Bull. Environ. Contam. Toxicol. (2005) 75:257–263
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DOI: 10.1007/s00128-005-0746-4

Bulletin of Environmental Contamination and Toxicology

Capillary Gas Chromatography Method for Alachlor in Pesticide Formulations

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Received: 19 November 2004/Accepted: 10 June 2005

Alachlor {2-chloro-N- (2, 6-diethylphenyl)-N- (methoxymethyl) acetamide} is a widely used herbicide for weed control in corn, soybean and many other crops (Chester et al. 1989). Furthermore it has frequently been detected in groundwater, since its leaching losses can be appreciable (Ferrer et al. 1997). Thus, it is frequently involved in groundwater contamination (Fernandez-Ourrusuno et al. 2000). Additionally, its losses by volatilization can also be substantial. These characteristics of alachlor may be considered responsible for the very low recoveries observed immediately after its application in the field (Helling et al. 1988). It has also been reported to be genotoxic (Ribas et al. 1996). For the abovementioned reasons alachlor shows special environmental and analytical interest. Monitoring the quality of formulations containing alachlor as active ingredient is becoming more and more important due to its widespread use in agriculture. The official CIPAC (Collaborative International Pesticides Analytical Council) method (CIPAC Handbook volume D 1988) for monitoring their quality could be considered outdated, since it involves the use of a GC-glass, packed column. Therefore the need for the development and validation of a convenient, non-laborious and accurate method, for the quality control of alachlor formulations is almost imperative.

Validation of analytical methods for pesticide formulations is based on a series of experimental procedures to establish specificity, linearity of response, repeatability and accuracy according to international guidelines (CIPAC guidelines 1999; EU guidelines 1991).

The purpose of the present study was the development and validation of a gas chromatography method with a Flame Ionization Detector (GC-F.I.D.), for the quantitative determination of alachlor in its commercially available EC formulations, widely used in Greece.

MATERIALS AND METHODS

The analytical standard of alachlor (99.8%) and the internal standard of dipentyl-phthalate (99%) were obtained from commercial sources. Five different batches of commercially available EC (Emulsifiable Concentrate) formulation and its

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blank formulation had been provided by the manufacturer together with the respective certificates.

An internal standard solution of dipentyl-phthalate (0.1512mg/ml) was used for preparing standard and sample solutions throughout the whole procedure.

Stock solution of alachlor (0.802mg/ml) was prepared with the dilution of the appropriate amount of the analytical standard with the internal standard solution and kept refrigerated at -18^0C. Alachlor stock solution was diluted to three different concentrations (0.201mg/ml, 0.322mg/ml and 0.403mg/ml), which were kept refrigerated at $< 5^0$C. These comprise the working solutions. The freshly prepared working solutions were used for establishing the chromatographic system's precision through repeatability testing and for defining the linearity of response to the analyte.

From the five different batches of alachlor the concentrated and diluted sample extracts were prepared according to the following procedures. The appropriate quantity of formulations, containing 80mg (±5%) of the active ingredient was added in a 100ml volumetric flask, followed by dilution to the volume with the internal standard solution. These solutions comprise the concentrated sample extracts. The concentrated blank formulations were prepared using the same weight of blank formulations as the weight of the commercial product (batch) used for the preparation of the concentrated sample extracts. The dilution of the blank formulation was made with acetone to the volume of 100ml, followed by evaporation of the solvent to the half of the initial volume. The concentrated sample extracts and the concentrated blank solutions were used for testing the specificity of the method. The five concentrated sample extracts were diluted to concentration in the range of the respective working solutions. These comprise the diluted sample extracts and were used for establishing the precision of the method and for its final evaluation.

The chromatographic system used was a ThermoFinnigan Trace GC equipped with: a split/splitless injector operated in the split mode, a Flame Ionization Detector (FID) and an autosampler (ThermoFinnigan AS 20000). The evaluation of GC runs and the instrument control was made via the chromcard software. Two columns of different polarity were used: a low polar CP-Sil 8Cb, 25m X 0.53mm X 1μm film thickness and a medium polar DB-1701, 25m X 0.53mm X 1μm film thickness. For both CP-Sil 8Cb and DB-1701 columns, helium was used as carrier gas. The operating conditions were: a) For the CP-Sil 8Cb column: pressure 45kPa, split flow 95ml/min, split ratio 13 and injection volume 0.5μl. b) For Db-1701 column pressure 13kPa, split flow 45ml/min, split ratio 10 and injection volume 0.5μl. Both injector and detector temperatures for both columns were set at 250^0C. The temperature program for achieving optimum separation of the compounds for CP-Sil 8Cb and Db-1701 was the following: constant temperature at 80^0C for 1min, then from 80^0C to 220^0C at a rate of 35^0C/min and then at 220^0C constant temperature for 8min.

RESULTS AND DISCUSSION

The validation of a new method for the quantitative determination of alachlor in its EC formulations is presented. Method validation was carried out by determining the parameters required by CIPAC and EU guidelines (CIPAC 1999 and EU 1991 guidelines). According to the abovementioned guidelines specificity, linearity, repeatability and accuracy (trueness/bias and method precision) were established (ISO Guide 5725 1986, 1994; Horwitz 1988).

The ability of the chromatographic system to resolve the analyte to be determined (specificity) from degradation products, metabolites and known additives was investigated (CIPAC guidelines 1999; EU guidelines 1991). For this purpose, concentrated sample extracts as well as concentrated blank solutions were analyzed. It was found that there was no interference, as there was no other peak at the regions of the pesticide and the targeted internal standard. Lack of interference was also demonstrated by the application of the abovementioned analyses to a second column of different polarity (DB-1701). Figure 1 shows chromatograms of instrument blank (acetone), concentrated blank solution, internal standard solution and concentrated sample extract demonstrating the specificity for column CP Sil 8Cb.

The linearity of response to the analyte must be demonstrated at least over the range of nominal analyte concentration ± 20% (CIPAC guidelines 1999; EU guidelines 1991). The linearity of response was determined by analyzing three working solutions with the following concentrations 0.201mg/ml, 0.322mg/ml and 0.403mg/ml. For each column the analytical curve (calibration) was made using three different concentrations with two replicates each (3X2injections per column) (Figure 2). For this purpose the ratio of the peak areas of the active ingredient and the internal standard was plotted against their concentration ratio. After having performed the multi-point calibration, correlation coefficient, slope and intercept with their confidence limits and standard deviation of relative residuals were determined for each column. In the case of pesticide formulations analysis the results are acceptable if the correlation coefficient is $r>0.997$ and the standard deviation of relative residuals is $Y_{rel.}\leq 0.01$. In that case calibration was considered acceptable as the correlation coefficient was 0.9999 for the column Db-1701 and 0.9996 for CP-Sil 8Cb and the standard deviation of relative residuals was 0.0029 for the column Db-1701 and 0.0074 for CP-Sil 8Cb. The linear regression and other calculations were simplified by using ANOVA (Miller and Miller 2000; Mullins E. 2003). Confidence interval of 95% was applied for all statistical evaluations.

The repeatability was tested for each column separately. Five replicate determinations of the working solution (0.322mg/ml) were made for each column. The mean value (average) and the relative standard deviation of the peak areas of the working standard, the internal standard and their ratio for both columns are presented in Table 1. In the case of pesticide formulations analysis the repeatability is acceptable if the relative standard deviation (%RSD) of the peak

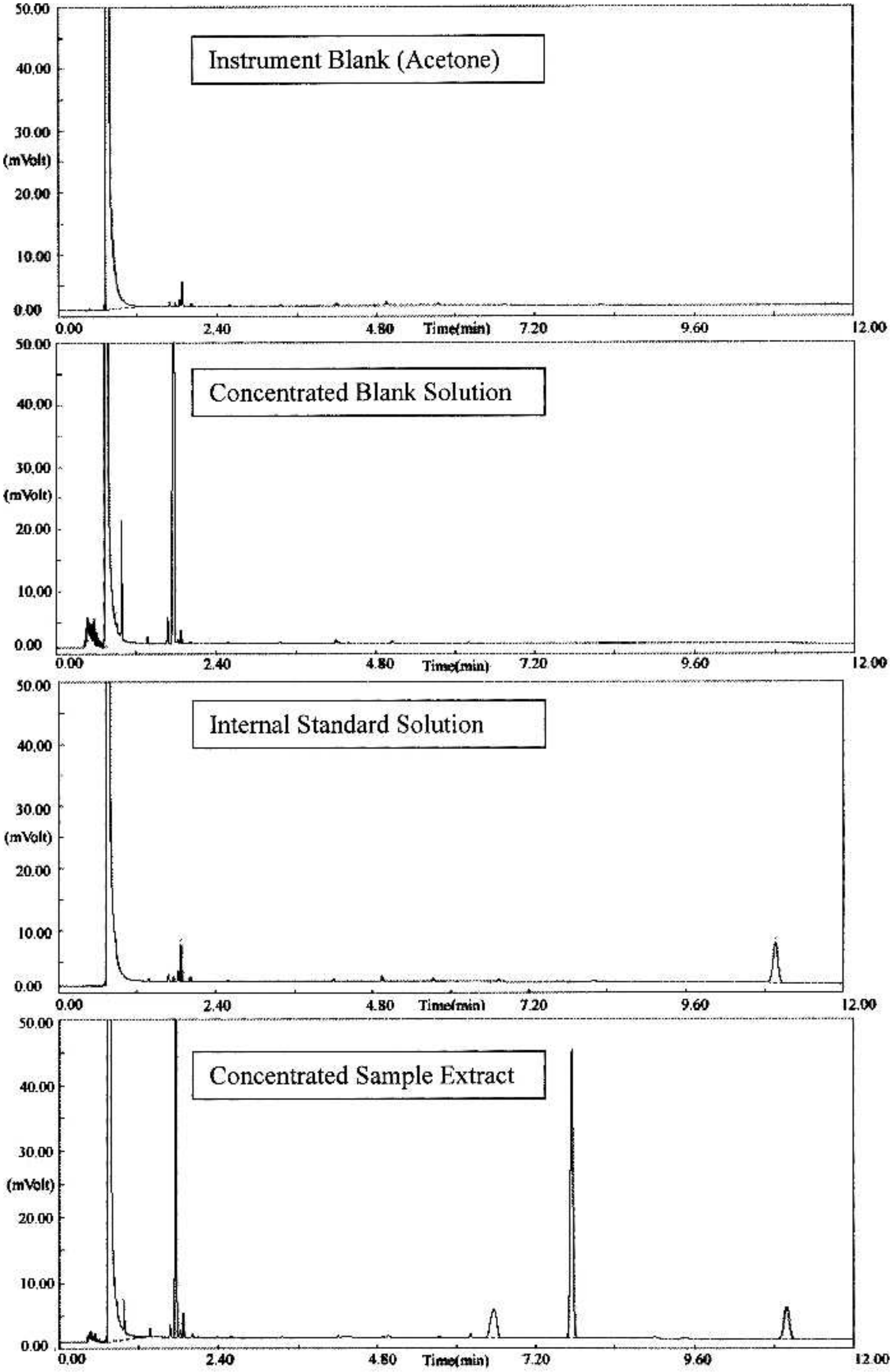

Figure 1. Specificity of the method for CP Sil 8Cb.

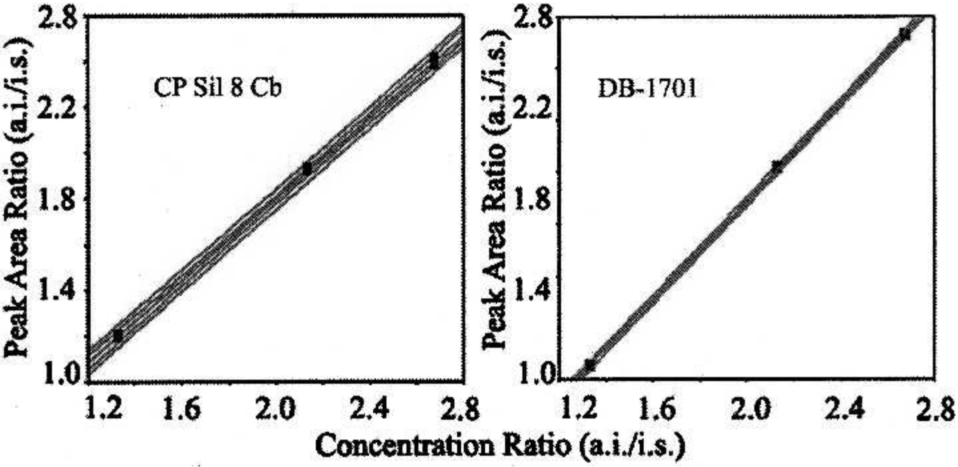

Figure 2. Confidence Interval for Linear Regression of Alachlor for CP-Sil 8Cb and DB-1701.

area ratios is less than 1%, as in that case.

The bias in the present study was calculated by measuring the concentrations (in duplicate) of the diluted sample extracts and comparing them with the values obtained from the respective Certificate of Analysis. The comparison was made using the paired t-test, in which the critical value ($t_{crit.}$) was found from statistical tables to be 2.776. The results are considered acceptable if the measured value ($t_{calc.}$) is less than or equal to $t_{crit.}$. In the present study for each column the results considered acceptable as it was found that $t_{calc.} \leq t_{crit.}$.

Table 1. Repeatability for CP-Sil 8Cb and Db-1701.

	Alachlor peak area (a.i.)	Internal standard (i.s.) peak area	Peak area ratio (a.i./i.s.)
CP-Sil 8Cb			
Mean Value	586957	307627	1.91
%RSD	0.7	0.7	0.2
DB-1701			
Mean Value	468605	233127	2.01
%RSD	0.7	1.1	0.6

Mean Value and Relative Standard (%RSD), of Active Ingredient (a.i.) area, Internal Standard (i.s) area and Ratio of a.i.area/i.s area.

Precision is the degree of agreement between independent analytical results obtained under specific circumstances (EU guidelines 1991). It is a measure of random errors, and may be expressed as repeatability and reproducibility (EU guidelines 1991). Precision is an important characteristic in the evaluation of all quantitative methods. Repeatability and reproducibility are expressed as relative standard deviation (R.S.D.) of a number of samples (CIPAC guidelines 1999; EU guidelines 1991). The expected repeatability and reproducibility values can be obtained from the Horwitz equation (equation 1) and the modified Horwitz equation (equation 2) (CIPAC guidelines 1999; EU guidelines 1991). The results are considered acceptable if they are smaller than the values calculated by the Horwitz equation.

$$RSD_R = 2^{(1-0.5\log C)} \qquad (1)$$

$$RSD_r\,(\%) = RSD_R\,(\%) \times 0.67 \qquad (2)$$

Where C is the concentration of the analyte in the sample expressed as a decimal mass fraction (1mg/kg=10^{-6}), RSD_R is the inter-laboratory relative standard deviation and RSD_r is the repeatability relative standard deviation. Data obtained from the analysis in duplicate of the samples were used to calculate the experimental RSD_r values. The Horwitz equation (Equation 1) and the modified Horwitz equation (Equation 2) were applied for the calculation of the expected values of RSD_R and RSD_r respectively. From the comparison of the experimental RSD_r values and the theoretical RSD_r values it was concluded that the repeatability of the method was acceptable as the measured values were not outside the recommended theoretical values.

For the final evaluation of the method the results obtained from the analysis in duplicate of the diluted sample extracts with the two columns were compared by using the paired t-test. The results obtained with the two columns were not significantly different as it was found that $t_{calc.} \leq t_{crit.}$ ($t_{calc.}$=0.042 and $t_{crit.}$=2.776). We conclude that our method is validated for alachlor.

Acknoweledgements. This work has been supported by the International Atomic Energy Agency (IAEA), Co-ordinated Research Project 'Quality Control of Pesticides' [Contract No 11772/RO]

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