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Multiresidue Determination of Insecticides and Fungicides in Fruits and Vegetables by Gas Chromatography

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Pesticides are applied to a broad variety of crops to reduce losses from weeds, insects and diseases. The widespread use of N-methyl carbamate and organophosphorus pesticides together with the high mammalian toxicity of some of them has created a need for an efficient method that world enable monitoring of these compounds in large numbers of different crop samples. These pesticides are heavily employed over the world for their broad insecticidal spectrum and short persistence in the environment and they are used either in the field or in commercial green houses (Nakamura et al. 1994; Sissons and Telling 1970].

In many cases, hazardous residues are left on fruit and also in different parts of the plants. The determination of these residues in fruits and vegetables is a formidable task, because the actual chemicals used on a crop are seldom known. In addition, residue testing of fruits and vegetables that are sold to the public must be rapid, because state and federal regulatory agencies must know if the pesticide contamination on a crop exceeds its allowable tolerance or if a pesticide is present on a crop where it is not allowed (Pylypiw 1993).

Several multiresidue procedures have been proposed for the dtermination of these pesticides in fruits and vegetables based on the use of G.C. with various selective and sensitive detectors (Dorea et al. 1996; Cai et al. 1995; Miyahara et al. 1994; Krause & August 1983; Sharp & Desmarchelier 1988; Seoud et al. 1995).

In the present study, a multi-residue method for the determination of carbamate and organophosphorus and some organoclorine and fungicides in fruits and vegetables from different places of Greece was established in order to find out the extent and magnitude of these insecticide residues.

The aim was to investigate a method, which provides accurate results for a variety of pesticides that are present in fruit and vegetable samples. The method uses a simple extraction. Pesticides are determined by capillary GC coupled with highly specific detectors for the analysed pesticides.

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MATERIALS AND METHODS

Samples of fruits and vegetables were processed as received in their raw, unwashed, and unpeeled form. The fruit and vegetable samples were cut and blended at high speed for 1-2 min. A 20 g portion of the fruit or vegetable sample was placed into a 250 ml wide neck bottle and 100 ml acetone was added. The sample was shaked for 1 h on a mechanical shaker. The suspension was filtered through a fast flow-rate filter paper in a Buchner porcelain funnel. The bottle and the filter cake were rinsed each with 20 ml acetone. The combined filtrate and washings were transfered with 20 ml sodium chloride solution and 500 ml dist water to a separator-y funnel. The separatory funnel was shaken vigorously three times with 75 ml portions of n-hexane. After separation, the water layer was drained off and discarded. The hexane phases were washed with 100 ml dist. Water and 20 ml sodium chloride solution. This final wash served to break any emulsions formed from the previous water washes.

The final was was discarded and the hexane phase was filtered through a cottonwool pad into a 300-ml round bottonet flask and evaporated in a rotary-evaporator. No clean-up step was required.

The fractions were evaporated to 1.0 ml in a gentle stream of nitrogen for GC injections.

A GC-MSD, QP 5000 Shimadzu equipped with capillary column 007 Quatrex-Methyl 5% phenylsilicone 30 x 0.32 mm x 0.5 vm was used at the following chromatographic conditions: Injector temperature 220 °C, Column programme of temperatures 55 °C (2min), 55-210 °C (5oC/min), 210 °C (20 min), 210-270 °C (20 °C/min), 270 °C (4 min). Helium was used as the carrier gas at 14 psi. The ion source and transfer were kept at 200 °C and 300 °C respectively. The spectra are obtained at 70 eV. Two ions for each pesticide were chosen for screening analysis in selected ion monitoring (SIM). The ions traces were divided into five groups that were recorded sequentially during the injection, on the basis of the retention times of the single substances.

A Shimadzu 14A capillary gas chromatograph equipped with flame thermionic detector (FTD) at 250 °C, was also used. The DB-1 column, 30m x 0.32mm i.d., used contained 5% methylsilicone (J & W Scientific, Folsom, CA). The column was programmed from 55 °C (2 min) to 210 °C (20 min) at 5 °C/min and to 270 °C (4 min) at 20 °C/min. The injection temperature was 220 °C. Helium was used as the carrier at 25cm/s and nitrogen was used as make-up gas at 25ml/min. The detector gases were hydrogen and air, and their flow rates were regulated according to results given through the simplex optimization of the analytical variables, in this instance air and hydrogen flow-rates in the detector. The ion source of FTD was an alkali metallic salt (Rb_2SO_4) bonded to a 0.2 mm spiral of platinum wire.

A Shimadzu 14A capillary gas chromatograph equipped with electron capture detector (ECD) at 250 °C, was also used. The DB-5 column, 30m x 0.32mm i.d., used contained 5% methylsilicone (J & W Scientific, Folsom, CA). The column was programmed from 55 °C (2 min) to 210 °C (20 min) at 5 °C/min and to 270°C (4 min) at 20 °C/min. The injection temperature as 220 °C. Helium was used as the carrier at 25cm/s and nitrogen was used as make-up gas at 25ml/min.

Quantification was performed by external and internal calibration using authentic standards. Sample analyses were run in either duplicate or triplicate and relative standard deviations of less than 15% were generally achieved. Recoveries of spiked pesticides from samples generally varied between 58.3 to 103.4% with relative standard deviations of approximately 4 to 12%. Appropriate corrections were made for recoveries. GC detectors used FTD, ECD and MSD afforded detection limits typically between 1 to 10 ng/g according to compound response for a 20 g sample.

Analysed pesticides and their activity, retention time, mean recoveries and detection limits are shown in table1.

Table 1. Analysed pesticides, activity, retention time and detection limits in GC-FTD system with DB-1 column.

No.	Pesticides	Activity[a]	Retention time	Recovery	Limit of
		t_r (min)	(%)	LOD (ng/g)	detection
1.	Cypermethrin	I	20.17	76.2	10
2.	Methomyl	I	24.35	87.5	5
3.	Pirimiphos ethyl	I	24.63	62.9	10
4.	Endosulfan	I	28.86	103.4	2
5.	Monocrotophos	I	29.36	85.4	2
6.	Terbufos	I	33.40	76.9	5
7.	Chlorothalonil	F	34.20	88.6	1
8.	Metalaxyl	F	37.75	62.8	5
9.	Parathion ethyl	I	40.74	88.5	1
10.	Captan	I	43.53	87.4	2
11.	Chlorpyrifos methyl	I	46.30	58.3	5
12.	Ethion	I	58.35	96.3	5

[a] I = insecticide; F = fungicide

RESULTS AND DISCUSSION

Recovery experiments were carried out by adding aliquots of pure standards in acetone to minced samples. In Table 1, pesticide recoveries in extracts from different crops are presented and show that, apart from a few cases, recoveries are above 80% (Table 1). The method described proved suitable and efficient for the survey in fruits and vegetables of most insecticides and fungicides commercially available in Greece.

Data presented in Table 2 demonstrate the concentration of the residues of pesticides in the examined fruits and vegetables. From the twenty two analysed vegetables, in twelve samples, residues were undetectable (n.d.) and in six samples they were below the detection limit (b.d.l.). From the twenty seven analysed fruits, only in eight samples, residues were estimated and in ten samples the concentrations were under the detection limit. Three different fungicides and four different insecticides were quantified.

Table 2. Summary of pesticides concentrations determined in vegetables and fruits during March and April 1998.

No. Sample	Pesticide determined	Concentration (ng/g)
Vegetables		
1.1. Cabbage (E. Thessaloniki)	Cypermethrin	23
	Methomyl	b.d.l[b]
1.2. Cabbage (W. Thessaloniki)	Methomyl	b.d.l
1.3. Cabbage (Sindos)	n.d.[a]	
2. Carrots (Thessaloniki)	n.d.	
3. Cauliflower	n.d.	
4. Chicory (W. Thessaloniki)	n.d.	
5. Cucumber (Creta)	n.d.	
6.1. Leek (E. Thessaloniki)	Methomyl	16
6.2. Leek (W. Thessaloniki)	n.d.	
6.3. Leek (Sindos)	n.d.	
7. Lemon (Argos)	n.d.	
8.1. Lettuce (W.Thessaloniki)	Chlorpyrifos methyl	b.d.l.
8.2. Lettuce (E. Thessaloniki)	Chlorothalonil	19
8.3. Lettuce (Langadas)	Cypermathrin	b.d.l
9. Pumpkin (E. Thessaloniki)	Methomyl	b.d.l.
10.1. Spinach (W. Thessaloniki)	n.d.	
10.2. Spinach (Agia Paraskevi)	n.d.	
11.1. Tangerine (Igoumenitsa)	n.d.	
11.2. Tangerine (Poros)	n.d.	
12. Tomatoes (Preveza)	Monocrotophos	14
	Cypermethrin	b.d.l

Fruits

13.	Apples		
	13.1. Alexandria	n.d.	
	13.2. Edessa	n.d.	
	13.3. Chrysoupolis	Parathion ethyl	b.d.l.
	13.4. Katerini	Cypermethrin	b.d.l.
		Parathion ethyl	14
		Captan	8
	13.5. Kastoria	Methomyl	18
	13.6. Naoussa	Methomyl	b.d.l.
	13.7. Pellla (Zervi)	n.d.	
	13.8. Ptolemaida	Chlorpyrifos methyl	14
		Ethion	b.d.l.
	13.9. Volos	Cypermethrin	b.d.l.
14.	Gooseberry		
	14.1. Epanomi	n.d.	
	14.2. Katerini	n.d.	
15.	Oranges		
	15.1 Argolida	n.d.	
	15.2. Arta	n.d.	
	15.3. Chania	Chlorpyrifos methyl	43
		Pirimiphos ethyl	b.d.l
	15.4. Ilia (Pyrgos)	Chlorpyrifos methyl	25
		Pirimiphos ethyl	b.d.l
	15.5. Igoumenitsa	Metalaxyl	35
		Chlorpyrifos methyl	b.d.l.
		Pirimiphos ethyl	b.d.l
	15.6. Lakonia	n.d.	
	15.7. Nafplio	cypermethrin	b.d.l.
16.	Pear		
	16.1. Giannitsa	n.d.	
	16.2. Italia	Captan	25

[a] n.d. = non detectable
[b] b.d.l. = below detection limits

The maximum residue value was estimated for oranges (Chlorpyrifos methyl 43 ng/g).

From the obtained data on fruits and vegetables from different areas of Greece, the concentrations of the pesticide residues were lower than permissible levels as they defined by different international organisations.

REFERENCES

Cai C, Liang M, Wen R (1995) Rapid multiresidue screening method for organophosphate pesticides in vegetables, Chromatographia 40: 417-420

Dorea H, Tadeo J, Sanchez-Brunete C (1996) Determination of organophosphorus pesticide residues in fruits by gas-chromatography with ITD and NPD detection. Chromatographia 43: 380-386

FAO/WHO (1992) Pesticide residues in food-evaluations, Part 1. Residues, Paper 118, p. 459, FAO Roma

Fytianos K, Vasilikiotis G, Weil L, Kavlendis E, Laskaridis N (1985) Preliminary study of organochlorine compounds in milk products, human milk and vegetables, Bull Environ Contam Toxicol 34: 504-508

Krause R, August M, (1983) Applicability of a carbamate insecticide multiresidue method for determining additional types of pesticides in fruits and vegetables, J Assoc Anal Chem 66: 234-240

Miyahara M, Okada Y, Takeda H, Saito Y (1994) multiresidue procedures for the determination of pesticides in food using GC/MS techniques, J Agric Food Chem 42: 2795-2802

Nakamura Y, Tonogai Y, Komakura K, To Y (1994) "Multiresidue analysis of 48 pesticides in agricultural products by capillary gas chromatoraphy", J Agric Food Chem 42: 2508-2518

Pylypiw H, (1993) Rapid gas chromatographic method for the multiresidue screening of fruits and vegetables for organochlorine and organophosphate pesticides, J of ADAC International 76: 1369-1373

Seoud M, Din A, Danial L, Ahmed S (1995) Residues and persistence of some organophosphorus insecticides applied to cabbage plants, Food Chemistry 54:137-140

Sharp G, Desmarchelier J (1988) Extraction and chromatographic determination of organophosphate and carbamate Insecticides in grain and grain products, Analyst 113: 1493-1507

Sissons D, Telling G (1970) Rapid procedures for the routine determination of organophosphorus insecticide residues in vegetables, J Chromatog 48: 468-477