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# Optimization of solid-phase extraction using developed modern sorbent for trace determination of ametryn in environmental matrices

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ABSTRACT

In recent years, there has been a significant increase in molecularly imprinted solid-phase extraction (MISPE) technique for the purification and clean-up of environmental samples. In this study, solid-phase extraction using the imprinted polymer has been optimized with the experimental design approach for a triazine herbicide, named ametryn with regard to the critical factors such as sample pH, sample concentration, sample flow-rate, sample volume, elution solvent, washing solvent and sorbent mass. These factors were evaluated statistically and also validated with spiked drinking water samples and showed a good reproducibility over six consecutive days as well as six within-day experiments. Also, in order to the evaluate efficiency of the optimized MISPE protocols, enrichment capacity, reusability and cross-reactivity of cartridges have been studied. Finally, a selective MISPE was successfully demonstrated for ametryn with a recovery of above 90% for spiked drinking water samples. It was concluded that the central composite design could prove beneficial for aiding the MIP and MISPE development.


## 1. Introduction

In recent decades, there has been notable development of different analytical chromatographic techniques for the determination of organic compounds such as pesticides; however, there has been no parallel development in sample preparation to the same extent [1]. Solid-phase extraction (SPE) has appeared as an alternative to liquid–liquid extraction (LLE) and the increased development of SPE has occurred with many improvements in formats, automation and introduction of new phases. One reason was the pressure to decrease organic solvent usage in laboratories which has encouraged the requirement for solvent-free procedures [2]. Also, many polar analytes are often partly soluble in water and cannot be extracted with good recoveries by LLE procedures whatever the organic solvent selected [3,4]. At the same time, the availability of cleaner and more reproducible sorbents than in the past has also helped its increasing acceptance by regulatory agencies. Other reasons for the growing interest in SPE techniques are a large choice of sorbents with the capability for new ones of trapping polar analytes, simplicity, cost and easy automation. However, a key SPE problem remains the method development. The primary decision for analysis is the selection of the type of sorbent able to solve the trace-analysis problems.

Nowadays, obtaining extracts free from matrix interferences in a few steps – one step if possible – has been recognized as an important goal and selectivity is included in the development of the SPE procedures. It is clear that, the more selective the SPE step is, the more sensitivity is obtained. Taylor-made sorbents such as immunosorbents and molecularly imprinted polymers (MIPs) have been introduced in that way. However, the obtainment of antibodies is difficult, time-consuming and expensive and in addition, it is difficult to guarantee its success. Also, it is important to point out that, after the antibodies have been obtained, they have to be immobilized on an adequate support, which may result in poor antibody orientation or even complete denaturation [1].

Recently, MIPs have been substituted for antibodies as specific binding reagents [5]. These polymers have the advantage of speed, relatively cheap, and ease of polymer preparation when compared with the preparation of immunosorbents [6,7]. Molecularly imprinted polymers (MIPs) are made by synthesizing highly cross-linked in the presence of a template. After polymerization, the template is removed by washing, leaving sites that are capable of selectively rebinding the target analyte. The mechanisms, by which these polymers specifically bind the template and related ligands, are attributed to the formation of functional groups in a specific arrangement within the polymer that corresponds to the

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template and to the presence of shape-selective cavities. Due to their compatibility with organic solvents, MIPs have many potential applications for the analysis of highly lipophilic compounds, such as many pesticides and other environmental pollutants [5].

Since MIPs provide good selectivity as separation materials, their use as preconcentration and clean-up sorbents in MISPE (molecularly imprinted SPE) has recently been evaluated [8]. MISPE has been discussed in specific reviews [9–14,1,8] and also in general reviews on the application of MIP to analytical separations [15–19]. Several general reviews have increasingly discussed MISPE as an emerging technique for highly selective clean-up of samples [20–25].

The most common method for preparing molecular imprinted polymers suitable for MISPE consists in bulk thermal-(or photo-) polymerization. Through this method a monolithic polymer is produced and has to be crushed and sieved to obtain particles of the desired size distribution.

MISPE has been successfully used in the determination of single analytes, such as sameridine [26], tamoxifen [27], and to less extent, in multianalyte determination, such as nicotine, and its oxidation products [28], and triazine herbicides [29–32]. For general use of the MISPE, the existing recognition elements need to be improved in order to meet the requirements in the given application. The large number of variables, coupled with the fact that they are dependent on each other, makes it an extremely difficult task to optimize an MISPE. The procedural optimization can be achieved in a traditional trial and error manner or with the assistance of chemometrics. Even using combinatorial methods under the best conditions, a few of the compositional variables can be explored. The complexity of these problems makes the application of chemometric methods as an ideal opportunity for the design and optimization of the MISPE columns [33]. The chemometric approach is based on the use of an optimum set of experiments (experimental design), which allows the simultaneous variation of all studied experimental factors [34]. Rather than making every combination in an *n*-dimensional matrix, these methods allow one to vary multiple parameters simultaneously.

In this article, the use of MIPs as specific binding matrix for solid-phase extraction of a triazine herbicide, named ametryn with regard to sample pH, sample concentration, sample flow-rate, sample volume, elution solvent, washing solvent and sorbent mass for environmental matrices was described. The aim of this work was optimization of the main factors, affecting the molecular recognition properties of the MISPE by a chemometric approach.

## 2. Experimental

### 2.1. Chemicals

Ametryn, with greater than 98.2% purity and other triazines (atrazine, cyanazine, simazine and propazine) were obtained from Riedel-de-Häen (Seelze, Germany), 2,4-dicholorophenoxyacetic acid (2,4-D) (Sigma–Aldrich–Fluka, Milan, Italy), methacrylic acid (MAA, functional monomer) and ethylene glycol dimethacrylate (EGDMA, co-monomer) were purchased from the Merck Company, Germany. 2,2-Azobisisobutyronitrile (AIBN, initiator) was obtained from the Acros Company, USA. All solvents (acetic acid, hydrochloric acid 32%, acetonitrile and methanol) and buffer solutions (citrate/hydrochloric acid, pH 4 and boric acid/potassium chloride-sodium hydroxide, pH 10) as well as sodium hydroxide pellets, were of analytical reagent grade (Merck, Darmstadt, Germany). Ultra pure water was prepared by Purite Purification System. Stock standard solutions (1 $\mu g\,mL^{-1}$) were made by weighing the solutes, their dissolution in 1 mL acetonitrile, then, deionized water and their storage at −18 °C.

### 2.2. Apparatus and analytical conditions

All measurements were performed by a reversed-phase HPLC system from the Knauer Company (Germany), consisting of a K-1001 series high pressure pump, a K-2006 photo diode-array detector and a VS injection valve, equipped with a 20 μL loop. The analytes were separated on a Chromolith Performance RR-$C_{18}$e 100 mm × 4.6 mm i.d. (Merch KGa A, Germany) and column guards (Chromolith Guard Cartridge Kit RP-$C_{18}$e & 5 cm × 4.6 mm i.d., 5 μm), using isocratic elution as follows: 60% acetonitrile and 40% purified water. Ametryn was monitored at 220 nm and quantified with external calibration using the peak area measurements ($R^2$ = 0.9998). Each sample was repeated three times to assure the chromatogram reproducibility. The flow-rate was set at 0.8 mL $min^{-1}$. Optimized chromatographic conditions for other triazines were shown in Table 1. The system was linked with a LaserJet 1200 series printer for recording the chromatograms, using a 1456-1 Chromogate Data System, Version 2.55. For the polymer synthesis, the used apparatus included soxhlets and a heater unit, a liquid extraction unit (S&S, Germany), a reactor heater system (Memmert, Germany), a nitrogen supply system, an ultrasonic shaker (Tecna-6, Italy), a syringe-filtration unit (FH-0.45 μm, Millipore Corp., USA), PTFE filters (0.2 μ, Sartorius, Germany), an oven (Memmert, Germany) and a shaker (Innova 4000). Also, for the MISPE procedures, a vacuum manifold (Tajhizteb, Iran), a Sibata vacuum pump (Hitachi Ltd., Japan), and a transformer (SE-300, Japan) were used. The amount of reagents was measured, using a digital balance (Sartorius-2024, Germany) for milligram quantities or less. Finally, adjustable-volume pipettors with disposable tips were used to load the sample, washing solvent and eluent into the cartridges (Socorex, Germany).

### 2.3. Preparation of molecularly imprinted SPE column

In this study, non-covalent bulk polymerization was employed, as one of the successful molecular imprinting protocols [35,36], to obtain glassy polymer blocks to be used as powder after being crushed, ground and sieved. The molecularly imprinted polymers were prepared as follows: ametryn and MAA were added to a 25 mL glass tube and, afterwards, the mixture was left for 5 min. Subsequently, EDMA, AIBN, and acetonitrile were added (see Table 2). The mixture was purged with nitrogen for 5 min and the glass tube was sealed under this atmosphere. It was then placed at a thermostated water bath at 55 °C to start the polymerization process. After 24 h, the tube was broken, the obtained polymer was ground and sieved, and the particles with sizes between 50 and 105 μm

**Table 1**
Chromatographic conditions for triazines analysis.

| Analyte | Mobile phase (%) | | Ammonium acetate (mmol) | Wavelength (nm) | Flow-rate (ml/min) | Injection volume (μL) |
|---|---|---|---|---|---|---|
| | Acetonitrile | Water | | | | |
| Atrazine | 50 | 50 | 1 | 226 | 1.4 | 20 |
| Cyanazine | 85 | 15 | – | 256 | 0.8 | 20 |
| Simazine | 40 | 60 | 1 | 226 | 1.2 | 20 |
| Propazine | 50 | 50 | 1 | 226 | 1.2 | 20 |

**Table 2**
Composition of the polymerization mixture.

| Ametryn | MAA | EDMA | Porogen | AIBN |
|---|---|---|---|---|
| 1 mmol | 4.49 mmol | 22.48 mmol | 6.41 mL | 2.03 mmol |

were collected. The template was removed by soxhlet extraction using a two step procedure (methanol:acetic acid washing (9:1 v:v) 18 h as a first step and methanol washing for 6 h as a second step). These procedures were optimized in our laboratory to generate MAA-based binding sites complementary to ametryn [37]. Finally, the produced powder was packed in cartridges. Furthermore, non-imprinted polymers (NIP) were prepared following the same procedure without the addition of the template molecule. Safety precautions were considered during the preparation of the polymerization mixture, the grinding, and the extraction of the polymer. These steps were performed in a safety cabinet, because they involve the handling of the toxic compounds of methacrylic acid, ethylene glycol dimethacrylate and 2,2-azobisisobutyronitrile.

### 2.4. Preliminary MISPE procedure

A 150-mg amount of dry imprinted and non-imprinted polymer was packed separately into empty SPE cartridges of 6 mL between two frits (length of 65 mm and i.d., 10 mm). The bleeding of residual template from the polymer was checked by washing the MISPE cartridges with methanol fractions (3× 1 mL each). The chromatograms of the all fractions were found to be free of ametryn at the sensivity of the UV detector. For the preliminary experiments on extraction, MISPE cartridges conditioned with 10 mL methanol followed by 10 mL LC-grade water to wet the polymer completely. After drying step (about 2 min), a 10 mL volume of a 100 ng $mL^{-1}$ solution of ametryn was passed through at 3 mL $min^{-1}$. After the loading, air was passed through the sorbents for drying the solid phase (10 min). Then, the cartridge was washed with 1 mL LC-grade water and 1 mL acetonitrile. A gentle vacuum was applied between each step (1 min). The target analyte was eluted from the cartridge with 3× 1 mL methanol and analyzed using an HPLC-UV system. It should be noted that, in order to optimize MISPE elution step, different methanol values of 1, 2, 2× 1, 3, 3× 1, 4, 4× 1, 5, and 5× 1 mL were applied. Also, the columns were washed with 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 mL acetonitrile and its influence on extraction recovery was examined. Another experiment performed during this section of study was evaluation of the different methanol and water values (9, 8, 7, 6, 5, 4, and 3 mL) on ametryn recovery. Finally, the effect of first vacuum time in washing step (5, 10, 15, 20, 25, and 30 min), on the ametryn recovery was investigated. The guideline for the experiments is shown in Table 3.

### 2.5. Qualitative optimization of MISPE procedure

In order to achieve the optimum MISPE conditions and remove non-specific interactions and interferences absorbed to the columns, the cartridges were washed with different acetonitrile volumes of 8 and 7 mL and different acetic acid (0 and 0.1 volume percentage with 8 mL and 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 volume percentage with 7 mL). However, care should be taken that, no analyte–sorbent bonding is broken during washing stage. Another experiment performed was evaluation of the elution solvent composition on ametryn recovery. Nine solvent compositions (methanol and acetic acid) were screened for their ability to produce optimum elution of the retained ametryn from the MIP columns. They were 2× 1 mL methanol (followed by 1%, 2%, 3%, 4%, and 5% acetic acid) and 3× 1 mL methanol (followed by 0, 1%, 2%, and 3% acetic acid). The MISPE procedure was used and examined (except for the solvents composition that was considered in the screening plan), following the same procedure with the preliminary MISPE procedure.

**Table 4**
Factor levels in the experimental designs.

| Variable | Low (−) | High (+) | Central (0) | $\alpha = 2$ | |
|---|---|---|---|---|---|
| | | | | Axial($-\alpha$) | Axial($+\alpha$) |
| Sample flow-rate (mL $min^{-1}$) | 2 | 4 | 3 | 1 | 5 |
| Sample concentration (ng $mL^{-1}$) | 65 | 145 | 100 | 10 | 190 |
| Sample volume (mL) | 7.5 | 12.5 | 10 | 5 | 15 |
| Sorbent mass (mg) | 100 | 200 | 150 | 50 | 250 |
| Sample pH | 4 | 10 | 7 | 1 | 13 |

### 2.6. Experimental design approach for SPE procedures on MIP

For the elimination of possible bias, the order of the running experiments was restrictedly randomized (restricted factor was the flow-rate of the sample). The standard approach to the analysis of the experimental design data was to evaluate a list of the main and interaction effects supported by an ANOVA Table, indicating which effects are significant [38]. The data were analyzed with the aid of the statistical software package, Minitab, Release 14, for windows [39]. The primary stage of the experimental design involved the selection of five factors which could influence the recovery efficiency. These factors could be the operational or compositional variables such as sample pH, sample concentration, sample flow rate, sample volume, and sorbent mass. Accordingly, a two-level full factorial design of $2^5$ was utilized following a linear and quadratic model, containing squared terms. This led to 32 basic experiments, undertaken in random order plus four central points. As the second stage of the experimental part, a central composite design was used with $\alpha$ values equal to 2 for the assessment of the $\alpha$ effects on the resulting data, adding ten star points to the above $2^5$ factorial design. For these reasons, 46 runs were selected. Table 4 depicts the values, corresponding to the high (+), low (−) and central (0) points and $\alpha$ values for each factor. For each run in the experiments, NIP columns were prepared. In order to optimize MISPE, the effect of sample flow-rate decreasing over the range of central composite design (1, 0.9, 0.8, 0.7, 0.6, 0.5, and 0.4 mL $min^{-1}$) on the ametryn recovery was investigated. Also, to obtain the best results for the extraction of ametryn from spiked drinking water samples, the effect of amount of 0.1 M HCl (1, 1.5, 2, 2.5, 3, 3.5,

**Table 3**
Guideline for experiments in the central status of the MISPE.

| Experiment | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Elution methanol (mL) | 1 | 2 | 2 × 1 | 3 | 3 × 1 | 4 | 4 × 1 | 5 | 5 × 1 | – |
| Washing acetonitrile (mL) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Conditioning (mL) Methanol and water | 9 | 8 | 7 | 6 | 5 | 4 | 3 | – | – | – |
| Vacuum time (min) | 5 | 10 | 15 | 20 | 25 | 30 | – | – | – | – |

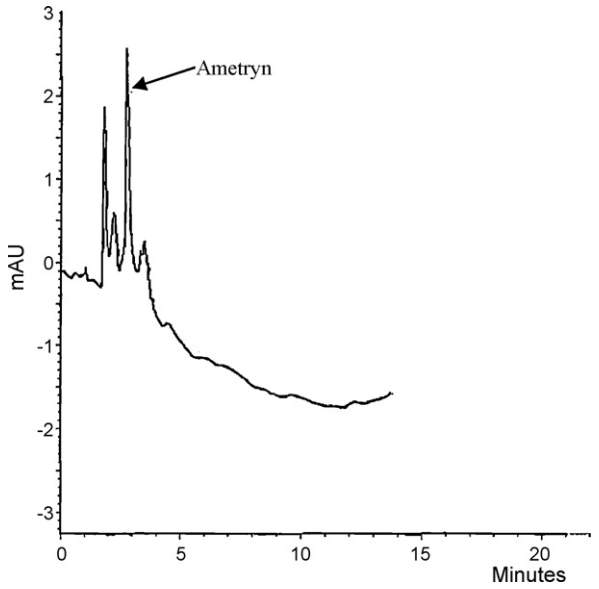


**Fig. 1.** The HPLC chromatogram of spiked drinking water ametryn at concentration of 100 ng mL$^{-1}$. Mobile phase, 60% acetonitrile and 40% purified water, flow-rate 0.8 mL min$^{-1}$, injection volume: 20 μL, the analytical column: performance RR-$C_{18}$e 100 mm × 4.6 mm i.d. (Merch KGa A, Germany) and column guards (Chromolith Guard Cartridge Kit RP-$C_{18}$e & 5 cm × 4.6 mm i.d., 5 μm), UV detection at 220 nm, the ambient temperature was used for the chromatographic system.

4, 4.5, and 5 mL) on the ametryn recovery was investigated. The same sequence of conditioning, loading and elution was used as explained beforehand. In order to evaluate the capacity of MISPE cartridges, different volumes of ametryn 10 μg L$^{-1}$ including 25, 50, 100, 200, 300, 400, 500, and 1000 mL were added to the MIP columns. Also, in order to evaluate the volume break-through, 1 mL sample of ametryn 0.1 μg mL$^{-1}$ was diluted into different volumes, including 25, 50, 100, 200, 300, 400, 500, and 1000 mL and added to the MISPE cartridges. The columns were washed and eluted according to the optimized method. Finally, the specificity of the ametryn MIP was determined via the cross-reactivity of similar and different pesticides.

### *2.7. Method validation and identification of ametryn in drinking water*

The drinking water samples were spiked with five different amounts of ametryn to reach a final concentration of 10, 65, 100, 145 and 190 ng mL$^{-1}$. They were stored at 4 °C and analyzed within 24 h after collection. The calibration graphs were constructed by plotting the peak area of the analytes versus their concentrations. The intra- and inter-day precision and accuracy data were obtained with the assay of spiked drinking water samples. Other triazines samples, atrazine, cyanazine, simazine and propazine were also examined to evaluate the selectivity.

## 3. Results and discussion

In order to achieve the optimum chromatographic conditions for the ametryn analysis, variables, including, mobile phase composition, UV wavelength, injection volume, and mobile phase flow-rate were optimized. Fig. 1 shows the ametryn chromatogram detected at 220 nm. The chromatograms of all fractions were found to be free of ametryn at the sensivity of the UV detector. The phenomenon of selective recognition, resulting from both polymer imprinting and the associated polymer recognition properties has been observed and reported in great detail. Despite the popularity in the literatures published within the past decades, the selectivity of MISPE mech-

**Table 5**
Mean recovery on the molecular imprinted and the non-imprinted polymers in the response surface methodology model.

| Run | Recovery (mean (%), $N$ = 3) | | Run | Recovery (mean (%), $N$ = 3) | | Run | Recovery (mean (%), $N$ = 3) | | Run | Recovery (mean (%), $N$ = 3) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | MIP (SD) | NIP (SD) | | MIP (SD) | NIP (SD) | | MIP (SD) | NIP (SD) | | MIP (SD) | NIP (SD) |
| 1 | 57.99 (0.72) | 21.26 (0.76) | 13 | 64.26 (0.17) | 18.26 (0.61) | 25 | 34.45 (0.36) | 4.95 (0.2) | 37 | 43.56 (0.35) | 11.70 (0.33) |
| 2 | 57.62 (0.44) | 21.85 (0.28) | 14 | 74.70 (0.39) | 39.21 (0.69) | 26 | 50.23 (0.22) | 9.66 (0.36) | 38 | 83.52 (0.44) | 29.71 (0.47) |
| 3 | 59.51 (0.37) | 14.37 (0.44) | 15 | 64.95 (0.48) | 34.16 (0.37) | 27 | 36.44 (0.22) | 23.71 (0.42) | 39 | 68.49 (0.35) | 25.74 (0.14) |
| 4 | 54.38 (0.35) | 29.46 (0.28) | 16 | 81.59 (0.28) | 23.76 (0.36) | 28 | 27.42 (0.39) | 20.11 (0.4) | 40 | 57.37 (0.26) | 17.53 (0.33) |
| 5 | 79.67 (0.2) | 23.69 (0.46) | 17 | 57.50 (0.3) | 14.60 (0.48) | 29 | 41.20 (0.33) | 18.60 (0.26) | 41 | 56.06 (0.17) | 21.66 (0.46) |
| 6 | 67.35 (0.28) | 34.50 (0.42) | 18 | 59.42 (0.3) | 33.84 (0.46) | 30 | 32.28 (0.79) | 4.54 (0.37) | 42 | 60.52 (0.26) | 21.75 (0.63) |
| 7 | 52.56 (0.36) | 33.41 (0.42) | 19 | 39.63 (0.33) | 8.84 (0.39) | 31 | 54.48 (0.35) | 14.04 (0.51) | 43 | 47.60 (0.63) | 35.65 (0.24) |
| 8 | 57.66 (0.22) | 34.16 (0.49) | 20 | 44.44 (0.28) | 24.80 (0.14) | 32 | 29.19 (0.57) | 19.69 (0.1) | 44 | 57.64 (0.44) | 13.35 (0.32) |
| 9 | 72.27 (0.24) | 19.73 (0.3) | 21 | 56.21 (0.57) | 23.15 (0.45) | 33 | 49.60 (0.32) | 28.80 (0.6) | 45 | 0 | 0 |
| 10 | 64.73 (0.42) | 18.25 (0.26) | 22 | 47.38 (0.39) | 10.38 (0.41) | 34 | 34.53 (0.36) | 23.59 (0.3) | 46 | 0 | 0 |
| 11 | 74.63 (0.56) | 19.04 (0.35) | 23 | 42.32 (0.36) | 24.81 (0.59) | 35 | 57.93 (0.24) | 21.46 (0.24) | | | |
| 12 | 76.57 (0.33) | 39.07 (0.17) | 24 | 51.28 (0.24) | 28.40 (0.22) | 36 | 57.86 (0.61) | 22.22 (0.33) | | | |

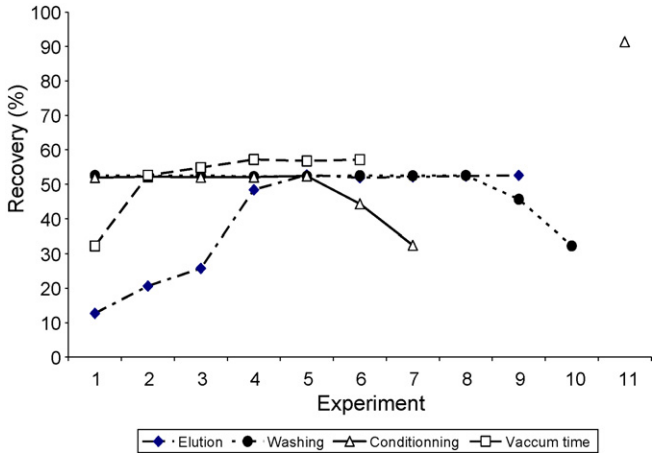


**Fig. 2.** Mean recovery of ametryn in different status of elution, washing, conditioning and first vacuum time in washing step ($n = 3$)

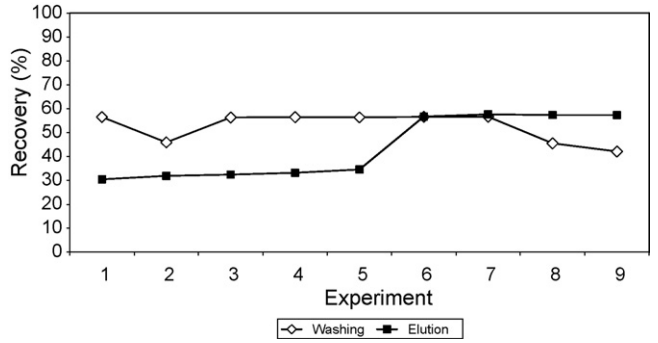


**Fig. 3.** Mean recovery of ametryn in different composition of elution and washing solvent ($n = 3$)

anisms and their rational control has not entirely been recognized and still is under question. Therefore, there is a need to optimize the extraction procedure in a more detailed fashion. Since all steps, including conditioning, loading, washing, and elution (both type and amounts) have a strong influence on the overall MISPE performance in terms of affinity, selectivity, loading capacity, etc., their proper selection (qualitative and quantitative) will ensure that polymers with appropriate properties have been obtained successfully.

In this study, the use of MISPE of a triazine herbicide, named ametryn with regard to qualitative and quantitative parameters for drinking water samples was described. Since the main aim of this work was to optimize the main factors, affecting the molecular recognition properties of the MISPE by a chemometric approach, the ametryn MIP was packed into cartridges as described in Section 2. In the first step and in agreement with the other studies [40,28], the bleeding of residual template from the polymer was checked by washing the MISPE cartridges with successive methanol fractions (3× 1 mL each). The chromatograms of all fractions were found to be free of ametryn at the sensivity of the UV detector. For ametryn, retention on the NIP column was lower than for the MIP column, which suggested that the polymer had been successfully imprinted (Table 5).

**Table 7**
The estimated response surface regression coefficients for the mean recovery on the MISPE.

| Term | Coefficient | $P_{value}$ | Term | Coefficient | $P_{value}$ |
|---|---|---|---|---|---|
| Constant | 55.38 | 0.001 | A × B | −2.26 | 0.637 |
| Flow-rate (A) | −23.43 | 0.001 | A × C | −1.19 | 0.78 |
| Concentration (B) | 7.32 | 0.002 | A × D | 0.74 | 0.862 |
| Volume (C) | −1.48 | 0.442 | A × E | 1.7 | 0.688 |
| Mass (D) | −5.48 | 0.008 | B × C | −1.53 | 0.749 |
| pH (E) | 11.29 | 0.001 | B × D | 2.59 | 0.589 |
| A × A | 14.26 | 0.004 | B × E | 2.19 | 0.647 |
| B × B | 12.49 | 0.014 | C × D | 1.008 | 0.813 |
| C × C | 9.01 | 0.059 | C × E | 1.55 | 0.716 |
| D × D | 3.34 | 0.471 | D × E | −2.23 | 0.601 |
| E × E | −49.28 | 0.001 | | | |

$S = 5.96$, R-Sq = 93.8%, R-Sq (adj) = 88.9%. The analysis was done using coded units.

### 3.1. Central status of the MISPE

As it has been mentioned in Section 2.4, in order to start the optimization process by an experimental design approach, a preliminary MISPE procedure was designed. From the results given in Fig. 2, it was deduced that, 3× 1 mL methanol as eluent could be applied for efficient elution in this stage. However, the eluent volume must be just sufficient to elute the compound of interest from the sorbent. Also, based on the obtained results (Fig. 2), the best acetonitrile volume for washing step of MISPE was 8 mL and appropriate methanol and water volumes in conditioning step was 5 mL. It should be noted that, water volume in the washing step was selected in accordance with the water volume in the conditioning step (5 mL). The retention of the analytes on a sorbent from an aqueous medium can be strongly affected by the presence of water embedded in the sorbent after passage of the sample [41]. Accordingly, the effect of drying time on affecting the extraction recoveries was studied. As the drying time increased up to 20 min, so did the extraction recovery of ametryn. Therefore, 20 min for first vacuum in washing step was selected.

**Table 6**
The experimental designs for MISPE.

| Factor[a] Run | A | B | C | D | E | Factor Run | A | B | C | D | E | Factor Run | A | B | C | D | E | Factor Run | A | B | C | D | E |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 | 13 | – | + | + | – | – | 25 | + | – | – | – | – | 37 | +α | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 14 | – | + | + | + | + | 26 | + | – | – | – | + | 38 | −α | 0 | 0 | 0 | 0 |
| 3 | – | – | – | – | – | 15 | – | + | – | + | – | 27 | + | + | – | + | – | 39 | 0 | +α | 0 | 0 | 0 |
| 4 | – | – | – | + | – | 16 | – | + | – | – | + | 28 | + | – | + | + | – | 40 | 0 | −α | 0 | 0 | 0 |
| 5 | – | + | + | – | + | 17 | – | – | + | – | – | 29 | + | + | – | – | – | 41 | 0 | 0 | +α | 0 | 0 |
| 6 | – | – | + | + | + | 18 | – | + | + | + | – | 30 | + | – | + | – | – | 42 | 0 | 0 | −α | 0 | 0 |
| 7 | – | – | + | + | – | 19 | + | + | + | – | – | 31 | + | + | + | – | + | 43 | 0 | 0 | 0 | +α | 0 |
| 8 | – | – | – | + | + | 20 | + | – | – | + | + | 32 | + | – | – | + | – | 44 | 0 | 0 | 0 | −α | 0 |
| 9 | – | – | + | – | + | 21 | + | + | – | – | + | 33 | + | + | + | + | + | 45 | 0 | 0 | 0 | 0 | +α |
| 10 | – | + | – | – | – | 22 | + | – | + | – | + | 34 | + | + | + | + | – | 46 | 0 | 0 | 0 | 0 | −α |
| 11 | – | – | – | – | + | 23 | + | – | + | + | + | 35 | 0 | 0 | 0 | 0 | 0 | | | | | | |
| 12 | – | + | – | + | + | 24 | + | + | – | + | + | 36 | 0 | 0 | 0 | 0 | 0 | | | | | | |

[a] A: flow-rate of sample, B: concentration, C: sample volume, D: mass of sorbent and E: pH of sample.

### 3.2. Qualitative optimization of the MISPE

In order to optimize the MISPE qualitatively, different composition of acetonitrile and acetic acid for washing step and different composition of methanol and acetic acid for elution step were considered to be studied. The results have been shown in Fig. 3, demonstrating that 7 mL acetonitrile plus 0.5% v/v acetic acid instead of the 8 mL acetonitrile can be used. Also, 3× 1 mL methanol plus 1% v/v acetic acid as eluent could be applied for efficient elution without acetic acid. However, it should be noted that, the enrichment of the analyte in MISPE is achieved by applying large volumes of samples and eluting the analyte in a minimum volume of eluent ideally.

### 3.3. Quantitative optimization of the MISPE

When the number of independent variables is small, then, overlying the response surfaces and choosing the optimum conditions constitute a simple and usually highly effective method. The obtained results are summarized in Tables 6 and 7. Table 6 exhibits the type of optimization design chosen in this work and the so-called response surface model: the central composite design (CCD), where the axial points are located on the sphere surrounding the two-level factorial design. As it has been mentioned in Section 2.6, in the experimental design, the evaluation of five factors was considered. Main effects plots (Fig. 4) depict the response surface plots for ametryn. The flow-rate of sample was the first variable. The amounts for the flow-rate were selected between 1 and 5 mL $min^{-1}$. The higher flow-rates were obtained using reduced pressure at the MIP-column outlet. Significant reduction of recovery was found for sample flow-rate from 1 to 5 mL $min^{-1}$ (Fig. 4). By combining the response surfaces, it was finally possible to suggest the optimum conditions for the flow-rate, i.e. 1 mL $min^{-1}$. It seems that using lower sample flow-rates would significantly increase the extraction recovery. From the result given in Table 8, it was deduced that, 0.6 mL $min^{-1}$ could be applied for sample flow-rate.

The next studied parameter was the sample concentration. The amounts for the sample concentration were selected between 10 and 190 ng $mL^{-1}$. Ideally, the extraction recovery should not be sample concentration dependent. In other words, for the method to be useful there should be no significant difference in recovery over the expected concentrations range of the compound to be analyzed. However, it was revealed that unusually selectivity and template affinity was better at higher concentration (Fig. 4). This

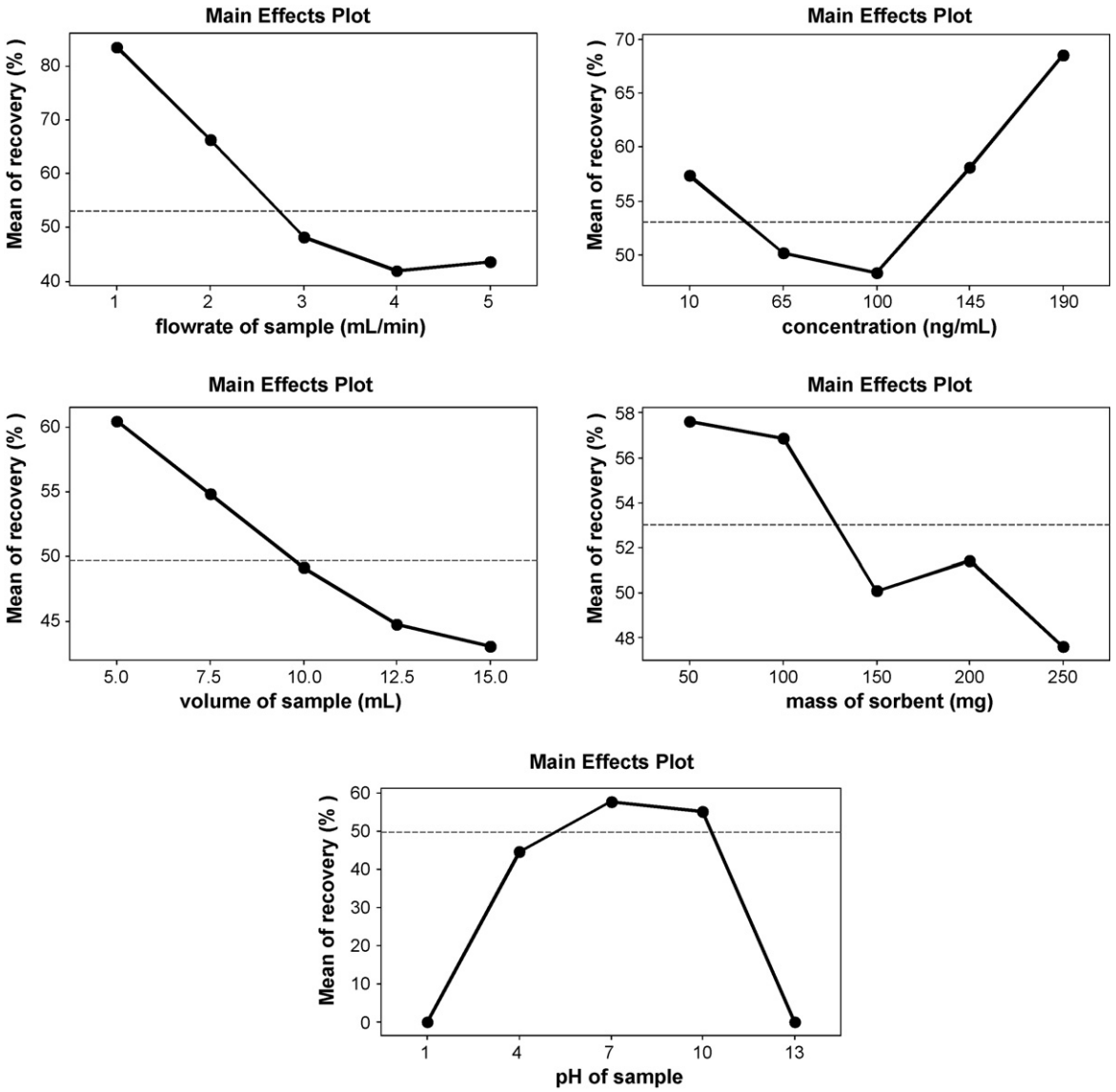


**Fig. 4.** Main effect plots of ametryn MISPE variables in the central composite design

**Table 8**
The effect of flow-rate of sample on recovery (%) of ametryn in MISPE.

| | Flow-rate (mL min$^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 0.9 | 0.8 | 0.7 | 0.6 | 0.5 | 0.4 |
| Mean ± SD ($N$ = 3) | 83.34 ± 0.21 | 85.78 ± 0.18 | 87.37 ± 0.27 | 89.49 ± 0.36 | 91.22 ± 0.13 | 90.76 ± 0.31 | 89.4 ± 0.34 |

**Table 9**
The effect of amount of 0.1 M hydrochloric acid on the ametryn recovery (%).

| | Amount (mL) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 1.5 | 2 | 2.5 | 3 | 3.5 | 4 | 4.5 | 5 |
| Recovery mean ($N$ = 3) | 78.36 | 80.05 | 83.55 | 86.7 | 89.35 | 90.85 | 88.56 | 85.46 | 80.55 |
| SD | 0.27 | 0.26 | 0.26 | 0.17 | 0.35 | 0.59 | 0.38 | 0.23 | 0.44 |

phenomenon has been previously shown [42] for atrazine, where it was proposed that, at higher concentrations the ability of atrazine to generate atrazine–atrazine complexes, both in solution and on the polymer surface, results in increased atrazine selectivity.

In order to evaluate the effect of sample volume on the MISPE performance, different volume of sample ranged from 5 to 15 mL as mentioned in Table 4 was prepared using deionized water. Significant reduction of recovery was found for sample volume from 5 to 15 mL min$^{-1}$ (Fig. 4). This phenomenon can be explained by the heterogeneous surface of the polymer involving the presence of binding sites or cavities of different energy levels. Application of a small volume of sample, allows to the analytes interacting with a larger number of binding sites than when a higher sample volumes are applied. It seems that, in the case of application a higher sample volume, i.e. 15 mL, the partial break-through volume for some binding site was attained. However, it should be noted that, the heterogeneity of the binding sites, is not a limiting factor for using MISPE, because, the retention remains always selective since the studied compound is not retained on the non-imprinted polymer.

Sample pH was another parameter investigated in this study. The amounts for the sample pH were selected between 1 and 13. Fig. 4 illustrates the effect of the sample pH on ametryn retention, showing the recovery obtained using different sample pH. The recoveries were found to be about similar at pH of 4–10. Ametryn is relatively stable in neutral, weakly acidic and weakly alkaline media and rapidly hydrolyzed to the 6-hydroxy derivatives in strong acids and alkalis. pH of (about) 7.0 proved to be optimum for the application of samples to the SPE cartridge containing the MIP to ametryn.

Finally, in order to evaluate the effect of sorbent mass on MISPE performance, different mass ranging from 50 to 250 mg was selected as mentioned in Table 4. To check the influence of the sorbent mass in the recovery values, a series of empty SPE cartridges were filled with different amounts of polymer. Based on the obtained results, the extraction recoveries were not significantly improved when the amount of sorbent was above 200 mg. It seems that, the difficulty in passing the sample through the system increases with the increase in polymer mass. On the other hand, problems with non-specific adsorption to the polymer can be reduced by the use of small amounts of MIP, thereby, reducing the polymer surface area available for non-specific adsorption. Accordingly, by combining the response surfaces it was finally possible to suggest the optimum condition for the amount of sorbent that was 120 mg.

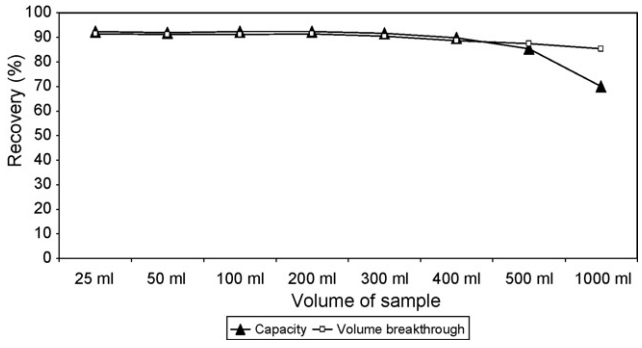


**Fig. 5.** Effect of different volume on the capacity and break-through of the MISPE columns

The data in Table 5 were evaluated by ANOVA at the 5% significance level. Regarding the results presented in Table 7, among the linear effects, the most crucial variables were the flow-rate of sample, sample pH, sample concentration and the sorbent mass as well as among quadratic effects; the effects of the flow-rate, sample concentration, and sample pH were significant ($P \leq 0.05$). Also, it can be derived that, the interaction between the studied variables (from A × B to D × E) was not significant ($P \leq 0.05$). Moreover, the R-Sq (adj) value was 88.9%, meaning that, the five studied factors could explain 88.9% of the variation in the recovery percentage. The results of the central composite design (CCD) were validated using ANOVA. The $P$-value for the model (0.001) was lower than the critical value of the significance set below 0.05. The $R^2$ value for a valid model is 0.6 or greater. For this model, the $R^2$ was 0.835, indicating that the model would be reasonably accurate.

The extraction recoveries were lower than 16% for the spiked drinking water samples, indicating a matrix effect. This loss of extraction recovery can be explained by the presence of cations in the water samples and an ion-exchange mechanism [31]. In order to eliminate matrix effect, regenerating the interaction site and achieving the best results for the extraction of ametryn from spiked drinking water samples, the effect of 0.1 M HCl (1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5 mL after sample loading) on the ametryn recovery was investigated. The results obtained from this experiment have been illustrated in Table 9 and showed that the smallest satisfactory volume for HCl was 3.5 mL.

The results of volume break-through and capacity of MISPE cartridges have been shown in Fig. 5, demonstrating that column capacity up to 400 mL of sample could be applied without significant loss of recovery (at least 89.83 ± 0.51 for 400 mL sample

**Table 10**
Recoveries of some triazines and non-related herbicide.

| | Recovery Mean (%) ± SD[a] | |
|---|---|---|
| | MIP | NIP |
| Ametryn | 91.42 ± 0.24 | – |
| Simazine | 93.54 ± 0.39 | 1.66 ± 0.57 |
| Cyanazine | 82.33 ± 0.2 | 2.66 ± 0.57 |
| Propazine | 87.63 ± 0.38 | – |
| Atrazine | 88.37 ± 0.32 | – |
| 2,4-D | – | – |

[a] SD, standard deviation for $n$ = 3. (–) Not detected.

**Table 11**
Day-to-day (D-day) and within-day (W-day) reproducibility of ametryn spiked in drinking water, sample volume: 10 mL, $N = 6$.

| | Concentration added (ng mL$^{-1}$) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 10 | | 65 | | 100 | | 145 | | 190 | |
| | D-day | W-day | D-day | W-day | D-day | W-day | D-day | W-day | D-day | W-day |
| Mean | 9.03 | 9.08 | 59 | 59.14 | 91.14 | 90.94 | 132.87 | 132.63 | 174.11 | 173.93 |
| SD | 0.1 | 0.08 | 0.86 | 0.35 | 0.41 | 0.26 | 0.2 | 0.36 | 0.33 | 0.28 |
| RSD | 1.11 | 0.88 | 1.45 | 0.59 | 0.45 | 0.29 | 0.15 | 0.27 | 0.19 | 0.16 |

volume) and for volume break-through no significant reduction of recovery was found in the total of the study range (25–1000 mL). Also, it should be noted that, based on a separate experiment, the ametryn MIP exhibited good stability and selectivity even after the 41 enrichment and desorption studies.

Usually the specificity of the ametryn MIP is determined via the cross-reactivity of several triazine herbicides (high or intermediate cross-reactivity) and some other pesticide compounds (low cross-reactivity) [43]. Based on the obtained results from Table 10 and similar to another work [42], atrazine, simazine, cyanazine and propazine are structurally similar and it was therefore unsurprising that their recoveries on the ametryn imprinted column were similar. The minor variation in recoveries observed can be accounted for by small variations in the interaction energies between the molecules and the recognition sites of the polymer [31], size, and the additional methyl substitution. Also, the compound with low cross-reactivity, 2,4-dicholorophenoxy acetic acid (2,4-D) was not retained on the ametryn MIP. This result confirms the high selectivity of the extraction on MIP. In addition, similar to another study [32] in order to decrease the non-specific interactions and obtain maximal selectivity, 1% methanol was added to the samples. Methanol was selected for its high eluting strength. However, this amount of methanol should be as low as possible because it showed a decrease in the retention of compounds retained on the surface of the polymer without affecting the overall retention in the imprints. The addition of 1% methanol causes a significant drop in extraction recoveries on the non-imprinted polymers (Table 10).

### 3.4. Method validation

More experiments were performed on spiked drinking water to validate the present method (Table 11). Spiked water sample can be a suitable model as it may contain interfering constituents similar to the real sample [44].

The spiked drinking water samples of 10 mL of ametryn were used for extraction followed by HPLC-UV determination. Linear standard curve (for extracted samples) over the range 10–190 ng mL$^{-1}$ was obtained each day ($n = 6$) with correlation coefficient of 0.994 or greater. The extraction procedure was reliable and reproducible from day-to-day and within-day (Table 11). The relative standard deviation (RSD) of 1.11, 1.45, 0.45, 0.15 and 0.19 was obtained for 10, 65, 100, 145 and 190 ng mL$^{-1}$, respectively, for day-to-day and 0.88, 0.59, 0.29, 0.27 and 0.16 at the same concentrations, respectively for within-day, showing suitable accuracy and precision. The detection limit of the method (signal/noise: 3:1) using spiked drinking water sample volume of 10 mL was 0.01 μg mL$^{-1}$ and reproducible and quantitative recoveries, ranging from 88% to 93% for triazine herbicides were possible.

## 4. Conclusion

This present study provides an improved understanding of the approaches available for the optimization of MISPE protocols. Although applications of this technique in the industrial and environmental fields are still rare, they have become more common in the recent years. The new MISPE studies are mainly based on developing imprinted polymers for new target analytes of biological and environmental interest. The use of the MISPE cartridges, might be limited because of their low yield of specific binding sites, so, in some cases these cartridges have low sample loading capacity and high non-specific binding. For these reasons, studies currently being developed involve the optimization of the protocols for improving the capacity and/or the selectivity of the MISPE sorbents. Chemometrics is frequently employed for analytical method optimization and it is believed that the central composite design could prove beneficial for aiding the MISPE protocol optimizations with variables such as the sample pH, sample concentration, sample flow-rate, sample volume, and sorbent mass. Future works may also consider using different parameters. Finally, the developed extraction protocol gave highly efficient and selective clean-up of drinking water samples. The authors are sure that, MISPE is still one of the most interesting applications within MIP field and based on the needs and facilities, these method protocols can be developed more in the near future.

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