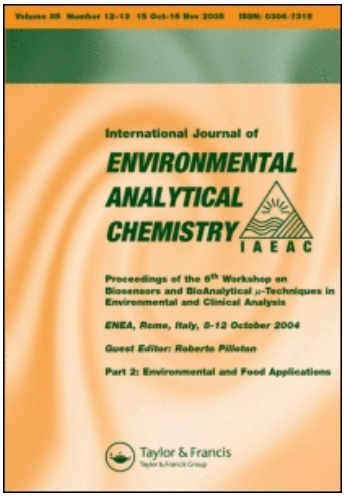

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Validation of a multi-residue method for the determination of pesticide residues in apples by gas chromatography

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Validation of a multi-residue method for the determination of pesticide residues in apples by gas chromatography

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The validation of a multi-residue method for the determination of 16 pesticides in apple fruits is described. The method involves the extraction of pesticides using acetone, dichloromethane, and petroleum ether (40–60°C) and subsequent determination by a gas-chromatographic system with an electron capture detector. Among the tested compounds, there is kresoxim methyl, which belongs to a strobilurin class of fungicides developed from the natural substance, strobilurin. Apple samples were fortified in two levels and pesticide residues were determined. Recoveries, standard deviations, and limits of quantification (LOQ) were calculated. The lowest calculated LOQ value was 0.01 mg kg^{-1} for the analyte λ-cyhalothrin, and the highest LOQ value was 0.15 mg kg^{-1} for the analytes triadimefon and triadimenol, limits that satisfy the MRLs set by EU.

Keywords: Pesticides; Residues; Apples; Food analysis

1. Introduction

Apple fruits are an important cultivation for Greece. There are a high number of pesticides applied in pome trees (mainly apples and pears) in order to protect them from predators and diseases. Apples are relatively inexpensive and are readily obtainable year round in most developed countries from local markets [1]. Currently, about 55 active ingredients (a.i.) are registered in Greece for use in the protection of apple trees [2].

In this work, a multi-residue method is presented which applies a simple treatment of samples before obtaining the final solution for injection. A gas-chromatographic system was used for the quantification and confirmation of six fungicides and 10 insecticides. For each compound, maximum residues levels (MRLs) have been set by the European Union, which vary from 0.1 to 3 mg kg^{-1}. Among the tested fungicides,

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there is kresoxim methyl that belongs to the stobilurins class of fungicide. It has protective, curative, eradicative, and long residual disease control, and acts by inhibiting spore germination [3]. The method was applied to the analysis of apples within a monitoring programme in Greece. The purpose of the study was to validate the method for determining testing compounds in apples with an acceptable recovery and reproducibility and to calculate the limits of quantification (LOQ) for each of them. It will always be necessary for the analyst to validate a method before it is applied in a practical situation. There is a further need for regular checks on the performance of the method in use at both the MRL and the lower limit of quantification [4]. In the laboratory, a method should be validated to provide evidence that it is suitable for the purpose for which it is to be used [5].

2. Experimental

2.1. *Chemicals and solvents*

Acetone, 2,2,4-trimethyl pentane, and toluene were used for the preparation of stock and working standard solutions. Acetone, dichloromethane, and petroleum ether were used in the extraction procedure. All solvents were of pesticide residue analysis grade and were obtained from Lab Scan (Ireland).

Pesticide standards of triadimenol (98%), λ-cyhalothrin (98.2%), deltamethrin (99%), myclobutanil (98.5%), fenarimol (98.5%), and α-cypermethrin (97.5%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany); phosalone (99.5%) and carbaryl (99.8%) from Rhone-Poulenc (Greece); captan (99.1%) and β-cyfluthrin (97.4%) from Alpha Agricultural Supplies SA; triadimefon (99.6%) from Bayer CropScience Hellas (Greece); tau τ-fluvalinate (93.4%) from Rieden de Haen (Greece); kresoxim methyl (99.9%) from Basg Agro Hellas (Greece); fenitrothion (98.65%) and chlorpyrifos methyl (99.8%) from Dow Agrosciences (Greece); and diazinon (99.5%) from Novartis (Greece).

Stock standard solutions of 1000 mg L^{-1} concentration were prepared in acetone for each of the 15 pesticides shown in table 1 and carbaryl, and stored at −20°C. Two standard solutions containing all the compounds were prepared in 2,2,4-trimethyl pentane/toluene (90/10) at proper concentrations as shown in table 2 and stored at −20°C. Working standard mixture solutions for measurement were prepared in extracts of apples, previously analysed twice for the absence of compounds interfering with the analytes. According to the European Commission's Document [5], the potential for matrix effects to occur should be assessed during the method validation. They are notoriously variable in occurrence and intensity, but some techniques are particularly prone to them. If the techniques used are not inherently free from such effects, calibration should be matrix-matched routinely, unless an alternative approach can be shown to provide equivalent or superior accuracy. The concentrations of the working standard mixture solutions were 60, 80, 100, 120, and 140% of the fortification concentrations of table 2. Quantification was performed by an improvement of single point determination and not by using a calibration curve. According to this technique the peak area of the sample solution was bracketed between two concentrations (not differing more than 20%) instead of a single point.

Table 1. Retention times, limits of quantification (LOQ) and maximum residue levels (MRLs) of the 15 pesticides studied.

Analyte	GC-ECD DB-05 ms	GC-ECD DB-17 ms	LOQ ($mg\,kg^{-1}$)	MRLs ($mg\,kg^{-1}$)
Diazinon	12.1		0.03	0.3
Chlorpyriphos methyl	14.0		0.04	0.5
Fenitrothion	15.57		0.04	0.5
Triadimenol	19.64/20.17		0.14	0.2
Kresoxim methyl	23.76		0.02	0.2
Phosalone	35.48		0.15	2
λ-Cyhalothrin	36.9		0.01	0.1
β-Cyfluthrin	41.92/42.15		0.02	0.2
tau τ-Fluvalinate	47.88/48.42		0.05	(0.5)
Deltamethrin	51.71		0.05	0.1
Triadimefon		21.5	0.15	0.2
Captan		30.3	2.2	3
Myclobutanil		32.22	0.1	0.5
Fenarimol		42.15	0.02	0.3
α-Cypermethrin		45.2/45.9	0.07	1

Table 2. Average recovery values and relative standard deviation for the 15 pesticides as derived from fortification experiments at concentrations equal to, and five to 10 times lower than, the MRL.

	1st fortification level ($n=6$)			2nd fortification level ($n=8$)		
Analyte	C ($mg\,kg^{-1}$)	Recovery (%)	RSD (%)	C ($mg\,kg^{-1}$)	Recovery (%)	RSD (%)
Diazinon	0.3	98.6	13.0	0.03	92.1	5.46
Chlorpyriphos methyl	0.5	95.3	6.39	0.05	96.5	7.33
Fenitrothion	0.5	92.9	8.12	0.05	90.4	6.2
Triadimenol	0.2	90.9	10.5	2	93	5.21
Kresoxim methyl	0.2	97.7	4.82	0.02	86	4.13
Phosalone	2	100	1.32	0.2	99.3	5.22
λ-Cyhalothrin	0.1	97.8	5.61	0.01	88.2	14.0
β-Cyfluthrin	0.2	97.8	6.70	0.02	97	5.32
tau τ-Fluvalinate	0.5	98.8	6.81	0.05	96.1	12.9
Deltamethrin	0.1	91.6	11.7	0.05	92.6	5.96
Captan[a]	3	101	1.07	0.3	–[b]	–[b]
Myclobutanil[a]	0.5	92.9	3.92	0.1	100	10.4
Fenarimol[a]	0.3	96.2	6.45	0.03	101	6.81
α-Cypermethrin[a]	1	98.9	6.77	0.1	90.8	2.78
Triadimefon[a]	0.2	98.6	5.27	–[b]	–[b]	–[b]

[a]$n=8$. [b]No data obtained at this level.

2.2. *Gas-chromatographic system*

For gas-chromatographic separation and determination, a Fisons HRGC 8560, series Mega 2 gas chromatograph with a splitless injector and an autosampler was used. For the first 10 analytes shown in table 1 and carbaryl, the analytical column DB-5 ms (30 m, 0.32 mm i.d. and 0.25 μm film thickness) was used, while for the other five analytes of table 1, the DB-17 ms (30 m, 0.3 mm i.d. and 0.25 μm film thickness) column was used, as the DB-5 ms column did not show a satisfactory sensitivity in their determination. Analytes were determined using an electron capture detector (ECD), except carbaryl, for which a nitrogen/phosphorous detector (NPD) was used. Instrument control, data acquisition, and integration of the compounds' peaks were

performed using Chrom-Card software. The temperature programme was from 50°C for 1 min to 180°C at 30°C min^{-1}, to 210°C at 1.8°C min^{-1}, and to 260°C at 30°C min^{-1} for 20 min.

2.3. *Extraction procedure*

An existing method was used for sample processing [6]: 30 mL of acetone was added in an aliquot of 15 g of the sample in a 250 mL PTFE centrifuge bottle (Nalgene, Rochester, NY) and stirred for 1 min in an ultra-turrax homogenizer at 15,000 rpm. Thirty millilitres of dichloromethane and 30 mL of petroleum ether (40–60°C) were added and then the mixture stirred for 1 min. The sample was centrifuged at 4000 rpm for 2 min. Twenty-five millilitres of the supernatant were evaporated to dryness on a water bath at 65–70°C, and then 5 mL of 2,2,4-trimethyl pentane/toluene (90/10) was added. The extract was placed in an ultrasonic bath for 30 s and transferred into a vial with a Teflon stopper, ready for chromatographic analysis.

2.4. *Preparation of fortified samples*

Control apple samples were prepared from fruits collected from untreated trees in the region of Korinthos, Greece. Samples were homogenized and analysed in duplicate and then stored at −20°C until analysis. Aliquots of 15 g of apple samples were fortified in two levels: one, the MRL set for each compound by the EU, and one, five or 10 times lower. The only exception was the analyte τ-fluvalinate, for which an MRL has not been set and therefore the concentration of 0.5 mg kg^{-1} was selected as the high level of the validation procedure. For detection of the 10 analytes in the MRL level, six replicates were used, while for all other cases, eight replicates were used (table 2).

3. Results and discussion

Acetone, dichloromethane and petroleum ether effectively extracted the tested compounds, and the chosen chromatographic programme separated most of them well. The chromatograms of the compounds are shown in figures 1 and 2. Carbaryl was not reproducibly determined in the nitrogen/phosphorous detector, and therefore it was not examined. Captan was successfully detected only at the MRL fortification level, as in the 10 times lower level the detector did not give any response.

Fortified apple samples were analysed to evaluate the effectiveness of the procedure. The method was evaluated by assessing the basic parameters, the accuracy, the precision, and the sensitivity. The accuracy was estimated by calculating the attained recovery, whereas the precision was estimated by assessing the relative standard deviation (RSD) values and the sensitivity by the limits of quantification (LOQ).

Mean recoveries of pesticides added at the MRL level were 91.6–101% (table 2), while the corresponding values at the lower fortification level were 88.2–101%. For a validated method, recoveries of 70–110% were acceptable, while in the case of routine analysis, the acceptable recoveries ranged between 60 and 140% [5]. Therefore, the calculated values indicate a good accuracy. Relative standard deviations at the MRL level were 2.78–14.0%, and at the lower fortification level 1.07–13.0%. These results are satisfactory for residue analysis [7] and indicate a good method precision.

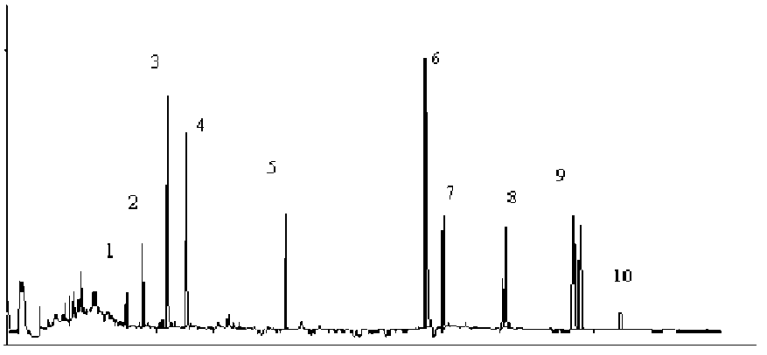

Figure 1. Chromatogram of the 10 compounds studied at the MRL level with the GC-ECD chromatographic system with a DB-5 ms column (1: diazinon; 2: chlorpyriphos methyl; 3: fenitrothion; 4: triadimenol; 5: kresoxim methyl; 6: phosalone; 7: λ-cyhalothrin; 8: β-cyfluthrin; 9: τ-fluvalinate; 10: deltamethrin).

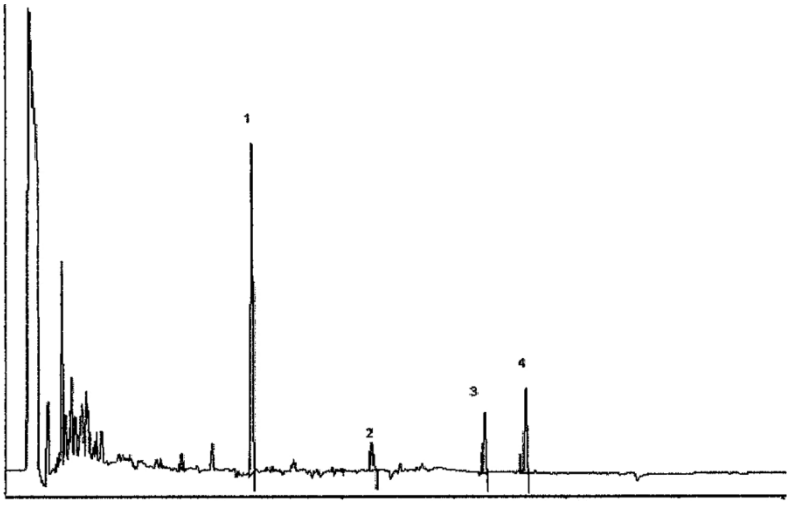

Figure 2. Chromatogram of the three compounds (2: myclobutanil; 3: fenarimol; 4: α-cypermethrin) studied at the low fortification level (five to 10 times lower than MRL) and of triadimefon (1) at the MRL level, with the GC-ECD chromatographic system with a DB-17 ms column.

The limits of quantification (LOQ) were calculated based on the criterion that the signal-to-noise ratio should be more than 10. The attained limits of quantification are shown in table 1 along with the retention times of the tested pesticides and their MRLs. The lowest calculated LOQ value was $0.01\,mg\,kg^{-1}$ for the analyte λ-cyhalothrin and the highest LOQ value was $0.15\,mg\,kg^{-1}$ for the analytes triadimefon and triadimenol, limits that satisfy the MRLs set by EU and give the method satisfactory sensitivity.

4. Conclusions

In this study, 16 of the 55 pesticides registered in Greece for the control of pests and diseases on pome fruits were included. The method is simple, fast, and suitable

for routine analysis. The tested compound carbaryl was not reproducibly determined in the the nitrogen phosphorous detector, possibly because of its decomposition in the chromatographic system to 1-naphthol [8] which cannot be detected with an NPD detecror, and it was not finally examined in the validation of the method. The proposed method of bracketing fits better with pesticide residue analysis, either for validation purposes or for real samples, as it does not suffer from an absence of linearity, especially at very low concentrations, as in the case of the second fortification level. The validation of the method resulted in a good accuracy, precision, and sensitivity, and this makes the method suitable, in terms of sensitivity, for routine analysis.

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