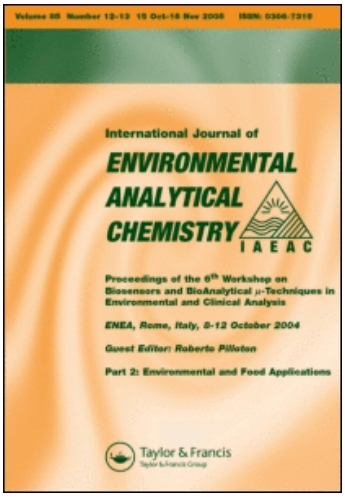

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Analysis of Pesticide Residues in Vegetables by Gas Capillary Chromatography

George E. Miliadis[a]; Panayota T. Malatou[a]
[a] Pesticide Residues Laboratory, Benaki Phytopathological Institute, Kifissia, Greece

To cite this Article Miliadis, George E. and Malatou, Panayota T.(1998) 'Analysis of Pesticide Residues in Vegetables by Gas Capillary Chromatography', International Journal of Environmental Analytical Chemistry, 70: 1, 29 — 36
To link to this Article: DOI: 10.1080/03067319808032602
URL: http://dx.doi.org/10.1080/03067319808032602

Intern. J. Environ. Anal. Chem., Vol. 70(1-4), pp. 29-36
Reprints available directly from the publisher
Photocopying permitted by license only

ANALYSIS OF PESTICIDE RESIDUES IN VEGETABLES BY GAS CAPILLARY CHROMATOGRAPHY

GEORGE E. MILIADIS* and PANAYOTA T. MALATOU

Pesticide Residues Laboratory, Benaki Phytopathological Institute, 8, Delta Str., Kifissia, 145 61 Greece

(Received 6 June, 1997; In final form 1 September, 1997)

A multiresidue analysis of 39 pesticides has been developed, as a rapid screening method for pesticide residues in vegetable samples. Gas chromatography with a) cold on column injection, DB-608 megabore column and nitrogen-phosphorus detection (NPD) and b) splitless injection, SE-52 capillary column and electron capture detection (ECD) was employed for the separation and identification of 15 compounds sensitive to NPD and 24 sensitive to ECD. The extraction methods included blending of small sample quantity with organic solvent, filtration and concentration.

The method's accuracy and precision were assessed in tomato matrix. Twelve target compounds, that are mainly used in the tomato cultivation in Greece, were selected from the 39 pesticides for this purpose, 6 of them sensitive to NPD and 6 sensitive to ECD. The recovery values for the NPD-sensitive compounds were 92.0–108.5% with relative standard deviations 0.6–8.4%, while recoveries for the ECD-sensitive compounds were 82.9–97.8% with relative standard deviations 0.81–14.8%. The estimated limits of detection for all studied compounds were between 0.001 and 0.01 mg/kg.

Keywords: Pesticide residues; gas capillary chromatography; vegetables

INTRODUCTION

Several hundred pesticides of diverse chemical nature are currently used for agricultural and other purposes. Published information show that the amount and number of pesticides released into the environment are continuously increasing[1]. This creates concern for the effect of residues of these chemicals that persist in the environment and may be taken by humans.

* Corresponding author. Fax No.: +30–1–8077506. E-mail: schatz@leon.nrcps.ariadne-t.gr

Monitoring programs for pesticide residues should be conducted routinely on a large scale to evaluate actual food contamination. This poses the demand for methods simple, fast and of high sensitivity, and with results of high reliability.

In this study a multiresidue analytical method has been developed, suitable for the simultaneous determination of 39 pesticides in vegetable samples. The extraction applied is a modified version of the Dutch analytical method [2] while the chromatographic separation and determination step were developed by the use of two chromatographic systems.

The aim of the study was to reduce the requirements for solvents, improving this way the extraction process. The results obtained demonstrate the applicability of the method as a rapid screening one for a great number of pesticide residues in vegetables samples.

EXPERIMENTAL

Reagents

a. Solvents: Ethyl acetate, toluene, propanol-2, acetone, all of "pesticide residue" grade (Lab-Scan Analytical Sciences).
b. Sodium sulphate, anhydrous (Merck).
c. Pesticide reference standards. Stock solutions of the compounds (1000 μg/mL) were prepared by proper dilution of analytical standards, purchased from commercial sources, with acetone. Working solutions (10, 1, 0.1 and 0.01 μg/mL) of the pesticides, as well as solutions of mixtures of them were prepared in acetone from the stock solutions.

Apparatus

A Fisons HRGC Mega 2 Series gas chromatograph was used with the following chromatographic systems:

a. a cold-on-column injector, with a 30m × 0.53mm i.d. DB-608 column, 0.83 μm film thickness and a nitrogen phosphorus detector (NPD) for the determination of the NPD-sensitive pesticides. Operating conditions were: helium carrier gas, set at 1 mL/min and ambient temperature for the injection port, preserved with secondary cooling, and 280°C for the detector.
b. a splitless injector, with a 30m × 0.32mm i.d. SE 52 capillary column, 0.25 μm film thickness and an electron capture detector (ECD) for the determination of the ECD-sensitive pesticides. Operating temperatures were:

210°C for the injector and 310°C for the detector. The temperature program for succeeding separation of the compounds was the same for both chromatographic systems, allowing this way simultaneous injection of the sample in the two injectors and subsequent separation with the following temperature program: from 75°C to 180°C at a rate of 30°C/min, increased to 210°C at 1.8°C/min and then to 260°C at 30°C/min, and remain there for 20 min.

Sample extraction

The general scheme proposed by the Dutch analytical method [2] was followed to establish the optimum conditions for the extraction of the pesticides from the samples. However, the amount of the sample processed, as well as the volume of the solvents used for the extraction, were reduced 5 and 2.5 times for the analysis of the NPD and the ECD-sensitive compounds, respectively, thus resulting in miniaturization of the extraction methods. The extraction of the 15 NPD-sensitive compounds was performed by adding 20mL of ethyl acetate and 10g of anhydrous sodium sulphate to 10g of the homogenized sample. The mixture was blended for 3 min in an omni mixer, at 3000 rpm and was then let to settle. The supernatant liquid was filtered through a Whatman No1 filter paper and 10mL of the filtrate were concentrated to 1 mL in a rotary evaporator, at 30°C. From the concentrated filtrate 1 μl was injected in the gas chromatograph.

The extraction of the 24 ECD-sensitive compounds was performed by mixing 20g of homogenized sample with 40mL toluene and 20 mL propanol-2. The mixture was blended for 3 min in an omni-mixer at 3000 rpm. The supernatant liquid was washed with 2 × 125 mL aqueous solution of 2% Na_2SO_4. The washed extract was then filtered through a Whatman No1 filter paper and 20 mL of the filtrate were concentrated in a rotary evaporator to 2mL from which 1 μl was injected in the gas chromatograph.

RESULTS AND DISCUSSION

The modification of the extraction method consisted of a significant reduction of the sample amount and of the solvent volumes used, restricting this way the cost of the analysis. Under the aspect of quantitative determination, and for separating labile compounds, direct on column injection has been proved to be a superior technique [3]. Cold-on-column injection, that was applied for the NPD-sensitive compounds, avoids losses caused by thermal degradation of components, as well as component discrimination and inaccuracies during the transfer of the sample to the column. This technique archieves lower detection limits and better reproducibility than the more traditional vaporisation injection techniques. On the

other hand, the major advantage of splitless injection, that was applied for the ECD-sensitive compounds, is the ease in handling samples, especially the "dirty" ones, such as pesticide residues and environmental samples. Splitless injection offers better precision and sensitivity compared to split injection for quantitative analysis.

The separation of the 39 compounds achieved by the two chromatographic systems was satisfactory enough, as shown from the relative retention times that are listed in Tables I and II, respectively. However, separation was not sufficient for the following couples of pesticides: opDDD – opDDT, cyhalothrin-ppDDT, captafol-endosulfan(β), fenchlorphos-chlorpyrifos methyl and metalaxyl-pirimiphos methyl, making difficult the simultaneous quantitation of them. For simultaneously quantitating these compounds a better separation was required and this was succeeded by slightly changing the second step of the temperature program of the chromatographic analysis from 210 to 230°C.

TABLE I Relative retention times (RRT), as to parathion ethyl (15.38 min), of the 24 pesticides sensitive to ECD with the SE-52 capillary column. Compounds with more than one RRT appeared in the chromatogram as multiple peaks

Compound	*RRT*
propyzamide	0.68
chlorothalonil	0.73
heptachlor	0.85
deltamethrin	0.93, 2.30
parathion ethyl	1.00
heptachlor epoxide	1.12
captafol	1.15, 1.25, 1.44
procymidone	1.20
α-endosulfan	1.27
DDE-pp	1.37
DDD-op	1.39
DDT-op	1.40
endrin	1.42
β-endosulfan	1.44
l-cyhalothrin	1.46, 1.56, 1.79, 1.82
DDD-pp	1.47
endosulfan sulfate	1.53
DDT-pp	1.55
iprodione	1.61
fenarimol	1.84
permethrin	1.94, 1.97
cyfluthrin	2.08, 2.10, 2.12, 2.13
cypermethrin	2.17, 2.20, 2.22, 2.23
fenvalerate	2.45, 2.53

TABLE II Relative retention times (RRT), as to chlorpyrifos ethyl (19.63 min.), of the 15 pesticides sensitive to NPD for DB-608 megabore column

Compound	*RRT*
methamidophos	0.27
acephate	0.39
propoxur	0.51
diazinon	0.65
fenchlorphos	0.88
chlorpyrifos methyl	0.89
metalaxyl	0.96
pirimiphos methyl	0.96
chlorpyrifos ethyl	1.00
bromophos ethyl	1.11
mecarbam	1.14
binapacryl	1.17
methidathion	1.20
benalaxyl	1.34
triazophos	1.38

The method's accuracy and precision were assessed in tomato matrix with twelve target pesticides, that were selected among the 39 compounds as the most commonly used in the tomato cultivation in Greece. Six were sensitive to NPD: ethoprop, diazinon, pirimiphos methyl, chlorpyrifos ethyl, mecarbam and methidathion, and 6 to ECD: quintozene, chlorothalonil, α-endosulfan, β-endosulfan, tetradifon and fenarimol.

The linearity of the 12 target compounds was tested over the range 0.008–0.4 ng for the NPD sensitive pesticides and 0.002–0.1 ng for the ECD sensitive ones. A linear relationship was found for all analytes sensitive to NPD with correlation coefficients $r \geq 0.999$ except for methidathion with $r = 0.995$, while for the analytes sensitive to ECD the correlation coefficients were found: 0.990 for α-endosulfan, 0.980 for β-endosulfan and chlorothalonil, 0.970 for fenarimol and quintozene and 0.960 for tetradifon.

For statistically validating the method's efficiency a recovery study was performed by spiking, in the laboratory, tomato samples with the 12 target compounds, at various concentration levels. Typical chromatograms of spiked tomato samples with the 6 ECD-sensitive and the 6 NPD-sensitive compounds are shown in Figures 1 and 2, respectively. The results of this study, as well as the repeatability of the method, are presented in Tables III and IV.

TABLE III Mean recoveries (%) ± relative standard deviation (N=3) for the NPD sensitive pesticides in spiked tomato samples, at various fortification levels (μg/g)

	Fortification level			
Compound	C_1*	2.5 C_1	5C_1	10 C_1
ethoprop	96.0 ± 3.2	99.9 ± 2.0	101.6 ± 4.3	98.5 ± 3.7
diazinon	99.4 ± 0.6	103.7 ± 6.1	92.0 ± 3.9	101.7 ± 3.4
pirimiphos methyl	106.4 ± 6.8	108.0 ± 3.4	99.0 ± 2.5	102.8 ± 1.0
chlorpyrifos ethyl	100.3 ± 7.3	106.2 ± 4.2	101.2 ± 6.8	101.1 ± 0.9
mecarbam	106.5 ± 3.0	103.5 ± 4.0	101.4 ± 2.9	104.3 ± 0.8
methidathion	105.6 ± 3.2	102.6 ± 8.4	106.8 ± 2.0	108.5 ± 5.4

C_1* = 0.008 μg/g for ethoprop and diazinon, 0.02 μg/g for chlorpyrifos ethyl and pirimiphos methyl and 0.04 μg/g for methidathion and mecarbam.

TABLE IV Mean recoveries (%) ± relative standard deviation (N=3) for the ECD sensitive pesticides in spiked tomato samples, at various fortification levels (μg/g)

	Fortification level		
Compound	C_2*	2C_2	40C_2
quintozene	90.9 ± 12	95.0 ± 1.4	89.2 ± 0.8
chlorothalonil	93.1 ± 7.9	85.0 ± 15	82.9 ± 6.2
α-endosulfan	88.0 ± 9.4	91.9 ± 6.5	93.1 ± 3.6
f-endosulfan	90.0 ± 4.5	86.0 ± 6.4	93.3 ± 2.9
tetradifon	94.6 ± 10	89.1 ± 9.3	91.8 ± 4.1
fenarimol	97.3 ± 6.7	87.0 ± 5.3	97.8 ± 3.2

C_2* = 0.01 μg/g for quintozene, α-endosulfan, f-endosulfan, and tetradifon, 0.02 μg/g for chlorothalonil, and 0.05 μg/g for fenarimol.

Quantification of the compounds in the fortified samples was carried out with a computer integrator, by comparing the detector response for the sample to that measured for the calibration standard within the linear range. As seen from Tables III and IV, average recoveries were from 92.0 to 108.5% for the NPD sensitive pesticides and from 82.9 to 97.8% for the ECD sensitive ones. The repeatability of the method was assessed by measuring the relative standard deviation (RSD) values from the recovery experiments. These values were from 0.6 to

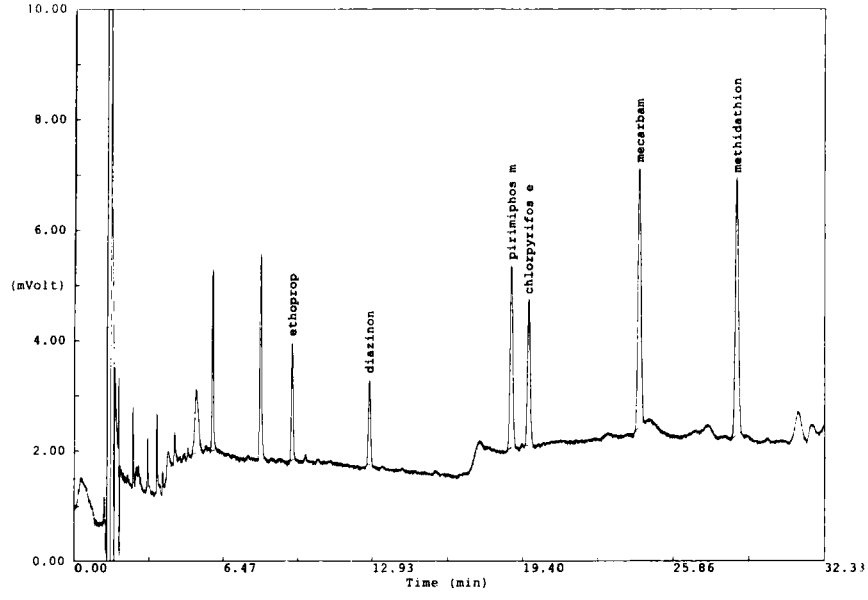

FIGURE 1 Gas chromatogram of 1 µl of spiked tomato sample with the 6 sensitive to NPD pesticides, at concentration 2.5 C_1 (see Table III)

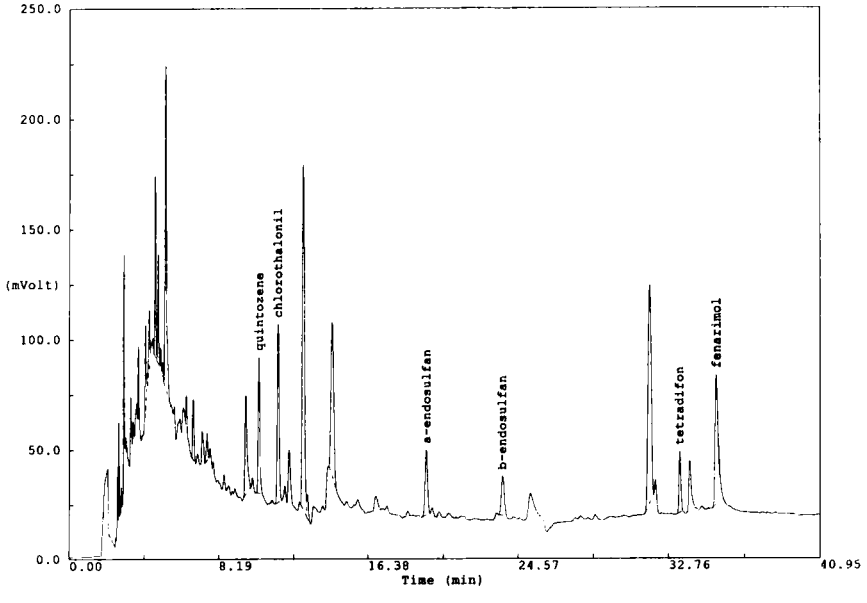

FIGURE 2 Gas chromatogram of 1 µl of spiked tomato sample with the 6 sensitive to ECD pesticides, at concentration C_2 (see Table IV)

8.4% and from 0.8 to 15% for the NPD and the ECD-sensitive pesticides respectively, values within the accepted range for residue determinations [4]. A conservative estimate of the method's detection limit is the product of the worst case standard deviation at the lowest validation level with the Student t-value [5], which is 6.96 at 99% confidence level and for 2 degrees of freedom (3 replicates). The detection limits, evaluated by this approach, were found 0.01 μg/g for all compounds sensitive to ECD, while for the NPD-sensitive ones were: 0.002 μg/g for ethoprop and diazinon, 0.001 μg/g for chlorpyrifos ethyl and pirimiphos methyl, and 0.01 μg/g for methidathion and mecarbam.

In conclusion the described method allows the fast, convenient and with low cost determination of an extended number of pesticides in agricultural products. The method was found accurate, precise and sensitive and can therefore be routinely applied.

Acknowledgements

The authors thank K. Bourou and G. Giannopoliti for processing samples and E. Pantazi for typing the manuscript.

References

[1] J.W. Readman, T.A. Albanis, D. Barcelo, S. Galassi, J. Tronzynski and G.P. Gabrielidis. *Mar. Pollut. Bull.* **26,** 613–619 (1993).

[2] Ministry of Welfare, Health and Cultural Affairs, Rijswijk, Netherlands in: *Analytical methods for residue of pesticides in foodstuffs.* (P.A. Greve, ed. Bilthoven, 1988) part I, pp 1–53.

[3] H.J. Stan and H. Goebel. *J. Chromatogr.*, **314,** 413–420 (1984).

[4] P.A. Greve, in: *Pesticide residue analysis* (A. Ambrus and R. Greenhalgh, eds, WHO/FAO, Rome, 1984) pp. 281.

[5] U.S.E.P.A. *Fed. Regist.*, **49,**198 (1984).