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Reversed migration micellar electrokinetic chromatography with off-line and on-line concentration analysis of phenylurea herbicides

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Abstract

Three environmentally important phenylurea herbicides (monuron, isoproturon, diuron) were separated in reversed migration micellar electrokinetic chromatography (RM-MEKC) using 50 m*M* sodium dodecyl sulfate, 50 m*M* phosphoric acid, and 15 m*M* γ-cyclodextrin. Three on-line concentration techniques are then evaluated to increase the detection sensitivity of the RM-MEKC system. Stacking with reverse migrating micelles (SRMM, water as the sample solvent) provided the best results among the focusing techniques studied. Using a z-shaped detection cell, more than 500-fold increase in peak height is obtained. As a sample preparation and off-line concentration method, solid-phase extraction (SPE) that further improved detection sensitivity was used in the analysis of spiked tap and pond water. For example, 1 parts per billion of each herbicide spiked in tap or pond water was detected by MEKC after SPE and SRMM.

Keywords: Micellar electrokinetic chromatography, reversed migration; Sample preparation; Pesticides; Phenylureas

1. Introduction

Herbicides and their degradation products are potential hazards to aquatic life and human health. Polar herbicides such as the phenylurea group can occur in environmental waters in the parts per trillion to the parts per billion (ppb) levels. They are usually extracted from the soil by rainwater, which transports them to nearby bodies of water such as lakes. Ingestion by humans may then be through the food chain or through contaminated drinking water supplies. Dedicated analytical methodologies are then required to monitor low levels of these compounds both in environmental and drinking waters.

For the determination of phenylurea herbicides in water, sample preparations using solid phase or liquid–liquid extractions followed by high-performance liquid chromatography (HPLC) analysis have been reported [1,2]. The low levels of analytes in the sample matrix necessitate the sample preparation step. As an alternative to HPLC, micellar electrokinetic chromatography (MEKC) has been utilized [3–5]. More studies have been devoted on the separation of these compounds by MEKC [6–11]. Principle of MEKC separation is based on analyte partitioning between the micellar and surrounding aqueous phase [12].

The objective of this study is to separate and concentrate on-capillary three test phenylurea herbicides (monuron, isoproturon, and diuron) utilizing reversed migration micellar electrokinetic chromatography (RM-MEKC) [13]. Three on-capillary or on-line concentration techniques, sweeping, stacking with reverse migrating micelles (SRMM), and stack-

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ing using reverse migrating micelles and a water-plug (SRW), are evaluated [14–17]. RM-MEKC is characterized by anionic micelles moving faster than the electroosmotic flow; thus positive potential is applied at the detector end in order to detect the analytes. Electroosmotic flow is reduced using low pH buffers [18]. Moreover, solid-phase extraction (SPE) was utilized as an off-line preconcentration step.

Sweeping is performed by preparing the sample in a matrix having a conductance similar to that of the background solution (BGS) and is void of the surfactant or micelle used in the BGS [14]. The focusing mechanism is based on the picking and accumulating of the analytes molecules found in the sample solution (S) zone by the micelles that enter and fill the S zone upon application of voltage. The greater the affinity of the analyte toward the micelle or the higher the retention factor of the analyte (k) [$k=K\ (V_{PS}/V_{aq})$, where K is the distribution coefficient, V_{PS} is the volume of the pseudostationary phase, V_{aq} is the volume of the aqueous phase) the greater the focusing effect [14,15]. Therefore, the sweeping phenomenon is useful for all types of analytes as long as the k is high. Moreover, pH of sample and separation buffers can be manipulated and a neutral or ordinary capillary may be used to improve the performance of the focusing method, and additives can be added to the sample matrix (e.g., organic solvent or nonionic surfactant) to enhance solubility. In this study, a low pH buffer and fused-silica capillary is utilized because they are readily available and cheap, and no additives are added to the sample matrix because the samples are soluble in 100% aqueous solution.

SRMM is performed by preparing the sample in a matrix having a conductance lower than that of the BGS (e.g., water) and is void of the surfactant used in the BGS [16]. The focusing mechanism is based on the abrupt change in analyte effective electrophoretic velocities at the stacking boundary. The stacking boundary separates regions of high and low electric fields [19,20]. The sweeping phenomenon is also partly responsible for the focusing effect in SRMM [21]. The advantage of SRMM is that the focusing process and the removal of the sample matrix that is inherent in large volume sample stacking occur upon application of voltage at negative polarity. Moreover, for low to moderate k, SRMM works better compared to sweeping alone (sample prepared in a same conductivity matrix compared to the BGS) [21]. Thus, SRMM is expected to work better for the test analytes studied here.

SRW is performed by preparing the sample in a matrix having a conductance lower than the BGS, however, a surfactant is added at a concentration slightly higher than the critical micelle concentration [17]. Unlike sweeping and SRMM, a water plug is first injected into the capillary before injection of the S. The focusing mechanism is primarily based on the abrupt change in analyte effective electrophoretic velocities at the stacking boundary. An advantage of SRW is that the presence of micelles in the sample matrix will aid in the solubilization of analytes. However, solubility is not a problem here since the test analytes are hydrophilic. Here, we will investigate whether SRW will provide greater enhancement in sensitivity compared to sweeping and specially SRMM.

2. Experimental

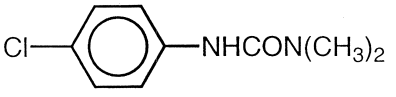
2.1. Apparatus

Preliminary studies on optimization of RM-MEKC separation conditions were carried out with an Otsuka Electronics CAPI-3200Z instrument (Hirakata, Osaka, Japan) equipped with a photodiode array detector. Stacking and sweeping experiments were carried out with a HP^{3D} capillary electrophoresis system (Hewlett-Packard, Waldbronn, Germany). Fused silica capillaries were obtained from Polymicro Technologies (Phoenix, AZ, USA) having 75 or 50 μm I.D. and 375 μm O.D. For the Otsuka instrument, 50 cm is the total length and 37.8 cm is the effective length of the capillary. For the Hewlett-Packard instrument, 64.5 cm is the total length and 56 cm is the effective length of the capillary. The high sensitivity detection cell (z-shaped detection cell) was obtained from Hewlett-Packard having a total length of 64.5 cm (56 cm effective length). Conductivity values were measured with a Horiba ES-12 conductivity meter (Kyoto, Japan). The pH of solutions was measured with the aid of a Beckman Φ

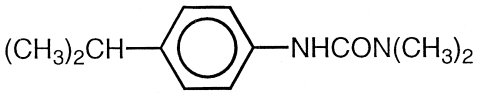
34 pH meter (Fullerton, CA, USA). Water was purified with a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Reagents and solutions

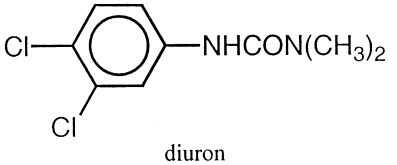
Most reagents (highest grade available) were purchased from Nacalai Tesque (Kyoto, Japan). Monuron and diuron were obtained from Tokyo Kasei (Tokyo, Japan) while isoproturon was obtained from Supelco (Bellefonte, PA, USA). Names and structures of the test herbicides are given in Fig. 1. Sample stock solutions of each herbicide were prepared with 50% aqueous acetonitrile. The concentration of each herbicide was 1000 parts per million (ppm). Appropriate amounts of the sample stock solutions were combined and diluted with the sample matrix to obtain sample solutions (S) in which the analytes had comparable peak heights. Stock solutions of 0.5-*M* sodium dodecyl sulfate (SDS) and 0.5 *M* phosphoric acid were used to prepare the background solutions (BGS). All Ss and BGSs were freshly prepared. All solutions were filtered through 0.45 μm filters (Toyo Roshi, Japan or Nippon Millipore, Japan) prior to use.



monuron
3-(4-chlorophenyl)-1,1-dimethylurea



isoproturon
3-(4-isopropylphenyl)-1,1-dimethylurea



diuron
3-(3,4-dichlorophenyl)-1,1-dimethylurea

Fig. 1. Names and structures of the test herbicides.

2.3. General electrophoresis and on-line concentration procedures

The capillary was flushed (~1 bar) prior to use with 1 *M* NaOH (20 min), followed by methanol (20 min), 0.1 *M* NaOH (20 min), purified water (20 min) and, finally, with the BGS (5 min). To ensure repeatability, the capillary was flushed between consecutive analyses with 0.1 *M* NaOH (1 min), methanol (1 min), purified water (2 min) and, finally, with the BGS (3 min).

For SRMM, test analytes prepared in water were injected into the capillary at the cathodic end using pressure. The injection times were much longer than what is usual (e.g., 1.2 s) for hydrodynamic injection. The electrode vials were filled with the BGS and the negative potential was applied at the injection-end of the capillary. For SRW, test analytes were prepared in 10 m*M* SDS in 1 m*M* phosphoric acid. A long water plug was injected at the cathodic end, followed by a long injection of S, then the BGS-containing vials were connected to both ends of the capillary and the separation potential was applied with negative polarity at the injection end. For the sweeping experiments, test analytes were prepared in phosphoric acid solution previously adjusted to the conductivity of the BGS. Resulting Ss were pressure injected into the capillary at the cathodic end. Then, the BGS-containing vials were connected to both ends of the capillary and the separation potential was applied with negative polarity at the injection-end. All injections were performed using 50 mbar pressure. A more detailed discussion of each technique can be found in the respective previous publications [14–17]. Other experimental conditions are stated in the figures or in the text.

2.4. Off-line preconcentration — solid-phase extraction

Tap or pond water was spiked with the test herbicides to a concentration of 1 ppb each. A 100 ml volume of this spiked solution was slowly passed

through a C_{18} SPE cartridge (Sep-Pak Light C_{18} Cartridge) from Waters (Milford, MA, USA). Flushing once with 2 ml methanol followed by 2 ml water previously activated the SPE cartridge [5]. After loading the sample into the SPE cartridge, the solid-phase material was dried by passing a gentle stream of nitrogen gas for 30 min. The retained herbicides were then eluted from the packing material using 2 ml of organic solvent (acetone, methanol, or ethanol) [5]. The organic solvent was then evaporated to dryness using a gentle stream of air. The residue was dissolved in 0.1 ml water and then filtered before injection into the capillary. Filtering before injection was done to remove particles that may have entered the sample during drying and reconstitution. Smaller volumes of water for reconstitution were not attempted because it is difficult to handle minute volumes of solution. Also, some solution after reconstitution may be lost during filtering.

3. Results and discussion

In finding the optimal conditions for separation, the starting BGS consisted of 50 m*M* SDS and 50 m*M* phosphoric acid. Incorporation of methanol, urea, or acetonitrile was unsuccessful to resolve all the test analytes. Addition of γ-cyclodextrin provided the best separation by forming inclusion complexes with the test analytes. The selected optimum BGS consisted of 50 m*M* SDS, 50 m*M* phosphoric acid, and 15 m*M* γ-cyclodextrin.

Fig. 2 shows the electropherograms obtained with sweeping (B), SRMM (C), and SRW (D) MEKC analysis. A usual injection (A) is included for comparison. The concentration of the samples in Fig. 2B, C, and D is a 10-fold lower compared to the concentration in Fig. 2A. Among the on-line concentration techniques, SRMM gave the highest improvement in detector response (>50-fold compared

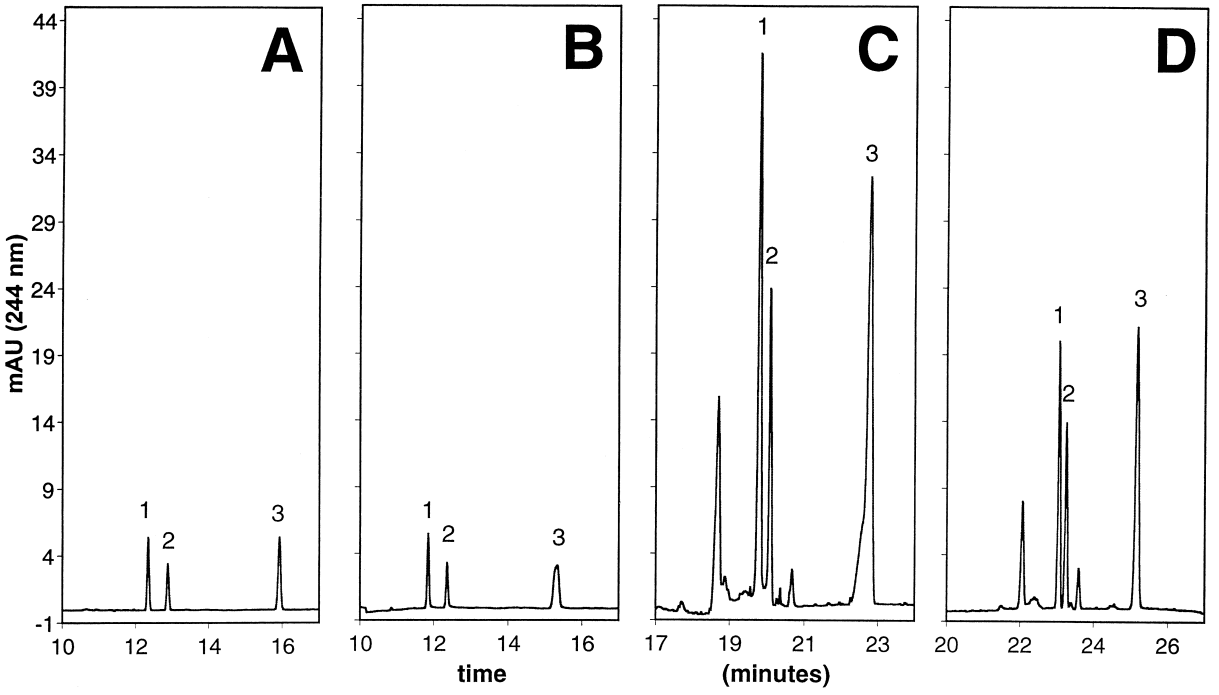


Fig. 2. Sample stacking and sweeping of phenylurea herbicides in MEKC. Conditions. BGS: 50 m*M* SDS–50 m*M* phosphoric acid–15 m*M* γ-cyclodextrin. Herbicides in S: isoproturon (1), diuron (2), monuron (3). Sample concentrations: 10 ppm each in the BGS (A); 1 ppm each in phosphoric acid solution having the same conductivity as the BGS (B); 1 ppm each in water (C); 1 ppm each in 10 m*M* SDS–1 m*M* phosphoric acid (D). Injection: 1.2 s (A); 15 s (B, sweeping); 110 s (C, SRMM); 80 s water and 35 s S (D, SRW). Separation conditions: applied voltage, −15 kV; capillary diameter, 75 μm I.D.

Table 1
%Relative standard deviations ($n=8$) with the on-line concentration techniques[a]

	RSD (%)		
	Isoproturon	Diuron	Monuron
(1) Sweeping			
Migration time	0.9	1.0	1.7
Peak height	0.1	10.3	5.6
C. peak area	4.1	10.0	3.2
(2) SRMM			
Migration time	7.0	6.8	5.4
Peak height	11.8	10.3	15.0
C. peak area	4.5	12.7	3.7
(3) SRW			
Migration time	8.2	8.2	7.5
Peak height	8.4	7.9	4.6
C. peak area	6.4	7.0	5.1

[a] Conditions: concentrations of samples, 1 ppm each, other conditions are the same as in Fig. 2.

to usual injection). Limit of detection for the SRMM technique is approximately 10–20 ppb for each test analyte. Moreover, the stacking techniques (SRMM and SRW) rendered better results compared to sweeping because the analytes are polar. Again, analytes having low to moderate k are better-concentrated using sample stacking than sweeping [21].

Table 1 lists the %RSD in migration time, peak heights, and corrected peak areas (peak area/migration time) with the on-line concentration techniques studied. Reproducibility in migration times is best using sweeping, probably because of the shorter injection time as compared to the other techniques. The poor reproducibility in migration time with SRMM and SRW may be explained by the difference in local electroosmotic flows in the S or water and BGS zones. In general, the high current observed (Joule heating) might explain the poor reproducibility (more than 80 μA using a 75 μm I.D. capillary).

To further improve the detection sensitivity, a z-shaped cell was used together with SRMM. Fig. 3 shows a usual injection (A) and a SRMM (B) MEKC analysis of the test analytes. Upon examination of Fig. 3A and B, signal enhancement was found similar to when a normal detection cell is used. Note that the concentration of the analytes in Fig. 3B is a 10-fold lower compared to those in Fig. 3A. However, if the peak heights obtained in Fig. 3B are compared to those in Fig. 2A, more than 500-fold

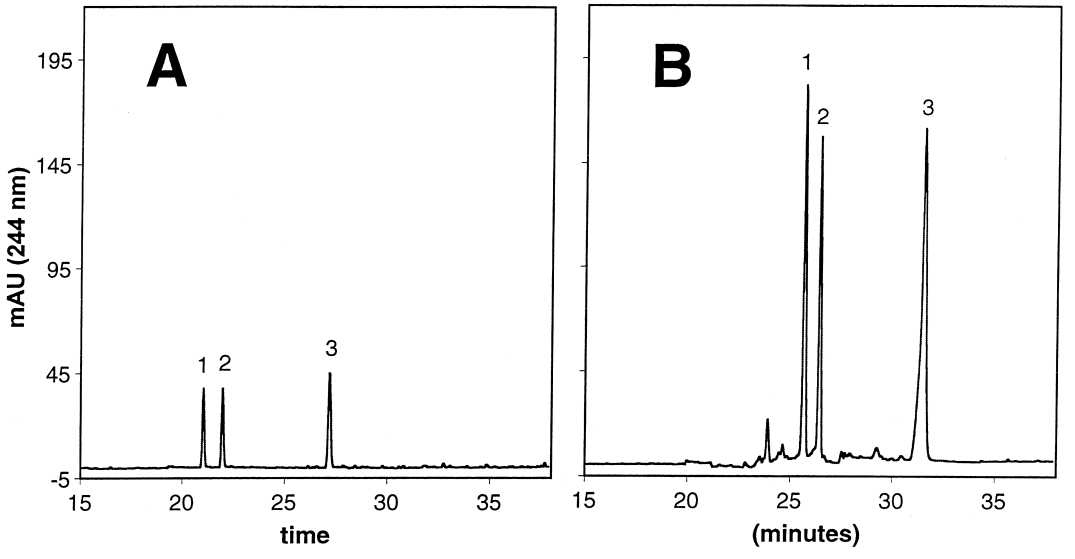


Fig. 3. SRMM-MEKC of phenylurea herbicides using a z-shaped detection cell. Conditions. Sample concentrations: 10 ppm each in the BGS (A); 1 ppm each in water (B). Injection: 1.5 s (A); 100 s (B, SRMM). Capillary diameter: 75 μm I.D. Other conditions and identification of peaks are the same as in Fig. 2.

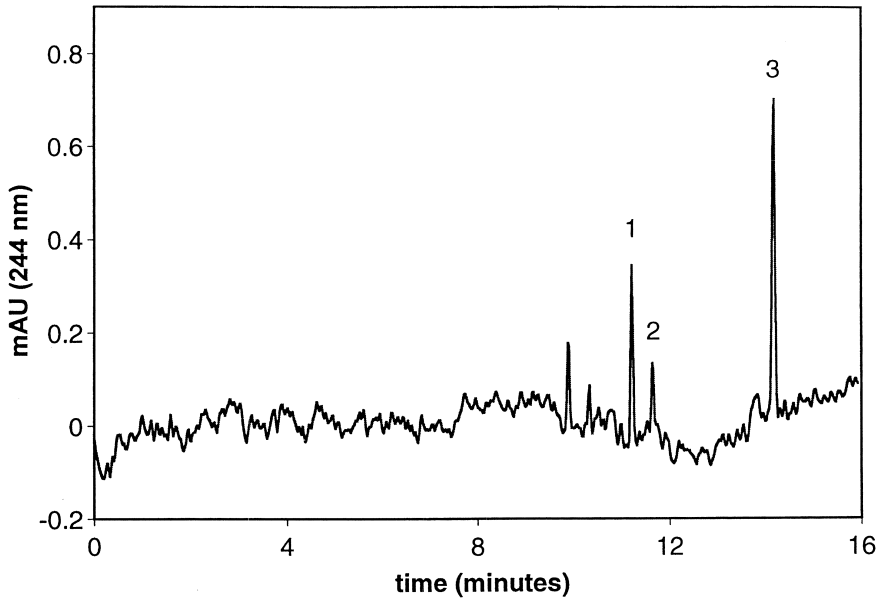


Fig. 4. Optimized SRMM-MEKC analysis of phenylurea herbicides. Conditions. sample solution, 0.1 ppm of each test herbicide in water; capillary diameter, 50 μm I.D.; injection, 100 s; separation voltage, −20 kV; other conditions and identification of peaks are the same as in Fig. 2.

improvement in peak heights are apparent. Although separation efficiency is good using the z-shaped detection cell, migration time increased twice compared to a usual capillary.

In an attempt to improve the focusing effect of sweeping without unfavorable effect on separation efficiency the concentration of the SDS in the BGS was increased to 100 m*M* (from 50 m*M*). Although it was possible to increase the injection time of sweeping to 65 s that rendered from 22 to 46-fold increase in peak heights, SRMM (for 100 s) still provided better results (50 to 60-fold increase in peak heights). The *k* values were still not high enough to provide better enhancements using sweeping.

Using a 50 μm I.D. capillary, the optimum SRMM injection time was 100 s (Fig. 4). The reproducibility and linearity of response of this method is summa-

Table 2
Reproducibility, linearity of response, and limit of detection (LOD) using SRMM[a]

	Isoproturon	Diuron	Monuron
(1) Reproducibility (n=11, using 1 ppm of each sample)			
Migration time	1.1	1.1	0.9
Peak height	3.6	6.3	1.8
C. peak area	6.0	8.0	7.2
(2) Linearity of response and LOD (S/N=3)			
Equation of line	$y=3.61x+0.03$	$y=1.99x-0.02$	$y=6.29x+0.14$
LOD (ppb)	17	30	10
Correlation coefficient	0.9988	0.9993	0.9941
(plots were obtained using 0.1–1 ppm concentrations)			
equation of the line: mAU=slope (ppm)+y-intercept			

[a] Conditions: the same as those found in Fig. 4.

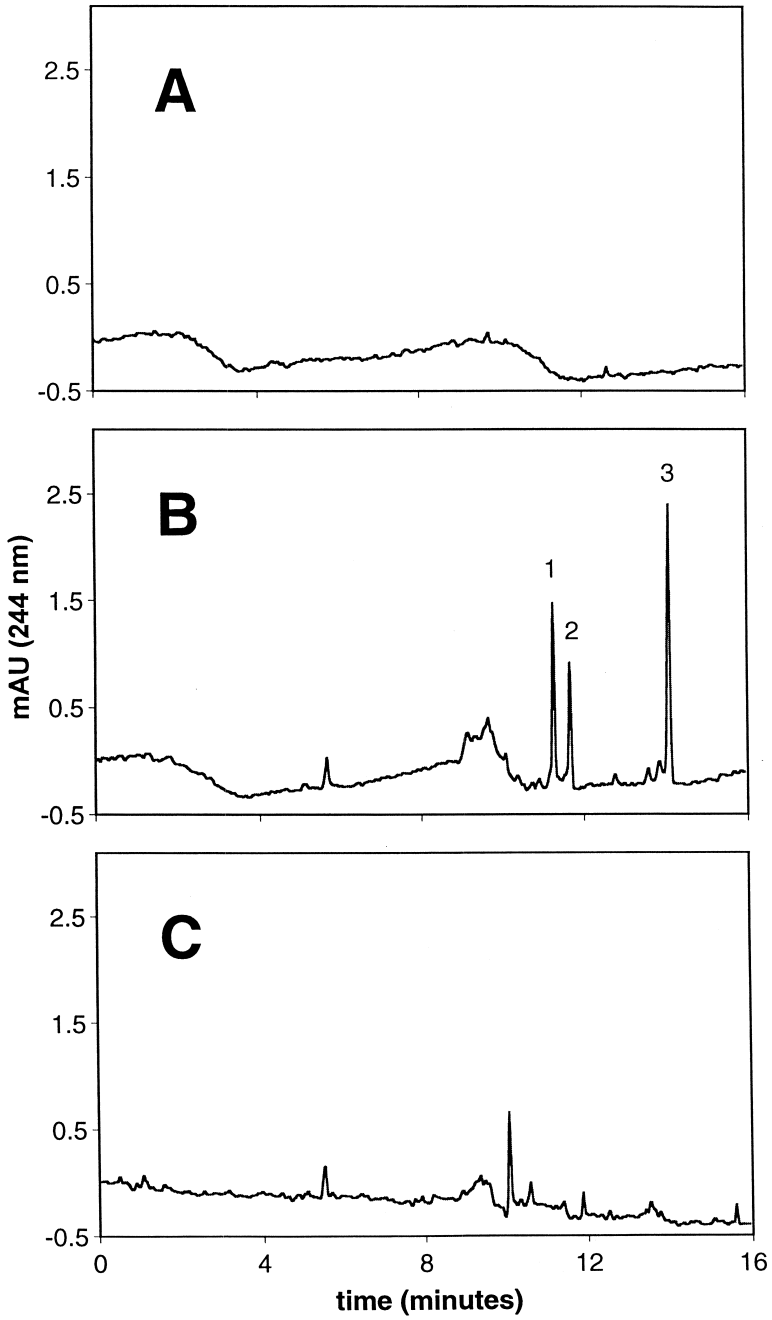


Fig. 5. SRMM-MEKC analysis of phenylurea herbicides spiked in tap water after solid-phase extraction: Conditions: concentration of herbicides in tap water, 1 ppb each (A, B), blank (C); injection, 1.2 s (A), 100 s (B, C); other conditions and identification of peaks are the same as in Fig. 4; SPE eluting solvent, acetone; other SPE conditions are stated in the Experimental section.

rized in Table 2. The reproducibility is relatively better than those obtained with a 75 μm I.D. capillary. Furthermore, the enhancements in detector response were comparable, the current was below 60 μA even when the applied voltage was increased to −20 kV, and the total electrophoresis time was below 16 min. Linearity of response is also acceptable.

Fig. 5 shows the electropherograms obtained with usual injection (A) and SRMM (B) MEKC analysis of tap water after SPE. It is clearly shown that without an on-line concentration step, the test analytes are not detected even after off-line concentration. The blank run electropherogram is shown in Fig. 5C, which indicates that there is no interference emanating from the sample matrix. In the analysis of pond water that contains more components than tap water, the signal-to-noise ratio was poor indicating that matrix components are also being concentrated by the SPE protocol. Moreover, three organic solvents for eluting the herbicides from the SPE packing material, namely acetone, methanol, and ethanol, were evaluated. It was observed that ethanol provided the best results, having recovery values slightly higher than methanol and 2–3 times higher than acetone.

In conclusion, the test phenylurea herbicides are best concentrated on-line using SRMM. The use of a z-shaped detection cell with SRMM improved detection sensitivity more than 500-fold. With normal cell configuration, the use of narrower capillaries is recommended. It is also demonstrated that coupling of SPE to SRMM further improved the concentration sensitivity of the MEKC method.

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