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Molecularly imprinted polymer cartridges coupled to high performance liquid chromatography (HPLC-UV) for simple and rapid analysis of fenthion in olive oil

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ABSTRACT

A combination of molecular modelling and a screening of the library of non-imprinted polymers (NIPs) was used to identify acrylamide as a functional monomer with high affinity towards fenthion, organophosphate insecticide, which is frequently used in the treatment of olives. A good correlation was found between the screening tests and modelling of monomer–template interactions performed using a computational approach. Acrylamide-based molecularly imprinted polymer (MIP) and non-imprinted polymer (NIP) were thermally synthesised in dimethyl formamide (porogen) using ethylene glycol dimethacrylate as a cross-linker and 1,1-azo-bis (isobutyronitrile) as an initiator. The chemical and physical properties of the prepared polymers were characterised. The binding of fenthion by the polymers was studied using solvents with different polarities. The developed MIP showed a high selectivity towards fenthion, compared to other organophosphates (dimethoate, methidathion malalthion), and allowed extraction of fenthion from olive oil samples with a recovery rate of about 96%. The extraction of fenthion using MIPs was much more effective than traditional C_{18} reverse-phase solid phase extraction and allowed to achieve a low detection limit (LOD) (5 $\mu g\ L^{-1}$).

1. Introduction

Organophosphorus pesticides (OPs) are comprised within the ten most widely used classes of pesticides all over the world [1]. OPs toxicants exhibit their effects by inhibition of acetylcholinesterase, which leads to the accumulation of the neurotransmitter acetylcholine in synapses and overstimulates the post synaptic cholinergic receptors with consequent signs of neurotoxicity [2–4]. These compounds, after being applied, remain in the environment for days and even months, depending on their intrinsic properties as well as climatic conditions. Therefore, non-target organisms, such as human, fish, bees and so on, are also threatened by these insecticides. Among this family of pesticides, fenthion occupies a prominent position since it is applied in many countries on a very large number of crops to combat agricultural buds and mosquito pests, respectively [5]. However, based on its high toxicity for birds, fenthion has been banned from the lists of plant protection products in the European Union countries, United States, Canada, and New Zealand.

Nevertheless, fenthion is still widely used in many countries and it is the most frequently found insecticide in Moroccan olive oils. Fenthion is slowly degraded in olives, the main degradation pathway consisting in an oxidation to fenthion-sulphoxide, which displays a higher biological activity than the parent compound [6]. Subsequent oxidation of sulphoxide to sulphone, a compound with lower biological activity, occurs with a slow rate in plants [5–7]. Another possible route of bioactivation consists in oxidative enzymatic desulphuration leading to fenthion-O-analogue (fenoxon) [5–7]. In the olive fruit pulp the major compounds found are fenthion and fenthion-sulphoxide, and small amounts of fenthion-sulphone and fenoxon. These metabolites tend to partition into the olive oil [8], where fenthion is usually found because of its lipophilic properties ($\log P_{oct/wat} = 4.8$) [9]. FAO, WHO and Codex Alimentarius Commission have established a maximum residue limit (MRL) of 1 $mg\ kg^{-1}$ for fenthion and its metabolites, determined separately or together and expressed as “fenthion” equivalent in olive oil [10,11].

For qualitative and quantitative purposes, the determination of fenthion in oils is frequently performed by gas chromatography coupled to tandem mass spectrometry (GC–MS/MS) [7,12–20].

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Since olive oil is a complex matrix, different clean-up procedures must be performed to avoid interferences with pigments, polyphenols and lipid material present in the olive oil. Liquid–liquid partitioning with different solvents is generally performed; acetonitrile being the most common one [21,22]. In other cases, a clean-up step by solid–liquid extraction is included [23], or sometimes olive oil samples are just diluted in cyclohexane. Other isolation methods include headspace solid-phase microextraction [13] and reversed-phase liquid chromatography/gas chromatography coupled with automated through-oven transfer adsorption–desorption interface [18].

Current trends for the control of the fenthion in olive oil are focused on the development of molecularly imprinted solid phase extraction (MISPE) which has already been successfully applied for pre-concentration and trace detection of other organophosphorus pesticides in the olive oil [24,25]. The present paper describes the synthesis and the evaluation of molecularly imprinted polymers (MIPs) for the selective extraction of fenthion. To the best of our knowledge, molecular imprinting has not been used before in order to pre-concentrate and purify these pesticides from such a complex matrix as olive oil. The obtained MIP was applied as a SPE sorbent to extract fenthion from olive oil, and the obtained results were compared with those achieved after conventional clean-up using reverse phase SPE.

2. Material and methods

2.1. Chemicals and stock solutions

The organophosphorus insecticides malathion, fenthion, dimethoate, fenthion-sulphoxide and methidathion were purchased from Sigma-Aldrich. Pesticide stock solutions (concentration 1 g L^{-1}) were prepared in acetonitrile (Sigma-Aldrich) and stored at 4 °C. Acrylamide, ethylene glycol dimethacrylate (EGDMA), 1,1-azo-bis (isobutyronitrile) (AIBN), trifluoroacetic acid (TFA), acetic acid, dimethyl formamide (DMF), hexane, toluene, chloroform, dichloromethane, methanol and acetonitrile were purchased also from Sigma-Aldrich (France). The 3-mL reservoir glass columns with frits were purchased from Chromabond (France) and the glass microplates containing a library of NIPs was prepared by S. Piletsky (UK).

2.2. Chromatographic evaluation

The quantification of pesticides was performed using an HPLC L-2000 series LaChrom Elite® system from Merck-Hitachi (VWR, France). Chromatographic separations were carried out with a C_{18} Supelcosil™ reverse phase column (250 × 4.6 mm, 5 μm) using a acetonitrile/water mixture (50/50, v/v) as a mobile phase at flow rate 1 mL min^{-1}. The injection volume was 20 μL. Methidathion, malathion, fenthion and dimethoate were detected at a wavelength of 220 nm, and data acquisition was performed using the EZchrom Elite software. Analysed samples (standards and recovered insecticide) were dissolved in the eluent phase (acetonitrile/water, 50/50, v/v). The quantification of OPs was conducted by measuring the peak area and comparing it with the relevant calibration curve. Standard solutions for the calibration curve were prepared by dilution of the stock solution with a concentration of 1 g L^{-1} in acetonitrile in the mobile phase solvent.

2.3. Computational screening of monomers capable to interacting with fenthion

The workstation used to simulate monomers/template interactions was Research Machines running the CentOS 5 GNU/Linux operating system. It was configured with a 3.2 GHz core 2 duo processor, 4 GB memory. This system was used to run the SYBYL 7.3 software suite (Tripos Inc., St. Louis, MO, USA). The structure of fenthion was minimised and screened against 20 functional monomers using the LEAPFROG algorithm as described earlier [26]. Based on the binding energy values of electrostatic, hydrophobic, Van der Waals forces, and dipole–dipole interactions, the monomers with the highest binding energies were selected for the polymer preparation [27].

2.4. Preparation of imprinted polymers

The preparation of imprinted polymers was performed as follows. The molar ratio of the template, functional monomer and cross-linker was 1:4:20, respectively. The polymer was synthesised by mixing 1 mM fenthion (template), 20 mM EGDMA (cross-linker), 40 mg of AIBN (initiator) and 4 mM of acrylamide (functional monomer) in 5 mL of DMF. The flask was sealed and after the mixture solution was degassed under nitrogen for 10 min, and then it was incubated in the oil bath at +80 °C for 16 h. The polymers' monoliths were crushed, ground and sieved through the sieve with pores around 45–100 μm diameter. The particles with size between 45 and 100 μm were collected. The polymers were washed with methanol/acetic acid (90:10, v/v) in a Soxhlet apparatus until no further template could be detected by HPLC-UV analysis. Washing with methanol was then used to remove the residual acetic acid, and the polymers were dried in an oven at +70 °C. The corresponding NIPs were prepared as a control in parallel in the absence of the template and treated in the same manner.

2.5. MISPE procedure

MIP and NIP particles (50 mg) were packed into 3-mL SPE cartridges, which were placed in a vacuum manifold, connected to a vacuum pump. The cartridges were conditioned with 2 mL of methanol and 1 mL of hexane. Samples of hexane or olive oil were spiked with 1 μg of fenthion and loaded into the cartridges. The cartridges were washed with 2 mL of dichloromethane containing 5% acetonitrile, and then eluted with 1 mL of methanol acidified with 2% TFA. Each extracted fraction was collected and evaporated at 50 °C using rotary evaporator and was reconstituted in 1 mL of acetonitrile/water (50/50, v/v) prior to HPLC-UV analysis.

2.6. Olive oil clean-up

2.6.1. Clean-up using MIP cartridge

Commercially available extra virgin olive oil was purchased in a local supermarket and spiked with various concentrations of pesticide in hexane. The mixture was incubated at +45 °C for 30 min, stirred and then 1-mL aliquot of oil was diluted in 9 mL of hexane, stirred for 5 min, and then filtered through SPE cartridges containing either NP or imprinted acrylamide-based polymers. All interfering components, which were present in olive oil, were removed using the optimised washing solution, and the elution was performed using 1 mL of methanol containing 2% TFA. The fraction was collected and evaporated to dryness. The residue was dissolved in 1 mL of a water–acetonitrile (50:50) mixture, and analysed using a reversed-phase analytical HPLC set-up.

2.6.2. Clean-up using C_{18} cartridge

The C_{18} cartridge was used for the clean-up of the olive oil in order to compare its selectivity with results obtained using imprinted polymers. The following procedure of extraction and quantification of fenthion from the olive oil using a C_{18} cartridge

was applied. In order to extract the insecticide from olive oil for the C_{18} experiment, 1 mL of olive oil containing 1 μg of fenthion was transferred into a separating funnel, the beaker was rinsed at least twice with 25 mL of acetonitrile. The separating funnel was agitated vigorously for at least 2 min and placed in a freezer in a horizontal position at 18 °C during 3 h. After that the phase separation acetonitrile fraction was collected and evaporated to approximately 3 mL using a rotary evaporator.

The solid phase extraction (SPE) of fenthion on the C_{18} column was made according to the following protocol: the cartridge was washed with 12 mL of methanol and conditioned with 12 mL of acetonitrile, and then 3 mL of the solution spiked with fenthion was loaded and filtered. The retained molecules were eluted with 1.5 mL of methanol. The eluted solution was evaporated and reconstituted in 1 mL of a water–acetonitrile (50:50) mixture which was analysed using HPLC.

2.7. Experimental validation

The absorption of pesticides from the model heptane solution was tested using a glass microtiter plate containing 20 NIPs which were immobilised on the surface of the wells. The binding affinity of polymers towards the organophosphorus pesticides was validated based on the measurement of the optical density of the solution of pesticide before and after 1-h incubation in the microtitre plate. Absorption measurements have been performed using a UV–vis spectrophotometer (Shimadzu, Tokyo, Japan) at a wavelength of 220 nm.

3. Results and discussion

3.1. Monomer screening

Since the selection of suitable functional monomers is a time-consuming stage in the development process of MIPs, computational modelling has become a versatile tool which allowed to reduce the time of experiments [28]. In the first step, a virtual library of nine functional monomers was created and screened for all possible interactions between the monomers and the template molecule in the pre-polymerisation complexes. The structure of the template molecule is presented in Fig. 1, and binding energies between the template and functional monomers are given in Table 1.

The selection of the suitable monomers is based on the rule that the monomer, which forms the most stable complex with a given template, would be suitable for the production of MIPs with good recognition properties [29,30]. According to the results of computational modelling, acrylamide was identified as the most promising monomer due to its strong interactions with fenthion (binding energy -29.07 kcal mol^{-1}) (Table 1). The two other functional monomers, which demonstrated high binding energies towards fenthion, were MBAA (binding energy -26.91 kcal mol^{-1}) and MAA (binding energy -24.37 kcal mol^{-1}).

The results of molecular modelling were then compared with the binding of fenthion by corresponding NIPs in glass microtitration plates. It was found that a good correlation was observed between experimental results and theoretical calculations (Table 1). Indeed, the polymers produced using monomers with the highest binding energy towards fenthion demonstrated the highest adsorption capacity, whereas the polymer prepared with DEAEM, resulting from the monomer with the lowest binding energy, showed the lowest adsorption. These experimental results confirmed the reliability of the computational method used in our studies. Therefore, acrylamide was selected as a suitable functional monomer for the synthesis of selective MIP for fenthion.

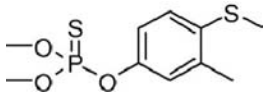

Fig. 1. Molecular structure of fenthion.

Table 1
Evaluation of the results obtained for fenthion using molecular modelling and experimental adsorption using a library of corresponding NIPs prepared in glass microplates.

Functional monomer	Binding energy, kcal mol^{-1}	Binding, %
Acrylamide	−29.07	89
N,*N*-Methylene-bis-acrylamide (MBAA)	−26.91	94
Methacrylic acid (MAA)	−24.37	87
Ethylene glycol methacrylate phosphate (EGMP)	−21.79	75
Acrylic acid (AA)	−16.33	72
Urocanic acid (UA)	−10.48	58
2-Vinyl pyridine (2-VP)	−6.70	33
o-Divinylbenzene (oDVB)	−5.84	25
N,*N*-Diethylamino ethyl methacrylate (DEAEM)	−4.98	16

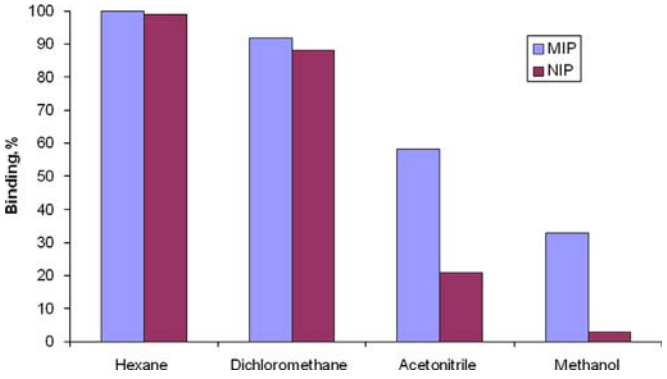

Fig. 2. Effect of the nature of loading solution on the binding capacity of fenthion on the MIP-based polymer and corresponding blank polymer NIP.

3.2. MISPE experiments

3.2.1. Effect of loading solvent

The choice of the loading solvent is crucial as it strongly affects template–MIP interactions. Solvents having different polarities were investigated, as shown in Fig. 2. The results presented show that the binding of fenthion to MIP was relatively low when using polar loading solvents like acetonitrile and methanol (less than 60% of binding). The same observation was made using NIP (less than 20% of binding). That behaviour might be due to the high solubility of fenthion in these polar solvents. The highest binding was obtained in hexane, and dichloromethane as loading solvents. This result suggested that the strongest interactions between the MIP and the template were obtained in apolar solvents. Hexane was thus chosen as a loading solvent in further experiments.

3.2.2. Optimisation of washing and elution conditions

The polymer's washing procedure was performed in order to reduce the concentration of interfering compounds in extracted solution as well as non-specific interactions between fenthion and MIP. Additionally, suitable washing process should be optimised in order to ensure the purity and high recovery of the extracted analyte. Initially, the polymer was conditioned with 5 mL of hexane and

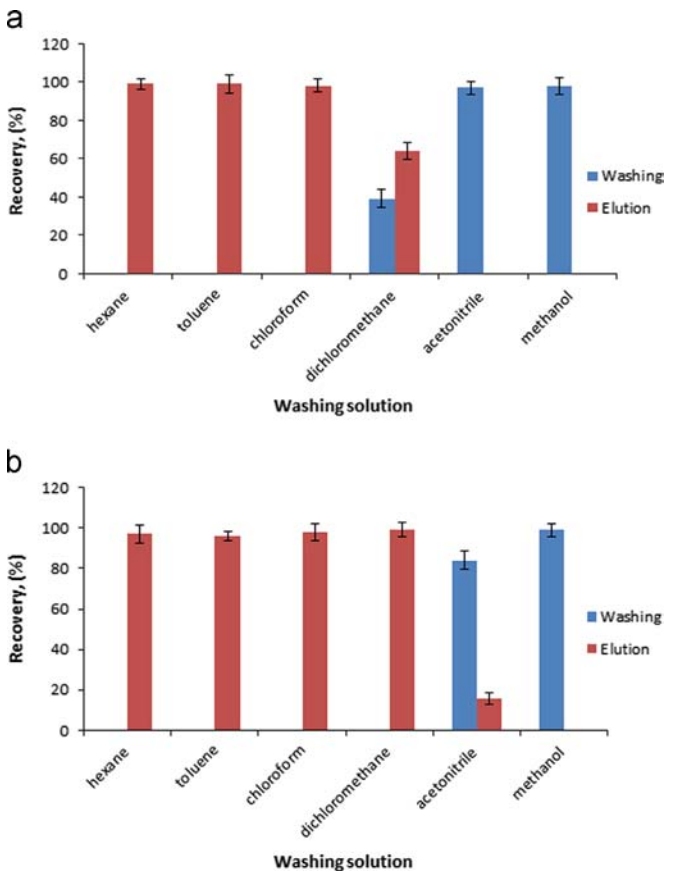

Fig. 3. Recovery of fenthion in the washing (blue) and elution (red) fractions after loading 1 mL of 1 mg L^{-1} pesticide on NIP (a) and MIP cartridges (b). Washing step: 2 mL of the solvents; elution step: 1 mL of methanol/2% TFA. (For interpretation of the references to colour in this figure caption, the reader is referred to the web version of this paper.)

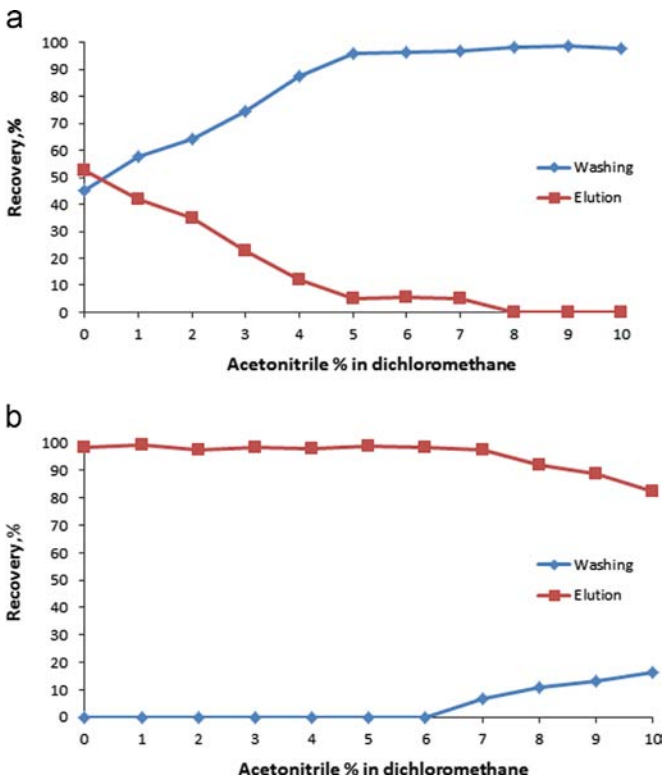

Fig. 4. Recovery of fenthion in the washing (blue) and elution (red) fractions after washing NIP (a) and MIP cartridges (b) with 2 mL of solution with different % of acetonitrile in dichloromethane followed by elution with 1 mL of methanol/2% TFA. (For interpretation of the references to colour in this figure caption, the reader is referred to the web version of this paper.)

tested with 1 mL of 1 mg L^{-1} fenthion solution in hexane. Different solvents were tested in order to optimise the washing procedure. About 2 mL of hexane, chloroform, toluene, dichloromethane, acetonitrile or methanol was applied onto SPE cartridges, followed by pesticide elution using 1 mL of methanol acidified with 2% TFA. Both the washing and elution fractions of the solvent were collected and analysed using reverse-phase HPLC. Results presented in Fig. 3 illustrate that washing of acrylamide-based NIPs (Fig. 3a) with chloroform, hexane and toluene has not removed any pesticide adsorbed on the polymer, as fenthion could not be detected in the washing solution. However, the use of dichloromethane as a washing solvent induced a partial removal of the pesticide. It seems that a low polar organic solvent did not affect the non-specific binding of fenthion. At the same time, the use of polar solvents (methanol and acetonitrile) allowed the complete removal of the pesticide from the cartridge, suggesting that non-specific binding could be efficiently reduced using washing with one of these solvents. The same experiment was performed on acrylamide-based MIPs and showed that methanol also strongly affected specific interactions (Fig. 3b). In contrast, the use of acetonitrile as the washing solvent allowed removing almost 80% of the loaded pesticide, while dichloromethane did not affect the binding of fenthion (Fig. 3b). Despite these results, acetonitrile was considered as a promising washing solvent as it showed a better efficiency for removing the molecules non-specifically bound to the NIP.

Since dichloromethane had no effect on specific interactions, a mixture of dichloromethane and acetonitrile was thus used to optimise the washing process and facilitate specific interactions between fenthion and MIP. Experiments were carried out using

Table 2
Recovery rates (%) of various pesticides loaded as 5-mL aliquotes of 1 mg L^{-1} solution onto acrylamide-based MIP and corresponding NIP. The calculations are based on triplicates, the RSD values are below 5%.

Analytes	MIP		NIP	
	Washing	Elution	Washing	Elution
Fenthion	4 ± 3.2	97 ± 4.1	95 ± 4.8	4 ± 2.1
Dimethoate	98 ± 3.7	nd*	97 ± 3.2	nd
Fenthion-sulphoxide	nd	93 ± 3.3	95 ± 4.4	nd
Methidathion	96 ± 3.5	6 ± 3.6	95 ± 3.6	nd
Malathion	97 ± 5	nd	98 ± 4.2	nd

nd* : not detectable.

different ratios (1–10%) of acetonitrile in dichloromethane as a washing solution. According to the obtained results (Fig. 4b), it was shown that increasing the ratio of acetonitrile in dichloromethane up to 6% did not affect elution of fenthion from MIP, but gradually increased its elution from NIP (Fig. 4a). When the concentration of acetonitrile in the dichloromethane varies from 5 to 6 %, all the molecules retained by not specific interactions on the NIP were completely removed after the washing step, whereas molecules specifically bound on the MIP remained bound. For this reason, dichloromethane containing 5% acetonitrile was selected as the washing solution for fenthion extraction in this work.

3.3. Selectivity

In order to investigate the selectivity of the imprinted polymer, MIP synthesised for fenthion and its corresponding NIP were used to study the binding capacity of other organophosphate insecticides,

including methidathion, dimethoate, malathion and fenthion-sulphoxide. These molecules were selected to investigate the selectivity of MIPs because of their structural similarity with fenthion. The binding capacities of MIP and NIP towards these molecules are summarised in Table 2. As can be seen from the results, the binding capacity of MIP to fenthion was much higher than the binding of any tested structural analogues of fenthion. Due to its very similar structure, the metabolite fenthion-sulphoxide was also efficiently retained on MIP, with a binding capacity close to 93%. Thus, the synthesised MIP was able to recognise both fenthion and fenthion-sulphoxide, as these molecules fitted well within the imprinted cavity. All the compounds tested in this work displayed a similar binding on MIPs and NIPs, except fenthion and fenthion-sulphoxide. These results show that the developed MIPs can be used for the selective extraction of fenthion and fenthion-sulphoxide from real samples.

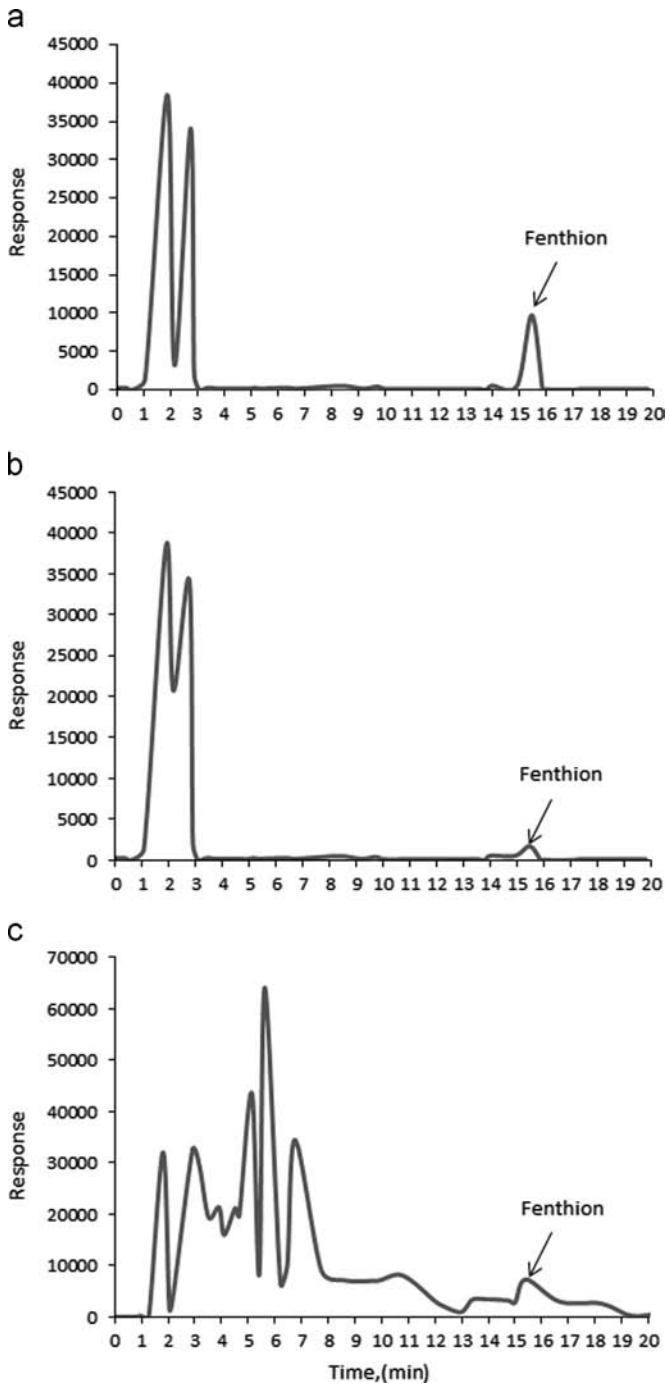

Fig. 5. Chromatograms of eluates obtained after extraction of 1 μg of fenthion from olive oil diluted in hexane using MIP cartridge (a), NIP cartridge (b), and C_{18}-silica cartridge (c) after liquid–liquid extraction with acetonitrile, a UV detection at 220 nm. Retention time: 15.5 min.

3.4. Extraction of fenthion from spiked olive oil

The MIP was used as a SPE adsorbent to selectively extract fenthion from olive oil. Olive oil samples are a very complex matrix: many triglycerides (98–99%) are present which subsequently can cause matrix effect during the extraction process. For this reason, a pre-treatment step using selective MIP was applied to isolate fenthion from olive oil sample before HPLC analysis. The chromatograms of fenthion after extraction using MIP and blank polymer NIP are presented in Fig. 5a and b, respectively. The results obtained using MIP showed a very satisfying average recovery rate of 96.1%. At the same time, the extraction using NIP led to a very low recovery rate, confirming that most of the template was lost during the washing step. In parallel, fenthion-spiked samples were also analysed following liquid/liquid extraction using acetonitrile, and SPE, which was performed using C_{18} column. The performances of MISPE and C_{18} cartridges are compared in Fig. 5c. As can be observed from the chromatograms, a worse clean-up was obtained when samples were extracted using C_{18} column (Fig. 5c). It was shown that the extraction using MIP-based SPE was much more selective and provided a cleaner extract containing predominantly fenthion (Fig. 5a). The limit of detection (LOD) and quantification (LOQ) in olive oil were 0.005 and 0.023 mg L^{-1} respectively.

4. Conclusion

In conclusion, two methodologies for the identification of monomers suitable for MIP preparation have been used: molecular modelling and screening of NIPs library. The obtained results show the efficiency of these methods in the selection of suitable functional monomers for designing MIPs. The selected monomer, acrylamide, was used to prepare a MIP capable of selective and efficient extraction of fenthion insecticide from olive oil. The MISPE method was shown to be more selective to fenthion than the conventional SPE method (C_{18}) and this method proved also to be highly accurate, quick and inexpensive.

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References

[1] L.G. Costa, Organophosphorus compounds, in Recent Advances in Nervous System Toxicology, in: C.L. Galli, L. Manzo, P.S. Spencer (Eds.), NATO ASI Series, vol. 100, Plenum Press, New York, NY, USA, 1988, pp. 203–246.
[2] M.M. Sanagi, H.H. Abbas, W.A. W. Ibrahim, H.Y. Aboul-Enien, Food Chem. 133 (2012) 557–562.
[3] D. Sharma, A. Nagpal, Y.B. Pakade, J.K. Katnoria, Talanta 82 (2010) 1077–1089.
[4] L.G. Costa, Clin. Chim. Acta 366 (2006) 1–13.
[5] P. Cabras, A. Plumitallo, L. Spanedda, J. Chromatogr. 540 (1991) 406–410.
[6] D.H. Hutson, T.R. Roberts, P.J. Jewess, Metabolic pathways of agrochemicals. Part 2: insecticides and fungicides, Royal Society of Chemistry, Cambridge, 1999.
[7] Y. Pico, M. Farré, C. Soler, D. Barcelo, Anal. Chem. 79 (2007) 9350–9363.
[8] E. Botitsi, P. Kormali, S. Kontou, A. Mourkojanni, E. Stavrakaki, D. Tsipi, Int. J. Environ. Anal. Chem. 84 (2004) 231–239.

[9] M. Lopez-Mesas, M. Crespi, Grasas Aceites 51 (2000) 183 (139).
[10] C. Lentza-Rizos, E.J. Avramides, Rev. Environ. Contam. Toxicol. 141 (1995) 111–134.
[11] F. Luchetti, Eur. J. Lipid Sci. Technol. 104 (2002) 559–563.
[12] R. Garcia-Repetto, M. Paz-Gimenez, M. Repetto, J. AOAC Int. 84 (2001) 342–349.
[13] C. Lentza-Rizos, E.J. Avramides, Analyst 115 (1990) 1037–1040.
[14] C. Tsoutsi, I. Konstantinou, D. Hela, T. Albanis, Anal. Chim. Acta 573 (2006) 216–222.
[15] G. Dugo, G. Di Bella, L. La Torre, M. Saitta, Food Control 16 (2005) 435–438.
[16] A. Di Muccio, A. Ausili, L. Vergori, I. Carmona, R. Dommarco, L. Gambetti, A. Santillo, F. Vergori, Analyst 115 (1990) 1167–1174.
[17] L. Rastrelli, K. Totaro, F. De Simona, Food Chem. 79 (2002) 303–305.
[18] B. Jongenotter, H.G. Janssen, LC-GC-Europe 15 (2002) 338–381.
[19] R. Sanchez, J.M. Cortes, J. Villen, A. Vazquez, J. AOAC Int. 88 (2005) 1255–1260.
[20] S. Barrek, O. Paisse, M.F. Grenier-Loustalot, Anal. Bioanal. Chem. 376 (2003) 355–359.
[21] L. Rastrelli, K. Totaro, F. De Simone, Food Chem. 79 (2002) 303–305.
[22] C. Yaguee, S. Bayarri, P. Conchello, R. Lázaro, C. Pérez-Arquillue, J. Agric. Food Chem. 53 (2005) 5105–5111.
[23] T. Galeano Diaz, A. Guiberteau Cabanillas, M.D. Lopez Soto, J.M. Ortiz, Talanta 76 (2008) 809–814.
[24] I Bakas, N. Ben Oujji, E. Moczko, G. Istamboulie, S Piletsky, E Piletska, I. Ait-Ichou, E. Ait-Addi, T. Noguer, R. Rouillon, Anal. Chim. Acta 734 (2012) 99–105.
[25] I. Bakas, N. Ben Oujji, E. Moczko, G. Istamboulie, S Piletsky, E Piletska, I. Ait-Ichou, E. Ait-Addi, T. Noguer, R. Rouillon, J. Chromatogr. A 1274 (2013) 13–18.
[26] S.A. Piletsky, K. Karim, E.V. Piletska, C.J. Day, K.W. Freebairn, C.H. Legge, A.P. F. Turner, Analyst 126 (2001) 1826–1830.
[27] F. Breton, R. Rouillon, E.V. Piletska, K. Karim, A. Guerreiro, I. Chianelli, S. A. Piletsky, Biosens. Bioelectron. 22 (2007) 1948–1954.
[28] S.A. Piletsky, E.V. Piletska, K. Karim, G. Foster, C.H. Legge, A.P.F. Turner, Anal. Chim. Acta 504 (2003) 123–130.
[29] Y. Liu, F. Wang, T. Tan, M. Lei, Anal. Chim. Acta 581 (2007) 137–146.
[30] W. Dong, M. Yan, M. Zhang, Z. Liu, Y. Li, Anal. Chim. Acta 542 (2005) 186–192.