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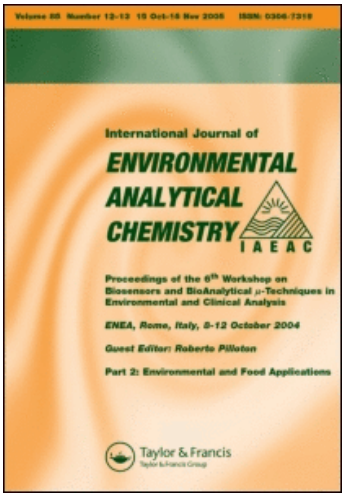

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Quality control data of fenthion and trifluralin determination in pesticide formulations

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QUALITY CONTROL DATA OF FENTHION AND TRIFLURALIN DETERMINATION IN PESTICIDE FORMULATIONS

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A "multi-pesticide" method, which makes possible the analysis of a number of active ingredients in formulated pesticides, is presented. Validated analytical methods and Quality Assurance/Quality Control (QA/QC) procedures were adapted for the determination of the active ingredient content of a number of pesticides. The same chromatographic conditions and columns were used for the analysis of trifluralin and fenthion aiming at the further application of the method to as many pesticide formulations as possible, in order to increase laboratory output and reduce the cost of analysis. The method involved gas chromatographic analysis (GLC) using a Flame Ionization Detector (FID). The results presented are statistically evaluated.

Keywords: Validation; Multi-pesticide; Gas chromatographic analysis; Fenthion; Trifluralin

INTRODUCTION

The analytical potential of modern laboratories has significantly increased in the past decade. As a result, the use of collaboratively tested methods is continuously being reduced and a move towards the development of validated "in-house" methods, provided that the laboratory implements appropriate quality assurance procedures, has become apparent [1].

Applying specific CIPAC or AOAC 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 has to be tested, the output of the laboratory is reduced by the need for frequent changing of columns (eluents) and consequent equilibration of the system.

To overcome these shortcomings, the concept of "multi-pesticide analytical methods" has been developed. In the case of pesticide formulation analysis, this briefly means that the pesticide samples are prepared for instrumental analysis according to one collaboratively tested or standard method. However, the instrumental determinations are carried

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out with just a few properly selected gas chromatographic columns and HPLC columns/elution systems. Naturally, there is no need to separate in one run a large number of active ingredients as in pesticide residue analysis, but only those substances that are present in a single sample. The elution temperature or the composition of the eluent is optimized for the pesticide present (or pesticides in the case of mixtures) to assure interference-free separation and accurate and precise detection. Therefore, these methods allow the analysis of a large number of pesticides, amenable to the technique, and the determination of several active ingredients or different members of classes of compounds, (e.g., phenoxy-alcanoic herbicides, sulfonyl ureas, "conazoles", etc.) by a limited number of determination procedures. In this way reduction of the diversity of stationary phases and internal standards used is achieved [1].

Since the operating temperature of a GC or the eluent composition of an HPLC can be easily and rapidly changed, each pesticide can be analyzed under optimum conditions, and various pesticides can be analyzed, one after the other, with relatively short equilibration times.

Application of multi-pesticide (MP) methods has two major advantages over the application of compound-specific CIPAC or AOAC methods: (1) the sample output of a laboratory with limited instrumentation is much larger; and (2) the operation cost of analysis is smaller (by applying uniform chromatographic methods to a limited number of columns).

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In order to apply the concept of MP methods, certain guidelines must be followed for the design of validated chromatographic analytical methods as well as for validated in-house methods. Method validation may be described as a set of tests used to establish and document the performance characteristics of a method and thereby demonstrate that the method is fit for a particular purpose [2].

In the field of pesticides, there is a continuous need for reliable analytical methods, which should be in compliance with national regulations as well as international requirements [2].

Methods for pesticide analysis generally comprise the following steps:

- Analyte extraction
- Chromatographic separation (for more than one active ingredients in the same sample) and
- Chromatographic measurements.

The aim of the present study is to develop and validate a novel "multi-pesticide" gas chromatographic method (GC-FID) for the determination of active ingredient(s) in pesticide formulations. Specificity, system precision (repeatability), accuracy and linearity were established.

EXPERIMENTAL

Materials and Methods

Analytical standards of trifluralin and fenthion (purchased from commercial sources) were certified to be 99.4 and 96.2% pure, respectively. Diisopentylphthalate was used as internal standard. Each of the reference compounds was supplied with a certificate stating the percentage purity, which had been determined by the supplier.

TABLE I Chromatographic conditions for two different columns

Conditions	*CP-SIL 8CB*	*DB-1701*
Temperature program	–	–
Initial temperature (°C)/time (min)	80/1	80/1
Programing rate (°C/min)	35	35
1st step temp (°C)/time (min)	230/12	230/10
Injector temperature (°C)	250	250
Split ratio	19	17
Detector temperature (°C)	300	300
Flow tate		
Carrier gas (He mL/min)	6.5	71
Hydrogen (mL/min)	22	22
Air (mL/min)	455	455
Injection volume (μl)	1	1

Individual stock solutions of 0.9, 1.2 and 1.5 mg/ml containing 1 mg/ml of internal standard, for both trifluralin and fenthion, were prepared in acetone (pesticide residue grade) and stored at −18°C in 100-ml volumetric flasks. The use of an appropriate internal standard minimizes the variability introduced by the calibration procedure.

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The gas chromatographic system used was a Fisons HRGC Mega 2 Series consisting of a split injector, a Flame Ionization Detector (FID) and an autosampler (FISONS AS800). The results of a new analytical method should be tested by comparing them with those obtained by using a second (perhaps a reference) method. For the purposes of this experiment the reference methods were the corresponding CIPAC validated methods [3, 4]. For that reason the samples were analysed in two different columns. A 25 m × 0.53 mm, i.d., 1 μm film thickness CP-SIL 8CB low polar column and a 15 m × 0.53 mm, i.d., 1 μm film thickness DB-1701 medium-polar column were used. The chromatographic conditions are listed in Table I. The evaluation of GC runs was performed with the use of the appropriate computer software.

Each sample was extracted according to the CIPAC methods [3, 4]. Sufficient sample (w_{mg}), containing about 120 mg of the active substance was weighed (to the nearest 0.1 mg) into a 100-mL volumetric flask. 10 ml of internal standard was added to the flask and then the solution was diluted to 100 ml with acetone [3].

RESULTS AND DISCUSSION

All sample extracts were analysed using the following sequence: Solvent (instrument blank), internal standard, blank formulation, sample extracts, concentrated sample extracts, five replicate injections of the stock solution of 1.2 mg/ml, Low Calibrated Level (LCL), Medium Calibrated Level (MCL), High Calibrated Level (HCL), sample 1 (duplicate injections), sample 2 (duplicate injections), sample 3 (duplicate injections), sample 4 (duplicate injections), sample 5 (duplicate injections). Sample extracts and concentrated sample extracts, as well as blank formulations, were used in order to check the existence of interfering peaks in the vicinity of the pesticide and the targeted internal standard. Blank formulations were extracted using the analytical procedure for the determination of the active ingredient content.

Before starting the analysis of the samples, performance evaluation was carried out with appropriate test compounds in order to ensure the suitability of the chromatographic system. For the evaluation of column performance, of the GC, retention

TABLE II Retention time (t_R), adjusted retention time (t'_R), retention factor (k), plate number/m (N_{eff}/m), peak width (Wh), tailing factor (T), resolution (Rs) and separation number (Trennzahl, TZ) for test compounds

Peak no.	*Compound*	t_R (s)	t'_R (s)	k (s)	N_{eff}/m (plates/m)	*Wh* (50%) (s)	*T*	*Rs*	*As* (10%)	*TZ*
1	2-Chlorophenol	117.0	62.4	1.14	562.3	1.6	1.07	–	1.01	
2	Dodecane	131.4	76.8	1.41	754.5	1.7	1.10	5.2	1.08	
3	2,4 Dimethylaniline	204.0	149.4	2.74	981.1	2.9	0.82	18.6	0.70	
4	Tetradecane	274.8	220.2	4.03	1016.1	4.2	0.87	11.8	0.75	
5	1-Methylnaphthalene	323.4	268.8	4.92	1159.3	4.8	0.95	6.4	0.86	
6	1-Undecanol	388.2	333.6	6.11	1222.9	5.8	0.95	7.2	0.91	
7	Pentadecane	424.8	370.2	6.78	1163.0	6.6	0.97	3.5	0.94	13
8	Methyl-dodecanoate	654.6	600.0	10.99	1385.7	9.8	0.99	16.5	0.96	

factor (k), number of effective theoretical plates (N_{eff}), resolution (Rs), peak assymetry (As), tailing factor (T) and Trennzahl separation number were used (Table II).

By the injection of 2 μL methane, the retention time of an unretained component (t_0) was found ($t_0 = 0.91$ min $= 54.6$ s) and was used for the calculation of corrected retention times. The following acceptance criteria were applied:

- Number of effective theoretical plates/m acceptance criterion: N_{eff}/m: 1200/m (for column 0.53 mm i.d.)
- Tailing factor acceptance criterion: T: 0.7–2.5
- Peak resolution acceptance criterion: Rs: > 1.0
- Peak asymmetry acceptance criterion: As: 0.7–1.7
- Trennzahl separation number acceptance criterion: Tz: 15 (for column 0.53 mm i.d.).

It is concluded (Table II) that the performance of the column is satisfactory because the values of the measured parameters do not exceed the theoretical limits and they do agree with the column specifications given by the manufacturers.

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Methods for Analysis of the Samples

Methods for the quantification of the active substance in the technical material and formulated products are required to be robust, accurate and precise according to Directive 91/414 EEC [5]. Taking into account the above requirements, for the validation of a "multi-pesticide" method of analysis, two representative compounds were selected: trifluralin and fenthion, both of which are widely used in Greece.

Trifluralin was prepared for instrumental analysis according to the collaboratively tested CIPAC method [3] while fenthion was prepared similarly to trifluralin, as there is no suitable CIPAC method [4] concerning fenthion sample preparation for GC analysis.

The laboratories performing analytical measurements have to assure that their results are providing true information on the measured parameters. It normally means that the results are unbiased, accurate and precise and the analyst can rely on the results obtained. In order to meet the above requirements the performance characteristics of the analytical methods used should be known. The values of these characteristics, which are derived experimentally, are used to assess the suitability of the method. The parameters to be tested for characterization of the methods applied for the determination

of active ingredients and impurities in pesticide products are listed below, together with the recommended procedure for their estimation, based on the CIPAC Guidelines [6].

Method Validation for the Active Substance

Method validation data should address the following issues [6]:

- Linearity of response for the analyte in the method
- An estimation of the precision of the procedure
- A demonstration of the accuracy of the procedure
- A demonstration of no interference from excipients
- A definition of the species being determined.

Linearity

Linearity of a test procedure is its ability (within a given range) to obtain test results proportional to the concentration (amount) of analyte in the sample [6]. The linearity of response to the analyte should be demonstrated at least over the range: nominal analyte concentration $\pm 20\%$. At least three concentrations should be measured with duplicate measurements for each [6]. After having performed the multi-point calibration (3×2 injections) (Fig. 1), correlation coefficient, slope and intercept with confidence limits and standard deviation (SD) of relative residuals were determined.

Calibration for both tested active substances is considered acceptable since correlation coefficient is > 0.997 and the standard deviation of relative residuals is ≤ 0.01 [7] for all of the determinations (Table III). The linear regression and other calculations can be simplified by using ANOVA. A confidence interval of 95% was applied for all statistical evaluations.

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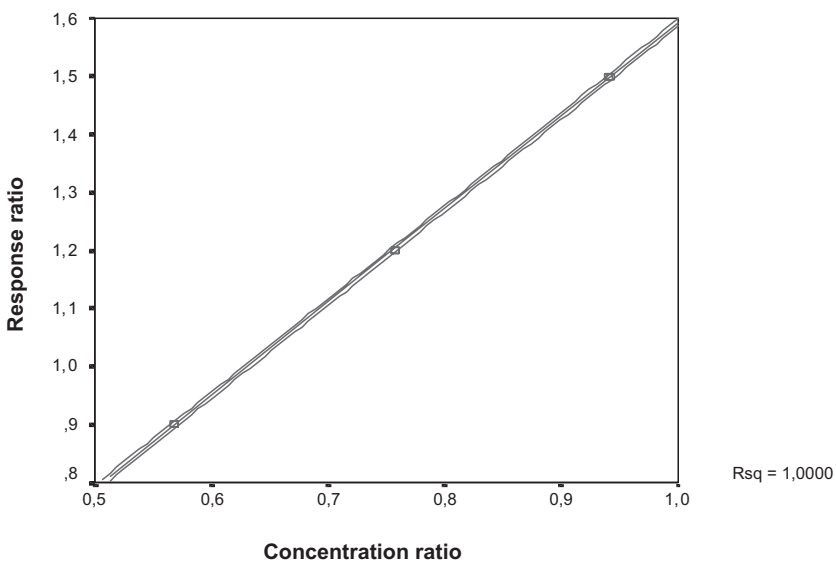

FIGURE 1 Confidence interval for linear regression of fenthion in DB-1701 column.

TABLE III Regression line, slope and intercept with confidence limits for fenthion and trifluralin (CP-Sil 8CB and DB-1701 columns)

Compound	$y = ax + b$	R^2	$a \pm SD_a$	$b \pm SD_b$
		CP-Sil 8CB column		
Fenthion	$y = 0.653 + 6.932 \times 10^{-3}$	0.9999	0.653 ± 0.002	$(6.932 \pm 3) \times 10^{-3}$
Trifluralin	$y = 0.597 + 4.626 \times 10^{-2}$	0.9983	0.597 ± 0.012	$(4.626 \pm 1.5) \times 10^{-2}$
		DB-1701 column		
Fenthion	$y = 0.623 + 8.4 \times 10^{-3}$	1.000	0.623 ± 0.002	$(8.4 \pm 2.0) \times 10^{-3}$
Trifluralin	$y = 0.618 + 3.55 \times 10^{-3}$	0.9994	0.618 ± 0.007	$(3.55 \pm 9) \times 10^{-3}$

TABLE IV Mean value, SD and %RSD for five replicate injections for trifluralin and fenthion in DB-1701 column

	t_R (min)	*Area*	t_R (min)	*Area*	*Ratio*
	Trifluralin		*Internal Standard*		
Mean value	6.95	1612478	11.11	2087619	0.77
SD	0.01	14724	0.02	15049	0.002
%RSD	0.1	0.9	0.2	0.7	0.2
	Fenthion		*Internal Standard*		
Mean value	10.54	1475201	11.09	1936598	0.76
SD	0.01	17187	0.01	20647	0.001
%RSD	0.1	1	0.1	1	0.1

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Precision

Precision is a measure of random errors, and may be expressed as repeatability [6]. This term is defined in ISO 5725-1986E [8]. Repeatability is the closeness of agreement between mutually independent test results obtained with the same method, on the identical test material, in the same laboratory, by the same operator using the same equipment within short intervals of time. A minimum of five replicate sample determinations must be made and the mean value, the percentage relative standard deviation (%RSD) and the number of determinations must be reported. The mean value (average), the standard deviation (SD) and the %RSD for fenthion and trifluralin are presented in Table IV. Chromatograms of five replicate injections for fenthion are shown in Fig. 2.

The acceptability of the $\%RSD_r$ (coefficient of variation, SD) should be based on the modified Horwitz equation [Eq. (2)], an exponential relationship between the among-laboratory relative standard deviation (RSD_R) and concentration (C):

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

For the estimation of repeatability (RSD_r), Eq. (1) is modified to

$$\%RSD_r = \%RSD_R \times 0.67 \tag{2}$$

In our case the calculated $\%RSD_r$ values were correct indicated that the repeatability was acceptable.

Accuracy

The accuracy of the procedure can be determined by the examination of a number of "samples" containing a known quantity of the analyte. These should be laboratory-

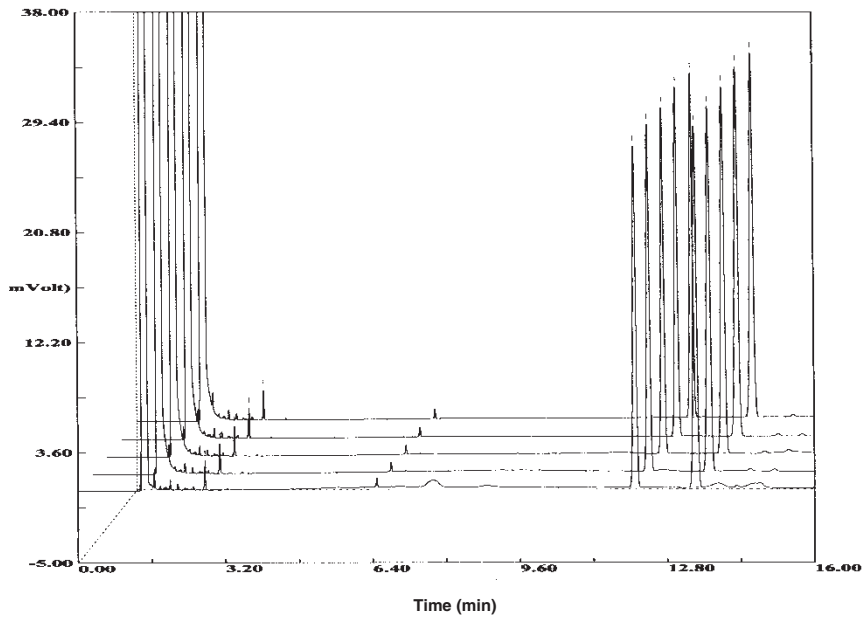

FIGURE 2 Five replicate determinations for fenthion in CP-Sil 8CB column.

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prepared co-formulant mixtures, in which a known quantity of analyte (corresponding to the quantity demanded by the method) is added. The analyte added should be a technical active ingredient of known purity. The whole sample should be analysed to eliminate sampling error. At least four recoveries are required, following the proposed procedure [6]. In our case it was not necessary to determine the accuracy, as the procedure for sample preparation is validated [3, 4].

Specificity

Specificity is the ability of an analytical method to distinguish the analyte to be determined from other substances present in the sample. A blank formulation and a sample to which a known amount of the analyte has been added may be analysed in order to check that there is no interference with the analyte from any expected compounds in the sample, degradation products, metabolites or known additives. In the case of pesticides analysis, a more concentrated extract of the blank may be analysed in order to demonstrate that no signal occurs. The specificity of the method is a definition of the species giving rise to the signal used for quantification [6].

Where specific impurities are known to occur in the technical active ingredient, it must be demonstrated that these do not contribute more than 3% to the total peak area measured for the analyte or internal standard under conditions used for the analysis [6].

For this purpose the standard analysis procedure was carried out on concentrated solutions and blank formulations. It was found that there was no interference as there were no other peaks in the region of the pesticide and the targeted internal standard.

Sample Analysis

Analyses of the samples (five batches of each pesticide product) with the MP method were carried out in duplicate.

It was necessary to check if duplicate injections were within the expected range based on the repeatability test. For that purpose the values of $(C_{max}-C_{min})$ and $(q_{crit} \times RSD_{GC} \times$ average response) were compared, where C_{max} and C_{min} are the maximum and the minimum concentrations respectively of the duplicate injections and q_{crit} is a theoretical value taken from statistical tables. The difference was smaller than the calculated extreme range, in all cases, as was expected from the method validation data. It is clear that the application of the method is under statistical control. It can also be concluded that the results are within the limits assigned from FAO specifications. The calculation of both the active ingredient content and the concentration was based on multi-point calibration.

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TABLE V Comparison of the results of trifluralin 48EC obtained with two different columns with the paired *t*-test

Sample no.	*Measured concentration*							*Difference*
	CP-Sil 8Cb			*DB-1701*				
	Replicate 1	*Replicate 2*	*Average*	*Replicate 1*	*Replicate 2*	*Average*		
1	0.74	0.73	0.735	0.74	0.74	0.74		0.005
2	0.76	0.76	0.760	0.77	0.77	0.77		0.010
3	0.73	0.73	0.730	0.75	0.75	0.75		0.020
4	0.73	0.73	0.730	0.74	0.74	0.74		0.010
5	0.72	0.72	0.720	0.75	0.75	0.75		0.030
Mean	0.74	0.73	0.740	0.75	0.75	0.75	Average	0.015
SD	0.014	0.015	0.015	0.012	0.120	0.01	SD_{dif}	0.010
RSD	0.02	0.02	0.02	0.02	0.02	0.02	RSD	0.667
							$t_{calc}=$	1.491
							$t_{crit}=$	2.776

TABLE VI Comparison of the results of fenthion 50EC obtained with two different columns with the paired *t*-test

Sample no.	*Measured concentration*							*Difference*
	CP-Sil 8Cb			*DB-1701*				
	Replicate 1	*Replicate 2*	*Average*	*Replicate 1*	*Replicate 2*	*Average*		
1	0.77	0.77	0.77	0.740	0.741	0.7405		0.0295
2	0.76	0.76	0.76	0.729	0.729	0.729		0.0310
3	0.77	0.77	0.77	0.739	0.737	0.738		0.0320
4	0.76	0.76	0.76	0.73	0.729	0.7295		0.0305
5	0.77	0.77	0.77	0.733	0.735	0.734		0.0360
Mean	0.77	0.77	0.77	0.73	0.73	0.73	Average	0.032
SD	0.005	0.005	0.005	0.005	0.005	0.01	SD_{dif}	0.003
RSD	0.010	0.007	0.007	0.007	0.007	0.01	RSD	0.08
							$t_{calc}=$	0.177
							$t_{crit}=$	2.776

Validation of the Method for the Tested Pesticides

The results obtained with the two methods both for trifluralin and fenthion were compared with the paired *t*-test.

It can be concluded (Tables V and VI) that the results obtained with the two methods are not significantly different, as t_{calc} is $\leq t_{crit}$ for both active ingredients, where t_{crit} is taken from statistical tables. So, the MP method, including chromatographic analysis on two columns, is validated for the tested pesticides.

Conclusions

The GC-FID generic system was found suitable for routine analysis of various pesticide formulations while method performance characteristics meet the requirements of a quantitative method as specified in the Guidelines [6].

Acknowledgements

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