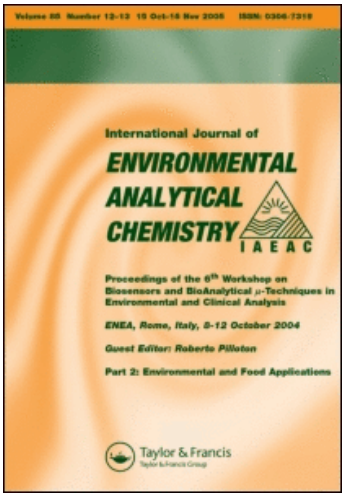

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'Single-laboratory' validation of a method of quantitative analysis of alachlor, chlorpyriphos-methyl, fenthion, and trifluralin

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A simple, new gas-chromatography method was developed and validated by a single-laboratory for quantitative determination of four active ingredients (alachlor, chlorpyriphos-methyl, fenthion, and trifluralin) in their commercially available emulsifiable concentrate formulations, widely used in Greece. This method enables the analysis of a number of pesticides with the same chromatographic conditions and internal standard. A capillary gas-chromatographic system equipped with a flame ionization detector and a split injector was used. The linearity of response, specificity, repeatability and other performance characteristics of the method are presented. As a result, the method is validated.

Keywords: 'Multi-pesticide' method; Gas chromatographic analysis; Fenthion; Alachlor; Chlorpyriphos-methyl; Trifluralin

1. Introduction

Monitoring the quality of pesticide formulations is becoming increasingly important due to the widespread use of pesticides in agriculture. The official methods for monitoring their quality are collaboratively tested methods, published by AOAC (Association of Official Analytical Chemists) and CIPAC (Collaborative International Pesticides Analytical Council) [1]. The use of specific CIPAC or AOAC chromatography methods with different columns, eluents, and internal standards is very expensive, as a diverse stock has to be maintained. Furthermore, when the instrumentation in a laboratory is limited but a large variety of pesticides have to be tested, the output of the laboratory is reduced by the need to frequently change columns, eluents, and consequent equilibration of the system.

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For these reasons, the need for the development of new methods with a higher sample output and lower analysis cost has become imperative. These methods can be considered as 'multi-pesticide' [2] methods, since they can be used for the determination of multiple pesticide formulations. For this study, the active ingredients are alachlor, chlorpyriphos-methyl, fenthion, and trifluralin.

Although a great number of 'multi-residue' methods have been developed [3, 4] for the determination of pesticide residues in various matrices, the concept of 'multi-pesticide' (MP) methods is surprisingly very recent. This explains the very small number of publications that can be found in the literature [2, 5].

A MP method is a method for the quantitative analysis of the active ingredients (a.i.) of several commercial pesticide formulations, using the same chromatographic column and elution system. The test portions are prepared and extracted according to a collaboratively tested CIPAC or AOAC or standard method, if available, in order to ensure comparable results with the standard procedures. Each sample is analysed separately from the sample of another active ingredient. In one sample, usually no more than two known active substances are to be separated from the impurities of the technical material and the components of formulations. This is the main difference from the 'multi-residue' analysis, in which multiple and unknown active ingredients are determined for each sample.

In the case of 'multi-pesticide' methods, instrumental determination is carried out with a notably limited number of properly selected gas-chromatography (GC) or high-performance liquid chromatography (HPLC) column/elution systems compared with the current CIPAC or AOAC methods. The elution temperature or the composition of the eluent is optimized for the pesticide to ensure interference-free separation as well as accurate and precise detection. Therefore, these methods allow the analysis of a large number of pesticides by a limited number of chromatographic columns, elution systems, and internal standards.

In order to apply the concept of MP methods, CIPAC [6] and EU [7] guidelines were followed for the development and validation of the method. Directive 91/414 EEC [7] as well as CIPAC [6] guidelines address the development of analytical methods and set the minimum validation requirements for pesticide analytical methods.

The aim of the present study is the development and validation of a 'multi-pesticide' GC method, GC-flame ionization detection (GC-FID), for the quantitative determination of four active ingredients (alachlor, chlorpyriphos-methyl, fenthion, and trifluralin) in their commercially available emulsifiable concentrate (EC) formulations, widely used in Greece. Although, the above-mentioned pesticides have been on the market for many years, the development and validation of an analytical method are almost imperative, as there is no CIPAC method for chlorpyriphos-methyl, whereas the method for trifluralin is not well specified [8]. In the case of fenthion [9] and alachlor [10], the CIPAC official methods could be considered outdated, since the method for alachlor involves the use of a GC-glass packed column, and the method for fenthion is a spectrophotometric method. This study is part of the 'Quality Control of Pesticides' Research Project co-ordinated and supported by the International Atomic Energy Agency. The aim of this project is to incorporate more pesticides compounds to the method in the future.

2. Experimental

2.1. *Materials and method*

The pesticide analytical standards chlorpyriphos-methyl (99.8%) and trifluralin (99.4%) were obtained as a donation from Dow AgroSciences Ltd, fenthion (96.2%) was from Bayer CropSciences, and alachlor (99.8%) was from Monsanto. Dipentyl-phthalate (99% pure; purchased from Neochema) was used as the internal standard. Each of the analytical standards as well as the internal standard was supplied with a certificate stating their concentration, determined by the supplier. For each active ingredient, five different batches of commercially available EC formulations Reldan 2E (chlorpyriphos-methyl 22% w/v) and Treflan 480EC (trifluralin 48% w/v) obtained as a donation from Dow AgroSciences, Lebaycid 500EC (fenthion 50% w/v) obtained as a donation from Bayer CropSciences, and Lasso EC (Alachlor 48% w/v) obtained as a donation from Monsanto), and their blank formulations had been provided by their manufacturers together with a certificate of analysis for each batch.

Internal standards (IS) were used to compensate for the variability in analytical conditions. An internal standard solution of dipentyl-phthalate (0.1512 mg mL^{-1}) was used for preparing all standard and sample solutions throughout the whole procedure.

Individual stock solutions of alachlor, chlorpyriphos-methyl, fenthion, and trifluralin (0.802 mg mL^{-1}, 0.808 mg mL^{-1}, 0.822 mg mL^{-1}, and 0.810 mg mL^{-1}, respectively) were prepared with the dilution of the appropriate amount of the respective analytical standard with the internal standard solution. The stock solutions were stored at $-18^{\circ}C$ in 100 mL volumetric flasks.

Each stock solution was diluted to three different concentrations (about 0.8, 1, and 1.2 times the nominal a.i. concentration of the commercial product) and kept refrigerated at $< 5^{\circ}C$. These constituted the working solutions.

The freshly prepared working solutions were used to establish the precision of the chromatographic system through repeatability testing and to define the linearity of response for each individual component.

From the five different batches of each formulation product, the concentrated and diluted sample extracts were prepared according to the following procedure.

The appropriate quantity of formulations, containing 80 mg ($\pm 5\%$) of the active ingredient, was added in a 100 mL volumetric flask, followed by dilution to the volume with the internal standard solution. These solutions comprise the concentrated sample extracts.

The concentrated blank solutions were prepared using the same weight of blank formulation as the weight of the respective commercial product (batch) used for the preparation of the concentrated sample extracts. The dilution of the blank formulation was made with acetone to a volume of 100 mL, 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 20 concentrated sample extracts were diluted to the concentration range of the respective working solutions. These comprise the diluted sample extracts and were used for establishing the precision and for the final evaluation of the method.

A single sample preparation procedure was validated for all four formulations due to the lack of collaboratively tested or standard methods (CIPAC or AOAC) for trifluralin, fenthion, and chlorpyriphos-methyl. In the case of trifluralin, the CIPAC method is not well specified [8], whereas in the case of fenthion, the CIPAC method is inappropriate for chromatographic analysis, since it is a spectrophotometric method [9]. For chlorpyriphos-methyl, there is no CIPAC method. CIPAC handbooks describe sample preparation for GC analysis for alachlor [10], but for reasons of conformity, the same preparation procedure as that for the three other a.i. was followed.

2.2. *Instrumentation*

The GC system used was a ThermoFinnigan Trace GC equipped with a split/splitless injector, operated in the split mode, a, an FID and an autosampler (ThermoFinnigan AS 2000). Two different columns were used: a low polar CP-Sil 8Cb, $25\,m \times 0.53\,mm \times 1\,\mu m$ film thickness and a medium polar DB-1701, $15\,m \times 0.53\,mm \times 1\,\mu m$ film thickness. Evaluation of GC runs and the instrument control were achieved using computer software.

The chromatographic conditions for CP-Sil 8Cb were: helium as a carrier gas set at pressure 45 kPa, split flow set at $95\,mL\,min^{-1}$, and split ratio of 13. The chromatographic conditions for DB-1701 were: helium as carrier gas set to a pressure of 13 kPa, split flow set to $45\,mL\,min^{-1}$, and split ratio of 10. Both detector and injector temperatures were set to 250°C. The injection volume was set to $0.5\,\mu L$.

The temperature programme appropriate for a good analysis of the compounds for CP-Sil 8Cb and DB-1701 was as follows: from 80 to 220°C at a rate of rate $35°C\,min^{-1}$. The temperature remained constant for 1 min at 80°C and for 8 min at 220°C.

3. Results and discussion

In the present analysis, the performance parameters of the columns used were checked. Also, for the active ingredients studied and for the chromatographic system used, signals were checked to confirm whether they were interference-free.

3.1. *System suitability test*

The GC performance parameters were checked to verify their suitability for the purpose of the analysis [11, 12]. For evaluating GC column performance characteristics, the column test mixture provided by the manufacturers was analysed. With the injection of $2\,\mu L$ of methane, the retention time of an unretained component was found ($t_0 = 36.8\,s = 0.613\,min$) and was used to calculate the corrected retention times. The following acceptance criteria were applied [2]:

- number of effective theoretical plates/m; acceptance criterion: N_{eff}/m: $1200\,m^{-1}$ (for column 0.53 mm i.d.);
- tailing factor; acceptance criterion: T: 0.7–2.5;
- peak resolution; acceptance criterion: R_s: >1.0;
- peak asymmetry; acceptance criterion: A_s: 0.7–1.7.

The parameters of interest for column DB-1701 are listed in table 1.

Table 1. System suitability test for the column DB-1701: retention time (t_R), adjusted retention time (t'_R), retention factor (k), number of effective theoretical plates per meter (N_{eff}/m), peak width (W_h), tailing factor (T), resolution (R_s), and asymmetry factor (A_s) for the mixture of the test compounds.

Peak no.	Compound	t_R (s)	t'_R (s)	k (s)	N_{eff}/m (plates m^{-1})	W_h (s)	T	R_s	A_s
1	Undecane	79.2	42.4	1.2	520.9	1.1	1.01	–	1.1
2	2,4-Dimethylphenol	181.9	145.1	3.9	1067.3	2.7	1.02	31.7	1.0
3	2,6-Dimethylaniline	205.8	169.0	4.6	1099.1	3.1	1.02	4.9	1.0
4	Tetradecane	257.6	220.8	6.0	981.6	4.3	1.01	8.3	1.0
5	1-Undecanole	296.8	260.0	7.1	1195.5	4.6	1.01	5.2	1.0
6	1-Methylnaphthalene	378.2	341.4	9.3	1147.0	6.1	1.00	9.0	1.0
7	Hexadecane	688.6	651.8	17.7	1060.3	12.2	1.00	20.0	1.0

It was concluded that the performance of the column was satisfactory as the values for the measured parameters did not exceed the acceptance criteria and were in accordance with the values given in column specifications by the manufacturers. Similar results were obtained for the column CP-Sil 8Cb.

3.2. *Method validation for the active substance*

The methods for quantification of the active substance in the technical materials and formulated products need to be robust, accurate, and precise according to Directive 91/414/EEC [7]. For this reason, a preliminary method validation was performed to determine whether the GC system would be acceptable with respect to specificity, repeatability, precision, and linearity [13, 14].

3.2.1. Specificity. The ability of the chromatographic system to resolve the analyte to be determined from degradation products, metabolites, or known additives was investigated [6, 7]. For this purpose, the concentrated sample extracts and the concentrated blank solutions were analysed. It was found that for all active ingredients, there was no interference as there were no other peaks in the region of the pesticide and the targeted internal standard. A lack of interference was also demonstrated with the above-mentioned procedure for a second column (DB-1701) of different polarity.

3.2.2. Repeatability of injections. The repeatability of injections was tested for each active ingredient and for each column separately [6, 7]. Five replicate injections of the medium calibrated level working solution were carried out for each column. The mean value (average) and the relative standard deviation (%RSD) of the peak area of the working standards and the internal standard, for all active ingredients, for column DB-1701 are presented in table 2.

In the case of pesticide formulations analysis, the repeatability of injections is acceptable if the relative standard deviation (%RSD) of the peak ratios is less than 1%, as is the case in the present study. Similar results were obtained using the column CP-Sil 8Cb.

Table 2. Repeatability of injections for DB-1701: mean value and relative standard deviation (%RSD), of active ingredient (a.i.) area, internal standard (IS) area, ratio of the area of a.i./IS, retention time (RT) of AS and IS for five replicate injections for alachlor, chlorpyriphos-methyl, fenthion, and trifluralin.

	a.i. peak area	IS peak area	Ratio a.i./IS ($=Y_i$)	RT a.i. (min)	RT IS (min)
	Alachlor	Dipentyl-phthalate			
Mean	468 605	233 127	2.01	8.10	11.43
%RSD	0.7	1.1	0.6	0.01	0.02
	Chlorpyriphos-methyl	Dipentyl-phthalate			
Mean	206 473	244 725	0.84	7.65	11.41
%RSD	1.3	1.5	0.7	0.02	0.02
	Fenthion	Dipentyl-phthalate			
Mean	363 506	214 621	1.69	9.22	11.44
%RSD	1.3	1.2	0.5	0.05	0.06
	Trifluralin	Dipentyl-phthalate			
Mean	429 247	224 152	1.92	5.84	11.42
%RSD	1.2	1.4	0.9	0.00	0.00

3.2.3. Linearity of response. The linearity of response to each analyte was determined by preparing three working solutions for each active ingredient [6, 7]. Each solution was of a known concentration, as mentioned in section 2. For each column, the working solutions were analysed with duplicate measurements (3×2 injections per column). After having performed the multi-point calibration, the correlation coefficient, slope, and intercept with their respective confidence limits and standard deviation (SD) of relative residuals were determined for each column.

Calibration is considered acceptable if the correlation coefficient is >0.997, and the standard deviation of relative residuals is ≤ 0.01. The linear regression and other calculations were simplified using ANOVA [14–16]. A confidence interval of 95% was applied for all statistical evaluations. The results obtained for columns DB-1701 and CP-Sil 8Cb are presented in tables 3 and 4, respectively.

3.2.4. Trueness – bias. 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 from the respective certificate of analysis. Comparisons were made using the paired *t*-test, where 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 this study, for each column and for each active ingredient, it was found that $t_{calc} \leq t_{crit}$.

3.2.5. Precision of the method. Repeatability and reproducibility are usually specified in terms of RSD [2, 6, 7]. The expected repeatability and reproducibility values can be obtained from the Horwitz equation (equation (1)) and the modified Horwitz equation (equation (2)) [2, 6, 7]:

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

$$\mathrm{RSD_r}(\%) = \mathrm{RSD_R}(\%) \times 0.67, \tag{2}$$

where C is the concentration of the analyte in the sample expressed as a decimal mass fraction, $\mathrm{RSD_R}$ is the inter-laboratory relative standard deviation, and $\mathrm{RSD_r}$ is the repeatability relative standard deviation.

Table 3. Linearity of response: equation of regression line, slope and intercept with confidence limits, correlation coefficient and correlation coefficient square, relative residuals (sY_{rel}) and concentration range for alachlor, chlorpyriphos-methyl, fenthion, and trifluralin (column DB-1701).

Active ingredient	Level of calibration	Equation of regression line	Slope $a \pm SD_a$	Intercept $b \pm SD_b$	R/R^2	sY_{rel}	Concentration range ($mg\,mL^{-1}$)
Alachlor	6	$y = 0.950x - 0.013$	0.950 ± 0.004	-0.013 ± 0.009	0.9999/0.9999	0.0029	0.2014–0.4030
Chlorpyriphos-methyl	6	$y = 0.386x + 0.022$	0.386 ± 0.020	0.022 ± 0.017	0.9998/0.9998	0.0045	0.2474–0.4109
Fenthion	6	$y = 0.720x + 0.017$	0.720 ± 0.011	0.017 ± 0.011	0.9995/0.9990	0.0094	0.2063–0.4106
Trifluralin	6	$y = 0.846x + 0.006$	0.846 ± 0.008	0.006 ± 0.018	0.9998/0.9997	0.0068	0.1966–0.4645

Table 4. Linearity of response: equation of regression line, slope and intercept with confidence limits, correlation coefficient and correlation coefficient square, relative residuals (sY_{rel}) and concentration range for alachlor, chlorpyriphos-methyl, fenthion, and trifluralin (column CP-Sil 8Cb).

Active ingredient	Level of calibration	Equation of regression line	Slope $a \pm SD_a$	Intercept $b \pm SD_b$	R/R^2	sY_{rel}	Concentration range ($mg\,mL^{-1}$)
Alachlor	6	$y = 0.893x + 0.012$	0.893 ± 0.011	0.012 ± 0.024	0.9996/0.9994	0.0074	0.2014–0.4030
Chlorpyriphos-methyl	6	$y = 0.351x + 0.024$	0.351 ± 0.003	0.024 ± 0.007	0.9998/0.9998	0.0040	0.2474–0.4109
Fenthion	6	$y = 0.679x + 0.024$	0.679 ± 0.006	0.024 ± 0.013	0.9998/0.9997	0.0051	0.2063–0.4106
Trifluralin	6	$y = 0.705x + 0.016$	0.705 ± 0.003	0.016 ± 0.008	0.9999/0.9999	0.0042	0.1966–0.4645

Table 5. Comparison of the results of Lasso 48EC (alachlor), Reldan 2E (chlorpyriphos-methyl), Lebaycid 500 EC (fenthion) and treflan 48EC (trifluralin), obtained with two different columns, with the paired *t*-test.

	Difference of the averages			
Sample no.	Alachlor	Chlorpyriphos-methyl	Fenthion	Trifluralin
1	0.107	0.0474	0.064	0.147
2	0.089	0.0481	0.062	0.028
3	0.094	0.0516	0.035	0.412
4	0.084	0.0615	0.056	0.144
5	0.092	0.0441	0.038	0.035
Average	0.093	0.051	0.051	0.128
SD_{dif}	0.009	0.007	0.014	0.182
RSD	0.097	0.137	0.275	1.420
t_{calc}	0.042	0.059	0.118	1.574
t_{crit}	2.776	2.776	2.776	2.776

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 used to calculate the theoretical values of RSD_R and RSD_r, respectively. From the comparison of the experimental RSD_r values and the theoretical RSD_r values, it can be concluded that the repeatability of the method is acceptable, as the measured repeatability was not outside the recommended theoretical values.

3.3. *Comparison of the results obtained with two different columns*

The results obtained from a duplicate analysis of the samples with the two columns were compared with the paired *t*-test. The results obtained with the two columns were not significantly different as it was found that $t_{calc} \leq t_{crit}$ for all the tested pesticides (table 5). Thus, the MP method, including a chromatographic analysis on two columns, is validated for the tested pesticides.

4. Conclusions

A new, simple, quick, and accurate method has been developed and validated for the determination of alachlor, chlorpyriphos-methyl, fenthion, and trifluralin in pesticide formulations. The new method is validated, as it meets the EU and CIPAC guidelines. This method was applied for the quality control of Reldan 2E, Treflan 480EC, Lebaycid 500EC, and Lasso EC, and proved to be simple, convenient, non-laborious and accurate.

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References

[1] FAO/IAEA. Principles of testing the chemical composition of pesticides. Paper presented at the International Workshop on Testing the Quality of Commercial Pesticides, Hungary (2001).
[2] H. Karasali, H. Anagnostopoulos, H. Ekonomopoulou, A. Hourdaki. *Int. J. Environ. Anal. Chem.*, **34**, 55 (2004).
[3] *Quality Control of Pesticides*. Available online at: http://www.iaea.org/trc/pest-research.htm (accessed 15 March 2003).
[4] T.A. Albanis, D.G. Hela. *J. Chromatogr. A*, **707**, 283 (1995).
[5] J.L. Bernal, M.J. Nozal, M.T. Martin, J.J. Jimenez. *J. Chromatogr. A*, **745**, 245 (1996).
[6] CIPAC. *Guidelines on Method Validation to be Performed in Support of Analytical Methods for Agrochemical Formulations. Document No. 3807*, Black Bear Press, Cambridge, UK (1999).
[7] Directive 91/414/EEC. *Methods of Analysis for Technical Material and Preparations* (Annex IIA, point 4.1 and Annex IIIa, point 5.1), European Commission, Brussels (1991).
[8] CIPAC. Handbook, Vol. H, pp. 293–298, Black Bear Press, Cambridge, UK (1998).
[9] CIPAC. Handbook, Vol. 1B, pp. 1830–1836, Black Bear Press, Cambridge, UK (1983).
[10] CIPAC. Handbook, Vol. D, pp. 4–6, Black Bear Press, Cambridge, UK (1988).
[11] E. Soboleva, A. Ambrus, O. Jarju. *J. Chromatogr. A*, **1029**, 161 (2004).
[12] D. Holcombe, B. King, A. Squirell, M. Walsh. *Eurachem Guide. The Fitness for Purpose of Analytical Method: A Laboratory Guide to Method Validation and Related Topics*, LGC, Teddington, UK (1996).
[13] W. Horwitz. *Pure Appl. Chem.*, **60**, 855 (1988); revised in W. Horwitz. *Pure Appl. Chem.*, **67**, 331 (1995).
[14] Analytical Methods Committee. *Analyst*, **113**, 1469 (1988).
[15] J.C. Miller, J.C. Miller. *Statistics for Analytical Chemistry*, 4th Edn, Pearson Education, London (2000).
[16] E. Mullins. *Statistics for the Quality Control Chemistry Laboratory*, 1st Edn, The Royal Society of Chemistry, Cambridge, UK (2003).