]. As a consequence, hair analysis has become more and more interesting since traces of many drugs are laid down in hair during keratinization and remain embedded throughout its life, especially in the case of cocaine [5]. Another possible source of cocaine in hair can be an external contamination, therefore hair must be decontaminated before its analysis to confirm cocaine consumption. Generally, the cocaine from an external source is easily eliminated by the decontamination step, but in order to really confirm the source of the cocaine in hair, it is recommended to analyze also its metabolites ratio even though metabolites enter the hair in a lesser extent than cocaine [6]. Gas chromatography coupled to mass spectrometry (GC-MS) has long been the analytical tool chosen for the analysis of cocaine and benzoylecgonine in biological matrices such as urine [7], blood [8], or hair [5] due to its selectivity and sensitivity, but derivatization of target compounds is often necessary and sample pre-treatment is generally laborious. Liquid chromatography (LC) has widespread since it also offers high separation efficiencies and does not need a derivatization of compounds. LC coupled to UV detection [9,10] and fluorescence detection [11,12] can also be used but mass spectrometry (MS) became essential due to its specificity, its versatility and its high sensitivity [13–15].

Due to the complexity of biological samples, purification is often necessary for quantitative analysis, particularly to avoid matrix

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effects on LC–MS techniques. Depending on the biological matrix tested, different sample treatments can be used such as protein precipitation [14], liquid–liquid extraction [16], solid phase extraction (SPE) [7,8,17], filtration [15], dilution [18], solid phase microextraction [19], or centrifugation [20]. In the case of hair analysis, a previous extraction or a digestion procedure must be performed in order to extract the drug from the hair fibers [5]. For liquid samples, the most common sample treatment is SPE because of its versatility. Nevertheless, SPE is mainly based on non-selective interactions and this may lead to the co-extraction of interfering substances. Therefore, selective sorbents based on a molecular recognition mechanism have been developed. Selective extraction can be performed by immunosorbents based on the high affinity and selectivity of antigen-antibody interactions. This kind of sorbents have demonstrated a great potential for sample pretreatment [21], but their development is time consuming and expensive. Recently, a new kind of selective sorbents has been developed based on aptamers which are single-stranded oligonucleotides that have a high affinity for a target molecule [22]. Cocaine was extracted from plasma by such a selective sorbent showing a great selectivity and high extraction recoveries [23]. Oligosorbents are less expensive than immunosorbents to produce, but the cost remains considerable. A third kind of selective sorbents are molecularly imprinted polymers (MIPs). These materials are synthetic polymers that possess specific cavities designed for a template molecule, therefore acting as a synthetic antibody. Firstly, a complex between a template and a functional monomer is formed. Then, this assembly is fixed through polymerization in the presence of a cross-linking agent. Finally, the template is removed from the polymer liberating specific binding cavities complementary to the template in size, shape and functionality. A non-imprinted polymer (NIP) is generally synthesized in the same conditions than the MIP but without the presence of the template molecule in order to confirm the presence of selective cavities in the MIP. MIPs have clearly shown their potential for selective SPE in biological samples [24].

MIPs for the selective retention of cocaine or benzoylecgonine have already been studied. A benzoylecgonine anilide MIP was prepared using methacrylic acid (MAA) as monomer, ethylene glycol dimethacrylate (EGDMA) in toluene and was evaluated in organic and aqueous media showing a good selectivity for benzoylecgonine in acetonitrile media. This sorbent was used as a SPE sorbent for the extraction of benzoylecgonine from an aqueous solution and a great selectivity was obtained since recovery was 80% in the MIP and only 20% in the NIP [25]. However, no applications to real samples were reported and cocaine's retention was not studied on this support. A cocaine MIP prepared using MAA and EGDMA in chloroform was applied in aqueous media after coating the surface of the MIP with mineral oil, i.e. a hydrophobic layer, to improve the recognition properties of the MIP in polar solvents [26]. An imprint factor (IF = k_{MIP}/k_{NIP}, where k_{NIP} and k_{MIP} are the cocaine retention factors in the NIP and the MIP respectively) of 1.8 was obtained in acetonitrile, thus demonstrating a satisfactory selectivity in this media. This support was not tested as SPE sorbent since the final objective was its use in a cocaine sensor. In another study, the structure and energy of the molecular complexes of cocaine and different functional monomers were studied by computational techniques [27]. The best results were obtained with acrylamide and itaconic acid as monomers, in dimethylformamide as porogen. Imprinting factor measured in chloroform with 7% acetic acid of this MIP was 1.2. Despite its satisfactory selectivity, no use of this MIP in SPE was envisaged. The ionization of functional monomers in solution, or in imprinted and non imprinted polymers under aqueous conditions was investigated by the same group [28]. An imprinting factor for cocaine of 2.6 was obtained in a 20% acetic acid aqueous solution for a cocaine MIP based on 2-trifluoromethacrylic acid functional monomer and toluene as porogen making this MIP suitable for the recognition of cocaine and benzoylecgonine in water. Again, the objective of this work was not the development of MIP-SPE procedure and no real samples were analyzed with this support.

These different studies have shown the feasibility of MIPs for the selective recognition of cocaine either in organic or in aqueous media, nevertheless, to our knowledge, MIPs have never been applied to the selective extraction of cocaine and benzoylecgonine to real samples.

The aim of this work was the synthesis of different cocaine MIPs and their characterization by studying the retention profile of cocaine and benzoylecgonine in organic media. The capacity of the most selective MIP was determined and a MIP-SPE procedure was applied to the selective extraction of these molecules from hair extract samples. The high selectivity of the MIP was demonstrated by comparing chromatograms obtained in LC–UV and LC–MS analyses obtained with and without a clean-up step of the extract on the MIP.

2. Materials and methods

2.1. Chemicals

Cocaine (COC) base, ammonium formate, formic acid and stock solutions of 1 g L^{-1} of cocaine in acetonitrile and 1 g L^{-1} benzoylecgonine (BZE) were provided by Sigma–Aldrich (Saint Quentin Fallavier, France). The working solutions with the concentration 0.1 mg L^{-1} were obtained by dilution of the stock solution (1 g L^{-1}) in HPLC-grade acetonitrile (MeCN) (Carlo Erba, Reagents, Val-de-Reuil, France). These solutions were stored at −20 °C. HPLC-grade methanol (MeOH), and 0.1 mol L^{-1} hydrochloric acid solution were purchased from Carlo Erba Reagent. Acetic acid was supplied by Sigma–Aldrich. Formic acid was supplied by Riedel-de Haën. High purity water was obtained from a Milli-Q purification system (Millipore, Saint-Quentin en Yvelines, France).

Methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), trimethylolpropane trimethacrylate (TRIM), 2-vinylpyridine (and divinylbenzene (DVB) were purchased from Sigma–Aldrich. EGDMA was washed twice with an equal volume of a solution of 10% NaOH in deionised water, and then washed twice with an equal volume of water. Afterwards, it was dried using an equal volume of saturated sodium chloride aqueous solution and next over Na_2SO_4. Washed EGDMA and MAA were distilled under vacuum in order to remove inhibitors and stored at −20 °C. Azo-*N*,*N*′-bis-isobutyronitrile (AIBN) was purchased from Acros Organics (Noisy-le-Grand, France). AIBN was of a high purity so it was used without further purification. The UV lamp used for the polymerization was an immersible mercury vapour lamp (TQ 150, 150 W, Heraeus, Hanau, Germany).

2.2. Apparatus and analytical conditions

An Agilent 1200 series (Agilent Technology, Massy, France) LC system equipped with a binary pump, an autosampler and a diode array detector controlled by Chemstation software was used. Cocaine and benzoylecgonine were separated on a Waters SymmetryShield RP18 column (150 mm × 2.1 mm i.d., 3.5 μm, Waters, Saint-Quentin-en-Yvelines, France) maintained at 35 °C with a column oven (Croco-cil, Interchim). An isocratic mode was used for the analysis consisting of 80% of ammonium format 5 mmol L^{-1} pH = 3.2 and 20% of MeCN. The analysis time was set to 7 min at a flow rate of 0.2 mL min^{-1}. The detection of cocaine and benzoylecgonine was carried out at 233 nm. The injection volume was set at 20 μL.

For hair extracts analyses, a tandem quadrupole mass spectrometer (Agilent Technologies, Massy, France) was coupled with

Table 1
Conditions of synthesis of MIPs using cocaine as template molecule, AIBN as initiator and using a template/monomer/cross-linker molar ratio of 1/4/20.

Name	Functional monomer	Cross-linker	Initiation	Solvent (mL per mmol of COC)
MIP 1	MAA	EGDMA	UV (24 h,15 °C)	MeCN 5.6 mL mmol^{-1}
MIP 2	MAA	EGDMA	Thermal (24 h, 60 °C)	MeCN 5.6 mL mmol^{-1}
MIP 3	MAA	TRIM	UV (24 h, 3 °C) and thermal (24 h, 60 °C)	CH_2Cl_2 10.9 mL mmol^{-1}
MIP 4	MAA	EGDMA/DVB 30/70	Thermal (24 h, 60 °C)	MeCN 5.6 mL mmol^{-1}
MIP 5	2-VP:MAA 1:1	EGDMA	UV (24 h, 15 °C)	MeCN 5.6 mL mmol^{-1}

an ESI source to the Agilent LC system. Data were acquired in the positive ion mode. Capillary voltage was 4000 V, gas flow was 10 L min^{-1}, gas temperature was 350 °C and nebulizer's pressure was 20 psi. COC and BZE were detected in MRM mode by measuring the fragmentation products of the [M+H]+ ions of cocaine (304.1 $m/z \rightarrow$ 182.1 m/z) and benzoylecgonine (290.1 $m/z \rightarrow$ 168 m/z). Cone voltage and collision energy were determined with the optimizer software (Agilent Technologies) for both cocaine and benzoylecgonine analytes. Cone voltage values were 110 V for cocaine and 115 V for benzoylecgonine. Collision energies obtained were 16 V for both analytes. Separation was carried out in an isocratic mode with a mix of 80% water and 20% acetonitrile both acidified with 0.1% (v/v) formic acid using the same column. The injection volume was set at 5 μL.

2.3. Synthesis of the MIPs

For the synthesis of the various MIPs, the template (cocaine), the monomers (MAA, 2-VP), the cross-linker (EGDMA, DVB, TRIM) and the initiator (AIBN, 50 mg mmol^{-1}) were mixed with dichloromethane or acetonitrile. The reagents used for the different MIPs are described in Table 1. A template/monomer/cross-linker molar ratio of 1/4/20 was used for the synthesis of each MIP. The solution was stirred and transferred into a glass tube (14 mm i.d.). The polymerization mixture was placed in an ice bath and was degassed with nitrogen for 15 min. The tube was sealed and transferred into a thermostated water bath (15 °C) and placed at a 10 cm distance from the UV lamp for photopolymerization. In the first 15 min, the tubes were turned at regular intervals to obtain a homogenous exposure to the UV light. Polymerization was achieved by irradiating the polymerization tube with the low-pressure mercury lamp for 24 h. For thermal polymerization, tubes were introduced in a thermostated bath during 24 h. After the polymerization, the tubes were crushed and the polymer was then ground with a ball mill and manually sieved. The particles size fraction of 25–36 μm was collected and slurried in MeOH/water (80/20 (v/v)) and then dried. Non-imprinted polymers (NIPs) were obtained by performing the same procedure in the absence of the template molecule in the polymerization mixture.

Twenty-five milligrams of each polymer were packed between two frits (1/16′, 20 μm, Interchim, Montluçon, France) into 1 mL empty propylene disposable cartridges (Interchim). In order to eliminate the remaining reagents from the packed polymers, particularly the template molecule in the case of the MIP, the following washing steps were performed: 50 mL of MeCN, 30 mL of MeOH/acetic acid (95/5 (v/v)), 30 mL of MeOH, 10 mL of high purity water, 2 mL of a 0.1 mol L^{-1} hydrochloric acid solution, 10 mL of high purity water, 5 mL of MeOH and finally 10 mL of MeCN. Finally they were conditioned with acetonitrile and kept at 4 °C.

Table 2
Optimized conditions of washing of the different MIPs/NIPs applied for obtaining elution profiles reported in Figs. 2 and 3.

MIP/NIP	Washing
MIP/NIP 1	1 mL MeCN + 1 mL MeCN/MeOH, 95/5 (v/v)
MIP/NIP 2	1 mL MeCN + 1 mL MeCN/MeOH, 99/1 (v/v)
MIP/NIP 3	1 mL MeCN +1 mL MeCN/MeOH, 97.5/2.5 (v/v)
MIP/NIP 4	1 mL MeCN
MIP/NIP 5	1 mL MeCN/MeOH, 97.5/2.5 (v/v)

2.4. Study of the elution profile of COC and BZE on MIPs

Before each use, the MIPs/NIPs were conditioned with a few milliliters of acetonitrile. Acetonitrile (1 mL) spiked with 0.1 μg of COC or BZE was percolated through the MIP and NIP cartridge. Different washing solutions containing MeOH and/or MeCN were percolated through the sorbent in order to limit the retention of the analytes by non-specific interactions on the MIP. These conditions are described in Table 2. The target analytes were then eluted from the cartridge with 1 mL of methanol containing 5% acetic acid. Each fraction was dried under a gentle stream of nitrogen. The residues were dissolved in 200 μL of the mobile phase. 20 μL of each fraction were analyzed by LC/UV. After each use, MIP and NIP were washed with 1 mL MeOH/acetic acid (95/5 (v/v)), 3 mL MeOH and 5 mL MeCN before to be stored.

For the study of the comparison of the MIP 1 with the MIP 2 and for the evaluation of capacity, the extraction procedure applied to the MIP 1 was as followed: samples of acetonitrile (1 mL) spiked with various amounts of cocaine (0.1 μg for experiments reported in Fig. 4 and 4–200 μg for experiments reported in Fig. 5) were percolated through the MIP and the NIP. A washing step was achieved using 1 mL of MeCN and then 1 mL of MeCN/MeOH (97.5/2.5 (v/v)) and the elution was carried out using 1 mL MeOH/acetic acid (95/5 (v/v)). This procedure was also applied to the treatment of hair extracts.

2.5. Treatment of hair samples

Prior to analysis, hair samples (around 60 hair locks) were decontaminated by incubation in water, water/methanol mixture (50/50 (v/v)) and CH_2Cl_2 successively. Hair was then dried for 2 h at 50 °C and thinly cut (1–2 mm). Samples of 50 mg were sonicated for 2 h with 2 mL of MeCN. Finally, 1 mL of extract was filtered, spiked 0.1 mg L^{-1} of COC and BZE (i.e. concentration in the extract equivalent to 4 ng mg^{-1} of hair) and stored at room temperature protected from light until its use. COC and BZE were spiked directly in organic hair extract in order to show the feasibility of the imprinted support. The hair organic extract was then percolated through the MIP 1 and the NIP 1. After a washing step with 1 mL of MeCN and 1 mL of a MeCN/MeOH (97.5/2.5 (v/v)) mixture, the target analyte was eluted with 2 mL of MeOH/acetic acid (95/5 (v/v)). For LC–UV analysis, each fraction was then concentrated up to dryness under a

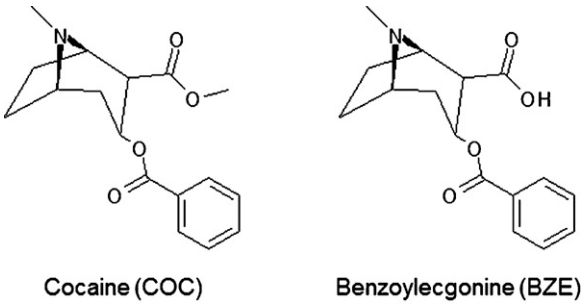

Fig. 1. Chemical structures of cocaine (COC) and its metabolite, benzoylegonine (BZE).

nitrogen stream and dissolved in 200 μL of the mobile phase and 20 μL were injected. For LC–MS analysis, 50 μL of the fractions were diluted with 950 μL of water and then 5 μL were injected.

3. Results and discussions

3.1. Choice of the conditions of synthesis of MIPs

For the MIPs synthesis, a non-covalent approach using cocaine (COC) as template molecule was selected. This polymerization method is based on the formation of a template–monomer complex through non-covalent bonds in an appropriate solvent. Then, polymerization around the template takes place in the presence of a cross-linker and an initiator. Finally, template molecules are removed from the highly cross-linked polymer leaving behind specific cavities complementary to the template in shape, size and functionality [28]. Owing to its basic properties and the polar groups constituting its structure (Fig. 1), COC can interact with the monomer by hydrogen bonds and/or electrostatic interactions, the nature of the interactions depending on the monomer used for the synthesis of the MIP and of the solvent used as porogen. Methacrylic acid (MAA), an acidic functional monomer, was selected to favor polar interactions during the synthesis of a MIP. For this, a slightly polar and non protic solvent, acetonitrile, was selected in order to enhance this type of interactions. To obtain a highly cross-linked structure, an excess of ethylene glycol dimethacrylate (EGDMA) was added to the polymerization mixture. The radical polymerization was initiated using azoisobutyronitrile (AIBN) either photochemically at 15 °C (MIP 1) or thermally at 60 °C (MIP 2). To study the effect of the nature of cross-linker on the selectivity of the MIP, a MIP was also synthesized using the same functional monomer, i.e. MAA, but using trimethylolpropane trimethacrylate (TRIM) as cross-linking agent, this "three-arm" monomer being supposed to modify the structure of the polymer thus favoring its porosity and the cavity formation. It was already used for the synthesis of a MIP using a metabolite of cocaine as template in toluene [25]. For this study, dichloromethane, whose polarity is closer to acetonitrile, was preferred to toluene for the synthesis of the MIP (MIP 3). The effect of a third cross-linker, divinylbenzene (DVB), in combination with EGDMA was also studied to synthesize a MIP (MIP 4), the objective being to favor π–π interactions as an additional retentive interaction. At last, MAA was associated to 2-vinylpyridine (2-VP) to synthesize a MIP (MIP 5). This basic monomer was selected to study its potential for the retention of the acid metabolite of COC, i.e. benzoylecgonine (BZE). The conditions of synthesis of those different MIPs are summarized in Table 1.

In all the cases, a non-imprinted polymer (NIP) was prepared, in the same conditions but without introducing the template molecule, to assess the presence of specific cavities in the corresponding MIPs. Since MIPs are expected to exhibit enhanced selectivity for their template in a solvent of a nature closed to the one used for the synthesis, MIPs were characterized in acetonitrile.

3.2. Selectivity of the MIPs toward cocaine and benzoylecgonine

The selectivity of a MIP results from the presence of specific binding sites in the polymeric network. By comparing the retention of the template molecules on the MIP and on the NIP, the presence of specific cavities can be confirmed. Thus, a selective off-line MIP SPE procedure was developed for the extraction of COC and BZE on each MIP. For this, a fraction of acetonitrile (1 mL) spiked with 100 ng of COC and BZE was loaded onto the MIPs and the NIPs packed in cartridges (25 mg of sorbent). The washing step was optimized to decrease non-specific interactions without disrupting the selective ones. Considering that target analytes might be mainly retained on MIP and NIP by hydrogen bonds, various proportion of methanol was added in acetonitrile to limit as much as possible the retention on NIP by non-specific interactions without affecting the retention with the cavities of the MIP. Finally, in order to recover COC and BZE from the supports, 1 mL of methanol containing 5% acetic acid was used to disrupt both polar interactions, i.e. hydrogen bonds and electrostatic interactions. The addition of acid in the elution fraction was necessary since some COC and BZE remained on some MIPs when pure methanol was used thus highlighting the contribution of electrostatic interactions in some cases. The optimal conditions of washing that provided a high amount of COC and BZE in the elution fraction of the MIP and a low one in the elution fraction of the corresponding NIP thus allowing to differentiate as much as possible the retention of the target analytes on MIPs and on NIPs, are summarized in Table 2. Resulting elution profiles of COC and BZE on MIPs and NIPs are reported in Figs. 2 and 3 respectively.

As can be seen by comparing the elution profiles, the presence of cavities was demonstrated for all the synthesized MIPS because a higher retention of at least one of the two target analytes was obtained on the MIP than on the NIP. However, the selectivity of the MIP 3 demonstrated by the retention of BZE is poor towards COC because its retention on the MIP 3 and on the corresponding NIP is similar. Therefore, the interest of using TRIM already demonstrated for the retention of BZE in acetonitrile [25] is poor for the selective retention of COC. Concerning results obtained using DVB associated to EGDMA as cross-linker (MIP 4), the selectivity is demonstrated for both molecules because a higher retention was obtained for both molecules on the MIP than on the NIP. However, the strength of the interactions between the target molecules and the cavities of the MIP is rather low as illustrated by their low amount recovered in the elution fraction. The expected π–π interactions did not strongly contribute to the selective retention of COC and BZE. A low strength of interaction between the target analytes and the MIP was also observed using MIP 5 that was synthesized using 2-VP associated to MAA as functional monomer. However, in this case, a higher selectivity was obtained than with MIP 4 as illustrated by the difference of retention observed for BZE between the MIP and the NIP. This basic monomer was selected to favor the selective retention of the acid metabolite, i.e. BZE that is confirmed by the obtained results. However, these conditions of synthesis are not well adapted to the simultaneous selective extraction of COC that is again poorly retained on the MIP 5 when applying the same washing solution. This is probably due to the ester function of the COC that makes this molecule less polar than BZE that has an acidic function instead, and therefore COC's retention is reduced. For both molecules, best results in terms of strength of retention and of selectivity were obtained using MAA as monomer and EGDMA as cross-linker either using thermal or UV initiation. Using both resulting MIPs, i.e. MIP 1 and MIP 2, a strong retention of COC and on BZE was obtained. Both molecules were recovered in the elution fraction of the MIP with

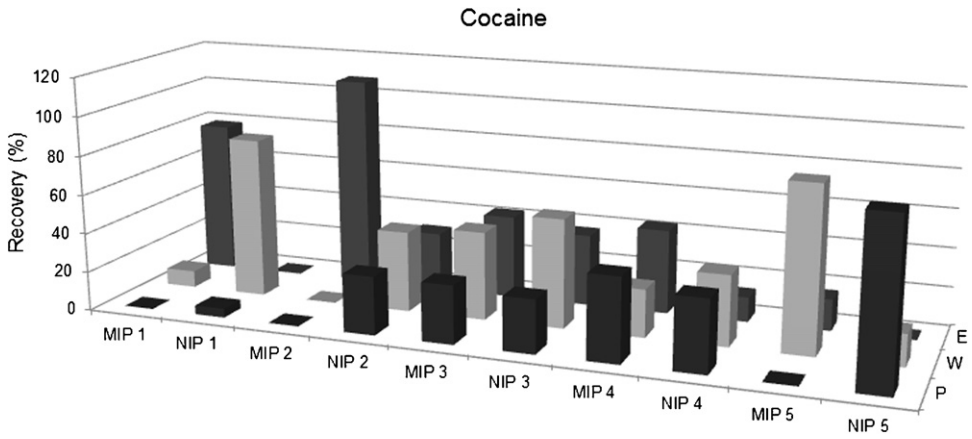

Fig. 2. Elution profile of COC from the different MIPs prepared according to conditions in Table 1 and from their corresponding NIPs when applying the condition of washing described in Table 2. P, percolation; W, washing; E, elution.

recoveries higher than 79%. A high selectivity was also obtained because the extraction procedure gave rise to an efficient removal of the target analytes from the NIP and then to recoveries lower than 30% for both molecules in the elution fraction. To confirm these good results and to check if the difference between the profiles observed on both MIPs (and on the corresponding NIPs) is significant, the extraction procedure was applied in three replicates on the MIP 1 and 2. For the MIP 1, the washing step was slightly modified by decreasing the elution strength of the washing step (using 2.5% MeOH instead of 5%) to limit the loss of molecules from the MIP during this step with the risk of decreasing the selectivity of the procedure. Results presented in Fig. 4 shows that a higher selectivity was obtained for both molecules on MIP 1 (Fig. 4(a)) than on MIP 2 (Fig. 4(b)). Indeed, recoveries of extraction of $79 \pm 9\%$ were obtained for COC using MIP 1 and no retention on the corresponding NIP and of $89 \pm 9\%$ and $4 \pm 3\%$ on the MIP 1 and the NIP 1 respectively for BZE. Concerning MIP 2, high recoveries of extraction were also obtained on the MIP for both molecules but the retention on the corresponding NIP is higher, with recoveries of $30 \pm 2\%$ for COC and $12 \pm 4\%$ for BZE thus highlighting the contribution of non specific interactions in the retention process on the MIP 2, particularly for cocaine. Therefore, these results showed that, for these molecules, decreasing the polymerization temperature gives rise to a higher selectivity and also a stronger retention than when applying high temperature. Therefore, the MIP 1 was selected and its performance was studied more in detail by measuring its capacity when applying the same extraction procedure.

3.3. Study of the capacity of the MIP 1

The capacity of a MIP corresponds to the maximum amount of a compound that can be retained on the imprinted polymer when applying particular extraction conditions. Therefore, the determination of the capacity was performed by measuring the amount of COC in the elution fraction of the MIP 1 after the loading of 1 mL acetonitrile samples spiked with various amounts of cocaine. Each percolation step was followed by a washing with 1 mL of acetonitrile and 1 mL of a mixture of acetonitrile with 2.5% (v/v) of methanol to disrupt non-specific interactions. Finally, COC was eluted from the MIP and this fraction was analyzed. The same study was performed on the NIP in order to check the low retention of COC on the NIP thus confirming a retention process on MIP only by the selective interactions of COC with the cavities of the MIP. The resulting curves are presented on Fig. 5. For the MIP, the shape of the curve presents two different parts. For the lowest amounts of cocaine loaded on the MIP, the trend is linear, meaning that there is a constant recovery of extraction of cocaine using the MIP. This recovery is given by the slope of this linear part corresponding to a recovery of 88%. This value is very close to the recoveries previously obtained for the MIP using the same extraction procedure (Fig. 4(a)). For higher amount of cocaine loaded on the MIP, the curve reaches a plateau. For the corresponding percolated amount of cocaine, recoveries decrease since the cartridge's capacity is exceeded. Considering the point where the two curves intercept as the maximum amount of cocaine retained on the MIP with constant recoveries,

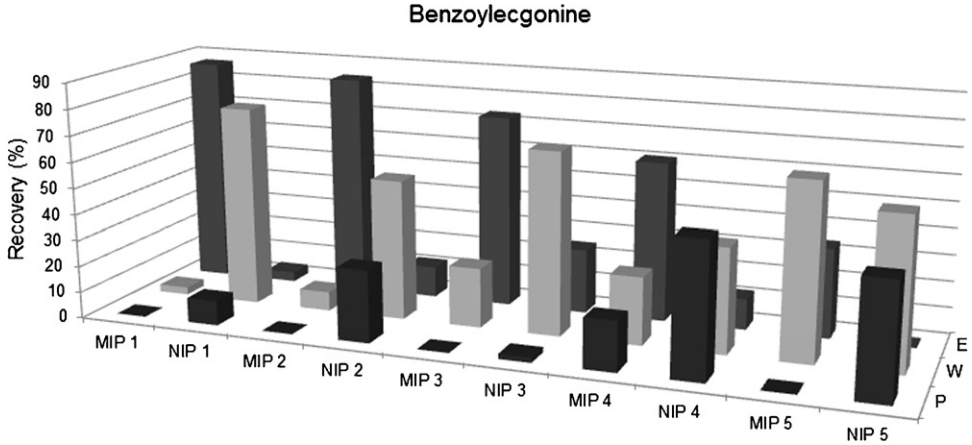

Fig. 3. Elution profile of BZE from the different MIPs prepared according to conditions in Table 1 and from their corresponding NIPs when applying the condition of washing described in Table 2. P, percolation; W, washing; E, elution.

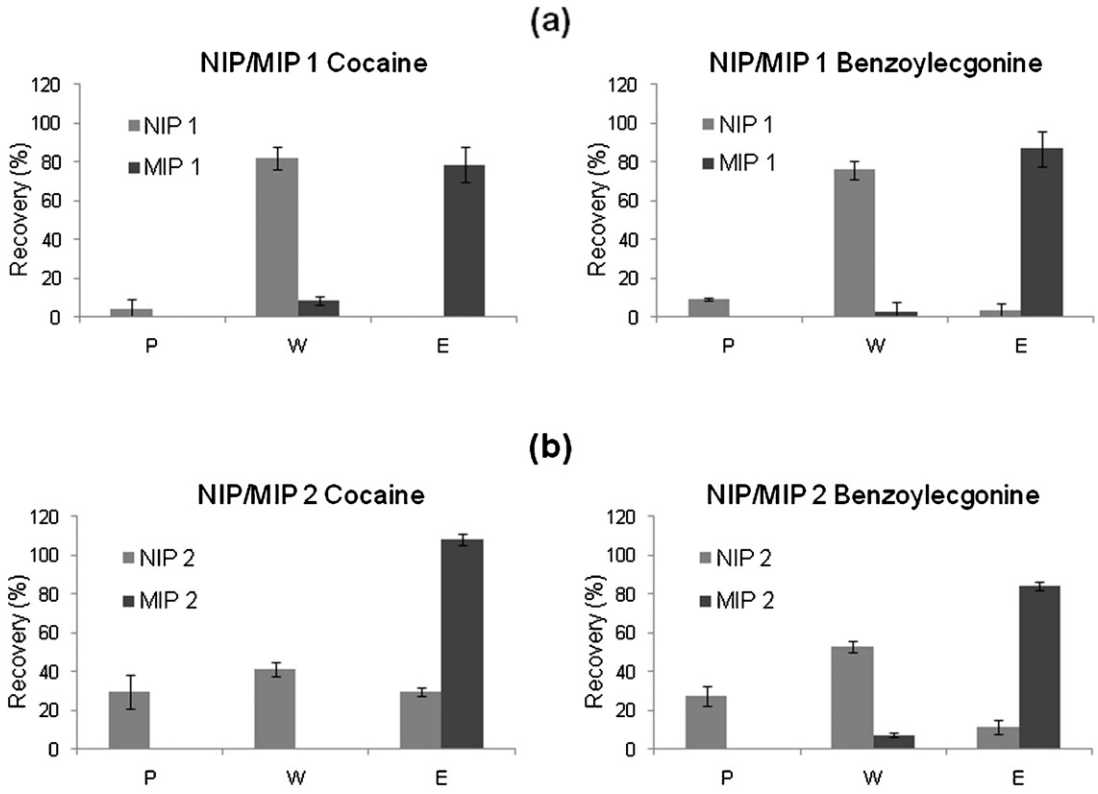

Fig. 4. Elution profiles ($n = 3$) of COC and BZE from MIP 1 (a) and MIP 2 (b) and from their corresponding NIPs when applying the protocol described in Table 2, except for the washing step of MIP 1 that was achieved using 2.5% MeOH instead of 5%. P, percolation; W, washing; E, elution.

the capacity can be estimated to 68 μg of cocaine for 25 mg of MIP that corresponds to 2.72 mg g^{-1} of MIP or to 8.96 μmol g^{-1}. Over this value, quantitative analyses are not reliable since there is a decrease in the recovery of extraction. This capacity value is in good agreement with the capacity values reported in the literature, between 1 μmol g^{-1} and 40 μmol g^{-1} [23]. This capacity is also enough for the trace analyses of cocaine in biological matrices. At least, it is important to notice again the high selectivity of the procedure because no retention of cocaine was observed on the NIP for percolated amount lowest to the capacity value thus highlighting the fact that this capacity value also corresponds to a number of specific cavities in the MIP that are available for the binding of cocaine in these conditions of use because there are no contribution of non specific interactions.

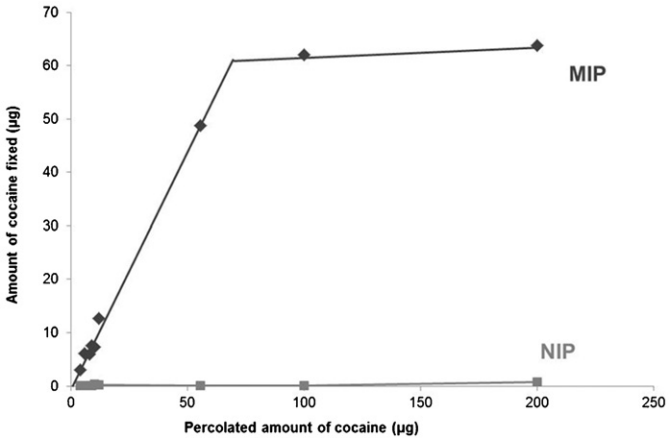

Fig. 5. Calibration curves obtained when plotting the amount of COC recovered in the elution fraction of the MIP 1 and the corresponding NIP after the percolation of various amounts of cocaine contained in 1 mL MeCN. Washing step: 1 mL MeCN and 1 mL MeCN/MeOH 97.5/2.5 (v/v). Elution: 1 mL MeOH/acetic acid 95/5 (v/v).

3.4. Extraction of cocaine and benzoylecgonine from hair extracts

Good results in terms of retention and selectivity were obtained in pure acetonitrile media for the MIP 1. This MIP was not only able to retain COC, that is the template molecule, but it also retained BZE. This selective retention of the BZE can be useful for hair analysis since the quantification of BZE content can help to differentiate between COC consumption or external contamination. The extraction procedure was then applied for the clean-up and the analysis of acetonitrile hair extract samples spiked with COC and BZE. For this, hair samples were decontaminated, cut into small pieces and extracted by sonication in acetonitrile. The selectivity was first tested with a concentration equivalent to 4 ng of each analyte per mg of hair. Resulting elution profiles are presented in Fig. 6. For both molecules, a high retention is still obtained for this extracts resulting from the treatment of a real matrix with recoveries of 89 ± 7% for COC and of 93 ± 5% for BZE on MIP ($n = 5$). The high selectivity of the procedure is also maintained because low recoveries between 0 and 2% were simultaneously obtained on the corresponding NIP. Those results are also in good agreement with results of Fig. 4(a) for MIP 1 obtained in pure media. Chromatograms corresponding to the LC/UV analysis of a hair extract spiked at 4 ng mg^{-1} with COC and BZE before and after a clean-up step on the MIP 1 are presented in Fig. 7. LC/UV is certainly not the method of choice for the trace analysis of these compounds in real samples because of its low sensitivity and its poor specificity. However, this lack of specificity allows illustrating the selectivity brought by the MIP. Indeed, without a MIP clean-up, the peak of BZE is co-eluted with an interfering compound that could interfere for its quantification. The use of the MIP prevents this co-elution as illustrated by the UV chromatogram

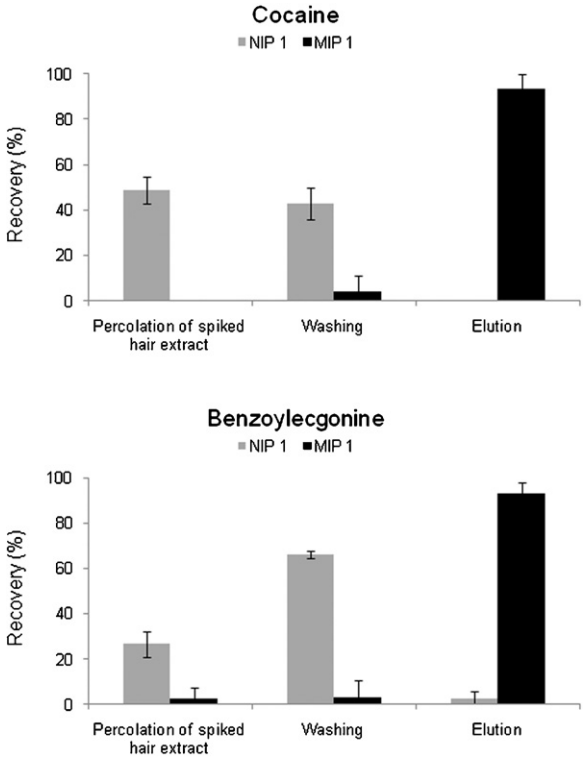

Fig. 6. Elution profile of COC and BZE from MIP 1 and from its corresponding NIP after the percolation of a 1 mL spiked MeCN hair extract ($n = 5$). Washing and elution conditions are similar to those of Fig. 5.

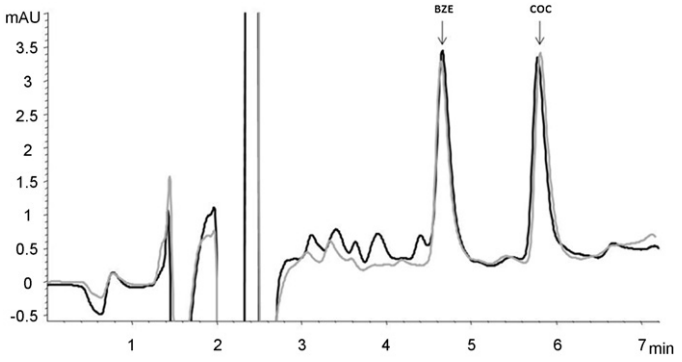

Fig. 7. LC–UV analysis of a hair extract spiked with COC and BZE at 4 ng g^{-1} before (black line) and after a clean-up step on MIP 1 (grey line).

corresponding to the analysis of the spiked hair extract purified on the MIP. The determination of cocaine and benzoylecgonine was also performed at a concentration equivalent to 0.5 ng of each analyte per mg of hair, which is the cut-off value used by the society of hair testing [6]. The resulting chromatograms obtained in LC/MS analysis in MRM mode are presented in Fig. 8. As expected, with this very specific mode of detection, very clean chromatograms were obtained with or without the use of MIP. However, by observing the results obtained for LC/UV chromatograms, MIP appears as a powerful tool to remove matrix components thus preventing the risk of co-elution in LC/UV analysis but also the risk of ion suppression or ion enhancement in the source of the MS often observed when applying LC/MS analysis to real samples and that affects the analyte quantification. Moreover, a UV detection can still be used as a fast and simple screening method, and the MIP extraction makes this analysis possible because the interferences are eliminated and also because it can preconcentrate the analytes.

The whole analytical method that includes a purification of the hair extract on MIP and the analysis of the elution fraction by LC/MS gave rise to a limit of quantification (LOQ) of 0.07 ng mg^{-1} and 0.04 ng mg^{-1} of hair for COC and BZE respectively, and the limits of detection (LOD) were 0.01 ng mg^{-1} and 0.02 ng mg^{-1} of hair for COC and BZE respectively. Those limits were estimated by considering, for the LOQ and the LOD, a signal-to-noise ratio of 10 and 3 respectively using the LC/MS chromatogram corresponding to the

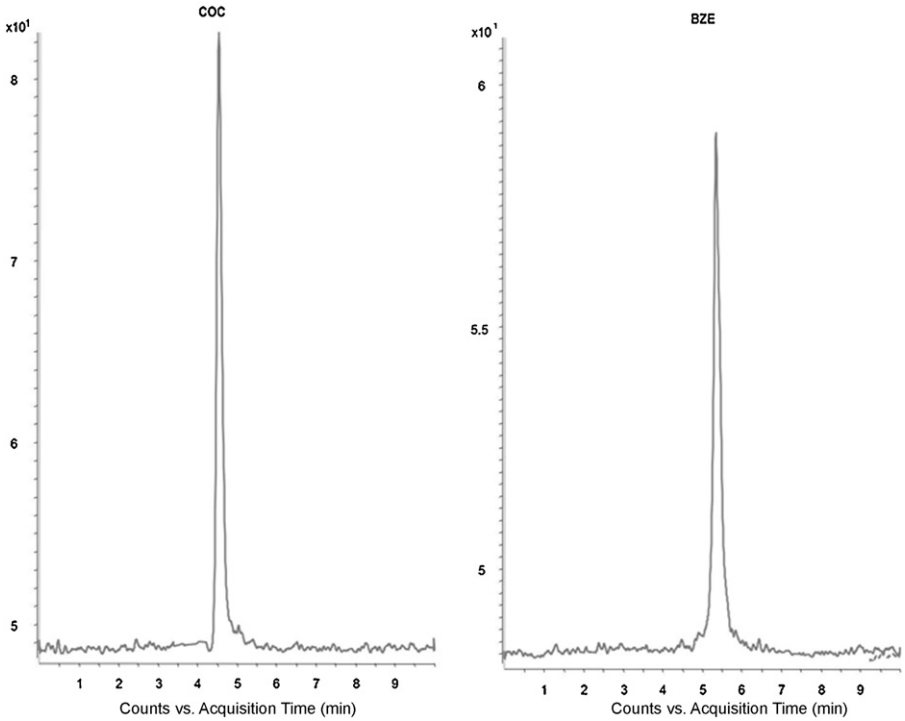

Fig. 8. LC/MS analysis in MRM mode of COC and BZE in a hair extract spiked with COC and BZE at 0.5 ng g^{-1} of hair after a clean-up step on MIP 1.

analysis of the hair extract spiked at 0.5 ng mg^{-1} and purified on the MIP. Lower limits could be reached because these limits were calculated with a chromatogram obtained by injecting an extract previously diluted by a factor 20 (with a solution of a nature close to the mobile phase). By replacing this dilution step by an evaporation of the extract and its reconstitution using a solution close to the mobile phase, lower LOQ close to 3 pg mg^{-1} and 2 pg mL^{-1} for COC and BZE respectively can be expected.

4. Conclusions

Several molecularly imprinted polymers have been synthesized for the selective extraction of cocaine and its acidic metabolite benzoylecgonine from hair extracts. The MIP resulting from the use of MAA as monomer and EGDMA as cross-linker in acetonitrile demonstrated a high potential for the selective retention of the target analytes in acetonitrile. This high selectivity was particularly demonstrated when using photopolymerization over thermal initiation during the polymerization step. The high capacity obtained for this MIP facilitates its applications to various types of samples containing up to 8.96 μmol of cocaine per gram of MIP. The selective potential of the MIP was illustrated by its application to a hair extract sample. A clean baseline was obtained in LC/UV analysis for the extract cleaned-up on the MIP. The MIP allows the removal of interfering compounds that are present in the chromatogram of the hair extract directly injected in LC/UV. Associated to LC/MS analysis, MIP provides a highly sensitive method allowing the determination of cocaine and benzoylecgonine with a limit of quantification lower than 0.07 ng mg^{-1}. The low sensitivity and also the cross-reactivity of the MIP for benzoylecgonine can be appreciable aspects for hair analysis especially when metabolites ratios are required for the confirmation of cocaine consumption.

Future development will concern the possibility to develop a selective extraction procedure in aqueous media for the direct application of MIP for the extraction of the target analytes from biological fluids such as plasma or urine. This will necessitate new developments in terms of synthesis or/and of extraction procedure, the polar interactions that have ensured the retention of the analytes on this MIP in acetonitrile being not favored in aqueous media.

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