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Hollow Fibre Membrane-Protected Molecularly Imprinted Microsolid-Phase Extraction (HFM-Protected-MI-MSPE) of Triazines from Soil Samples

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Abstract: In this work, a combination of molecularly imprinted polymer (MIP) technology with micro solid-phase extraction in a hollow fibre device is described. MIP microspheres were synthesized and packed into polypropylene hollow fibre (HF) segments. The proposed device was used directly for trace enrichment and clean-up of triazines in soil sample extracts by hollow-fibre membrane-protected molecularly imprinted micro solid-phase extraction (HFM-protected-MI-MSPE). Analytes were extracted from soil by ultrasonic assisted extraction, evaporated to dryness and reconstituted in toluene. The proposed device was immersed in the toluene extracts, and migrations of analytes through the walls of the hollow fibre was achieved with the help of external agitation. Then, selective recognition of the target analytes by MIP-microspheres took place in the inner part of the HF. All parameters affecting the extraction were optimized. Under optimum conditions, quantitative recoveries were obtained for simazine, cyanazine, atrazine, propazine and terbutylazine in soil samples, with relative standard deviations lower than 11%. The detection limits (LODs) were lower than 5 ng g^{-1} in all cases.

Keywords: triazines; imprinted polymers; micro-solid phase extraction; miniaturization; hollow fibre membrane

1. Introduction

Over the last decade, new goals have been established in sample preparation, such as the use of smaller initial samples sizes, improving the selectivity of the extraction process, and minimizing the amount of glassware and organic solvents used [1]. In this regard, the solid-phase micro-extraction technique (SPME) [2], where the analytes are retained in a micro-extraction phase and then desorbed for their analytical determination as concentrated extracts, has undergone huge development. The use of SPME has been widespread; however, it is not free of some disadvantages, such as difficulties for direct immersion of the fibre coating in complex matrices that can damage the sorbent, as well as the limited selectivity of the available sorbents.

Molecular imprinting is a well-known technology that provides improvement to the selectivity of the extraction process. Molecularly imprinted polymers (MIPs) are synthetic materials obtained by copolymerizing a monomer with a cross-linker in the presence of a template molecule. Removal of the template once polymerization is complete leaves cavities that are complementary in size, shape and functionality to the analyte, allowing its further selective rebinding in a complex matrix. Thus, MIPs can selectivity rebind the template and related compounds that are separated from matrix-interfering compounds [3–5].

Koster et al. described for the first time the preparation of a MIP-coated silica fibre for the SPME of brombuterol from human urine [6]. Later, Martín-Esteban and coworkers [7,8] and Djozan and Baheri [9] proposed another approach, where the MIP fibre is prepared inside fused silica capillaries. Just recently, the use of a hollow fibre (HF) membrane to protect the MIPs was described in the literature [10]. This approach has the advantage that the organic solvent is immobilized within the pores of the membrane of the HF, which contains the MIP polymer in its lumen. In this way, a supported liquid membrane between the sample and the MIP is created, and is used to protect the polymer from the sample matrix. Thus, the analytes are firstly extracted from the samples into the organic solvent, where the MIPs subsequently perform selective recognition. The polymer inside the HF can be in the shape of a monolith, as described for the analysis of thiabendazole in orange juices [11] and triazine pesticides in lake water [12]. Alternatively, MIP can be prepared as microspheres to be introduced into the lumen of the HF; this methodology was developed in our laboratory, and has been used for the determination of sulfonamides [13] and triazines [14] in environmental waters.

In the present work, MIP microspheres were prepared by precipitation polymerization, packed inside the hollow fibre, and used for selective extraction of triazines in soil sample complex extracts. MLRs of triazines in these sample have not been established; however, several data can be found in the literature [15] estimating the PNCEs (Predicted Non-Effect Concentration) in the low ng g^{-1} concentration range.

2. Materials and Methods

2.1. Reagents

Simazine (SIM), cyanazine (CYA), atrazine (ATZ), propazine (PPZ) and terbutylazine (TER) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock standard solutions (1 g L^{-1}) were prepared in acetonitrile and stored at −22 °C. HPLC-grade toluene, acetonitrile (ACN), methanol (MeOH), and acetone were purchased from Scharlab (Barcelona, Spain). Methacrylic acid (MAA), divinylbenzene-80 (DVB-80) and 2,2′-azo-bis-isobutyronitrile (AIBN) were purchased from Sigma-Aldrich (St. Louis, MO, USA). MAA was freed from stabilizers by distillation under reduced pressure, and DVB-80 was purified with a neutral alumina column (Aldrich, St. Louis, MO, USA). AIBN was recrystallized within MeOH prior to use. All other chemicals were used as received. Purified water was obtained from a Milli-Q purification system (Millipore, Molsheim, France). Acetic acid (HOAc) was purchased from Panreac (Barcelona, Spain). The Q3/2 polypropylene hollow fibre used to support the organic phase was purchased from Membrana (Wuppertal, Germany) with an internal diameter of 600 µm, 200 µm of wall thickness and 0.2 µm pores. Sample stirring was performed in a Vibramax 100 agitator (Heidolph, Kelheim, Germany). The ultrasonic water bath (290 W, 50/60 Hz, S 40 H Elmasonic) was supplied by Elma (Singem, Germany).

2.2. Polymer Preparation

The precipitation polymerization procedure was firstly described by Wang et al. [16], and ensures the obtention of polymeric microspheres under certain polymerization conditions. Briefly, PPZ (0.34 mmol), MAA (1.36 mmol), DVB-80 (6.78 mmol), and AIBN (0.44 mmol) were dissolved in 25 mL of a 25:75 (*v*/*v*) toluene: acetonitrile mixture in a 40 mL glass tube. Polymerization was developed in an incubator equipped with a roller (Barloworld Scientific, Staffordshire, UK) that provided slow rotation (24 rpm). The temperature was ramped from room temperature to 60 °C over 2 h, and then maintained at 60 °C for 24 h. The polymer microspheres were separated from the polymerization mixture by vacuum filtration on a nylon membrane. Polymerization mixture residues were washed with ACN (80 mL), and the template was removed by Soxhlet extraction using 150 mL of a MeOH:HOAc 1:1 (*v*/*v*) mixture during 8 h. Finally, the obtained polymers were washed with MeOH (80 mL) and ACN (80 mL) and dried before storage at room temperature. The non-imprinted polymer (NIP) microspheres were prepared as described for MIP, but without the addition of template in the polymerization mixture.

2.3. Sample Preparation

Agricultural soil samples were collected from different locations of Spain: an experimental plot located in the region of Madrid, rice fields from Natural Park of L'Albufera in Valencia and agricultural fields from Murcia. Soil samples were sieved and stored at room temperature until analysis. For sample extraction, 10 g of soil were placed inside a glass column with a polyethylene frit placed at the bottom. Subsequently, 20 mL of ACN were added and the extraction was carried out in an ultrasonic bath for 15 min. Later, the extracts were collected with the help of a vacuum manifold and filtered through a 0.45 µm PTFE syringe filter from Scharlab (Barcelona, Spain). Finally, extracts were evaporated under a gentle air stream and then reconstituted with 4.5 mL of toluene.

2.4. Preparation of MIP Membranes

Polypropylene hollow fibre membranes were received in pieces of 53.2 cm and were cut into small 6 cm pieces and cleaned with acetone for 5 min in an ultrasonic bath. Then, one end was sealed by mechanical pressure, and polymeric microspheres were introduced at the other end. For that purpose, a slurry of 100 mg of MIP homogenized in 1 mL of ACN with an ultrasonic bath was first prepared. Subsequently, the slurry was injected into the hollow fibre with the help of a medical syringe with needle. The solvent leaked trough the pores of the membrane leaving the MIP microspheres inside the lumen. After filling, the other end of the fibre was closed by mechanical pressure, and both ends were immediately tied together with a thread to ensure the seal. The same procedure was performed for NIP microspheres. Packed fibres were stored at room temperature, being stable both in dry conditions or immersed in toluene for at least three months.

2.5. Hollow-Fibre Membrane-Protected Molecularly Imprinted Microsolid-Phase Extraction (HFM-Protected-MI-MSPE)

All the steps for the extraction procedure were performed under orbital stirring in a Vibramax 100 agitator (Heidolph, Schwabach, Germany). For conditioning, the packed fibres were immersed in a conventional 4.5 mL screw cap glass vial (Supelco, Belefonte, PA, USA) containing 4.5 mL of toluene and stirred at 750 rpm during 5 min. Then, the conditioned fibres were immersed in 4.5 mL of toluene soil extracts for 45 min at 750 rpm. Subsequently, fibres were washed for 5 min with 4.5 mL of a mixture Toluene /ACN 98:2 (v/v) at 750 rpm. Then, fibres were dried at 60 °C for 30 min and introduced in HPLC inserts containing 450 µL of a mixture of MeOH:HOAc 99:1 (v/v) for elution for 30 min at 1050 rpm.

2.6. HPLC Analysis

HPLC analysis was performed with an Agilent Technologies 1200 series instrument equipped with a quaternary high-pressure pump, a vacuum degasser, an autosampler, and a diode-array detector (Wilmington, DE, USA). Separation was performed in a Kromasil 100 C18 3.5 µm (100 × 4.0 mm i.d.) analytical column from Scharlab (Barcelona, Spain). A sample volume of 100 µL was injected, and the analytes were separated by linear gradient elution from 85% A (purified water) and 15% B (Acetonitrile) to 30% A and 70% B in 15 min. Then, solvents were switched to initial conditions over 2 min and were maintained for other 2 min before starting the next run. The flow-rate was 1 mL min^{-1} for a total run analysis of 19 min. Column temperature was set at 25 °C, and the analytes were monitored at 220 nm.

3. Results and Discussion

3.1. Optimization of HFM-Protected-MI-MSPE in Soil Samples

The objective of the present work was to study the performance HFM-protected-MI-MSPE in the analysis of triazines in soil samples by HPLC-UV. These herbicides strongly sorb to soil, thus requiring exhaustive extraction methods [17], which inevitably co-extract high amount of matrix interferents (e.g., humic and fulvic acids, particulated matter, etc.). These facts prevent the possibility

of using simple UV detectors for routine analysis, thus requiring more expensive selective alternatives, such as c or tandem MS-MS for the final quantification. For this purpose, all parameters involved in HFM-protected-MI-MSPE of triazines from soil extracts were optimized. A scheme of the working protocol is shown in Figure 1.

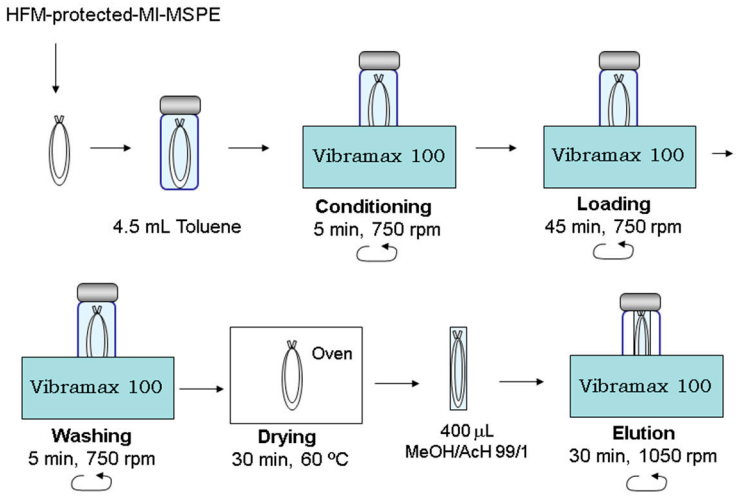

Figure 1. Scheme of the hollow-fibre membrane-protected molecularly imprinted microsolid-phase extraction procedure.

The first experiments were focused on promoting the migration of analytes from the acetonitrile crude soil sample extracts to the fibre, and on improving selective retention in the MIP microspheres. A set of parallel experiments using MIP and NIP fibres was carried out using 4.5 mL volume standards containing 250 ng of each triazine selected for this study, and the results are shown in Figure 2. It can be seen that the recoveries obtained by HFM-protected-MI-MSPE in acetonitrile solutions were very low (5 to 9%, depending upon the triazine). This fact can be easily understood, taking into account the fact that the interaction of triazines with methacrylic-based MIPs is mainly governed by hydrogen bond interactions, which are always favoured in highly hydrophobic media [18]. For that reason, a good strategy for increasing recoveries was to dilute the acetonitrile extract with toluene (the solvent used as porogen during polymerization) in different proportions. As can be observed, retention on the fibre increased as the content in the toluene increased, being quantitative in all cases for dilutions higher that 1/8. Another successful alternative was to evaporate extracts to dryness, and then redissolve them in 4.5 mL of toluene. Both alternatives gave similar recoveries, and could be selected depending on the concentration range analysed. For high-concentration samples, taking only an aliquot of 500 µL of acetonitrile extract and 1/8 dilution avoids the use of a large volume of toluene. However, for trace analysis where a high concentration factor is needed, evaporation of the complete acetonitrile extract (5 mL) and redissolution in 4.5 mL of toluene should be the chosen protocol, since not only would this increase sensitivity, but it would also avoid the consumption of large amounts of toluene.

A washing step was included in order to favour selective recognition of triazines by MIP microspheres, which, at the end, would provide a better sample clean-up. In this sense, and on the basis of previous studies carried out in our laboratory [14], different mixtures of toluene with more polar solvents, such as acetonitrile and methanol in low percentages, were evaluated as washing solutions capable of disrupting non-specific interactions without affecting (if possible) specific interactions. The same conditions described above were used for this set of experiments, but including a washing step of 5 min at 750 rpm with 4.5 mL of toluene mixtures with percentages of ACN or MeOH varying within the 1 to 10% range. According to the obtained results, the mixture consisting of 2% of ACN in toluene was selected as the optimum washing solution, since it permitted almost complete removal of

non-specific interactions with minimal disturbance of specific interactions. Recoveries for all triazines were negligible in NIP fibres, whereas they remained quantitative in MIP fibres, clearly demonstrating an evident imprint effect responsible of the selective molecular recognition.

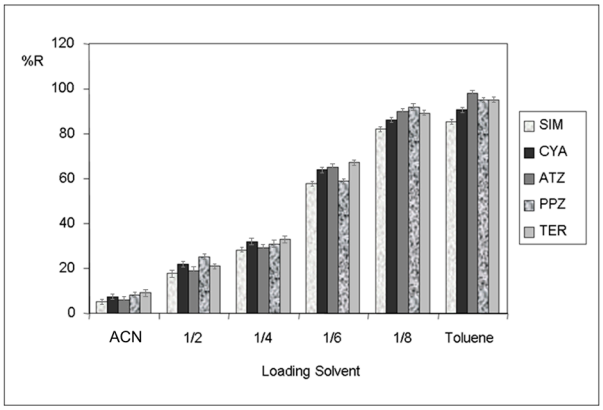

Figure 2. Mean recoveries (%R for $n = 3$) obtained for the optimization of the retention of the different triazines in the MIP fibre.

Finally, and after drying the fibres as mentioned in the experimental section, quantitative elution of analytes was achieved using a mixture of MeOH:HOAc 99:1 (v/v). This last step was carried out directly in 450 µL HPLC inserts with the aim of increasing sensitivity and reducing sample manipulation.

3.2. Analytical Performance and Application of the HFM-Protected-MI-MSPE of Triazines in Soil Samples

The developed HFM-protected-MI-MSPE procedure was then applied for the determination of triazines in soil samples. In order to demonstrate the feasibility of the proposed method, soil samples of different Spanish locations were selected. Spiked (10 ng g^{-1} of each triazine) and non-spiked crude soil extracts were prepared as described above and analysed with and without performing selective HFM-protected-MI-MSPE.

Figure 3 shows a comparison of the results obtained for the analysis of the agricultural soil samples from the Murcia region. Figure 3a,b shows the HPLC-UV analysis of a spiked soil sample extract without and with performing the selective HFM-protected-MI-MSPE, respectively. As can be seen, direct analysis of spiked crude soil extract provides a chromatogram with a big hump of organic matter and a highly noisy baseline with a lot of interfering peaks. Consequently, identification of the selected triazines by means of the UV spectrum comparison tool provided by the diode-array software was only possible for cyanacine (peak number 2), since the match factors obtained for the peaks appearing at the retention times of the other triazines were lower than 950. In contrast, by using the proposed HFM-protected-MI-MSPE procedure (Figure 3b), detection and quantification of all triaizines could easily be accomplished, thanks to the high selectivity of the MIP microspheres. The high degree of clean-up achieved resulted in a chromatogram almost free of matrix interference, in which match factors obtained by spectral comparison were higher than 980 for all the selected triazines. Finally, analysis of non-spiked samples by HFM-protected-MI-MSPE permits the conclusion that triazines were not present in that soil (Figure 3c).

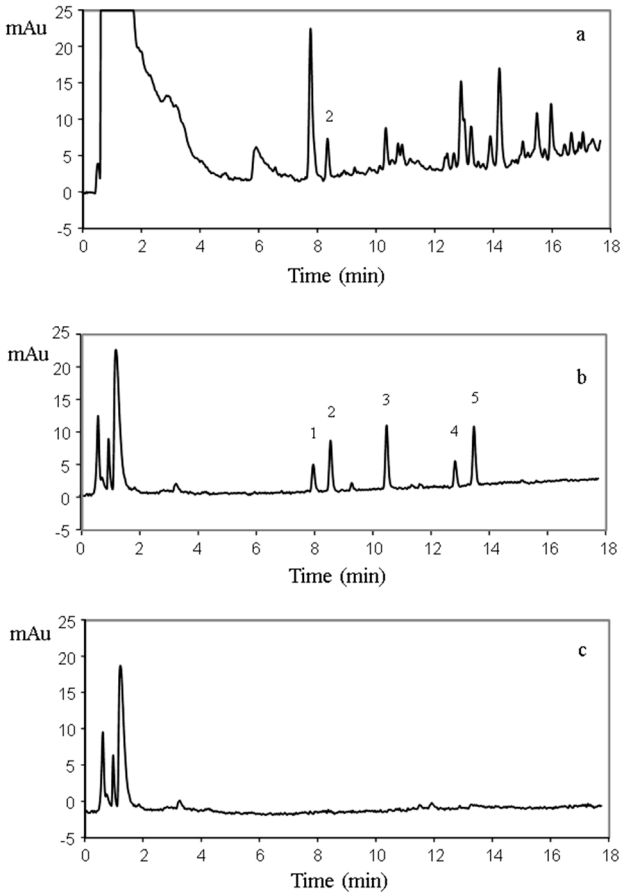

Figure 3. Comparison of chromatograms obtained in the analysis of soil samples from Murcia: (**a**) Spiked crude soil sample extract (10 ng g^{-1}); (**b**) Spiked soil sample extract (10 ng g^{-1}) after performing HFM-protected-MI-MSPE; (**c**) non-spiked soil sample extract after performing HFM-protected-MI-MSPE. Peak assignment: 1. SIM; 2. CYA; 3. ATZ; 4. PPZ; 5. TER.

Another illustrative example of the selectivity provided by the developed method can be seen in Figure 4. In this case, the chromatograms correspond to the analysis of soil samples collected in L'Albufera (Valencia, Spain). Spiked soil sample extracts (10 ng g^{-1}) analysed directly or after HFM-protected-MI-MSPE are shown in Figure 4a,b, respectively; Figure 4c corresponds to the analysis of a non-spiked sample under the proposed procedure. The obtained results were very similar, with the identification of atrazine, propazine and terbutylazine in the crude sample extract being, in this case, impossible; whereas the match factors obtained following the proposed procedure (Figure 4b) surpassed 975 for all the triazines selected in this work. Finally, non-agricultural soil samples collected in Madrid were also analysed, and the results were as satisfactory as those obtained for both agricultural soils described above.

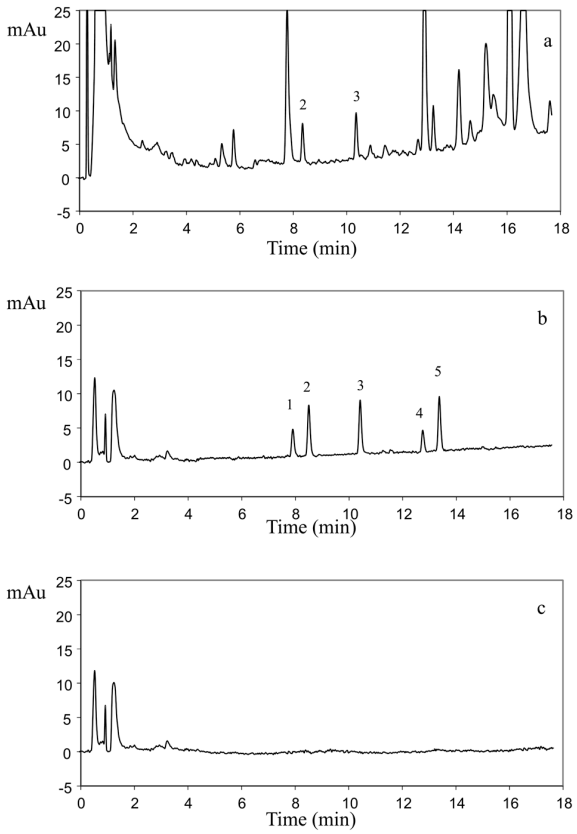

Figure 4. Comparison of chromatograms obtained in the analysis of soil samples from Albufera: (**a**) Spiked crude soil sample extract (10 ng g^{-1}); (**b**) Spiked soil sample extract (10 ng g^{-1}) after performing HFM-protected-MI-MSPE; (**c**) non-spiked soil sample extract after performing HFM-protected-MI-MSPE. Peak assignment: 1. SIM; 2. CYA; 3. ATZ; 4. PPZ; 5. TER.

Table 1 summarizes all the obtained results and the analytical performance of the proposed method in the different soil samples. For the recovery and precision study, three different samples of each soil were enriched with 10 ng g^{-1} of each triazine and analysed by the proposed procedure. Mean recovery values varied between 72% and 103%, with RSDs lower than 11% proving the high accuracy and repeatability of this method. The limits of detection were calculated as three times the signal of the background noise obtained in the analysis of non-spiked samples at the retention times of the corresponding analytes, and are also included in Table 1. The obtained values varied within a 1.2 and 3.7 ng g^{-1} concentration range, which is low enough to prove the suitability of the developed method for routine laboratory analysis of selected triazines at real trace concentration levels in soil samples from different origin.

Table 1. Analytical performance and application of HFM-protected-MI-MSPE for the determination of triazines in soil sample from different origin.

	Murcia			**Valencia**			**Madrid**		
	%R	**RSD (%) [1]**	**LOD [2]**	**%R**	**RSD (%) [1]**	**LOD [2]**	**%R**	**RSD (%) [1]**	**LOD [2]**
SIM	77.3	9.2	3.7	72.8	9.0	3.4	76.1	8.9	2.9
CYA	85.9	8.9	1.9	94.6	7.1	1.5	101.1	6.4	1.3
ATR	102.3	10.0	1.5	82.5	9.5	1.4	85.8	5.8	1.2
PPZ	93.7	9.5	3.6	86.7	9.0	3.6	92.4	7.3	3.4
TER	99.2	10.7	1.6	96.2	7.4	1.2	84.3	8.2	1.3

[1] $n = 5$; [2] ng g^{-1}.

4. Conclusions

A HFM-protected-MI-MSPE procedure for the determination of triazines in soil samples has been developed in this work. The liquid membrane supported in the hollow fibre protects MIP microspheres placed inside the lumen, which prevents deterioration of the sorbent, thus allowing several analyses to be performed with the same fibre. The developed analytical method offers multiple advantages such as low organic solvent consumption, miniaturization, and a high enrichment and clean-up of the sample. In fact, it exhibited an excellent selectivity leading to chromatograms almost free of co-extractives allowing the use of an UV detector for soil analysis. The analytical performance of the proposed method, in terms of %R, RSDs and LODs, proved the suitability of the developed procedure for trace analysis of triazines in soil samples.

Author Contributions: M.D.-Á., A.M.-E. and E.T. conceived and designed the experiments; E.T. and M.D.-Á. performed the experiments and analyzed the data; E.T. and M.D.-Á. wrote the paper draft which was corrected to the final version by A.M.-E.

Conflicts of Interest: The authors declare no conflict of interest.

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