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Multiresidue method for analysis of pesticides in pepper and tomato by gas chromatography with nitrogen–phosphorus detection

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Abstract

An analytical multiresidue method for the simultaneous determination of various classes of pesticides in vegetables (pepper and tomato) was developed. Vegetable samples are extracted with acetone and the pesticides are partitioned into ethyl acetate/cyclohexane. Final determination was made by gas chromatography with nitrogen–phosphorus detection. Confirmation analysis of pesticides was carried out by gas chromatography coupled with mass spectrometry in the selected ion monitoring (SIM) mode. The identification of compounds was based on retention time and on comparison of the primary and secondary ions. Recovery studies were performed at 0.05, 0.1 and 0.02 mg kg^{-1} fortification levels of each compound and the recoveries obtained ranged from 70.1% to 128.5% with relative standard deviations lower than 7%. The method showed good linearity over the range assayed 50–1500 μg l^{-1} and the detection and quantification limits for the pesticides studied varied from 0.1 to 4.4 μg kg^{-1} and 0.4 to 14.5 μg kg^{-1}, respectively. The proposed method was used to determine pesticides levels in peppers and tomatoes grown in experimental greenhouses.
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Keywords: Multiresidue; Pepper; Tomato; Pesticides; Gas chromatography

1. Introduction

The application of a large number of pesticides is essential in modern agricultural practices to control pest and diseases that damage vegetables and fruit. These compounds help to obtain an increase in the quality and harvest productivity. However, it has the drawback of pesticide residues which remain on fruit and vegetables, constituting a possible risk to consumers (Conacher & Mes, 1993). Therefore, governments and international organizations have established maximum residue levels (MRLs), limiting the amount of pesticides in foods.

Analysis of multiple pesticide residues in fruits and vegetables is often a time-consuming, labour-intensive, and expensive process due to the complexity of the many analytes and matrices involved. A large variety of methods have been used in the determination of different pesticides in these foods. A wide variety of techniques have been used to extract and to purify pesticides from fruits and vegetables, including liquid–liquid extraction (LLE) (Luke, Forberg, & Masumoto, 1975), solid-phase extraction (SPE) (Hsu, Biggs, & Saini, 1991; Rotich, Zhang, & Li, 2003), accelerated solvent extraction (ASE) (Adou, Bontoyan, & Sweeney, 2001), gel permeation chromatography (GPC) (Stan, 2000; Andersson & Palsheden, 1998), microwave-assisted extraction (MAE) (Barriada-Pereira et al., 2005), matrix solid-phase dispersion (MSPD) (Torres, Pico, & Manes, 1995) and supercritical fluid extraction (SFE) (Valverde Garcia, Fernandez Alba, Contreras, & Aguera, 1996).

The most frequently used technique for analysis of pesticide residues in fruits and vegetables is gas chromatography with different selective detectors as flame photometric (FPD) (Ueno, Oshima, Saito, & Matsumoto, 2003), pulsed flame photometric (PFPD) (Podhorniak, Negron, & Griffith, 2001), nitrogen–phosphorus (NPD) (Ueno, Oshima,

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Saito, & Matsumoto, 2001), and electron-capture detectors (ECD) (Gelsomino, Petrovicova, Tiburtini, Magnani, & Felici, 1997; Ueno, Oshima, Saito, Matsumoto, & Nakazawa, 2004). Numerous method use gas chromatography coupled with mass spectrometry (GC-MSD) (Gamón, Lleó, Ten, & Mocholí, 2001; Lehotay, de Kok, Hiemstra, & van Bodegraven, 2005), due to the possibility of confirming pesticide identity in these matrices.

In the case of non-volatile and/or thermally instable and/or polar pesticides and metabolites, liquid chromatography (LC) with diode array (DAD) (Lagana, D'Ascenzo, Fago, & Marino, 1997) and fluorescence detection (Fillion, Hindle, Lacroix, & Selwyn, 1995) has been also employed. Liquid chromatography coupled with mass spectrometry (LC–MS) (Pous, Font, & Picó, 2001; Picó, Font, Moltó, & Mañes, 2000) or with tandem mass spectrometry (LC–MS–MS) (Frenich, Vidal, Lopez, Aguado, & Salvador, 2004; Mol, van Dam, & Steijger, 2003) has lately become a powerful analytical technique for the identification and quantification of residues in fruits and vegetables.

The principal aim of this work was to develop a rapid multiresidue method for the analysis of 39 pesticides in pepper and tomato (M.A.P.A. Registro de Productos Fitosanitarios, 2006), commonly used in this cultivation in Spain (Vademécum 2004). The paper describes a simple and effective procedure for sample extraction, using a low volume of organic solvent and without cleanup. Residue levels in pepper and tomato, two of the main cultivation activities in the Region of Murcia (Spain), were determined by gas chromatography (GC) with nitrogen–phosphorus detection (NPD) with confirmation by gas chromatography (GC) with mass-selective detection (GC-MSD).

2. Materials and methods

2.1. Materials and standards

Reference pesticide standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany) with purity ranging from 94% to 100%. Acetone, acetonitrile, dichloromethane, ethyl acetate, cyclohexane and *n*-hexane, of special grading for the pesticide residue analysis, were obtained from Scharlau (Barcelona, Spain).

Pesticides stock solutions (1000 μg ml^{-1}) of individual pesticide standards were prepared by dissolving 0.025 g of the pesticide in 25 ml of ethyl acetate/cyclohexane (1/1, v/v).

A pesticide intermediate standard solution (10 μg ml^{-1}) was prepared by transferring 1 ml from each pesticide solution to a 100 ml volumetric flask and diluting to volume with ethyl acetate/cyclohexane (1/1, v/v) to obtain a concentration of 10 μg ml^{-1}. Several standard solutions, with concentrations of 0.05–2 μg ml^{-1}, were injected to obtain the linearity of detector response and the detection limits of the pesticides studied.

2.2. Apparatus

GC-NPD analysis was performed with an Agilent (Waldbronn, Germany) model HP 6890 gas chromatograph equipped with a nitrogen–phosphorus detector and automatic split–splitless injector model Agilent 7683. An HP-5MSI fused silica capillary column (30 m × 0.25 mm i.d.) and 0.25 μm film thickness, supplied by Agilent Technologies, was employed. Operating conditions were as follows: injector and detector temperatures, 250 and 325 °C, respectively; nitrogen as makeup gas at 25 ml min^{-1} and helium as carrier (constant pressure eluting, bromophos 20.08 min); hydrogen and air as detector gases at 3 and 60 ml min^{-1}. The column temperature was maintained at 70 °C for 2 min and then programmed at 25 °C min^{-1} to 150 °C, increased to 200 °C at a rate of 3 °C min^{-1} followed by a final ramp to 280 °C at a rate of 8 °C min^{-1}, and held for 10 min. The total analysis time was 41.87 min. The volume of sample injected in splitless mode was 1 μl. The concentration of each compound was determined by comparing the peak areas in the sample with those found for mixtures of pesticide standards of known concentration.

An Agilent model HP 6890 gas chromatograph equipped with a model 5973N mass spectrometric detector was operated in electron impact ionization mode with an ionizing energy of 70 eV, scanning from m/z 50 to 500 at 3.21 s per scan. The ion source temperature was 230 °C and the quadrupole temperature 150 °C. The electron multiplier voltage (EM voltage) was maintained at 1300 V, and a solvent delay of 4.5 min was employed. Gas chromatography was performed under the same conditions used in GC/NPD.

Analysis was performed with selected ion monitoring (SIM) mode using primary and secondary ions. Table 1 lists the pesticides along with their retention times, molecular mass, the target and qualifier ions, and their qualifier to target abundance ratios. The target and qualifier abundances were determined by injection of individual pesticide standards under the same chromatographic conditions using full scan with the mass/charge ratio ranging from m/z 50 to 500. Pesticides were confirmed by their retention times, the identification of target and qualifier ions, and the determination of qualifier-to-target ratios. Retention times had to be within ±0.1 min of the expected time, and qualifier-to-target ratios had to be within a 10% range for positive confirmation.

For the extraction of samples, a Polytron PT2000 homogenizer (Kinematica AG, Lucerne, Switzerland) was used.

An Eppendorf model 5810R centrifuge (Hamburg, Germany) and a Büchi model R-205 rotavapor (Flawil, Switzerland) was used in the centrifugation and evaporation to dryness of samples, respectively.

2.3. Sample preparation

Vegetable samples. Pesticide-free vegetables grown in two organic greenhouses of pepper and tomato localized

Table 1
Retention time (RT, min), molecular weight (MW), target (T), qualifier ions (Q_1, Q_2 and Q_3) (m/z) and abundance ratios (%) of qualifier ion/target ion (Q_1/T, Q_2/T and Q_3/T)[a] of the studied pesticides

Pesticide	RT	MW	T	Q_1	Q_2	Q_3	Q_1/T	Q_2/T	Q_3/T
1 Propyzamide	13.95	256.1	173	175	145	255	62.3	29.2	23.3
2 Pyrimethanil	14.13	199.3	198	199	200	77	46.5	6.0	6.2
3 Diazinon	14.47	304.3	179	137	152	199	96.8	67.8	59.0
4 Pirimicarb	15.69	238.3	166	72	238	167	50.4	25.3	10.5
5 Chlorpyrifos methyl	16.59	322.6	286	288	125	290	68.6	48.5	16.7
6 Tolclofos methyl	16.81	301.1	265	267	125	266	38.6	20.2	11.5
7 Pirimiphos methyl	18.31	305.3	290	276	305	233	80.1	36.9	31.4
8 Malathion	18.80	330.4	173	127	125	93	85.3	83.5	60.9
9 Chlorpyrifos ethyl	19.23	350.6	197	199	314	97	93.2	70.1	71.3
10 Triadimefon	19.39	293.8	57	208	85	210	76.5	28.9	26.1
11 Cyprodinil	20.54	225.3	224	225	210	226	62.8	10.3	8.7
12 Pendimethalin	20.99	281.3	252	253	281	162	14.9	12.7	12.4
13 Tolyfluanid	21.25	347.3	137	238	106	63	41.3	40.2	29.2
14 Triadimenol I	21.67	295.8	112	168	128	70	85.2	58.6	25.6
15 Triadimenol II	22.05	295.8	112	168	128	70	84.3	60.8	24.9
16 Fludioxonil	24.06	248.2	248	127	154	182	25.3	23.5	15.4
17 Buprofezin	24.58	305.5	105	106	104	172	48.2	46.7	41.0
18 Oxyfluorfen	24.73	361.7	252	302	331	361	43.2	41.5	32.3
19 Kresoxim methyl	24.98	313.4	116	206	131	222	66.1	58.3	44.4
20 Benalaxyl	26.75	325.4	148	91	206	204	42.6	27.8	21.0
21 Tebuconazole	27.43	307.8	125	250	70	83	99.6	46.0	47.7
22 Phosalone	29.68	367.8	182	121	184	367	37.5	30.8	24.1
23 Pyriproxyfen	29.93	321.4	136	96	78	137	10.7	10.2	10.2
24 λ-Cyhalothrin I	30.09	449.9	181	197	208	209	77.5	51.8	46.8
25 λ-Cyhalothrin II	30.37	449.9	181	197	208	209	83.6	53.6	47.6
26 Acrinathrin	30.71	541.4	181	208	93	289	63.3	52.6	40.5
27 Pyridaben	31.52	364.9	147	117	148	132	13.2	12.7	11.8
28 Cyfluthrin I	32.22	434.3	163	206	165	227	69.3	65.9	52.9
29 Cyfluthrin II	32.36	434.3	163	206	165	227	71.0	66.2	47.4
30 Cyfluthrin III	32.48	434.3	163	206	165	227	67.2	66.8	52.3
31 Cyfluthrin IV	32.54	434.3	163	206	227	199	65.7	52.4	46.5
32 Cypermethrin I	32.69	416.3	181	163	165	77	87.2	75.3	35.3
33 Cypermethrin II	32.84	416.3	181	163	165	209	95.0	80.3	37.8
34 Cypermethrin III	32.97	416.3	163	181	165	209	81.2	65.9	45.2
35 Cypermethrin IV	33.02	416.3	163	181	165	209	81.4	64.2	46.3
36 Fluvalinate-tau I	34.72	502.9	250	252	209	181	33.6	29.3	25.0
37 Fluvalinate-tau II	34.85	502.9	250	252	209	181	35.0	28.6	24.1
38 Deltamethrin	36.00	502.2	181	253	251	255	66.5	41.9	32.7
39 Azoxystrobin	36.72	403.4	344	388	345	372	30.4	28.7	15.8

[a] Q/T (%) ratios are the results of abundance values of the qualifier ion (Q_1, Q_2, Q_3) divided by the abundance of the target ion (T) × 100.

in Campo de Cartagena and Aguilas, (Murcia, Spain) respectively, were used as blank to spike samples for recovery studies.

Real samples were taken in six experimental greenhouses of pepper and tomato from the Region of Murcia.

Procedure. A 10 g representative portion of the sample was transferred into a 100 ml beaker and homogenized with 20 ml of acetone by means of an Polytron mixer for 2 min. After homogenization, 20 ml of ethyl acetate/cyclohexane (1/1, v/v) were added and then centrifuged for 10 min at 4000*g*. Extract was filtered quantitatively through glass funnel containing a filter paper DP302, 150 mm diameter (Albet, Barcelona, Spain). The organic phase was concentrated to dryness using rotary vacuum evaporation. The residue was redissolved in 5 ml of ethyl acetate/cyclohexane (1/1, v/v) and an aliquot analyzed using GC-NPD under conditions described above.

3. Results and discussion

3.1. Gas chromatographic determination

Pesticides residue levels were determined by GC-NPD. Fig. 1 shows representative chromatograms of a standard pesticide mixture, a pepper sample and a tomato sample spiked with the compounds of the standard solution. Three solvents (acetonitrile, acetone and ethyl acetate) were tested as extractants, and the best results were obtained with acetone for all compounds. In liquid–liquid extraction, ethyl acetate and the mixtures of ethyl acetate with *n*-hexane and cyclohexane were tested. The test with ethyl acetate was not continued because of

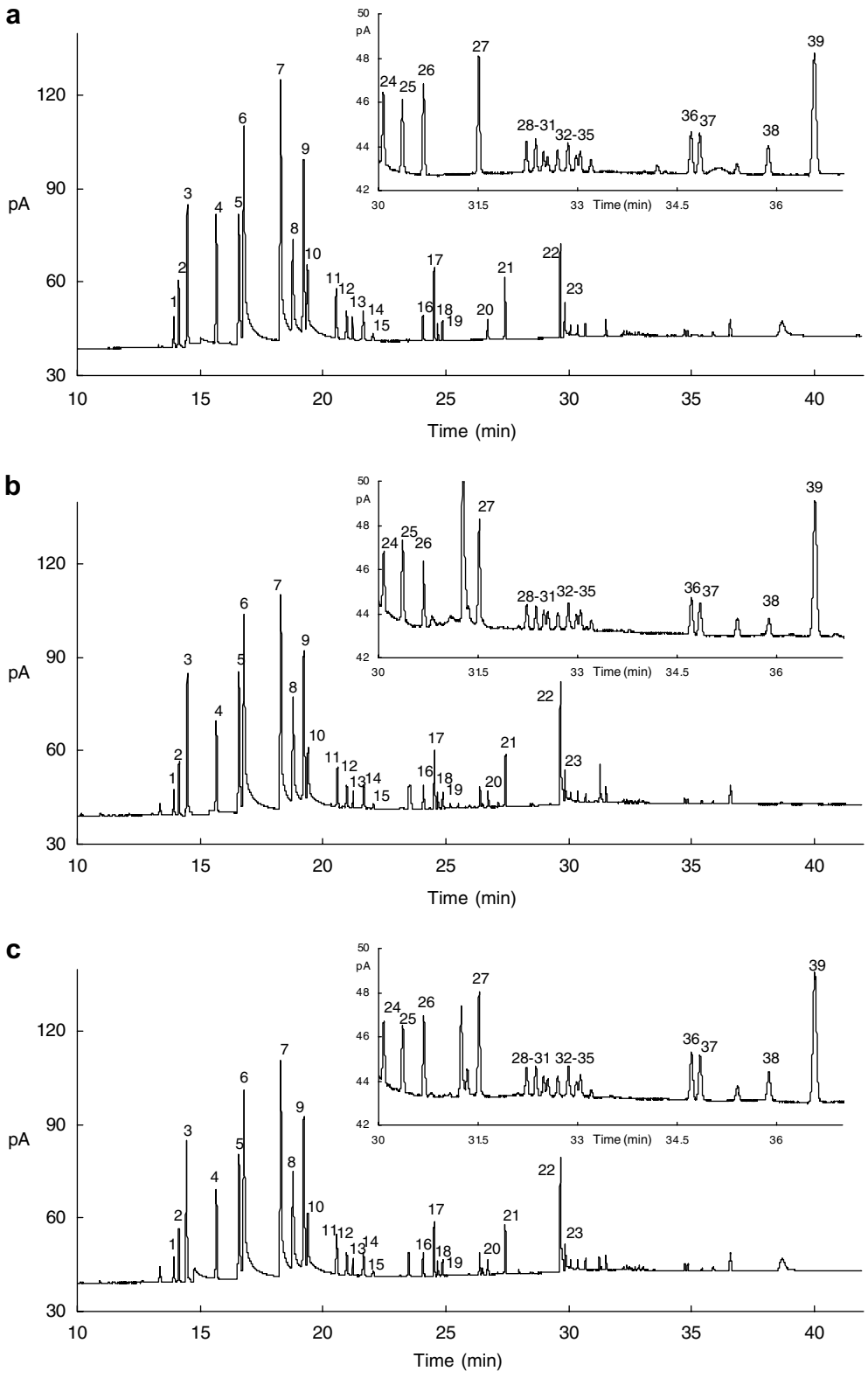

Fig. 1. Chromatograms (NPD) obtained for: (a) Standard solution (0.25 mg kg^{-1}, except propyzamide, tolyfluanid and oxyfluorfen 0.5 mg kg^{-1}). (b) Spiked red pepper sample (0.25 mg kg^{-1}, except propyzamide, tolyfluanid and oxyfluorfen 0.5 mg kg^{-1}). (c) Spiked tomato sample (0.25 mg kg^{-1}, except propyzamide, tolyfluanid and oxyfluorfen 0.5 mg kg^{-1}). For peak numbers see Table 1.

higher residual amounts of water in the organic phase after liquid–liquid partitioning. Good results were obtained with ethyl acetate/cyclohexane and ethyl acetate/*n*-hexane mixtures. Finally, a ratio acetone/ethyl acetate/cyclohexane of 2/1/1 was chosen. All pesticides were satisfactorily separated with high sensitivity and selectiv-

ity. The absence of coextracted interferences was confirmed by blank extract analysis (Fig. 2). The developed method provides clean blank extracts without interferences during GC and, therefore, cleanup of vegetable samples was not required.

3.2. Method validation

Linearity. The NPD response for all pesticides was linear in the concentration assayed (0.05–2 μg ml^{-1}) with determination coefficients >0.999 for all pesticides.

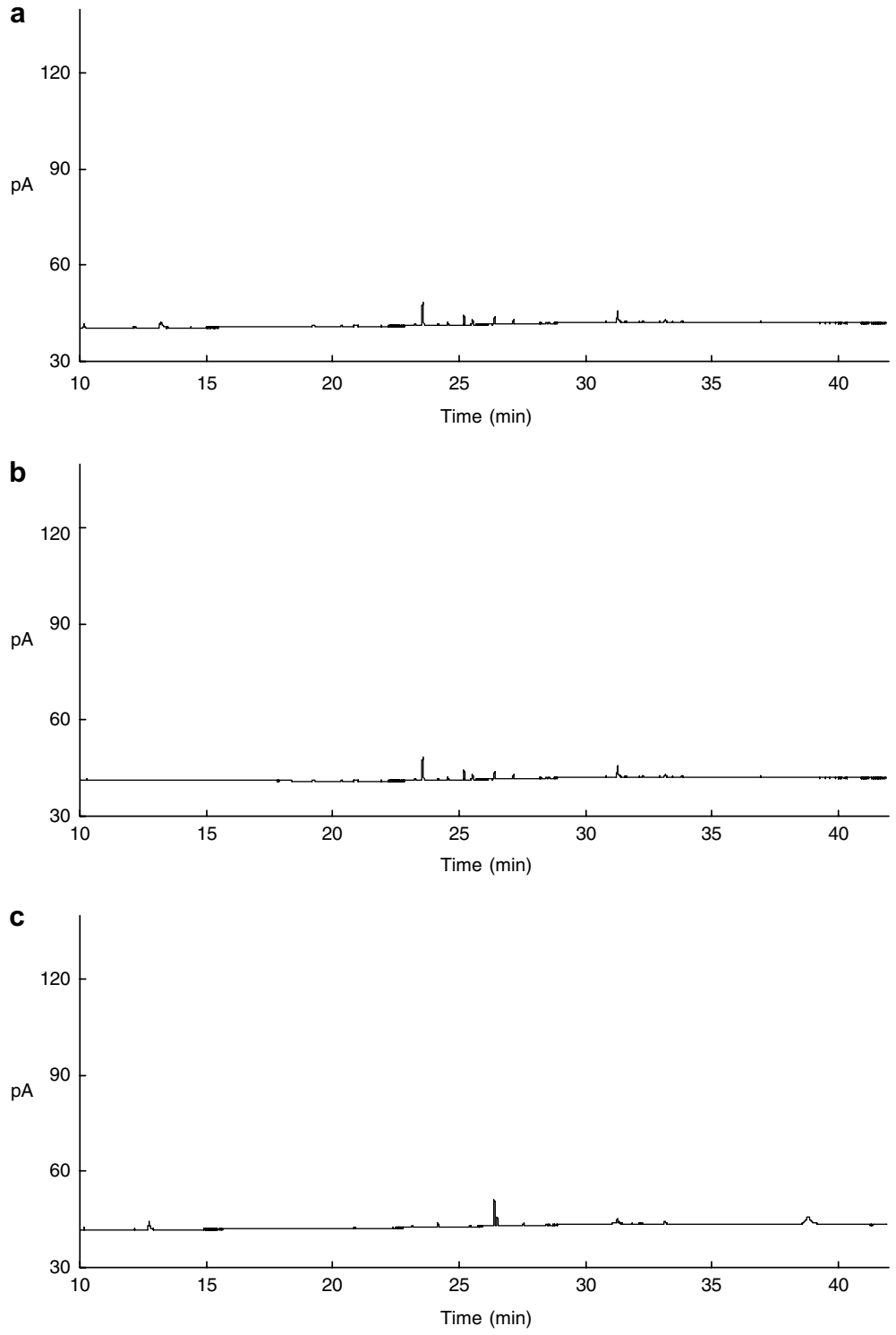

Fig. 2. Chromatograms (NPD) obtained for: (a) A control green pepper sample. (b) A control red pepper sample. (c) A control tomato sample.

Detection and quantification limits. The limits of detection (LOD) of the proposed method were determined at a signal-to-signal ratio of 3 for the individual pesticides in vegetables by GC-NPD, whereas the limits of quantification were obtained at a signal-to-signal ratio of 10. The LODs for all compounds range between 0.1 and 4.4 $\mu g\,kg^{-1}$ (Table 2). The range of LOD achieved is in the lower end of that obtained by other authors (Cook, Beckett, Reliford, Hammock, & Engel, 1999; Torres, Pico, & Manes, 1996; Gelsomino et al., 1997).

Recovery. A study of recoveries for each pesticide at three different fortification levels was carried out in order to assess the extraction efficiency of the proposed method. For that, five uncontaminated vegetables (red pepper, green pepper and tomato) samples were spiked with 0.05, 0.1 and 0.2 $\mu g\,g^{-1}$ of pesticide and processed as described. Average recovery data and relative standard deviations (RSD) obtained are shown in Table 3. The recoveries obtained for all pesticides ranged from 70.1% to 128.2% for red pepper, 70.3–128.5 for green pepper and 71.6–120.2 for tomato. The relative standard deviation (RSD) was <7.0% in the most unfavourable case.

3.3. Determination in real samples

Vegetables from experimental greenhouses from the Region of Murcia were sampled and analyzed following the extraction methods described above. Pesticide levels encountered in the collected samples are shown in Table 4. Analysis of real samples showed the validity of method used, which allowed the determination and identification of pesticides present in the samples.

The results of this study show that the proposed method, to determine residues of pesticides in various vegetables, is rapid, simple, sensitive and uses small volumes of solvents, reducing the risk for human health and the environment. Similar results have been obtained for green pepper and red pepper samples for most compounds. Good recovery and low detection through method were obtained for all the pesticides studied, including new generation pesticides, since their decomposition is quicker and has a less damaging effect on the environment. The method shows advantages compared with other conventional methods given the use of a low volume of organic solvent in the sample extraction as it avoids the use of a chlorinated hydrocarbon, short extraction time and the fact that a cleanup is not

Table 2
Limits of detection (LOD, $\mu g\,kg^{-1}$) and limits of quantification (LOQ, $\mu g\,kg^{-1}$) of pesticides assayed by GC-NPD

Pesticide	Limits of detection (LOD, $\mu g\,kg^{-1}$)			Limits of quantification (LOQ, $\mu g\,kg^{-1}$)		
	Red pepper	Green pepper	Tomato	Red pepper	Green pepper	Tomato
Propyzamide	1.4	1.2	0.8	4.6	3.9	2.6
Pyrimethanil	0.1	0.3	0.2	0.5	1.1	0.5
Diazinon	0.1	0.1	0.1	0.5	0.3	0.2
Pirimicarb	0.2	0.3	0.1	0.6	0.9	0.5
Chlorpyrifos methyl	0.1	0.2	0.2	0.5	0.7	0.8
Tolclofos methyl	0.3	0.3	0.2	0.9	0.9	0.6
Pirimiphos methyl	0.1	0.2	0.1	0.4	0.7	0.2
Malathion	0.7	0.8	0.9	2.3	2.6	2.9
Chlorpyrifos ethyl	0.5	0.8	0.4	1.6	2.5	1.4
Triadimefon	0.4	0.7	0.6	1.4	2.3	1.9
Cyprodinil	0.2	0.2	0.3	0.6	0.5	1.0
Pendimethalin	0.9	1.3	0.8	3.0	4.3	2.5
Tolyfluanid	3.2	4.0	3.3	10.5	13.2	10.9
Triadimenol	1.0	0.6	0.4	3.5	2.1	1.4
Fludioxonil	3.0	1.9	0.9	9.8	6.4	3.0
Buprofezin	0.6	0.8	0.3	1.9	2.8	1.1
Oxyfluorfen	2.1	2.8	2.3	7.1	9.2	7.7
Kresoxim methyl	1.4	1.3	1.6	4.7	4.5	5.3
Benalaxyl	1.0	1.4	0.8	3.5	4.6	2.6
Tebuconazole	0.6	0.3	0.7	2.0	1.0	2.2
Phosalone	0.6	1.1	0.3	2.0	3.6	1.0
Pyriproxyfen	0.6	0.8	0.7	2.0	2.6	2.2
λ-Cyhalothrin	2.8	3.4	3.0	9.4	11.4	10.0
Acrinathrin	4.2	3.3	2.9	13.8	11.0	9.7
Pyridaben	2.4	2.4	2.4	8.1	8.1	8.1
Cyfluthrin	2.6	2.3	3.1	8.6	7.8	10.4
Cypermethrin	3.8	3.1	3.4	12.6	10.4	11.2
Fluvalinate-tau	1.9	2.5	2.1	6.4	8.3	7.1
Deltamethrin	3.8	4.4	3.4	12.6	14.5	11.2
Azoxystrobin	0.7	0.7	0.5	2.5	2.3	1.8

Table 3
Recovery of pesticides from spiked vegetables samples[a]

Pesticide	Fortification level ($\mu g\ g^{-1}$)	Mean recovery ± RSD[b] (%)[a]		
		Red pepper	Green pepper	Tomato
Propyzamide	0.05	87.2 ± 4.5	79.6 ± 3.2	78.1 ± 3.9
	0.10	83.4 ± 3.1	78.1 ± 3.6	76.6 ± 3.5
	0.20	78.9 ± 3.7	89.7 ± 3.6	84.0 ± 4.8
Pyrimethanil	0.05	83.3 ± 3.8	88.0 ± 2.9	85.1 ± 3.5
	0.10	83.6 ± 2.8	81.5 ± 3.6	77.3 ± 1.6
	0.20	82.5 ± 2.9	90.3 ± 3.9	85.8 ± 6.8
Diazinon	0.05	77.1 ± 4.3	90.7 ± 4.9	76.8 ± 3.5
	0.10	81.4 ± 3.9	80.2 ± 4.2	73.2 ± 2.9
	0.20	81.3 ± 4.6	87.4 ± 3.6	76.5 ± 4.1
Pirimicarb	0.05	76.4 ± 3.8	70.3 ± 3.7	71.6 ± 2.5
	0.10	78.2 ± 3.1	72.9 ± 3.3	75.0 ± 2.6
	0.20	77.1 ± 3.6	77.0 ± 3.9	78.1 ± 3.3
Chlorpyrifos methyl	0.05	104.0 ± 3.9	106.6 ± 3.8	97.9 ± 3.5
	0.10	102.6 ± 3.0	104.3 ± 4.6	90.6 ± 2.7
	0.20	99.0 ± 7.0	108.8 ± 4.1	105.7 ± 4.3
Tolclofos methyl	0.05	80.5 ± 2.5	79.1 ± 3.1	98.6± 3.9
	0.10	81.2 ± 3.3	79.8 ± 4.5	85.8 ± 6.7
	0.20	85.5 ± 2.8	88.1 ± 3.7	83.9 ± 5.2
Pirimiphos methyl	0.05	83.6 ± 2.6	81.7 ± 3.8	83.6 ± 4.2
	0.10	84.3 ± 4.2	83.3 ± 4.9	78.1 ± 3.5
	0.20	81.6 ± 5.0	88.9 ± 3.6	87.6 ± 3.2
Malathion	0.05	108.4 ± 3.6	111.5 ± 2.4	110.1 ± 4.0
	0.10	102.5 ± 4.2	108.6 ± 2.3	104.2 ± 3.1
	0.20	115.0 ± 2.2	111.4 ± 3.2	106.9 ± 2.9
Chlorpyrifos ethyl	0.05	89.4 ± 3.7	95.1 ± 2.9	90.4 ± 4.3
	0.10	90.0 ± 5.2	92.6 ± 4.5	83.9 ± 5.9
	0.20	86.3 ± 3.9	95.2 ± 5.2	91.7 ± 4.6
Triadimefon	0.05	83.3 ± 4.5	80.8 ± 5.7	81.2 ± 3.8
	0.10	81.2 ± 3.4	81.8 ± 3.7	75.7 ± 3.1
	0.20	80.6 ± 3.1	85.2 ± 4.5	83.3 ± 3.6
Cyprodinil	0.05	80.7 ± 4.5	83.7 ± 4.9	84.5 ± 3.9
	0.10	81.4 ± 3.7	80.8 ± 3.0	77.1 ± 3.8
	0.20	83.4 ± 2.8	86.5 ± 4.5	84.2 ± 2.2
Pendimethalin	0.05	80.7 ± 4.9	77.8 ± 4.2	85.8 ± 4.5
	0.10	77.0 ± 3.8	75.8 ± 3.5	78.7 ± 3.3
	0.20	75.6 ± 2.4	80.0 ± 3.7	83.2 ± 2.8
Tolyfluanid	0.05	71.3 ± 2.2	71.6 ± 2.2	90.1 ± 4.6
	0.10	72.6 ± 3.2	91.1 ± 3.5	105.0 ± 6.5
	0.20	79.2 ± 2.9	86.7 ± 3.2	108.9 ± 2.7
Triadimenol	0.05	87.2 ± 4.5	80.6 ± 4.2	89.9 ± 3.3
	0.10	77.1 ± 5.2	78.4 ± 3.6	79.8 ± 3.2
	0.20	78.2 ± 2.8	78.2 ± 2.0	82.0 ± 2.7
Fludioxonil	0.05	95.8 ± 3.4	71.5 ± 4.3	86.9 ± 3.1
	0.10	91.7 ± 3.3	88.4 ± 3.6	83.4 ± 3.0
	0.20	88.2 ± 4.3	95.4 ± 3.0	86.4 ± 2.9
Buprofezin	0.05	77.5 ± 3.2	79.9 ± 4.1	77.9 ± 3.1
	0.10	79.7 ± 3.0	83.7 ± 3.4	76.6 ± 3.5
	0.20	77.2 ± 2.3	86.1 ± 3.0	84.2 ± 2.4
Oxyfluorfen	0.05	88.1 ± 2.9	75.8 ± 4.6	76.0 ± 4.9
	0.10	84.4 ± 3.9	86.4 ± 3.6	77.6 ± 3.9
	0.20	85.5 ± 4.6	84.7 ± 2.9	76.5 ± 1.9
Kresoxim methyl	0.05	92.4 ± 5.2	78.3 ± 4.7	84.0 ± 5.6
	0.10	82.4 ± 4.0	86.4 ± 3.6	81.4 ± 2.5
	0.20	86.4 ± 4.7	85.4 ± 4.3	80.0 ± 4.7
Benalaxyl	0.05	84.0 ± 3.5	71.6 ± 3.0	91.5 ± 4.8
	0.10	87.6 ± 3.9	72.8 ± 3.6	81.8 ± 3.5
	0.20	84.2 ± 3.8	91.4 ± 4.3	85.9 ± 3.5
Tebuconazole	0.05	79.9 ± 3.1	71.4 ± 3.5	92.9 ± 4.6
	0.10	80.7 ± 5.4	76.6 ± 3.0	83.0 ± 4.5
	0.20	80.1 ± 2.9	81.3± 2.6	82.6 ± 3.2
Phosalone	0.05	128.2 ± 3.4	126.6 ± 3.4	109.3 ± 6.3
	0.10	127.2 ± 3.6	128.5 ± 2.1	120.2 ± 3.0
	0.20	121.3 ± 4.8	127.2 ± 2.9	115.2 ± 4.0
Pyriproxyfen	0.05	107.3 ± 5.9	77.6 ± 3.5	82.6 ± 4.2
	0.10	97.8 ± 3.6	75.3 ± 2.5	77.6 ± 3.7
	0.20	87.3 ± 4.1	86.0 ± 2.9	83.9 ± 3.3
λ-Cyhalothrin	0.05	85.2 ± 3.9	93.5 ± 5.5	98.2 ± 4.8
	0.10	90.4 ± 3.3	103.9 ± 2.1	85.7 ± 3.5
	0.20	94.1 ± 3.8	90.6 ± 5.3	89.8 ± 4.4
Acrinathrin	0.05	70.1 ± 2.2	89.7 ± 3.5	92.1 ± 3.2
	0.10	71.3 ± 3.1	78.4 ± 3.9	93.1 ± 6.5
	0.20	70.8 ± 2.8	73.8 ± 3.3	97.9 ± 1.8
Pyridaben	0.05	76.9 ± 6.2	70.8 ± 4.0	90.7 ± 5.9
	0.10	72.1 ± 4.5	72.5 ± 3.6	75.7 ± 4.1
	0.20	78.4 ± 5.3	88.0 ± 3.1	82.6 ± 3.6
Cyfluthrin	0.05	103.2 ± 5.1	115.0 ± 3.9	107.5 ± 3.0
	0.10	93.8 ± 3.7	93.0 ± 3.1	98.6 ± 4.1
	0.20	94.4 ± 4.0	98.4 ± 3.3	100.1 ± 3.6
Cypermethrin	0.05	99.4 ± 3.8	80.3 ± 2.3	106.3 ± 3.0
	0.10	85.4 ± 4.2	90.5 ± 3.6	94.8 ± 3.9
	0.20	84.2 ± 3.8	84.9 ± 2.4	96.3 ± 3.6
Fluvalinate-tau	0.05	73.0 ± 3.5	93.3 ± 2.0	103.9 ± 2.6
	0.10	73.6 ± 3.0	93.5 ± 3.6	105.7 ± 4.8
	0.20	73.8 ± 2.8	103.9 ± 2.5	103.9 ± 3.6
Deltamethrin	0.05	72.3 ± 3.7	72.4 ± 2.4	80.6 ± 4.3
	0.10	71.2 ± 3.2	98.5 ± 3.6	109.5 ± 3.0
	0.20	74.6 ± 2.8	90.8 ± 2.4	92.8 ± 2.7
Azoxystrobin	0.05	103.3 ± 5.5	102.2 ± 3.3	112.9 ± 5.3
	0.10	100.1 ± 3.9	96.4 ± 3.6	95.1 ± 3.5
	0.20	98.0 ± 4.1	100.4 ± 2.7	101.1 ± 4.2

[a] $n = 5$.
[b] Relative standard deviation.

required. Another advantage of the method is the application to the analysis of pesticides in pepper and tomato samples collected in experimental greenhouses from the Region of Murcia, where several pesticides were found. Finally, our method is versatile and is capable of allowing the inclusion of new pesticides used in these agricultural growing.

Table 4
Pesticide residues found in real vegetable samples

Sample	Pirimicarb[a] (μg g^{-1})	Pyriproxyfen[a] (μg g^{-1})	Tebuconazole[a] (μg g^{-1})	Buprofezin[a] (μg g^{-1})
Red pepper 1	0.01 ± 0.002			
Red pepper 2		0.02 ± 0.005		
Green pepper 1	0.03 ± 0.006			0.02 ± 0.004
Green pepper 2				0.01± 0.003
Tomato 1			0.01 ± 0.002	0.03 ± 0.006
Tomato 2				0.04 ± 0.004

[a] Mean of four determinations ± RSD.

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