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Journal of Chromatography A, 896 (2000) 373–379

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Optimisation of alachlor solid-phase microextraction from water samples using experimental design

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Abstract

We have tested screening and response surface experimental designs to optimise the solid-phase microextraction (SPME) of the widely used herbicide alachlor. Extraction time and sample volume were the only statistically significant factors from those studied. In the final optimised conditions the procedure was applied to the SPME–HPLC analysis of alachlor in spiked water samples with excellent figures of merit.

Keywords: Factorial design; Experimental design; Water analysis; Alachlor; Pesticides

1. Introduction

Alachlor [2-chloro-2′, 6′-diethyl-*N*-(methoxymethyl)acetanilide] was introduced in 1969 as a selective herbicide for corn, soybeans, peanuts, cotton, annual grasses and many broadleaf species [1], and it is still one of the most widely used herbicides that can be found in groundwater as well as in surface water. Although the persistence of alachlor in the environment is limited (approximately 15 days), its complete mineralisation to CO_2, H_2O and NH_3 is difficult under natural conditions, as it was demonstrated by the very little ring-labeled [^{14}C] alachlor that is converted to $^{14}CO_2$ in soil [2]. To date, no single microbial culture has been identified that can completely mineralise alachlor, demonstrating its extremely high persistence to biodegradation. Thus, bioaugmentation strategies to enhance remediation of chloroacetamide-contaminated soils may be limited [3].

Alachlor is a Restricted Use Pesticide (RUP). In accordance with the US Environmental Protection Agency (EPA) proposed guidelines for Carcinogen Risk Assessment, alachlor was characterised as a probable carcinogen for humans (group B2) [4]. The Commission of the European Union has classified this compound among the high priority pesticides, including those products used in amounts over 50 tonnes per year and with some potential to leach [5]. Therefore, reliable extraction, identification and quantitation methods for this chloroacetamide type compound are required.

Extraction techniques that have already been applied to alachlor include solid-phase extraction (SPE) [6–9], combined SPE–SFE (supercritical fluid extraction) [10], micro-liquid–liquid extraction [11] and pressurized liquid extraction (PLE) [12,13]. Solid-phase microextraction (SPME), first introduced by Pawliszyn and his group [14], integrates into a single step sampling, extraction and concentration,

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0021-9673/00/$ – see front matter
PII: S0021-9673(00)00679-8

being an alternative to the more conventional sample extraction techniques. SPME has been already applied to some chloroacetamides (especially metolachlor) [15,16] coupled to gas chromatography (GC) but not to alachlor. In this study, SPME followed by high-performance liquid chromatography–diode array detection (HPLC–DAD) analysis of alachlor is presented. Unlike in GC, where the injector provides the means for thermal desorption of analytes from the fibre, in HPLC analytes are desorbed from the fibre using the mobile phase (solvent desorption). This required the development of a dedicated interface and implies some disadvantages in comparing the performance with SPME–GC (mainly slower diffusion rates and loss of some preconcentration factor). On the contrary, HPLC attainable detection limits for these type of pesticides compares favourably with GC. Figures of merit that will be shown demonstrate that the combination of SPME–HPLC techniques is useful for the analysis of alachlor in water samples.

The measured and estimated Henry's law constant (H) for this herbicide at ambient temperature is in the range $3.2\cdot10^{-3}$–$1.2\cdot10^{-5}$ Pa m^3/mol [4], so volatilisation of alachlor from water will not be significant. Therefore, no headspace experiments have been attempted and only aqueous extraction by direct fibre immersion has been considered.

The optimisation of the main factors affecting the process was carried out by means of experimental design [17]. The essential difference between the classical one-variable-at-a-time method and the experimental design is that, in the latter case, the values of all the factors are varied in each experiment in a programmed and rational way. Many of the factors studied will probably have no influence, only a few will act upon the response; thus, the influencing factors can be detected while keeping the number of trials to a minimum [18]. In this work, two- and three-level factorial designs have been applied to optimise the SPME process of alachlor from water. Operational variables studied were: extraction time, pH, temperature, volume, stirring-speed and desorption time. The results of the chemometric study as well as the application of the optimal conditions to the extraction and determination of alachlor from water samples are described.

2. Experimental

2.1. Chemicals and reagents

Alachlor was purchased from Riedel-de Häen (Sigma–Aldrich, Madrid, Spain). A stock solution (1238.61 μg/g) was prepared in methanol (gradient HPLC-grade, Scharlau) and diluted when necessary with water (purified with a Milli-Q system; Millipore, Mildford, MA, USA). The buffer (25 m*M* acetic acid–acetate, pH=4) was prepared from 100% glacial acetic acid (Merck) adjusting the pH with a 0.5 *M* sodium hydroxide (BDH) solution. The pH 8 buffer was prepared mixing 96.9 ml of a 0.0666 *M* sodium phosphate dibasic — $Na_2HPO_4\cdot2H_2O$, (Merck) — solution and 3.1 ml of a 0.0666 *M* potassium dihydrogenphosphate — KH_2PO_4 (Merck) — solution.

Standards for calibration were prepared adding the necessary amount from the alachlor stock to a 100-ml calibrated flask, adjusting methanol content (to keep it constant in all the standards) and filling it up with ultrapure water. All concentrations were calculated by mass.

Standards and samples were placed into vials (25- and 100-ml vials were used for 20-ml and 60–100-ml samples, respectively) and pH fixed according the particular experiment in the design to be carried out. A magnetic stirring bar (25 mm long×7 mm in diameter, for large volume samples, and 7 mm long×3 mm in diameter, for small volume ones), was placed into the vial, which was immediately sealed with a cap and a pre-punched septum. Vials were placed inside a heating bath over a magnetic plate (Agimatic-N, Selecta) and let equilibrate. Temperature and agitation degree were adjusted and maintained according the values imposed by the particular experiment in the design. Then, the fibre is immersed into the solution, during the desired time (also imposed by the design). All samples were covered with aluminium foil to avoid alachlor photodegradation.

2.2. Instrumentation and chromatographic conditions

The HPLC system, assembled from modular com-

ponents, consisted of a model 600E pump (Waters); a Symmetry C_{18} column (15 cm×3.9 mm, 4 μm, Waters) and a diode array detector (Hewlett-Packard, HP series 1100). Two injectors in series: a Rheodyne injector (model 7725i) fitted with a 20 μl sample loop for direct injections; and a Supelco SPME–HPLC interface were used to inject the samples. Data acquisition was done by means of a HP Chemstation (Rev. A.06.01 (403)).

All experiments were carried out in isocratic elution mode, using a mobile phase water–MeOH (20:80) at a constant flow-rate of 1 ml/min. The UV detection wavelength was 220 nm.

2.3. Solid-phase microextraction

The SPME fibre assembly and SPME–HPLC interface were purchased from Supelco (Bellefonte, PA, USA). The SPME–HPLC interface consists of a six-port injection valve and a desorption chamber which replaces the injection loop in the HPLC system. The SPME fibre is a 60-μm partially crosslinked polydimethylsiloxane–divinylbenzene (PDMS–DVB) for HPLC use (Supelco). Before the first use, the fibre was conditioned in static mode following the supplier instructions. After sample extraction, the fibre is introduced into desorption chamber under ambient pressure when the injection valve is in the ''load'' position. For static desorption, the fibre was soaked in the desorption solvent, (methanol–water, 1:1). After the desired desorption period (typically 15 min, except when indicated in the text), the valve is switched to the ''inject'' position and the analytes are delivered to the column by the mobile phase flow-rate. One minute later, the valve is returned to the ''load'' position, the fibre is taken out from the interface and inserted into a heated cleaner — adapted from an injection port of a GC —, keeping it at 250°C for 3 min. It was proved that there is no carryover at all by handling the fibre in this way.

2.4. Experimental design

Initially, five factors were selected as potentially affecting the extraction efficiency, namely: extraction time, pH, temperature, volume and stirring-speed. To screen the relative influence of these factors and their possible interactions in the experimental domain, a quarter fraction factorial design (2^{5-2}) was chosen, which will study the effects of the selected five factors in eight runs. The order of the experiments was fully randomised. This will provide protection against the effects of lurking variables. Two center-points were added to ensure enough degrees of freedom for error evaluation. The values corresponding to the upper (+) and lower (−) levels taken by each variable in this design are listed in Table 1.

After this first design was analysed, a second experimental design was planned to evaluate the optimum. Only extraction time (the most significant variable among those listed above), and a new one: the desorption time, were now studied by applying a three-level factorial design (3^2), which will study the effects of these two factors in nine runs taken in a random order giving approximation enough to the optimum location and characteristics. Table 1 shows

Table 1
Factor levels in the designs for Alachlor SPME optimisation

Factor (units)	Low (−)	High (+)
Screening (2^{5-2}) fractional factorial design		
Extraction time (min)	15	45
Volume (ml)	20	100
pH	4.0	8.0
Temperature (°C)	25	45
Agitation (rpm)	100	900
Response surface (3^2) design		
Extraction time (min)	45	120
Desorption time (min)	15	45

Table 2
Design matrix and response values in the screening design (2^{5-2})

Run	Stirring speed (rpm)	pH	Temperature (°C)	Volume (ml)	Time (min)	Peak area (arbitrary units)
1	900	8	45	100	45	158.4
2	100	8	45	20	15	36.2
3	900	8	25	100	15	80.4
4	100	4	25	100	45	154.7
5	500	6	35	60	30	142.2
6	500	6	35	60	30	102.7
7	900	4	45	20	45	133.1
8	100	4	45	100	15	90.8
9	100	8	25	20	45	76.1
10	900	4	25	20	15	47.7

the upper and lower values for both factors in this second design. In all cases, data analysis was performed by means of the statistical package Statgraphics Plus for Windows V. 3.3 [19].

3. Results and discussion

3.1. Optimization of the SPME extraction: factorial designs

SPME conditions were optimised using a constant sample concentration of 600 μg/l in all the first design experiments. Table 2 shows the corresponding experimental design matrix. Response was evaluated in terms of alachlor peak area. The analysis of these results showed that not all the initially selected variables produced significant effects and that no significant interactions between factors were apparent. If interactions are assumed to be negligible the screening quarter fraction design has enough degrees of freedom to give insight into the main factors considered. Fig. 1 shows the main effects plot for SPME of alachlor. The main effects plot shows the estimated variable as a function of each experimental factor. In each plot the factor of interest varies from its lowest level to its highest level, while all the other factors remain constant at their central values.

As can be seen, extraction time and the sample volume were the factors with greatest effect (in fact, the only statistically significant factors). As can be expected, the peak area for alachlor increases when the sample volume and extraction time increase.

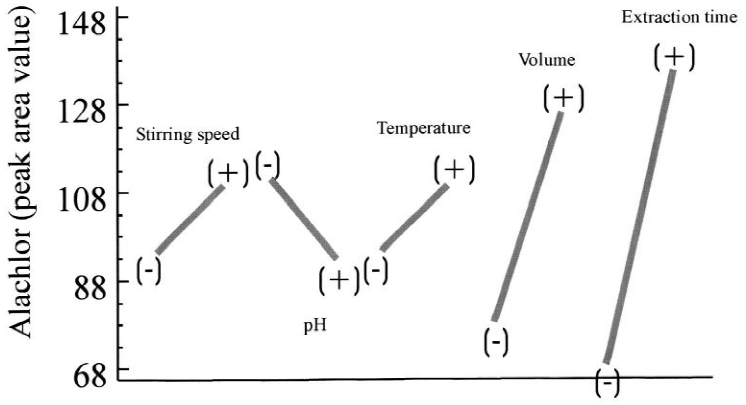

Fig. 1. Main effects influence on the solid-phase microextraction of alachlor. The lines indicate the magnitude and sign (increase or decrease) of the extraction efficiency variation with the factor level (from low to high).

Temperature and stirring speed were not significant so ambient temperature and a medium stirring speed (ca. 500 rpm), were adopted in subsequent experiments. Working at ambient temperature avoids the need of dedicated heating baths, thus simplifying the operational set-up. On the other hand, SPME from aqueous matrices require stirring to facilitate rapid extraction by transporting analytes from the bulk of the solution to the vicinity of the fibre, and to reduce the effect caused by the ''depletion zone'' produced close to the fibre as a result of fluid shielding and slow diffusion coefficients of analytes in liquid matrices [20]. Finally, pH of the solutions was fixed at 4 for further experiments. Although not statistically significant, apparent lower results were obtained when working at higher pH values.

Fig. 2 shows the estimated response surface for alachlor SPME based on the two statistically significant factors. The higher the sample volume and the higher the extraction time, the better the response. Obviously, sample volume reaches the maximum feasible value when using 100-ml vials. However, it can be seen that the highest value of the extraction time tested remains insufficient to reach an optimum extraction value. Since the objective of the SPME experiments was to reach distribution equilibrium in the system, in order to optimise the SPME process, the experimental design need to be shifted to higher values for the extraction time factor.

Given the above results, the optimal extraction time could be found by univariate search. However, it was decided to carry out a second experimental design considering a new variable (desorption time), not taken into consideration previously. Some experiments, carried out to study and avoid fibre carryover, indicated that desorption times used in the first screening design could not be long enough to efficiently remove all the retained alachlor. Also, it was decided to use lower sample concentration: 60 μg/l. Levels of the extraction time were shifted to higher figures and those of desorption time fixed to warrant that a complete desorption could be obtained. Table 1 shows these values. Remainder variables were set as described above. The corresponding experimental matrix and results are depicted in Table 3. The data analysis of this matrix produced the estimated response surface shown in Fig. 3, in which the extraction time only appeared statistically significant, because the lower level for desorption time (15 min) is long enough to produce the complete desorption of the alachlor extracted by the fibre. No significant interactions were detected. In the graph it can be seen that the extraction time line is getting curve at its upper end so the estimated

Table 3
Design matrix and response values in the response surface design (3^2)

Run	Extraction time (min)	Desorption time (min)	Peak area (arbitrary units)
1	120	15	45.8
2	120	30	47.3
3	45	30	7.3
4	45	15	10.6
5	82.5	30	35.8
6	120	45	45.8
7	82.5	15	30.4
8	82.5	45	37.4
9	45	45	21.4

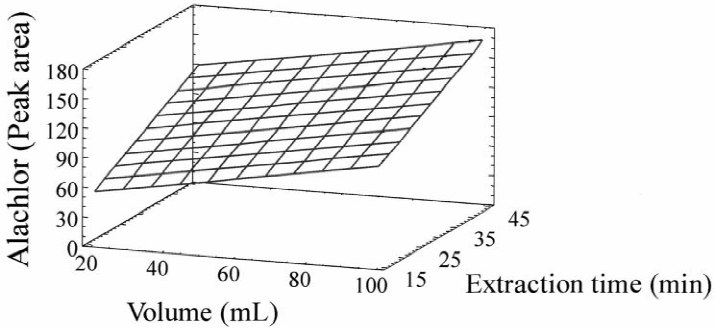

Fig. 2. Estimated response surface for the solid-phase microextraction of alachlor obtained by plotting the two main statistically significant factors.

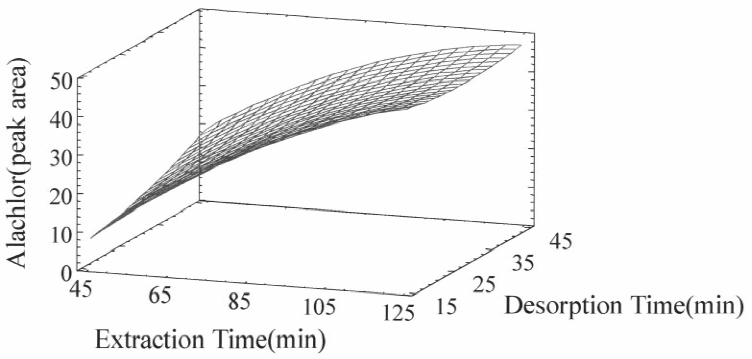

Fig. 3. Estimated response surface from the 3^2 factorial design, obtained by plotting the two studied factors.

response surface exhibit a plateau near the maximum value (120 min), indicating that the optimum extraction time that will warrant reproducible results has been reached.

3.2. Solid-phase microextraction of alachlor from water samples

Finally, the following optimal values were adopted for the extraction of alachlor from water samples: extraction time, 120 min; sample volume, 100 ml; pH, 4; ambient temperature (25°C) and medium stirring speed (500 rpm). A typical chromatogram of the extract (λ=220 nm) is depicted in Fig. 4. A 12-fold concentration factor was obtained when comparing peak area from direct and SPME injections. In all experiments the identification of alachlor was done by means of spectra comparison using a diode array detector–spectral library, created in the laboratory by direct injection of the standard. As can be seen in the graph in Fig. 4, the alachlor standard produced two peaks by itself. Also a peak due to fibre bleeding appears always, even in blank runs. This peak does not overlap with the alachlor peak and it is probably caused by the relatively high

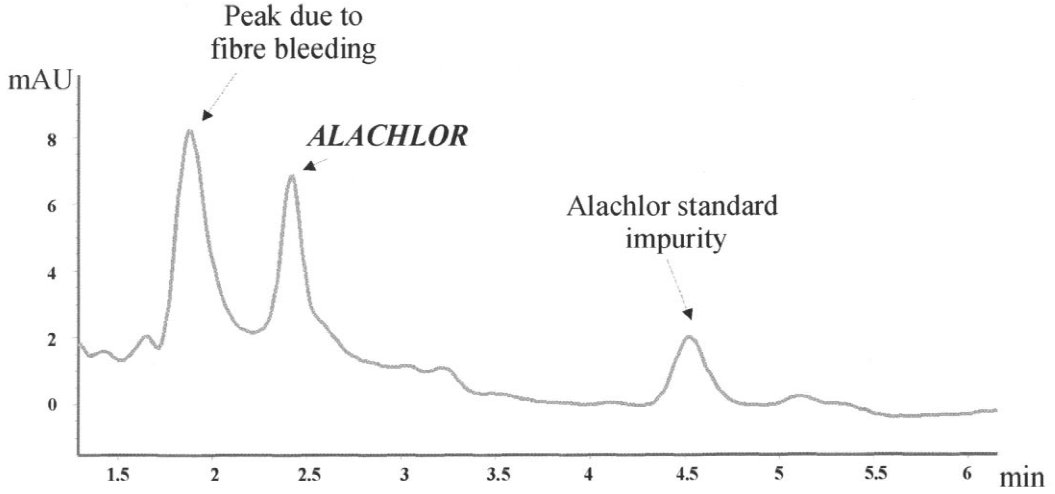

Fig. 4. SPME–HPLC chromatogram of a 60-ng/ml alachlor-spiked water sample obtained under the optimised conditions (wavelength=220 nm).

strength of desorption solvent. It does not affect significantly the fibre lifetime, estimated approximately in 40–50 extraction and desorption processes.

In optimal conditions, a three-point standard calibration (each one by triplicate) in the range 15–60 ng alachlor/g water was performed ($n=9$). Chromatographic peak areas were fitted by linear regression giving a correlation coefficient of 0.99997 for the following calibration curve: $y=0.622088x+1.70257$. To statistically validate the regression analysis, an analysis of variance (ANOVA) was applied, passing both linearity and lack of fit Fisher tests. Peak area reproducibility was evaluated by means of seven extractions series carried out on different dates, giving a relative standard deviation (RSD) of 16% (within the typical SPME–HPLC reproducibility range — from 2 to 21%). Retention time reproducibility was evaluated from the same set of injections to be about 2.5%. The limit of detection (LOD), calculated for a signal to noise (S/N) ratio equal to 3, is 0.27 ng/g. The limit of quantification (LOQ) ($S/N=10$) is 0.91 ng/g, better than the prescribed USA maximum contaminant level (MCL) of 0.002 mg/l [4]. With the same detection scheme but direct injection of standards, LOD and LOQ were 13.8 ng/g and 46.1 ng/g, respectively.

4. Conclusions

The optimal conditions were successfully applied to the extraction of alachlor from water samples, giving excellent detection and quantification limits and, therefore, making the process suitable for the alachlor determination in the framework of the established legal environmental limits.

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