Yingying Wen[1,4]
Lingxin Chen[1]
Jinhua Li[1]
Yanling Ma[1,3]
Shoufang Xu[1,4]
Zhong Zhang[1]
Zongliang Niu[1]
Jaebum Choo[2]*

[1]Key Laboratory of Coastal Zone Environmental Processes, Yantai Institute of Coastal Zone Research (YIC), Chinese Academy of Sciences (CAS); Shandong Provincial Key Laboratory of Coastal Zone Environmental Processes, YICCAS, Yantai, P. R. China
[2]Department of Bionano Engineering, Hanyang University, Ansan, South Korea
[3]College of Chemistry and Chemical Engineering, Qufu Normal University, Qufu, P. R. China
[4]Graduate University of Chinese Academy of Sciences, Beijing, P. R. China

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Research Article

Molecularly imprinted matrix solid-phase dispersion coupled to micellar electrokinetic chromatography for simultaneous determination of triazines in soil, fruit, and vegetable samples

A simple and sensitive method for the simultaneous determination of four triazines from soil, strawberry, and tomato samples was developed by selective molecularly imprinted matrix solid-phase dispersion (MI-MSPD) coupled to micellar electrokinetic chromatography (MEKC). Using atrazine as template, the synthesized molecularly imprinted polymers (MIPs) were employed as the dispersion sorbent of MSPD to successfully extract atrazine and its analogs of simazine, ametryn, and propazine from the three different real samples, while matrix interferences were effectively eliminated simultaneously under the optimum extraction conditions. Excellent separation was achieved within 7 min by using an optimized buffer system composed of 30 mmol/L ammonium acetate, 20 mmol/L SDS, and 15% ACN at pH 9.45, obtained by orthogonal design. Good linearity was obtained in a range of 0.5–25 μg/g with the correlation coefficients $R^2 \geq 0.9991$ except for strawberry sample within 1–25 μg/g, and limits of detection were between 12.9–31.5 ng/g in all the three samples. The average recoveries of the four triazines at three different spiked levels were ranged from 53.5 to 98.4% with the relative standard deviations of 1.28–4.89%. This method was proved convenient, costeffective, and environmental benign and could be used as an alternative tool to the existing methods for analyzing the residues of triazines in soil, fruit, and vegetable samples.

Keywords:
Matrix solid-phase dispersion / Micellar electrokinetic chromatography / Molecularly imprinted polymers / Orthogonal design / Triazines
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1 Introduction

Triazine herbicides are widely used for weed control in several crops and their activity is based upon their ability to inhibit photosynthesis in plants [1]. But their prolonged use involves the risk of their retention in crops and soils. Because triazines and their degradation products are very toxic and survivable for many years in the environment [2, 3], there are increasing environmental and healthy concerns for these compounds. Many countries, including United States, European Union members, Japan, and China, have established maximum residue limits (MRLs) for triazine herbicides in vegetables and corps, i.e. 50–250 ng/g [4–6]. Various high-efficiency analysis methods are desired for monitoring the presence and determining the levels of triazines. The most frequently used methods for the determination of triazines are HPLC [3, 7] and GC [8, 9], which always includes sample pretreatment procedures, such as solid-phase extraction (SPE) [10], solid-phase microextraction (SPME) [11], cloud point extraction [12] and dispersive liquid–liquid microextraction [13]. However, all the above pretreatment methods could not be directly applied for semisolid and solid samples that must be pretreated into solution to adapt those extraction procedures. Besides, quantification of these

Correspondence: Professor Lingxin Chen, Key Laboratory of Coastal Zone Environmental Processes, Yantai Institute of Coastal Zone Research (YIC), Chinese Academy of Sciences (CAS); Shandong Provincial Key Laboratory of Coastal Zone Environmental Processes, YICCAS, Yantai 264003, P. R. China
E-mail: lxchen@yic.ac.cn
Fax: +86-535-2109130

Abbreviations: AA, acetic acid; **AE**, acetic ester; **AME**, ametryn; **ATR**, atrazine; **DCM**, dichloromethane; **MIP**, molecularly imprinted polymer; **MSPD**, matrix solid-phase dispersion; **NIP**, nonimprinted polymer; **PRO**, propazine; **SIM**, simazine

*Additional corresponding author: Professor Jaebum Choo, E-mail: jbchoo@hanyang.ac.kr

triazines in solid and semisolid samples is often more difficult because matrix interferences are usually coextracted/coeluted. Moreover, there are growing requirements to search for more effective and simpler methods capable of using smaller amounts of solvents and sample sizes. Matrix solid-phase dispersion (MSPD) is such a method that can effectively eliminate these matrix interferences in dealing with solid and semisolid samples.

MSPD has found particular applications as an analytical process for the simultaneous disruption, cleanup and extraction of solid, semisolid, and highly viscous biological samples [7], with flexibility and selectivity, resulting in rapid pretreatment and low solvent consumption [8]. This technology involves mechanically blending a small amount of sample matrix with an appropriate sorbent followed by washing and elution of compounds with a small volume of solvent. Many materials such as octadecylsiloxane (C_{18}, C_8, etc.), underivatized silicates (silica gel, sand, etc.), and other organic (graphitic fibers) or inorganic (Florisil, alumina, etc.) solids are available as dispersants/sorbents of MSPD [9, 14–17]. However, the common sorbents lack special selectivity, and consequently MSPD is still confronted with difficulty of effective extraction targeted analytes from complicated matrices. Recently, a polymer material, i.e. molecularly imprinted polymers (MIPs) [18], due to its specific molecular recognition properties for a given compound and its analogs, high stability, as well as low cost and easy preparation, has been increasingly utilized to enhance the selectivity of MSPD [19–22]. Yan et al. synthesized water compatible ofloxacin MIPs as dispersion sorbent in MSPD, and realized the simultaneous isolation of five fluoroquinolones in chicken eggs and swine tissues [19]. Guo et al. utilized chloramphenicol (CAP) MIPs as selective MSPD sorbent and completed the efficient determination of CAP in fish tissues [20]. Qiao et al. synthesized ofloxacin MIPs as a dispersant of MSPD, which showed high affinity to enrofloxacin and ciprofloxacin in aqueous environment and could selectively enrich them from chicken tissue matrix [21]. Yan et al. synthesized a kind of aniline–naphthol MIP microsphere, applied as a selective sorbent of miniaturized MSPD, and successfully attained the simultaneous determination of four Sudans in egg yolk samples [22].

Meanwhile, MIPs-based triazines analysis has also been increasingly reported. They usually involve SPE coupled with HPLC or CE [10, 23–29]. For instance, the SPE procedure using propazine-MIPs as sorbent was applied to the cleanup of drinking and groundwater, soil, and corn sample extracts, and the triazines were determined by MEKC [27]. Lara et al. used and evaluated MIPs as in-line concentrators in CE for the analysis of atrazine and its metabolites in complex matrix with a minimum of sample treatment [28]. Also, we have just recently prepared the porous atrazine MIPs and applied them to selective SPE of triazines in soil samples followed by HPLC [29]. The prepared MIPs contributed to the sample cleanup and extraction enhancement and therefore improved method performances for triazines. However, little attention has been paid to quantitative determinations of triazines based on the use of MIPs as sorbents/dispersants for the MSPD method.

Herein, we employ MIPs as MSPD dispersants (MI-MSPD) for concurrent cleanup of samples and extraction of triazines at trace levels, followed by MEKC. To the best of our knowledge, this work represents the first attempt of using MIPs as selective MSPD sorbents to develop a new MI-MSPD-MEKC method for selective extraction and simultaneous determination of four triazines in three different samples. The MIPs were prepared through bulk polymerization by using atrazine as the template molecule, methacrylic acid as the functional monomer and ethylene glycol dimethacrylate as the cross-linking in the porogen of toluene. Additionally, a kind of fractional factorial design (i.e. orthogonal design) was used to aid optimizing the separation conditions of MEKC. Also, key factors affecting the MSPD efficiency were systematically investigated. Excellent analytical performance of the MI-MSPD-MEKC was attained and this method could be potentially applied for the determination of triazines in complicated samples.

2 Materials and methods

2.1 Chemicals, solutions, and samples

Four triazines standards of simazine (SIM), atrazine (ATR), ametryn (AME), and propazine (PRO) were purchased from Sigma-Aldrich (Steinheim, Germany). Furazolidone and diethylstilbestrol were purchased from J&K Technology Limited (Beijing, China). Methacrylic acid (MAA) and ethyleneglycol dimethacrylate (EGDMA) were also purchased from Sigma-Aldrich (Steinheim, Germany) and distilled in vacuum prior to use. 2,2'-azo-bis-isobutyronitrile (AIBN) was purchased from Shanghai Chemical Reagents Company (Shanghai, China) and recrystallized in methanol prior to use. SDS and sodium borate were purchased from Aladdin (Shanghai, China). Chromatographic grade ACN, methanol (MeOH), toluene, dichloromethane (DCM), and acetic ester (AE) were purchased from J&K Technology Limited (Beijing, China). Other affiliated chemicals such as sodium hydroxide, anhydrous sodium sulfate, ammonium acetate, acetic acid (AA), and ammonia water were all obtained from local suppliers (Yantai, China). All the chemicals were of analytical grade. Deionized water used throughout the work was produced by a Milli-Q Ultrapure water system (Millipore, Bedford, MA, USA).

Standard stock solutions containing 1000 μg/mL of individual triazines were prepared by dissolving the required amounts of the standards in ACN. They were stored in a refrigerator at 4°C. Working solutions were prepared by diluting the stock solutions with Milli-Q water.

Soil samples, collected in the local field (Yantai, China), were air-dried for 24 h at room temperature, and then grounded and sieved through a mesh gauge with a grain size of 2 mm. Strawberry and tomato samples were kindly provided by Yantai Inspection and Quarantine Bureau (Yantai, China), and then were prepared with a food processor

and mixed thoroughly and stored in a refrigerator at −20°C. Before used, they were slightly thawed.

2.2 Apparatus and software

All experiments were performed on a P/ACE MDQ CE system (Beckman Coulter, Fullerton, CA, USA) in conjunction with a diode-array detector (DAD) monitoring at 222 nm. Separation was performed at 25°C, using an applied voltage of 22 kV. Samples were hydrodynamically (5 s, 0.5 psi) injected at the anode. Bare fused-silica capillaries (Yongnian Optic Fiber Factory, Hebei, China) were used for triazines separation, with 75 μm id, 375 μm od, total length of 50.2 cm, and effective length of 40 cm. An Ion 510 pH meter (Ayer Rajah Crescent, Singapore) was used to adjust pH values. New capillaries were initialized by flushing with water (10 min), 1.0 M NaOH (40 min), water (10 min), and run buffer (30 min) before use. Between analyses the capillary was rinsed with run buffer (2 min). All the samples were passed through microporous nylon filters of 0.45-μm pore sizes in diameter.

2.3 Synthesis of the atrazine MIPs

We employed the traditional, mature polymerization method of bulk polymerization to obtain the atrazine MIPs. The synthesis procedure was as follows. A total of 215.7 mg of atrazine and 0.346 mL of methacrylic acid were dissolved in 2.5 mL of toluene to prepare an atrazine prepolymer solution. The solution was stored in a refrigerator at 4°C for 12 h and then 50 mg AIBN and 3.017 mL of EGDMA were added and dissolved adequately in a sonicating bath for 5 min. The solution was degassed with nitrogen for 5 min and the tubes were closed and sealed under this atmosphere. The monolithic polymers were obtained in a water bath at 60°C for 24 h, and would be powdered to particles by a grinder, with the size of about 10 μm, prior to use. And then the resultant polymeric particles were washed by Soxhlet extraction with MeOH/AA solution (9:1, v/v) (i.e. 10% AA) and MeOH to remove both the template molecules and residual monomers. This procedure was performed repetitiously until atrazine could not be detected in the soaking solution by MEKC. Finally, the MIPs particles were dried to constant weight under vacuum at 40°C for use.

Following the above procedure except the absence of atrazine, nonimprinted polymers (NIPs) were prepared.

2.4 Morphology and characterization of the MIPs

The morphological evaluation was performed by scanning electron micrography (SEM, JSM-5600LV, operating at 20 kV, Japan). All samples were sputter coated with gold before SEM analysis. The MIPs were also characterized by nitrogen adsorption experiments and CE. Nitrogen adsorption–desorption results were recorded using AUTOSORB 1 (Quantachrome Instruments, Germany). The Brunauer, Emmett, and Teller (BET) method [30] was used to determine the specific surface area. The samples were degassed in a vacuum at 300°C prior to adsorption measurements.

The binding capacity was investigated as follows. Twenty milligrams of polymer particles were dispersed in a 5-mL flask containing 2.0 mL atrazine solutions of various concentrations. After shaking for 24 h at room temperature, the samples were centrifuged. The concentration of the supernatant solution was determined using CE. The amount of atrazine adsorbed onto the MIPs was calculated by subtracting the amount of unbound compounds from the amount of compounds added to the mixture. Moreover, selectivity experiments were carried out by using its structural analogs of ametryn, simazine and propazine, and two references of furazolidone and diethylstilbestrol.

2.5 MSPD procedure

The MSPD procedure is schematically shown in Supporting Information Fig. S1. Soil sample (0.2 g) was transferred to a glass mortar, and a suitable amount of standard solution was added to the sample to evaluate recovery. After 5 min, a portion of 0.2 g MIPs was gently blended with the sample using a pestle to obtain a completely homogeneous mixture. This mixture was introduced into an empty syringe (0.05 g of C_{18} and 1 g anhydrous sodium sulfate were prepacked at the bottom), rinsed with 5.0 mL of water, and then eluted with 5.0 mL of MeOH. The eluent was evaporated to dryness under a gentle flow of nitrogen, and the residue was reconstituted into 0.4 mL of run buffer for MEKC analysis.

For strawberry and tomato samples, 0.2 g sample was blended with 0.6 g MIPs, and eluted by 5 mL AE and 10 mL DCM, respectively. The other procedures were followed as above.

3 Results and discussion

3.1 Characterization of the atrazine MIPs

As seen from Fig. 1, the prepared atrazine MIPs show dense, homogenous rough surface with numerous, larged-dimension pores, and satisfactory mechanical strength. The uniform and open structure is obviously favorable for the embedding of the template molecules and mass transfer. And the specific surface areas were attained of 130.6 m^2/g for MIPs and 73.6 m^2/g for NIPs, respectively. The results indicated that larger specific area leads to higher binding capacity of MIPs than NIPs, as can be seen from Supporting Information Fig. S2.

As for the selectivity of the MIPs, the binding of triazines and reference compounds were investigated by equilibrium-binding experiments at an initial concentration of 40 μg/mL. As seen from Fig. 2, the binding capacities for the four triazines are close while they are much higher than two references; the capacity for ATR is the largest followed by AME, and SIM similar to PRO. So, the obtained ATR-MIPs could

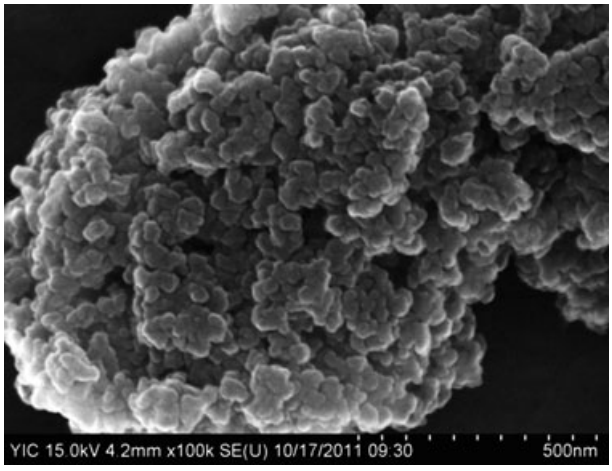

Figure 1. SEM of the prepared atrazine MIPs by bulk polymerization.

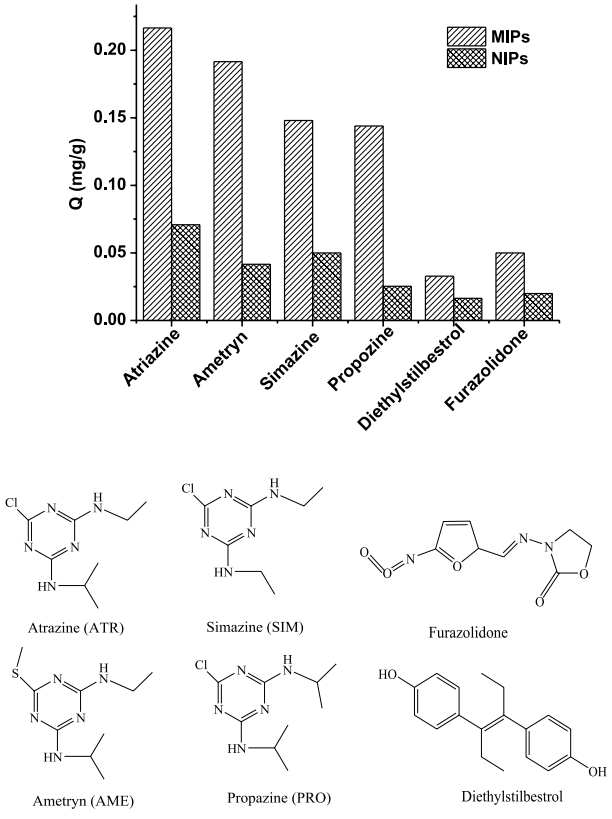

Figure 2. Binding capacities of MIPs and NIPs for four triazines, furazolidone and diethylstilbestrol, and their chemical structures. Measurement conditions: Polymer, 20 mg; $C_0 = 40$ mg L^{-1}; $V =$ 2.0 mL; adsorption time, 24 h; room temperature.

selectively bind triazines from other herbicides. In contrast, the NIPs gave the similar binding capacity for all the compounds (Fig. 2), showing there were no specific binding sites. Therefore, the MIPs could be used as selective sorbent for triazines.

Table 1. Experimental design chart including five variables, five levels orthogonal design and R_s (peak resolution of AME and PRO), t and R_s/t data

No.	C_1 (mM)	C_2 (mM)	C_3 (%)	V (kV)	pH	t (min)	R_s	R_s/t (1/min)
1	10	5	10	22	7.45	4.08	0.00	0.00
2	10	10	15	24	8.05	4.20	0.67	0.16
3	10	20	20	26	8.45	4.81	1.25	0.26
4	10	30	25	28	9.05	4.41	1.18	0.27
5	10	40	30	30	9.45	4.43	0.76	0.17
6	15	5	15	26	9.05	2.83	0.00	0.00
7	15	10	20	28	9.45	2.90	0.00	0.00
8	15	20	25	30	7.45	4.86	0.10	0.02
9	15	30	30	22	8.05	6.83	0.78	0.11
10	15	40	10	24	8.45	14.31	4.53	0.32
11	20	5	20	30	8.05	3.12	0.00	0.00
12	20	10	25	22	8.45	4.52	0.00	0.00
13	20	20	30	24	9.05	4.53	0.00	0.00
14	20	30	10	26	9.45	7.89	3.63	0.46
15	20	40	15	28	7.45	20.94	5.22	0.25
16	25	5	25	24	9.45	3.42	0.00	0.00
17	25	10	30	26	7.45	4.53	0.00	0.00
18	25	20	10	28	8.05	8.81	3.42	0.39
19	25	30	15	30	8.45	7.24	3.41	0.47
20	25	40	20	22	9.05	9.08	3.47	0.38
21	30	5	30	28	8.45	3.54	0.00	0.00
22	30	10	10	30	9.05	3.75	1.41	0.38
23	30	20	15	22	9.45	6.45	3.04	0.47
24	30	30	20	24	7.45	18.23	5.17	0.28
25	30	40	25	26	8.05	9.92	4.46	0.45

3.2 Optimization of MEKC separation conditions

For MEKC, there are many conditions to be optimized, such as buffer concentration, SDS concentration, buffer pH, and organic solvent concentration as well as applied voltage, all of which can significantly affect the separation efficiency. During the optimization process, an orthogonal design, the mainly used type of fractional factor design, would be adopted to simultaneously study the influences of main parameters in a short time.

Selection of suitable background electrolyte is important for CE separation. In this system, three electrolyte solutions including sodium borate, phosphate buffers and ammonium acetate were tested, respectively. Finally, ammonium acetate was chosen as the background electrolyte. On the basis of the preliminary experimental results, the concentration of ammonium acetate (C_1) and SDS (C_2), the content of ACN (C_3), the applied potential (V), and buffer pH (pH) were selected as variables. The ranges and intervals of the five variables were also determined in preliminary experiments, which are indicated in Table 1. The results showed that it was difficult to separate AME and PRO and the migration time of PRO was the longest in all the four triazines. Therefore,orthogonal design was selected to obtain an optimum separation conditions for AME and PRO in a short time.

Table 2. Linear relations and detection limits of triazines for soil, strawberry, and tomato samples

Sample	Triazines	Linear range (μg/g)	Slope (RSD[a],%)	Intercept (RSD,%)	R^2	LOD (ng/g)	LOQ (ng/g)
Soil	SIM	0.5–25	+2254.5 (13.8)	−858.1 (6.9)	0.9997	13.5	45.0
	ATR	0.5–25	+1989.2 (10.2)	−1417.2 (9.4)	0.9991	12.9	43.0
	AME	0.5–25	+2233.8 (15.7)	−1138.3 (10.1)	0.9999	18.3	61.0
	PRO	0.5–25	+2397.9 (16.2)	−535.4 (5.8)	0.9995	16.0	53.3
Strawberry	SIM	0.5–25	+1573.0 (9.6)	+690.5 (6.1)	0.9998	20.9	69.7
	ATR	1–25	+1366.9 (8.5)	−497.5 (5.0)	0.9996	21.1	70.3
	AME	1–25	+1226.9 (9.1)	−53.4 (2.2)	1.0000	21.4	71.3
	PRO	1–25	+1288.3 (7.4)	−467.3 (6.5)	0.9999	24.4	81.3
Tomato	SIM	0.5–25	+1318.8 (8.2)	+507.4 (3.9)	0.9992	31.3	104.3
	ATR	0.5–25	+1224.9 (6.9)	+68.4 (1.9)	0.9991	16.4	54.7
	AME	0.5–25	+1509.2 (8.3)	+154.3 (2.8)	0.9992	26.1	87.0
	PRO	0.5–25	+1457.9 (7.9)	−55.5 (1.8)	0.9996	31.5	105.0

a) Relative standard deviation, $n = 3$.

Table 3. Precision of migration time and peak area for the MEKC-UV determination of triazines

Sample	Triazines[a]	Intraday RSD (%)[b]		Interday RSD (%)[c]	
		Migration time	Peak area	Migration time	Peak area
Soil	SIM	1.21	3.21	1.74	5.65
	ATR	0.14	0.89	2.45	7.41
	AME	0.50	2.09	1.77	4.40
	PRO	0.52	0.93	1.66	5.03
Strawberry	SIM	0.61	2.19	1.21	1.53
	ATR	0.91	3.61	1.78	5.32
	AME	1.31	3.55	1.64	4.01
	PRO	1.35	4.17	2.14	5.51
Tomato	SIM	0.40	2.64	1.72	5.49
	ATR	0.66	4.83	1.00	4.95
	AME	0.95	3.12	1.89	2.67
	PRO	1.02	3.14	1.19	2.68

a) Spiking triazines individual at 12.5 μg/g.
b) $n = 6$.
c) $n = 6$.

A five-level five-factor orthogonal design was built for the separation of the triazines and a total of 25 experiments were performed, as shown in Table 1. The aim was to see which factor had predominant influence on peak resolution (R_s) of AME and PRO as well as to determine the optimum operation conditions in a short time. The R_s data, migration time of PRO (t) and R_s/t were shown in Table 1. The data process is indicated in Supporting Information, similar to that described in our previous work [31]. The effect of each variable on R_s is shown in Supporting Information Fig. S3. An estimation of the effects of the variables is performed on the response. From the figure, it can be seen that the concentration of SDS is the most important factor affecting the separation of AME and PRO. The migration time of PRO, peak of which is the last one in the electropherogram, increased with increasing concentrations of SDS when the other factors were fixed. The content of ACN is the second important factor for that the addition of organic modifier to the buffer is an effective way of improving separation selectivity, efficiency, and resolution. And the concentration of the ammonium acetate, the applied potential, and buffer pH are the three factors that do not have very important effect on the separation of AME and PRO compared with the other two. From Table 1, it can be seen that there are several experimental combinations in which baseline separation can be realized ($R_s > 1.5$). However, there are some combinations in which R_s even exceeds 4 but the migration time of PRO are too long such as No. 10, 15, and 24. So in order to obtain an excellent separation in a short time, R_s/t was selected as the final index. And it is very interesting that the R_s/t value of No. 19 and 23 were the same. Finally, the optimum separation conditions were chosen as No. 23 from Table 1 for its shorter migration time compared with No. 19, as follows: 30 mmol/L ammonium acetate, 20 mmol/L SDS, 15% acetonitrile, 22 kV applied voltage, and pH 9.45. According to the above conditions, a typical electropherogram in which four triazines were baseline-separated within 7 min is shown in Supporting Information Fig. S4.

3.3 Optimization of MSPD extraction conditions

The selectivity of MSPD procedure depends on the combination of dispersant/sorbent and solvent used. Three main parameters were optimized including sorbent, ratio of MIPs sorbent to sample, and elution solvent and volume.

The usual solid supports for the MSPD procedure, commercial C_{18}, Florisil, and PestiCard, were investigated, as well as the prepared ATR-MIPs. Results showed that C_{18} failed to extract the studied triazines. The extraction efficiencies of Florisil and PestiCard were lower compared with that of the MIPs. Take Florisil as an example for the extraction efficiency (indicated by peak area in electropherograms, A) comparing with MIPs, and for an instance in the strawberry matrix, as can be seen from Supporting Information Fig. S5. The results showed that the ATR-MIPs sorbent exhibited much higher extraction efficiencies for the triazines compared with that of the Florisil, suggesting the MIPs open a promising way as MSPD sorbent to selective preconcentration of triazines.

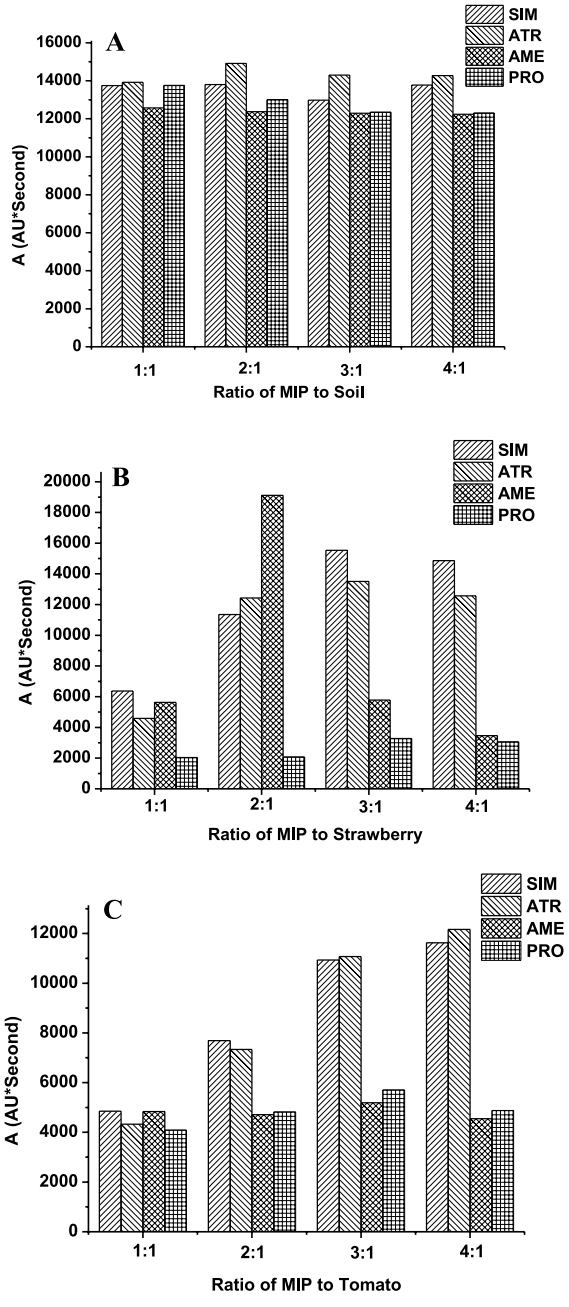

Figure 3. Effect of the ratio of MIPs sorbent to samples on the response peak area of triazines in (A) soil, (B) strawberry, and (C) tomato. The spiked concentrations of triazines were 12.5 μg/g. MEKC separation conditions: run buffer, 30 mmol/L ammonium acetate, 20 mmol/L SDS, and 15% ACN (pH 9.45); applied voltage, 22 kV; wavelength, 222 nm. MSPD conditons: ATR-MIPs as dispersion sorbents; 5 mL MeOH for soil sample, 5 mL AE for strawberry sample and 10 mL DCM for tomato sample.

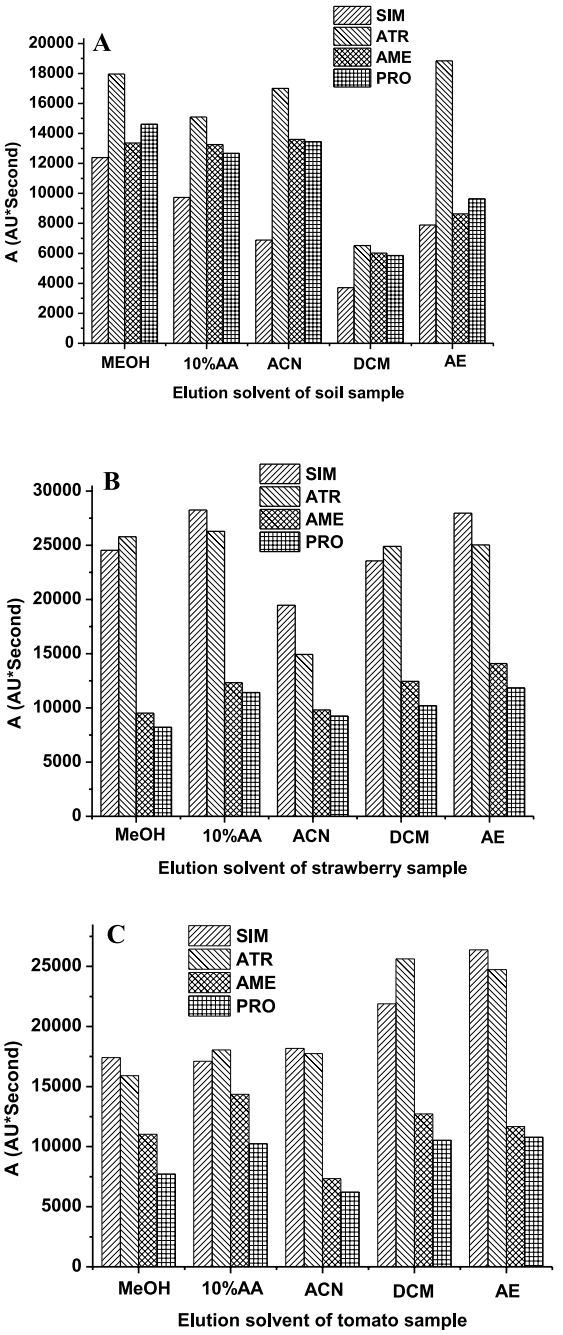

Figure 4. Effect of elution solution on the response peak area of triazines in (A) soil, (B) strawberry, and (C) tomato. The spiked concentrations of individual triazines were 12.5 μg/g. MEKC separation conditions were the same as those described in Fig. 3. MSPD conditions: volume of elution solution were 5 mL, 5 mL, and 10 mL and MIPs sorbent to sample ratios were 1:1, 3:1, and 3:1 for soil, strawberry, and tomato, respectively.

Furthermore, a significant difference was observed between MIPs and NIPs. The MIPs showed high affinity toward the triazines especially for ATR as template molecules. Notably, there were still some coextracted interfering substances when MIPs were used as the sorbent. So, C_{18} was employed as the cleanup sorbent, owing to its weak adsorption for the triazines and wide-general adsorption for various substances. Therefore, the MIPs were selected as the MSPD extraction sorbent followed by C_{18} cleanup.

During the blending process, the MSPD sorbents act as an abrasive and as a bound solvent that can break the sam-

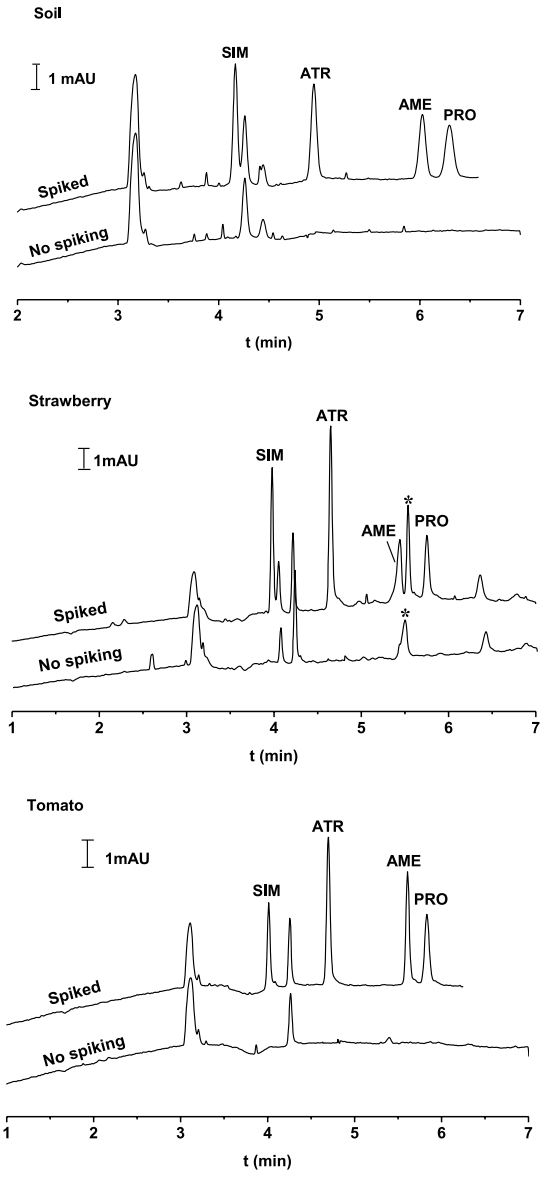

Figure 5. Typical electropherograms of different samples with MI-MSPD. The soil, strawberry, and tomato samples no spiking and spiked with 12.5 μg/g individual triazines were treated with MI-MSPD. The peak with "*" is an unknown peak and might be the matrix peak of an interfering substance contained in the strawberry samples. MEKC separation conditions were the same as those described in Fig. 3. MSPD conditions: 5 mL MeOH for soil sample, 5 mL AE for strawberry sample and 10 mL DCM for tomato sample and MIPs sorbent to sample ratios were 1:1, 3:1, and 3:1 for soil, strawberry, and tomato, respectively.

ple architecture, disperse its components, and promote more effective interactions between them and the analytes [14]. Therefore, the ratio of sorbent to sample can also affect extraction efficiency. As shown in Fig. 3A, when the ratio was 1:1, the extraction efficiencies for AME and PRO spiked soil were higher than that at other ratios. Although the extraction efficiencies of SIM and ATR were a little higher when the ratio was 2:1 compared with 1:1, the peak areas were almost the same. Therefore, the ratio of 1:1 was selected as the best ratio for MIPs to soil sample. For strawberry sample, extraction efficiencies increased with increasing ratio of MIPs to sample from 1:1 to 3:1 (Fig. 3B). However, when the ratio was 2:1, the efficiency of AME was much higher than that at 3:1, while other triazines showed lower efficiency. So, in order to extract all the four triazines together with satisfactory efficiencies, the ratio of 3:1 was chosen for strawberry sample. For tomato sample, extraction efficiencies increased with increasing the ratio of the MIPs to sample from 1:1 to 3:1 for AME and PRO (Fig. 3C). Also, the extraction efficiencies of SIM and ATR were a little higher when the ratio was 4:1 compared with 3:1 same as the soil sample, and the elution pressure increased a lot with increasing the ratio from 3:1 to 4:1. Therefore, 3:1 was the ratio selected for tomato sample.

To optimize the elution condition, generally used elution solvents of MeOH, ACN, DCM, and AE were tested. Moreover, referring to our previous work [25] for atrazine analysis, methanol/acetic acid solution (9:1, v/v), i.e. 10% (v/v) AA, was also adopted as elution solution for the optimization test. High extraction efficiency was found by using MeOH for soil sample, shown in Fig. 4A. For strawberry sample, after eluted with the above solutions, respectively, the extraction efficiencies of AME and PRO by AE and 10% AA were higher than others (Fig. 4B). Although the efficiencies of SIM and ATR eluted by 10% AA were a little higher than AE, the attained peaks eluted by the latter were narrower and had little interference. Therefore, AE was selected as the elution solution for strawberry. Tomato sample, like strawberry, was eluted by the above elution solutions. As shown in Fig. 4C, the elution efficiency of the DCM was higher than that of AE. So DCM was selected as the elution solution. Moreover, effect of the elution solution volume was also tested (Supporting Information Fig. S6). Overall, after testing for the soil, strawberry, and tomato samples, the optimum elution solutions were chosen, 5 mL MeOH, 5 mL AE, and 10 mL DCM, respectively. This suggested that different matrices usually required different elution conditions for MSPD.

3.4 Analytical performance of the MI-MSPD-MEKC method

Analytical performance of the MI-MSPD-MEKC was evaluated in three different matrices. Calibrations curves were assessed by using samples fortified at six different concentration levels, between peak-area and the corresponding concentrations of the four triazines. Good linear relationships were attained within 0.5–25 μg/g or 1.0–25 μg/g ($R^2 \geq 0.9991$) (Table 2). And the regression equations for triazine standards were obtained as shown in Table S1. The LODs calculated as the triazine concentration based on the peak height being three times the background noise (S/N = 3), were 12.9–31.5 ng/g (Table 2). And based on the peak height being ten times the background noise (S/N = 10), the LOQs

Table 4. Method recoveries for triazines in soil, fruit, and vegetable samples

Sample	Triazines	Added (μg/g)	Recovery[a)] (%)	RSD[b)] (%)	Added (μg/g)	Recovery[a)] (%)	RSD[b)] (%)	Added (μg/g)	Recovery[a)] (%)	RSD[b)] (%)
Soil	SIM	0.50	86.1	1.32		76.2	2.06		96.5	4.89
	ATR	0.50	90.3	3.49		94.7	2.99		89.6	2.02
	AME	0.50	77.3	1.88		78.0	2.16		80.2	2.15
	PRO	0.50	60.8	2.01		71.1	3.87		79.3	1.88
Strawberry	SIM	0.50	72.3	3.65		79.8	2.98		85.2	2.78
	ATR	1.00	89.8	2.46	2.50	97.4	3.76	12.5	98.4	4.06
	AME	1.00	72. 6	1.28		80.2	1.89		76.6	2.57
	PRO	1.00	57. 9	2.33		65.1	1.96		80.0	1.65
Tomato	SIM	0.50	59.0	2.00		76.6	2.36		79.8	1.97
	ATR	0.50	63.7	1.56		72.7	2.17		89.5	2.11
	AME	0.50	55.4	1.79		68.4	1.64		73.7	1.93
	PRO	0.50	53.5	1.47		66.5	1.56		70.9	1.39

a) $n = 6$.
b) $n = 6$.

Table 5. Method comparisons for analysis of triazines

	Ref. [10]	Ref. [12]	Ref. [13]	Ref. [32]	Present study
Triazine[a)]	3	1	5	3	4
Sample	Lettuce and apple	Water and soil	Water and soil	Soil	Soil, strawberry, and tomato
Pretreatment method	SPE	CPE[b)]	DLLME[c)]	SPME	MIP-MSPD
Analytical method	HPLC-UV	HPLC-UV	HPLC-UV	GC-MS	MEKC-UV
Migration time	<13min	<6 min	<14 min	<15 min	<6.5 min
LOD	22–38 ng/g	3.5 μg/L	0.05–0.2 ng/mL	0.13–1.12 ng/g	12.9–31.5 ng/g
RSD (%)	0.72–1.55	0.80–2.38	2.9–5.6	7.0–12.8	0.89–4.83
Comment	Long migration time	Just effective for water solutions	Long migration time, just effective for water solutions	Poor reproducibility, expensive SPME fibers, long migration time	Short migration time, good repeatability, low analytes/solvents consumption, efficient cleanup, and high-selective extraction, applicable to solid and semisolid matrices

a) The number of triazines detected.
b) Cloud point extraction.
c) Dispersive liquid–liquid microextraction.

were also obtained of 43.0–105.0 ng/g (Table 2). The attained LODs are lower than the MRLs of 50–250 ng/g regulated by United States, European Union, Japan, and China, as well as LOQs are also acceptable for MRLs detection. The developed method proved capable to monitor the triazine residues in the selected samples below the MRLs legislated.

To determine intraday precision, we performed the replicate analyses ($n = 6$) at spiked concentration of 1.0, 12.5, and 25 μg/g on the same day. The procedure was repeated on different days ($n = 6$) at the same concentration to determine the interday precision. The precision was given by the intraday and interday RSD. As shown in Table 3, the intraday precisions (spiking triazines individual at 12.5 μg/g) in terms of migration time and peak area obtained were found to be excellent for the four triazines with the RSD values falling in the range of 0.14–1.35% and 0.89–4.83%, respectively, based on six consecutive injections. The interday precisions in migration time and peak area were in the range of 1.00–2.45% and 1.53–7.41%, respectively. As for 1.0 μg/g, in terms of migration time and peak area, the intraday precisions were 0.86–1.38% and 2.97–4.02%, respectively, and the interday precisions were 1.32–1.99% and 3.23–6.00%, respectively. And as for 25.0 μg/g, in terms of migration time and peak area, the intraday precisions were 0.92–1.40% and 2.05–3.87%, respectively, and the interday precisions were 1.76–2.00% and 2.88–5.91%, respectively. For simplicity, the related data tables are not shown here. Therefore, the method was demonstrated applicable for sensitive and accurate quantitative determination of the triazines in complicated samples.

3.5 Recovery study in different samples

In order to validate the potential applications of the MI-MSPD procedure for selective extraction of triazines in real samples, three sample solutions spiked the mixture standards of SIM, ATR, AME, and PRO individual at 12.5 μg/g, respectively, were pretreated using the MI-MSPD. As shown in Fig. 5, four compounds were remarkably detected by the MI-MSPD, which was attributed to the fact that the MI-MSPD has high cleanup ability, and thereby the matrix effects could be significantly reduced. On the other hand, the endogenous triazines were not detected in the soil, strawberry, and tomato samples (Fig. 5). Also, as seen from the figure, there is an unknown peak between AME and PRO at the migration time of 5.5 min for the strawberry samples with and without spiking triazine standards. It is very likely to be a matrix peak of an interfering substance contained in the strawberry samples.

The further validation of the method was performed by examining recoveries of the spiked samples using the optimized procedures. The results are listed in Table 4. Satisfactory recoveries were obtained, such as 65.1–97.4% with precision of 1.56–3.87% at 2.5 μg/g. This demonstrated the MI-MSPD-MEKC greatly applicable for the selective extraction, sample cleanup, and simultaneous separation and accurate quantitation of trace triazines in different matrices.

4 Concluding remarks

In this work, a MI-MSPD extraction coupled to MEKC separation was successfully developed and applied for the simultaneous determination of trace level triazines in soil, fruit, and vegetable samples. The MSPD based on ATR-MIPs offered efficient cleanup and selective extraction for the four triazines in different samples, which could significantly eliminate the matrix interferences. The developed method appears to be more advantageous over other methods [10, 12, 13, 32] as shown in Table 5. And the employment of an experimental design for MEKC optimization helped to greatly simplify the total experimental process. The developed MI-MSPD-MEKC with simple UV detection obtained similar or higher detection sensitivity to/than some hyphenation methods with simple instrumental setup and obviously low costs, as a rapid, simple, accurate, and environmental friendly analysis methods. Given the advantages, further research focusing on excellent MIPs as novel MSPD dispersants will be promising for routine monitoring of trace triazines and other persistent organic pollutants (POPs) in environmental and food samples.

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