Journal of Chromatography A, 1218 (2011) 3935–3939

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Short communication

# Novel liquid–liquid–solid microextraction method with molecularly imprinted polymer-coated stainless steel fiber for aqueous sample pretreatment

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ARTICLE INFO

*Article history:*
Received 16 March 2011
Accepted 25 April 2011
Available online 6 May 2011

*Keywords:*
Liquid–liquid–solid microextraction
Molecularly imprinted polymer
Aqueous sample
Chloroacetanilide herbicide

ABSTRACT

A novel liquid–liquid–solid microextraction (LLSME) method was developed to overcome the well-known water-compatibility problem of molecularly imprinted polymers (MIPs). The enrichment factors with MIP-LLSME method were within 70–210 for trace chloroacetanilide herbicides under optimized extraction conditions. The method was characterized by simplicity, low solvent-consumption and high selectivity, and it was suitable for the one-step pretreatment of various aqueous samples such as river water and farm water.



## 1. Introduction

Sample pretreatment is critical to the identification, confirmation and quantification of analytes in various samples [1]. Solid-phase microextraction (SPME), pioneered by Belardi and Pawliszyn [2] in 1990s, is a novel, simple, time-saving and solvent-free pretreatment technique. Recently, many research interests have been poured into the development of novel SPME coating with enhanced selectivity. Notably, molecularly imprinted polymers (MIPs), acclaimed as an artificial antibody, was an attractive alternative of selective SPME coating over the past several years [3–6].

However, the application of MIPs was disturbed by the water-compatibility problem as a result of the competition between analytes and water for the recognition cavities in MIP [7]. Though some strategies were utilized to solve this problem through the improvements in MIP synthesis [8–11], the consequent restrictions in monomer, solvent or recognition mechanism prevented these strategies from becoming a conventional MIP preparation method. In our previous works [12–15], analytes should be transferred from aqueous samples into a non-polar solvent to avoid the water-compatibility problem found in MIP-coated SPME method, and then multiple procedures were needed such as liquid–liquid extraction (LLE), filtration, reduced pressure distillation and dissolving. It would result in a large consumption of organic solvent, tedious operations, potential impurity introduction and analyte losing. Recently, Li's group reported the protection of the MIP-coated SPME fiber through a disposable porous polypropylene hollow fiber membrane which was filled with a small volume (about 6 μL) of organic solvent, and then the direct pretreatment of aqueous samples could be achieved [6].

In this contribution, a novel liquid–liquid–solid microextraction (LLSME) device was developed to provide a routine solution to the water-compatibility problem of the MIP-coated SPME fiber, which integrated traditional LLE and SPME into one single operation. This device was reusable and easier to operate, and the bigger volume of organic solvent (about 100 μL) was helpful to enhance sensitivity compared with Li's method [6].

## 2. Experimental

### 2.1. Chemicals and reagents

Metolachlor, propisochlor, acetochlor and butachlor were purchased from Kesai Chemical Industry Corporation (Jinan, China). Alachlor and pretilachlor were purchased from Sigma–Aldrich (St. Louis, MO, USA). Metolachlor metabolites of hydroxymetolachlor, deschlorometolachlor and desmethylmetolachlor were kindly provided by Shenzhen Bureau of Quality and Technical Supervision (Shenzhen, China). Trimethylolpropane trimethacrylate (TRIM) was purchased from Sigma–Aldrich. Methacrylic acid (MAA) and azo(bis)-isobutyronitrile (AIBN) were purchased from Damao Reagent Plant (Tianjin, China). The HPLC grade methanol and ace-

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doi:10.1016/j.chroma.2011.04.069

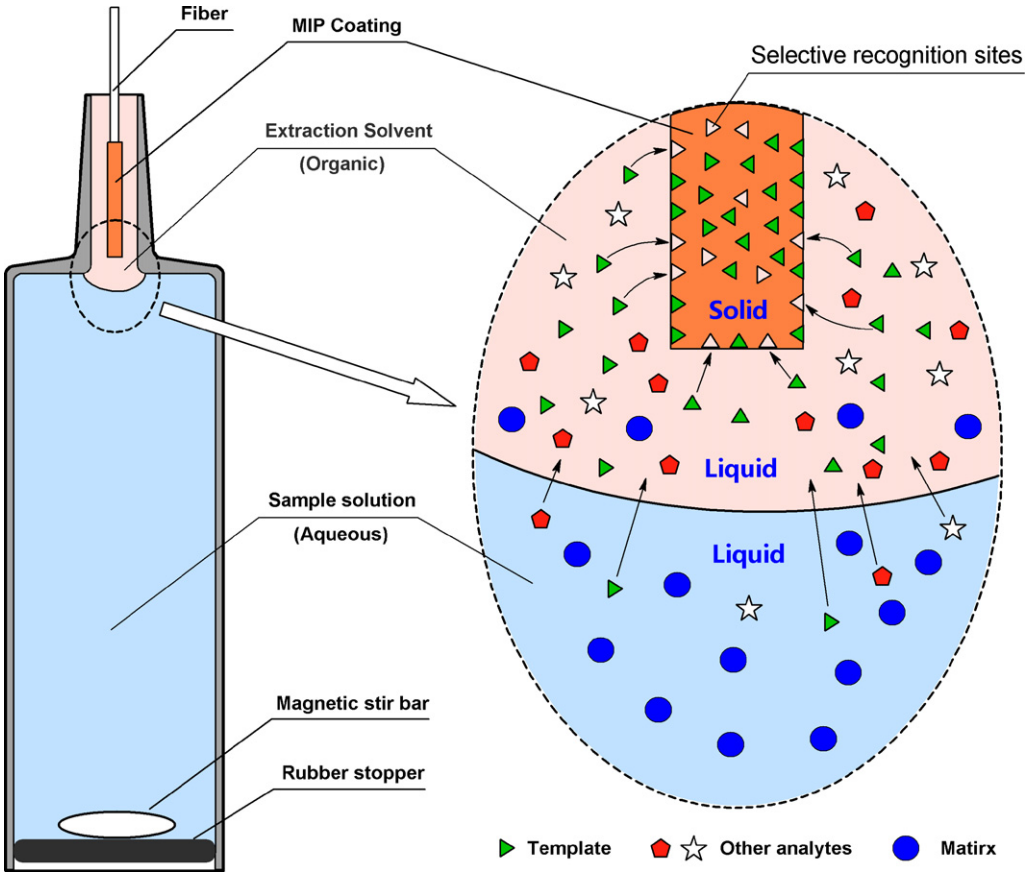


**Fig. 1.** Schematic representation of MIP-LLSME device and extraction mechanism of template analytes through liquid–liquid and liquid–solid phase interfaces.

tonitrile were purchased from Merck (Darmstadt, Germany). Water was double distilled. All other reagents were of analytical grade.

### 2.2. MIP-LLSME device preparation

The preparation of metolachlor MIP-coated stainless steel fiber was according to our previous work [15]. A schematic representation of liquid–liquid–solid microextraction device is described in Fig. 1. A glass syringe without plunger is utilized for the extraction chamber which is divided into two rooms. The lower room (about 10 mL in volume) sealed with a rubber stopper in bottom is used to load an aqueous solution, and the upper room (about 100 μL in volume and 1.5 cm in height) is used to load a water-immiscible organic solvent floating on the aqueous solution as a result of less density. Meanwhile, a MIP-coated stainless steel fiber is immersed into the organic solvent and fixed with a holder. When extracted, analytes are firstly enriched through a liquid–liquid interface between the aqueous solution and the organic solvent, and then separated selectively through a liquid–solid interface between the organic solvent and the MIP coating.

### 2.3. MIP-LLSME procedure

A 10-mL aqueous standard solution or sample solution and a Teflon magneton were added into the lower room of LLSME device, and then the rubber stopper was utilized to adjust the liquid level of the aqueous solution to the boundary between upper and lower room. After that, 100 μL of toluene was loaded into the upper room, and this water/toluene two-phase system was conditioned for 60 min at the stir speed of 1000 rpm to reach liquid–liquid microextraction equilibrium. Subsequently, a MIP- or NIP-coated stainless steel fiber was immersed into toluene for 20-min extraction under stirring. Finally, the fiber was pulled out and immersed into a SPME–HPLC coupling interface (Supelco, Bellefonte, PA, USA) for 10-min desorption with about 60-μL methanol. After desorption, the fiber was pulled out and all desorption solution was injected into a Dikma $C_{18}$ (250 mm × 4.60 mm I.D., 5 μm packing, Beijing, China) column for the analysis with C-10ATvp HPLC (Shimadzu, Japan) and an ultraviolet detector. The chromatographic and detection conditions were according to our previous work [15]. After 30-min condition in toluene, this fiber would be ready for next extraction.

As comparison, the commercial SPME with polyacrylate (PA), polydimethylsiloxane (PDMS) and polydimethylsiloxane/divinylbenzene (PDMS/DVB) coatings (Supelco) was performed with 80-min extraction in 10-mL aqueous solution at the stir speed of 1000 rpm and 10-min desorption in 60-μL methanol in the SPME–HPLC coupling interface. The traditional LLE method was perform with 30-min shaking of 10-mL aqueous solution and 30-mL toluene in a separating funnel, and then the upper toluene solution was separated and condensed to 1.0 mL.

### 2.4. MIP-LLSME condition and performance investigations

The MIP-LLSME conditions such as the ionic strength and pH of aqueous solution, organic solvent, stirring speed, condition time, extraction time and desorption time were optimized. The initiate conditions were as following: pH of aqueous solution was 7 and no salt was added, stirring rate of 750 rpm, organic solvent of hexane, condition time of 60 min, extraction time of 60 min and desorption time of 10 min. A metolachlor, propisochlor and butachlor mixed

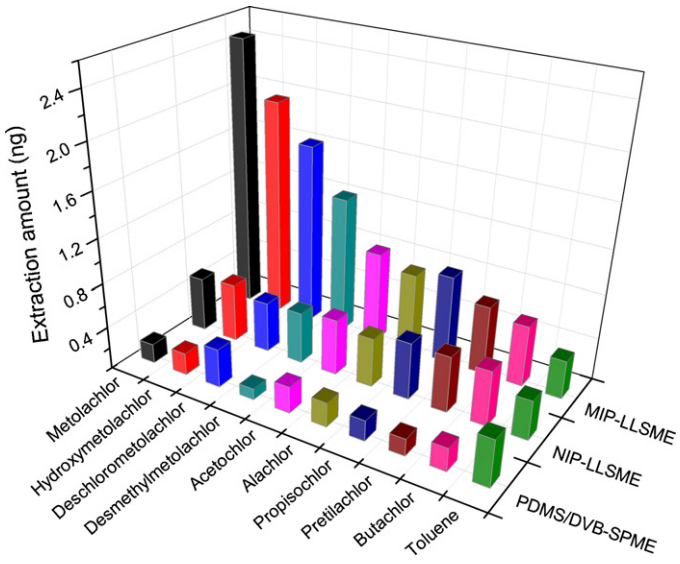


**Fig. 2.** Selectivity comparison among MIP-LLSME, NIP-LLSME and PDMS/DVB-SPME methods over individual metolachlor, hydroxymetolachlor, deschlorometolachlor, desmethylmetolachlor, acetochlor, alachlor, propisochlor, pretilachlor, butachlor or toluene standard solution at 50 μg $L^{-1}$ level.

standard solution of 50.0 μg $L^{-1}$ in water was used as a model sample.

### 2.5. Sample pretreatment

The river and farm water samples, polluted seriously and defined as class V in water quality, were collected from the Pearl River and a farmland near Guangzhou Higher Education Mega Center (Guangzhou, China), respectively. After collection, these samples were placed in a refrigerator at 4 °C for 24 h natural sedimentation, and then the supernatants were isolated and stored in a glass bottle at 4 °C prior to use.

## 3. Results and discussion

### 3.1. LLSME device

To solve the water-compatibility problem of MIP coating, a novel LLSME device was developed. As shown in Fig. 1, a MIP-coated fiber is immersed into 100-μL organic solvent to perform solid-phase microextraction, and simultaneously, an organic solvent is suspended on a large-volume aqueous solution to perform liquid–liquid microextraction (LLME). Consequently, the analytes are firstly enriched through LLME, and then extracted with MIP-coated SPME. Compared with traditional MIP-coated SPME method [12–15], this novel LLSME device brings many advantages, such as negligible organic solvent consumption, one-step operation, enhanced sensitivity and high flexibility for various MIPs suffered from the water-compatibility problem.

### 3.2. Investigation of extraction conditions of MIP-LLSME

#### 3.2.1. Organic extraction solvent

Water-immiscible organic solvents including benzene, toluene, hexane, cyclohexane, ethyl acetate and 1-butanol were studied. The results indicated that the extraction amounts of chloroacetamide herbicides with toluene were higher than those with other solvents. The low polarity of toluene was helpful to avoid interference to hydrogen-bond interaction [16]. Moreover, the rigid recognition cavities in MIP coating prepared in toluene would not be distorted by swelling to ensure extraction capacity and selectivity [17]. In contrast, the extraction amounts with 1-butanol were the lowest. Finally, toluene was applied through following investigations.

#### 3.2.2. Ionic strength, pH and stir speed of aqueous sample

Ionic strength investigation was conducted using various ionic strengths (in terms of NaCl), ranging from 0 to 25%. It was revealed that the extraction amounts of metolachlor, propisochlor and butachlor decreased remarkably when ionic strength was enhanced, which was in good agreement with the data from the literature dealing with same analytes by single-drop microextraction method. It was believed that the addition of salt may change the Nerst diffusion film physical properties, reduce the diffusion rates of solutes from water to the organic solvent, and then affect the extraction amount [18]. Consequently, salt addition was not used in the following experiments.

The impact of pH in aqueous matrix was studied with the range of 2.6–8.9 adjusted through HAc–NaAc or Tris–HCl buffer solution. There were not noticeable differences when pH was lower than 6. However, the extraction amounts of three herbicides reduced remarkably under the alkaline condition, and the declines of 34–58% were observed when pH was 8.9. This was probably because three herbicides were all weakly acidic, and the alkaline condition would result in the ionization of herbicides, which was disadvantageous to the extraction with the organic solvent. Consequently, the aqueous sample media was adjusted to pH 5 to enhance the extraction efficiency.

The investigation of stir speed was performed at 0, 100, 250, 500, 750 and 1000 rpm. It was revealed that the extraction amounts of three chloroacetanilide herbicides were enhanced with the increase of stir speed. The effective mixing is important to ensure sample homogeneity and quicken the diffusion of analytes, so 1000 rpm was selected as a result of the high stability of 100-μL organic solvent suspended in the top room of LLSME device.

#### 3.2.3. Condition time, extraction time and desorption time

The condition time was investigated to achieve the distribution equilibrium of analytes between the aqueous sample solution and the organic solvent of toluene. The results indicated that the distribution of three herbicides reached equilibrium after about 40 min, and the extraction amounts were increased 155–180% compared with the results without the condition procedure. Moreover, the effect of interface area between water and toluene was investigated, and it was indicated that a higher area was advantageous to the reducing of the distribution equilibrium time. When a plane interface with the lowest area was adopted, the equilibrium time

**Table 1**
Enrichment factors[a] of 50.0 μg $L^{-1}$ metolachlor, propisochlor and butachlor with LLE and different SPME strategies.

| | MIP-LLSME | LLE | MIP-SPME | PDMS/DVB-SPME | PDMS-SPME | PA-SPME |
|---|---|---|---|---|---|---|
| Metolachlor | 207.5 | 21.4 | 17.1 | 18.7 | 6.8 | 4.9 |
| Propisochlor | 81.6 | 18.5 | 13.1 | 17.1 | 2.6 | 2.1 |
| Butachlor | 70.4 | 25.8 | 11.1 | 18.5 | 2.1 | 3.6 |

[a] Enrichment factor was defined as the ratio of analyte concentration in extraction solution of LLE or desorption solution of SPME, which was calibrated with standard solution, to that of original sample solution.

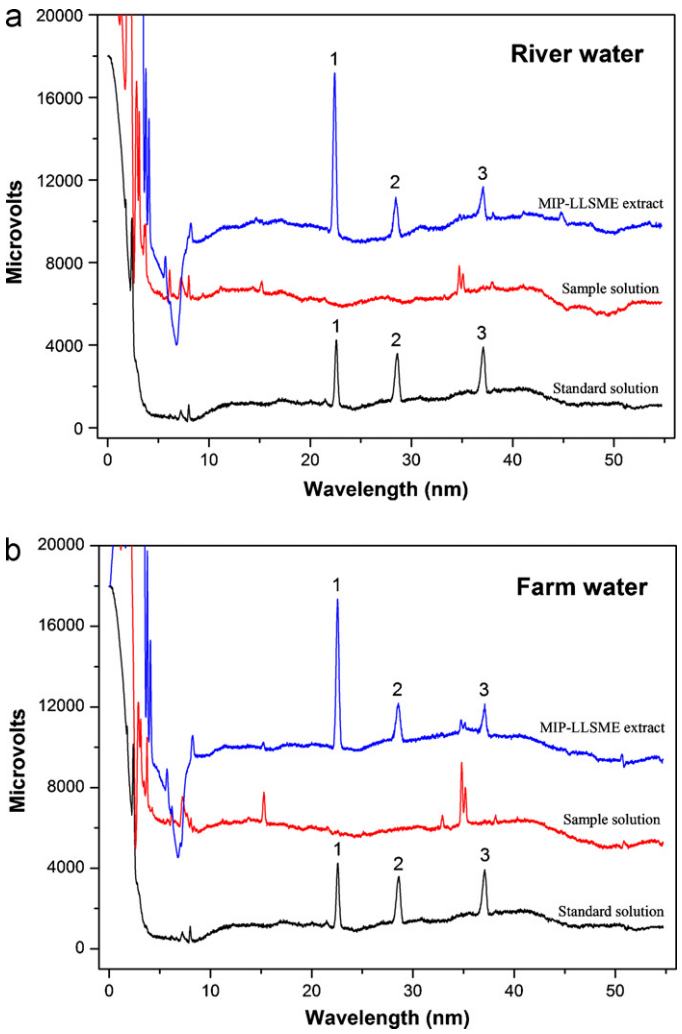


**Fig. 3.** Chromatograms of 1.0 mg $L^{-1}$ metolachlor, propisochlor and butachlor mixed standard solution, 10.0 μg $L^{-1}$ spiked river water and farm water sample solutions and their extracts with MIP-LLSME. (1) Metolachlor, (2) propisochlor, (3) butachlor, injection volume for standard solution and spiked sample solutions: 10 μL.

was prolonged to 60 min. The adequate condition of 60 min was selected to eliminate the negative impact from the interface area variation.

For extraction time study, it was observed that the adsorption of metolachlor reached equilibrium only after 15 min, and by contrast, propisochlor and butachlor needed about 25 min as a result of weaker affinities to MIP coating. Compared with our previous work (adsorption equilibrium time of 30 min for metolachlor with the same MIP-coated fiber) [15], this quicker adsorption was probably caused by the faster mass transfer resulting from the lower volume of 100-μL extraction solvent and the higher analyte concentration resulting from the previous condition procedure. Without the condition treatment, the adsorption equilibrium time of metolachlor was found to be about 90 min, and the significance of condition treatment could be confirmed. To ensure the extraction efficiency, extraction time of 20 min was finally selected.

With about 60-μL methanol as solvent, the desorption of three herbicides was directly performed in the SPME–HPLC coupling interface. The effect of desorption time was studied, and it was revealed that within 1 min, about 89%, 82% and 79% of the adsorbed metolachlor, propisochlor and butachlor could be quickly desorbed, respectively, and the desorption equilibrium was achieved only after 10 min.

### 3.3. Coating extraction performance

The extraction capacities of the metolachlor MIP coating were investigated with a series of metolachlor, propisochlor and butachlor mixed standard solutions of 0.2–500 μg $L^{-1}$. It was indicated that, resulting from the tailor-made recognition cavities, the MIP coating possessed high extraction capabilities to metolachlor and its structural analogues of propisochlor and butachlor. When the concentration was up to 200 μg $L^{-1}$, the extraction saturation was achieved and the extraction capacities of metolachlor, propisochlor and butachlor were about 4.9, 2.4 and 2.0 ng, respectively.

The selectivities of MIP coating were evaluated with chloroacetanilide herbicides, metolachlor metabolites and toluene which were prepared individually with the concentration of 50 μg $L^{-1}$. As shown in Fig. 2, it was indicated that the MIP coating possessed specific selectivities to metolachlor, metolachlor metabolites and other chloroacetanilide herbicides. The selectivity coefficients, defined as the extraction amount ratio of MIP to NIP coating, were 4.9, 4.1, 3.7 and 2.8 for metolachlor, hydroxymetolachlor, deschlorometolachlor and desmethylmetolachlor, respectively. In contrast, weaker selectivities were found to other chloroacetanilide herbicides with the coefficients lower than 1.65 due to their lower similarities in molecular size, shape and functional groups with metolachlor. The extraction of toluene was based on the non-specific adsorption, and no marked difference in the extraction amount was observed between MIP and NIP coating. The commercial direct SPME with PDMS/DVB coating was used as the comparison, and the results indicated its remarkable weaker extraction capabilities and selectivities for chloroacetanilide herbicides and metolachlor metabolites.

### 3.4. Comparison with traditional LLE and SPME methods

To valuate the enrichment capability of MIP-LLSME method, traditional LLE and SPME were used for comparison with different strategies as following: (i) LLE; (ii) MIP-coated SPME in water solution expressed as MIP-SPME; (iii) commercial PA, PDMS or PDMS/DVB coating SPME in water solution expressed as PA-SPME, PDMS-SPME and PDMS/DVB-SPME, respectively.

The enrichment factors were listed in Table 1, and as expected, the water-compatibility problem affected the performance of MIP-SPME strategy, resulting from the interference of aqueous media to the hydrogen-bond based recognition. The LLE strategy could achieve better enrichment effect, but at the cost of large organic solvent consumption and tedious operations. In contrast, the commercial SPME strategies with PA, PDMS or PDMS/DVB coating were solvent-free and highly efficient, but their weak selectivities limited their enrichment performance. Clearly, the highest enrichment effect was achieved with the novel MIP-LLSME strategy proposed in this work. The enrichment factors of three herbicides by MIP-LLSME were about 70–210 which were much higher than those with MIP-SPME, LLE and commercial SPME strategies. Furthermore, the organic solvent consumption of MIP-LLSME was reduced to negligible 100 μL, and only one-step extraction operation was all need for the aqueous sample before HPLC analysis.

### 3.5. Linearity, limit of detection and precision

A series of metolachlor, propisochlor and butachlor mixed standard solutions were used to study the linearity of MIP-LLSME method, and the linear ranges of 1–100, 5–100 and 5–100 μg $L^{-1}$ were achieved for metolachlor, propisochlor and butachlor with the correlation coefficients of 0.9983, 0.9988 and 0.9974, respectively.

The limits of detection (calculated with the signal-to-noise ratio of 3) were 0.2, 1.4 and 2.8 $\mu g\,L^{-1}$ for metolachlor, propisochlor and butachlor, respectively, which were much lower than the metolachlor maximum residue limit of 70 $\mu g\,L^{-1}$ recommended by the United States environmental protection agency (USEPA) lifetime health advisory [19]. The method precision was monitored with 50.0 $\mu g\,L^{-1}$ mixed standard solution and the RSDs of extraction amounts of metolachlor, propisochlor and butachlor were 3.1, 4.3 and 4.5% ($n = 6$), respectively.

### 3.6. Real sample analysis

The practical applicability of MIP-LLSME method was validated with the spiked real river water and farm water samples, as shown in Fig. 3. The results demonstrated the high enrichment capabilities and selectivities of MIP-LLSME method for trace metolachlor, propisochlor and butachlor in aqueous samples. The recoveries of three herbicides in the spiked river water and farm water samples at three levels of 10, 50 and 70 $\mu g\,L^{-1}$ were 75.6–95.4 and 79.3–97.9%, respectively.

## 4. Conclusions

In this paper, a novel LLSME device was designed to solve the water-compatibility problem of the MIP-coated SPME fiber. With the characteristics of high selectivity, enhanced efficiency and negligible organic solvent consumption, MIP-LLSME method would provide an exciting low-cost and environment-friendly solution for the simple, selective and sensitive pretreatment of trace metolachlor and other chloroacetamide herbicides in various aqueous samples.

## Acknowledgements

The authors would like to thank the National Natural Science Foundation of China for the financial support of this research under the grant number of 20905026.

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