Optimization of solid phase microextraction conditions for determination of triazines

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Abstract. A simple, solvent-free, and field-compatible approach to sample preparation is provided by the basic process known as solid-phase micro extraction (SPME). The aim of this study was to improve the circumstances under which triazines were extracted from aqueous matrices. The complex technique enabled the detection of the analytes in the water at a physiological level. An evaluation of the practicability of the most commercially available fiber coatings has been conducted. The porous materials (Carbowax/DVB and PDMS/DVB) extracted more efficiently than homogeneous ones (PDMS and polyacrylate). Further investigation has been focused on 65 mm PDMS/DVB fiber. Solid phase microextraction is a technique based on the partition equilibrium of analytes between the sample and the fiber. Therefore, 60 minutes were required to reach equilibrium at room temperature. The desorption efficiency at 3 temperature levels had been compared and 270 °C was retained. Experiments were performed with a Finnigan MAT GCQ Model installed with a 30-m x 0.32-mm ID, ZB-5 (0.25-mm film thickness) fused-silica capillary column, and Helium as a carrier gas (at 30 kPa), The ion trap mass spectrometer was operated in the electron ionization (EI) mode. Keywords: Fiber coating, Gaz chromatography, Mass spectrometry, Pesticides, Water matrices, Analysis.

1 Introduction

Since the concentrations of the substances to be determined in environmental samples are very low (µg/L range) and each substance can only be detected up to a certain limit, they must be concentrated in an enrichment procedure before analysis.
In this work, selected pesticides were extracted from the water matrices. In the literature, different extraction options, e.g., liquid-liquid extraction (LLE), solid-phase extraction (SPE) or solid-phase micro-extraction (SPME) are described for the selected substances [1-2].
There are advantages and disadvantages to each method. The biggest disadvantage of LLE is time consuming and the large amounts of organic solvents used. Due to these disadvantages, LLE has already been replaced by SPE in most typical applications [3-4].
For sample preparation using SPE, less organic solvent is usually consumed than with LLE. SPME is another alternative technique for sample preparation developed by Pawliszyn in 1989 [5].
The advantage of this preparation compared to the previous SPE with packed columns is the integrated use of one instrument for all necessary steps of sample preparation prior to their instrumental analysis by means of capillary gas chromatography (GC).
By combining the known extraction agents from classical SPE with a special geometric arrangement in the form of a fiber, time- and material-consuming steps can be saved [6]. However, the fiber can be reused up to 70-100 times. The cost analysis of the SPME method is substantially lowered comparable to the SPE [4-7-8].

Based on the advantages of the SPME (solvent-free, easy handling, low equipment requirements and rapid method), pesticide analysis using SPME is carried out. SPME is based on a partial extraction of the analyte by means of a polymer film immobilized on a fused silica fiber.
The operating principle of the SPME device is fairly simple, it is illustrated as follows: Inside a cannula there is a steel wire to the end of which the "fused silica fiber" coated with a polymer film is glued. For extraction, the fiber is pushed out of the cannula and exposed to the liquid phase or vapor space of the sample. During the removal process the analyte is adsorbed or absorbed on the fiber.
The fiber is then withdrawn into the cannula and the cannula is used to puncture the septum in the gas chromatographic injector. After pushing the fiber out of the cannula, the sample is released by thermodesorption of the analyte in the injector. This is followed by the usual gas chromatographic separation and detection [5,9].
The coating of the fiber can be either liquid or solid, so that the predominant sorption mechanism may be adsorption or absorption. For polar analytes (e.g. triazines) a polyacrylate coating is mainly used. This is a partially cross-linked polymer with high polarity. The solid, crystalline state changes to the liquid state at a

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desorption temperature above 200 °C. The predominant sorption process in this case is absorption [10-11]. A coating of polydimethylsiloxane (PDMS) is used for non-polar analytes. The nonpolar PDMS is a highly viscous, rubbery liquid with varying degrees of cross-linking. Absorption also plays the predominant role in this fiber coating.
The term chromatography covers a series of microanalytical separation methods used to separate individual compounds from a given mixture of substances. Within the scope of this work, quantitative and/or qualitative analyses of the substances were carried out using chromatographic method (GC), with Mass spectrometry detection system. [12-13].
This study monitored pesticides based on their use and persistence [14].
The pesticides selected were: Atrazine, simazine, ametryne, propazine, terbutylazine, sebutylazine, trietazine, prometryne and terbutryne. Since pesticides are easily transformed, their metabolites' environmental fate is even more important when considering the ultimate fate of the applied pesticides.
The metabolites we have worked with were (mother compounds in parenthesis): deethyl-atrazine “DEA” and deisopropyl-atrazine “DIA”, (atrazine).

2 Experimental

2.1 Chemicals

Pure analytical pesticide standards (purity higher than 98%), atrazine, deethyl-*atrazine*, deisopropyl-*atrazine*, simazine, ametryne, propazine, terbutylazine, sebutylazine, trietazine, prometryne, terbutryne, and the internal standard malathion (D6) were obtained from Dr. Ehrenstorfer (Augsburg, Germany).
To prepare standard stock solutions of each compound, we weighed the solutes and dissolved them in acetone in 10 mL volumetric flasks; these were then stored at 4ºC in the dark.
Dilute solutions were prepared and water samples were spiked to the required concentrations. Solvents were obtained from Merck (Darmstadt, Germany) and were all analytical grades.

2.2 Equipment

Experiments were performed by using a Finnigan MAT GCQ Model, equipped with a 30-m x 0.32-mm ID, DB-5.MS (0.25-μm film thickness) fused-silica capillary column (J&W Scientific, USA) and an Optic2, Ai, Cambridge split–splitless injector, (Mode: splitless (1 min), Initial pressure: 8 psi (55.16 kPa), Final pressure: 14 psi (96.53 kPa)). Helium (5.0) was used as a carrier gas.
The oven temperature program was as follow: initial temperature 65°C (held for 1 min) increased at 30°C/min to 165°C for 7 min, at 6°C/min to 200°C for 11 min, at 25°C/min to 225°C for 3 min.
The ion source temperature was 175°C. The MS transfer line was kept at 275°C to prevent the analytes from recondensing.
The electron impact (EI) ionisation conditions were the following: ion energy 70 eV and mass range 50-500 in the full scan mode. The scan-rate was 0.4 scans s^{-1}.
The selected ions for quantification of the analytes are as follows (m/z in parentheses): atrazine (200, 215), simazine (186, 201), propazine (214, 229), terbuthylazine (173, 214), trietazine (186, 200), sebuthylazine (200, 202), ametryn (212, 227), prometryn (184, 241), terbutryn (185, 226), DEA (172, 187) and DIA (158, 173).

2.3 Solid phase microextraction

For the solid-phase microextraction, glass vials (from Supelco) of different volumes with screw caps and Teflon-coated septa were used as sample vessels. In order to prevent pesticide contamination of glass sample vials, all glass vials were rinsed with nitric acid and demineralized water.
The glass vials were then dried at 110°C in an oven for 5 hours [15].
The vials were rinsed with acetone and dried before loading the sample.
The purity of the sample vials was checked by blindex extraction (removal without the addition of pesticides). Before each series of measurements, the SPME fibre was baked out at an injector temperature of 250°C in order to remove any contaminants sorbed from the ambient air.
7 mL glass vials containing a magnetic stir bar were used for the extraction each filled with 5 mL sample solution. All experiments were carried out at room temperature with stirring (750 rpm) on a magnetic stirrer (from Variomag Elektronik Multipoint MP).

3 Results and discussion

3.1 Optimisation of the SPME method

3.1.1 Chromatographic separation

Using a GC–MS system and an injection volume of 1 μL of a standard mixture in acetone, atrazine, DEA, DIA, simazine, ametryne, propazine, terbutylazine, sebutylazine, trietazine, prometryne, and terbutryne were separated. As can be seen in Figure 1, all analytes are resolved.

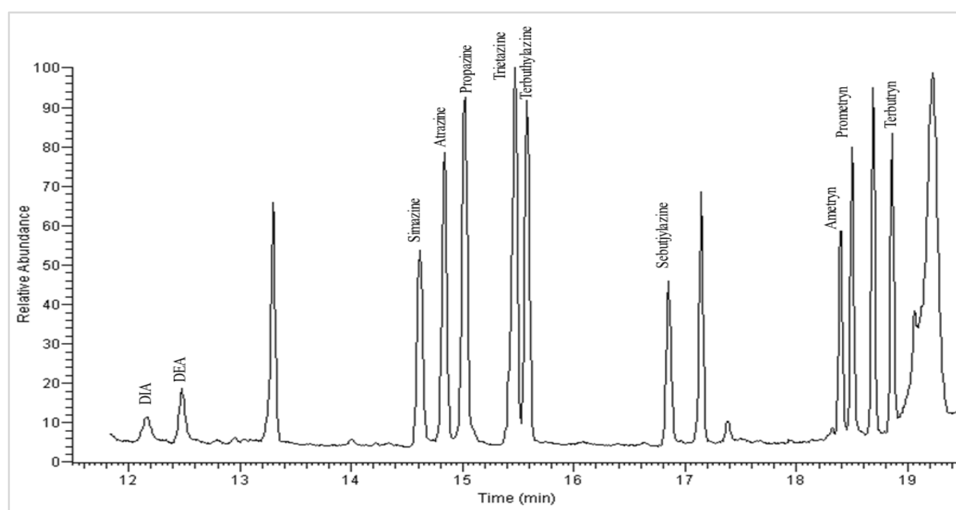

Fig. 1. GC/MS chromatogram of a standard mixture.

3.1.2 Choice of the SPME fiber

We systematically investigated the effects of different parameters on the efficiency of the microextraction and desorption steps. An optimized procedure for group determination of 11 analytes was implemented. Adsorption time, desorption temperature; time, and solvent influence and ionic strength are optimized for the selected fibres. Selecting the appropriate coating fiber is one of the most important steps in the development of the SPME method. In the first optimization step, the effects of four different coating fibers (PDMS, PDMS/DVB, Polyacrylate, and CW/DVB) on triazine concentrations were compared by direct extraction, at room temperature, for 60 min [16]. Experiments were performed using two different solvents (acetone and methanol). As can be seen (Figure 2), the best overall results were obtained with methanol, giving a higher peak area for all analytes than acetone, except the most polar species of the group, DEA and DIA (logKow 1.51 and 1.15, respectively). The removal efficiency was lower for PDMS and Polyacrylate fibers respectively, followed by PDMS-DVB fiber. By far, the highest responses for all compounds were obtained from CW-PDMS fibers (see Figure 3). However, we noticed significant degradation of the CW-PDMS fibers after several extractions (see figure 4). Therefore, PDMS–DVB fibers were selected for the removal of these analytes [17]. However, the PDMS–DVB, known as the semipolar phase, is also capable of extracting more polar analytes [18]. For multi-residue analysis of pesticides with varying polarity and physicochemical properties, a PDMS/DVB SPME fiber was used as the extraction phase. The new SPME-GC/MS approach was thoroughly verified in terms of analytical figures of merit and found to be suitable for routine quality control of 29 of 46 pesticides in drinking and surface waters [16].

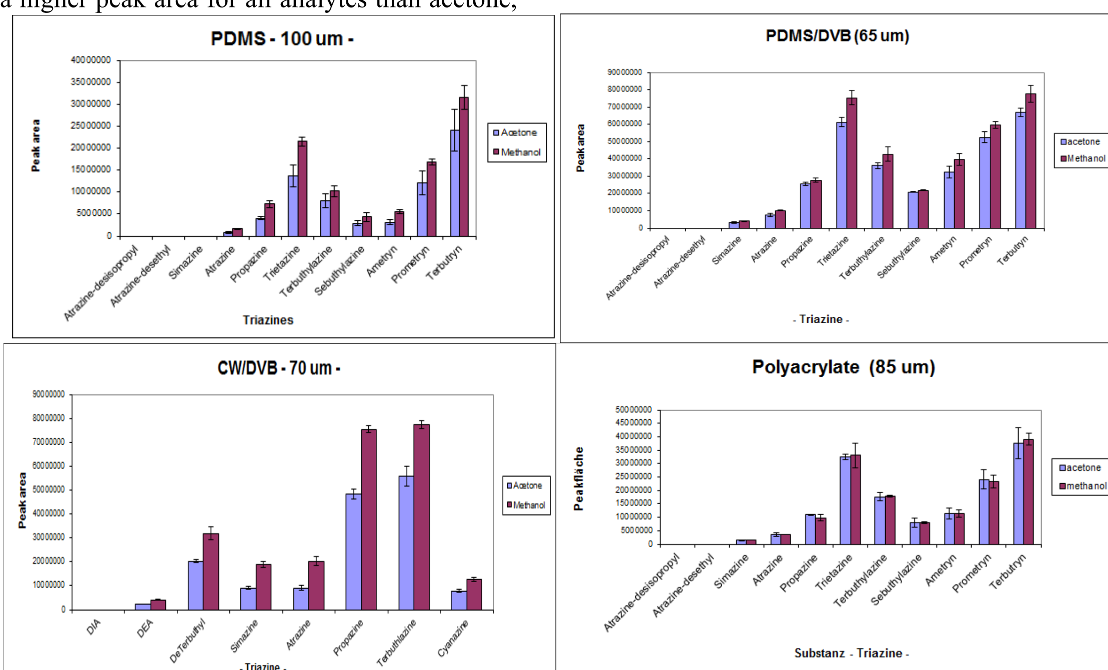

Fig. 2. Efficiencies of different solvents and SPME yields. Eight analytes were used to test CW/DVB fiber.

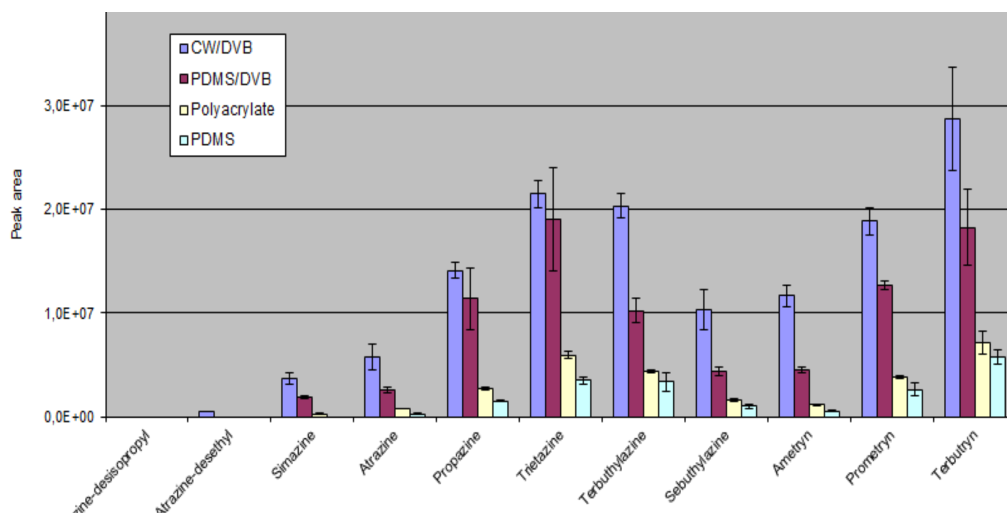

Fig. 3. Comparison of SPME efficiencies for CW/DVB, PA, PDMS-DVB and PDMS fibers.

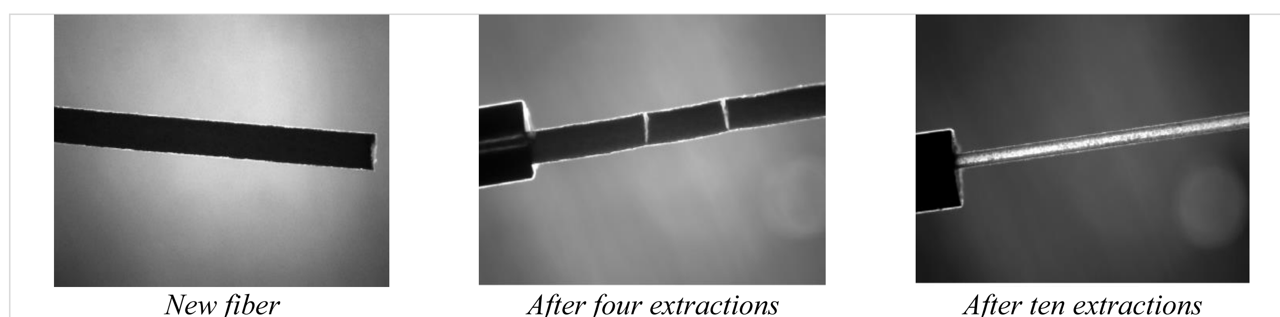

Fig. 4. Rapid deterioration of the SPME fiber.

3.1.3 Desorption time and temperature.

The desorption temperature and the desorption time should be considered when desorbing analytes in a GC instrument. For immediate release of analytes, the fibers must be desorbed at high temperatures. In this context, time and temperature were optimized to maximize desorption efficiency of PDMS-DVB fibers. However, the supplier recommends a maximum temperature to avoid degradation of the polymeric fiber. Variable injector temperatures were tested at three levels: 220, 250 and 270 °C to determine the optimal desorption temperature (Figure 5). A temperature of 270 °C was chosen as a compromise value to prevent thermal degradation of the fiber and extend its lifetime. Various desorption times (from 1 to 12 minutes) were tested, with the best results obtained within 10 minutes because after this time no significant changes in the response for eight analytes were observed. However, after 10 minutes, we noticed a slight decline in the terbutryne, trietazine and ametryne responses (Figure 5). Each time a sample is injected, an empty vial is injected to ensure that the contact conditions are sufficient to allow compounds to desorb from the fiber completely. The carryover effects were not observed when desorption conditions were optimal [19]. In other study, SPME has been used to determine different classes of pesticides in aqueous samples. An 85-mm polyacrylate coated silica fiber was used. The effect of several parameters on SPME has been investigated. The optimum conditions for SPME Were found to be a temperature of 55°C and the time of 45 min for the absorption process and 250°C for 2 min for the desorption process [20].

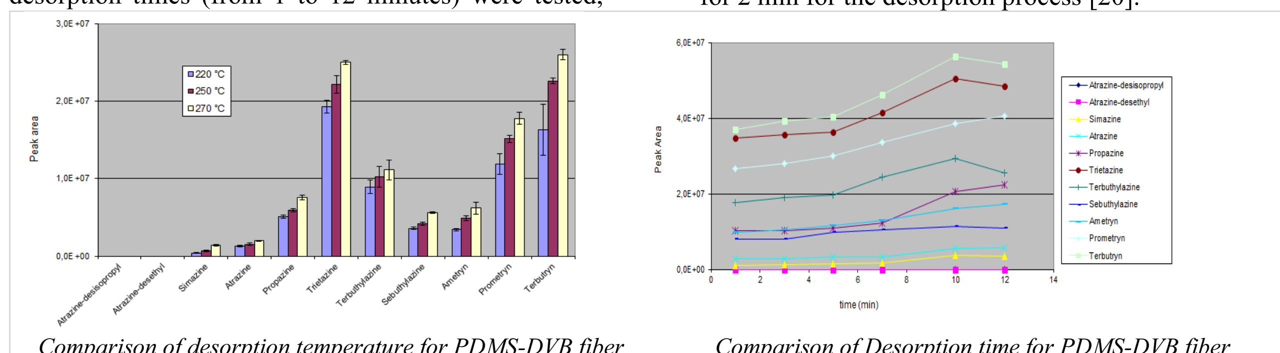

Fig. 5. Comparison of desorption temperature and time for PDMS-DVB fiber.

To increase the retention of water-soluble components in solid phase microextraction (SPME), the "salting out effect" approach is used to reduce the solubility of analytes in water and favor extraction. An increase in ionic strength can be achieved by adding salts, such as sodium chloride. The effect of ionic strength on PDMS/DVB fiber extraction yield was investigated using samples with increasing sodium chloride concentration from 10% to 30%. Triazine group behaved in three distinct ways. For the most polar species, DEA, DIA, simazine, atrazine and ametrine, the removal yield increased steadily with sodium chloride concentration.The removal yield of propazine, terbuthylazine, and cebutylazine increased up to 10% sodium chloride and remained stable thereafter. In contrast, salt addition did not affect the SPME recovery of the other three components, triethazine, terbutrin and prometryne (fig 6). In SPME, sodium chloride affects both kinetics and thermodynamics. Considering the salting-out effect, the improved removal efficiency can be explained thermodynamically, which is particularly associated to polar species. On the other hand, the presence of salt increases the viscosity of the sample and slows the diffusion of analytes to the fibers. The last effect is responsible for the decreased response of ametryn, prometryn and terbutryn, observed at high salt concentrations of 30%. In view of the above results and since no significant benefit of salt addition below 30% was observed for the two metabolites of atrazine (DEA and DIA), the concentration of NaCl added to the water samples was set at 30% (0.3 g/mL).

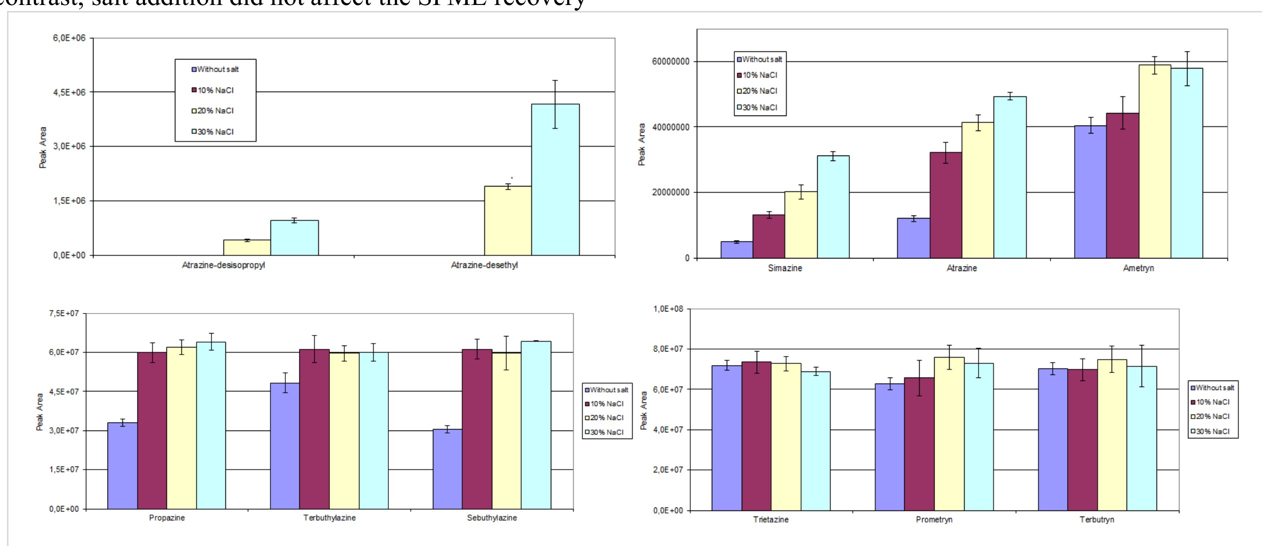

Fig. 6. Effect of different salt concentrations (10-30% w/v) on removal efficiency for triazine pesticides.

3.1.4 Equilibrium conditions

During solid-phase microextraction, analytes are partitioned according to their partition coefficient between a liquid sample and a polymeric phase. The extraction kinetics was determined by using standard solutions and different exposure times. Extraction time profiles are shown in Figure 7. The equilibration time ranged from 1 min to 840 min. Equilibrium was reached after 240 min. To work in a reasonable timeframe, the extraction time for further experiments was set to 1 hour, allowing multiple extractions per day. Quantitation was then based on the timed accumulation of analytes in the coating.

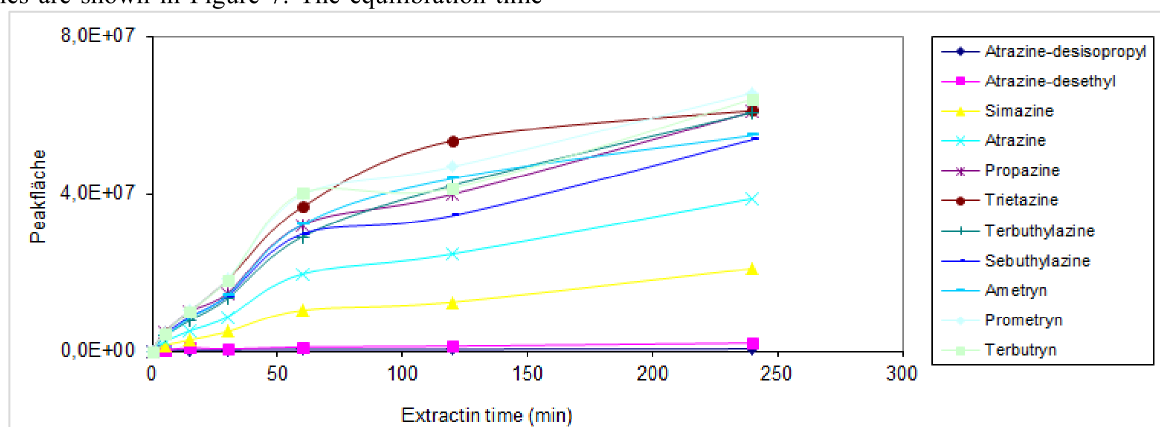

Fig. 7. Extraction time profiles for triazines studied in the concentration range between 2 and 2.4 µg/l, 30% NaCl(w/v). Standard deviation ranged between 1 and 10%.

Figure 8 shows an example of a calibration curve for Atrazine, simazine, ametryne, propazine, terbutylazine, sebutylazine, trietazine, prometryne, terbutryne and the metabolites DEA and DIA. Using this method, the triazines studied were analyzed efficiently.

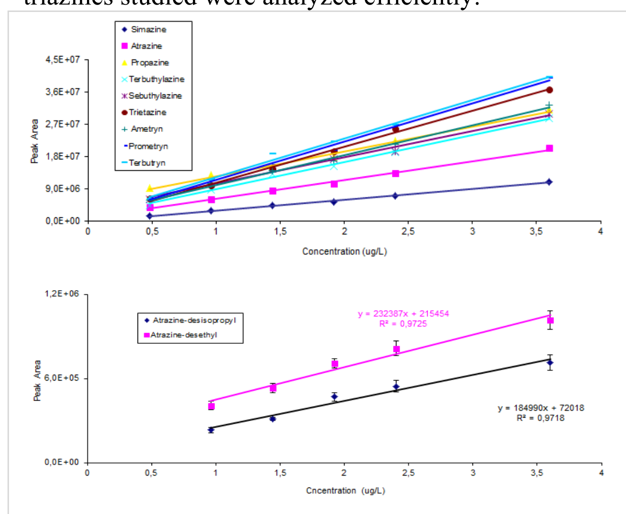

Fig. 8. Calibration curves for triazine pesticides in deionized water.

4 Conclusion

The SPME-method was developed for the determination of atrazine, DEA, DIA, simazine, ametryne, propazine, terbutylazine, sebutylazine, trietazine, prometryne, terbutryne in drinking water as well as a variety of environmental samples. Requirement of only small sample volume (5 mL) for the extraction, makes the optimized method easy to use at low cost. A groundwater sample from the Rmel area (north-west of Morocco) was analyzed to test the applicability of the optimized method. Triazines can be detected in small environmental water samples using the optimized SPME method, nevertheless, fibers should be handled with care since they are fragile and easily break, as well as the fiber coating is susceptible to damage during insertion into the sample or agitation.
In terms of SPME fibers, the great bulk of work has been done with PDMS and PA fibers, primarily because these were the first commercially available. The current tendency is to employ more polar fibers, such as Carbowax-divinylbenzene, Carbowax-templated resin, or Carboxen-PDMS, which were recently commercialized. These fibers should enable the extraction of more polar chemicals, which is particularly relevant in pesticide residue analysis for analytes that differ from the majority of organochlorine and organophosphorus pesticides [15].

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