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Short communication

Simplified multiresidue method for the determination of organophosphorus insecticides in olive oil

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Abstract

A simple and rapid method for the determination of 13 organophosphorus insecticides and their metabolites in olive oil by GC is described. The pesticide was extracted from oil with acetonitrile and no cleanup was needed. GC–nitrogen–phosphorus detection response factors of pesticides were affected by solvents and coextractive substances. Pesticides in hexane showed on average higher response factors. Standards were prepared in the residue-free oil extract solubilized in hexane to handle effects of matrix and solvent. The low amount of coextractive substances does not decrease the column efficiency, even after a few hundred analyses. Recovery at three fortification levels (ca. 0.1, 1.0 and 3.0 mg/kg) ranged from 74 to 118%, with coefficients of variation ranging from 1 to 16.

Keywords: Olive oil; Environmental analysis; Food analysis; Organophorphorus compounds; Pesticides

1. Introduction

The olive fruit fly (*Dacus oleae*) is the key insect pest of olives in the Mediterranean area. To control this pest many insecticides are used mainly belonging to the organophosphorus pesticides class. Many gas chromatographic methods for fatty matrices are generally used in the determination of their residues in olive oil. A recent review by Lentza-Rizos and Avramides summarises the available literature on those methods through 1993 [1]. Most are based on partitioning between hexane or light petroleum and acetonitrile, followed by cleanup and GC determination. The simplest and most rapid technique was that used by Morchio et al. who injected oil samples, previously diluted 1:1 with acetone, directly into a gas chromatograph [2]. This method is good if a few samples are to be analyzed; its limitation is due to a decrease in column resolution efficiency after a few analyses with the gas capillary technique, leading to washing of the column and injector at the end of each working day. The aim of this work was to develop a simple and rapid multiresidue method which allowed the determination of main organo-

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phosphorus insecticides and their metabolites, commonly used in olive-growing.

2. Experimental

2.1. Chemicals and materials

Pesticide analytical standards were purchased from Ehrenstorfer (Augsburg, Germany). Triphenylphosphate (99%) was used as the internal standard (I.S.) and was of analytical grade (Janssen, Geel, Belgium). Acetone, acetonitrile, chloroform, hexane and methanol were HPLC grade; anhydrous sodium sulfate was analytical grade (Carlo Erba, Milan, Italy). Stock standard solutions of the pesticide (ca. 500–1000 mg/kg each) were prepared in methanol. An intermediate solution containing all pesticides at ca. 50 mg/kg was prepared by dilution in methanol. Working standard solutions of insecticides were obtained by intermediate solution which, after evaporation of methanol, were taken up with the three solvents containing the I.S. at 0.3 mg/kg. Oil matrix standard solutions in hexane were prepared by adding working standard solutions to untreated olive oil extract and evaporating to dryness under a nitrogen stream.

2.2. Apparatus and chromatography

An HRGC Mega 5160 gas chromatograph (Carlo Erba) was employed. It was fitted with an NPD-40 nitrogen–phosphorus detector, an AS 550 autosampler (Carlo Erba) and a split-splitless injector, connected to an HP 3396-II reporting integrator (Hewlett-Packard, Avondale, PA, USA). A Durabond fused-silica column (30 m×0.25 mm I.D.) (J&W Scientific, Folsom, CA, USA) was employed, with DB 1701 (14% cyanopropylphenyl–methylpolysiloxane) liquid-phase (film thickness 0.25 μm). The injector and detector were operated at 250 and 280°C, respectively. The sample (2 μl) was injected in the splitless mode (60 s), and the oven temperature was programmed as follows: 90°C for 1 min, raised to 180°C (15°C/min), to 250°C (5°C/min), to 280°C (10°C/min), and held for 13 min.

2.3. Extraction procedure

Two grams of olive oil were weighed in a 30-ml screw-capped tube; 2 ml of hexane and after agitation another 10 ml of acetonitrile were added. The tube was agitated in a rotatory shaker for 30 min. The acetonitrile layer was allowed to separate, then 7.5 ml were poured into a 10-ml beaker and let to evaporate to dryness under a nitrogen stream. The residue was taken up with 1.5 ml of organic solvent containing I.S. and injected for GC analysis.

2.4. Recovery assays

Untreated oil samples were fortified on average at ca. 0.1, 1.0 and 3.0 mg/l by adding intermediate pesticide solutions in hexane. Samples were allowed to equilibrate for 30 min prior to extraction, and were processed according to the above procedure. The recovery assays were replicated four times.

3. Results and discussion

In this work 13 organophosphorus insecticides were selected among those commonly used for controlling the olive fruit fly (*Dacus oleae*) in Italy [3]. Two of these insecticides, methamidophos and omethoate, are metabolites of acephate and dimethoate, respectively.

3.1. GC determination

At first we evaluated the effect of solvents on the response factors of the pesticides, a problem generally neglected in pesticide analysis. The same aliquot of intermediate pesticide solution was diluted in three solvents (hexane, chloroform and acetone) containing internal standard at 0.3 mg/kg, to obtain three working solutions at the same concentration (ca. 1.1 ppm). The solutions were injected in the gas chromatograph. Table 1 shows relative response (RR) factors to chloroform which on average yielded lower signals. Pesticides in hexane on average showed higher response factors than other solvents with RR values ranging between 1.61 and 0.85; methidathion and omethoate showed higher RR

Table 1
GC–NPD relative response (RR) values[a] for pesticides in different solvents and in oil matrix at ca. 1.5 mg/kg

Pesticide	In solvent[b]			Oil matrix[c]
	Hexane	Chloroform	Acetone	
Dichlorvos	0.87	1	0.86	1.41
Metamidophos	1.26	1	1.17	3.75
Acephate	0.85	1	1.18	4.66
Omethoate	1.60	1	1.39	5.70
Diazinon	1.22	1	1.01	1.27
Dimethoate	1.32	1	1.03	1.92
Parathion methyl	1.21	1	0.97	1.39
Fenitrothion	1.26	1	1.02	1.46
Fenthion	1.32	1	1.08	1.22
Quinalphos	1.46	1	1.25	1.37
Phenthoate	0.85	1	1.00	1.20
Methidathion	1.61	1	1.46	1.31
I.S.	1.31	1	1.07	1.51
Azinphos methyl	1.34	1	1.24	2.00

[a] Mean of triplicate analysis.
[b] Peak height in solvent/peak height in chloroform.
[c] Peak height in oil matrix/peak height in hexane.

values (1.6), while dichlorvos, acephate and phenthoate showed lower values (ca. 0.9).

After the oil extraction procedure described in Section 2.3, the residue was taken up with solvent and injected in GC. The amount of lipid present in solution was low (16±1 μg/μl of coextractives) and the column resolution efficiency was good after a few hundred analyses. The peak height of the pesticides was generally enhanced with respect to that of solvents and hexane showed a higher increase. Matrix effects on peak response were known in pesticide laboratories, but it is only recently that this issue has been investigated [4–6].

To confront peak heights at the same concentration in oil matrix and solvent alone, the residue-free oil extract was taken up with working solution of pesticides in solvent alone at ca. 1.1 mg/kg. Table 1 shows GC–nitrogen–phosphorus detection (NPD) response relative values for the pesticides in oil matrix and in hexane and Fig. 1 its chromatograms.

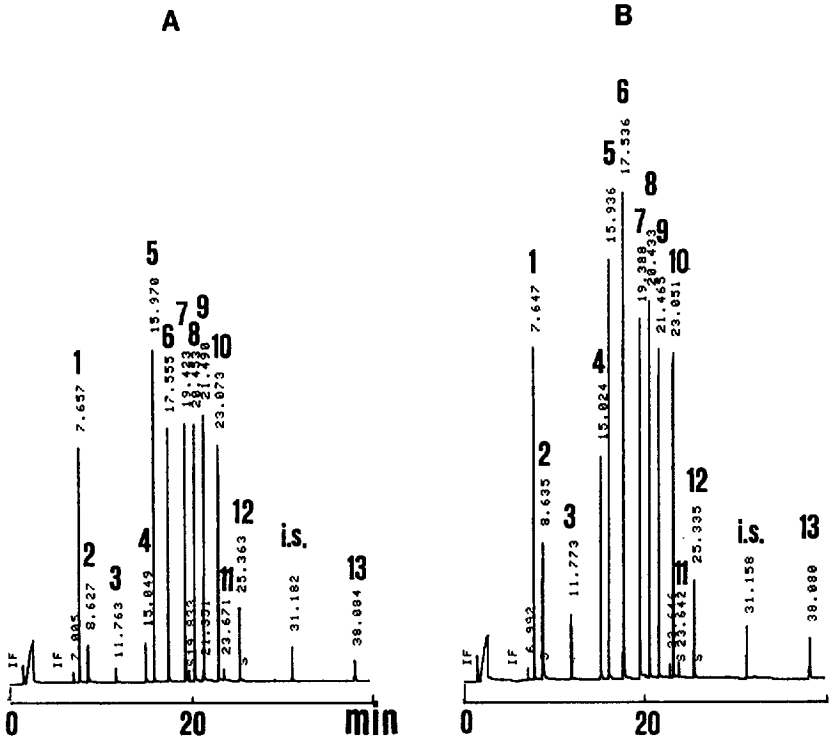

Fig. 1. Comparison of organophosphorus insecticides in hexane (A) and spiked to a residue-free oil extract (B) at ca 1.1 mg/kg. For GC conditions see Section 2.2. (1) Dichlorvos, (2) methamidophos, (3) acephate, (4) omethoate, (5) diazinon, (6) dimethoate, (7) parathion methyl, (8) quinalphos, (9) fenitrothion, (10) fenthion, (11) phenthoate, (12) methidathion, (13) azinphos methyl.

Some compounds (methamidophos, acephate, omethoate and dimethoate) showed a very high increase in response (from 1.92 to 5.70 times); pesticides more soluble in water had a higher increase in response factor. This matrix effect decreased the limits of detection [7] that ranged between 0.001 and 0.02 mg/kg for the studied compounds, with the higher value for phenthoate and the lower value for dimethoate.

3.2. Linearity

Under the chromatographic conditions described in Section 2.2 the calibration graphs in residue-free oil extract (internal standard mode) were constructed by plotting peak heights vs. concentrations; a good linearity was achieved in the range 0.01–3.50 mg/kg, with correlation coefficients between 0.9995 and 0.9999.

3.3. Recovery and repeatability

To extract pesticide residues, a micro-extraction method with acetonitrile was used, as described in Section 2.3. This extraction solvent was used by other authors [1].

The gas chromatograms of untreated oil extracts were all free from interfering peaks and indistinguishable from those obtained with pure solvents, therefore no cleanup was necessary. Since coextractive substances modified the peak height, the quantification of residues was performed by measuring fortified oil samples against matrix-matched standards using hexane as a solvent since it increased the response more than other solvents.

The recovery and repeatability data are summarized in Table 2. All pesticides were extracted from matrices fortified at ca 0.1, 1.0 and 3.0 mg/kg with recovery ranging from 74 to 118. The accuracy was acceptable; coefficients of variation ranged from 1 to 16%.

4. Conclusions

The described method allows a simple and rapid determination of pesticides in olive oil. Since the gas chromatograms of untreated oil extracts were all free from interfering peaks and indistinguishable from those obtained with pure solvents, no cleanup was needed. Since solvents and coextractive substances modified the peak heights, to handle this matrix effect it was necessary to prepare standards in the residue-free oil extract solubilized in hexane, which increased the response more than other solvents. The low amount of coextractive substances does not decrease the column efficiency even after a few hundred analyses.

Table 2
Recoveries (% ±S.D.) of pesticides in olive oil at different fortification levels (mg/kg)

Pesticide	Fortification level (mg/kg)	Recovery (% ±S.D).	Fortification level mg/kg)	Recovery (% ±S.D).	Fortification level (mg/kg)	Recovery (% ±S.D).
Dichlorvos	2.05	92±7	0.82	74±6	0.08	117±1
Methamidophos	2.50	89±7	1.01	83±11	0.10	96±8
Acephate	2.45	118±10	0.98	114±11	0.10	110±9
Omethoate	2.65	95±7	1.06	116±11	0.11	85±10
Diazinon	2.30	91±5	0.92	91±6	0.09	94±5
Dimethoate	3.13	110±8	1.24	107±8	0.12	106±4
Parathion methyl	2.50	102±9	1.00	97±4	0.10	101±4
Fenitrothion	2.55	100±6	1.02	95±5	0.10	105±2
Fenthion	2.30	93±9	0.92	82±5	0.09	98±2
Quinalphos	3.00	91±12	1.20	90±4	0.12	102±1
Phenthoate	3.35	106±15	1.34	97±6	0.13	77±16
Methidathion	2.45	104±14	0.98	108±4	0.10	107±1
I.S.	0.3		0.3		0.3	
Azinphos methyl	3.15	109±4	1.26	104±9	0.13	98±12

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