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Molecularly imprinted polymer cartridges coupled on-line with high performance liquid chromatography for simple and rapid analysis of dextromethorphan in human plasma samples

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ABSTRACT

In this paper, a novel method is described for automated determination of dextromethorphan in biological fluids using molecularly imprinted solid-phase extraction (MISPE) as a sample clean-up technique combined with high performance liquid chromatography (HPLC). The water-compatible molecularly imprinted polymers (MIPs) were prepared using methacrylic acid as functional monomer, ethylene glycol dimethacrylate as cross-linker, chloroform as porogen and dextromethorphan as template molecule. These imprinted polymers were used as solid-phase extraction sorbent for the extraction of dextromethorphan from human plasma samples. Various parameters affecting the extraction efficiency of the MIP cartridges were evaluated. The high selectivity of the sorbent coupled to the high performance liquid chromatographic system permitted a simple and rapid analysis of this drug in plasma samples with limits of detection (LOD) and quantification (LOQ) of 0.12 ng/mL and 0.35 ng/mL, respectively. The MIP selectivity was evaluated by analyzing of the dextromethorphan in presence of several substances with similar molecular structures and properties. Results from the HPLC analyses showed that the recoveries of dextromethorphan using MIP cartridges from human plasma samples in the range of 1–50 ng/mL were higher than 87%.

1. Introduction

Dextromethorphan ((+)-3-methoxy-17-methyl-(9α,13α,14α)-morphinan) (Fig. 1), is as a cough suppressant, for the temporary relief of cough caused by minor throat and bronchial irritation (such as commonly accompanies the flu and common cold), as well as those resulting from inhaled irritants. In addition, a combination of dextromethorphan and quinidine has been shown to alleviate symptoms of easy laughing and crying (pseudobulbar affect) in patients with amyotrophic lateral sclerosis and multiple sclerosis. Dextromethorphan is also being investigated as a possible treatment for neuropathic pain and pain associated with fibromyalgia [1]. The drug has been quantified using different methods such as LC–MS-MS [2], GC–MS [3], HPLC [4,6], potentiometric sensor [5], spectrophotometry [7], capillary electrophoresis [8], and fluorimetry [9].

Analysis of pharmaceutical compounds in biological matrices such as plasma requires sample preparation to clean-up the sample before the chromatographic separation that can be usually completed by solid-phase extraction (SPE). However, classical SPE sorbents mainly develop non-specific hydrophobic interactions that lead to the co-extraction of interfering compounds often preventing an easy and reliable quantification of the analyte. To overcome this lack of selectivity, selective materials giving rise to a mechanism of retention based on molecular recognition can be used. They are composed of immunosorbents (ISs) whose affinity and selectivity stem from antigen–antibody interactions. Therefore, they allow a selective extraction of the target analyte and of similarly structured compounds [10,11]. Their direct integration in on-line system is also feasible if the solid support used is pressure resistant [12,13]. Despite their important interest as a selective sample pretreatment sorbents, the development of ISs is time consuming and relatively expensive. These drawbacks have contributed to the development of molecularly imprinted polymers (MIPs). MIPs are synthetic materials possessing specific cavities specially designed for the recognition of an analyte of interest. Their synthesis procedures for SPE use are mainly based on strong non-covalent interactions (such as hydrogen bonds or ionic interactions) between a target molecule (template) with functional monomers, followed by polymerization in the presence of a cross-linker, usually in a non-protic and weakly polar solvent. Once the template is removed, selective molecular recognition sites, often

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described as three-dimensional shapes in the polymer, are available for the selective rebinding of the target molecule and some structurally related compounds. The first application was carried out by the group of Sellergren in 1994 for the extraction of pentamidine present at low concentration level in urine [14]. Nowadays, MIPs have been largely applied to the extraction or to the clean-up of target analytes from various complex matrices [15,16]. Concerning the pharmaceutical field, many examples deal with the extraction of a drug by MIP from tablets [17–19], plasma [18–24], or from urine [23–26] samples. Most of these applications were based on off-line procedures. A very few applications were carried out in on-line mode [26–28] while trends in analytical chemistry are for high throughput approaches that require to minimize the time spent performing analysis. Therefore, MIP cartridges coupled on-line with high performance liquid chromatography (HPLC) can allow a straightforward and fast pretreatment due to their specific recognition properties.

Recently, we applied MIPs as artificial receptors in potentiometric detection of hydroxyzine [18], cetirizine [19], drug delivery of dipyridamole [29], and off-line solid-phase extraction of verapamil [30], bromhexine [23], metoclopramide [24], and tramadol [31] in human plasma and urine samples. In this work a novel automated method has been presented for the selective chromatographic determination of dextromethorphan based MIPs as selective SPE cartridge sorbents for efficient sample clean-up of the analyte from complex biological matrices. Polymer selectivity was also obtained in the presence of several substances with similar molecular structures and properties. This new SPE cartridge sorbents allows the sensitive, simple and inexpensive extraction and determination of the drug in human plasma samples while various pharmaceutical compounds found in the biological fluid were not retained on the MIP support.

2. Experimental

2.1. Chemicals and reagents

Methacrylic acid (MAA) from Merck (Darmstadt, Germany) was distilled in vacuum prior to use in order to remove the stabilizers. Ethylene glycol dimethacrylate (EGDMA) and 2,2′-azobis isobutyronitrile (AIBN) as the initiator of polymerization from Sigma–Aldrich (Steinheim, Germany) were used without any purification. All solvents used in chromatography analyses were HPLC grade and purchased from Merck.

All solvents used in chromatography analyses were HPLC grade and supplied by Merck Inc. Dextromethorphan HBr (Divi's Laboratories Limited) was used for preparing stock and standard solutions. A standard stock solution of dextromethorphan (1 mg/mL) was prepared in distilled water. This solution was freshly prepared each day and stored at 4 °C. Aliquots of standard stock solution of dextromethorphan were dispensed into 10 mL volumetric flasks and the flasks made up to volume with the mobile phase to give final concentrations range of 0.05–200 ng/mL.

2.2. HPLC apparatus

An Alliance HPLC instrument from Waters Company was used to separate and analyze dextromethorphan in aqueous samples. The chromatographic system was composed of a multi solvent gradient Waters pump, a Waters 2996 photodiode array detector and an online degasser. A Rheodyne model 7725i injector with a 250 μL loop was used to inject the samples. Chromatographic separation was achieved on an ACE C18, 5 μm, 4.6 mm × 250 mm column. For the mobile phase, a degassed mixture of methanol:phosphate buffer (0.05 M, pH 5.0) (90:10) was prepared and delivered in isocratic mode at flow rate of 1.0 mL/min. All of the analyses were carried out at an operation wavelength of 227 nm and HPLC data were acquired and processed using a PC and Millennium 2010 chromatogram manager software (Version 2.1 Waters).

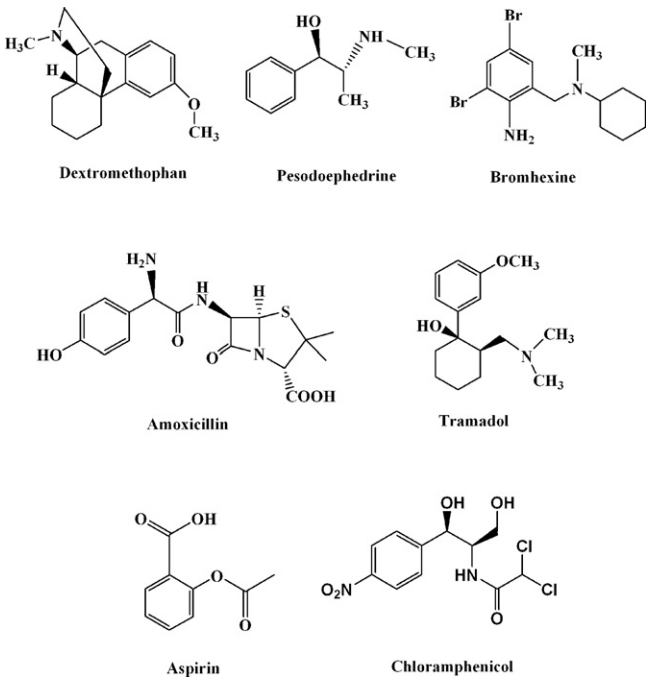

Fig. 1. Structures of the drugs used in this study.

2.3. Operation of the on-line SPE-HPLC system

Exactly 120 mg of the optimized MIP polymer was packed in a polypropylene cartridge, which was incorporated in a flow system prior to the HPLC analytical instrumentation. The principle of sequential injection was utilized for a rapid automated and efficient SPE procedure on the MIP. Samples, buffers, washing and elution solvents were introduced to the extraction cartridge via a vacuum pump. This cartridge was conditioned with 1 mL methanol, 1 mL ultra-pure water, 1 mL 25 mM ammonium phosphate, adjusted to pH 3.0. Extraction experiments involved loading the cartridge with 5 mL of sample containing 50 ng/mL dextromethorphan at a flow rate of 1.0 mL/min. After loading, cartridge was washed with 1 mL HCl 0.1 M, 1 mL ultra-pure water, applied full vacuum through the cartridge for 20 min to removed residual moisture from cartridge, then 1.5 mL dichloromethane, after that applied vacuum for 2 min to removed residual solvent. The elution phase was performed by passing by used 3 × 1 mL methanol:phosphate buffer applied a gentle vacuum between each fraction through the cartridge. This solution subsequently eluted into the injection loop by reversing the pump flow. Finally each eluted sample was injected into the analytical column and analyzed on HPLC.

2.4. MIP and NIP preparation with precipitation polymerization

For the preparation of the dextromethorphan imprinted polymer, the template (73 mg, 0.27 mmol) was dissolved in chloroform in a 20 mL thick walled glass tube. The functional monomer (MAA) (0.242 mL, 3.5 mmol), the cross-linking monomer (EGDMA) (2.6 mL, 14 mmol), and the initiator (AIBN) (0.057 mg, 0.164 mmol) were then added to the above solution. The mixture was spontaneously exposed by sonication for 30 min to remove oxygen. Then the solution was placed in a water bath at 60 °C for 18 h. The

hard polymers were obtained and then crushed. After the polymerization procedure and drying, the polymer particles were put in methanol:phosphate buffer (0.05 M, pH 5.0) (90:10) for 18 h until remove target molecule and then washed with distilled water three times to remove any solvent. The complete removal of template was followed by HPLC–UV. In order to verify that retention of template was due to molecular recognition and not to non-specific binding, a control, non-imprinted polymer (NIP) were also prepared according to the above procedures except for the absence of template during polymerization.

2.5. Morphologic and porosity analysis

Scanning electron microscopy (SEM, PhilipsXL30 scanning microscope, Philips, Netherlands) was employed to determine the shape and surface morphology of the produced polymer particles. Polymeric particles were sputter coated with gold prior to the SEM measurement. The polymer structures were characterized by means of N_2 adsorption–desorption isotherm measurements at 77 K with a BELSORP-mini, BEL Japan, Inc.

2.6. Batch rebinding experiments

Batch adsorption experiments were used to evaluate the binding affinity of the imprinted polymer as reported before [32]. The general procedure for extraction of dextromethorphan by the MIP was as follows: The polymer beads were suspended in aqueous solutions and the pH was adjusted at 8.0. Then 100 mg of the polymer particles were added in 10-mL flask containing dextromethorphan solutions of various concentrations. The mixtures were thermo stated at 25 °C for 8 h under continuous stirring and then was filtrated on a paper filter (flow rate = 50 mL/min by applied vacuum). The free concentration of dextromethorphan after the adsorption was recorded by HPLC–UV at 227 nm. Three replicate extractions and measurements were performed for each aqueous solution. The adsorbed dextromethorphan was desorbed from the MIP by treatment with 2 mL of methanol:phosphate buffer (90:10, v/v). The imprinted polymer containing dextromethorphan was placed in the desorption medium and stirred continuously at 600 rpm and room temperature for designated time. The final dextromethorphan concentration in the aqueous phase was determined. The same procedure was followed for NIP particles.

2.7. Extraction procedure for human plasma samples

Drug-free human plasma was obtained from the Iranian blood transfusion service (Tehran, Iran) and stored at −20 °C until use after gentle thawing. Due to possibility of protein-bonding for dextromethorphan and reducing the recoveries in solid-phase extraction processes, it is necessary to have some treatments with plasma before extraction with MIP particles. So, the plasma samples was diluted with 25 mM ammonium acetate (pH 5.0), then centrifuge 20 min at 8000 rpm to remove excess of proteins. Then the supernatant was filtered through a cellulose acetate filter (0.20 μm pore size, Advantec MFS Inc., CA, USA). The filtrate was collected in glass containers and stored at −20 °C until the analysis was performed. 2 mL of the filtered supernatant were collected to be directly percolated through the MIP or the NIP cartridges.

3. Results and discussion

3.1. Optimal MIP formulation and progenic solvent

There are several variables, such as amount of monomer or nature of cross-linker and solvent that affects the final characteristics of the obtained materials in terms of capacity, affinity and selectivity for the target analyte. Thus, by achieving an optimum combination of cross-linker and functional monomer, non-specific binding should be able to be minimized. Primary experiments revealed that the imprinted polymers prepared in chloroform show better molecular recognition ability than acetonitrile (AN) and methanol in aqueous environment (Fig. 2). Thus, in chloroform, different formulations for the obtainment of MIPs with improved molecular recognition capabilities have been used. Generally, proper molar ratios of functional monomer to template are very important to enhance specific affinity of polymers and number of MIPs recognition sites. High ratios of functional monomer to template result in high non-specific affinity, while low ratios produce fewer complexation due to insufficient functional groups [33]. Seven molar ratios of the monomer MAA to the template of 2:1, 4:1, 6:1, 8:1, 10:1. 13:1 and 15:1 were used in this study. The optimum ratio of functional monomer to template for the specific rebinding of dextromethorphan was 13:1 (MIP6, Table 1), which had the best recovery of 92%, while that of the corresponding NIP was low at 23%. The specific adsorption recovery of dextromethorphan at 13:1 was 69%, while those at 2:1, 4:1, 6:1, 8:1, 10:1 and 15:1 were 17%, 13%, 38%, 31%, 42%, and 31%, respectively. Therefore, a typical 1:13:52 template:monomer:cross-linker molar ratio was used for further studies.

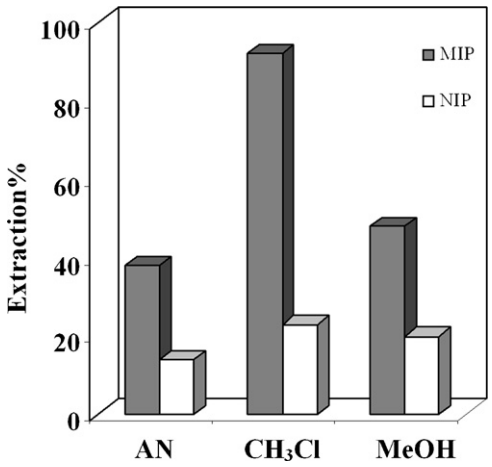

Fig. 2. Recoveries obtained using the MIP and NIP polymers synthesized in different organic solvents. Batch experiments with 50 mg of polymer particles; sample volume, 5 mL; pH, 8.0; dextromethorphan concentrations, 50 ng/mL.

3.2. Study of morphology and porosity of microspheres

Fig. 3 shows the SEM images of MIP and NIP particles. The microspheres were obtained by chloroform, appropriate amount of template/functional monomer/cross-linker agent (molar ratio of 1:13:52) and gentle mixing during the precipitation polymerization. The difference in size and morphology of imprinted and non-imprinted polymers is due to influence of template compound on the particle growth during the precipitation polymerization [34].

The porosities of the microspheres were determined by nitrogen adsorption/desorption analysis of Brunnauer–Emmet–Teller (BET) adsorption measurements. The corresponding BET surfaces were 229 and 214 $m^2\,g^{-1}$ for MIP and NIP, respectively. Upon extraction with a good solvent, here methanol:phosphate buffer, only the specific surface of the imprinted polymer changed significantly to 254 $m^2\,g^{-1}$.

Table 1
Compositions and comparisons of the extraction of dextromethorphan from dextromethorphan standard solution (5 mL, 50 ng/mL) using 50 mg of various polymers as sorbents at pH 8.0, elute: 2 mL methanol:phosphate buffer (0.05 M, pH 5.0) (90:10).

MIP	MAA (mmol)	Dextromethorphan (mmol)	EGDMA (mmol)	AIBN (mmol)	Extraction (%) (mean ± SD)[a]
MIP1	0.54	0.27	14	0.056	36 (± 1.3)
MIP2	1.08	0.27	14	0.056	32 (± 2.6)
MIP3	1.62	0.27	14	0.056	58 (± 2.8)
MIP4	2.16	0.27	14	0.056	51 (± 3.2)
MIP5	2.7	0.27	14	0.056	64 (±1.4)
MIP6	3.51	0.27	14	0.056	92 (±3.1)
MIP7	4.05	0.27	14	0.056	54 (±1.7)
NIP1	0.54	–	14	0.056	19 (± 1.9)
NIP2	1.08	–	14	0.056	19 (± 2.1)
NIP3	1.62	–	14	0.056	20 (± 2.4)
NIP4	2.16	–	14	0.056	20 (± 2.6)
NIP5	2.7	–	14	0.056	22 (± 1.4)
NIP6	3.51	–	14	0.056	23 (± 2.3)
NIP7	4.05	–	14	0.056	23 (± 2.1)

[a] Average of three determinations.

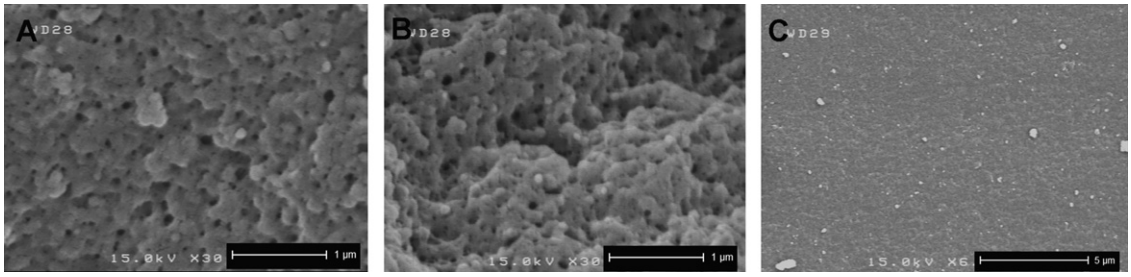

Fig. 3. Scanning electron micrographs of unleached MIP (A) leached MIP (B) and NIP (C) particles.

3.3. Effect of pH

It has been demonstrated that efficient imprint rebinding is possible in aqueous buffer solutions, showing high binding affinity and selectivity as a result of hydrophobic interactions. The effect of pH on the rebinding efficiency of dextromethorphan was investigated by varying the solution pH from 1.0 to 11.0. Several batch experiments were performed by equilibrating 50 mg of the imprinted particles with 20 mL of solutions containing 100 ng/mL of dextromethorphan under the desired range of pH. It was observed that dextromethorphan underwent complete rebinding/elution at neutral pHs (6.0–8.0) (Fig. 4). The lower responses observed at lower and higher pHs may be attributed to the protonation of the amine group of dextromethorphan and deprotonation of carboxyl groups of the polymer, respectively. However, due to the better selectivity coefficients at pH 8.0 (Section 3.7), this pH was chosen for further studies.

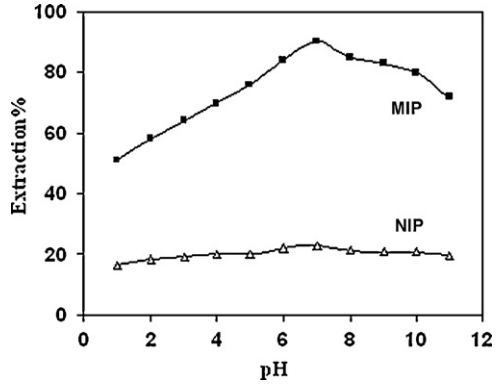

Fig. 4. Effect of pH on extraction of MIP and NIP particles in batch experiments.

3.4. Choice of loading, washing and eluent solution

Commonly, the synthetic polymers have binding ability with both specific and non-specific interactions. The specific interactions may originate mainly from the imprinting procedure, which creates selective recognition sites for the template. The non-specific interactions were assessed by measuring the binding of the non-imprinted polymer. At first, cartridges were conditioned with 1 mL methanol, 1 mL of ultra-pure water and 1 mL of 25 mM ammonium phosphate at pH 3.0. Water samples were then loaded onto the cartridges at a flow rate of 1 mL/min and the wash procedure was assessed for obtaining maximum recovery of the analytes using a variety mixtures including; ultra-pure water, hydrochloric acid 0.1 M, acetonitrile, acetone, tetrahydrofuran, dimethyl formamide (DMF), acetonitrile–acetone, dichloromethane (DCM) and acetonitrile–methanol. In order to investigate the usefulness of the washing step, 5 mL of 50 ng/mL dextromethorphan aqueous solution (pH 8.0) was loaded on the MIP and NIP cartridges separately, followed by desorption with the washing solvent. The results showed that in the most excellent protocol, washing with 1 mL of hydrochloric acid (0.1 M) and 1 mL of ultra-pure water, full vacuum through the cartridge for 20 min to removed residual moisture from cartridge and then washing with 1.5 mL dichloromethane, the recovery of dextromethorphan in NIP cartridge was decreased up to 14%, while the recovery of dextromethorphan by the MIP cartridges was not reduced (89%).

It is important to note that, washing with hydrochloric acid is necessary for removal of ionic species (ex. metal ions) from complex matrices such as human plasma. DCM wash should be done prior to elution to have selective retention of dextromethorphan. Note that we need to dry the SPE cartridge thoroughly after the washing steps by vacuum application. For the recovery of strongly bounded dextromethorphan, the polymeric sorbents were eluted with 3 × 1 mL of methanol:phosphate buffer (0.05 M, pH 5.0) (90:10). In addition,

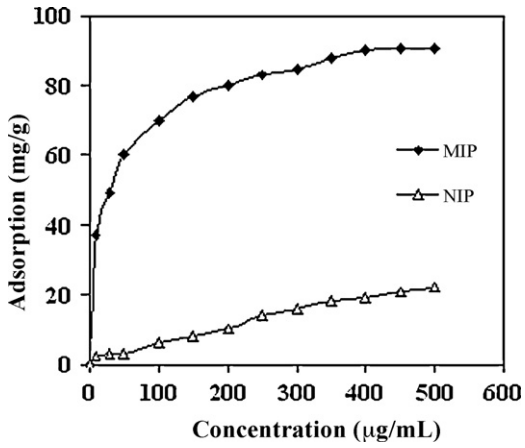

Fig. 5. Effect of dextromethorphan concentrations on the retention capacities of MIP and NIP particles at pH = 8.0.

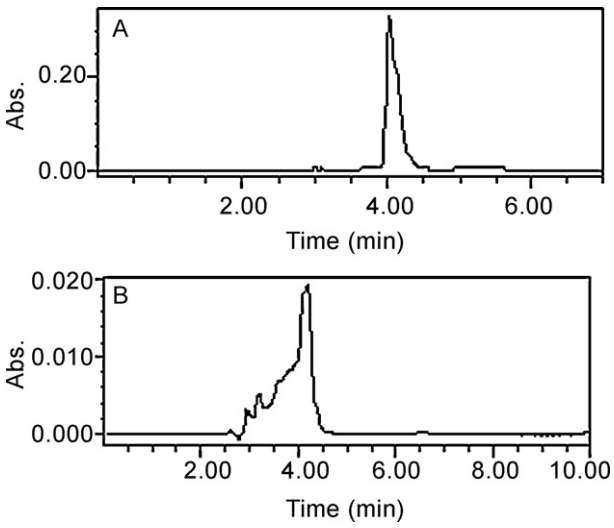

Fig. 6. HPLC chromatogram obtained on-line after clean-up a 50 ng/mL solution of dextromethorphan in human plasma samples with MIP (A) and (B) NIP monitored at 227 nm; conditions: column ACE C18, 5 μm, 4.6 mm × 250 mm, eluent: methanol:phosphate buffer (0.05 M, pH 5.0) (90:10) at flow rate of 1.0 mL/min.

increasing of the sample volume up to 50 mL, just had a light effect on the extraction of dextromethorphan.

3.5. Calibration curve and precision

For designing calibration curve of plasma samples, the area under the curves of dextromethorphan were plotted versus its concentration and linear regression analysis performed on the resultant curve. A correlation coefficient of 0.999 across the concentration range was obtained following linear regression analysis. Results from the on-line SPE-HPLC analyses showed that the MIP extraction of dextromethorphan is linear in the range of 0.3–150 ng/mL. The reproducibility and repeatability of the method were evaluated from run-to-run MISPE experiments (10 ng/mL standard solution, $n = 7$) and different batch experiments (fore batches) and RSDs of 3.2% and 4.4% for the extraction amounts of dextromethorphan were obtained, respectively.

3.6. Equilibrium batch rebinding and adsorption capacity

Equilibrium batch rebinding is one of the most general methods to evaluate the presence of cavities. A well-known amount of template is introduced in a vial with a given amount of MIP or NIP. Once the system has come to equilibrium, the amount of free template in the solution is measured to determine the amount of adsorbed template.

In the measurement of the adsorption capacity of MIP and NIP absorbents, the absorbents (25 mg) were added into 100 mL dextromethorphan solutions at concentrations of 10–500 μg/mL, and the suspensions were mechanically shaken for 30 min at room temperature, followed by centrifugally removing of the absorbents. The remained dextromethorphan in the supernatant was measured by HPLC, and the isothermal adsorptions are plotted in Fig. 5. According to these results, the maximum amount of dextromethorphan that can absorb by MIP was found to be 90 mg/g at pH 8.0. For higher dextromethorphan amounts, a slight increase of retained dextromethorphan was observed on MIP capacity curve. As all the accessible specific cavities of the MIP are saturated, the retention of the analyte is only due to non-specific interactions which can be approximately identical for MIP and NIP polymers.

3.7. Study of MIP selectivity

For surveying selectivity of dextromethorphan molecularly imprinted polymer, some drugs such as pseudoephedrine, aspirin, acetaminophen, diphenhydramine, tramadol, bromhexine and amoxicillin were selected. Their molecular structures are shown in Fig. 1. Solutions of all compounds were prepared individually with the concentration of 50 ng/mL. Elution solvent was methanol:phosphate buffer.

Batch rebinding experiment and chromatographic evaluations are the methods most customarily used to verify the selectivity of the imprinted materials [35]. For equilibrium batch rebinding experiments, a known mass of template in solution is added to a vial containing a steady mass of polymer. When the system came to equilibrium, the concentration of free template in solution is measured and the mass of template adsorbed to the MIP calculated [25]. The initial concentrations of drugs (50 ng/mL, 5 mL) were extracted by imprinted material at pHs of 6.0, 7.0 and 8.0 on MIP and NIP particles. The distribution ratio, selectivity coefficient and relative selectivity coefficients of dextromethorphan were determined by equation reported previously [31]. The results showed that the selectivity coefficients at pH of 8.0 were better than the others.

Distribution ratio (K_D), selectivity coefficient (k^{Sel}) and relative selectively coefficient (k') values of MIP and NIP material for these different drugs at pH of 8.0 are listed in Table 2. The data in Table 2 show that MIP exhibit moderately affinity for pseudoephedrine, tramadol, amoxicillin, aspirin, chloramphenicol, and bromhexine with the relative selectively coefficient of 14.0, 15.4, 11.8, 21.2, 12.7 and 16.3 respectively. This could be simply clarified by their close similarity to dextromethorphane in the way of the arrangement of the functional groups or the size of the three-dimensional structure.

3.8. Real sample analysis

The applicability of the extraction method to demonstrate the potential of MIP for the selective sample clean-up and on-line determination of the drug in real samples was examined by extraction and determination of dextromethorphan in the human plasma samples. The plasma samples were prepared according to Section 2.7. The results showed that the procedure can elute interferences and avoid contaminating HPLC column. The chromatograms obtained for plasma samples are compared in Fig. 6. This efficient method allowed cleaner extracts to be obtained and interfering peaks arising from the complex biological matrices to be suppressed. Results from the HPLC analyses showed that the MIP extraction of dextromethorphan for plasma samples have good pre-

Table 2
Distribution ratio (K_D), selectivity coefficient (k^{Sel}) and relative selectively coefficient (k') values of MIP and NIP material for different drugs.

Drug	K_D (MIP) (mL/g)	K_D (NIP) (mL/g)	k^{Sel} (MIP)	k^{Sel} (NIP)	k'
Dextromethorphan	2125	64	–	–	–
Pseudoephedrine	150	63	14.2	1.01	14.0
Tramadol	130	60	16.4	1.06	15.4
Amoxicillin	136	48	15.6	1.33	11.8
Aspirin	74	47	28.8	1.36	21.2
Bromhexine	139	68	15.3	0.94	16.3
Chlormphenicol	174	67	12.2	0.96	12.7

Table 3
Assay of dextromethorphan in human plasma by means of the described on-line SPE-HPLC procedure.

Sample	Spiked value (ng/mL)	Found[a] (ng/mL)	Recovery ± SD%	
			MIP	NIP
Human plasma	1	0.87	87.4 ± 4.2	12.7 ± 1.0
	5	4.55	91.0 ± 3.3	14.0 ± 1.2
	10	8.94	89.4 ± 3.2	13.3 ± 1.1
	50	46.0	92 ± 3.0	14.3 ± 1.1

[a] Average of three determinations.

cision (4.8% for 50.0 ng/mL) and recoveries (between 87.4 and 92%) (Table 3). The LOQ and LOD were determined based on a signal-to-noise ratios and were based on analytical responses of 10 and 3 times the background noise, respectively. The LOD was found to be 0.12 ng/mL with a resultant %R.S.D. of 3.35 ($n = 4$). The LOQ was found to be 0.35 ng/mL.

4. Conclusions

In this paper, for the first time, dextromethorphan-molecularly imprinted polymers were synthesized and were used for the preparation of MIP cartridges coupled on-line with high performance liquid chromatography. The automated SPE-HPLC method based on MIP has been developed for simple and rapid analysis of dextromethorphan in human plasma samples. This efficient method allowed cleaner extracts to be obtained and interfering peaks arising from the complicated biologic samples to be suppressed. The method was applied to the trace dextromethorphan determination at fore levels, and the recoveries for the spiked human plasma samples were higher than 87% in the range of 1–50 ng/mL. It could be concluded that the technique has great potential in developing selective extraction and on-line determination of other important compounds.

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