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Retention mechanism of analytes in the solid-phase extraction process using molecularly imprinted polymers Application to the extraction of triazines from complex matrices

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Abstract

Two molecularly imprinted polymers (MIPs), prepared in dichloromethane with terbutylazine and ametryn as template molecules, were evaluated for the selective extraction of triazines from complex matrices. Various parameters affecting extraction recoveries on MIPs were studied in order to obtain an optimized extraction procedure allowing to reduce non-specific interactions. In order to test the selectivity of the MIPs, the same procedure was applied to the extraction of compounds possessing the same polarity and size as the triazines. By means of molecular modeling, the effects of the electric charge distribution and of the size of the molecules on the retention mechanism were studied. The value of capacity for terbutylazine MIP was also measured. At last, the high selectivity resulting from the use of MIPs was clearly demonstrated by their applications to the clean-up of grape juice and soil extracts spiked with triazines. In addition, the soil extract was cleaned-up by immunoextraction allowing the comparison of both approaches in terms of selectivity.

Keywords: Retention mechanism; Molecularly imprinted polymers; Triazines

1. Introduction

The analysis of organic contaminants in complex matrices at low level of concentration requires a procedure of pretreatment in order to extract and preconcentrate the analytes. At present, solid-phase extraction (SPE) is routinely used as well for the extraction of compounds from liquid matrices as for the purification from solid matrices. The principle of the extraction is generally based on non-selective hydrophobic or polar interactions between the target molecule and the sorbent. However, as the environmental samples are often complex, a partial co-extraction of interfering substances can take place. In order to enhance the selectivity of the extraction, new selective materials involving a mechanism of molecular recognition were recently developed. A first approach consisted of the development of antibodies covalently bonded an appropriate sorbent to form a so-called immunosorbent (IS). The resulting immunoextraction method is based on the high affinity and the selectivity of antigen–antibody interactions that allows to selectively extract the target compound, and compounds possessing a similar structure, from complex matrices with high recoveries. Several reviews have been published in recent years reporting the interest of the immunoextraction as a selective sample pretreatment method [1–3]. This approach has been successfully applied to the selective extraction of the studied compounds, i.e. the triazines, from environmental liquid samples [4–6] and for the clean-up of soil extracts [7]. Nevertheless, the development of an IS is expensive and takes a long time. These drawbacks have led to the recent development of synthetic antibody mimics, so-called molecularly imprinted polymers (MIPs).

Molecularly imprinted polymers are new selective sorbents with molecular recognition sites designed for a particular analyte. Methods of imprinting are generally divided into covalent or non-covalent categories, depending on the nature of the interactions that were developed between the template molecule and the polymer during the synthesis [8]. The most common approach consists of a non-covalent

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imprinting that results from the complexation in solution of a template molecule with functional monomers, through non-covalent bonds. The choice of the solvent of polymerization and of the monomers happened to be critical for the resulting selectivity of the MIP [9–12]. The most commonly used monomer is methacrylic acid. The appropriate solvent of polymerization is generally an aprotic and a non- or weakly-polar solvent. Consequently, the main interactions that are developed between the template and the molecular imprint are hydrogen bonds or dipole–dipole interactions.

MIPs offer some advantages compared to ISs: they are easily and rapidly synthesized with a low cost and present a higher thermal and chemical stability [13] than ISs.

It has been largely demonstrated that MIPs offer the highest selectivity when samples were in the solvent used for the MIP preparation [12]. Consequently, when a methacrylate-based MIP is used for solid-phase extraction, a selective procedure involves the transfer of analytes in the appropriate organic solvent. In numerous procedures, a liquid–liquid extraction or a solid-phase extraction on classical sorbent is performed prior to the MIP application. In those cases, MIPs are just used as clean-up sorbents. MIPs have already been applied to biological samples [14–20] or environmental samples such as surface waters, soil extracts or red wine [21–24]. Several studies reported the development of MIPs for triazines showing the potential of using MIPs as class-specific sorbents [25–32]. For instance, the selectivity of a MIP for ametryn and other related triazines in real water was shown, by performing a first extraction onto a C18 silica cartridge before the clean-up on the MIP, but low extraction recoveries were obtained [30]. The operation could be automated by an on-line method using a similar approach involving two sorbents [26,28].

Therefore, the use of the MIP for solid-phase extraction does not appear so straightforward for providing both good recoveries and selectivity. The objective of this study was to optimize the extraction procedure for the class-extraction of the triazines group and metabolites. This optimization required a good knowledge of the retention mechanism in order to identify the nature of the interactions developed between the analytes and the MIP during the extraction process. Two methacrylate-based polymers synthesized in dichloromethane with terbutylazine and ametryn as templates were used for this study. A non-imprinted polymer was used for assessing the specificity of the interactions. Values of extraction recoveries were correlated to results from molecular modeling to explain the interactions involved in the retention process. The different parameters affecting the extraction recoveries in a SPE process such as the capacity, the solvent of percolation, the sample volume and the flow-rate were studied.

Finally, the selectivity of the extraction procedure was demonstrated by applying the MIP for the clean-up of grape juice and soil extract. This selectivity was also compared to the one obtained when applying immunoextraction to the same soil extract.

2. Experimental

2.1. Chemicals

Pesticides (triazines and phenylureas) were obtained from C.I.L. (Saint-Foy-la-Grande, France). Stock standard solutions of 100 mg/l were prepared by weighing the solutes and dissolving them in methanol or in a water–methanol (50:50) mixture for some degradation products of triazines. The stock solutions were stored at 4 °C. A standard solution of 5 mg/l was obtained by dilution in methanol from the stock solution. Methacrylic acid (MAA) and ethylene glycol dimethacrylate (EDMA) were purchased from Aldrich-Chemie (Steinheim, Germany) and distilled before use. Azo-*N*,*N*′-diisobutyronitrile (AIBN) was obtained from Janssen Chemica (Beerse, Belgium) and recrystallized from methanol before use. The UV-lamp used for the polymerization was a medium-pressure mercury vapor lamp (Philips, HPK 125 W). HPLC-grade acetonitrile and methanol were purchased from Mallinckrodt Baker (Deventer, the Netherlands) and dichloromethane was from Pestipur SDS (Peypin, France). High purity water was obtained from a Milli-Q purification system (Millipore, Saint-Quentin en Yvelines, France).

2.2. Apparatus and analytical conditions

The HPLC system was equipped of a Varian ProStar 400 autosampler, a Varian 230 solvent delivery unit and a Varian 330 Polychrom diode-array detector (Varian, Les Ulis, France). The triazines were monitored at 220 nm and the phenylureas at 244 nm. The reversed-phase column was an Equisil ODS 5 μm, 250 mm × 4.6 mm i.d. (CIL) that was connected to a precolumn (Hypersil 5 μm, 20 mm × 2.1 mm i.d., Colochrom, Gagny, France). The mobile phase was a mixture of acetonitrile and phosphate buffer (5×10^{-3} M, pH 7). The flow rate was set at 1 ml/min.

2.3. Synthesis of molecular imprinted polymers

EDMA (20 mmol), MAA (4 mmol), template (1 mmol) and AIBN (0.24 mmol) were added to the porogen (5.6 ml of dichloromethane) and transferred to glass polymerization tubes. The polymerization mixture was then purged with nitrogen for 10 min and placed into a water bath at 15 °C and allowed to equilibrate for 10 min. Polymerization was achieved by irradiating the solutions with a low pressure mercury lamp. After 24 h, the tubes were crushed, the polymers were ground and sieved. The 25–36 μm fraction (for the terbutylazine MIP) and the 25–50 μm fraction (for the ametryn MIP) were then slurried in MeOH/H_2O 90/10.

A non-imprinted polymer was obtained by performing the overall procedure in the absence of template.

2.4. SPE procedure

2.4.1. Study of the retention mechanism in dichloromethane

Cartridges of 3 ml were packed either with 170 mg of the terbutylazine and ametryn MIPs or with 170 mg of the non-imprinted polymer. Before each use, the sorbent was conditioned with 10 ml of dichloromethane. A solution of dichloromethane (10 or 25 ml) spiked with 500 ng of each pesticide was percolated through the cartridge. The second step of the extraction consisted of the removal of the remaining interfering compounds by percolating 10 ml of dichloromethane. The target analytes were eluted from the cartridge with 3 ml of methanol. This fraction was then concentrated up to dryness by a nitrogen stream and dissolved in 1 ml of a water–methanol (4:1) mixture; 100 μl were analyzed by reversed-phase HPLC.

2.4.2. Optimized extraction procedure on MIP of triazines from dichloromethane

Volumes of 10 ml of dichloromethane with 1% of methanol, spiked with 500 ng of each pesticide were percolated through the cartridge packed with 170 mg of terbutylazine and ametryn MIPs or with 170 mg of non-imprinted polymer. Except for the percolating step, the extraction procedure was carried out following the above procedure: 10 ml of dichloromethane are percolated through the sorbent for the washing step and the compounds are eluted by 3 ml of methanol. The eluted fractions were concentrated up to dryness by a nitrogen stream and dissolved in 1 ml of a methanol–water (1:4) mixture; 100 μl of each fraction were analyzed by reversed-phase HPLC.

2.4.3. Extraction of triazines from grape juice and soil extract

Extraction of triazines from grape juice: 10 ml of filtered grape juice (Whatman filter GF/C, 47 mm, 1.2 μm, Maidstone, England) were spiked at 10 μg/l with each triazine and percolated through a classical polymeric sorbent (SDB, 100 mg, 1080 m^2/g, J.T. Baker, Deventer, the Netherlands). At first, the sorbent was conditioned with 5 ml of methanol and 5 ml of pure water. After percolation of the sample, the sorbent was washed with 2 ml of pure water. The eluted step was carried out using 4 ml of methanol. This eluted fraction was concentrated up to dryness by a nitrogen stream and dissolved in a volume of 700 μl of a methanol–water (1:4) mixture; 100 μl of the fraction were analyzed by reversed-phase HPLC.

Extraction of triazines from a soil: 5 g of dried and sieved soil were spiked at 20 ng/g with a mixture of triazines and extracted by Microwave Assisted Solvent Extraction (Soxwave 100, Prolabo, Nogent sur Marne, France). The procedure was previously optimized in the laboratory: the extraction was carried out in 40 ml of a dichloromethane–methanol (9:1) mixture during 30 min by applying a power of 90 W. The extract obtained was evaporated up to dryness by a nitrogen stream and the dry residue was dissolved in a volume of 200 μl of a methanol–water (1:4) mixture; 50 μl of the fraction were analyzed by reversed-phase HPLC.

2.4.4. Clean-up on MIP of grape juice and soil extract

After the extraction of triazines from grape juice by the polymeric sorbent and from soil by solvent, the extracts were concentrated to dryness by a nitrogen stream and dissolved in a volume of 10 ml of dichloromethane with 1% of methanol in order to be percolated through the MIP. Ten milliliters of dichloromethane were percolated for the washing step and the compounds were eluted by 3 ml of methanol. The eluted fraction of the ametryn MIP used for the clean-up of grape juice was concentrated up to dryness by a nitrogen stream and dissolved in 700 μl of a water–methanol (3:1) mixture; 100 μl were analyzed by reversed-phase HPLC. The eluted fraction of the terbutylazine MIP used for the clean-up of soil extract was concentrated up to dryness by a nitrogen stream and dissolved in 200 μl of a methanol–water (1:4) mixture; 50 μl of the fraction were analyzed by reversed-phase HPLC.

2.4.5. Clean-up on immunosorbent of soil extract

An immunosorbent (IS) was used for the clean-up of the soil extract in order to compare both approaches in terms of selectivity. The procedure of extraction of triazines from the soil extract by Microwave Assisted Solvent Extraction was described in Section 2.4.3. The extract obtained was evaporated up to dryness by a nitrogen stream and the dry residue was dissolved in a volume of 5 ml of a methanol–water (2:98) mixture in order to be percolated through the immunosorbent.

The IS was based on polyclonal antibodies specific of triazines and packed in a 3 ml-cartridge: 2.5 mg of antibodies (CovalAb, Oullins, France) were immobilized on 250 mg of silica. The first step of the immunoextraction procedure consists of the conditioning of the sorbent with 5 ml of PBS (phosphate-buffered saline solution made on phosphate buffer 5×10^{-3} M containing 0.15 M sodium chloride) then 10 ml of pure water. After percolation of the sample through the cartridge, 3 ml of water were percolated to remove interfering compounds that are not specifically retained on the IS. The target analytes were eluted by 3 ml of a water–methanol (3:7) mixture. The eluted fraction was then concentrated up to dryness by a nitrogen stream and dissolved in 200 μl of a water–methanol (4:1) mixture; 50 μl of the fraction were analyzed by reversed-phase HPLC.

The IS was regenerated with 10 ml of water then 5 ml of PBS containing 0.1% azide and was stored at 4 °C.

2.5. Molecular modeling

Results of molecular modeling used in this work have been previously described [33]. This approach was per-

formed using the HyperChemPro 6.0 software package (Hypercube Inc., Gainesville, Florida, USA). First of all, molecular mechanic has permitted to find conformations of low energy; they are refined using semi-empirical mechanic. Finally, the conformation that possesses the lowest energy was refined with ab initio mechanic in order to obtain the electronic distribution.

2.6. Effect of the sample flow-rate and evaluation of the capacity of the terbutylazine MIP

An amount of 130 mg of terbutylazine MIP was packed into a column (50 mm × 4.6 mm i.d.) and connected to a Varian 2010 pump. The first step of the procedure consisted of conditioning the MIP with 10 ml of dichloromethane. Various volumes of a dichloromethane–methanol (99:1) mixture containing increasing amount of atrazine were percolated through the MIP. 1.3 ml of a dichloromethane–methanol (99:1) mixture was then percolated to provide maximal selectivity. The elution was carried out using 3 ml of methanol. This fraction of elution was then concentrated to dryness by a nitrogen stream and dissolved in various volume of a water–methanol (4:1) mixture, 100 μl were analyzed by reversed-phase HPLC.

3. Results and discussion

3.1. Selection of the analytes

Fig. 1 and Table 1 report the structure of the 14 selected triazines and commonly found degradation products. Chloro-, thiomethyl- and methoxy- triazines have been chosen for representing the triazines group. Triazines have a common structure; they only differ by the nature of the group in 2-position (Cl, SMe, OMe) in the heterocycle and the nature of the alkyl group of amine functions. The dealkylated degradation products, namely, deethylterbutylazine (DET), deethylatrazine (DEA), deisopropylatrazine (DIA), differ from chloro-triazines by their secondary amino-group substituent. Hydroxyatrazine (OHT) is the hydroxylated metabolite and differs by the nature of the substituent in the 2-position.

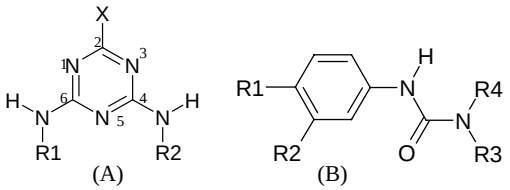

Fig. 1. General structures of triazines and phenylureas. (A) Triazines: X: Cl, OMe, SMe, OH; R1, R2: H, alkyl groups, CN. (B) Phenylureas: R1–R4: Cl, OMe, H, alkyl groups.

Table 1
Structure of studied triazines and their metabolites

Compounds	X	R1	R2
Ametryn	MeS	C_2H_5	$CH(Me)_2$
Prometryn	MeS	$CH(Me)_2$	$CH(Me)_2$
Terbutryn	MeS	$CH(Me)_2$	C_2H_5
Simazine	Cl	C_2H_5	C_2H_5
Cyanazine	Cl	C_2H_5	CMe(CN)
Atrazine	Cl	$CH(Me)_2$	C_2H_5
Sebutylazine	Cl	C_2H_5	CHMe(Et)
Propazine	Cl	$CH(Me)_2$	$CH(Me)_2$
Terbutylazine	Cl	$C(Me)_3$	C_2H_5
Prometon	MeO	$CH(Me)_2$	$CH(Me)_2$
DET	Cl	$CH(Me)_2$	H
DIA	Cl	H	C_2H_5
OHT	OH	$CH(Me)_2$	C_2H_5

3.2. Retention mechanism in dichloromethane

The studied MIPs were synthesized in dichloromethane with methacrylic acid as monomer. With this solvent, the main interactions that are developed between the template and the molecular imprint are hydrogen bonds and dipole–dipole interactions. As MIPs are known to offer the highest selectivity when samples are in the solvent used for polymerization, our first experiments have been carried out with dichloromethane samples spiked with each triazine or degradation product. Two samples of 10 and 25 ml of dichloromethane were spiked with 500 ng of each analyte and were percolated, respectively, through the terbutylazine MIP, the ametryn MIP and the non-imprinted polymer. After a washing step using 10 ml of pure dichloromethane, the elution step was accomplished using 3 ml of methanol. The role of this polar and protic solvent is to strongly interact, via hydrogen bonds, with the polymer to disrupt these interactions initiated by the compounds.

The recoveries obtained using the ametryn MIP are reported in Fig. 2a. Similar results have been obtained with the terbutylazine MIP. Recoveries obtained for all compounds were higher than 90% for both volumes. The recoveries obtained using the non-imprinted polymer are reported in Fig. 2b. Recoveries are low for all triazines except for prometon and for the three dealkylated metabolites for both sample volumes and close to 100% for the hydroxy-metabolite. Furthermore, the recoveries on the non-imprinted polymer decrease when the sample volume increases from 10 to 25 ml.

The comparison of the results obtained with the imprinted and the non-imprinted polymers allows to conclude on the specificity of the interactions between the analytes and the MIP. All triazines are specifically retained on the MIP except for prometon and four compounds that are retained by non-specific interactions since their retention occurs on the non-imprinted polymer. In order to understand the effect of the structure of compounds, the partial charge brought by the atoms and their molecular volume were calculated for each molecule by molecular modeling. The detailed results have already been published in [33]. The mean values of

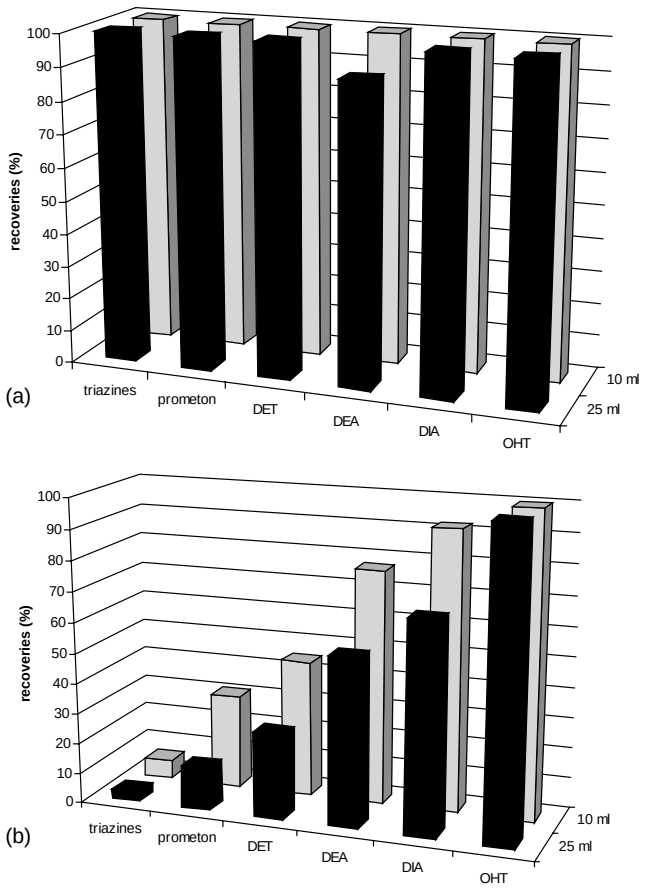

Fig. 2. Extraction recoveries (%) obtained for triazines (mean values for the whole group of studied triazines) and their metabolites after the percolation of 10 and 25 ml of dichloromethane spiked with 500 ng of each analyte on the: (a) ametryn MIP and (b) non-imprinted polymer. For 10 ml, $n = 3$, R.S.D. varied between 0 and 8%.

the partial charges are reported in Table 2. The molecular modeling method shows that there is a few delocalization of pairs of electrons of the nitrogen atoms since the three nitrogen atoms of the heterocycle and both nitrogen atoms of the secondary amine function have a partial negative charge. So, each nitrogen atom of the molecules is a potential hydrogen bond acceptor. Concerning the partial charge of the groups in 2-position, the molecular modeling indicates a positive charge for the chlorine atom. This positive value can be explained by the ratio between inductive and mesomeric effect that is in favor of the mesomeric effect: it possesses a strong electronegative inductive effect but a conjugation with the PI system of the heterocycle also occurs. The thiomethyl group also carries a positive charge, which is also due to the delocalisation of the pair of electrons on the PI system. The oxygen atom of the hydroxyl group of OHT and methoxy group of prometon bear a negative charge. The methoxy group can only be an acceptor of hydrogen bonds, whereas the hydroxyl group is both a donor and an acceptor.

Table 2
Main results from molecular modeling using the HyperChemPro 6.0 software package [33]

Mean value of the charge brought by the atom		Ability to form hydrogen bonds
Nitrogens of cycle	−0.740	Acceptor
Nitrogen of amine group	−0.860	Acceptor
Sulfur of thiomethyl group	+0.546	No hydrogen bond
Chlorine	+0.270	No hydrogen bond
Oxygen of methoxy group	−0.690	Acceptor
Oxygen of hydroxyl group	−0.695	Acceptor and donor

In pure dichloromethane, the retention of compounds due to binding with imprints or due to adsorption to polymer surface is based on the same mechanism: compounds develop in each case hydrogen bonds but with different energy levels. Triazines (except for prometon) are specifically retained on the imprinted polymers by several points of hydrogen bonding. By the result of molecular modeling, we can assume that these interaction sites were created during the synthesis by the development of hydrogen bonds between the basic nitrogen atoms of the template and the hydrogen of the carboxylic groups. To create several hydrogen bonds in the imprint, there is relatively strict geometric requirements such as the angle and the distance between donor and acceptor functionalities that are crucial for the overall gain in binding energy. For the non-imprinted polymer, triazines can develop hydrogen bonds but these interactions will always be of lower energy because there are no predefined interactions sites. Hence, triazines develop certainly hydrogen bonds with the non-imprinted polymer but these interactions are not strong enough for a retention. Nevertheless, a retention occurs with prometon on the non-imprinted polymer. This retention can be explained by the addition of an acceptor group, which is able to provide a strong hydrogen bond on polymer surface. The three dealkylated metabolites are also non-specifically retained. The studied metabolites have a primary amine function. The basic nitrogen of this function is more accessible than the nitrogen atom of the secondary amine function in triazines to be able to develop hydrogen bonds. As for prometon, the occurrence of a stronger interaction with the polymer involves a retention on the polymer surface without requiring several points of attachment. The effect of this accessibility to the nitrogen atom was demonstrated by the recoveries obtained for the three metabolites on the non-imprinted polymer. DET, which has the bulkiest alkyl group, i.e. tertiobutyl group, presents the lowest extraction recovery compared to the two other metabolites. The hydroxyl group of OHT is both a strong donor and a strong acceptor of hydrogen bonds. Consequently, it is strongly retained by the non-imprinted polymer.

The influence of samples volumes on recoveries on the non-imprinted polymer can be easily explained because it behaves as a normal-phase SPE sorbent. In SPE process, there is an analogy with liquid chromatography. The analyte retention occurs as long as the analyte is not eluted by the mobile phase, i.e. dichloromethane. Elution occurs when the breakthrough volume is overloaded thus explaining that the recovery decreases when the sample volume increases.

Breakthrough has occurred with 10 ml samples for prometon, DEA and DIA, and recoveries decrease when the sample volume is 25 ml. But concerning the OHT, no breakthrough occurs for 25 ml due to a strong interaction by hydrogen bond between the hydroxyl group and the polymer network.

Two parameters govern the formation of hydrogen bonds: the nature of the chemical group in 2-position, i.e. its residual charge that defines its ability to develop hydrogen bonds, and the accessibility of the nitrogen atom that belongs to the primary amine function. Selectivity is, therefore, limited when applying MIP to compounds possessing chemical groups such as hydroxyl group able to initiate a strong hydrogen bond. In these conditions, the compound do not need to interact with the different sites of recognition within imprints because the developed hydrogen bond with the polymer network is strong enough. With regards to other analytes that can be present in real samples, one can expect a strong retention of some polar analytes having functional groups able to provide hydrogen bonds with the carboxylic groups. Therefore, it is important to decrease as much as possible these non-specific interactions.

3.3. Optimisation of the extraction procedure to reduce the amount of non-specific interactions

In order to decrease the non-specific interactions and obtain maximal selectivity, 1% methanol is added to dichloromethane during the percolation of the sample. Methanol was selected for its high eluting strength. However, this amount of methanol should be as low as possible because it has to decrease the retention of compounds retained on the residues of monomers at the surface of the polymer without affecting the overall retention in the imprints. So, in the second series of experiments, 10 ml of a dichloromethane–methanol (99:1) mixture spiked with 500 ng of each triazine are percolated on the MIP cartridge followed by a washing step carried out with 10 ml of dichloromethane and by an elution with 3 ml of methanol. The extraction recoveries obtained on both terbutylazine and ametryn MIPs and on the non-imprinted polymer are reported in Table 3. The addition of 1% of methanol causes a significant drop in extraction recoveries on the non-imprinted with 0% extraction recoveries for all compounds except for OHT. The strong retention of OHT on the non-imprinted polymer was previously explained by the presence of the hydroxyl group in the 2-position developing very strong hydrogen bonds with the monomers. For other compounds, the addition of 1% of methanol allows the removal of non-specific interactions meaning that their retention on the MIP results from specific interactions with the imprints.

The comparison of the extraction recoveries obtained on both MIPs demonstrates the difference in specificity between the two MIPs. The ametryn MIP retains strongly all triazines and their metabolites because high extraction recoveries were obtained in the 72–100% range for each analyte (with the exception of terbutryn with 60% extraction recovery). In contrast, low extraction recoveries were obtained for the thiotriazines on the terbutylazine MIP which is more specific to the chlorotriazines and their metabolites. Thiotriazines possess a thiomethyl group that is larger than the chlorine atom of the terbutylazine template. A steric hindrance phenomenon limits the access to the designed cavities. In addition, the electric charge distribution in thiotriazines contributes to a poor development of hydrogen bonds involving a lower retention on the MIP when 1% methanol is added to dichloromethane.

Table 3
Extraction recoveries (%) obtained on the ametryn and terbutylazine MIPs and on the non-imprinted polymer for triazines and metabolites after the percolation of 10 ml of a dichloromethane–methanol (99:1) mixture spiked with 500 ng of each analyte ($n = 3$, R.S.D. varied between 0 and 8%)

MIP	Ametryn	Terbutylazine	Non-imprinted
Ametryn	78	26	0
Prometryn	81	31	0
Terbutryn	60	25	0
Simazine	93	100	1
Cyanazine	82	100	1
Atrazine	88	100	0
Sebutylazine	72	98	1
Propazine	87	100	0
Terbutylazine	77	100	1
Prometon	100	100	1
DET	100	100	2
DIA	98	97	9
OHT	100	100	100

The specificity of retention on the terbutylazine MIP was also demonstrated by studying the retention of eight phenylurea herbicides having a molecular weight and a polarity similar to triazines and able to develop hydrogen bonds. The structure of phenylureas are presented in Fig. 1. Samples made of 10 ml of a dichloromethane–methanol (99:1) mixture spiked with 500 ng of each phenylurea were percolated on the terbutylazine MIP cartridge followed by a washing step carried out with 10 ml of dichloromethane and an elution step with 3 ml of methanol. Phenylureas were not retained on the non-imprinted polymer, demonstrating thus that the conditions of percolation (1% of MeOH in dichloromethane) allows to avoid retention by non-specific interactions as for triazines. Concerning the retention on the imprinted polymer, the presence of specific cavities designed for the template and compounds belonging to the same structural family is demonstrated because the phenylureas are also not retained on the terbutylazine MIP. This result confirms the high selectivity of the extraction on MIP that requires a perfect match between the structure of the molecule and the imprints.

3.4. Effect of the sample flow-rate

As the slow mass transfer characteristic of polymers was already demonstrated concerning the MIPs, a kinetic study was done following the protocol described in Section 2.6.

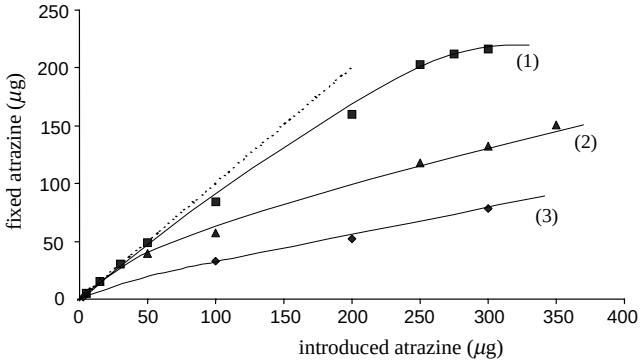

Fig. 3. Curves of capacity obtained after the percolation of: (1) 5 ml, (2) 10 ml and (3) 25 ml of a dichloromethane–methanol (99:1) mixture spiked with increasing amount of atrazine on the terbutylazine MIP (130 mg). The dotted line corresponds to a slope of 1 meaning an extraction recovery of 100%.

For this, 5 ml of a solution spiked with 50 μg of atrazine were percolated through the column and extraction recoveries were measured with several flow rates of percolation (0.3–2 ml/min). The same extraction recovery was obtained for all experiments with an average of 88% of recovery and a R.S.D. of 6%. This result showed that, within the studied range, the flow rate had no effect on the extraction recoveries. A flow rate of 0.5 ml/min was selected for each and every experiment.

3.5. Evaluation of the capacity of the terbutylazine MIP: evidence for heterogeneous interactions sites

The complete characterization of a MIP requires the measurement of its capacity. The capacity corresponds to the maximum amount of a compound that can be retained on a MIP in given conditions (nature and volume of the percolated sample). Therefore, the determination of the capacity was performed by measuring the extraction recoveries after the percolation of a dichloromethane–methanol (99:1) mixture containing various amount of atrazine on the terbutylazine MIP. The addition of methanol guarantied the removal of non-specific interactions with the MIP (as previously described) thus ensuring retention on the MIP only by specific interactions. The terbutylazine-template was replaced with atrazine in order to solve the problem of leakage of the template trapped in the polymer network and altering experimental results. These experiments were carried out at a constant flow rate of 0.5 ml/min. Three curves of capacity were drawn for different volumes of percolation (5, 10 and 25 ml). Fig. 3 shows the amount of atrazine bound on the terbutylazine MIP according to the amount introduced in the column during the percolation process. The curve obtained with a percolated volume of 5 ml presents a linear part for the lowest percolated amounts of atrazine and tends to reach a plateau for the highest ones. The linear part (observed for percolated amounts of 0–60 μg) corresponds to the extraction of atrazine with constant recoveries of 100%. In this range, a quantitative extraction can be carried out. Passed this range, i.e. 60 μg, a decrease in recoveries is observed. Therefore, for a reliable quantitative method, the total amount of triazines in unknown samples should not be higher than 60 μg or 460 μg/g. By comparison with the immunosorbents, the capacity value is on average thirty times higher than the capacity of an IS [1]. It is another advantage of using MIP instead of IS for a selective extraction. This value is also in accordance with other studies that report capacity values in 1 and 40 μmol/g range [34–37]. It is important to notice that our experiments are carried out by percolating the sample through the MIP, consequently, no isotherm as a Scatchard plot analysis could be carried out in order to estimate dissociation constants [35,38] because the equilibrium that governs the partition of the studied compound between the MIP and the percolated solution is never reached.

For a percolated amount higher than 60 μg, the extraction recoveries decrease and the fixed amount tend to reach a plateau at 220 μg which appears to be the amount at which binding cavities are saturated. When increasing the percolated volume up to 10 ml, the upper limit of the calibration is slightly lower than for 5 ml sample. Then, the curve tends to reach a plateau with a lower introduced amount than for 5 ml percolated volume. This phenomenon can be explained by the heterogeneous surface of the polymer involving the presence of binding sites or cavities of different energy levels. In fact, the experimental plotted curve of capacity can be considered as the sum of different curves of capacity, each one corresponding to one type of binding sites. The percolation of a small volume of sample, i.e. 5 ml, allows to the analytes to interact with a larger number of binding sites than when percolating a higher volume of sample as 10 ml. In the last case, the breakthrough volume for some binding sites was attained. Consequently, the retention is only ensured by the binding sites of higher energy of interaction. When increasing the volume up to 25 ml, the number of accessible binding sites decrease and still causes decreases in the capacity. So, the upper limit of the calibration range estimated at 60 μg corresponds to the saturation of a group of selective binding sites while the plateau observed for 220 μg corresponds to the saturation of other binding sites of lower energy yet specific for the retention of the target analytes because no retention is observed on the non-imprinted polymer.

To conclude, the heterogeneity of the binding sites is again demonstrated but it is not a limiting factor for using MIP in selective SPE. In fact, even if the retention mechanism of triazines results from interactions with different binding sites, the retention remains always selective since the studied compound is not retained on the non-imprinted polymer. And to establish a quantitative extraction method, the analyst just has to check that the level of contaminants in the sample stays in the range of concentration that corresponds to the linear part of the curve. For higher amount of compounds, the sample has to be diluted or the amount of MIP in the cartridge must be increased.

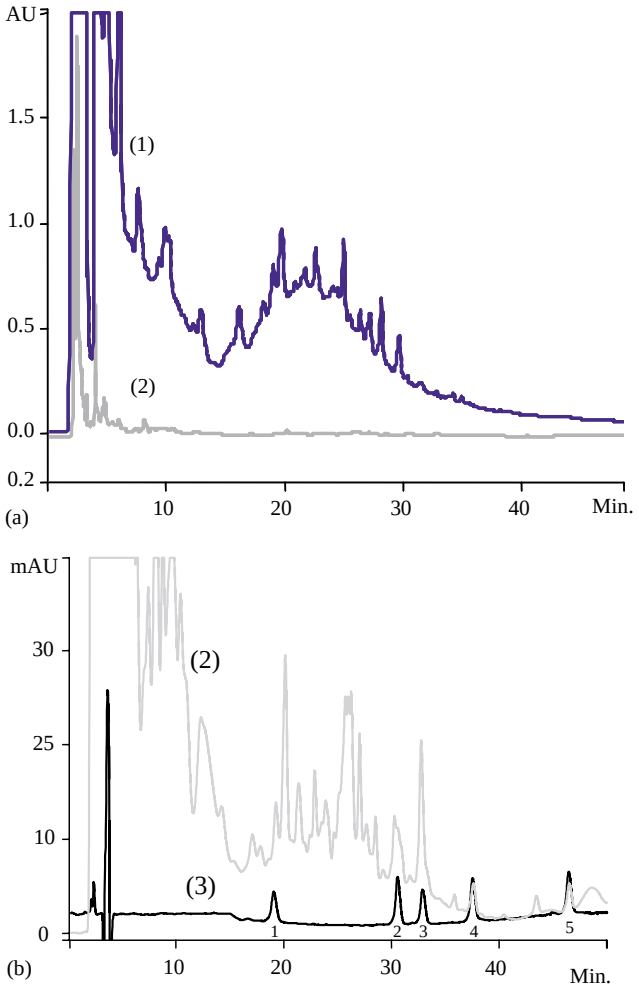

Fig. 4. (a) Chromatograms obtained after the preconcentration of 10 ml of grape juice spiked at 10 μg/l of a mixture of triazines through the SDB sorbent: (1) without and (2) with a clean-up on the ametryn MIP. (b, 2) Chromatograms resulting from the clean-up on MIP (2) and of the direct injection of standard solution containing 15 ng of each compound (3). (1) DEA; (2) simazine; (3) DET; (4) atrazine; (5) terbutylazine. UV detection at 220 nm.

3.6. Selective extraction of triazines from complex matrices

To demonstrate the potential of MIPs for the selective clean-up of complex matrices, they were applied to the purification of a grape juice and a soil extract. At first, 10 ml of filtered grape juice spiked with 10 μg/l of each triazine were preconcentrated on a classical styrene divinylbenzene (SDB) sorbent. The resulting extract was then purified on the ametryn MIP (see Section 2.4.3). The resulting chromatogram is compared to the direct injection of the SDB extract (without the clean-up on MIP) in Fig. 4a. By using only the SDB sorbent, many interfering compounds are co-extracted and appeared in the chromatogram thus preventing a thorough detection of the triazines. In contrast, the purification of the SDB extract on the ametryn MIP allowed to remove most of the interfering compounds. The comparison, in the Fig. 4b, of the chromatograms resulting of the purification on MIP and the standard injection shows that the identification and quantification of the target analytes are easier. It demonstrates that a real contribution of selectivity is brought by the MIP when applied to a complex matrix such as a grape juice.

The potential of the MIP was also tested for the purification of a solid matrix extract and results were compared to those obtained using an immunosorbent based on anti-triazines polyclonal antibodies immobilized onto sil-

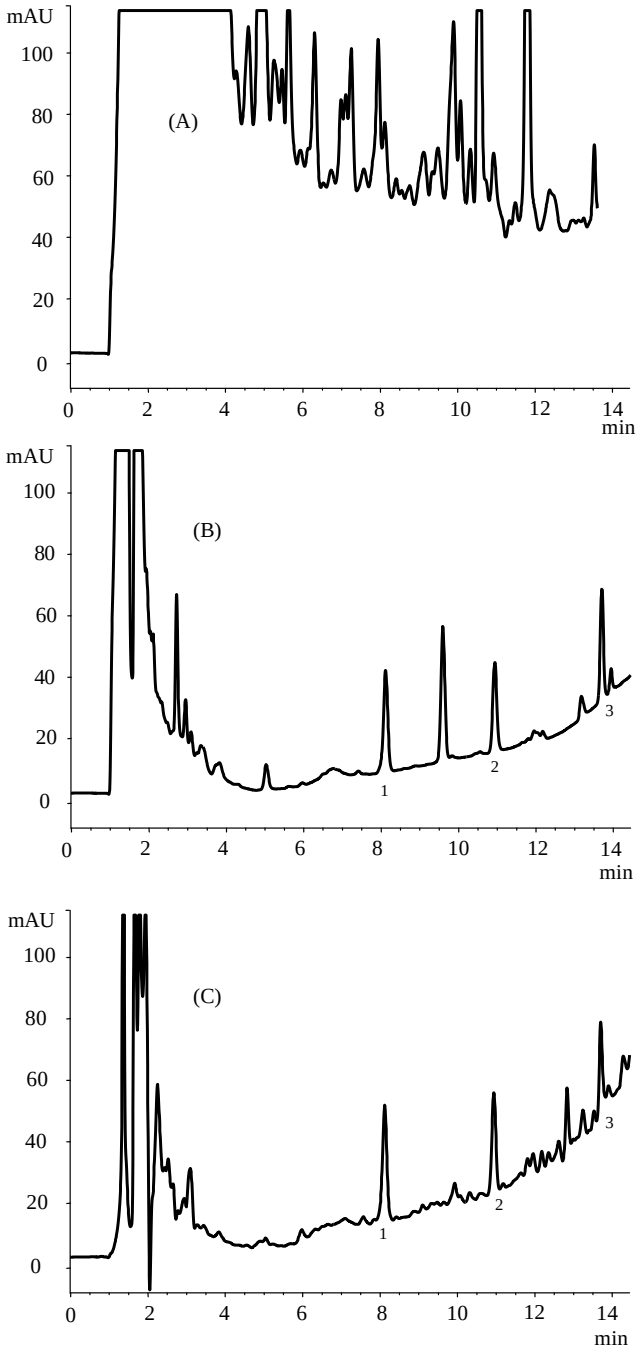

Fig. 5. Chromatograms obtained after the injection of a soil extract containing 20 ng/g of triazines (A) without and (B) with a clean-up on the terbutylazine MIP and (C) on the anti-triazines immunosorbent. (1) atrazine; (2) simazine; (3) terbutylazine. UV detection at 220 nm.

ica. It was then interesting to compare this selective support with the MIP in terms of selectivity because, at present, IS are more used than MIP for multi-residue analysis of pesticides. A soil from the Burgundy area (France) was spiked at 20 ng/g with a mixture of triazines and was extracted by Microwave Assisted Solvent Extraction (see Section 2.4.3). The chromatograms obtained after direct injection in HPLC of the soil extract, after the clean-up using the terbutylazine MIP, or using the immunosorbent are presented in Fig. 5. By comparing the clean-up of the MIP with the direct injection of the extract, the potential of MIP for the clean-up is largely demonstrated. As a matter of fact, the chromatogram shows a cleaner baseline with the clean-up on MIP. It allows an easy identification and quantification of the three spiked compounds. Also, the chromatogram obtained by immunopurification showed that the benefit in selectivity from MIP is comparable to the selectivity brought by the immunosorbent. In conclusion, for this application, MIP can be considered as real synthetic antibodies.

4. Conclusion

This study has shown the potential of MIPs for the rapid clean-up of extracts from complex samples. The ametryn MIP was shown to be highly class-selective for triazines and their degradation products. This selectivity can only be obtained after the removal or reduction of non-specific interactions on the MIP. It requires a drastic selection of the organic solvent or mixture used to dissolve the extract before the percolation on MIP. Molecular modeling provided a better understanding of the retention mechanism involved in the SPE procedure and was a useful guide for reducing non-specific interactions.

With an optimized procedure, a high selectivity can be obtained for the treatment of complex samples and it is similar to that obtained with immunosorbents. MIPs present a number of advantages compared to antibodies with respect to their ease, cost and time of preparation. In addition, their high capacity indicates a great potential for the miniaturization of the analytical system.

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