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Pesticide analysis by micellar electrokinetic capillary chromatography

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Abstract

On-capillary sample concentration using sample stacking for the improvement of detection limits for various pesticides separated by micellar electrokinetic capillary chromatography was examined. The dependence of the stacking on different parameters was investigated. An approximately 30-fold preconcentration was achieved by applying sample stacking. Employing a two-step enrichment process (solid-phase extraction combined with sample stacking), detection limits were improved and the sample volume for SPE was reduced. In addition, the total time for the analysis was considerably reduced. Detection limits were between 0.01 and 0.1 ng/ml under these enrichment conditions.

Keywords: Micellar electrokinetic capillary chromatography; Environmental analysis; Sample preparation; Water analysis; Pesticides

1. Introduction

The intensive use of pesticides in recent years to increase agricultural productivity has led to pesticide residues in natural waters at concentration levels which exceed the legal limits. Pesticides with different chemical structures can be found in ground and surface waters, e.g., triazines, carbamates or organophosphorus compounds (OPs). The European Drinking Water Directive demands a limiting value of 0.1 ng/ml for a single pesticide and 0.5 ng/ml for the sum of all pesticides owing to their toxicological hazardous potential.

Micellar electrokinetic capillary chromatography (MEKC) can be employed to separate and determine various pesticides [1]. The disadvantage of this method is the poor detection limit of about 0.1–0.5 μg/ml, which restricts its applicability to environmental problems. A decrease in the detection limit was accomplished by sample preconcentration using solid-phase extraction (SPE). Applying SPE (250-ml water sample), the detection limit was enhanced about 500-fold (0.2–1 ng/ml) [2]. To reach higher enrichment factors, large sample volumes of up to 1000 ml are necessary, causing long extraction times of up to 8 h. Consequently, the extraction time should be minimized to make the method feasible for routine analysis.

This paper considers the possibility of a further decrease in the detection limit whilst avoiding an increase in the sample volume for the SPE procedure. The sample stacking technique [3–7]

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to give an on-capillary concentration effect was investigated for applicability to pesticide analysis. The dependence of pesticide stacking on parameters such as injection technique, voltage and time period under reversed polarity conditions was examined. Quantitative results of a combination of SPE and sample stacking as a two-step enrichment procedure are discussed.

2. Experimental

2.1. Micellar electrokinetic capillary chromatography

For all measurements, a SpectraPhoresis 1000 capillary electrophoresis system (Thermo Separation Products, San Jose, CA, USA) equipped with a multiple-wavelength, fast-scanning UV–Vis detector (190–365 nm UV and 366–780 nm Vis) was used. Untreated fused-silica capillaries (CS Chromatographie Service, Langerwehe, Germany) of 75 μm I.D. and an effective length of 63 cm (total length 70 cm) filled with borate–SDS buffer (pH 8) were employed. Sample injection was performed by vacuum, applied at the cathode end for different times. Variable voltages for the separation experiments (between 20 and 30 kV) and for the stacking experiments (between −10 and −2 kV) were applied. The operating temperature was 25°C. Detection was effected in the UV range and for data acquisition SpectraPhoresis 1.04 software was used.

2.2. Solid-phase extraction

For sample preconcentration by SPE, a vacuum manifold (J.T. Baker, Gross-Gerau, Germany) was used, equipped with SPE cartridges filled with C_{18} (200 mg) as sorbent (Alltech, Unterhaching, Germany). The columns were activated and conditioned before use with 3 ml of methanol and 3 ml of water. The pH of the water samples was adjusted to 6–7 and 2–3 (for enrichment of 2,4D) by addition of HCl and buffer–HCl, respectively. Sample suction was performed at a rate of about 3 ml/min. After loading, the columns were washed with 2 × 1 ml of water and then dried. The pesticides were eluted with 4 × 0.5 ml of methanol within 10 min. The combined eluates were concentrated by vacuum evaporation and then the volume was adjusted to 0.5 ml with water or buffer.

2.3. Materials

All reagents for electrolyte buffers were obtained from Fluka (Neu-Ulm, Germany) and NaOH for rinsing the capillaries, HCl for pH adjustment and methanol as solvent from Merck (Darmstadt, Germany). Purified water from an Elgastat unit (Elga, High Wycombe, UK) was used for buffer preparation. Prior to use, the buffer solutions were filtered through a 0.45-μm membrane filter.

The following pesticides were investigated: prophame and carbofuran (carbamates), parathion ethyl and chlorfenvinphos (OPs), atrazine, simazine and desmetryn (triazines), 2,4D (phenoxycarboxylic acid) and diuron (phenylurea). All standards were purchased form Riedel-de Haën (Seelze, Germany). Stock solutions (100 μg/ml) prepared by dissolution in methanol were used for preparation of spiked water samples.

3. Results and discussion

The above pesticides were separated and determined in a spiked drinking water sample under optimized conditions according to our previous work [1,2]. Fig. 1 shows the electropherogram which was obtained after SPE of a 250-ml sample (extraction time ca. 2 h) and subsequent MEKC.

For these pesticides, the recoveries were between 80 and 90% [relative standard deviation (R.S.D.) ca. 10%]. A detection limit, including the 500-fold enrichment, of ca. 0.2–1 ng/ml (R.S.D. = 10–15%) was achieved. This value is not sufficient to determine the maximum permissible concentration of 0.1 ng/ml). Higher enrichment factors require the use of at least a 500–1000-ml sample volume, which is disadvantageous with regard to the extraction time. The

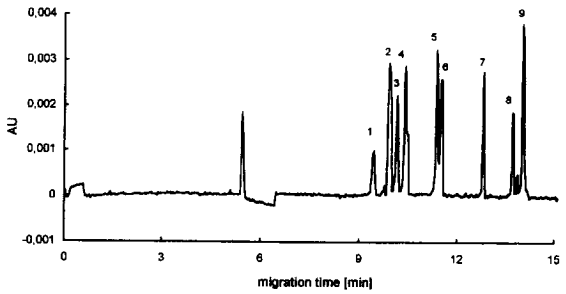

Fig. 1. Separation of pesticides after preconcentration by SPE. Sample: 250 ml of drinking water, spiked with 1 μg of each pesticide (4 ng/ml). 1 = 2,4D; 2 = carbofuran; 3 = simazine; 4 = prophame; 5 = desmetryn; 6 = atrazine; 7 = diuron; 8 = parathione ethyl; 9 = chlorfenvinphos. Conditions for SPE: 500-fold enrichment; procedure as described under Experimental; volume adjusted to 0.5 ml of buffer. Conditions for MEKC: buffer, 5 m*M* borate–30 m*M* sodium dodecylsulphate (SDS) (pH 8); 20 kV; capillary, 63/70 cm × 75 μm I.D.; injection, vacuum 2 s.

applicability of sample stacking might maintain the possibility of using a small sample volume and even to increase the enrichment factor as a result of a further, multiplying preconcentration effect.

3.1. Sample stacking effect

In sample stacking, which is based on the dependence of electrophoretic velocity on electrical field strength, a large sample zone at low conductivity compared with that of the buffer is introduced into the capillary. The phenomena during the stacking process can be divided into four steps (see Fig. 2). In Fig. 2a, the largest possible volume of pesticide sample, dissolved in water–SDS (30 s vacuum applied, ca. 50 nl, zone length ca. 1 cm), was injected. The pesticides were distributed between SDS micelles and the water zone. The negatively charged micelles experienced a rapid movement against the electroosmotic flow (EOF) towards the stationary boundary (sample/buffer) when a high voltage with reversed polarity was applied (Fig. 2b). This was due to the higher electric field strength in the sample compared with that in the buffer zone. The micelles slowed down after passing the boundary (lower field strength) and stacked into a zone shorter than the original. At the same

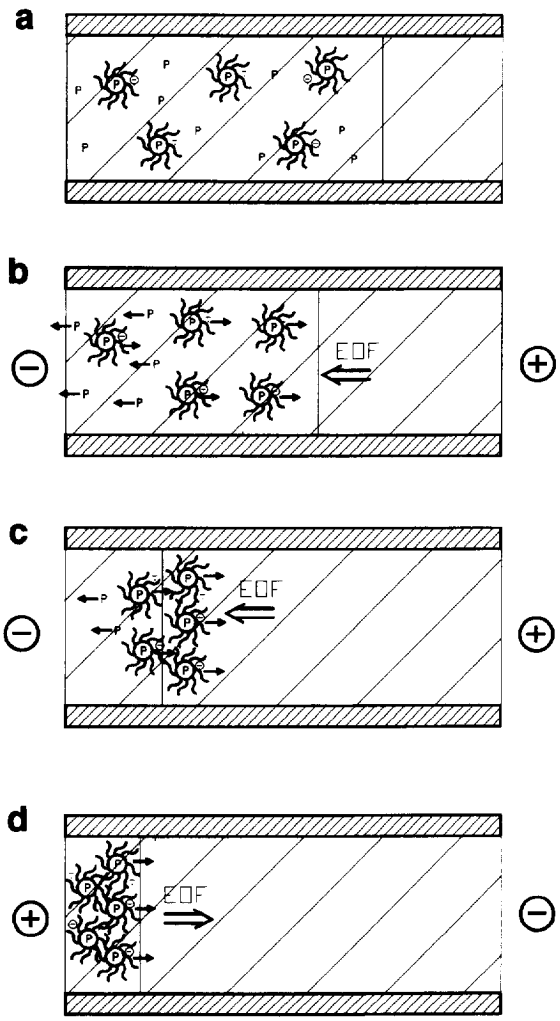

Fig. 2. Sample stacking of pesticides with matrix removal using EOF. P = pesticide. (a) Injection of a large pesticide sample; distribution of pesticides between micelles and water zone. (b) Application of high voltage with reversed polarity; movement to the boundary water (sample)/buffer zone; $v_- > v_{eo}$. (c) Removal of water zone; compression of the sample–matrix; (d) Switching back the polarity; start of the separation; $v_- < v_{eo}$.

time, the water zone was pushed out of the capillary by the EOF (Fig. 2c). When the concentrated pesticide zone had reached the beginning of the capillary, the polarity was switched back and the electrophoretic migration and separation started (Fig. 2d).

Different parameters influence this preconcentration procedure, as follows.

3.2. Influence of injection technique and conditions

An increase in injection times on applying normal injection from buffer resulted in higher peak areas because of the increased injection volume. A further increase was achieved by injection from water (sample stacking) (factor *A*, Table 1). This is due to the difference in the electrical field strength which now occurs and results in the stacking and concentration of the pesticide zone as described in Fig. 2b. There is no removal of the water zone because the polarity is in the normal position and so the EOF is directed to the cathode. This stacking procedure achieved a larger effect at higher injections times.

Both contributions to the peak-area enhancement (increased injection volume and stacking) cannot be utilized owing to the dramatic loss in resolution, *R*, which was calculated with the following equation [8]:

$$R = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k_2}{1 + k_2} \cdot \frac{1 - \frac{t_{e0}}{t_{mc}}}{1 + \left(\frac{t_{e0}}{t_{mc}}\right) k_1} \qquad (1)$$

where N = plate number, α = separation factor, k_1 and k_2 = capacity factors of components 1 and 2 and t_{e0} and t_{mc} = migration times of EOF and micelles. The value of the resolution under optimized conditions with a 2-s injection from buffer was about 1.6 and decreased to about 0.4 under the stacking conditions with a 30-s injection from water (Fig. 3). This is the result of the mismatch between the electroosmotic flow velocities on both sides of the sample/buffer boundary, as reported by others [3]. It created strong zone broadening and worked against the stacking.

To increase the peak area (and thus the determinable amount of pesticide) and even maintain the high resolution, sample stacking under reversed polarity conditions was employed. Hereby subsequent sample–matrix removal by the EOF is evoked, as described in Fig. 2c. This procedure led to a further concentration effect (factor *B*, Table 1) and full maintenance of resolution (Figs. 3 and 4).

The factor *B* for the various pesticides was found to be about fivefold. The above-mentioned peak dispersion by injecting a larger volume was taken into account for the evaluation of the entire preconcentration effect. Hence comparable peak areas are those where the resolution was maintained. Therefore, a comparison has to be made between the conditions at stacking with reversed polarity (30 s) and at optimum separation with normal injection from buffer (2 s). A mean maximum enhancement factor *C* of about 30-fold was found in this way (Table 2).

Table 1
Influence of injection technique and conditions on peak area of prophame

Injection time (s)	Enhancement factor A[a]	Enhancement factor B[b]
5	1	0.9[c]
10	1.3	1.5
20	1.4	3.5
30	1.5	3.9

[a] Factor A: enhancement of peak area by sample stacking relative to peak area with normal injection.
[b] Factor B: enhancement of peak area by sample stacking under reversed polarity relative to peak area with normal injection.
[c] Loss of prophame.

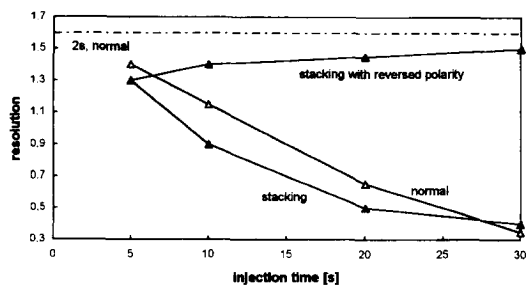

Fig. 3. Influence of injection technique on resolution. Calculated between peaks 2 and 3 in Figs. 1 and 4.

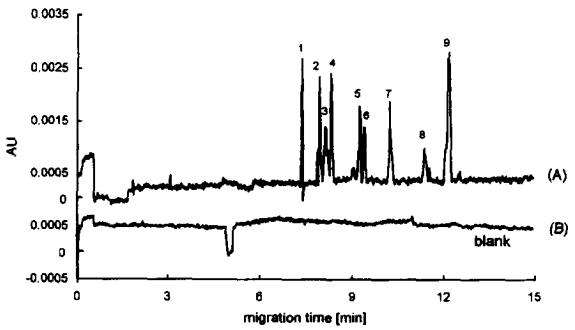

Fig. 4. (A) Separation of pesticides after preconcentration by SPE and sample stacking with matrix removal. Sample, 125 ml of drinking water, spiked with 31.25 ng of each pesticide (0.25 ng/ml); numbers as in Fig. 1. Conditions for SPE: 250-fold enrichment, procedure as described under Experimental; volume adjusted to 0.5 ml of water. Conditions for MEKC: buffer and capillary as in Fig. 1; 25 kV; injection, vacuum 30 s; Injection: sample stacking with matrix removal under reversed polarity conditions (−2 kV for 0.5 min) (ca. 30-fold preconcentration). (B) Electropherogram of blank: drinking water without spiked pesticides after the same SPE and MEKC procedures.

3.3. Influence of various stacking periods and voltages

The investigations demonstrated that the concentration effect depends strongly on the chosen period and voltage during the stacking step with reversed polarity. The right moment for switching back the polarity is very important because a back-flush of the pesticides finally causes a loss of their amount. It is determined by the electrophoretic velocity of SDS micelles, the electroosmotic velocity and the distribution of the pesticides between the micelle and water phases. The highest enhancement of the peak areas were obtained at shorter stacking periods and lower stacking voltages. An optimum value was observed when the voltage polarity at 5 kV for 0.5 min was reversed. The concentration effect was reduced dramatically at −10 kV for 1 min.

Table 2
Maximum enhancement of peak area by stacking with reversed polarity

Pesticide (selected)	Factor C^a
2,4D	25.4
Diuron	29.6
Prophame	31.6
Atrazine	42.9
Parathion ethyl	35.9

[a] Factor C: enhancement of peak area by sample stacking under reversed polarity relative to peak area with normal injection and optimum resolution.

3.4. Influence of velocities and pesticide structure

The peak-area enhancement which is caused by the increased injection volume is similar for all the pesticides. However, as can be seen in Table 2, the concentration factors C differ from each other. This means that the stacking phenomena do not effect all analytes in the same way. It was stated that the triazines and OPs higher values were reached than for carbamates or ureas. This is according to their polarity. The more hydrophobic compounds, interacting more strongly with the micelles, migrate within them against the EOF towards the sample/buffer boundary. In contrast, the more polar compounds can be influenced more strongly by the EOF and can lead to a sample loss faster. The differences in the C factors are in an acceptable range.

3.5. Two-step enrichment process

The demonstrated stacking results were applied to the separation of pesticides which had been enriched by SPE. Thus, a combination of SPE and sample stacking with matrix removal under reversed polarity conditions was carried out as a two-step enrichment process. Fig. 4 shows the electropherogram which was obtained after this procedure and subsequent MEKC separation. Although the original concentration in the sample was 16 times lower than in that in

Fig. 1, the preconcentration by stacking reduces the required sample volume for the SPE (125 ml, extraction time ca. 45 min). Finally, the signals were as those in Fig. 1. The differences in peak areas are due to the discussed differences in enhancement factors. The entire enrichment effects of various pesticides differ from each other when sample stacking is employed as a concentration technique. Furthermore, the different matrix conditions (buffer and water) during injection led to variations in peak shape, especially for peaks 1, 3 and 8.

Under these enrichment conditions, detection limits lower than the maximum permissible concentration of 0.1 ng/ml were determined. For the application of the present MEKC method, it can be concluded that no further enrichment factor is required. The process without any enrichment needs a factor of up to 5000-fold. Table 3 presents the results for some pesticides. The lowest detection limit was found for atrazine.

In contrast to the improvement in detection limit, the precision of the determination deteriorates. Reproducibility of the MEKC analysis with sample stacking is compared with that for analysis without stacking in Table 4. There are slight variations in the R.S.D.s of migration times but substantial variations in the R.S.D.s of concentrations.

4. Conclusion

Applying sample stacking with matrix removal under reversed polarity conditions, an approximately 30-fold preconcentration of various pesticides (carbamates, OPs, triazines, phenylureas, phenoxycarboxylic acids) can be achieved. The on-capillary enrichment effect obtained in this way depends on the electrophoretic and electroosmotic velocities, the distribution of the pesticides between SDS micelles and the sample matrix (i.e. their polarity), the injection conditions, (i.e. injection time, normal or reversed polarity at/after hydrodynamic injection), the voltage during the reversed polarity step and the period of the polarity step.

Performing a combination of SPE and sample stacking (two-step enrichment process) improves the detection limit and requires smaller sample volumes for SPE, so that the procedure takes only half the time. After the combined enrichment procedure and the subsequent MEKC separation of the pesticides, the detection limits were between 0.01 and 0.1 ng/ml.

The process offers the possibility of determining pesticides according to the European Drinking Water Directive within acceptable times. These studies demonstrate that MEKC can be advantageously employed in pesticide analysis in

Table 3
Comparison of detection limits

Pesticide (selected)	MEKC without enrichment (μg/ml)	MEKC with SPE (ng/ml)	MEKC with SPE and sample stacking (ng/ml)
2,4D	0.5	2	0.08
Diuron	0.6	2.4	0.08
Prophame	0.14	0.6	0.02
Atrazine	0.16	0.6	0.014
Parathion ethyl	0.36	1.4	0.04
Achieved Enrichment factor	–	250	~5000–10000

Detection limit calculated as the amount of a reagent blank signal plus three times the standard deviation of the blank signal ($n = 5$).

Table 4
Reproducibility of migration times and concentrations

Pesticide (selected)	MEKC without enrichment		MEKC with SPE		MEKC with SPE and sample stacking	
	R.S.D. of time (%)	R.S.D. of conc. (%)	R.S.D. of time (%)	R.S.D. of conc. (%)	R.S.D. of time (%)	R.S.D. of conc. (%)
2,4D	1.7	2.8	1.9	9.3	1.9	34.1
Diuron	1.7	3.1	1.7	12.5	1.8	25.9
Prophame	1.6	5.1	1.5	16.4	1.6	35.7
Atrazine	1.8	3.5	1.6	12.7	1.7	21.6
Parathion ethyl	2.0	2.1	2.1	8.9	2.4	30.9
Procedure conditions	As in Fig. 1 (without SPE)		As in Fig. 1		As in Fig. 4	
Number of experiments	$n = 7$		$n = 5$		$n = 5$	
Sample concentrations	2 μg/ml		4 ng/ml		0.25 ng/ml	

terms of speed, low cost, low sample volume and simple feasibility.

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