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Multiresidue analysis of pesticides in fruits and vegetables by gel permeation chromatography followed by gas chromatography with electron-capture and mass spectrometric detection

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Abstract

A wide range screening method for multiresidue analysis of seventy-seven pesticides (twelve organohalogens, forty-five organonitrogens, eleven organophosphorus and nine pyrethroids) in agricultural products is proposed. Pesticide residues were extracted from crop samples with acetone followed by dichloromethane partitioning. Crop extracts were cleaned-up by gel permeation chromatography equipped with a 10 mm diameter column. Analytical screening was by gas chromatography using long, narrow-bore fused-silica open-tubular columns equipped with electron-capture detection (ECD). Recoveries of majority of pesticides from spiked samples of carrot, melon and tomato at fortification levels of 0.04–0.10 mg/kg ranged from 70 to 108%. The lowest recovery was for chlormephos (51.5%). Limits of detection were less than 0.01 mg/kg for ECD. Confirmation of pesticide identity was performed by gas chromatography–mass spectrometry in selected-ion monitoring mode. The multiresidue procedure was applied in routine crop analysis: a 9-month period data are reported.

Keywords: Fruits; Vegetables; Food analysis; Environmental analysis; Pesticides

1. Introduction

Pesticides are applied worldwide to a broad variety of crops both for field and post-harvest protection. Increasing public concern in recent years about possible health risks from pesticide residues in the diet, has deeply modified the strategy for crop protection, with emphasis on food quality and safety, and the widespread concern for the health of society has led to strict regulation of maximum residue limits (MRLs) and total dietary intakes of pesticide residues in food commodities [1].

Compliance monitoring is most requested in food production, processing and trade. An increasing number of food processing companies, consumer organizations and supermarket food companies devote resources to official and private laboratories for analytical testing of pesticide residues to determine

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compliance with national maximum residue limits (MRLs).

Standard multiresidue procedures for fruits and vegetables are described by many monitoring agencies in their screening programs [2] and are officially accepted in many countries: Holland and Malcolm [3] in their review article report the official methodologies currently adopted in some European and oversea countries. In Italy the regulatory environment for residue tolerance in food is that of the European Union [4], but official national methodology is still lacking. An official European method is presently being elaborated by the European Committee for Standardization (CEN) [5]: the document (prEN 12393) would represent a guide protocol for multiresidue analysis throughout the European Union.

Chromatographic methods are the most suitable for residue analysis [6–9] in particular gas chromatography (GC) using long, narrow-bore capillary columns equipped with selective and sensitive detection methods such as electron-capture detection (ECD) [10,11], nitrogen–phosphorus detection (NPD) [12,13] and flame-photometric detection (FPD) [14,15] according to different classes of pesticides. An emerging strategy in multiresidue methodology is the search for universal detection systems, e.g., in GC coupled with mass-selective detectors [16–19].

Recently many Italian laboratories have developed their own multiresidue methods for monitoring pesticides in food, particularly organophosphorus (OP) [18,20,21] and fungicide [22] residues. However, many of the proposed screening methods cover a limited range of pesticides. A modern trend in multiresidue methodology is moving to the development of reliable procedures capable of determining as many pesticides as possible in the most rapid and accurate manner. GC–ECD is the favoured technique for the determination of majority of pesticides. Confirmation of identity of pesticide residues may be performed by GC coupled with mass spectrometry (GC–MS) [23–26].

Special care must also be paid to the extraction and clean-up steps. A broad variety of solvent extraction and partitioning systems have been proposed for crop sample extraction: acetone, methanol, acetonitrile and ethyl acetate are commonly used in solvent-based extraction methods. Hovewer, multiresidue procedures based on acetone extraction followed by dichloromethane partitioning have shown high effectiveness for a very wide range of pesticide residues and crops [3]. Moreover, occurring of interfering coextractives from sample matrix requires extensive clean-up. Gel permeation chromatography is a widely used efficient technique for purification and large molecule removal from sample extracts [27–29]. Additional advantages are reproducibility and compatibility of eluting solvents with GC detectors.

The aim of the present work was to develop a rapid and accurate multiresidue method to determine organohalogen, organonitrogen, pyrethroid and some organophosphorus pesticides in routine testing of raw agricultural products (fruits and vegetables). The paper describes a simple and effective procedure for sample extraction and partitioning using a modified Luke extraction method [30]. Clean-up was based on gel permeation chromatography (GPC) followed by high-resolution GC–ECD for simultaneous determination of seventy-seven pesticide residues. Eleven phosphorus-containing pesticides were also included: in the preliminary step of our pilot study we found an adequate ECD response to OP compounds although more accurate methodology requires FPD. Confirmatory analysis was carried out by means of GC–MS in selected-ion monitoring (SIM) mode.

The proposed method was applied for compliance monitoring of fruits and vegetables entering local market. Preliminary data from the monitoring activity are reported; critical discussion of the described method is provided.

2. Experimental

2.1. Chemicals

2.1.1. Pesticide standards

Pesticide reference standards with the purity of 95–100% were all purchased from LabService Analytica (Bologna, Italy) and Chebios (Rome, Italy). Pesticides investigated are listed in Table 1. Official common names were adopted from The Pesticide Manual [31].

Table 1
Standard mixes, retention times, recoveries and detection limits of tested pesticides

Pesticide (CAS RN)	Mix	t_R (min)	Spiking level (mg/kg)	Recovery[a] (%)			LOD (mg/kg)
				Carrot	Melon	Tomato	
Organohalogens							
Aldrin (309-00-2)	A	29.24	0.08	88.0	90.5	90.6	0.001
Bromopropylate (18181-80-1)	A	40.96	0.09	89.6	85.3	91.5	0.002
Chlorfenson (80-33-1)	A	35.77	0.07	88.1	93.5	84.2	0.001
Chlorothalonil (1897-45-6)	A	27.72	0.05	79.3	83.5	77.8	0.001
Chlorthal-dimethyl (1861-32-1)	A	30.58	0.07	85.4	81.3	96.4	0.001
Diclofop-methyl (51338-27-3)	A	39.90	0.07	86.8	79.7	87.3	0.004
Dicofol (115-32-2)	B	31.25	0.07	88.1	86.3	86.6	0.003
Endosulfan[b] (115-29-7)	B	33.75 37.66	0.04	81.3	90.0	84.0	0.001
γ-HCH[b] (58-89-9)	C	15.75 25.59	0.06	95.4	84.9	91.8	0.001
Hexachlorobenzene (118-74-1)	C	22.61	0.06	76.9	74.3	76.8	0.001
Methoxychlor (72-43-5)	C	42.54	0.07	99.1	99.8	107.1	0.002
Tetradifon (116-29-0)	A	43.45	0.07	89.1	88.5	88.5	0.001
Organonitrogens							
Anilazine (101-05-3)	A	35.93	0.06	73.1	74.5	76.2	0.003
Benfluralin (1861-40-1)	A	19.53	0.07	88.1	82.4	92.3	0.001
Bitertanol (55179-31-2)	A	45.83	0.07	86.5	–	80.1	0.010
Buprofezin (69327-76-0)	A	36.21	0.06	74.3	78.1	–	0.006
Captafol (2425-06-1)	A	18.10	0.07	74.5	80.4	85.1	0.008
Chinomethionat (2439-01-2)	A	34.59	0.05	80.7	70.9	81.5	0.003
Chlozolinate (72391-46-9)	A	32.15	0.07	88.5	85.8	84.0	0.002
Clofentezine (74115-24-5)	A	9.41	0.06	91.6	88.3	79.0	0.008
Cymoxanil (57966-95-7)	B	12.63	0.06	77.3	71.0	76.7	0.010
Cyproconazole[b] (113096-99-4)	B	36.83 36.95	0.06	79.8	81.8	88.3	0.008
Dichlofluanid (1085-98-9)	B	31.02	0.07	90.9	90.5	78.1	0.002
Dicloran (99-30-9)	B	25.00	0.04	90.7	93.5	90.3	0.001
Etaconazole (60207-93-4)	A	37.80	0.06	81.0	77.8	79.4	0.002
Fenarimol (60168-88-9)	C	45.48	0.06	98.0	95.0	–	0.001
Fenoxaprop (73519-55-8)	C	44.79	0.10	99.1	99.4	101.5	0.007
Folpet (133-07-3)	C	18.07	0.06	95.1	97.3	97.7	0.004
Haloxyfop-etotyl (87237-48-7)	C	38.63	0.09	86.5	79.5	88.1	0.002
Hexaconazole (79983-71-4)	C	34.52	0.06	99.3	98.9	99.4	0.001
Hexythiazox (78587-05-0)	C	35.90	0.07	89.2	90.5	91.8	0.007
Imazalil (35554-44-0)	C	35.15	0.06	99.8	99.5	103.7	0.002
Iprodione (36734-19-7)	C	40.43	0.07	95.4	97.3	98.3	0.005
Linuron (330-55-2)	C	11.04	0.05	99.3	95.6	101.2	0.006
Metamitron (41394-05-2)	C	39.46	0.04	98.9	105.3	95.8	0.007
Metobromuron (3060-89-7)	C	9.54	0.05	95.0	94.0	102.3	0.003
Metolachlor (51218-45-2)	C	30.12	0.05	96.8	95.1	94.3	0.006
Metribuzin (21087-64-9)	C	29.64	0.05	106.3	88.6	99.1	0.002
Monolinuron (1746-81-2)	C	7.98	0.05	90.3	88.1	86.8	0.010
Myclobutanil (88671-89-0)	C	36.38	0.06	100.8	99.5	100.6	0.005
Nuarimol (63284-71-9)	B	40.54	0.06	105.6	101.5	101.9	0.001
Penconazole (66246-88-6)	B	32.38	0.06	92.1	95.4	88.8	0.002
Pendimethalin (40487-42-1)	B	31.97	0.06	78.8	91.4	92.3	0.002

(Contd.)

Table 1. Continued

Pesticide (CAS RN)	Mix	t_R (min)	Spiking level (mg/kg)	Recovery[a] (%)			LOD (mg/kg)
				Carrot	Melon	Tomato	
Prochloraz (67747-09-5)	C	46.89	0.08	86.2	86.3	82.6	0.004
Procymidone (32809-16-8)	C	33.31	0.05	102.5	99.0	89.5	0.003
Propachlor (1918-16-7)	B	21.01	0.05	83.4	81.7	85.4	0.007
Propiconazole[b] (60207-90-1)	C	38.98	0.06	97.8	96.5	94.0	0.003
		39.17					
Propyzamide (23950-58-5)	B	24.37	0.05	98.3	97.0	95.7	0.003
Quizalofop-ethyl (76578-14-8)	C	50.54	0.07	99.8	103.5	101.8	0.006
Teflubenzuron (83121-18-0)	A	7.98	0.10	73.2	65.3	60.1	0.010
Tebuconazole (107534-96-3)	B	39.66	0.06	80.8	79.5	82.1	0.008
Terbuthylazine (5915-41-3)	B	25.49	0.04	79.5	82.9	85.4	0.010
Triadimefon (43121-43-3)	C	30.42	0.06	97.3	96.7	97.0	0.002
Triadimenol (89482-17-7)	C	32.53	0.06	100.1	93.1	96.0	0.007
Trifluralin (1582-09-8)	A	19.33	0.07	92.4	84.9	91.5	0.001
Triforine (26644-46-2)	B	8.03	0.09	86.3	81.5	96.5	0.005
Vinclozolin (50471-44-8)	B	27.82	0.06	78.9	91.3	71.3	0.002
Organophosphorus							
Bromophos (2104-96-3)	A	31.81	0.07	88.3	85.0	87.1	0.001
Bromophos-ethyl (4824-78-6)	B	33.17	0.08	85.3	86.3	82.2	0.001
Carbophenothion (786-19-6)	A	38.87	0.07	89.5	–	86.7	0.001
Chlormephos (24934-91-6)	A	15.43	0.06	55.4	51.5	52.6	0.005
Chlorpyrifos (2921-88-2)	B	30.78	0.07	87.8	88.2	87.7	0.001
Chlorpyrifos-methyl (5598-13-0)	A	28.76	0.06	81.2	79.1	76.7	0.001
Diazinon (333-41-5)	B	25.33	0.06	83.4	90.1	88.4	0.006
Dichlorvos (62-73-7)	B	9.29	0.06	65.2	70.1	66.3	0.008
Dimethoate (60-51-5)	B	26.41	0.05	92.8	93.5	94.2	0.007
Phosalone (2310-17-0)	B	43.67	0.08	90.7	91.7	89.5	0.003
Tolclofos-methyl (57018-04-9)	B	29.61	0.05	91.5	90.0	88.5	0.003
Pyrethroids							
Cyfluthrin[b] (68359-37-5)	A	47.33	0.07	68.5	70.1	71.4	0.005
		47.59					
		47.93					
λ-Cyhalothrin[b] (91465-08-6)	B	42.17	0.07	–	106.1	107.9	0.002
		42.38					
		42.69					
Cypermethrin[b] (52315-07-8)	B	48.92	0.05	86.4	89.3	85.3	0.003
		49.45					
		49.61					
Deltamethrin[b] (52918-63-5)	B	57.76	0.05	96.6	91.3	79.1	0.003
		59.06					
Esfenvalerate[b] (66230-04-4)	B	53.56	0.07	83.4	82.4	83.5	0.003
		54.78					
Fenpropathrin (64257-84-7)	C	41.31	0.07	97.1	104.2	90.0	0.004
Fenvalerate[b] (51630-58-1)	A	53.54	0.07	66.5	65.1	72.4	0.004
		54.76					
Permethrin[b] (52645-53-1)	B	45.73	0.06	81.1	88.9	79.0	0.005
		46.10					
Tetramethrin (7696-12-0)	C	41.93	0.07	100.1	102.1	97.8	0.008

[a] Mean of three replicates. Standard deviations are 1–8%.

[b] Compounds with two or more isomeric forms. The sum of the peak areas was applied for the quantification.

2.1.2. Pesticide solutions

Pesticide stock solutions (2 m*M*) were prepared by dissolving of pesticide standards in mixture acetone–*n*-hexane (1:1) and storing in a freezer at −18°C in glass bottles with PTFE-faced screw caps. Three pesticide working solutions (mix A, B and C) (Table 1) with single concentrations ranging from 0.07 to 0.08 m*M* (corresponding to the range 0.12 to 0.37 μg/ml) were prepared by dilution of stock solutions in acetone–*n*-hexane (1:1). The pesticide working solutions were prepared for recovery test and for GC method development.

2.1.3. Organic solvents and reagents

Acetone, *n*-hexane, dichloromethane, ethyl acetate and cyclohexane of special grade for pesticide residue analysis were purchased from Carlo Erba Reagenti (Milan, Italy). Organic solvents, particularly dichloromethane which is toxic, were handled with care observing safety precautions, using efficient fume hoods and wearing protective gloves. Sodium chloride (NaCl) and anhydrous sodium sulphate (Na_2SO_4) from Carlo Erba Reagenti were of RPE analytical grade. Whatman No.1 filter papers were from Carlo Erba Reagenti.

2.1.4. Crop samples

A broad variety of fruit and vegetable samples (apple, grapes, kiwi, melon, peach, strawberry, watermelon, carrot, kohl-rabi, lettuce, spinach, tomato, zucchina) were collected from local farmers for routine pesticide analysis.

2.2. Equipments

2.2.1. Gel permeation chromatography system

Sample clean-up was performed on a Dedicated Sample Cleanup System (DSCS) Model 18L-MLS

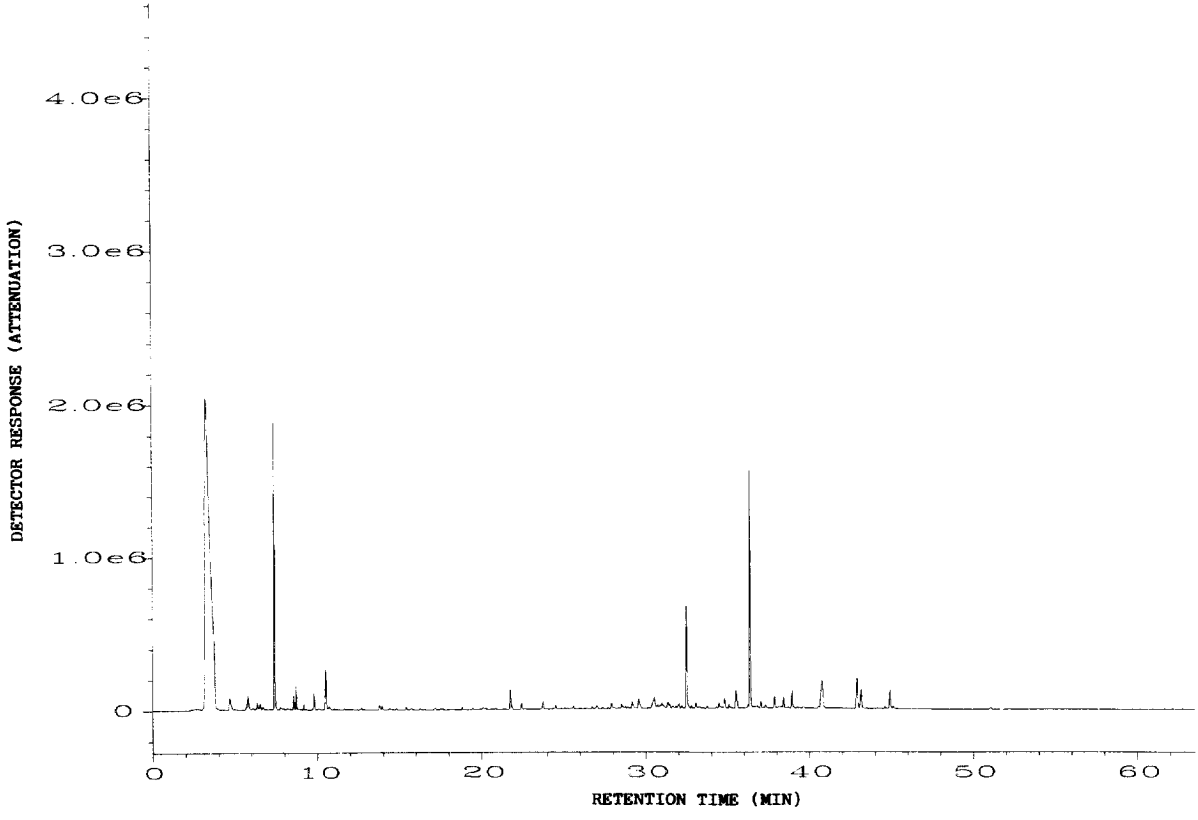

Fig. 1. GC–ECD chromatogram of carrot extract. GC conditions as described in Section 2.2.2.

(LabService Analytica) equipped with 1-ml sample loop and a glass column Vario Outfit (450 mm×10 mm I.D.) slurry-packed with 8.5 g of Envirosep SX3 styrene–divinylbenzene copolymer (200–400 mesh) (LabService Analytica) in ethyl acetate–cyclohexane (1:1) and compressed to a bed length of ca. 30 cm. Operating conditions were: mobile phase, ethyl acetate–cyclohexane (1:1); flow-rate, 1 ml/min; dump cycle, 16 min; collect cycle, 15 min; wash cycle, 10 min.

2.2.2. GC–ECD

A Hewlett-Packard Model 5890 Series II Plus (Palo Alto, CA, USA) with electronic pressure control equipped with a ^{63}Ni electron-capture detector, an HP 7673 autosampler and a fused-silica capillary column SPB-608 (30 m×0.25 mm I.D.; film thickness 0.25 μm) from Supelco (Bellefonte, PA, USA) was used. Operating conditions were as follows: initial temperature 50°C (1 min), increased at 20°C/min to 150°C, held for 5 min, then increased at 4°C/min to 280°C and finally held at 280°C for 20 min; injector temperature 275°C; carrier gas He; column flow-rate 1 ml/min; detector temperature 300°C; make-up gas N_2; operated in the splitless mode (electronic pressure control); purge off time 1 min; injection volume 1 μl.

2.2.3. GC–MS

A Hewlett-Packard Model 5890 Series II gas chromatograph equipped with an HP 5972 mass-selective ion detector (quadrupole) and a fused-silica capillary column Supelco PTE-5 (30 m×0.25 mm I.D.; film thickness 0.25 μm) was used for confirmatory analysis. GC conditions were as follows: initial temperature 50°C (1 min), increased at 20°C/min to 150°C, held for 5 min, then increased at 4°C/min to 280°C, the final temperature being held for 30 min; injector temperature 250°C; carrier gas He; flow-rate

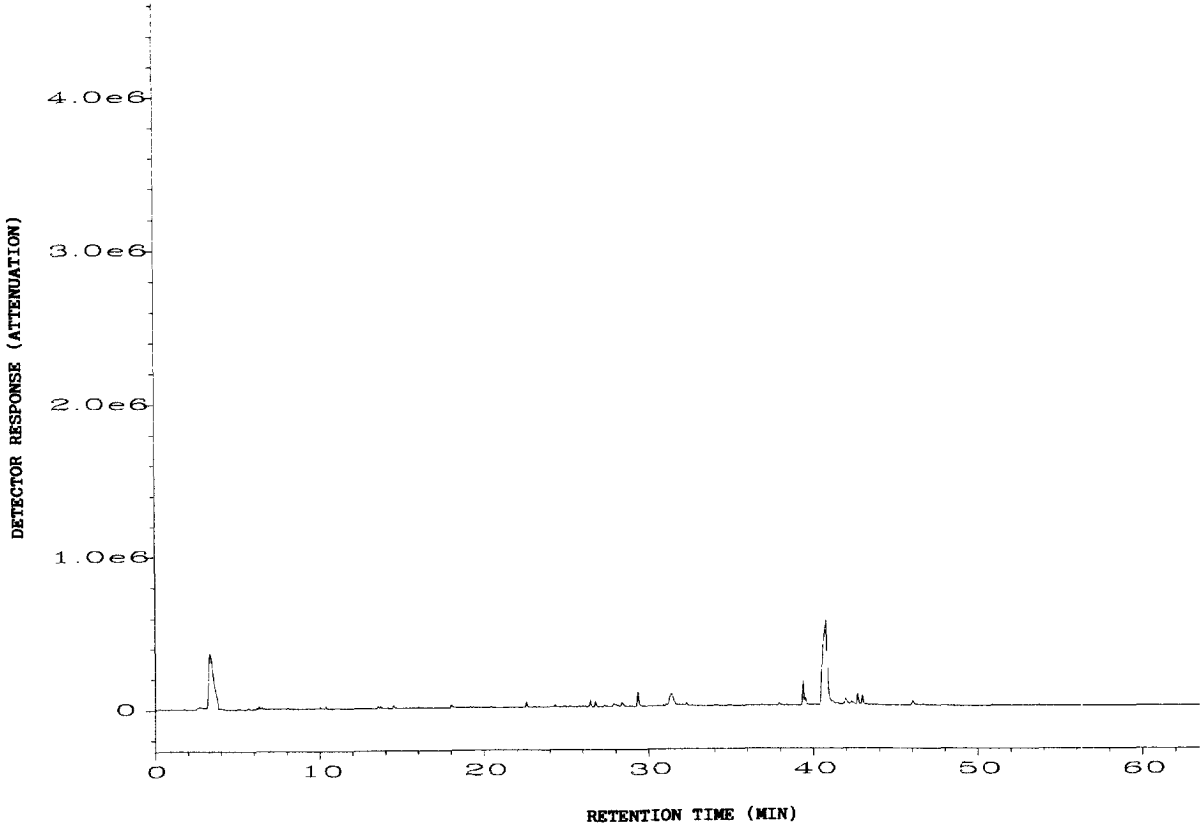

Fig. 2. GC–ECD chromatogram of lettuce extract. GC conditions as described in Section 2.2.2.

1 ml/min; detector temperature (GC–MS transfer line) 280°C; operated in the splitless mode; purge off time 1 min; injection volume 1 μl. MS conditions were: solvent delay 3 min; electron impact ionization voltage 70 eV; scan rate 1.5 scans/s; scanned-mass range 50–550 *m/z*.

For both GC–ECD and GC–MS instrumental control, data acquisition and processing were provided by a Vectra 486/33VL computer equipped with a Hewlett-Packard G1034C ChemStation data system.

2.3. Sample extraction and clean-up

Fruits and vegetables were processed as specified in the European Union legislation [32]. Ca. 1 kg of crop sample was chopped and then blended for 3–5 min. A 100-g portion of the homogenate was extracted with 200 ml of acetone in a ultrasonic bath for 30 min and then filtered under vacuum through a Buchner funnel fitted with a Whatman No. 1 filter paper. A 50-ml volume of saturated NaCl solution and 500 ml of distilled water were added to the filtrate followed by liquid–liquid partitioning with 2×100 ml of dichloromethane. The organic phases were combined, dehydrated by passing through a filter containing a bed of anhydrous Na_2SO_4 and concentrated using a vacuum rotary evaporator equipped with a 30–35°C water-bath; the sample was dried under a gentle stream of pure nitrogen. The residue was dissolved in 3 ml of GPC mobile phase (ethyl acetate–cyclohexane, 1:1) and injected into the GPC column. The purified organic fraction, as determined from the calibration procedure with corn oil according to US Environmental Protection Agency (EPA) method No. 3640, was collected, concentrated using the rotary evaporator (water-bath set at 30–35°C) and completely dried under a gentle stream of nitrogen. The final volume was adjusted to

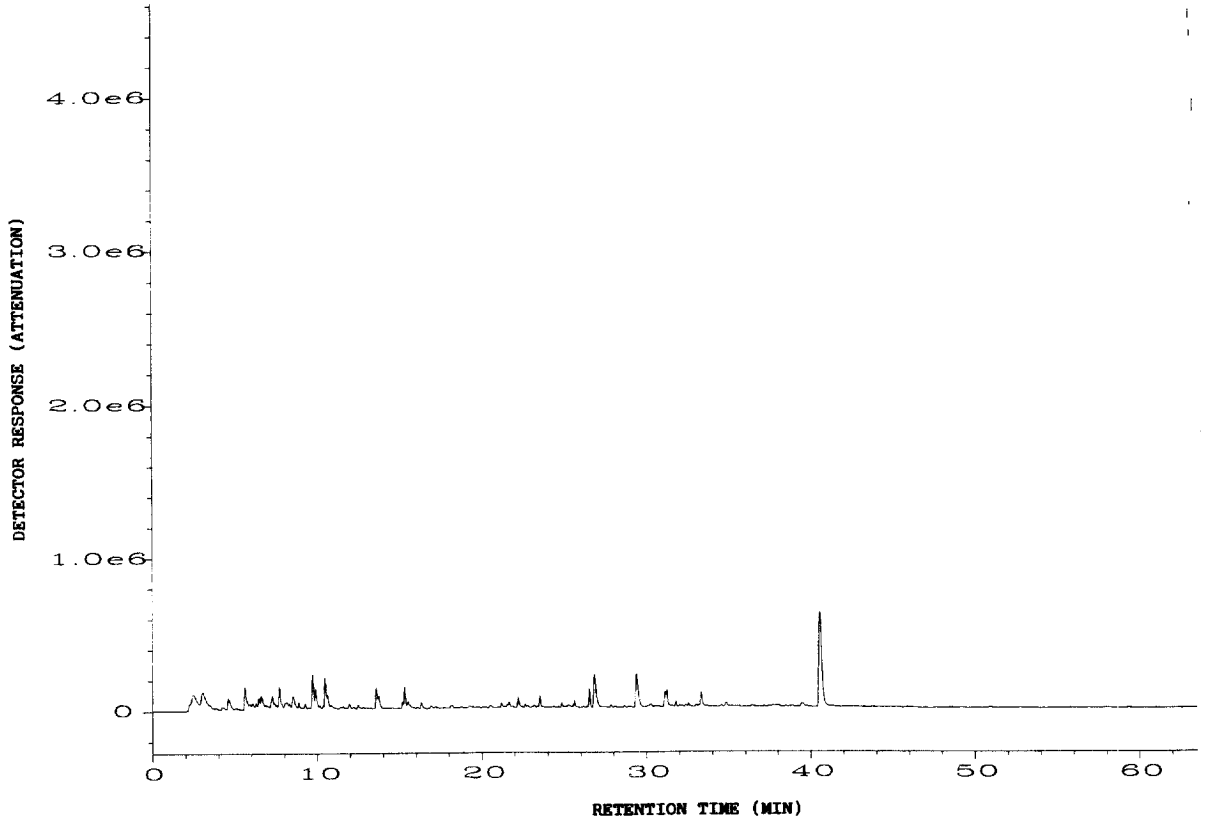

Fig. 3. GC–ECD chromatogram of melon extract. GC conditions as described in Section 2.2.2.

1 ml with the acetone–*n*-hexane mixture (1:1) before GC analysis.

2.4. Recovery study

Recoveries of tested pesticides were determined in triplicate at one fortification level in spiked samples of carrot, melon and tomato. A 100-g amount of chopped and blended crop sample was fortified in triplicate with each working solution and immediately was processed as described above in Section 2.3. Fortification levels for each pesticide, ranging from 0.04 to 0.10 mg/kg, are reported in Table 1.

2.5. GC calibration

Each group of standards was calibrated as one-point linear external standard and sample data were processed by using peak area for each component. The sum of the peak areas of all isomers of some pyrethroids (cyfluthrin, λ-cyhalothrin, cypermethrin, deltamethrin, esfenvalerate, fenvalerate, permethrin), some conazoles (organonitrogen pesticides: cyproconazole and propiconazole) and some organohalogen pesticides (endosulfan and γ-HCH) was applied for calibration and calculation of recovery.

2.6. Limits of detection

The minimum detectable quantities were estimated from the signal-to-noise ratios (*S/N*) of pesticide peaks of at least of 3 in the recovery test at 0.005–0.050 mg/kg and from background signals for the extracts of carrot, melon and tomato. Injection volume was 1 μl.

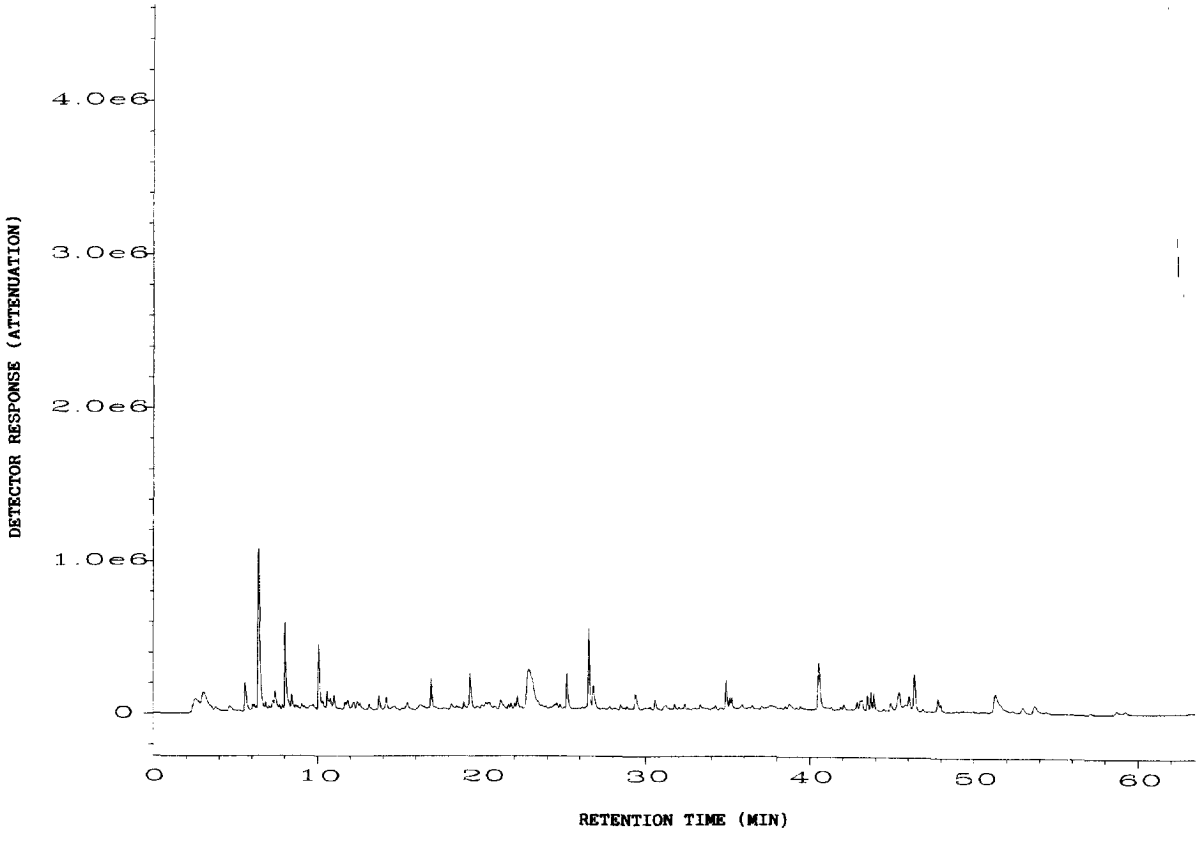

Fig. 4. GC–ECD chromatogram of tomato extract. GC conditions as described in Section 2.2.2.

3. Results and discussion

3.1. Sample extraction and clean-up

Several procedures have been proposed for the extraction and the enrichment of crop samples. Some of them have been recently reviewed [3].

Extraction of pesticides from fruits and vegetables by using a solvent such as acetone which is miscible in plant materials, is the usual approach of most multiresidue methods. However, acetone will also extract many interfering compounds from the sample matrix. For this reason, a further clean-up procedure is required [33]. After extraction with acetone crop samples were purified by liquid–liquid partitioning with dichloromethane to remove hydrophilic interfering coextractives. Addition of NaCl solution as an ionic strength modifier was necessary to improve recovery of less hydrophobic compounds [34,35]. For removal of lipidic and high-molecular-mass compounds GPC was applied: a modified Patterson's procedure [36], which suggested a reduced column system (10 mm I.D., 1-ml injection volume and 1 ml/min eluent flow-rate), has been adopted. The author gave a valuable contribution toward the development of miniaturized GPC systems which are able to reduce typical drawbacks like solvent consumption, disposal problems, health hazards and large-volume sample. However, in our method dichloromethane in the mobile phase was replaced with the less harmful ethyl acetate; the cleaning-up time was reduced to nearly 41 min/sample corresponding to 41 ml of mobile phase/sample; automation could also permit high efficiency of analysis. Figs. 1–5 show the chromatograms from some unspiked representative crop samples (carrot, lettuce, melon, tomato and zucchina), showing a satisfactory clean-up of crop extracts.

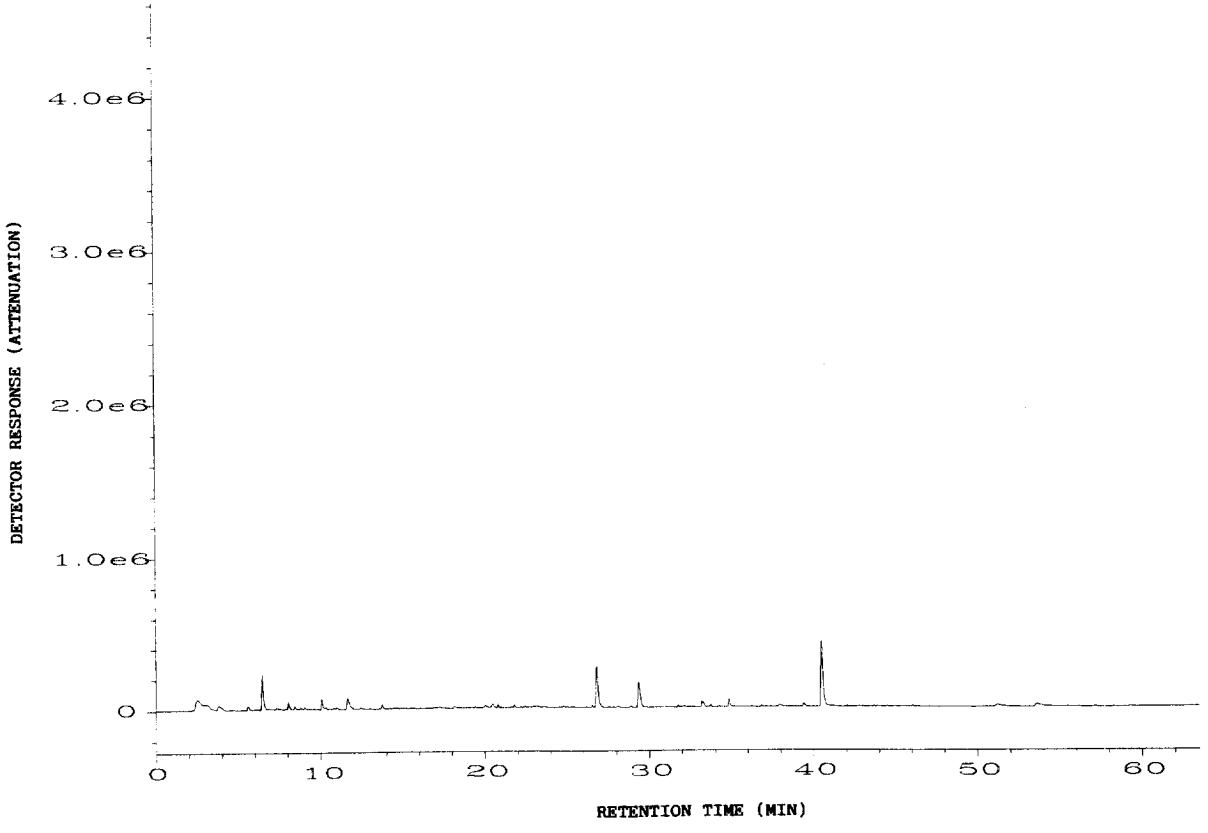

Fig. 5. GC–ECD chromatogram of zucchina extract. GC conditions as described in Section 2.2.2.

Due to the reduced column diameter frequent GPC maintenance was required.

3.2. Detection and quantification

The pesticides tested were selected by screening those most widely applied for crop protection in central Italy and the most required for compliance monitoring. They belong to organohalogen, organonitrogen, organophosphorus and pyrethroid groups of pesticides.

The main problem faced was resolving the pesticide peaks from possible interfering coextractives from sample matrices. For this reason, in the process of development of GC methods we were looking for high resolution of chromatographic peaks and to reach lower limits of detection. High-resolution GC using capillary columns enabled us to achieve good separation performances in an adequate time of analysis. Selective and sensitive detectors, as in ECD, provided good responses even to very low concentrations. In the case of some halogen-containing organophosphorus pesticides we found an adequate ECD response. In many cases MS detection has been employed for quantitation [16–19]: in our work, however, we only performed MS for confirmation of peak identity. Electronic pressure control allowed for good chromatography throughout the

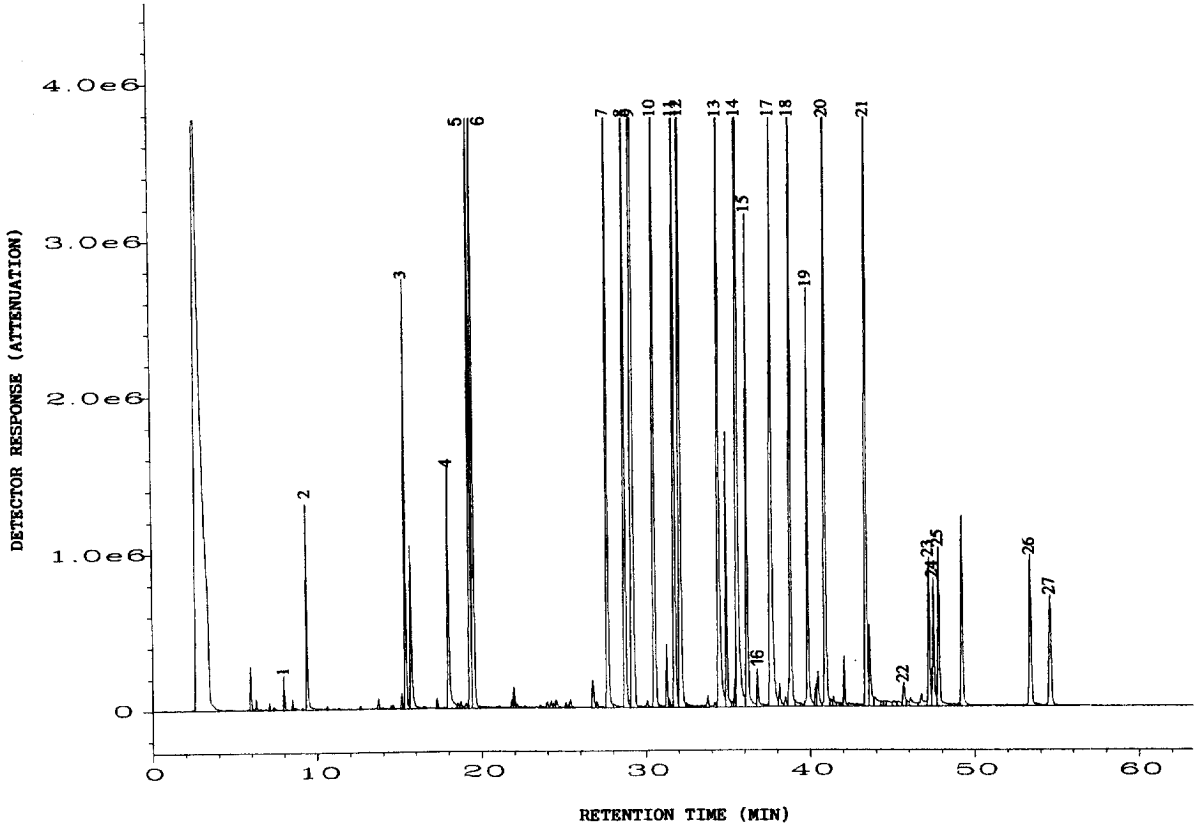

Fig. 6. GC–ECD chromatogram of melon extract fortified with mix A. Peaks: 1=teflubenzuron (0.31 mg/kg); 2=clofentezine (0.25 mg/kg); 3=chlormephos (0.20 mg/kg); 4=captafol (0.29 mg/kg); 5=trifluralin (0.28 mg/kg); 6=benfluralin (0.28 mg/kg); 7=chlorothalonil (0.22 mg/kg); 8=chlorpyrifos-methyl (0.27 mg/kg); 9=aldrin (0.30 mg/kg); 10=chlorthal-dimethyl (0.28 mg/kg); 11=bromophos (0.31 mg/kg); 12=chlozolinate (0.28 mg/kg); 13=chinomethionat (0.20 mg/kg); 14=chlorfenson (0.25 mg/kg); 15=anilazine (0.23 mg/kg); 16=buprofezin (0.25 mg/kg); 17=etaconazole (0.27 mg/kg); 18=carbophenothion (0.29 mg/kg); 19=diclofop-methyl (0.28 mg/kg); 20=bromopropylate (0.36 mg/kg); 21=tetradifon (0.30 mg/kg); 22=bitertanol (0.28 mg/kg); 23=cyfluthrin I; 24=cyfluthrin II; 25=cyfluthrin III (sum of three isomers: 0.36 mg/kg); 26=fenvalerate I; 27=fenvalerate II (sum of two isomers: 0.35 mg/kg). Injection volume, 1 μl. GC conditions are described in Section 2.2.2.

run. Late-eluting compounds showed narrow, well-shaped peaks comparable with those from early-eluting compounds. This would facilitate integration and quantitation.

Optimal chromatographic conditions for multiresidue analysis of different families of pesticides were studied. Due to the large number of tested compounds pesticide reference standards were grouped into three different working solutions (Table 1) for reaching better distribution of their chromatographic peaks, which was helpful for calibration and recovery tests. For qualitative analysis crop sample chromatograms were compared with those from standard mixtures. Single pesticide retention time windows (±0.02 min) were selected from standard chromatograms for peak matching in crop samples. Retention times of pesticides investigated are listed in Table 1. Chromatograms of fortified melon extracts are reported in Figs. 6–8.

Concentrations were calculated on the base of peak areas. Calibration was performed weekly. Due to the large number of pesticides, it was impractical

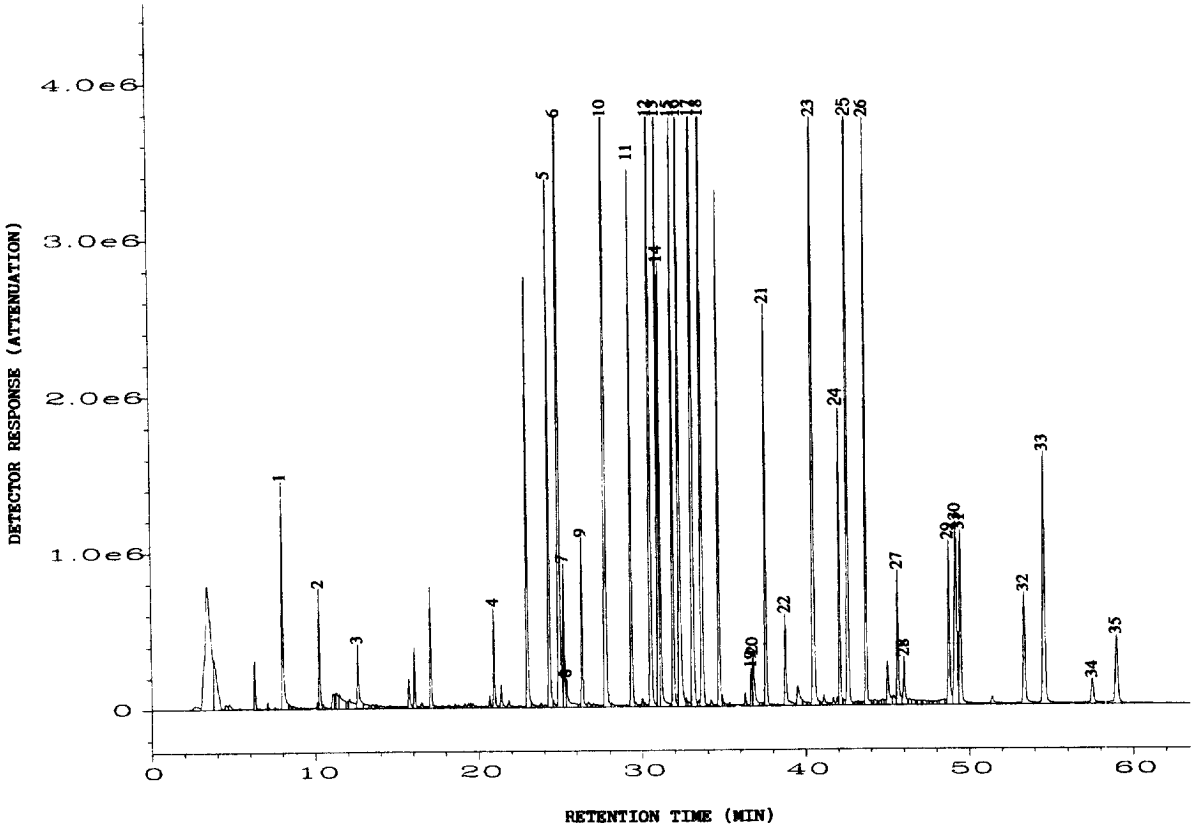

Fig. 7. GC–ECD chromatogram of melon extract fortified with mix B. Peaks: 1=triforine (0.27 mg/kg); 2=dichlorvos (0.17 mg/kg); 3=cymoxanil (0.22 mg/kg); 4=propachlor (0.16 mg/kg); 5=propyzamide (0.19 mg/kg); 6=dicloran (0.15 mg/kg); 7=diazinon (0.23 mg/kg); 8=terbuthylazine (0.17 mg/kg); 9=dimethoate (0.17 mg/kg); 10=vinclozolin (0.21 mg/kg); 11=tolclofos-methyl (0.22 mg/kg); 12=chlorpyrifos (0.26 mg/kg); 13=dichlofluanid (0.25 mg/kg); 14=dicofol (0.27 mg/kg); 15=pendimethalin (0.21 mg/kg); 16=penconazole (0.21 mg/kg); 17=bromophos-ethyl (0.33 mg/kg); 18=endosulfan I; 19=cyproconazole I; 20=cyproconazole II (sum of two isomers: 0.22 mg/kg); 21=endosulfan II (sum of two isomers: 0.30 mg/kg); 22=tebuconazole (0.23 mg/kg); 23=nuarimol (0.23 mg/kg); 24=λ-cyhalothrin I; 25=λ-cyhalothrin II (sum of two isomers: 0.33 mg/kg); 26=phosalone (0.27 mg/kg); 27=permethrin I; 28=permethrin II (sum of two isomers: 0.29 mg/kg); 29=cypermethrin I; 30=cypermethrin II; 31=cypermethrin III (sum of three isomers: 0.31 mg/kg); 32=esfenvalerate I; 33=esfenvalerate II (sum of two isomers: 0.31 mg/kg); 34=deltamethrin I; 35=deltamethrin II (sum of two isomers: 0.37 mg/kg). Injection volume, 1 μl. GC conditions are described in Section 2.2.2.

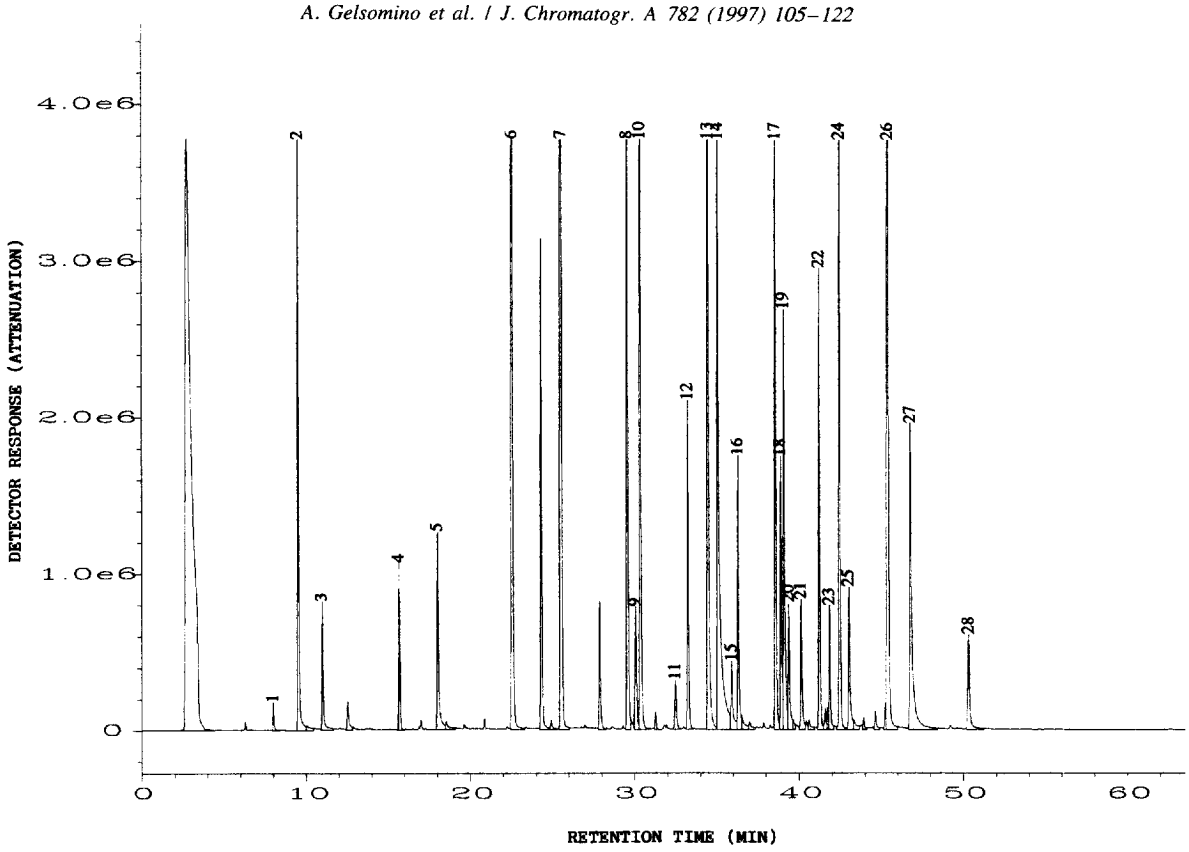

Fig. 8. GC–ECD chromatogram of melon extract fortified with mix C. Peaks: 1=monolinuron (0.17 mg/kg); 2=metobromuron (0.20 mg/kg); 3=linuron (0.19 mg/kg); 4=γ-HCH I; 5=folpet (0.23 mg/kg); 6=hexachlorobenzene (0.22 mg/kg); 7=γ-HCH II (sum of two isomers: 0.22 mg/kg); 8=metribuzin (0.17 mg/kg); 9=metolachlor (0.22 mg/kg); 10=triadimefon (0.23 mg/kg); 11=triadimenol (0.23 mg/kg); 12=procymidone (0.22 mg/kg); 13=hexaconazole (0.24 mg/kg); 14=imazalil (0.27 mg/kg); 15=hexythiazox (0.27 mg/kg); 16=myclobutanil (0.22 mg/kg); 17=haloxyfop-etotyl (0.33 mg/kg); 18=propiconazole I; 19=propiconazole II (sum of two isomers: 0.26 mg/kg); 20=metamitron (0.16 mg/kg); 21=iprodione (0.25 mg/kg); 22=fenpropathrin (0.27 mg/kg); 23=tetramethrin (0.26 mg/kg); 24=methoxychlor (0.27 mg/kg); 25=fenoxaprop (0.28 mg/kg); 26=fenarimol (0.26 mg/kg); 27=prochloraz (0.29 mg/kg); 28=quizalofop-ethyl (0.29 mg/kg). Injection volume, 1 μl. GC conditions are described in Section 2.2.2.

to carry out more than a one-point calibration because of the large number of injections that would be required for the three groups of standards.

Limits of detection (LODs) were estimated from extracts of carrot, melon and tomato. Because recoveries of over 90% of spiked pesticides from the three matrices were very similar, the values were combined to generate LODs which are reported in Table 1. Detection limits were less than 0.01 mg/kg for ECD which is compliant with the legislative minimum detectable quantity [1,4]. These limits are conservative in most cases ($S/N>3$, no significant interferences).

3.3. Recovery study

The occurrence of interfering coextractives from sample matrices was assessed in previous experiments during the development of the enrichment procedure. Carrot, melon and tomato were chosen as reference matrices for the recovery assay. Spiking levels and mean recoveries from fortified crop samples in triplicate experiments for each matrix are shown in Table 1. Of the seventy-seven pesticides tested almost 94% gave recoveries between 70 and 108% which is considered optimal basis for method validation. Other compounds (teflubenzuron, di-

Table 2
Retention times, main ions and relative abundances of pesticides detected by GC–MS

Pesticide	t_R (min)	Main ions, m/z (relative abundance, %)		
Organohalogens				
Aldrin	22.57	263 (68)	293 (26)	329 (6)
Bromopropylate	35.65	149 (100)	167 (30)	279 (10)
Chlorfenson	37.40	111 (79)	175 (100)	302 (66)
Chlorothalonil	18.67	109 (23)	229 (10)	266 (100)
Chlorthal-dimethyl	23.44	223 (18)	301 (100)	332 (32)
Diclofop-methyl	32.47	253 (100)	281 (41)	340 (82)
Dicofol	34.19	111 (39)	139 (10)	251 (72)
Endosulfan[a]	26.30	237 (100)	265 (61)	339 (36)
	29.38			
γ-HCH	17.15	145 (22)	181 (100)	219 (81)
Hexachlorobenzene	15.88	214 (17)	249 (24)	284 (100)
Methoxychlor	34.40	227 (100)	274 (5)	374 (3)
Tetradifon	35.15	159 (100)	229 (53)	356 (37)
Organonitrogens				
Anilazine	24.81	178 (33)	239 (100)	276 (10)
Benfluralin	15.22	264 (18)	292 (100)	335 (5)
Bitertanol	36.27	112 (13)	170 (100)	212 (4)
Buprofezin	28.38	105 (100)	172 (38)	305 (11)
Captafol	32.36	79 (100)	183 (10)	349 (4)
Chinomethionat	25.67	116 (62)	206 (100)	234 (77)
Chlozolinate	25.20	188 (100)	259 (73)	331 (57)
Clofentezine	6.13	75 (20)	102 (32)	137 (100)
Cymoxanil	13.96	170 (54)	198 (44)	216 (100)
Cyproconazole	29.17	125 (25)	139 (54)	222 (100)
Dichlofluanid	22.39	123 (100)	167 (33)	224 (30)
Dicloran	16.07	124 (100)	176 (90)	206 (80)
Etaconazole	29.84	173 (100)	191 (33)	245 (67)
Fenarimol	36.88	139 (100)	251 (66)	330 (38)
Fenoxaprop	37.87	261 (29)	288 (100)	361 (91)
Folpet	25.38	104 (100)	260 (88)	295 (18)
Haloxyfop-etotyl	33.24	288 (78)	302 (100)	433 (38)
Hexaconazole	27.29	83 (100)	214 (47)	231 (17)
Hexythiazox	26.48	156 (100)	184 (63)	227 (65)
Imazalil	27.50	173 (98)	215 (100)	296 (6)
Iprodione	33.73	187 (51)	245 (20)	314 (100)
Linuron	22.26	61 (100)	160 (16)	248 (15)
Metamitron	29.30	104 (80)	174 (40)	202 (100)
Metobromuron	19.57	61 (100)	170 (15)	258 (13)
Metolachlor	22.98	162 (100)	211 (9)	238 (53)
Metribuzin	20.33	144 (20)	198 (100)	214 (5)
Monolinuron	16.83	61 (100)	126 (22)	214 (15)
Myclobutanil	28.25	150 (52)	179 (100)	288 (12)
Nuarimol	32.18	107 (100)	235 (65)	314 (37)
Penconazole	24.85	159 (100)	248 (78)	281 (8)
Pendimethalin	25.06	162 (10)	252 (100)	281 (11)
Prochloraz	40.68	180 (100)	266 (24)	308 (90)
Procymidone	25.74	96 (100)	255 (8)	283 (53)
Propachlor	13.08	120 (100)	176 (27)	211 (9)
Propiconazole[a]	31.42	173 (100)	221 (62)	259 (58)
	31.74			
Propyzamide	17.86	145 (34)	173 (100)	254 (20)

(Contd.)

Table 2. Continued

Pesticide	t_R (min)	Main ions, m/z (relative abundance, %)		
Quizalofop-ethyl	41.19	243 (37)	299 (100)	372 (96)
Teflubenzuron	7.48	113 (42)	141 (100)	157 (49)
Tebuconazole	32.72	125 (86)	250 (100)	307 (7)
Terbuthylazine	17.65	173 (46)	214 (100)	229 (25)
Triadimefon	23.44	57 (100)	208 (42)	293 (3)
Triadimenol	25.56	112 (100)	128 (47)	168 (57)
Trifluralin	15.10	263 (73)	306 (100)	335 (8)
Triforine	6.53	203 (100)	303 (70)	321 (20)
Vinclozolin	20.71	187 (100)	212 (99)	285 (76)
Organophosphorus				
Bromophos	23.91	125 (100)	316 (9)	331 (100)
Bromophos-ethyl	26.15	97 (100)	303 (58)	359 (55)
Carbophenothion	31.04	157 (100)	199 (19)	342 (26)
Chlormephos	9.12	121 (100)	154 (50)	234 (55)
Chlorpyrifos	23.18	97 (100)	197 (78)	314 (46)
Chlorpyrifos-methyl	20.56	109 (59)	125 (94)	286 (100)
Diazinon	18.35	88 (100)	179 (70)	304 (37)
Dichlorvos	6.72	109 (100)	185 (33)	220 (9)
Dimethoate	16.11	87 (100)	125 (53)	229 (8)
Phosalone	35.51	121 (18)	182 (100)	367 (50)
Tolclofos-methyl	20.81	125 (31)	250 (13)	265 (100)
Pyrethroids				
Cyfluthrin[a]	40.07	163 (100)	206 (78)	226 (50)
	40.32			
	40.50			
	40.58			
λ-Cyhalothrin	36.86	181 (100)	197 (79)	449 (6)
Cypermethrin[a]	40.79	163 (100)	181 (86)	209 (24)
	41.05			
	41.22			
	41.32			
Deltamethrin	44.93	181 (100)	209 (24)	253 (96)
Esfenvalerate	43.58	125 (100)	167 (81)	419 (18)
Fenpropathrin	34.62	97 (100)	181 (73)	349 (9)
Fenvalerate[a]	43.06	125 (100)	167 (85)	419 (17)
	43.58			
Permethrin[a]	38.51	163 (21)	183 (100)	390 (3)
	38.81			
Tetramethrin	34.31	123 (26)	164 (100)	227 (30)

[a] Compound with two or more isomeric forms.

chlorvos, cyfluthrin, fenvalerate) gave recoveries in the range 60–70% which could still be considered acceptable. Lower recovery of the water-soluble organophosphorus pesticide chlormephos (50–60%) was probably due to loss during the liquid–liquid partitioning. Moreover, some compounds gave better recoveries in certain matrices than in others: this could be probably due to the phenomenon known as a "matrix-induced chromatographic response enhancement". This fact has been extensively investigated by Erney et al. [37]. The standard deviations ranging from 1–8% and the recovery results suggest that the extraction and the clean-up procedure could be considered reliable enough for routine multiresidue screening. No correction factor was applied for calculation of analytical results.

In any case, recovery must be checked in every matrix when applying the method to a different commodity. In our screening activity other crops were tested: apple, grapes, kiwi, peach, strawberry, watermelon, kohl-rabi and spinach; no interfering peaks were noted in the sample extracts.

3.4. Confirmation by GC–MS

Due to the large probability of false positive results obtained by GC–ECD screening methods, confirmatory analysis was needed.

MS was carried out as screening method for confirmation of all positive and ambiguous results from GC analysis. As a primary step in the MS method development, pesticide working solutions were analysed by GC–MS scanning in full-scan mode with a scan range from 50 to 550 *m/z*. The total-ion chromatograms (TICs) for all pesticide standards were obtained and all spectral data were stored in the computer library. For confirmation of peak identity GC–MS operating in SIM, which allows higher instrumental sensitivity and lower detection limits, was applied. The three most representative fragments (a target ion and two qualifiers) and retention time window were selected for each pesticide peak from the TICs stored in the computer library. Single pesticide retention times, characteristic ions and their relative abundances, which were applied in SIM mode, are reported in Table 2. Target compounds in crop sample were identified by matching their retention times and characteristic ions with those of standards. A ±0.05 min retention time window was chosen for confident peak matching.

Some compounds showed two or more chromatographic peaks (endosulfan, propiconazole, cyfluthrin, cypermethrin, fenvalerate and permethrin) due to the presence of isomeric forms. In this case all isomers retention time windows were useful for compound identification. Esfenvalerate and fenvalerate showed identical spectral data as they are structural isomers.

A GC–MS-SIM chromatogram for a positive grape sample is reported in Fig. 9.

3.5. Applications of the method developed

Fig. 10 illustrates a chart summarizing the proposed procedure for multiresidue analysis. The method has been successfully applied for analytical testing of fruits and vegetables to determine compliance with the maximum residue limits. The procedure demonstrated acceptable performance for analysis of other commodities such as apple, grapes, kiwi, peach, strawberry, watermelon, kohol-rabi and

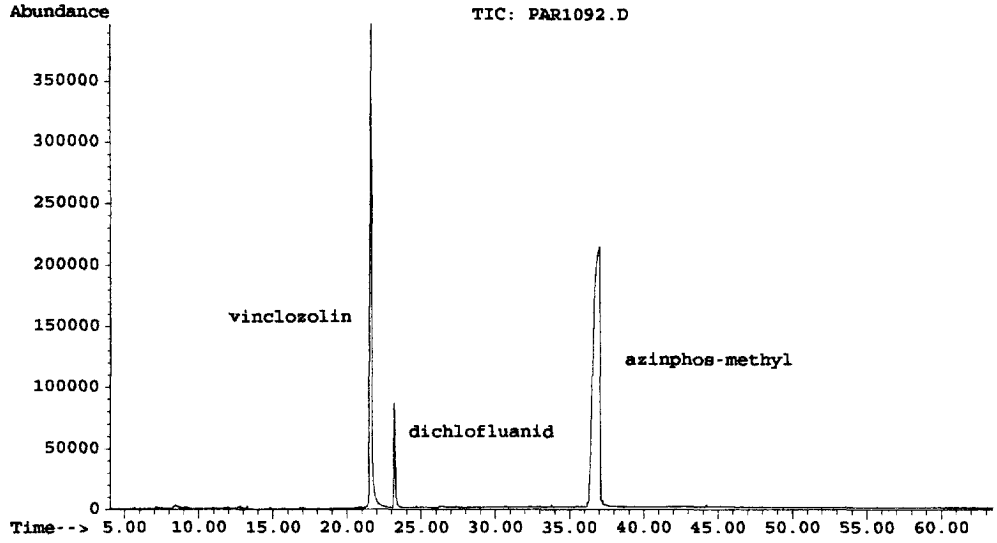

Fig. 9. GC–MS-SIM chromatogram for grape sample positive for vinclozolin (1.8 mg/kg), dichlofluanid (10.9 mg/kg) and azinphos-methyl (1.5 mg/kg; not included in the present study). Injection volume, 1 μl. GC–MS conditions are described in Section 2.2.3.

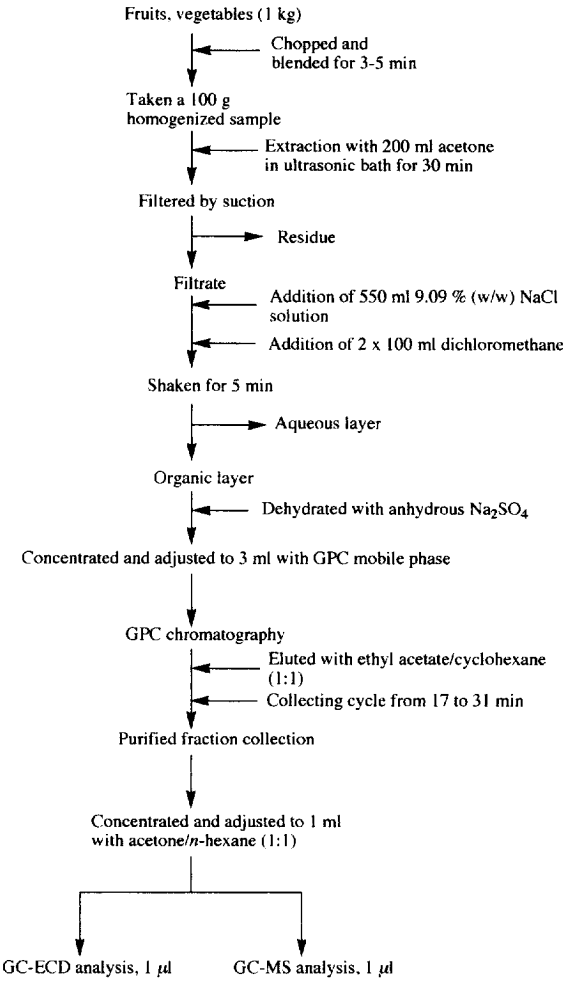

Fig. 10. Flow chart of the proposed method for multiresidue analysis of fruits and vegetables.

spinach. During a 9-month period (February–October 1996) 300 agricultural samples were screened for pesticide residues. Among all samples analysed nearly 35% gave positive results and 18% exceeded the MRL tolerance. Fungicide residues were the most frequently found and may be explained by their large application for post-harvest protection. Tolclofos-methyl and procymidone, an organophosphorus and an organonitrogen fungicide, respectively, were the most abundant and in many cases the latter exceeded the legislative limits. In a few cases residues from illegally sprayed crops were found.

Fig. 11 shows a typical GC–ECD chromatogram of a carrot sample positive for tolclofos-methyl and procymidone.

4. Conclusions

The increasing concern about food safety and the emergence of new analytical technologies are promoting the development of more accurate procedures for multiresidue analysis.

The aim of the paper was to contribute to a research field which is moving toward the development of very wide range screening methods [17,19,38]. Acetone extraction and dichloromethane partitioning showed high effectiveness for most tested compounds. However, toxicity of the chlorinated partition solvent poses the problem of searching for safety alternative methods. An interesting contribution has been recently given by Specht et al. [39]. GPC equipped with a 10 mm diameter column reduced solvent consumption and increased clean-up efficiency. Capillary column GC was successfully applied for achieving good resolution of compounds. GC analysis was carried out as primary screening method and for pesticide residue quantitation. Although GC–ECD showed good accuracy for all tested pesticides, a higher sensitivity and selectivity could be achieved by using FPD for P-containing compounds. MS operating in SIM mode has been used as secondary screening method for confirmation of pesticide identity.

The proposed method was applied in routine crop analysis. The pilot study also provided some useful indications which could constitute the scope of further research: necessity of using more specific detectors, increasing the number of tested matrices, expanding the range of investigated pesticides.

Acknowledgments

The authors are grateful to Consorzio Agrital Ricerche for financial support.

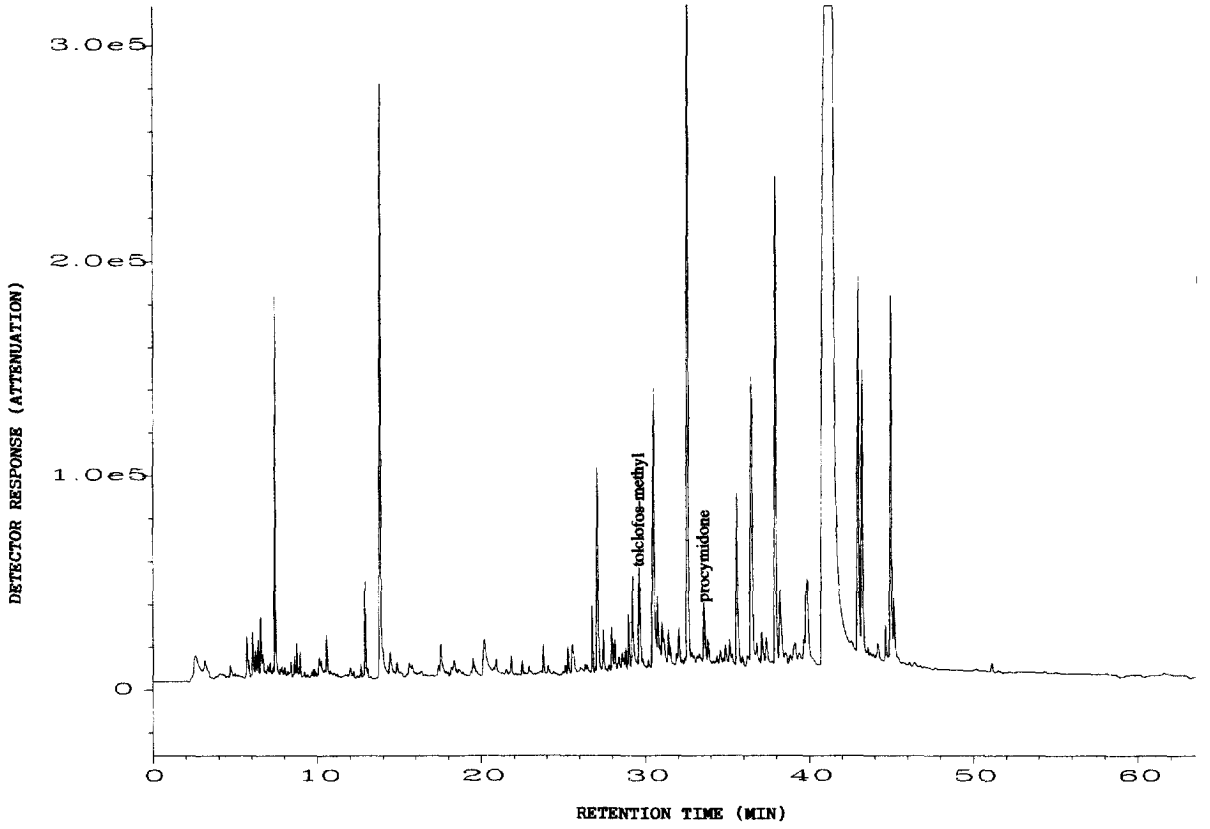

Fig. 11. Chromatogram of carrot extract obtained by GC–ECD. Sample was positive for tolclofos-methyl (0.07 mg/kg) and procymidone (0.03 mg/kg). Injection volume, 1 μl. GC conditions are described in Section 2.2.2.

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