TECHNICAL COMMUNICATIONS

Multiresidue Determination of Pesticides in Fruit and Vegetables by Gas Chromatography/Tandem Mass Spectrometry

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Pesticide residues in fruit and vegetables were determined by gas chromatography/tandem mass spectrometry (GC/MS/MS). Electron impact (EI)/MS/MS and chemical ionization (CI)/MS/MS were developed for 80 compounds, including organochlorine, organophosphorus, organonitrogen, and pyrethroids, providing unambiguous spectral confirmation for these complex matrixes. Residues were extracted from samples with acetone followed by a mixture of dichloromethane–petroleum ether. Two injections per sample were required for analysis of the entire pesticide list by EI/MS/MS and CI/MS/MS. Initial steps involving cleanup and concentration of extracts were eliminated. The excellent selectivity and good linearity allowed quantification and identification of low levels of pesticides in the most difficult matrixes. The method has been used for routine analysis of many vegetables.

The presence of pesticide residues in foods, especially in vegetables, is a growing concern for Spanish producers, traders, and consumers. Monitoring programs and export controls are needed for the protection of consumers and for quality evaluation of commodities. For many years, the Laboratorio Agroalimentario of Valencia has analyzed thousands of fruit and vegetable samples for organophosphorus, organochlorine, and pyrethroid pesticides at low levels by gas chromatography (GC) using selective detectors: flame photometric, nitrogen phosphorus, and electron capture detectors (FPD, NPD, and ECD; 1–6). Although these detectors are sufficiently sensitive for compliance with maximum residues limits (MRLs) in European Union regulations, they provide poor specificity for confirmation in these matrixes; therefore, GC coupled to mass spectrometry (MS) was required.

Many studies have reported the use of GC/MS to control pesticide residues in matrixes such as fruit, vegetables, milk, and soils, with either full scan or selected ion monitoring (SIM; 7–14). However, GC/MS in full scan provides low sensitivity due to interferences in vegetable matrixes, and SIM gives poor spectral information.

GC/MS/MS offers various advantages in selectivity and sensitivity at low quantities in dirty extracts such as vegetables, soils, sediments, and biological matrixes (15, 16). Several authors performed conditions for electron impact (EI)/MS/MS determination of pesticide residues in agricultural samples, and compared the concentrations in selective detectors and MS/MS (17–22). These studies showed that GC/MS/MS can be used in routine analysis with few difficulties and good reliability.

Recently, Lehotay (23) studied a direct sample introduction (DSI; or "dirty sample injection") system coupled with GC/MS/MS detection for 22 representative pesticides in fruit and vegetables. Reproducibility of retention times, effect of injection volume in DSI, and matrix effects were compared with those of carbofrit and DSI injection systems.

We developed a GC/MS/MS method to analyze pesticide residues in vegetable material by either EI/MS/MS or chemical ionization (CI)/MS/MS, injecting large volumes of noncleaned extracts of complex matrixes.

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Experimental

Chemicals

(**a**) *Reagents.*—Pesticide standards certified (Labor Dr. Ehrenstorfer-Schäfers, Augsburg, Germany). Acetone, dichloromethane, cyclohexane, toluene, methanol, and petroleum ether were all pesticide residue quality (Scharlau, Barcelona, Spain). Triphenyl phosphate (TPP), internal standard, purity 99.5% (Ehrenstorfer). Internal standard (IS) solution of TPP was prepared at 0.2 mg/L in cyclohexane.

(**b**) *Standard materials.*—The pesticides listed in Tables 1 and 2 were of the highest purity available. Each stock standard was prepared in toluene at 1 mg/mL and stored at 5ºC. Working standards solutions (0.01–0.50 mg/L) were prepared by appropriate dilutions in internal standard solutions.

Instruments

(**a**) *Homogenizer.*—Heidolph Diax 600 (Schawabach, Germany).

(**b**) *Food chopper.*—Dito-Sama K-55 (Aubusson, France).

Table 1. EI/MS/MS conditions

Retention time, min	Compound	Segment	Channel	Parent ion, *m/z*	Quantitation ion, *m/z*	CID amplification, v	Excitation storage level, v	LOD,[a] ppb
11.62	Diphenylamine	2	1	168	139	90	80	0.4
13.26	Hexachlorobenzene	3	1	284	177	94	90	1.5
13.66	Dichloran	3	2	176	148	71	70	0.6
14.56	Lindane	4	1	219	180:185	70	100	0.5
14.85	Fonofos	4	2	246	137	30	80	1.1
15.51	Chlorothalonil	4	3	266	133	65	71	4.5
18.00	Chlorpyrifos methyl	5	1	286	208	73	85	3.3
18.35	Vinclozolin	5	2	212	109	71	72	1.4
18.53	Tolclofos-methyl	5	3	265	219	84	100	8.4
20.25	Dicofol-o,p	6	1	250	139	46	70	7.0
20.30	Fenitrothion	6	2	260	125	60	71	8.3
20.68	Dichlofluanid	6	3	224	123	65	95	5.2
21.35	Chlorpyrifos	7	1	314	258	94	172	0.9
21.40	Aldrin	7	2	263	191	96	90	1.2
22.43	Dicofol-p,p´	7	3	250	215	42	70	5.4
24.06	Isofenfos	8	2	213	185	49	90	0.6
24.08	Tolylfluanid	8	1	238	137	70	105	0.8
24.27	Chlorfenvinphos	8	3	267	159	83	100	8.5
24.58	Quinalfos	8	4	298	190	72	110	7.8
24.97	Folpet	9	1	260	232	54	85	10.5
25.33	Bromophos-ethyl	9	2	359	303	78	140	0.7
25.55	Chinometionat	9	3	234	206	46	85	0.7
26.15	Endosulfan-α	10	1	339	263:269	53	125	4.3
26.83	Fenamiphos	11	1	303	195	55	95	0.3
27.00	Hexaconazol	11	2	214	172	80	80	6.8
27.32	Profenofos	12	1	339	267:269	37	75	2.8
27.94	Myclobutanil	12	2	179	125	64	80	5.3
29.47	Endosulfan-β	13	1	339	263:269	53	125	6.4
31.55	Endosulfan-sulphate	14	1	387	285:291	34	71	4.9
32.47	TPP (IS)	15	1	327	169	64	80	0.1
33.91	Pyridaphenthion	16	1	340	199	65	130	1.3
34.07	Iprodione	16	2	314	245	85	125	0.4
34.32	Bifenthrin	17	2	181	165	40	50	1.1
34.37	Bromopropylate	17	1	341	181:187	46	70	15.0
34.82	Fenpropathrin	17	3	265	210	81	110	3.1
36.95	Cyhalothrin-lambda	18	1	181	62	60	152	1.9
37.30	Fenarimol	18	2	330	139	73	120	10.4
37.38	Pyrazofos	18	3	265	210	53	80	0.6
37.45	Acrinathrin	18	4	289	261	74	95	5.8
38.90	Permethrin-I	19	1	183	168	68	75	4.2
39.27	Permethrin-II	19	1	183	168	68	75	3.7
40.57	Cyfluthrin	20	1	206	151	80	75	3.8
43.32	Fenvalerate	21	1	225	119	48	71	1.4
43.60	Fluvalinate	21	2	250	200	59	71	1.6
45.18	Deltamethrin	22	1	253	91:93	50	70	3.8

[a] LOD = limit of detection expressed as minimum concentration determined at S/N = 5.

Table 2. CI/MS/MS conditions

Retention time, min	Compound	Segment	Channel	Parent ion, *m/z*	Quantitation ion, *m/z*	CID amplification, v	Excitation storage level, v	LOD,[a] ppb
7.77	Methamidophos	2	1	142	126	48	60	0.7
7.81	Dichlorvos	2	2	221	145	73	90	1.1
9.20	Acephate	3	1	143	141	51	60	5.3
10.47	Molinate	4	1	188	98	56	70	0.3
10.67	Heptenofos	4	2	251	215	67	100	0.3
13.22	Thiometon	5	1	89	61	36	40	0.2
13.47	Dimethoate	5	2	230	199	48	100	1.3
14.82	Diazinon	6	1	305	169	86	120	0.1
15.82	Etrimfos	7	1	293	265	90	120	0.1
16.40	Pirimicarb	7	2	239	182	70	100	0.3
18.50	Parathion-methyl	8	1	264	172	78	100	10.2
19.12	Metalaxyl	8	2	280	220	49	100	0.4
20.12	Pirimiphos-methyl	8	3	306	246	80	100	2.1
20.97	Malathion	9	1	285	127	29	100	0.9
21.67	Fenthion	9	2	279	247	67	100	0.6
21.88	Parathion-ethyl	9	3	292	236	62	110	0.1
23.90	Penconazole	10	1	284	173	61	100	1.1
24.08	Chlozolinate	10	2	332	304	53	120	0.3
24.38	Mecarbam	11	1	227	171	52	90	2.3
24.48	Phenthoate	11	2	247	157	60	100	3.6
24.73	Procymidone	11	3	284	256	73	110	0.05
25.37	Methidathion	12	1	145	85	35	60	0.7
27.98	Buprofezin	13	1	191	134	47	80	0.2
28.07	Bupirimate	13	2	317	210	80	120	0.4
29.78	Oxadixyl	14	1	279	219	60	140	3.2
29.85	Ethion	14	2	199	143	50	80	0.5
30.72	Triazophos	15	1	314	162	78	120	1.2
31.18	Carbophenotion	15	2	343	199	62	150	20.2
32.33	Nuarimol	16	1	315	252	90	120	0.6
32.47	TPP (IS)	16	2	327	247	60	80	0.01
34.07	Phosmet	17	1	318	160	50	130	10.5
35.60	Tetradifon	18	1	357	195:197	69	100	0.7
36.05	Azinphos-methyl	18	2	160	132	23	60	35.0
37.57	Azinphos-ethyl	19	1	160	132	39	60	3.7
41.00	Cypermethrin	20	1	191	127	62	80	3.2

[a] LOD = limit of detection expressed as minimum concentration determined at S/N = 5.

(**c**) *Centrifuge*.—Hereaus Sepatech Model Labofuge GL (Hanau, Germany).

(**d**) *Rotary evaporator*.—Büchi Model R-114 (Flawil, Switzerland).

(**e**) *GC/MS/MS system*.—Saturn 2000 (Varian Corp., Walnut Creek, CA).

(**f**) *Capillary column*.—CP Sil 8CB 30 m × 0.25 mm id, 0.25 μm film thickness (Varian-Chrompack, Middleburg, The Netherlands).

Instrumental Conditions

Varian Model Saturn 2000 GC/MS/MS system with CP-3800 gas chromatograph equipped with 1079 injector with electronic flow control (EFC). Saturn 2000 MS/MS detector equipped with CI and liquid CI, and CP8200CX autosampler with 100 μL syringe.

GC operating conditions: 1079 temperature-programmable injection port with carbofrit inserted in the liner: initial tempera-

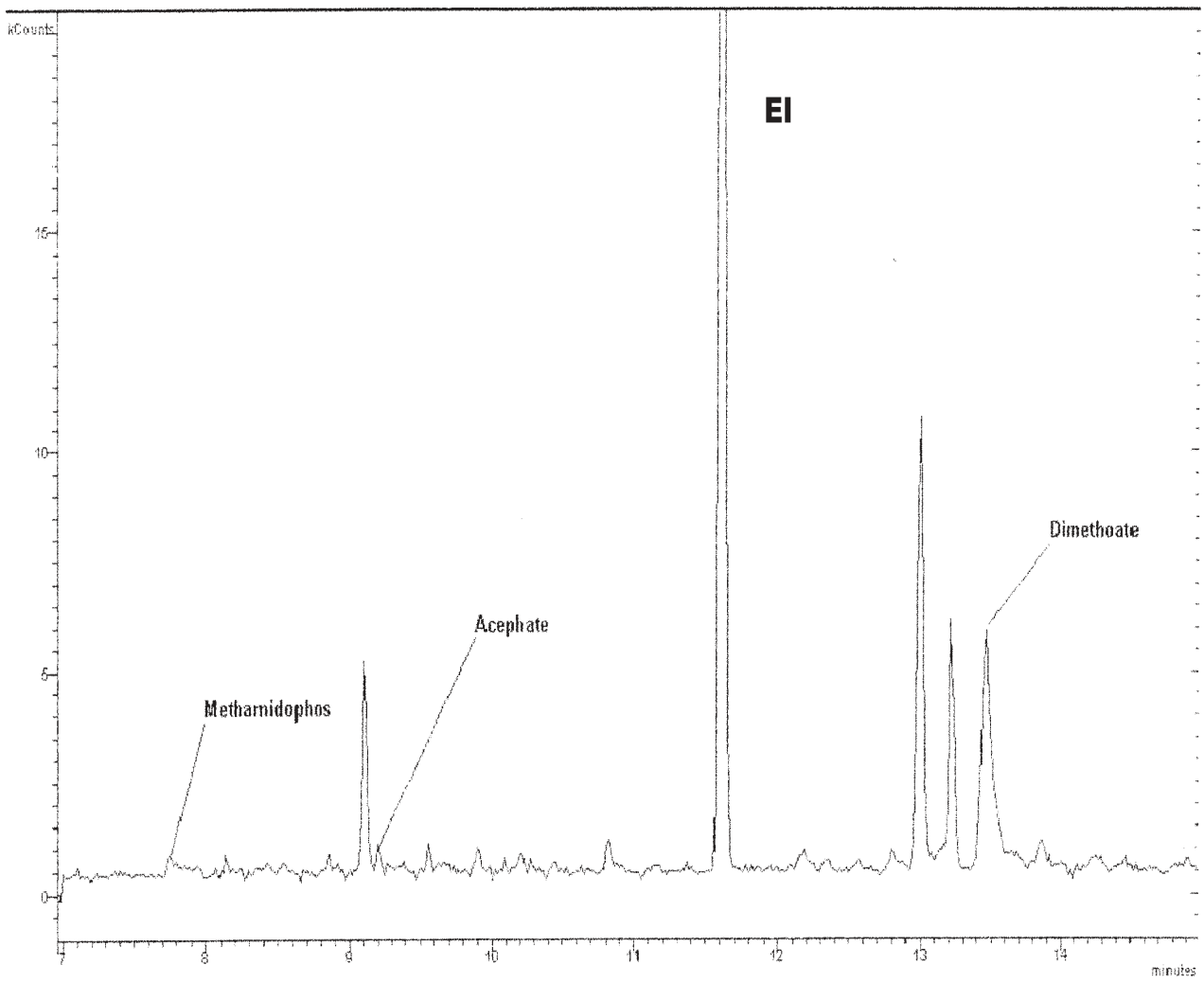

Figure 1. Full scan EI chromatogram of spiked orange extract corresponding to 0.10 mg/kg methamidophos, acephate, and dimethoate.

ture, 70°C; initial time, 0.50 min; 100°C/min rate; 310°C final temperature, 10 min final time, then cooling to initial temperature. Column constant flow was 1 mL/min; split ratio, initial: open 100:1, closed 0.50 min at 3.5 min, ratio 100:1 open. Column oven program, initial temperature at 70°C for 3.5 min, ramp to 180°C at 25°C/min, hold for 10 min; then ramp to 300°C at 4°C/min, and hold for 10 min.

Carbofrit is conditioned inside injector port by heating at 300°C for 2 h; 2 m × 0.25 mm id precolumn was also used.

MS operating conditions: Trap temperature, 200°C; manifold, 50°C; transfer line, 280°C; EI and methanol CI tuned per factory recommendations.

Autosampler in sandwich mode, needle residence time, 0.10 min; solvent plug, 10 μL; pause time, 5 s; uptake speed, 30 μL/s; vial needle depth, 90%; injection rate, 10 μL/s; and sample volume 10 μL.

Analytical Procedure

Extraction.—After homogenization of 2 kg fruit or vegetables, a 15 g portion was weighed into a 250 mL Teflon centrifuge bottle and then homogenized with 30 mL acetone for 30 s. A 60 mL volume of dichloromethane–petroleum ether (1 + 1) was added, and the mixture was homogenized for 1 min, after centrifuging at 4000 rpm for 5 min. The organic phase was decanted into a graduated flask, and volume of extract was measured (usually ca 85 mL). An aliquot of extract (10 mL) was concentrated to dryness in a rotary evaporator with water bath at 35°C. The residue was dissolved in 2 mL internal standard solution. Concentration factor was ca 1 g/mL sample. Then, 10 μL of this solution was injected into the GC/MS system.

Analysis.—Each sample was analyzed in 2 different injections, one in EI/MS/MS mode and the other in methanol

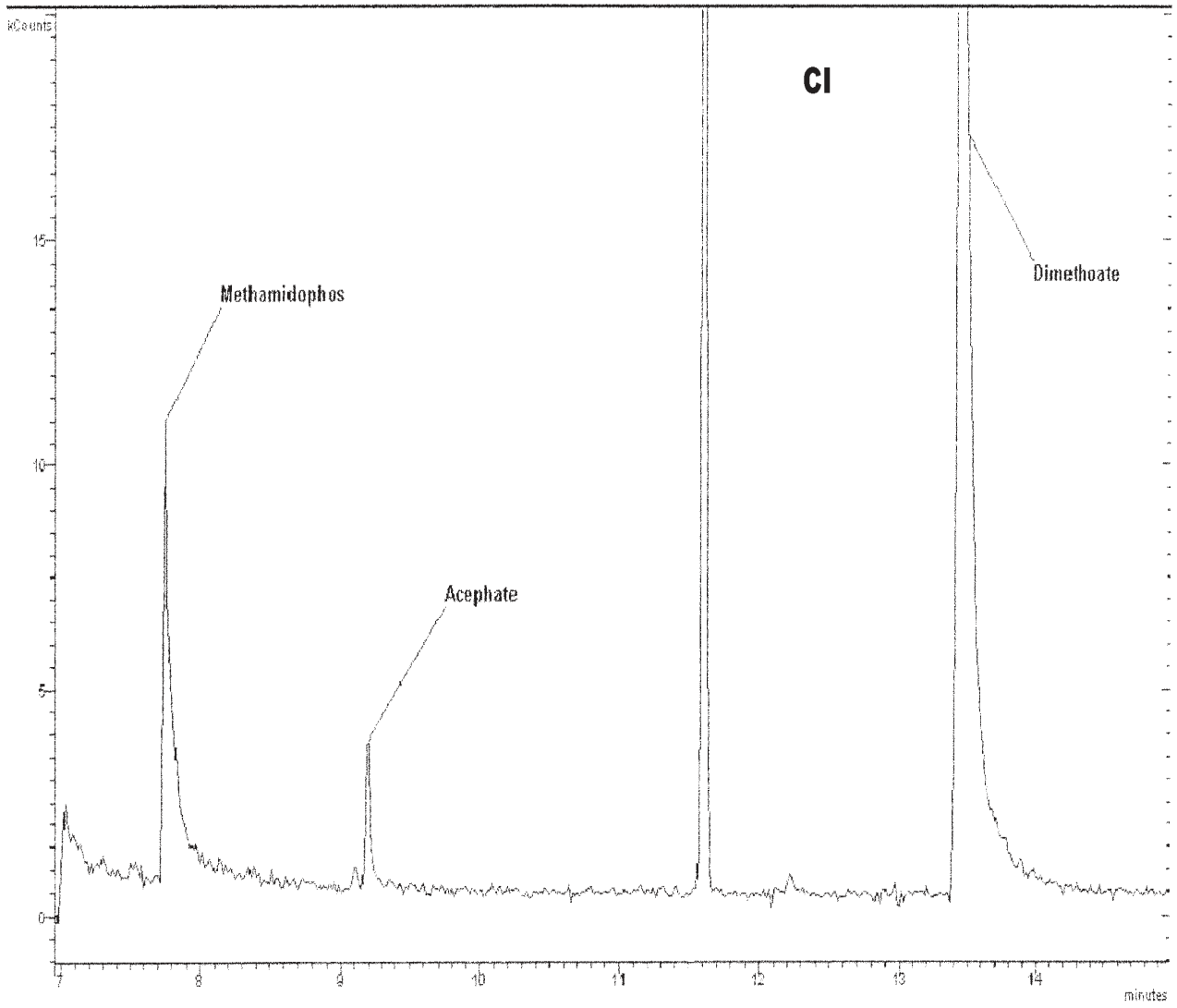

Figure 2. Full scan CI chromatogram of spiked orange extract corresponding to 0.10 mg/kg methamidophos, acephate, and dimethoate.

CI/MS/MS mode. MS/MS conditions are summarized in Tables 1 and 2, respectively. These conditions were developed experimentally to obtain the best sensitivity and spectral identification for each of the analyzed pesticides.

Results and Discussion

The proposed method attempts to resolve the problems involved in analysis of pesticide residues in vegetable extracts. The main problems are related to complex matrixes. Previously, the matrix effect was handled by very complicated and difficult sample preparation and cleaning procedures. With those procedures, pesticide recoveries were poor and sensitivity was diminished. Today the trend is to minimize sample preparation and make the analysis selective. Several factors have been considered to avoid the problems related to this analysis: injection, selectivity for organochlorine pesticides, selectivity, and sensitivity for organophosphorus pesticides.

Injecting vegetable extracts is very complicated and must be carefully considered. Standard isothermal splitless injection gives good results for chlorinated pesticides but not for phosphorus pesticides. In addition, parts on the injection port must be cleaned or changed frequently to maintain sensitivity of the system. The column near the injector port must be scored to eliminate background interference. All of these problems are minimized with the large volume injection (LVI) technique. Injection of 10 μL increases sensitivity enough to improve the detection limit of the method for determining maximum residue limits (MRLs). The introduction of carbofrit (20) improves the inertness of the injector port. In such injection, injector port initial temperature must be maintained at the solvent boiling point while the split vent is on; after 0.5 min, the split vent is closed and the injector is heated

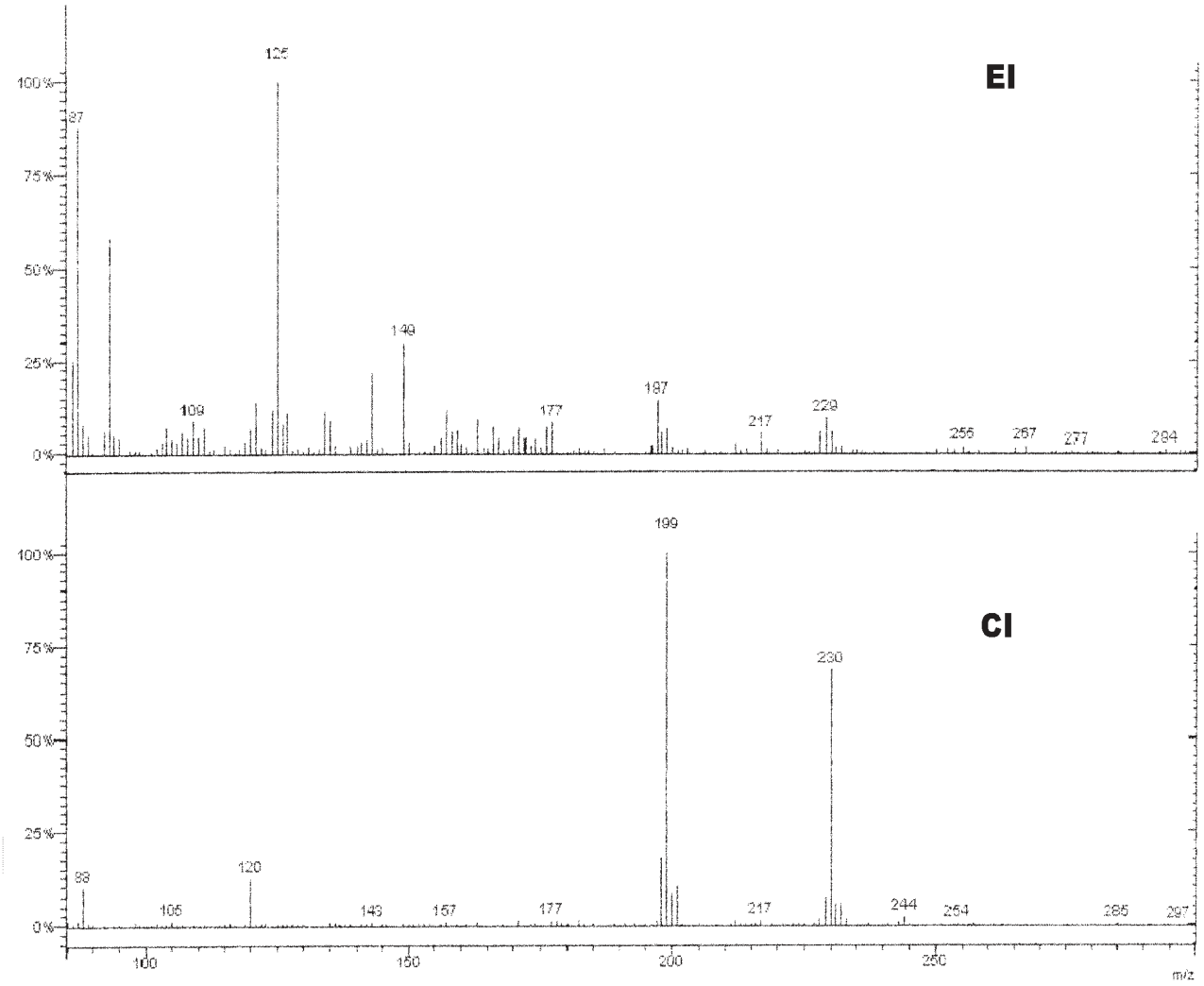

Figure 3. Full scan EI and CI spectra of dimethoate.

until it reaches the final temperature. Meanwhile, column temperature begins at the boiling point temperature for 3.5 min to provide complete entrance of the analytes into the column. The temperature is then increased to 180°C with a slow ramp of 4°C/min to 300°C to completely elute all compounds from the column. With this injection technique, we achieved very good sensitivity, and background was reduced by absorption of the sample matrix by the carbofrit. Each carbofrit has a long lifetime of up to 800 injections depending on the matrix content of the sample. Changing the carbofrit and precolumn on a regular basis will give the analytical column a lifetime of at least 2 years or more.

Regarding sensitivity to organochlorine pesticides, several authors (15–20) reviewed the advantages of EI/MS/MS detection with increased sensitivity and decreased background by isolation of one ion and a second fragmentation. For quantitation we used an ion produced in the second fragmentation that did not exist in EI; the signal-to-noise (S/N) ratio was maximized instead in EI where all the background was present. Table 1 presents the conditions used for the compounds analyzed by EI/MS/MS.

Fragmentation of organophosphorus pesticides, mainly pesticides showing low mass ions, is very different from that of organochlorines. Intense ions in the spectra of these expressed compounds are usually less than 120 uma and EI becomes very difficult because of the high background in this range produced by the sample matrix. Chromatograms are shown of orange extract-spiked methamidophos, acephate, and dimethoate in EI (Figure 1) and in CI (Figure 2). The increased sensitivity to pesticides in CI compared with EI was produced by using Varian's Selective Ejection Chemical Ionization, which provided clean positive chemical ionization spectra and, thus, substantially decreased the background. Methanol was used as reagent gas in CI, making it possible to obtain clean and reproducible spectra.

Figure 3 shows that very intense and higher mass ions were obtained for dimethoate unlike the unspecific ions of EI for organophosphorus pesticides. The choice of the CI reagent gas is also very important. Choosing methanol instead of stan-

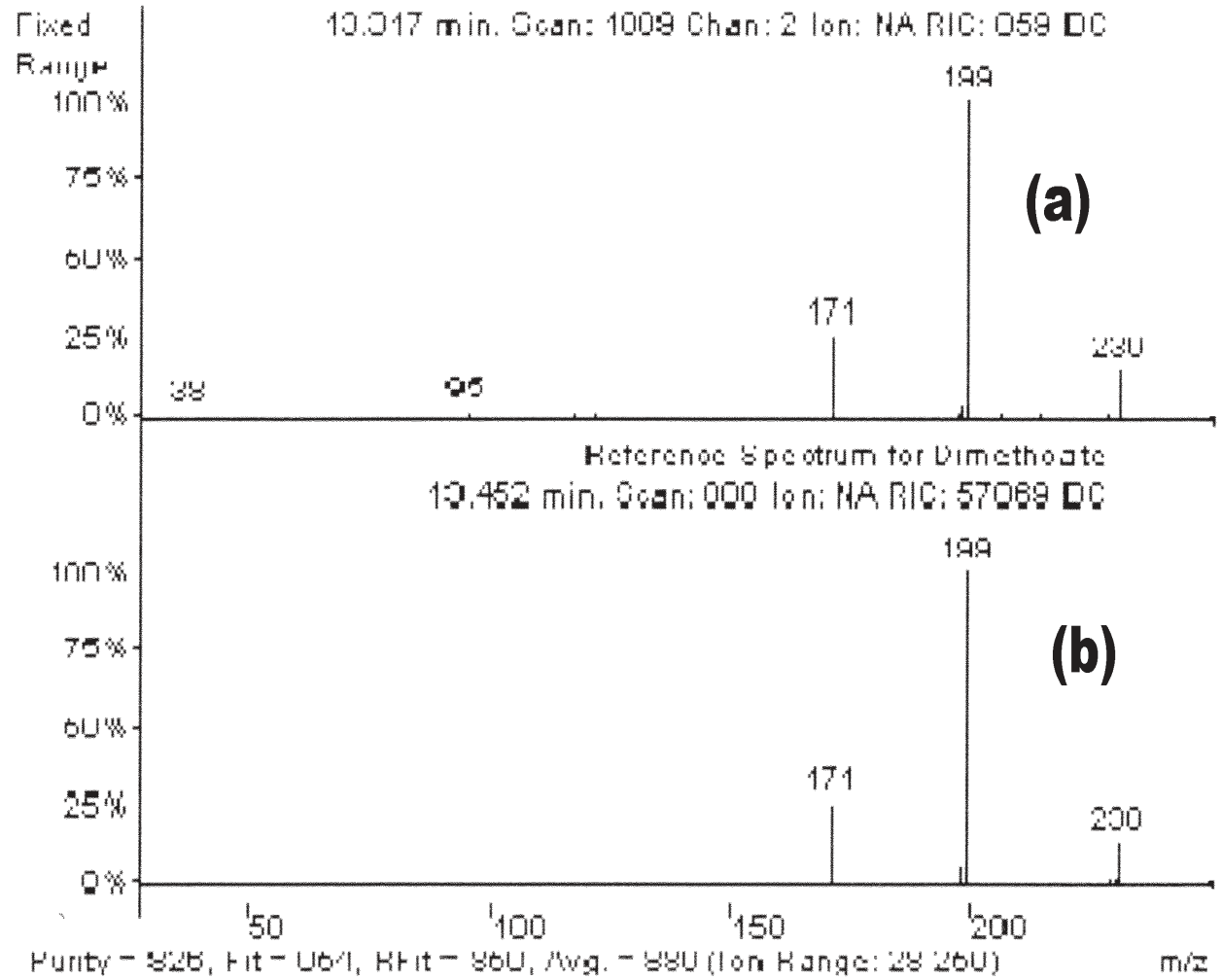

Figure 4. CI/MS/MS spectra of dimethoate in (a) orange sample and (b) reference spectrum.

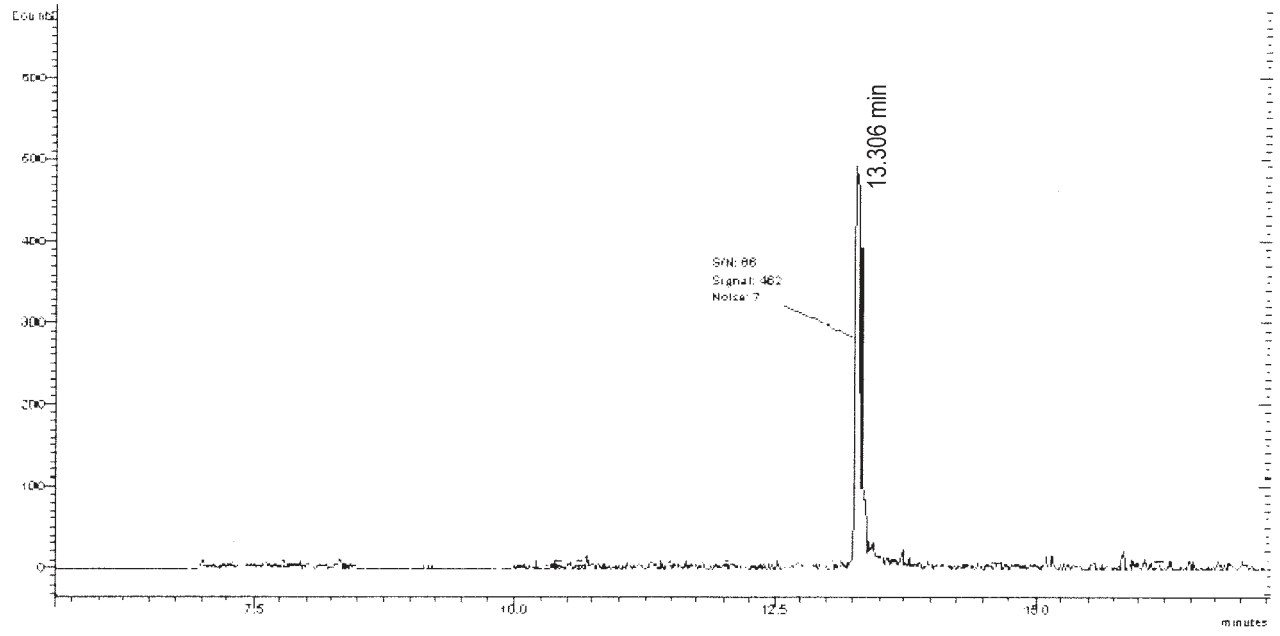

Figure 5. CI/MS/MS chromatogram of dimethoate 0.01 mg/kg in orange sample.

dard gases such as methane or isobutane provides very high M+1 ions and not much fragmentation.

Although the CI spectrum does not provide enough spectral information for sufficient confirmation security, these high mass ions are very good as parent ions for MS/MS purposes, providing extra unequivocal confirmation by the improved selectivity, as shown in Figure 4 for dimethoate. Table 2 lists all the conditions used for the compounds analyzed by CI/MS/MS.

Good sensitivity was achieved for all organophosphorus pesticides in orange extract spiked samples, as shown in Table 2 and Figure 5 for dimethoate (0.01 mg/kg), where the S/N ratio was >50. Notice that there are no chromatographic interferences present. The increase in sensitivity even competes with NPDs for many of the pesticides studied.

Even with positive trace analysis, an MS/MS unequivocal confirmation was obtained, as shown Figure 4. In this spectrum very close matching >900 was achieved. The removal of background spectral interferences demostrates the selectivity of the MS/MS methods.

Tables 1 and 2 provide the limits of detection (LODs) calculated from software-reported S/N ratios (S/N > 5) for the pesticide calibration standards in sample extracts. The LODs were not verified by injecting low-level concentrations, although they were estimated by extrapolation for linear calibration plots with correlation coefficients of ≥ 0.99.

Calibration curves were obtained after injections of the standard mixtures in matrixes matched for 5 concentrations (2 injections each) ranging from 0.01 to 0.50 μg/mL with correlation coefficients between 0.995 and 1.00.

Conclusions

The proposed method attempts to resolve the problems involved in analysis of pesticide residues in vegetable extracts. Several factors were considered, such as injection, selectivity for organochlorine pesticides, selectivity, and sensitivity for organophosphorus pesticides. The introduction of the carbofrit improves the inertness of the injector port, giving very clean chromatograms. In addition, it allows large volume injection with high sensitivity.

EI/MS/MS is a very good technique for analysis of organochlorine pesticides. CI/MS/MS completely solves the sensitivity problem related to organophosphorus pesticide analysis in vegetable extracts, even when compared with NPD detection, mainly for early eluting pesticides such as methamidophos, acephate, and dimethoate. The excellent selectivity and sensitivity allows quantification and identification of low levels of pesticides in vegetable samples. Large volume injection and GC/MS/MS combination can be used in routine analysis to provide good results.

References

(1) De Paoli, M., Barbina, M.T., Damiano, V., Fabbro, D., & Bruno, R. (1997) *J. Chromatogr. A* **765**, 127–131

(2) Andersson, A., & Ohlin, B. (1986) *Var Foeda Suppl.* **38**, 79–109

(3) Luke, M.A., & Masumoto, H.T. (1986) *Anal. Methods Pest. Plant Growth Regul.* **15**, 162–186

(4) *Analytical Methods for Pesticide Residues in Foodstuffs*, 6th Ed. (1996) Inspectorate for Heath Protection, The Hague, The Netherlands

(5) McMahon, B., & Hardin, N. (1994) *Pesticide Analytical Manual*, Vol. 1, 3rd Ed., U.S. Food and Drug Administration, Washington, DC

(6) Coscolla, R., & Gamón, M. (1997) *Pestic. Sci.* **50**, 155–159

(7) Bennett, D.A., Chung, A.C., & Lee, S.M. (1997) *J. AOAC Int.* **80**, 1065–1077

(8) Feigel, C., Varian Appl. Note 26

(9) Fillion, J., Hindle, R., Lacroux, M., & Selwing, J. (1995) *J. AOAC Int.* **78**, 1252–1266

(10) Cook, J., Engel, M., Wylie, P., & Quimby, B. (1999) *J. AOAC Int.* **82**, 313–326

(11) Lacassie, E., Dreyfuss, M.F., Daguet, J.L., Vignaud, M., Marquet, P., & Lachatre, G. (1998) *J. Chromatogr. A* **805**, 319–326

(12) Cabras, P., Angionai, A., Garau, V.L., Pirisi, F.M., & Brandolini, V. (1998) *J. AOAC Int.* **81**, 1185–1189

(13) Andersson, A., & Palsheden, H. (1998) Pesticide Analytical Methods in Sweden, Uppsala, Sweden, National Food Administration, Rapport 17/98, Part 1, 9-41

(14) Fillion, J., Sauvé, F., & Selwin, J. (2000) *J. AOAC Int.* **83**, 698–713

(15) Plomley, J.B., Koester, C.J., & March, R.E. (1994) *Anal. Chem.* **66**, 4437–4443

(16) Hayward, D.G., Hooper, K., & Andrzejewski, D. (1999) *Anal. Chem.* **71**, 212–220

(17) Feigel, C., Varian Appl. Note 28

(18) Feigel, C., Varian Appl. Note 30

(19) Feigel, C., Varian Appl. Note 40

(20) Cost, M., Ingram, R., & Lynn, B., Varian Appl. Note 53

(21) Schachterle, S., & Feigel, C. (1996) *J. Chromatogr. A* **754**, 411–422

(22) Sheridan, R.S., & Meola, J. (1999) *J. AOAC Int.* **82**, 982–990

(23) Lehotay, S.J. (2000) *J. AOAC Int.* **83**, 680–697