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Selective mixed-bed solid phase extraction of atrazine herbicide from environmental water samples using molecularly imprinted polymer

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ABSTRACT

A novel approach for the selective extraction of organic target compounds from water samples has been developed using a mixed-bed solid phase extraction (mixed-bed SPE) technique. The molecularly imprinted polymer (MIP) particles are embedded in a network of silica gel to form a stable uniform porous bed. The capabilities of this method are demonstrated using atrazine as a model compound. In comparison to conventional molecularly imprinted-solid phase extraction (MISPE), the proposed mixed-bed MISPE method in combination with gas chromatography–mass spectrometry (GC–MS) analysis enables more reproducible and efficient extraction performance. After optimization of operational parameters (polymerization conditions, bed matrix ingredients, polymer to silica gel ratio, pH of the sample solution, breakthrough volume plus washing and elution conditions), improved LODs (1.34 $\mu g\ L^{-1}$ in comparison to 2.25 $\mu g\ L^{-1}$ obtained using MISPE) and limits of quantification (4.5 $\mu g\ L^{-1}$ for mixed-bed MISPE and 7.5 $\mu g\ L^{-1}$ for MISPE) were observed for the analysis of atrazine. Furthermore, the relative standard deviations (RSDs) for atrazine at concentrations between 5 and 200 $\mu g\ L^{-1}$ ranged between 1.8% and 6.3% compared to MISPE (3.5–12.1%). Additionally, the column-to-column reproducibility for the mixed-bed MISPE was significantly improved to 16.1%, compared with 53% that was observed for MISPE. Due to the reduced bed-mass sorbent and at optimized conditions, the total amount of organic solvents required for conditioning, washing and elution steps reduced from more than 25 mL for conventional MISPE to less than 2 mL for mixed-bed MISPE. Besides reduced organic solvent consumption, total sample preparation time of the mixed-bed MISPE method relative to the conventional MISPE was reduced from more than 20 min to less than 10 min. The amount of organic solvent required for complete elution diminished from 3 mL (conventional MISPE) to less than 0.4 mL with the mixed-bed technique shows its inherent potential for online operation with an analytical instrument. In order to evaluate the selectivity and matrix effects of the developed mixed-bed MISPE method, it was applied as an extraction technique for atrazine from environmental wastewater and river water samples.

1. Introduction

Sample preparation is an important step in most analytical procedures, whereby the sample is treated prior to its analysis in order to pre-concentrate the analytes, remove matrix compounds, so as to improve the selectivity of an analytical procedure. Significant trends in sample preparation are simplification, miniaturization and automation of analytical techniques. According to the principles of “Green Chemistry”, the minimization of solvent and reagent use is also an actual argument for developing improved extraction methods. Most existing extraction techniques are laborious, time-consuming and use large quantities of organic solvents [1]. Therefore, modifications were made to certain extraction techniques to optimize the methods, either for a group of analytes [2,3] or even for a single analyte [4–6].

Solid phase extraction (SPE) is certainly the most popular and widely-used sample preparation technique for liquid samples that can be applied in off-line or online systems, with the advantage of possible automating. Over time, significant efforts have been devoted to develop various sampling formats and sorbent materials to improve simplicity, selectivity, sorption capacity and chemical or physical–mechanical stability [7,8].

A new class of selective sorbent materials is molecularly imprinted polymers (MIPs) that are based on molecular recognition. MIPs are tailor-made polymers in which recognition sites are imprinted in the polymer matrix according to the size, shape and

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the functional groups of a template molecule. MIPs operate as artificial specific receptors that can be used as a powerful tool in the development of highly selective analytical methods. In addition to the high selectivity of MIPs, their simplicity of production and furthermore, less strict operation conditions compared to immunosorbents, make their applications remarkably widespread [9–12]. So far, MISPE is the most used technical application of MIPs [13]. New miniaturized MISPE formats like MIP grafted to porous polyethylene [4], microextraction by packed sorbent (MEPS) [14], porous membrane protected micro-solid phase extraction (μ-SPE) [15] and molecularly imprinted monolith μ-SPE [16] were developed and optimized. The key advantage of these extraction techniques is the minimized usage of organic solvents. In some cases, on-line connection to gas chromatography and liquid chromatography is possible and allows automated operation and minimal labor effort.

Solid phase disk extraction (SPDE) is an innovative format of SPE in which a disk-shaped matrix is loaded with the solid sorbent [17]. While SPDE devices are only commercially available for a limited range of sorbent types, SPE cartridges can be easily prepared in the laboratory [7].

The objective of this study was to develop a simple technique that embeds MIPs in a specific sorbent for combining the innovative features of SPDE with those of miniaturized solid-phase extraction and the selectivity of imprinted polymers. Expected advantages of this approach include significant reduction of channeling and voiding effects (that can provide increased column-to-column reproducibility), increased precision, and reduction of organic solvent and time demand. Atrazine herbicide was selected as a model compound to compare the benefits of the new mixed-bed MISPE approach with already reported conventional MISPE.

2. Experimental

2.1. Chemicals

The chemicals used for the polymer synthesis and extraction experiments were acetic acid, methanol, acetone and chloroform of GC grade purity provided by MERCK (Darmstadt, Germany). Dichloromethane (DCM), ethylene glycol dimethacrylate (EGDMA), methacrylic acid (MAA), 2,2′-azobisisobutyronitrile (AIBN) and atrazine (ATZ) were obtained from Sigma-Aldrich (Steinheim, Germany). MAA and AIBN were purified by distillation under reduced pressure and methanol recrystallization, respectively. The other chemicals were used as delivered, due to their high purity.

The stock standard solution of atrazine was prepared in methanol at a concentration of 2000 mg L^{-1} and stored in the refrigerator. Other standard solutions were prepared daily via dilution of stock solution using pH adjusted deionized water.

2.2. Instrumentation for polymer preparation and extraction procedure

KDS100 syringe pump from KD Scientific (Holliston, USA) was used to generate defined flows through the prepared cartridges. GFL water bath (Burgwedel, Germany) and VL-6LM UV-lamp (6 W, 312 nm) were used for the synthesis of the imprinted polymers. 8 mL BAKERBOND SPE glass columns (inside diameter: 12 mm, length: 91 mm), 3 mL BAKERBOND SPE glass columns, polytetrafluoroethylene (PTFE) frits, speedisk cartridge (H_2O-Philic DVB), SDB cartridge (Styrene-divinylbenzene), octadecyl cartridge (C_{18}) and 24-fold vacuum extraction box were purchased from J.T.Baker (Deventer, Holland). Silica gel S (0.063–0.1 mm) was obtained from Riedel-de Haën (Seelze, Germany).

2.3. SPE procedure with styrene-divinylbenzene sorbent

A styrene-divinylbenzene (SDB) extraction column containing 200 mg of sorbent was used for the comparison of developed method with an available commercial extraction method. The extraction procedure was adapted from Mendas et al. [18] with slight modification. The SDB cartridge was conditioned by the passage of 3 mL of acetone and 5 mL of deionized water. A vacuum was applied to draw the acetone and water through the cartridge. A 10 mL spiked sample was passed through the previously-conditioned cartridges at a flow rate of about 3 mL min^{-1}. The loaded cartridge was washed with 10 mL of deionized water and dried using the SPE vacuum manifold for 20 min. The dried cartridge was eluted with 5 mL acetone. The resulting eluate was evaporated under a gentle stream of inert gas at 25 °C and then reconstituted with 0.1 mL of chloroform.

2.4. Mixed-bed MISPE preparation

The preparation of the atrazine MIP was adapted from Matsui et al. [19]. In summary, MIP is prepared using 0.032 g of atrazine, 0.011 g of AIBN, 0.827 g of EGDMA and 50 μL of MAA dissolved in 2.2 mL chloroform as porogen. Polymer mixture was deaerated with helium for 15 min and photochemically polymerized at 25 °C for 4 h using the UV-lamp at 312 nm. The non-imprinted polymer (NIP) mixture was prepared in the same way without the target molecule. Following polymerization, the material was ground and sieved through a 40-μm sieve, followed by soxhlet extraction with methanol and acetic acid (99:1). Finally, the polymer particles were dried under vacuum conditions and stored in a desiccator at room temperature until use.

As mentioned in Section 1, significant efforts have been devoted to developing various MISPE formats. Fig. 1 shows the schematics of conventional MISPE, miniaturized MISPE, MISPE with reduced mass bed and the mixed-bed MISPE. For the preparation of mixed-bed MISPE cartridges, one PTFE frit was inserted inside a glass cartridge. In order to avoid sorbent loss, the atrazine MIP and silica gel particles were weighted directly inside the one-side closed cartridge. The particles were mixed thoroughly with a fine laboratory spatula for 5 min. Then the lower part of the cartridge that contains the mixed particles was connected to the SPE vacuum manifold. Along with a gentle vacuum, 1 mL methanol was poured onto the mixed particles and the other PTFE frit was inserted to the cartridge for fixing the porous bed.

3 mL empty glass cartridges were used as received. 8 mL empty glass cartridges were cut from 91 to 45 mm to reduce the dead volume of water sample during sampling step. To make the MISPE extraction columns, the atrazine MIP particles were weighted and packed in the same way as described for mixed-bed MISPE.

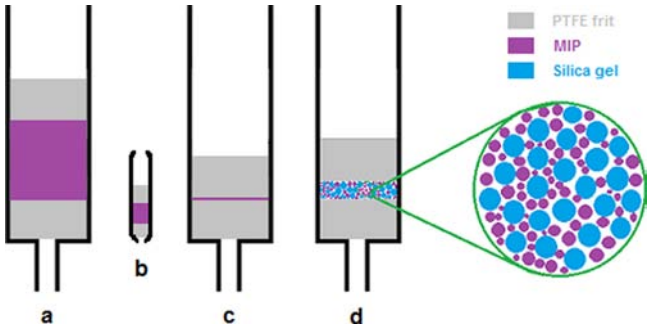

Fig. 1. Schematic diagrams of different procedures for MISPE preparation. (a) Conventional MISPE. (b) Miniaturized MISPE. (c) MISPE with reduced mass-bed (this work). (d) Mixed-bed MISPE (this work).

2.5. Mixed-bed MISPE extraction procedure

As shown in Fig. 2, the open part of the prepared mixed-bed MISPE cartridge was closed with a Teflon nozzle that can be easily connected to the different needle sizes of syringes. A syringe pump was used for liquid delivery along the tubes. In order to have a uniform sample flow through the mixed-bed MISPE, the sample was inserted through the Teflon nozzle located at the bottom-up design. Regarding to reduce the consumed organic solvent, the conditioning step was done from the lure tip of the prepared cartridges. The mixed-bed MISPE cartridges were conditioned with 0.5 mL of methanol followed by 0.5 mL of distilled water to wet the polymer completely. Water samples were passed through the previously conditioned cartridges. In order to remove non-specific adsorbed target molecules and to eliminate potential matrix effects, washing was carried out using 10 mL of distilled water and 1 mL of dichloromethane. The cartridge was immediately washed after sample loading, with 10 mL water using the SPE vacuum manifold. Since dichloromethane is not miscible with water, the vacuum was continued for 5 min to dry the mixed-bed completely. The reduction in water content was followed by weighing. Then 1 mL of dichloromethane was passed through the dried cartridge and vacuum for 30 s to remove the remaining dichloromethane. Finally, the cartridges were eluted with 0.4 mL of methanol–acetic acid mixture (99:1). The resulting eluates were evaporated under a gentle stream of inert gas at 25 °C and then reconstituted with 0.1 mL of chloroform. Dichloromethane was easily collected from the reservoir part of mixed-bed MISPE cartridges, while eluent solvent was forced to pass through with a rubber piston. All of the experiments were repeated three times, unless otherwise stated.

2.6. GC–MS determination

The analysis was performed using a GC–MS system (Agilent GC 7890A, MSD 5975C) coupled with an Agilent Technologies CTC Analytics Combi PAL autosampler. For separation, a HP-1MS capillary column (30 m, 0.25 mm ID, 1 μm film) and helium gas as the carrier at a constant flow rate of 1 mL min^{-1} were applied. 1 μL of the samples were injected in the injection port of the GC that was maintained at a temperature of 260 °C. The oven temperature of the GC was initially held at 60 °C for 1 min, then increased to 280 °C at a rate of 15 °C min^{-1} and remained at this level for 2 min. Mass spectrometric detection was carried out using electron impact (70 eV) ionization. After recording full scan spectra, we monitored atrazine and brombenzol-d5 (internal standard) using their typical ions (*m*/*z* 200 and 215 for atrazine and *m*/*z* 82, 161 and 163 for brombenzol-d5).

2.7. Real samples

The analytical applicability of the proposed method was assessed using three different water samples. Laboratory tap water, river water and influent from the municipal wastewater treatment plant in Leipzig (500,000 PE) were used for preparing the atrazine solutions which were applied in the experiments to show the influence of matrix components on atrazine extraction. Prior to spiking, all samples were checked with styrene-divinylbenzene column to ensure that they are free of atrazine. Each spiked sample was prepared freshly before the extraction experiments. Tap water without any other treatment was used for preparing atrazine solutions applied in the experiments. River water and wastewater were filtered with a 45 μm glass fiber filter and were stored in refrigerator for experiments. The pH of all samples was checked to be in the range of 6–7. Both wastewater and the river water proved to be challenging examples for samples with complex matrices provoking the selectivity and efficiency of the developed mixed-bed MISPE extraction.

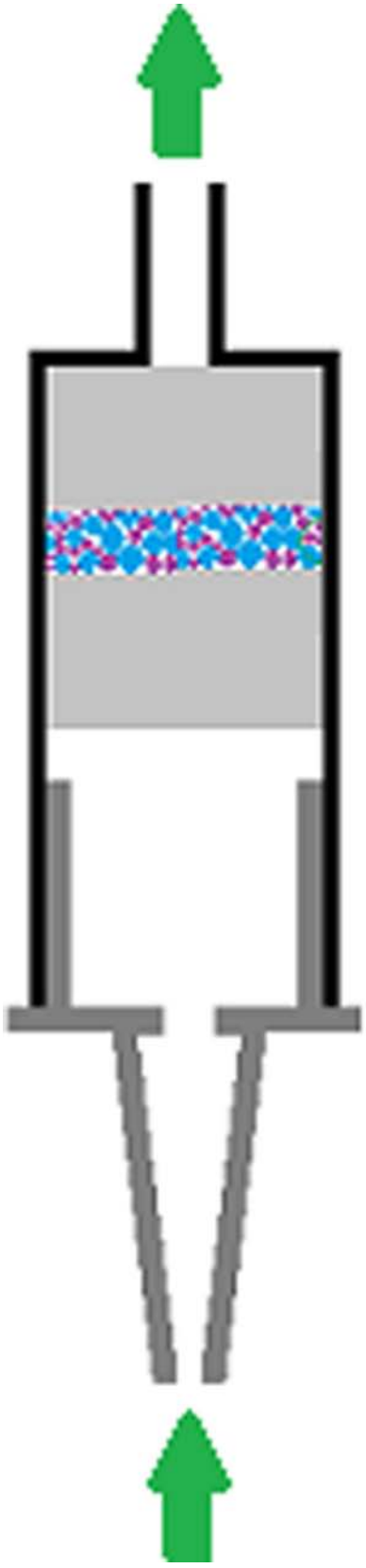

Fig. 2. Schematic diagram of selective mixed-bed solid phase extraction procedure.

2.8. Calculations

In order to evaluate the proposed method, percentage recovery of atrazine was calculated according to the following equation:

$$\%R = \frac{\mu_e}{\mu_0} \times 100 = \frac{C_e \times V_f}{C_0 \times V_0} \times 100$$

where μ_0 is the initial amount of atrazine (μg) in the sample and μ_e is the amount of extracted atrazine (μg) using the whole extraction procedure. C_e and V_f are the concentration and volume of the final extract that is used for injection to the instrument. C_0 and V_0 are the initial concentration and volume of the sample.

3. Results and discussion

3.1. Preparation of mixed-bed MISPE

3.1.1. Polymerization conditions for MIP/NIP preparation

There are many variables which can potentially impact upon the chemical, morphological and molecular recognition properties of the imprinted polymers [20]. Two important factors which can modify the selectivity and capacity of the synthesized molecularly imprinted polymers are the temperature in which the polymerization is carried out and the volume and type of porogen used [21]. Polymerization was carried out using thermally polymerization at 60 °C (MT) and photochemically polymerization at 25 °C (MU1) for 4 h. In order to evaluate the effect of the porogen volume on the selectivity and efficiency of extraction, it was increased from 2.2 mL (MU1) to 4.4 mL (MU2). In order to prepare a conventional MISPE, a 3 mL cartridge must usually be packed with more than 50 mg of sorbent. 3 mL cartridge that was packed with 50 mg of synthesized polymers resulted in very high back pressure. Finally, 10 mg MIP and NIP materials were used to pack 3 mL cartridges. A 10 mL water sample with 0.4 mg L^{-1} atrazine was passed through the conditioned sorbents with a flow rate of 2 mL min^{-1}. Fig. 3 shows atrazine recovery of polymers synthesized in different conditions. The results (Fig. 3) indicated that synthesis at 25 °C (MU1) provided a MIP material with the best and most reproducible recovery and selectivity for atrazine. As previous studies discovered [21], a higher temperature has a negative impact on the complex stability during the imprinting process and the polymerization reaction is hard to control resulting in low reproducibility of MIPs [20]. Thus, a polymerization temperature of 25 °C under ultraviolet irradiation was preferred. Furthermore, the experiments indicated a lower recovery and selectivity when a higher porogen volume (4.4 mL chloroform) was used for MIP synthesis. It is known that the nature and the amount of porogenic solvent determine the strength of the non-covalent interactions between the target substance and the monomers. Additionally, the porogen influences the polymer morphology that affects the performance of MIP [20]. Under our synthesis conditions, a higher porogen volume caused more non-specific sites whereby the MIP materials reflected lower selectivity compared to relative NIP materials. Finally, the porogen volume of 2.2 mL and the polymerization temperature of 25 °C under ultraviolet irradiation were selected for the synthesis due to the enhanced recovery and selectivity of the synthesized polymers.

3.1.2. Selection of matrix ingredient to be mixed with the MIP material

Commercially available SPDEs are prepared by blending PTFE with sorbent particles to produce thin membranes and finally SPE-disks [22]. The type and amount of matrix ingredients are factors that affect the performance of this technique directly. The matrix ingredients should be inert towards the target molecule, so that the affinity of PTFE and silica gel particles for extracting atrazine could be evaluated. PTFE (< 100 μm) particles were prepared by grinding and sieving PTFE frits. Silica gel S (S) (63–100 μm) was obtained from Riedel-de Haën (Seelze, Germany). SPE glass columns were packed with 10 mg of the aforementioned ingredients and used for the extraction of atrazine from standard water samples. A 10 mL water sample with 0.1 mg L^{-1} atrazine was passed through the previously-conditioned cartridges at a flow rate of 2 mL min^{-1}. Both silica gel and PTFE particles showed very low attraction (recovery < 2%) for the extraction of atrazine (which was washed completely during washing step). In comparison to PTFE, the silica gel consists of more rigid and uniform particles, therefore allowing higher flow rates at lower back pressure when packed in a cartridge. Therefore, silica gel was selected for our further investigations.

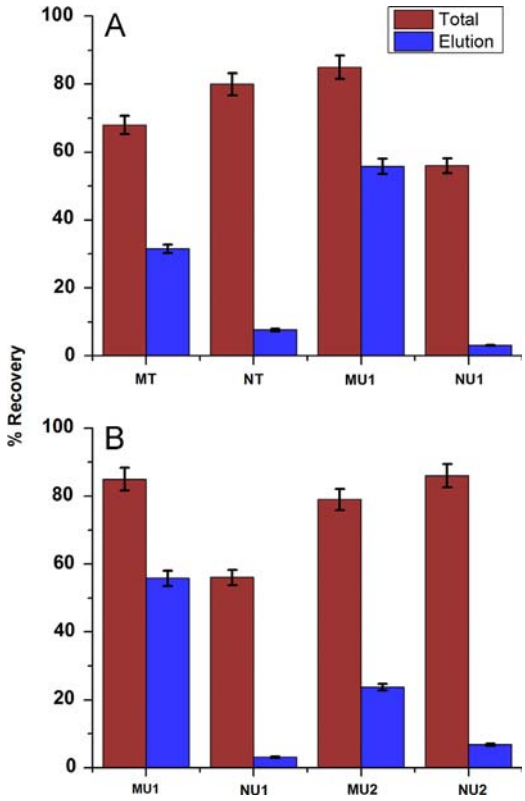

Fig. 3. Influence of the polymerization conditions of imprinted polymers for the recovery of atrazine. (A) Temperature effect. (B) Porogen volume effect. "Total" is percentage recovery values for washing plus elution steps and "Elution" represents the percentage recovery for the elution step only.

3.1.3. Optimization of the MIP-silica gel composition

In SPE, the mass and type of sorbent predetermine the performance of SPE, its capacity, extraction time and the type and volume of organic solvent required. Unlike silica gel, MIP materials swell when they come into contact with solvents [9]. This causes a reduction in the number of available selective sites for interaction with the target analytes, increased channeling and voiding effects, elevated back pressure and run times. Reducing the amount of MIP material as a SPE sorbent will reduce back pressure and the required volume of organic solvents for conditioning, washing and elution steps. In this case, the time required for extraction decreases. However, the cartridge's capacity may also decrease as well. For this purpose, different ratios of MIP/silica gel material mixtures were evaluated. The best ratio guarantees that the MIP particles are totally homogenized and

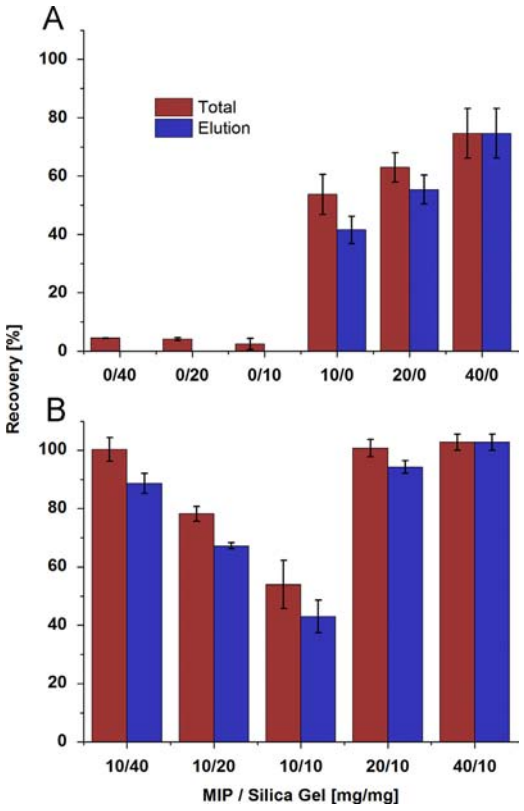

Fig. 4. Percentage recovery evaluation of different imprinted polymer to silica gel ratios. (A) Pure silica gel and imprinted polymer. (B) Mixture of silica gel and imprinted polymer.

dispersed in the silica gel matrix, making it possible to operate with a higher capacity per milligram of sorbent. A 10 mL water sample with 0.1 mg L^{-1} atrazine was passed through the conditioned sorbents at a flow rate of 2 mL min^{-1}. As shown in Fig. 4, by adding 10 mg of silica gel to 20 mg and 40 mg of imprinted polymers, the recovery of atrazine increased to 96% and 100%, respectively. Using 10 mg of MIP, the recovery of atrazine increased from 43% (only MIP) to more than 85% when 40 mg of silica gel was added. In an extra experiment, it was demonstrated that 40 mg of silica gel does not remarkably extract atrazine because the corresponding analysis did not detect atrazine above the LOD. The results suggest the recovery improvement seems to be attributable to the higher number of imprinted sites that become available for binding atrazine when the MIPs have been homogenously dispersed in the silica gel. Due to the reduced amount of MIP sorbent, required organic solvents for washing and elution steps can be reduced (Section 3.2.3). For further method developments, a ratio of 10:40 was applied allowing higher flow rates, less back pressure and the possibility of percolating the real samples directly without any other pre-filtration.

3.1.4. Extraction efficiency performance of mixed-bed MISPE in comparison with commercial sorbents

In order to validate the extraction efficiency of the new prepared mixed-bed MISPE, different commercial sorbents commonly used for atrazine were compared. For this purpose, 10 mg of the following commercial sorbents: styrene-divinylbenzene copolymer (SDB) (43–123 μm), octadecylsilane bonded to silica gel (ODS) (47–60 μm) and H_2O-Philic divinylbenzene (DVB) (15 μm) were packed into SPE cartridges and utilized for the extraction of 1 μg atrazine from 10 mL water sample. As depicted in Fig. 5, from

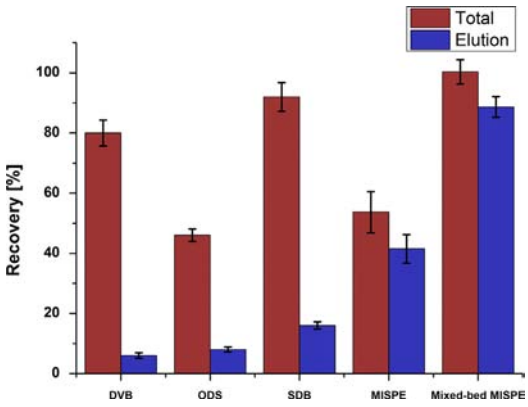

Fig. 5. Percentage recovery evaluation of different sorbents. (SDB: styrene-divinylbenzene, DVB: divinylbenzene, ODS: octadecyl group bonded type silica gel, and PTFE: polytetrafluoroethylene.)

all tested sorbents, mixed-bed MISPE shows the best extraction efficiency for atrazine.

3.2. Optimization of mixed-bed MISPE conditions

3.2.1. pH of the sample solution

The binding of atrazine molecules onto MIP is mainly based on hydrogen bonds that are expected to be formed between atrazine and MAA. In order to strengthen these interactions, the pH condition of the medium has to be properly adjusted. Therefore, pH value of the sample solution is one of the most important parameters for the selective extraction of atrazine [23]. In our investigation, the pH of the standard solution was adjusted to values ranging from pH 2 to pH 9. The recovery of atrazine decreased at higher pH and in highly acidified solutions. These conditions vary strongly from those applied for the imprinting process carried out under neutral conditions. Thus, the cavities of the polymer are shaped for the neutral atrazine molecule. At basic or acidic conditions, the ionic form of atrazine and the functional groups of the MIP fit less well in these polymer sites. For instance, under strong acidic conditions, the carboxyl groups as active sites in the cavities are in their protonated form as atrazine itself. The recovery of atrazine with NIP particles was very low for all pH conditions, which shows non-specific adsorption of analyte. According to these results, a pH range of 6–7 was selected as the optimum for further experiments.

3.2.2. Sample flow rate and breakthrough volume

Sample flow rate is an important parameter that influences the extraction yield of an analyte and the extraction time. A 10 mL water sample with 0.1 mg L^{-1} atrazine was passed through the conditioned mixed-bed MISPE cartridges at flow rates of 1, 2, 3 and 5 mL min^{-1}. Lower flow rate means more time for mass transfer, which was reflected in an increased recovery of atrazine. An approximate plateau at 89% recovery is reached for flows less than 3 mL min^{-1}. With the intention of reducing the extraction time, the flow rate was set at 3 mL min^{-1} for subsequent developments.

In order to determine the optimal volume for sample loading; 10, 20, 30 and 40 mL water samples, each containing 1 μg of atrazine, were loaded onto the mixed-bed sorbent at 3 mL min^{-1}. The recovery of atrazine decreased with increasing volume, which can be attributed to the promoted diffusion of the analyte in small sample volumes [4]. In order to determine trace concentration of pesticides in groundwater and surface waters at low part-per-billion range, a large sample volume must be preconcentrated. Applying the mixed bed sorbent, 10 mL of water samples are sufficient to detect

atrazine above the LOQ (4.5 μg L^{-1}) (see Sections 3.3 and 3.4). A loading volume of 10 mL was therefore found to be most efficient for atrazine extraction and was used for further optimizations.

3.2.3. Optimization of washing and elution steps

Although MIPs offer the highest selectivity when samples are administrated in the solvent used for the polymerization [24], in our experiments water was used as the loading solvent to avoid any other pre-concentration step. Due to the retention of the analytes via non-specific hydrophobic interactions during percolation of water samples, the washing step plays an important role in demonstrating the selectivity of the synthesized polymer [25]. Non-selective sites and those cavities with incomplete or irregular shape are able to adsorb molecules less tightly than the specific imprinted areas of the polymer. So, optimization of the washing step is essential because it simultaneously allows the interactions of target molecules with the specific cavities and removal of the co-extracted non-target substances [25]. To optimize the washing step, different solvents were examined and dichloromethane as a weakly polar and aprotic solvent was used [24,25]. Besides the washing step, the drying step also affects the selectivity of MIPs. Pap et al. [24] showed that small amounts of water that remain after sample application due to incomplete drying can change the binding affinity of the MIP material to atrazine. The authors reported that at least 30 min were needed to remove the adsorbed water from 50 mg of MIP. Furthermore, the application of the organic solvent before complete drying caused high variance in their experiments [24]. In our experiments, within 20 s, 10 mL distilled water was passed through the atrazine-loaded mixed-bed sorbent via a SPE vacuum manifold and was further dried until no water loss was measured (by weighting). 5 min was required to completely dry the mixed-bed. The dried cartridge was then connected from its lure tip to a syringe containing dichloromethane. For washing, 1 mL dichloromethane was passed through the cartridge at a flow rate of 2 mL min^{-1} and easily collected from the reservoir part of mixed-bed MISPE cartridges for further analysis. A vacuum was then applied for 30 s to remove any remaining dichloromethane via the SPE vacuum manifold. In order to elute the mixed-bed, methanol–acetic acid (99:1) was used as the eluent solvent. Successive portions of 0.2 mL eluted solvent were collected from the lure tip of the cartridge and used for further analysis. The results showed that 89% of atrazine was contained within the first elution fraction. In order to be sure about complete elution of target analyte, 0.4 mL of methanol–acetic acid (99:1) was finally selected as the eluent volume.

3.3. Method validation

To validate the proposed method, standard atrazine solutions in water (pH 6) at a range of 5–200 μg L^{-1} were prepared and analyzed at optimized conditions with the developed mixed-bed MISPE-GC/MS. In comparison, atrazine was extracted by pure MIP sorbent in SPE mode (MISPE). Standard atrazine solutions in chloroform at concentrations ranged 0.52–2.06 mg L^{-1} were also prepared and analyzed directly with GC–MS. Linear regression analyses were performed using the peak area obtained by mixed-bed MISPE, MISPE and directly-injected samples against the corresponding concentrations. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated at signal-to-noise ratios of 3 and 10, respectively. The calibration curve obtained for the concentration ranges mentioned above was linear with correlation coefficients of R^2=0.9988 for directly injected sample, R^2=0.9651 for MISPE and R^2=0.9925 for mixed-bed MISPE. LOD and LOQ for the mixed-bed MISPE were 1.34 μg L^{-1} and 4.5 μg L^{-1}, respectively. The relative standard deviations (RSDs) for the concentration range of 5–200 μg L^{-1} were 3.5–12.1% for MISPE and 1.8–6.3% for mixed-bed MISPE. Besides sensitivity enhancement and the improvement of the RSD with the developed method, column-to-column reproducibility was also evaluated. 10 different cartridges were prepared for each of the MISPE and the mixed-bed techniques and assessed via loading the water sample containing atrazine at a concentration of 0.1 mg L^{-1}. The results obtained showed that the percentage recovery levels (±RSD %) were 48 (±53%) and 82 (±16.1%) for MISPE and mixed-bed MISPE techniques, respectively.

The operational parameters and analytical characterization of mixed-bed MISPE, corresponding MISPE, conventional MISPE and the SPE procedure with SDB sorbent are summarized in Table 1. The data emphasized that with the new mixed-bed approach, the LODs could be improved while the amount of organic solvent and the extraction time can be significantly reduced.

3.4. Matrix influence

The applicability of the method was evaluated for the determination of atrazine in real water samples with different complex matrix backgrounds. Before atrazine was added, all samples were analyzed for atrazine using styrene-divinylbenzene column described in [18]. All samples were found to be atrazine-free. Tap water, river water and an influent obtained from the central municipal wastewater treatment plant in Leipzig were spiked with atrazine to adjust a concentration of 5 μg L^{-1}. The results of the analyses summarized in Table 2 indicate a low matrix influence on the extraction process. Even the complex composition of wastewater did not significantly reduce atrazine recovery. The wastewater sample was used as an example to compare the selectivity and "cleanup" performance of the new MIP procedure with that of a commercial SPE protocol involving SDB sorbent. The extracts of both methods were analyzed by GC–MS in full scan mode to obtain an overview of the extracted components. The total ion current chromatogram of the extract obtained by the standard SPE procedure using a commercial SDB cartridge (Fig. 6)

Table 1
Operational parameters and analytical performance of extraction techniques for atrazine in water samples.

	SDB-SPE	MISPE	Reduced mass-bed MISPE	Mixed-bed MISPE
Adsorbent (mg)	200	200	10	10 (MIP); 40 (silica gel)
Conditioning solvent (mL)	2	25	0.5	0.5
Drying time (min)	n.a.	>20	5	5
Elution solvent (mL)	2	3	0.4	0.4
RSD (%)	12	n.a.	3.5–12.1	1.8–6.3
Column-to-column RSD (%)	n.a.	n.a.	53	16.1
LOD (μg L^{-1})	10	n.a.	2.25	1.34
Ref.	[13]	[25]	This work	This work

n.a.: Data not available from literature.

Table 2
Determination of atrazine spiked into samples with different matrices.

Sample	Spiked (μg L^{-1})	Found[a] (μg L^{-1})	Recovery[a] (%)	RSD[a] (%)
Tap water	5	5.15	103	0.353
River water	5	4.9	98	1.131
Wastewater	5	4.4	88	1.562

[a] Number of sample=3.

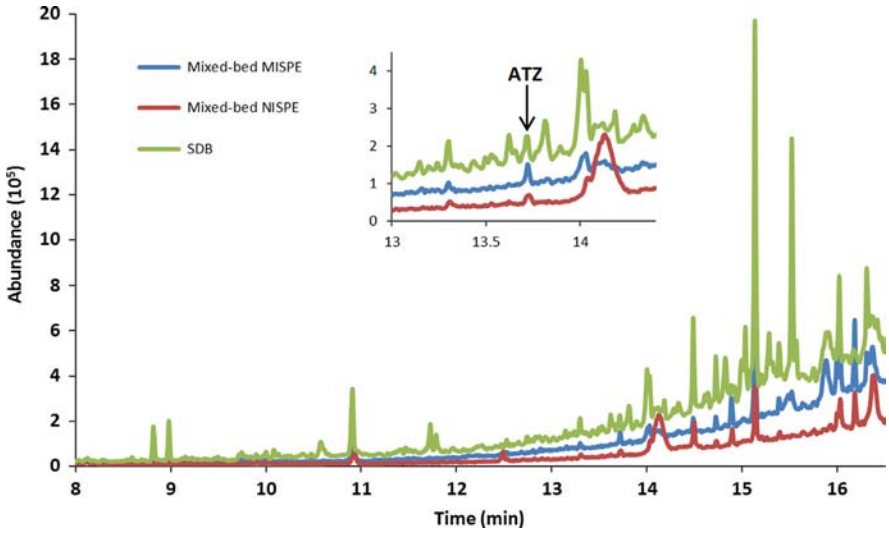

Fig. 6. GC–MS chromatograms of spiked wastewater (5 μg L^{-1}) obtained by styrene-divinylbenzene (SDB) column and mixed-bed MISPE method. Inset graph shows the same chromatogram with different *y*-axis scale.

indicates a very complex mixture of substances, while the corresponding chromatogram of the mixed-bed MISPE extract contained much less signals, therefore emphasizing the higher selectivity of the extraction process. The specific molecular interaction enabled by the MIP polymer reduces the number of co-extracted matrix molecules significantly.

4. Conclusions

A novel preparation protocol was developed for the construction of an atrazine-selective mixed-bed MISPE which was validated for the extraction of atrazine from water samples. Besides its simplicity, the developed method enabled rapid extraction with reduced organic solvent consumption. The reduced volume of elution solvent reveals the inherent potential of the method for automation and online coupling with an instrumental method (such as large volume injection-GC–MS). In addition to the improvements in LOD and precision, column-to-column reproducibility was increased in comparison to the relative MISPE method. High selectivity for atrazine, cleanup functionality and high levels of robustness are further characteristics of the developed mixed-bed method.

References

[1] M. Tankiewicz, J. Fenik, M. Biziuk, Talanta 86 (2011) 8–22.
[2] F. Chapuis, V. Pichon, F. Lanza, B. Sellergren, M.C. Hennion, J. Chromatogr. B 804 (2004) 93–101.
[3] E. Turiel, A. Martin-Esteban, P. Fernandez, C. Perez-Conde, C. Camara, Anal. Chem. 73 (2001) 5133–5141.
[4] F. Barahona, E. Turiel, A. Martin-Esteban, J. Chromatogr. A 1218 (2011) 7065–7070.
[5] T. Alizadeh, M.R. Ganjali, M. Zare, Anal. Chim. Acta 689 (2011) 52–59.
[6] T. Alizadeh, M.R. Ganjali, P. Nourozi, M. Zare, Anal. Chim. Acta 638 (2009) 154–161.
[7] C.F. Poole, Trends Anal. Chem. 22 (2003) 362–373.
[8] F. Augusto, L.W. Hantao, N.G.S. Mogollon, S.C.G.N. Braga, Trends Anal. Chem. 43 (2013) 14–23.
[9] M. Zarejousheghani, M. Moder, H. Borsdorf, Anal. Chim. Acta 798 (2013) 48–55.
[10] A. Martin-Esteban, Trends Anal. Chem. 45 (2013) 169–181.
[11] Y.L. Hu, J.L. Pan, K.G. Zhang, H.X. Lian, G.K. Li, Trends Anal. Chem. 43 (2013) 37–52.
[12] H. Borsdorf, T. Mayer, M. Zarejousheghani, G.A. Eiceman, Appl. Spectrosc. Rev. 46 (2011) 472–521.
[13] F.G. Tamayo, E. Turiel, A. Martin-Esteban, J. Chromatogr. A 1152 (2007) 32–40.
[14] A. Prieto, S. Schrader, C. Bauer, M. Moder, Anal. Chim. Acta 685 (2011) 146–152.
[15] T.P. Lee, B. Saad, W.S. Khayoon, B. Salleh, Talanta 88 (2012) 129–135.
[16] W.P. Zhang, Z.L. Chen, Talanta 103 (2013) 103–109.
[17] D.F. Hagen, C.G. Markell, G.A. Schmitt, D.D. Blevins, Anal. Chim. Acta 236 (1990) 157–164.
[18] G. Mendas, V. Drevenkar, L. Zupancic-Kralj, J. Chromatogr. A 918 (2001) 351–359.
[19] J. Matsui, Y. Miyoshi, O. Doblhoffdier, T. Takeuchi, Anal. Chem. 67 (1995) 4404–4408.
[20] H.Y. Yan, K.H. Row, Int. J. Mol. Sci. 7 (2006) 155–178.
[21] D.J. Oshannessy, B. Ekberg, K. Mosbach, Anal. Biochem. 177 (1989) 144–149.
[22] ⟨http://www.sigmaaldrich.com/analytical-chromatography/sample-preparation/spe/3m-empore.html⟩.
[23] D. Djozan, B. Ebrahimi, Anal. Chim. Acta 616 (2008) 152–159.
[24] T. Pap, V. Horvath, A. Tolokan, G. Horvai, B. Sellergren, J. Chromatogr. A 973 (2002) 1–12.
[25] F. Chapuis, V. Pichon, F. Lanza, S. Sellergren, M.C. Hennion, J. Chromatogr. A 999 (2003) 23–33.