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Application of gas chromatography–tandem mass spectrometry to the analysis of pesticides in fruits and vegetables

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Abstract

A new analytical method was devised using gas chromatography with tandem mass spectrometry (GC–MS–MS) for the routine analysis of 31 multi-class pesticide residues and approximately 8000 fresh fruit and vegetable samples (green bean, cucumber, pepper, tomato, eggplant, watermelon, melon, and marrow). Extraction of the pesticides with dichloromethane was carried out. The optimal ionization mode, either electron impact or chemical ionization, was selected for each pesticide in the same run. Carbofrit was used in the liner and combined with the selectivity of the detector this avoided additional clean-up. Thus, not only was money and time saved, the uncertainty of the method was decreased in its application to routine analysis. The average recoveries in cucumber obtained for each pesticide ranged between 71 and 119% at two different fortification levels ($n=10$ each) that ranged between 7 and 300 ng g^{-1} (depending on the pesticide). The relative standard deviation was lower than 19% for all compounds tested. The calculated limits of detection and quantification were typically <1 ng g^{-1} which were much lower than the maximum residue levels established by European legislations.

Keywords: Fruits; Vegetables; Food analysis; Pesticides

1. Introduction

Pesticides are essential in modern agricultural practices but, due to their biocide activity and potential risk to consumers, the control of the presence of pesticide residues in foods is a growing source of concern for the general population [1]. Governments and international organizations are regulating the use of pesticides setting the maximum residue levels (MRLs) in foods. Pesticide monitoring programs are established by governments in order to assess and control the quality of vegetables, and thereby evaluate and enforce the proper usage of pesticides in agricultural practices.

To detect the large number of pesticides applied to crops typically requires the use of analytical separation techniques such as gas chromatography (GC) or high-performance liquid chromatography (HPLC). Both techniques have been widely used with class-selective detection methods, especially for GC with

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electron-capture (ECD), flame photometric (FPD), and nitrogen-phosphorus (NPD) detection. None of these detectors are confirmatory (do not provide unambiguous results) [2,3] and all them are subject to the interference of the matrix. For this reason mass spectrometry (MS) has become very popular in pesticide residue laboratories. It can quantify and confirm the results by its full scan or selected ion monitoring (SIM) spectra. Unfortunately, full scan often does not provide enough sensitivity in real samples but SIM, which improves sensitivity, reduces considerably the qualitative information, thus increasing the risk of false positives [4]. The combination of GC with tandem mass spectrometry (MS–MS) has been shown to be applicable to the analysis of trace amounts of contaminants like pesticides or metabolites in complex samples such as biological fluids [5–7], waters [8,9] or fruits and vegetables [10,11].

The increased selectivity of this technique reduces the influence of the matrix, and lowers the limits of detection [12–14]. The degree of trust in the results is improved because the quality of the information obtained is not reduced as in SIM. The benchtop ion trap detector offers this MS–MS technology at a reasonable price and personnel training [15,16].

Extraction strategies in multi-class pesticide residues analysis are varied [1,17] but the use of organic solvent extractions are preferred in routine laboratory analysis because of their simplicity, speed, and high recoveries for compounds in a wide range of polarity [18]. Clean-up steps usually accompany this process, which reduces the amount of interferences and their negative influences on the selectivity of the analytical signal and maintenance of instruments [19–23]. However, these clean-up processes are expensive and time-consuming and increase the imprecision of the method.

This paper proposes a new method to determine 31 multi-class pesticides in fresh vegetables suitable for routine analysis. It is based on a fast and simple dichloromethane extraction of the vegetable without clean up and GC–MS–MS analysis. The method was validated and applied, using quality control criteria established in the laboratory of pesticide residues CUAM, to the analysis of 8000 samples of fruits and vegetables grown in greenhouses of El Ejido (Almería, Spain).

2. Experimental

2.1. Chemicals

Dichloromethane, cyclohexane, hexane, acetone, and methanol (for analysis of pesticide residues) from Scharlau (Barcelona, Spain) were used as received. Pesticide analytical standards of the pesticides and caffeine, which was used as an internal standard (I.S.), were purchased with the purity certified from Dr. Ehrenstorfer (Ausburg, Germany). Stock solutions of individual pesticides were prepared in acetone at concentrations that ranged between 75 and 550 mg l^{-1} and stored in a freezer at −30 °C (1 year of maximum storage time). Working solutions were obtained by appropriate dilutions with cyclohexane and stored in a refrigerator (4 °C) (2 months of maximum storage time). No degradation was observed for the compounds in the above-mentioned storage times. Anhydrous sodium sulfate for residue analysis was obtained from Panreac (Barcelona, Spain).

2.2. Apparatus

A Saturn 2000 GC–MS–MS system (Varian, Walnut Creek, CA, USA) was used for all work. A Varian 8200 autosampler was used to perform 10-μl injections using a 100-μl syringe. The gas chromatograph (model CP-3800) was fitted with a split/splitless programmed temperature injector 1079 operated in the large volume injection mode and an electronic flow control (EFC) system. The glass liner contained a Carbofrit (Restek, Bellefonte, PA, USA) plug. A fused-silica untreated capillary column 2 m×0.25 mm I.D. from Supelco (Bellefonte, PA, USA) connected to an DB-5MS 30 m×0.25 mm I.D., 0.25 μm film thickens analytical column from J&W Scientific (Folsom, CA, USA) were used. The ion trap electrodes were SilChrom coated to reduce chemical interaction with the surfaces. The mass spectrometer was operated in electron impact (EI) and chemical ionization (CI) modes and the MS–MS option was used. An MS–MS library was created for the target analytes at our experimental conditions and commercial MS libraries are available for additional helpful information [24,25]. Helium (purity 99.999%) was used as carrier gas.

2.3. Sample collection and storage

Fresh vegetables were sampled and transported following the 79/700/CEE directive [26]. Samples were analyzed within 24 h being stored at 4 °C until the moment of the extraction.

2.4. Analytical procedure

2.4.1. Extraction procedure

Two-kg vegetable samples were chopped with an appropriate chopper (Hamilton Beach, Washington, USA). A 50.0-g aliquot was weighed into a glass container and homogenized with 105 ml of dichloromethane in Polytron PT2100 (Kinematica, Littan/Luzern, Switzerland) for 2 min. Anhydrous sodium sulfate (80 g) was then added. The mixture was allowed to rest for 2 min, then filtered through a 12-cm Büchner funnel and filtered again through paper filter with anhydrous sodium sulfate into a spherical flask. Evaporation of the solvent to dryness was done in a rotary evaporator (35–40 °C). The dried residue was re-dissolved with 5 ml of cyclohexane and 1 ml of this solution was added to a 2-ml volumetric flask along with 50 μl of I.S. solution. The final 2-ml volume was reached with cyclohexane and 10 μl of this final extract was injected in the analyzer using a sandwich injection technique.

2.4.2. Instrumental conditions

The injector temperature was programmed from 70 °C (hold 0.5 min) to 300 °C at 100 °C min^{-1} (hold 10 min). The split vent was initially open then closed at 0.5 min to transfer the analytes to the column for another 3 min. The carrier gas head pressure was set initially at 8 p.s.i. (hold 26 min). Then it was reduced from 8 to 6 p.s.i. with a gradient of 2 p.s.i. min^{-1} (hold 13 min), and then raised to 9 p.s.i. at 3 p.s.i. min^{-1} (hold 15.1 min) (1 p.s.i.= 6894.76 Pa). The oven temperature was ramped from 70 °C (hold 3.5 min) to 150 °C at 50 °C min^{-1}, then to 180 °C at 5 °C min^{-1} (hold 5 min), then to 205 °C at 4 °C min^{-1} (hold 5 min) and finally to 300 °C at 4 °C min^{-1} (hold 5 min). Total time for the GC analysis was 56.1 min.

The mass spectrometer was calibrated weekly following the autotune test of the software. Air and water were checked daily as well as the pressure of CI reagent (methanol). All the compounds were analyzed using a non-resonant wave form type. Depending on the analyte, two ionization modes (CI and EI) were used. The common parameters for both modes were multiplier voltage (1×10^5 gain) of 1700 V, multiplier offset +200, and trap, manifold and transferline temperatures were 200, 50 and 280 °C, respectively. Automatic gain control (AGC) was turned on. For CI mode the emission current was set to 30 μA, prescan ionization time was 100 μs and the AGC target value was 2000 counts. In EI mode those values were 80 μA, 1500 μs, and 5000 counts, respectively. Specific MS–MS conditions for each analyte are listed in Table 1.

3. Results and discussion

3.1. Effect of experimental variables

3.1.1. Instrumental variables

The GC conditions were optimized to separate the pesticides studied. For that, different temperature and gas flow programs were tested in order to resolve the analytes of the standard mixture in a reasonable time. It must be mentioned that the detector can determine up to four pesticides that co-elute in the MS–MS mode. Volumes of 10 μl were injected by a typical large-volume technique in order to potentially decrease the detection limits [27].

For the MS, sensitivity was maximized by means of optimizing the amount of target ions into the trap with the AGC. Higher AGC target values than we use can cause electrostatic interactions between the ions providing worse signals and therefore lower sensitivity. The parent ion was selected for each analyte considering its m/z and relative abundance (both as high as possible) in order to improve sensitivity and selectivity. The optimization of collision-induced dissociation (CID) parameters was carried out in order to generate MS–MS spectra with a relative abundance of the parent ion between 10 and 30%. The base peak obtained was selected for quantification in most cases, except for some analytes that presented various intense peaks (metalaxyl) or the main ion was a cluster (bromopropylate). In these cases, the sum of the main ions was used to

Table 1
MS–MS conditions

Compound	Start time (min)	m/z range	Parent ion (m/z)	Quantif. ions (m/z)	Isolation window (m/z)	Excitation storage level (m/z)	Excitation amplitude (V)
Methamidophos[a]	7	70–230	142	126+141	5	45	40
Acephate[a]	9	70–160	143	79+157	5	60	56
Ethoprophos[a]	11.5	80–260	243	131+173	5	80	52
Lindane	15	90–320	219	180:185	3	100	70
Chlorthalonil	17.15	80–305	266	133	3	85	86
Etrimphos	17.15	80–305	292	181	3	70	45
Pirimicarb	17.15	80–305	166	83	3	53	49
Caffeine (I.S.)	17.15	80–305	194	120	3	60	58
Methyl-parathion	19.5	80–300	263	136	3	80	48
Vinclozoline	19.5	80–300	285	241	3	105	44
Metalaxyl	19.5	80–300	206	132+162	3	75	55
Fenitrothion	21.3	70–325	260	125	3	71	59
Malathion	21.3	70–325	173	99	3	75	54
Triadimefon	23	70–265	208	144	3	75	62
Pendimethalin	24.2	90–340	252	208+162	3	95	65
Chlozolinate	24.2	90–340	331	259	3	145	58
Procymidone	26.4	65–300	283	253:257	3	80	57
Triflumizole	26.4	65–300	218	183	3	76	68
α-Endosulfan	27.7	90–255	241	170:172	3	80	84
Fenamiphos	29.5	80–320	303	195	3	95	56
Myclobutanil	31.3	80–260	179	125	3	80	65
β-Endosulfan	31.3	80–260	241	170:172	3	80	84
Ethion	31.3	80–260	231	175	3	100	63
Carbofenothion	34.5	80–360	342	296	3	131	65
Endosulfan-sulfate	34.5	80–360	272	235:238	3	80	64
Tebuconazole	37.2	90–265	250	125	3	75	63
Bromopropylate	38.8	90–350	341	181:187	3	70	46
Fenpropathrin	38.8	90–350	265	210	3	95	69
Acrinathrin	41.5	90–275	181	152	3	80	90
Pyrazophos	41.5	90–275	265	210	3	80	53
Cypermethrin	45	90–180	163	127	3	70	53
Difenoconazole	48.5	90–240	323	265	3	122	84

[a] Chemical ionization mode (methanol).

quantify. Fig. 1 shows the MS–MS spectra of the mentioned examples.

3.1.2. Extraction variables

An extraction with organic solvent was selected to extract the pesticides from the fresh vegetables because of its simplicity and suitability to routine analysis [28]. Dichloromethane was chosen because of its extractive capacity of pesticides with different chemical and physical properties. Nevertheless, the low selectivity of dichloromethane led to the co-extraction of several matrix interferences that usually necessitate additional clean-up steps. Such interferences typically reduce the selectivity of the detection method and dirty the instrument increasing time for instrument maintenance. However, we have used a simpler and less expensive alternative to clean-up steps using the combination of Carbofrit glass liner packing with MS–MS detection as others have done [27]. Carbofrit reduces the amount of low volatile and interference substances in the instrument and the MS–MS increases the selectivity of the analytical signal. This combination is very convenient in the application of the method to the routine analysis of a large number of samples. It can be observed that the change of glass liner, carbofrit and guard column is recommended after every 180–200 samples. However, the absence of Carbofrit generated an incre-

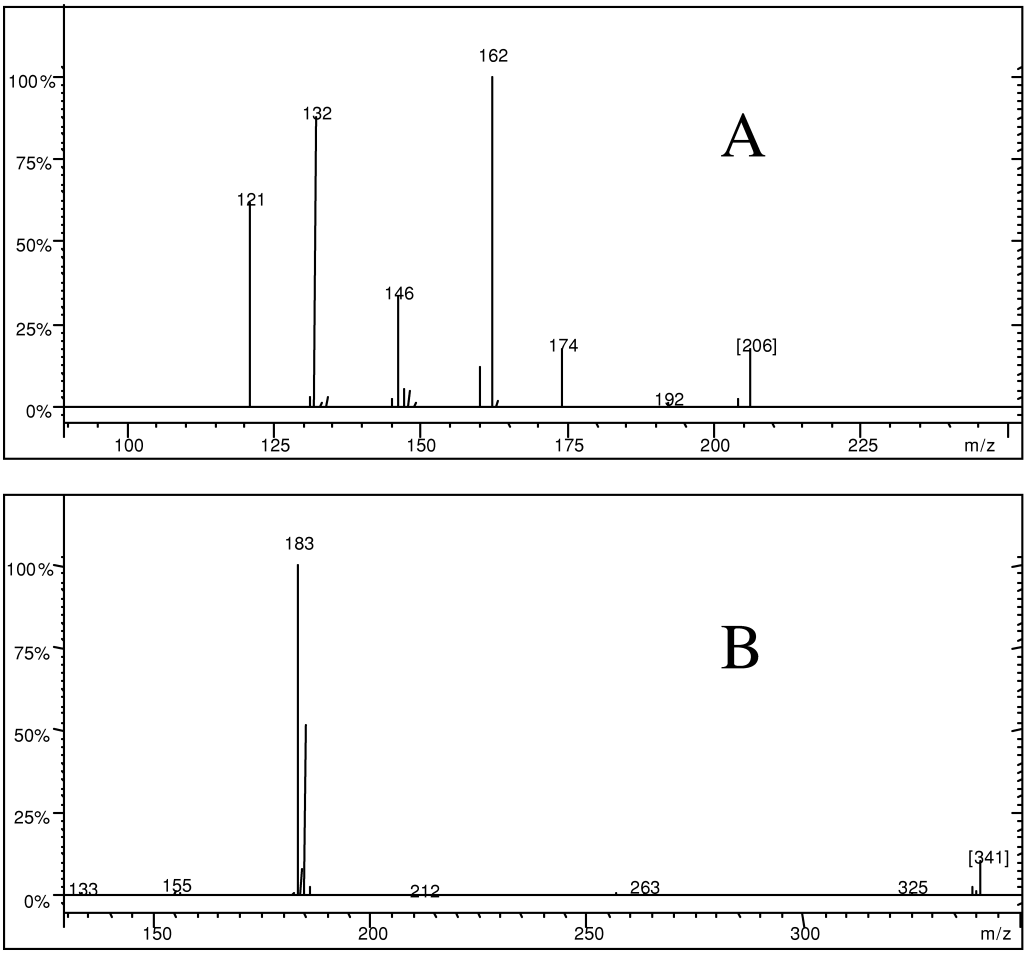

Fig. 1. (A) Spectrum of metalaxyl; ions 132 and 162 selected as quantification ions. (B) Spectrum of bromopropylate; cluster of ions 181:187 selected as quantification ions.

ment in the frequency of maintenance of the instrument, especially to the analytical column (column clogging that required to cut a few centimeters to the inlet end).

3.2. Validation of the method

3.2.1. Identification of target analytes

Retention time windows (RTWs) were defined as retention time average ±10 standard deviations of the retention time of 10 blank samples spiked at a mid-level calibration standard for each compound. The target analytes were searched in the appropriate RTW. Additionally, inter-day repeatability of the retention time is shown in Table 4.

3.2.2. Quantification of target analytes

Blank sample extracts spiked with the pesticides at three different concentration levels were injected to perform instrument calibration. The linearity of the calibration curves was studied for each pesticide considering area of peaks relative to the I.S. Better quantification results were obtained when the origin point (0,0) was included in the linear regressions. Good linearity of the response was found for all pesticides at concentrations within the tested interval, with linear regression coefficients higher than 0.967.

Table 2 shows the results. A study of recoveries for each pesticide at two different fortification levels was carried out in order to assess the extraction efficiency of the proposed method. For that, 10 uncontaminated cucumber samples were spiked with the pesticides and processed as described. Average recovery data and relative standard deviations (RSDs) obtained are shown in Table 3. Recoveries of the compounds between 70 and 120% have been set as a criterion for validation of the method. All pesticides presented gave acceptable recoveries within the cited interval of validation and the RSD was lower than 18% in all cases. The signals from the chromatograms of 10 blank cucumber samples extracted and injected were evaluated as recommended by IUPAC [29] to estimate the limits of detection (LOD) and quantification (LOQ). The results obtained are also shown in Table 3. Inter-day data for recovery and repeatability ($n=10$) are shown in Table 4. The data were obtained from measures on different days and using different calibration curves. The recovery data were within the validation interval and the RSD was lower than 19% in all cases.

3.2.3. Confirmation procedure

The confirmation of a compound previously identified by retention time only was done comparing the MS–MS spectra obtained in the sample with those previously stored as reference spectra. The reference spectra were daily obtained and stored injecting a blank cucumber sample spiked at the concentration of the second calibration level. The comparison

Table 2
Average retention times and typical calibration parameters

Analyte	t_R (min)	Calibration range (mg kg^{-1})		Calibration		
				Slope	Intercept	R^2
Methamidophos	7.68	0.005	0.025	2.641	−0.016	0.990
Acephate	10.10	0.010	0.050	0.878	−0.009	0.998
Ethoprophos	12.76	0.005	0.025	4.539	−0.090	0.995
Lindane	16.87	0.100	0.500	0.840	−0.257	0.985
Etrimfos	17.85	0.010	0.050	0.435	−0.041	0.999
Chlorthalonil	18.52	0.100	0.500	0.262	−0.153	0.955
Pirimicarb	18.53	0.020	0.100	1.023	−0.034	0.980
Vinclozoline	20.41	0.100	0.500	0.441	−0.007	0.999
Methyl-parathion	20.50	0.020	0.100	0.721	0.010	0.998
Metalaxil	20.73	0.050	0.250	1.729	0.014	0.999
Fenitrothion	21.90	0.100	0.500	2.487	0.029	0.997
Malathion	22.18	0.020	0.100	2.060	0.183	0.983
Triadimefon	23.63	0.020	0.100	1.011	−0.019	0.996
Pendimethalin	24.90	0.020	0.100	6.326	−0.259	0.991
Chlozolinate	25.82	0.020	0.100	0.989	−0.010	0.998
Procimidone	26.97	0.100	0.500	0.844	−0.074	0.996
Triflumizole	27.11	0.020	0.100	0.155	−0.005	0.982
α-Endosulfan	28.32	0.100	0.500	0.057	−0.004	0.993
Fenamiphos	30.56	0.020	0.100	9.444	−0.141	0.995
Myclobutanil	32.03	0.005	0.025	0.760	−0.019	0.998
β-Endosulfan	33.61	0.100	0.500	0.250	−0.002	0.996
Ethion	33.78	0.020	0.100	12.227	−0.364	0.997
Carbofenothion	35.75	0.005	0.025	0.481	−0.010	0.990
Endosulfan-sulfate	36.39	0.100	0.500	0.026	−0.005	0.997
Tebuconazole	37.97	0.020	0.100	1.272	−0.030	0.997
Bromopropylate	39.74	0.010	0.050	3.905	1.351	0.990
Fenpropathrin	40.10	0.010	0.050	0.750	−0.018	0.987
Acrinathrin	41.93	0.005	0.025	1.500	0.071	0.994
Pyrazophos	42.73	0.005	0.025	0.752	−0.043	0.949
Cypermethrin	47.10	0.020	0.100	3.084	−0.048	0.993
Difenoconazole	50.40	0.010	0.050	2.014	−0.094	0.970

Table 3
Recoveries, precision, threshold fit, LOD and LOQ

Analyte	First fort. level (n=10)			Second fort. level (n=10)			Confirmation threshold fit[a]	LOD (ng g^{-1})	LOQ (mg g^{-1})
	Conc. (ng g^{-1})	Avg. recov. (%)	RSD (%)	Conc. (ng g^{-1})	Avg. recov. (%)	RSD (%)			
Methamidophos	7	86	11	15	79	12	656	0.06	0.19
Acephate	13	85	12	30	73	17	629	0.21	0.69
Ethoprophos	7	83	9	15	105	15	716	0.01	0.04
Lindane	130	88	10	300	74	11	676	0.32	1.10
Etrimfos	13	87	10	30	98	8	693	0.02	0.08
Chlorthalonil	130	89	16	300	83	13	626	0.54	1.80
Pirimicarb	26	106	6	60	90	16	630	0.50	1.70
Vinclozoline	130	84	5	300	100	10	650	0.42	1.40
Methyl-parathion	26	119	9	60	92	13	589	2.60	8.60
Metalaxil	65	114	6	150	98	7	562	2.30	7.70
Fenitrothion	130	84	10	300	111	14	579	0.27	0.91
Malathion	26	87	5	60	95	14	514	0.34	1.10
Triadimefon	26	100	4	60	94	5	687	0.69	2.30
Pendimethalin	26	108	4	60	88	8	584	0.07	0.23
Chlozolinate	26	95	4	60	98	6	682	0.05	0.16
Procimidone	130	106	4	300	100	5	700	0.22	0.72
Triflumizole	26	95	11	60	96	12	492	0.68	2.30
α-Endosulfan	130	98	4	300	93	7	707	0.29	0.96
Fenamiphos	26	90	12	60	97	6	726	0.01	0.02
Myclobutanil	7	80	8	15	100	6	672	0.14	0.45
β-Endosulfan	130	98	5	300	90	7	666	0.77	2.60
Ethion	26	111	6	60	104	6	726	0.01	0.02
Carbofenothion	7	88	12	15	111	18	585	0.31	1.00
Endosulfan-sulfate	130	82	17	300	84	14	503	0.70	2.30
Tebuconazole	26	109	4	60	91	8	565	0.36	1.20
Bromopropylate	13	98	4	30	98	6	641	0.02	0.08
Fenpropathrin	13	101	7	30	92	9	462	0.05	0.17
Acrinathrin	7	118	15	15	95	7	704	0.60	2.00
Pyrazophos	7	98	6	15	71	11	658	0.04	0.12
Cypermethrin	26	110	15	60	84	7	519	0.16	0.53
Difenoconazole	13	90	5	30	89	12	619	0.47	1.56

[a] Mass spectral match factor.

results (fit) are scaled to 1000 for the best match (identical spectra). To set the fit threshold for each pesticide, the average fit of the MS–MS spectra from 10 injections of the mid-level standard was determined and 250 was subtracted from the average. The instrument software then automatically would confirm the presence of the pesticide if fit exceeded the threshold value and the signal/noise ratio was greater than 3. Table 3 lists threshold fit values obtained for each compound.

3.2.4. Quality criteria

In order to assure quality results in routine analysis, several quality criteria have been established. For that, a blank extract, a blank sample extract spiked at the concentration of the second calibration level, and a calibration curve were processed daily with the set of samples. The blank extract minimizes the chance for false positives due to a possible contamination in the extraction process or chemicals. The efficiency of the extraction procedure is checked with a matrix spike which also helps to detect anomalies due to the extraction or instrumental causes. Analysis of samples within the sequence was carried out if recoveries were between 60 and 130% in the matrix spike. Calibration curve is used to check the linearity in the working range of concentration and to avoid quantification mistakes due to matrix effects or instrumental

Table 4
Inter-day recoveries, precision and t_R

Analyte	First fort. level (n=10)			Second fort. level (n=10)			t_R second fort level (n=10)		
	Conc. (ng g^{-1})	Avg. recov. (%)	RSD (%)	Conc. (ng g^{-1})	Avg. recov. (%)	RSD (%)	Average (min)	RSD (%)	RTW (min)
Methamidophos	7	79	16	15	88	16	7.83	0.05	7.3–8.3
Acephate	13	80	14	30	84	19	10.30	0.04	9.9–10.7
Ethoprophos	7	84	13	15	85	17	12.95	0.07	12.3–13.7
Lindane	130	78	15	300	89	17	16.99	0.08	16.2–17.8
Etrimfos	13	77	16	30	91	17	18.05	0.10	17.1–19.1
Chlorthalonil	130	79	19	300	89	15	18.77	0.09	17.9–19.7
Pirimicarb	26	99	12	60	96	15	18.80	0.08	18.0–19.6
Vinclozoline	130	80	15	300	87	12	20.64	0.09	19.7–21.5
Methyl-parathion	26	106	16	60	99	14	20.73	0.06	20.1–21.3
Metalaxil	65	109	8	150	99	8	20.97	0.05	20.5–21.5
Fenitrothion	130	89	10	300	110	14	22.15	0.04	21.8–22.6
Malathion	26	86	6	60	85	19	22.42	0.04	22.0–22.8
Triadimefon	26	97	7	60	95	8	23.89	0.04	23.5–24.3
Pendimethalin	26	101	6	60	97	13	25.17	0.05	24.7–25.7
Chlozolinate	26	92	9	60	104	13	26.10	0.05	25.6–26.6
Procimidone	130	107	12	300	96	9	27.27	0.06	26.7–27.9
Triflumizole	26	91	18	60	95	15	27.40	0.07	26.7–28.1
α-Endosulfan	130	93	9	300	94	6	28.60	0.11	27.5–29.7
Fenamiphos	26	93	14	60	94	17	30.82	0.09	29.9–31.7
Myclobutanil	7	78	13	15	98	8	32.34	0.09	31.4–33.2
β-Endosulfan	130	93	10	300	92	8	33.94	0.11	32.8–35.0
Ethion	26	108	9	60	97	10	34.10	0.03	33.8–34.4
Carbofenothion	7	81	18	15	92	19	36.08	0.04	35.7–36.5
Endosulfan-sulfate	130	84	18	300	84	14	36.70	0.04	36.3–37.1
Tebuconazole	26	93	11	60	97	8	38.29	0.05	37.8–38.8
Bromopropylate	13	91	15	30	91	14	40.50	0.05	40.0–41.0
Fenpropathrin	13	89	13	30	94	9	40.47	0.06	39.9–41.1
Acrinathrin	7	97	18	15	101	14	42.34	0.09	41.4–43.2
Pyrazophos	7	88	14	15	81	14	43.15	0.05	42.7–43.7
Cypermethrin	26	96	19	60	110	10	47.40	0.04	47.0–47.8
Difenoconazole	13	87	12	30	77	13	50.75	0.07	50.1–51.5

variations. Table 5 summarizes information on parameters and criteria used.

3.3. Application of the method

In the period September 2000–July 2001, approximately 8000 samples of different fresh fruit and vegetable matrices (green bean, cucumber, pepper, tomato, eggplant, watermelon, melon, and marrow) were analyzed with the analytical method in CUAM located in El Ejido (Almería, Spain). Using the automatic search and identification menus of the instrument's data systems programmed with the routine analysis quality criteria selected can be carried out identification, quantification and confirmation of the pesticides. During 2000 and 2001, this method has demonstrated to be very useful in the control of pesticide residues in several fresh fruits and vegetables even at concentrations lower than the MRLs established by the EU and different states laws. Fig. 2 shows an example of positive determination in a real sample and Table 6 summarizes the results found above the limits permitted by the legislation of Spain and EU. It must be pointed out that a low percentage of positives occurred, which can be attributed to the studied area has become aware of the proper use of pesticides. In part, this was caused by the detection and control in the use of pesticides by independent laboratories like CUAM. The pesticide most frequently found over EU MRLs

Table 5
Validation work and criteria

	Identification	Quantification	Confirmation	Others
Parameter studied	Retention time	Recovery	Threshold fit	Calibration curve
Injection	10 spiked blanks	10 spiked blanks	10 spiked blanks	Three points in
Levels	Second point of calibration curve	Second point of calibration curve 30% over first point of calibration curve	Second point of calibration curve	cucumber matrix Three levels of concentration
Items established	Average retention time	Average recovery at two fortification levels	MS–MS reference spectra	Calibration curve
	Retention time window	Standard deviation	Average fit	Regression coefficient (R^2)
			Threshold fit	
To validate	–	Recovery between 70	–	R^2>0.9
Quality criteria for routine work	t_R within RTW	and 120% Recovery between 60 and 130%	Sample fit> threshold fit	R^2>0.9

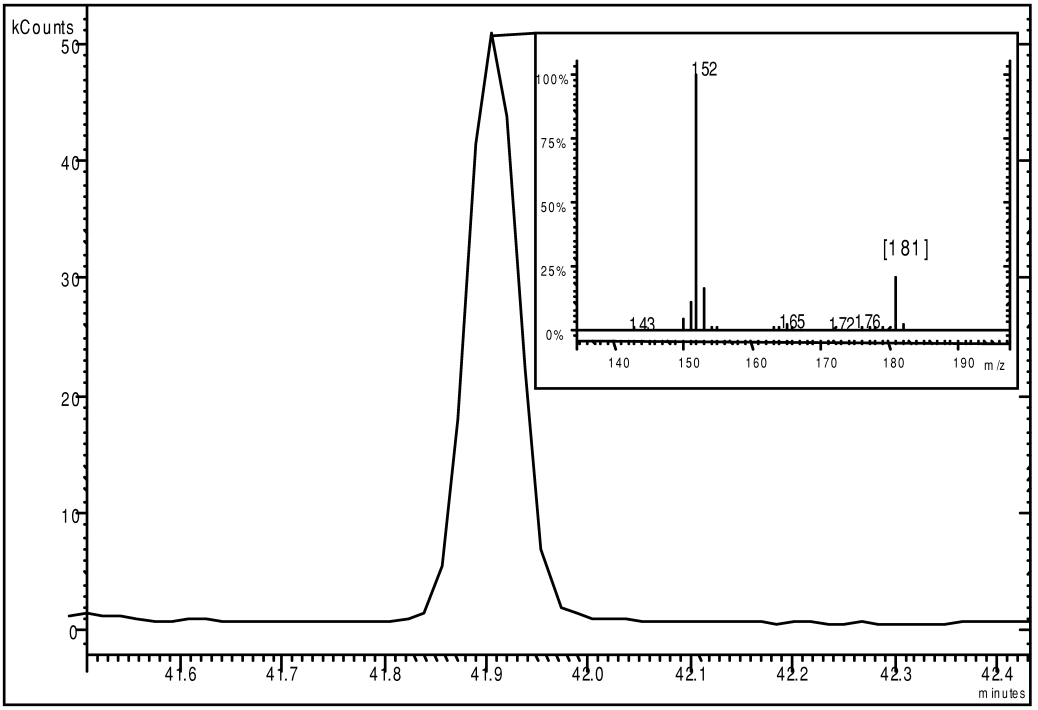

Fig. 2. Acrinathrin chromatogram and spectrum in a positive sample of pepper. Concentration found 0.104 mg kg^{-1}. S/N=43.

Table 6
Summary of the results of the analysis of real samples

Matrix	No. of samples	Over MRL of Spain	Over MRL of EU
Pepper	3839	10	64
Melon	352	2	0
Cucumber	1008	14	38
Marrow	884	5	2
Tomato	1119	2	7
Eggplant	306	0	0
Watermelon	81	0	0
Bean	339	5	1
Pea	3	0	0
Total	7931	38	112

in pepper matrix was metalaxyl (37 cases), followed by cypermethrin and methamidophos (10 cases each) and endosulfan (four cases). In cucumber matrix the analyte found in most cases at concentrations over the EU limits was metalaxyl (37 cases). Acrinathrin exceeded the Spanish MRL in pepper in seven samples and nine samples for cucumber. Tebuconazole (five cases) was the another analyte found in cucumber.

4. Conclusions

A new GC–MS–MS method has been proposed to determine 31 pesticides often used in Almería (Spain) in fresh fruit and vegetable samples. The use of Carbofrit in the glass liner injector reduced the amount of low volatile compounds from the matrix that reached the analytical column and improved the large volume injection (10 μl) of extracts. The use of MS–MS has proven its capability to increase selectivity of the detection and quantification including valuable qualitative information for confirmation of results. In this way, a clean up step is not necessary thus reducing costs, time, and uncertainty. The method is very suitable to routine analysis. Approximately 8000 samples of various kinds of fruits and vegetables grown in greenhouses of El Ejido (Almería, Spain) were analyzed with the proposed method finding that less than 0.5% of the samples presented pesticide residues above the Spanish MRL or 1.4% above the EU MRL.

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