RESIDUES AND TRACE ELEMENTS

Applicability of Gradient Liquid Chromatography with Tandem Mass Spectrometry to the Simultaneous Screening for About 100 Pesticides in Crops

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A method was developed for screening crops for a range of pesticide residues by liquid chromatography/tandem mass spectrometry (LC/MS/MS). A complete set of LC, electrospray ionization (ESI), and tandem MS acquisition parameters was established for the determination of 108 analytes; these parameters were used for the simultaneous acquisition of 98 analytes in the positive ESI mode and 10 analytes in an additional MS/MS method in the negative ESI mode. The entire procedure involves extraction of residues with methanol–water and partition into dichloromethane. The utility of the method is demonstrated by the analysis of crops of 5 matrix types (water-containing, acidic, dry, sugar-containing, and fatty). Of 108 pesticides/metabolites tested, 104 showed sufficient stability in most matrixes for determination by LC/MS/MS. These analytes belong to 20 chemical classes, which demonstrate the general applicability of the method for multiclass analysis. By using matrix-matched standards, 67 compounds could be determined in most matrixes with recoveries of 70–120% and a relative standard deviation of ≤25% at the 0.01 mg/kg level.

Currently, more than 800 pesticides (active ingredients) are sold worldwide. For many of these compounds, legal action levels (e.g., maximum residue limits or tolerances) in food have been established and must be enforced. For this type of target analysis, multiresidue analytical methods are preferred to reduce workload and costs. Until now, all established multiresidue methods, which allow the determination of hundreds of pesticides, have used gas chromatography (GC) for final determination (1–4). For pesticides that are not GC-amenable, several methods that use liquid chromatography (LC) with UV or fluorescence detection are available (5–8). However, these methods often suffer from insufficient selectivity and sensitivity or need sophisticated cleanup of sample extracts.

Received October 22, 2002. Accepted by JS February 17, 2003.
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Recently, tandem mass spectrometry (MS/MS) was found to be far superior to other LC detection techniques for the determination of residues of aryloxyalkanoic acids (9–12), benzoylureas (13), benzimidazoles (14–17), carbamates (18–20), sulfonylureas (21–23), and more polar organophosphates (24–26). However, in most cases the published methods are suitable for a small group of compounds only or compounds belonging to the same chemical class.

Our aim was to develop a generally applicable LC/MS-based multiresidue method for the determination of a large number of pesticides from distinct chemical classes after fast and inexpensive extraction and cleanup. In a first step, information about the most suitable ionization technique was needed as well as knowledge about the largest number of compounds that can be analyzed simultaneously with commercial triple-quadrupole mass spectrometers. The complete analytical procedure was validated for the compounds finally selected for the MS/MS determinative step.

Experimental

Reagents

(**a**) *Solvents.*—Acetonitrile and methanol were LC grade; dichloromethane and ethyl acetate were analytical reagent grade (Merck, Darmstadt, Germany).

(**b**) *Pesticide standards.*—Sources of the pesticide standards are summarized in Table 1. In addition, azadirachtin, cymoxanil, fentin hydroxide, fipronil, bromoxynil octanoate, ioxynil octanoate, MCPA 2-ethylhexyl ester, MCPA butoxyethyl ester, MCPA ethyl ester, MCPA thioethyl ester (all from Ehrenstorfer Laboratories GmbH, Augsburg, Germany), MCPA methyl ester (Riedel-de-Haën, Seelze, Germany), cyhexatin (Dow Chemicals, Indianapolis, IN), and fenbutatin oxide (Shell Research, Kent, UK) were tested but not included in the final test.

(**c**) *Purified water.*—Prepared by using a Milli-Q water purification system (Millipore, Schwalbach, Germany).

(**d**) *Disposable ChemElut extraction columns.*—20 mL sample capacity, Part No. 1219-8008, and 5 mL sample capacity, Part No. 1219-8006 (Varian GmbH, Analytical Instruments, Darmstadt, Germany).

(**e**) *Membrane filters.*—13 mm, 0.45 μm polytetrafluoroethylene (PTFE; Amchro, Hattersheim, Germany).

Table 1. Analyte-specific parameters and suppliers of the 108 pesticides/metabolites used

				First transition			Second transition				
Analyte	ESI	RT, min[a]	Precursor ion	*m/z*	DP, V[b]	CE, V[c]	*m/z*	DP, V	CE, V	Supplier[d]	References
2,4-D	–	10.8	$[M–H]^-$	219→161	–21	–14	219→125	–16	–34	3	9–11, 23, 27
3,4,5-Trimethacarb	+	13.5	$[M+H]^+$	194→137	61	15	194→122	61	35	1	28
3-Hydroxycarbofuran	+	8.6	$[M+H]^+$	238→163	16	19	238→181	16	15	1	20, 29
5-Hydroxyclethodim sulfone	+	8.4	$[M+H]^+$	408→204	16	27	408→150	51	25	3	
5-Hydroxythiabendazole	+	9.1	$[M+H]^+$	218→191	71	35	218→147	66	43	1	
Acephate	+	1.9	$[M+H]^+$	184→143	6	13	184→125	6	25	4	24, 25
Aldicarb	+	10.3	$[M+NH_4]^+$	208→89	1	21	208→116	11	13	1	23, 28
Aldicarb sulfoxide	+	2.4	$[M+H]^+$	207→89	36	17	207→132	31	11	1	20, 28
Aldoxycarb	+	3.0	$[M+NH_4]^+$	240→76	1	19	240→166	11	17	1	28
Amidosulfuron	+	7.0	$[M+H]^+$	370→261	21	19	370→218	21	31	1	
Atrazine	+	13.0	$[M+H]^+$	216→174	21	25	216→104	21	37	2	10, 23, 30–32
Azoxystrobin	+	14.1	$[M+H]^+$	404→372	36	19	404→344	31	29	1	
Bendiocarb	+	11.8	$[M+H]^+$	224→167	6	13	224→109	11	21	4	28
Bensulfuron-methyl	+	13.6	$[M+H]^+$	411→149	51	27	411→119	41	51	1	22, 23
Bromoxynil	–	9.18	$[M–H]^-$	276→81	–46	–36	278→81	–56	–40	1	9, 10, 27
Butocarboxim	+	10.1	$[M+NH_4]^+$	208→75	1	15	208→116	1	11	4	28
Butocarboxim sulfoxide	+	2.2	$[M+H]^+$	207→75	31	19	207→132	6	11	1	28
Butoxycarboxim	+	2.8	$[M+NH_4]^+$	240→106	6	19	240→177	11	15	1	28
Carbaryl	+	12.4	$[M+H]^+$	202→145	11	15	202→127	11	35	2	15, 19, 20, 23, 28, 33
Carbendazim	+	9.9	$[M+H]^+$	192→160	41	25	192→132	21	41	2	14–16, 23, 28–30, 34
Carbofuran	+	11.8	$[M+H]^+$	222→165	16	17	222→123	16	29	1	10, 19, 20, 23, 28, 29, 32
Chlorsulfuron	+	9.1	$[M+H]^+$	358→141	51	23	358→167	51	25	1	21–23
Cinosulfuron	+	9.6	$[M+H]^+$	414→183	36	23	414→215	31	21	1	
Clethodim	+	14.6	$[M+H]^+$	360→164	41	25	360→268	46	17	3	
Clethodim-imin sulfone	+	8.9	$[M+H]^+$	302→98	71	41	302→208	66	27	3	
Clethodim-imin sulfoxide	+	9.0	$[M+H]^+$	286→208	26	21	286→166	31	31	3	
Clethodim sulfone	+	10.3	$[M+H]^+$	392→164	1	33	392→208	56	27	3	
Clethodim sulfoxide	+	10.4	$[M+H]^+$	376→206	1	19	376→164	51	29	3	
Cyprodinil	+	15.8	$[M+H]^+$	226→93	61	45	226→77	61	63	1	
Daminozide	+	1.2	$[M+H]^+$	161→143	46	15	161→61	46	19	4	35
Demeton-S-methyl	+	12.0	$[M+NH_4]^+$	248→89	6	17	248→61	11	47	2	28
Demeton-S-methyl sulfone	+	4.3	$[M+H]^+$	263→169	66	21	263→109	71	37	1	23, 28
Desmedipham	+	13.8	$[M+NH_4]^+$	318→182	31	19	318→136	16	33	1	36
Desmethylformamido-pirimicarb	+	11.9	$[M+H]^+$	253→72	11	25	253→225	16	15	1	37
Desmethyl-pirimicarb	+	10.8	$[M+H]^+$	225→72	16	27	225→168	16	19	1	37
Diflubenzuron	–	15.4	$[M–H]^-$	309→156	–36	–12	309→289	–16	–8	3	13, 38
Dimethoate	+	8.3	$[M+H]^+$	230→199	16	13	230→125	11	29	1	15, 23, 29
Diuron	+	13.5	$[M+H]^+$	233→72	66	31	233→160	66	33	2	10, 23, 29, 31, 34
Ethiofencarb	+	12.5	$[M+H]^+$	226→107	16	21	226→164	11	13	3	19, 28, 29
Ethiofencarb sulfone	+	6.7	$[M+NH_4]^+$	275→107	11	25	275→201	11	15	1	28
Ethiofencarb sulfoxide	+	7.1	$[M+H]^+$	242→107	41	23	242→185	41	13	1	28

Table 1. (*continued*)

				First transition			Second transition				
Analyte	ESI	RT, min[a]	Precursor ion	*m/z*	DP, V[b]	CE, V[c]	*m/z*	DP, V	CE, V	Supplier[d]	References
Fenhexamide	+	15.0	$[M+H]^+$	302→97	91	33	302→55	91	57	1	
Fenoxycarb	+	15.5	$[M+H]^+$	302→88	66	29	302→116	61	17	1	19, 28
Fenpropimorph	+	19.5	$[M+H]^+$	304→147	46	39	304→117	61	71	1	
Flazasulfuron	+	10.2	$[M+H]^+$	408→182	41	25	408→227	36	25	1	
Florasulam	+	8.6	$[M+H]^+$	360→129	71	29	360→192	81	23	1	
Fluazifop-P-butyl	+	16.9	$[M+H]^+$	384→282	61	25	384→328	61	21	1	
Fludioxonil	–	14.4	$[M–H]^-$	247→126	–56	–42	247→169	–56	–42	1	
Flufenoxuron	+	18.2	$[M+H]^+$	489→158	86	27	489→141	86	57	1	13
Fosthiazate	+	12.7	$[M+H]^+$	284→104	61	27	284→228	61	15	1	
Furathiocarb	+	17.0	$[M+H]^+$	383→195	51	23	383→252	46	19	1	28
Haloxyfop-ethoxyethyl	+	16.8	$[M+H]^+$	434→316	81	25	434→288	81	35	1	
Haloxyfop-methyl	+	16.3	$[M+H]^+$	376→316	91	23	376→288	86	33	1	
Hexaflumuron	–	16.5	$[M–H]^-$	459→439	–6	–14	459→276	–16	–22	1	
Imazalil	+	15.7	$[M+H]^+$	297→159	26	31	297→201	21	23	1	16, 28
Imidacloprid	+	7.7	$[M+H]^+$	256→209	51	21	256→175	46	25	1	14, 28, 29, 39
Indoxacarb	+	16.3	$[M+H]^+$	528→203	76	51	528→56	76	55	3	
Iodosulfuron-methyl	+	11.3	$[M+H]^+$	508→167	36	27	508→141	41	35	1	
Ioxynil	–	11.3	$[M–H]^-$	370→127	–46	–36	370→243	–16	–26	1	10, 27
Iprovalicarb	+	14.8	$[M+H]^+$	321→119	46	23	321→203	51	13	3	
Isoproturon	+	13.3	$[M+H]^+$	207→165	46	19	207→72	46	33	3	10, 23, 30, 34
Isoxaflutole	+	13.3	$[M+NH_4]^+$	377→251	26	25	NO[e]				
Linuron	+	14.2	$[M+H]^+$	249→160	66	23	249→182	71	21	2	23, 28, 32, 34
MCPA	–	10.9	$[M–H]^-$	199→141	–46	–18	NO				9–11, 27
Mecoprop-P	–	12.1	$[M–H]^-$	213→141	–51	–14	NO				9, 10, 23
Metalaxyl	+	13.3	$[M+H]^+$	280→220	46	19	280→160	51	31	1	39
Metamitron	+	8.1	$[M+H]^+$	203→175	56	23	203→104	51	29	1	10
Methamidophos	+	1.7	$[M+H]^+$	142→94	26	19	142→125	26	19	1	24, 25
Methiocarb	+	14.3	$[M+NH_4]^+$	243→169	11	17	243→121	11	27	1	28
Methomyl	+	3.9	$[M+H]^+$	163→88	36	13	163→106	36	15	1	18, 20, 28
Metolachlor	+	15.2	$[M+H]^+$	284→252	16	19	284→176	11	35	3	23, 31
Metsulfuron-methyl	+	8.0	$[M+H]^+$	382→167	36	21	382→199	31	27	1	21, 23
Monocrotophos	+	5.5	$[M+H]^+$	224→127	46	21	224→98	46	17	1	25
Nicosulfuron	+	6.4	$[M+H]^+$	411→182	61	25	411→106	51	45	1	22
Omethoate	+	2.1	$[M+H]^+$	214→125	51	29	214→109	51	35	1	25
Oxamyl	+	3.0	$[M+NH_4]^+$	237→72	1	21	237→90	1	13	1	20, 28
Oxydemeton-methyl	+	3.6	$[M+H]^+$	247→169	21	19	247→109	41	35	4	
Phenmedipham	+	13.9	$[M+H]^+$	301→136	56	25	301→168	56	15	3	36
Pirimicarb	+	12.8	$[M+H]^+$	239→72	16	31	239→182	16	21	1	15, 18, 19, 29, 37
Primisulfuron-methyl	+	13.2	$[M+H]^+$	469→254	71	25	469→199	71	25	1	22, 23
Promecarb	+	14.4	$[M+H]^+$	208→109	11	21	208→151	11	13	3	28, 32
Propamocarb	+	3.5	$[M+H]^+$	189→102	16	23	189→144	16	17	1	
Propoxur	+	11.6	$[M+H]^+$	210→111	11	19	210→168	6	11	4	15, 19, 20, 28
Prosulfuron	+	12.5	$[M+H]^+$	420→141	56	27	420→167	61	25	1	
Pymetrozin	+	6.0	$[M+H]^+$	218→105	56	27	218→79	51	47	1	

Table 1. (*continued*)

Analyte	ESI	RT, min[a]	Precursor ion	First transition			Second transition			Supplier[d]	References
				m/z	DP, V[b]	CE, V[c]	*m/z*	DP, V	CE, V		
Pyridate	+	20.3	$[M+H]^+$	379→207	6	21	379→351	41	17	4	
Pyridate metabolite (6-chloro-3-phenylpyridazine-4-ol)	+	6.3	$[M+H]^+$	207→104	66	31	207→77	71	43	4	
Pyrimethanil	+	14.2	$[M+H]^+$	200→107	61	33	200→82	51	35	2	
Quinmerac	+	4.7	$[M+H]^+$	222→204	21	23	222→141	26	43	1	
Quizalofop-ethyl	+	17.0	$[M+H]^+$	373→299	71	25	375→301	71	23	3	
Rimsulfuron	+	8.4	$[M+H]^+$	432→182	46	29	432→325	56	21	1	23
Spiroxamine	+	15.4	$[M+H]^+$	298→144	41	27	298→100	41	41	3	
Tebuconazole	+	15.7	$[M+H]^+$	308→70	21	39	308→125	21	47	3	30
Tebufenozid	+	15.4	$[M+H]^+$	353→133	41	23	353→297	41	15	3	
Teflubenzuron	–	17.4	$[M-H]^-$	379→339	–6	–12	379→359	–11	–8	1	
Thiabendazole	+	11.3	$[M+H]^+$	202→175	56	35	202→131	61	43	4	14–16, 28, 34, 39
Thiacloprid	+	10.2	$[M+H]^+$	253→126	81	29	253→186	76	19	3	
Thifensulfuron-methyl	+	8.1	$[M+H]^+$	388→167	36	21	388→205	21	33	1	21, 23, 40
Thiodicarb	+	13.0	$[M+H]^+$	355→88	26	21	355→108	26	21	1	28
Thiofanox	+	12.7	$[M+H]^+$	219→57	16	17	219→61	11	15	3	
Thiofanox sulfone	+	7.6	$[M+NH_4]^+$	268→57	16	29	268→76	6	17	1	
Thiofanox sulfoxide	+	6.8	$[M+NH_4]^+$	252→104	6	17	252→178	6	15	1	
Thiophanate-methyl	+	12.0	$[M+H]^+$	343→151	26	25	343→192	26	21	4	14, 16, 28
Triasulfuron	+	10.3	$[M+H]^+$	402→167	46	25	402→141	41	29	1	21–23
Tribenuron-methyl	+	6.9	$[M+H]^+$	396→155	51	21	396→181	61	27	1	23, 40
Triflumuron	–	15.9	$[M-H]^-$	357→154	–16	–14	357→176	–16	–22	1	
Triflusulfuron-methyl	+	13.3	$[M+H]^+$	493→264	46	29	493→238	46	29	1	
Vamidothion	+	8.6	$[M+H]^+$	288→146	16	17	288→118	16	31	1	24, 25, 28

[a] RT = Retention time obtained with standards in solvent on a Phenomenex Aqua, 5 μm, C18, 125 Å, 50 × 2 mm column.
[b] DP = Declustering potential (similar to the cone voltage of other manufacturers).
[c] CE = Collision energy.
[d] 1 = Ehrenstorfer, 2 = Riedel-de-Haen, 3 = registration applicant, and 4 = other.
[e] NO = Second product ion not observed.

(**f**) *Ammonium formate*.—Analytical grade (Fluka, now Sigma-Aldrich, Seelze, Germany).

(**g**) *Sodium chloride solution*.—20 g NaCl/100 mL water (Merck).

Apparatus

(**a**) *Homogenizer*.—Ultra Turrax T25 (Jahnke und Kunkel, Staufen/Breisgau, Germany).

(**b**) *Centrifuge*.—Varifuge GL, Model 4100 (Heraeus-Christ, Osterode, Germany).

(**c**) *Vacuum rotary evaporator*.—Buchi Rotavapor Model R110 (Buchi Labortechnik, Essen, Germany).

(**d**) *Liquid chromatograph*.—Agilent 1100 system equipped with G1322A degasser, G1312A binary pump, G1313A autosampler, and G1316A column oven (Agilent Technologies Deutschland, Waldbronn, Germany).

(**e**) *Analytical columns*.—Luna, 5 μm, C18, 50 × 2 mm; Luna, 3 μm, C18(2), 50 × 2 mm; and Aqua, 5 μm, C18, 125 Å, 50 × 2 mm (Phenomenex, Aschaffenburg, Germany).

(**f**) *MS/MS system*.—Applied Biosystems API 2000 triple-quadrupole mass spectrometer (Applera Deutschland, Weiterstadt, Germany) equipped with either a TurboIonSpray (electrospray ionization; ESI) or a heated nebulizer atmospheric pressure chemical ionization (APCI) interface.

Extraction

All experiments were conducted with 5 different matrixes: tomato, lemon, avocado, raisins, and wheat flour. The samples were obtained from local supermarkets without paying special

Table 2. API source parameter sets

Parameter	ESI+	ESI–	1st APCI+/–	2nd APCI+/–	3rd APCI+/–
Curtain gas (nitrogen), psi	35	35	35	35	35
Heater gas temperature, K	400	350	325	375	425
Collision gas (nitrogen), psi	2	2	2	2	2
Ion spray voltage (ESI), V	5500	–4200	—	—	—
Nebulizer gas (ESI), psi	60	60	—	—	—
Heater gas (ESI), psi	60	60	—	—	—
Auxilliary gas (APCI), psi	—	—	70	70	70
Nebulizer gas (APCI), psi	—	—	35	35	35
Needle current, μA	—	—	2	2	2

attention to obtaining "noncontaminated" sample materials, which were tested as blanks.

(**a**) *Recovery experiments.*—Fresh fruit and vegetable samples were cut into small pieces to avoid the loss of juice. In the case of tomato, lemon, or avocado, the spiking solutions were added to a 10 g portion of the thoroughly mixed samples. The amount of spiked sample for raisins or wheat flour was 5 g. Water was added to all samples 1 h after fortification to obtain 10 mL as the sum of the natural and added water. To 10 g tomato (water content, 95%), lemon (water content, 90%), or avocado (water content, 70%), 0.5, 1, or 3 mL water was added, respectively. In the case of raisins (5 g sample; water content, 20%) and wheat flour (5 g sample; water content, 10%), the amounts of water added were 9 and 9.5 mL, respectively. These water-containing test portions were homogenized in centrifuge tubes (glass) with 20 mL methanol for 2 min by using an Ultra Turrax T25 tissue dispenser. The homogenate was centrifuged at ca 3000 × *g*.

(**b**) *Other extraction solvents.*—By using the above procedure, additional sample extracts were produced with 20 mL acetone, acetonitrile, or water, which replaced the 20 mL methanol. Extraction with 20 mL ethyl acetate required no addition of water. The nonvolatile residue of 3 mL extract thus obtained was weighed after evaporation to dryness at 110°C.

Cleanup

A 6 mL aliquot of the methanol–water extract was mixed well with 2 mL NaCl aqueous solution. A 5 mL aliquot was transferred to a ChemElut column with 5 mL sample capacity. After 5 min equilibration, the column was washed with 16 mL dichloromethane. The eluate collected was evaporated to dryness at 40°C. The residue was redissolved in 250 μL methanol with the help of an ultrasonic bath, and the solution was further diluted with 1 mL water. The resulting final extract (1.25 mL) contained the residues of 1 g water-containing (tomato, avocado, lemon) or 0.5 g dry sample (wheat flour, raisins) per milliliter. Finally, the extract was filtered through a 0.45 μm PTFE filter into a glass vial.

Two blank extracts used for the preparation of matrix-matched standards were produced with a mixture of 15 mL sample extract and 5 mL NaCl solution for each. The partition was performed with 64 mL dichlormethane in a ChemElut column with 20 mL sample capacity. Each residue was redissolved in 1 mL methanol and 4 mL water.

Liquid Chromatography

In all experiments, mobile phase A was water–methanol (80 + 20), and mobile phase B was water–methanol (10 + 90). Mobile phases A and B both contained 5mM ammonium formate. The flow rate was 0.2 mL/min, and the injection volume was 20 μL. The mobile phase composition was changed during a run as follows: Starting with 0%, the percentage of mobile phase B was increased linearly to 100% over 11 min and then kept constant for another 12 min. Equilibration time before the next injection was 15 min.

Mass Spectrometry

(**a**) *General.*—The effluent from the LC system was introduced into an API 2000 triple-quadrupole mass spectrometer equipped with either a TurboIonSpray (ESI) or a heated nebulizer (APCI) interface. The analytes were directly infused for optimization procedures using the syringe pump of the API 2000. ESI source parameters in the positive ion mode were optimized for 3 different analytes (pirimicarb, bendiocarb, and dimethoate). Because no distinct variation of optimum source parameters was observed between the 3 analytes, the ESI source parameters were kept constant for all analytes of this study.

The APCI source was optimized with some analytes by starting from the settings proposed by the manufacturer for optimum performance. For final optimization of the heated-nebulizer temperature, the responses of all analytes obtained at different temperatures were determined simultaneously in LC/MS/MS runs. Table 2 summarizes the ESI and APCI source parameters finally used for both the positive and the negative ionization modes.

(**b**) *Selection of multireaction mode (MRM) transitions.*—The transitions in the MRM of the tandem mass spectrometer were selected and tuned by using solutions of individual analytes in water–methanol (1 + 1) with 5mM ammo-

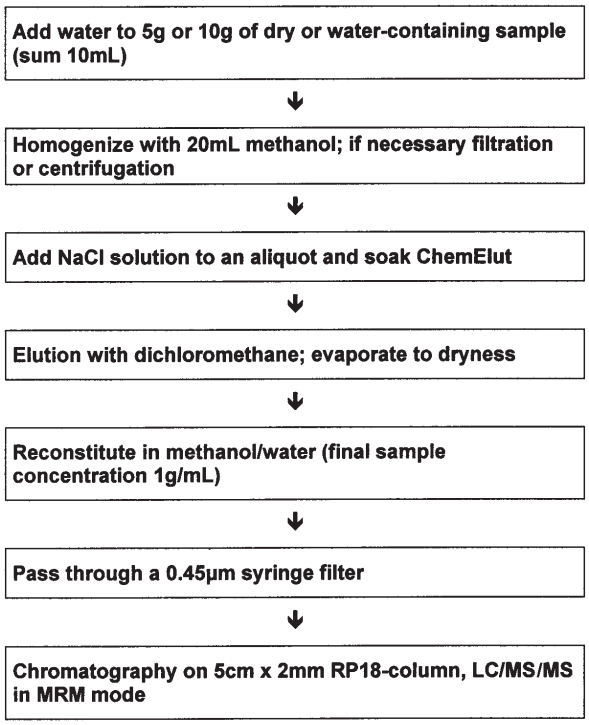

Figure 1. Flow chart of the method.

nium formate at a concentration of 100 ng/mL. These solutions were introduced into the mass spectrometer via a syringe pump at a flow rate of 0.2 mL/min.

(**c**) *ESI versus APCI.*—The whole set of compounds was analyzed simultaneously in subsequent LC/MS/MS runs with the LC gradient mentioned above. The electrospray source and the APCI source were operated with the selected spray conditions (Table 2). All injection parameters [20 µL injection volume, 0.2 mL/min flow rate, and methanol–water (1 + 1) with 5mM $HCOONH_4$] and all analyte-dependent parameters of the ion path were kept constant.

(**d**) *Interference check.*—The flow injection experiments were performed with solutions of individual analytes [20 µL injection volume, 0.2 mL/min flow rate, and 100 ng/mL in methanol–water (1 + 1) with 5mM $HCOONH_4$]. After injection, all MRM transitions covered by the method were recorded.

(**e**) *Number of simultaneously detectable MRM transitions (dwell-time experiment).*—A mixture of ethiofencarb sulfone, ethiofencarb sulfoxide, imidacloprid, metsulfuron-methyl, nicosulfuron, pymetrozin, rimsulfuron, thiofanox sulfoxide, and thifensulfuron-methyl, at a concentration of 100 ng/mL each, was subjected to a positive ESI (ESI+) flow injection analysis using decreasing dwell times (conditions were the same as those for the interference check).

(**f**) *Final acquisition methods.*—A first method contained the parameter set for 98 positive MRM transitions of precursor ions produced with electrospray (Table 1), i.e., one transition for each pesticide to be detected. A dwell time of 25 ms per transition and the source parameters listed in Table 2 were used. The second method summarized 10 negative MRM transitions with a dwell time of 150 ms each. Thus, 2 injections per run were used. For confirmation purposes, a second transition was identified for each analyte (Table 1), but these transitions were not used during method validation.

Recovery Experiments

(**a**) *Sample set.*—The whole methodology (Figure 1) was validated by using 11 samples for each matrix: 2 blanks, 3 samples fortified at 0.01 mg/kg, and 2 samples fortified at each of the following levels: 0.05, 0.1, and 1 mg/kg. A stock solution (1 µg/mL in methanol) containing most of the analytes was prepared and kept at –78°C. Because of the relatively fast decomposition of tribenuron-methyl and primisulfuron in methanol, a second stock solution of these compounds was prepared in water (pH adjusted to 8 with ammonia). Extracts were prepared as described under *Extraction* and *Cleanup*, with methanol–water (2 + 1) as the extraction solvent. The final extracts of tomato, lemon, and avocado represented 1 g sample in 1 mL solvent (methanol–water [20 + 80], 5mM $HCOONH_4$), whereas the extracts prepared from raisins and wheat flour represented 0.5 g sample in 1 mL solvent. The extracts were filtered into glass vials through a 0.45 µm PTFE filter for LC/MS/MS analysis.

(**b**) *Calibration.*—In accordance with the European Union Guidelines for Residue Monitoring (41), calibration was achieved by preparing matrix-matched standards from the extracts of blank samples. For comparison purposes (determination of matrix effects), standards in solvent also were used. As a consequence of the 2 weights of the test portions (10 or 5 g), depending on the water content of the individual matrix, each particular spiking level corresponded to 2 different standard concentrations. The concentrations of the standards prepared are summarized in Table 3. Analytes were quantified by using a 3-point calibration with those matrix-matched standards appropriate for the spiked concentration. For example, samples

Table 3. Analyte concentration in standard mixtures

	Standard concentration, ng/mL	
Spiking level, mg/kg	For 10 g test portion[a]	For 5 g test portion[b]
0.005	5	2.5
0.010	10	5
0.025	25	12.5
0.050	50	25
0.100	100	50
0.500	500	250
1.000	1000	500

[a] Tomato, lemon, and avocado.
[b] Raisins and wheat flour.

Table 4. Relative response factors (RRFs) of all analytes compared with the ESI response of imazalil[a]; comparison of ESI (under optimized conditions) and APCI at various heated-nebulizer temperatures

		RRF			
Analyte	Polarity	ESI 400°C	APCI 325°C	APCI 375°C	APCI 425°C
2,4-D[b]	–	0.017	0.026[b]	0.032[b]	0.038[b]
3,4,5-Trimethacarb	+	5.414	0.018	0.016	0.019
3-Hydroxycarbofuran	+	0.312	0.129	0.077	0.054
5-clethodim sulfone	+	0.076	0.005	0.005	0.008
5-Hydroxythiabendazole	+	1.166	0.067	0.099	0.077
Acephate[b]	+	0.405	0.775[b]	0.540[b]	0.389
Aldicarb	+	2.298	0.151	0.106	0.059
Aldicarb sulfoxide	+	0.740	0.007	0.002	0.002
Aldoxycarb	+	0.611	0.078	0.036	0.017
Amidosulfuron	+	0.130	—[c]	0.001	0.001
Atrazine	+	2.004	0.751	0.660	0.559
Azoxystrobin	+	2.451	0.112	0.091	0.077
Bendiocarb[b]	+	0.100	0.137[b]	0.101[b]	0.075
Bensulfuron-methyl	+	0.405	0.014	0.008	0.004
Bromoxynil	–	0.112	0.013	0.013	0.014
Butocarboxim	+	3.627	0.206	0.113	0.060
Butocarboxim sulfoxide	+	0.777	0.048	0.029	0.014
Butoxycarboxim	+	1.721	0.174	0.096	0.065
Carbaryl	+	0.325	0.255	0.207	0.158
Carbendazim	+	8.410	0.562	0.387	0.263
Carbofuran	+	2.495	1.710	1.373	0.997
Chlorsulfuron	+	0.123	0.003	0.001	0.001
Cinosulfuron	+	0.722	0.001	0.002	0.002
Clethodim	+	0.370	0.029	0.020	0.019
Clethodim-imin sulfone	+	0.270	0.063	0.072	0.057
Clethodim-imin sulfoxide	+	1.852	0.265	0.222	0.163
Clethodim sulfone	+	0.098	0.005	0.006	0.007
Clethodim sulfoxide	+	0.350	0.009	0.011	0.012
Cyprodinil	+	1.122	0.111	0.115	0.097
Daminozide	+	1.111	0.003	0.005	0.004
Demeton-S-methyl	+	2.037	0.003	0.002	0.001
Demeton-S-methyl sulfone	+	1.111	0.034	0.017	0.010
Desmedipham	+	2.614	0.017	0.010	0.006
Desmethylformamido-pirimicarb	+	7.756	1.438	1.176	0.931
Desmethyl-pirimicarb	+	5.251	0.867	0.749	0.625
Diflubenzuron	–	0.018	0.005	0.003	0.002
Dimethoate	+	1.111	0.366	0.312	0.259
Diuron	+	1.122	0.043	0.040	0.031
Ethiofencarb	+	2.102	0.528	0.437	0.327
Ethiofencarb sulfone	+	4.270	0.043	0.017	0.018
Ethiofencarb sulfoxide	+	3.617	0.064	0.026	0.019
Fenhexamide	+	0.420	0.064	0.058	0.050
Fenoxycarb	+	1.863	0.050	0.037	0.030
Fenpropimorph	+	4.869	0.326	0.295	0.279

Table 4. (*continued*)

Analyte	Polarity	RRF			
		ESI 400°C	APCI 325°C	APCI 375°C	APCI 425°C
Flazasulfuron	+	0.508	0.004	0.004	0.003
Florasulam	+	0.589	0.057	0.049	0.048
Fluazifop-P-butyl	+	2.255	0.131	0.131	0.111
Fludioxinil	–	0.103	0.014	0.014	0.013
Flufenoxuron	+	0.580	0.038	0.025	0.030
Fosthiazate	+	3.562	0.011	0.006	0.006
Furathiocarb	+	2.647	0.053	0.035	0.025
Haloxyfop-ethoxyethyl	+	0.836	0.006	0.005	0.004
Haloxyfop-methyl	+	1.166	0.034	0.032	0.026
Hexaflumuron	–	0.017	0.008	0.006	0.006
Imazalil (reference)	+	(1.000)	0.098	0.099	0.091
Imidacloprid	+	0.361	0.089	0.075	0.064
Indoxacarb	+	0.145	0.007	0.004	0.002
Iodosulfuron-methyl	+	0.505	0.003	0.002	0.000
Ioxynil	–	0.292	0.024	0.023	0.023
Iprovalicarb	+	3.889	0.142	0.093	0.073
Isoproturon	+	0.596	0.031	0.029	0.027
Isoxaflutole	+	0.380	0.005	0.004	0.004
Linuron	+	0.440	0.041	0.038	0.037
MCPA	–	0.044	0.013	0.015	0.018
Mecoprop-P	–	0.059	0.007	0.008	0.008
Metalaxyl	+	2.876	0.058	0.047	0.038
Metamitron	+	0.657	0.055	0.050	0.045
Methamidophos	+	0.514	0.411	0.344	0.307
Methiocarb	+	5.261	0.053	0.047	0.040
Methomyl	+	1.176	0.045	0.037	0.031
Metolachlor	+	4.227	0.995	0.886	0.836
Metsulfuron-methyl	+	0.359	0.002	0.001	0.002
Monocrotophos	+	1.569	0.009	0.010	0.005
Nicosulfuron	+	0.109	—[c]	—[c]	—[c]
Omethoate	+	1.405	0.029	0.013	0.012
Oxamyl	+	3.617	0.184	0.099	0.087
Oxydemeton-methyl	+	3.105	0.272	0.149	0.088
Phenmedipham	+	0.752	0.004	0.003	0.002
Pirimicarb	+	7.636	1.329	1.231	1.111
Primisulfuron-methyl	+	0.105	0.010	0.004	0.002
Promecarb[b]	+	0.691	0.813[b]	0.680	0.533
Propamocarb	+	7.625	1.004	0.605	0.504
Propoxur	+	1.209	0.648	0.536	0.373
Prosulfuron	+	0.115	0.014	0.003	0.002
Pymetrozin	+	2.397	0.216	0.204	0.179
Pyridate	+	1.133	0.036	0.025	0.019
Pyridate metabolite	+	1.078	0.192	0.144	0.130
Pyrimethanil	+	0.757	0.163	0.141	0.145
Quinmerac	+	2.723	0.034	0.064	0.091

Table 4. (*continued*)

Analyte	Polarity	RRF			
		ESI 400°C	APCI 325°C	APCI 375°C	APCI 425°C
Quizalofop-ethyl	+	1.318	0.051	0.041	0.042
Rimsulfuron	+	0.036	—[c]	—[c]	—[c]
Spiroxamine	+	12.96	0.448	0.413	0.377
Tebuconazole	+	0.951	0.481	0.461	0.427
Tebufenozid	+	4.989	0.085	0.057	0.042
Teflubenzuron	–	0.024	0.010	0.008	0.009
Thiabendazole	+	3.769	0.359	0.286	0.283
Thiacloprid	+	3.148	0.190	0.187	0.169
Thifensulfuron-methyl	+	0.344	—[c]	0.003	0.002
Thiodicarb	+	0.916	0.016	0.015	0.019
Thiofanox	+	1.050	0.110	0.059	0.022
Thiofanox sulfone	+	1.765	0.020	0.013	0.007
Thiofanox sulfoxide	+	3.377	0.033	0.016	0.011
Thiophanate-methyl	+	1.209	0.057	0.069	0.079
Triasulfuron	+	0.265	0.002	0.001	0.001
Tribenuron-methyl	+	0.625	0.002	0.001	0.000
Triflumuron[b]	–	0.047	0.099[b]	0.073[b]	0.073[b]
Triflusulfuron-methyl	+	0.010	—[c]	—[c]	—[c]
Vamidothion	+	2.778	0.419	0.259	0.194
Median of RRFs		1.025	0.053	0.039	0.031

[a] Approximately 900 000 counts after injection of 20 μL standard solution at 100 ng/mL.
[b] Analytes and response factors indicating those few analytes giving a better response with APCI than with ESI.
[c] — = Signal was too small to measure.

spiked at a level of 0.01 mg/kg were quantified with standards corresponding to 0.005, 0.01, and 0.025 mg/kg. Samples and standards were measured immediately after preparation.

Results and Discussion

Selection of MRM Transitions

Suitable transitions from precursor to product ions (MRM transitions) were identified with the help of the automatic tune function of the instrument software. Usually, transitions from most abundant precursor to most abundant product ions were selected. Small fragments with *m/z* ratios of <80 were generally omitted if alternative product ions were available. In order to (*1*) achieve a stable and high abundance of precursor ions, (*2*) select 2 suitable mass transitions, and (*3*) optimize the yield of product ions, each analyte was tuned individually. Because preliminary experiments showed an influence of the flow rate on the declustering potential, the syringe pump was operated at a flow rate of 0.2 mL/min. To detect interference with such solvent clusters that may occur during an LC run, water–methanol (1 + 1) with 5mM ammonium formate was chosen as the solvent for tuning.

The most important analyte-dependent parameters, declustering potential (DP) and collision energy (CE), thus found are summarized in Table 1. As indicated, in a few cases the $[M + NH_4]^+$ ion was chosen as the precursor ion because of the higher ionization yield compared with that of the $[M + H]^+$ ion. These tune parameters were used for >6 months without any necessity of retuning, i.e., no striking decrease in sensitivity was observed. It should be noted that several analytes tested gave only a very low ESI response or no signal at all (i.e., bromoxynil octanoate, ioxynil octanoate, 1,1-dimethylhydrazine, ethephon, fipronil, maleic acid, azadirachtin, cymoxanil, and esters of MCPA).

A comparison between the individual MRM transitions chosen here and those of other researchers (*see* references in Table 1) has, in many cases, shown the same ions to be most suitable for tracing a given analyte irrespective of the type of spectrometer and the source parameters. This fact is impressively illustrated especially when a comparison is made with the multiresidue method presented by Jansson et al. (28). Using a Micromass Quattro spectrometer and an almost identical eluant system (methanol–water with 10mM $HCOONH_4$), Jansson et al. (28) studied 32 of the analytes included in our method. For 21 compounds, exactly the same transitions were found to be most suitable, and for an additional 7 analytes, the transitions chosen by us as qualifier transitions were used.

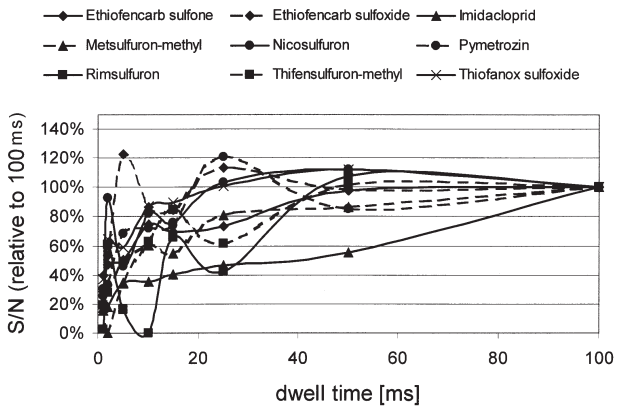

Figure 2. Dependence of S/N (relative to the S/N at a dwell time of 100 ms) on dwell time.

ESI versus APCI

Table 4 summarizes the relative response factors based on the electrospray response (LC peak area) of imazalil. This pesticide was chosen as the reference because its response factor represents the median of the response factors of all analytes investigated. When the optimized source parameters reported in Table 2 and identical standard concentrations (100 ng/mL) were used, most analytes exhibited a better response with ESI. Only the 5 pesticides 2,4-D, acephate, bendiocarb, triflumuron, and promecarb (Table 4) produced higher signal intensities with APCI compared with ESI. In addition, the ESI response factors presented in Table 4 show a high variation between analytes of 3 orders of magnitude. The best APCI response in terms of the median of relative response of all analytes was obtained with the source operated at 325°C. This shows that the most advantageous heated-nebulizer temperature for APCI was lower than the analogous temperature selected for electrospray. One reason for this surprising result is the use of a heater-gas temperature of 400°C in the electrospray experiment. Such a high temperature is recommended by the manufacturer for flow rates of 0.2 mL if the LC eluant consists mainly of water. Because an LC gradient was used, which starts with 80% water and ends with 90% methanol, spray conditions could not be the best for all analytes. The reason we chose a high temperature was the observation that small peaks more often occur in the early part of the chromatogram, i.e., with high water content in the eluant. The use of this high heater–gas temperature resulted in an improved signal intensity of the early (smaller) peaks at the expense of some decrease in intensity of the later-eluting compounds, which are more easily detected.

Irrespective of the parameter chosen, the median of the relative response factors was significantly smaller when APCI was used. This finding is in contrast to results obtained by Thurman et al. (42), who reported a less-sensitive detection of many neutral and basic pesticides, using ESI with an Agilent HP1100 liquid chromatograph/mass spectrometer. One apparent reason is the higher flow rate (0.3–0.4 mL) applied in their study, which usually enhances APCI response and decreases ESI response. Additionally, the ion source of the

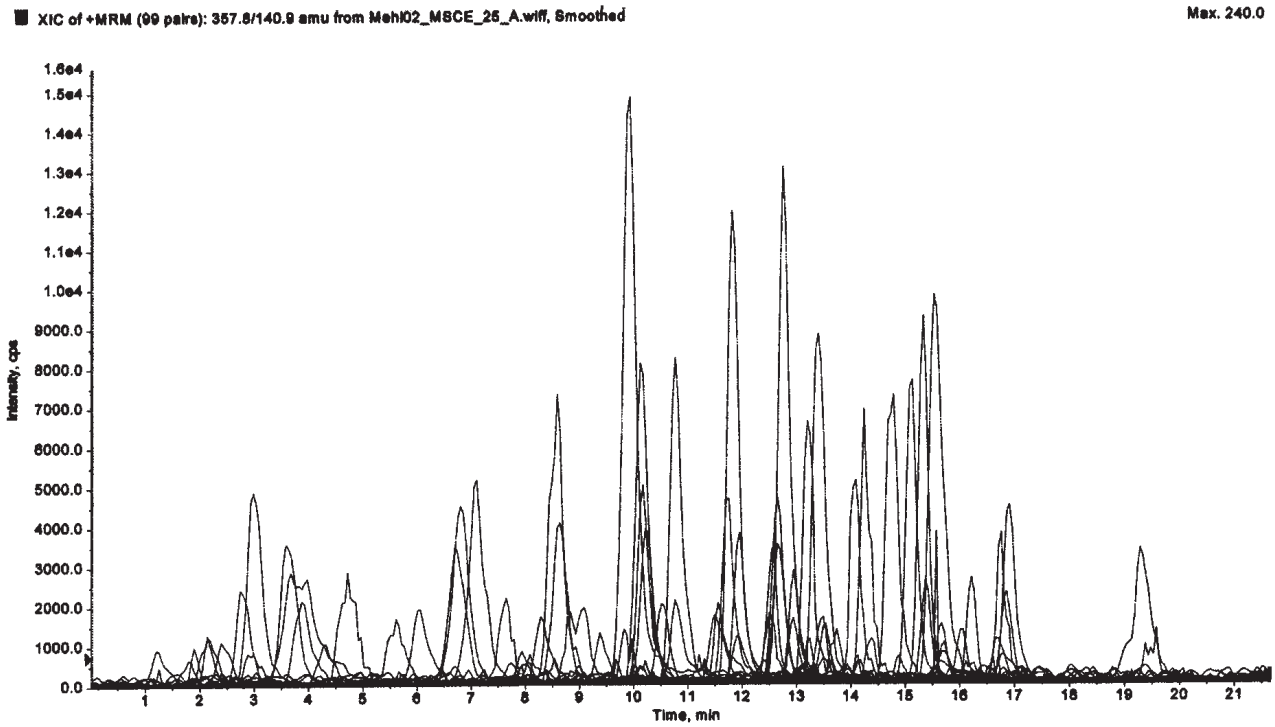

Figure 3. Chromatogram of a standard with 98 pesticides at a level of 0.025 mg/kg prepared in wheat flour blank extract.

Table 5. Amounts (mg) of coextracted matrix components for the different extraction solvents tested

Matrix	Matrix type	Amount of extracted sample, g	Acetone–water (2 + 1)	Acetonitrile–water (2 + 1)	Methanol–water (2 + 1)	Water	Ethyl acetate
Tomato	Water-containing	1.0	38	36	34	33	8
Orange[a]	Acidic	1.0	79	69	81		3
Wheat flour	Dry	0.5	31	12	52	33	5
Raisins	Sugar-containing	0.5	370	148	333	302	4
Avocado	Fatty	1.0	33	40	30	43	332

[a] Orange used instead of lemon.

HP1100 instrument shows some significant differences, compared with the ESI source of the API 2000, for example, the orthogonally positioned spray device and the use of a drying gas that acts like a combination of the turbo and curtain gas of the API 2000 ion source.

Number of Simultaneously Detectable MRM Transitions (Dwell-Time Experiment)

The dependence of signal intensity (measured as peak area in counts per second) and signal-to-noise ratio (S/N) on dwell time was tested with a mixture of ethiofencarb sulfone, ethiofencarb sulfoxide, imidacloprid, metsulfuron-methyl, nicosulfuron, pymetrozin, thiofanox sulfoxide, thifensulfuron-methyl, and rimsulfuron, each at a concentration of 100 ng/mL. This mixture was subjected to an ESI+ flow injection analysis (conditions were the same as those for the interference check). The dwell time used for these analytes was varied as follows: 100, 50, 25, 15, 10, 5, 2, and 1 ms. To keep the cycle time constant, a 10th "dummy" transition was introduced into the MS/MS acquisition method. The dwell time of this dummy transition was rising from 450 ms for a 100 ms analyte dwell time to 900, 1125, 1215, 1260, 1305, 1332, and 1341 ms, respectively, for the other experiments. Together with a settling time of 700 ms (minimum value automatically set) and a pause between each transition of 5 ms, a constant cycle time of 2.1 s was obtained in these MS/MS experiments.

A dwell-time reduction to 20 ms was accompanied by only a minor reduction in time-normalized signal intensity (measured in counts per second). As demonstrated in Figure 2, the S/N decreased in the worst 2 cases to about 40% of the 100 ms value. However, this decreased S/N compares with an absolute ratio of >100 for the compounds investigated. This experiment was performed at a concentration of 100 ng/mL; thus, quantification of even lower levels seems possible. Consequently, with a cycle time of approximately 3 s, a simultaneous measurement of about 100 MRM transitions is possible. In cases of sufficiently high pesticide concentrations, even measurements with a minimum dwell time of 10 ms should be feasible, allowing the simultaneous observation of about 200 MRM transitions.

The desirable simultaneous detection of analytes with ESI+ and negative ESI (ESI–) requires switching the polarity of all parameters. Thus, 2 times a settling time of ≥700 ms for each acquisition cycle (i.e., single data point of the chromatogram) is required. A reduction by 50% of the time effectively available for analyte detection would be the consequence. Therefore, separate ESI+ and ESI– acquisition methods, i.e., 2 injections per sample, are proposed. Finally, a method with 98 MRM transitions (ESI+) with a dwell time of 25 ms was tested. Figure 3 presents the chromatograms of a matrix-matched standard (tomato; 0.025 mg/kg) obtained with this method. A second acquisition method with ESI– was

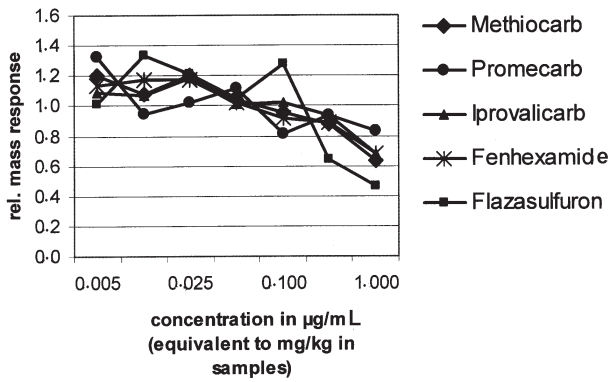

Figure 4. Trend of response factors. Mass response is normalized to the mean mass response of each pesticide. Matrix: lemon.

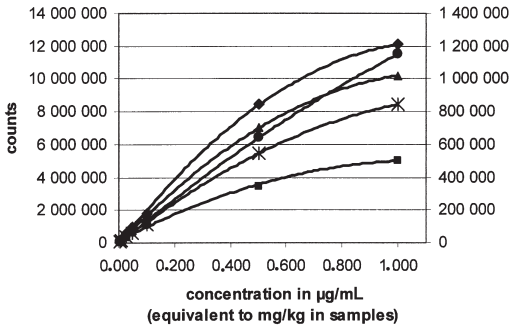

Figure 5. Usual calibration calculated with polynomial regression (2nd order) based on the data of Figure 4. Left axis: methiocarb and iprovalicarb. Right axis: promecarb, fenhexamide, and flazasulfuron. *See* Figure 4 legend.

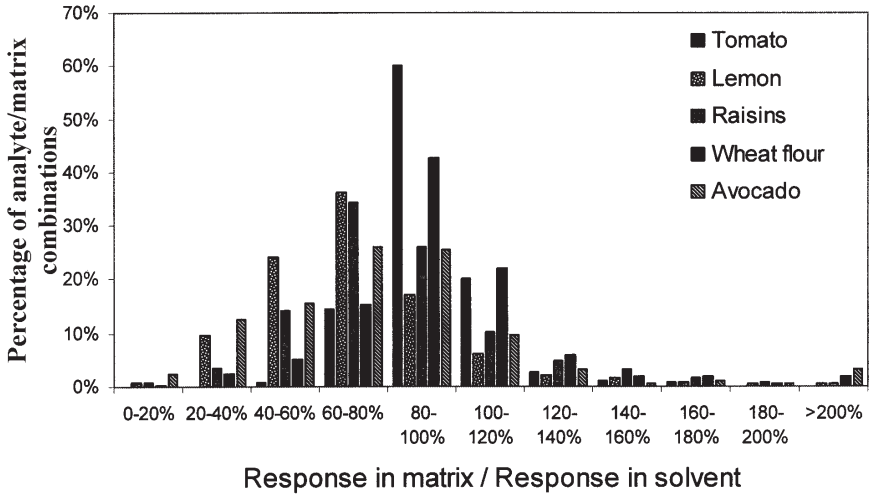

Figure 6. Magnitude of matrix effects observed with different matrixes.

established for another 10 pesticides (Table 1). Here, a dwell time of 150 ms was used because of the low number of analytes.

To enhance the dwell time for analytes eluting in a certain time window, separate acquisition periods usually are used. Switching to the next period is performed in a part of the chromatogram without elution of analytes. However, as demonstrated in Figure 3, such "empty" regions do not exist and, unfortunately, API 2000 software does not allow the overlap of such acquisition periods or the combination of MRM traces of 2 periods. To acquire complete peaks of all analytes, 2 consecutive injections with overlapping periods would be necessary. Because MRM experiments with a dwell time of 25 ms produced chromatographic peaks of an intensity sufficient to detect very low pesticide levels, we decided to avoid using more than one acquisition period.

Interference Check

As described above for each analyte, specific MRM transitions were selected (Table 1). To avoid any misinterpretation of detected signals, the aim of a first interference check was an examination of the selectivity of these transitions, i.e., the extent to which the injection of a certain pesticide results in additional peaks in the MRM traces of the other analytes. This examination was performed by flow injection analysis without separation on an LC column. Therefore, the check was able to identify potential problems, which must be avoided by an appropriate LC separation. The 74 first-tuned analytes were subjected to such an interference check. Additional peaks larger than 10% of the analyte peak were detected in 9 cases only. Most often, they were due to partial decomposition (e.g., thiophanate-methyl to carbendazim, thiodicarb to methomyl; *see* discussion below) or to common transitions. Such common transitions were found for the following pairs of analytes with identical molecular weights: *m/z* 411→182 for nicosulfuron and bensulfuron-methyl, *m/z* 226→93 for cyprodinil and methiocarb, and *m/z* 243→169 for methiocarb and ethiofencarb. The quasi molecular ion of desmethylformamido-pirimicarb (*m/z* 253) generates a fragment ion of *m/z* 225 in the orifice region of the interface between the ion source and the vacuum region. A subsequent breakdown of this fragment to a product ion of *m/z* 72 in the collision cell simulates the presence of desmethyl-pirimicarb, which is detected in our method by the transition *m/z* 225→72.

In a final interference check, a mixture containing all analytes was analyzed by ESI–MS/MS after LC separation. All interfering additional transitions resulted in peaks that were well separated from the target analyte. Because 34 analytes were not included in the first flow injection experiment, 2 additional MS/MS interferences could be detected. These are monocrotophos ($[M + H]^+$; *m/z* 224) simulating bendiocarb in its MRM trace *m/z* 224→167, and pyridate (*m/z* 379→207) simulating its metabolite (*m/z* 207→104). Additional peaks in the traces of clethodim, clethodim sulfoxide, and clethodim sulfone are presumably due to isomers (43).

A systematic study of crosstalks requiring an acquisition method based on sorted Q3 masses was not performed. However, such a crosstalk was observed by coincidence for haloxyfop-ethoxyethyl and haloxyfop-methyl (common product ion 316), because this pair of analytes was measured directly one after the other. This crosstalk did not cause any problem because the retention times of both analytes were sufficiently different. Even in the case of identical retention times, crosstalks can be circumvented by choosing an appropriate order of acquisition that avoids the use of an identical product ion mass in 2 successive transitions.

Unexpectedly, in nearly 50% of the individual standard solutions injected, a weak MRM transition, *m/z* 248→89, was observed, suggesting the presence of demeton-S-methyl. In freshly prepared solutions, the intensity of the detected signal corresponded to the very low concentration of 1 ng/mL, but it increased rapidly with time when vials with perforated septa were allowed to stand. In a separate experiment with a vial septum in pure solvent, the signal-producing compound was found to be released from these caps.

Table 6. Stability of some pesticides in matrix-matched standards

Pesticide	Matrix	Formation of degradation products (%)[a] after 12 h	1 day	4 days	Remaining parent (%)[b] after 4 days
Clethodim	Tomato	1	2	2	83
Demeton-S-methyl		0	0	0	89
Ethiofencarb		0	1	3	111
Thiodicarb		5	19	45	47
Thiofanox		1	1	1	101
Thiophanate-methyl		3	7	13	39
Clethodim	Lemon	1	2	1	80
Demeton-S-methyl		0	0	0	96
Ethiofencarb		1	1	3	109
Thiodicarb		7	9	23	63
Thiofanox		1	1	2	86
Thiophanate-methyl		2	3	6	69
Clethodim	Raisins	3	3	6	78
Demeton-S-methyl		0	0	1	89
Ethiofencarb		4	5	16	79
Thiodicarb		8	13	37	60
Thiofanox		2	3	8	100
Thiophanate-methyl		8	10	17	42
Clethodim	Wheat flour	3	3	7	91
Demeton-S-methyl		0	1	1	93
Ethiofencarb		4	6	16	107
Thiodicarb		6	10	24	69
Thiofanox		2	4	8	89
Thiophanate-methyl		6	9	14	53
Clethodim	Avocado	13	22	41	58
Demeton-S-methyl		3	4	8	67
Ethiofencarb		49	82	174[c]	24
Thiodicarb		16	26	53	48
Thiofanox		21	33	75	84
Thiophanate-methyl		23	33	43	6

[a] In blank matrixes spiked with clethodim, demeton-S-methyl, ethiofencarb, thiodicarb, thiofanox, and thiophanate-methyl, the formation of clethodim sulfoxide, oxydemeton-methyl, ethiofencarb sulfoxide, methomyl, thiofanox sulfoxide, and carbendazim was calculated on the basis of separate matrix-matched standards containing all degradation products only.

[b] The disappearance of analytes was determined by comparison of signal intensities measured with a matrix-matched standard stored 4 days in a refrigerator and a freshly prepared matrix-matched standard.

[c] The reason for this "recovery" that exceeds 120% could not be identified.

LC Conditions

The separation efficiency of Luna, 5 μm, C18; Luna, 3 μm, C18(2); and Aqua, 5 μm, C18, 125 Å was investigated. The behavior of all columns was quite similar. The longest retention times were observed on the Luna C18 (2). Because we planned to start the gradient with a very high water content, the Phenomenex Aqua, 5 μm, C18 column was chosen for further investigation. Only 2 compounds (fenbutatin oxide and cyhexatin) were excluded from further experiments because of high or unstable retention under the LC conditions chosen. The retention times obtained on this column for standards in solvent are summarized in Table 1.

Usually, the built-in divert valve can be used to reduce ion-source contamination by early-eluting matrix components, provided that first-eluting analytes are sufficiently separated from the matrix. Unfortunately, such conditions are not found here. At least 3 pesticides (daminozide, methamidophos, and acephate) elute 1.2–1.9 min after injec-

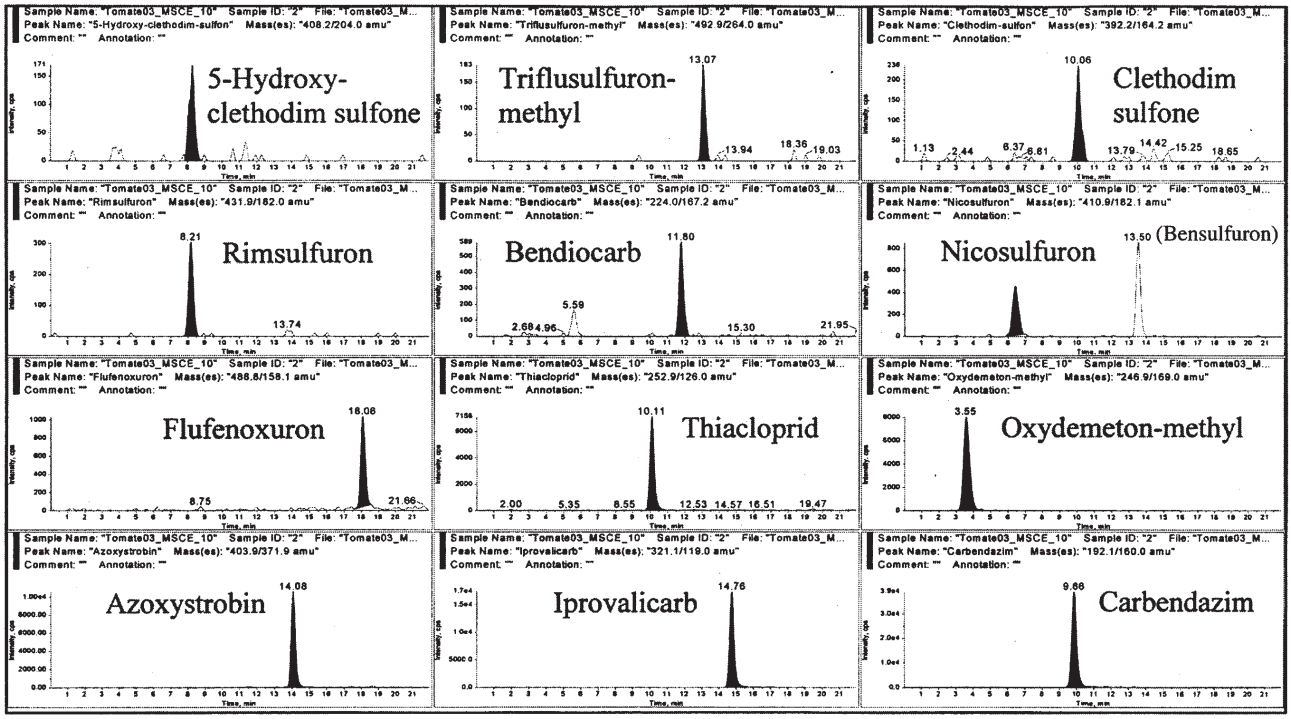

Figure 7. Matrix-matched standard (tomato) with 98 pesticides corresponding to a level of 0.01 mg/kg. For the upper 6 chromatograms, the analytes selected were those showing the poorest response of all (*see* Table 4).

tion. For this reason, and when the dead time of the column (0.9 min) is taken into account, the divert valve is ineffective in reducing ion-source contamination.

Additional peaks identified in the interference check showed retention times sufficiently different to separate them from the analyte peaks. As a result, the unambiguous identification of all pesticides under investigation was not disturbed by other analytes. Figure 3 shows the chromatogram obtained from a tomato extract fortified at 0.025 mg/kg. The chromatogram contains the signals of all 98 MRM transitions of the ESI+ method.

Selection of Extraction Solvent

Five different extraction solvents (acetone–water [2 + 1], acetonitrile–water [2 + 1], methanol–water [2 + 1], water, and ethyl acetate) were tested for their (unwanted) ability to coextract matrix constituents, which may pollute the ion source and reduce the ionization yield of analytes. This ability was measured as the nonvolatile residue after evaporation of solvent from the extracts (Table 5). The results for the 3 mixtures of organic solvent with water and water only did not strikingly differ from each other, but they did differ from the results for ethyl acetate. For most of the matrixes tested, ethyl acetate is a very suitable extraction solvent because of the very low amount of coextracted matrix constituents. Therefore, it offers a very simple way to obtain extracts well suited for LC/MS/MS analysis (28). On the other hand, it does not work well with fatty matrixes such as avocado, and requires an evaporation/reconstitution step if extracts are analyzed without cleanup. Therefore, we decided not to use ethyl acetate. From the remaining water-miscible solvents, methanol was selected because of the use of methanol in the mobile phases for LC. This choice had offered the option to analyze raw extracts directly after dilution with water (to obtain 80% water in the methanol–water mixture, which corresponds to mobile phase A). However, later tests had shown that <60% of the analytes could be quantified with the API 2000 instrument when this approach was used.

Calibration

(**a**) *Check of (non)linearity*.—Figure 4 shows the dependence of relative response factors on concentration levels. For this most efficient presentation of calibration data, the response (peak area) is divided by the injected amount. In a second step, the response factors obtained are based on the mean response factor of each pesticide. A perfect linear calibration graph results in a straight line at the relative mass response of 1. This special kind of presentation was chosen to demonstrate even slight deviations from linearity. Furthermore, it allows a direct comparison of analytes with strikingly different response factors without any special scaling of the response axis. Compared with the more usual presentation of calibration data in Figure 5, the graphic representation in Figure 4 does not suffer from the large concentration range.

The R^2 values of the calibration curves in Figure 5 are 0.9996 for methiocarb, 0.9994 for promecarb, 0.9997 for iprovalicarb, 0.9996 for fenhexamide, and 0.9913 for flazasulfuron. However, because the lower concentration lev-

Table 7. Recovery data[a] for 67 analytes with recoveries of 70–120% and relative standard deviations of ≤25% for ≥3 matrixes

	Tomato		Lemon		Raisins		Wheat flour		Avocado		All matrixes and all levels[c]		
Analyte	Mean, %	RSD, %[b]	Mean, %	RSD, %	Mean, %	RSD, %	Mean, %	RSD, %	Mean, %	RSD, %	*n*	Mean, %	RSD, %
3,4,5-Trimethacarb	110	4	96	10	85	6	77	7	101	2	43	96	12
Acephate	67	7	87	16	58	30	85	12	74	8	43	85	20
Aldicarb[d]	87	4	45	12	10	39	86	5	34	34	43	68	44
Aldicarb sulfoxide[e]	132	4	138	14	171	8	94	3	174	9	43	133	23
Aldoxycarb	114	3	95	15	86	7	85	16	100	2	43	100	16
Sum of aldicarb, aldicarb sulfoxide, and aldoxycarb	111	3	92	8	89	6	88	6	102	3	43	100	13
Amidosulfuron	119	12	88	18	70	64	40	14	109	15	43	90	31
Atrazine	109	7	90	14	70	16	84	11	97	12	43	95	15
Azoxystrobin	97	4	78	8	74	4	59	11	87	12	43	84	14
Bensulfuron-methyl	99	7	86	13	69	1	74	13	125	4	43	98	23
Bromoxynil	115	3	84	14	87	22	19	6	54	18	43	75	46
Butocarboxim[d]	80	1	35	9	7	63	91	6	15	53	43	60	55
Butocarboxim sulfoxide[e]	116	6	107	6	137	4	105	9	144	5	43	119	17
Butoxycarboxim	121	1	91	3	100	6	92	1	93	4	43	103	13
Sum of butocarboxim, butocarboxim sulfoxide, butoxycarboxim	106	2	78	5	81	4	96	2	84	5	43	94	14
Carbaryl	106	14	78	20	84	12	68	30	109	13	43	99	21
Chlorsulfuron	100	20	86	6	48	10	50	38	93	6	43	78	30
Cinosulfuron	105	3	71	16	48	38	81	7	62	9	43	75	33
Clethodim[d]	72	16	4	173	0	—	93	11	NC[f]	—	34	48	89
Clethodim-imin sulfone	115	1	103	17	69	93	125	6	87	1	43	100	22
Clethodim-imin sulfoxide	121	3	91	11	63	38	90	8	91	2	43	94	20
Clethodim sulfone	136	8	107	17	102	45	130	35	136	34	43	116	27
Clethodim sulfoxide[e]	244	3	145	25	158	19	123	14	198	4	43	168	28
5-Hydroxyclethodim sulfone	114	33	135	25	51	88	24	173	59	14	43	83	56
Sum of clethodim and 5 metabolites	133	2	97	12	74	21	98	12	NC[f]	—	34	101	22
Demeton-S-methyl[d]	47	4	7	6	0	—	63	7	0	—	43	38	89
Oxydemeton-methyl[e]	120	2	110	5	86	8	99	7	113	5	43	111	14
Demeton-S-methyl sulfone	109	5	92	4	85	6	98	10	107	5	43	108	19
Sum of demeton-S-methyl, oxydemeton, and demeton-S-methyl sulfone	92	1	70	2	57	7	86	8	73	5	43	86	20

Table 7. (*continued*)

	Tomato		Lemon		Raisins		Wheat flour		Avocado		All matrixes and all levels[c]		
Analyte	Mean, %	RSD, %[b]	Mean, %	RSD, %	Mean, %	RSD, %	Mean, %	RSD, %	Mean, %	RSD, %	*n*	Mean, %	RSD, %
Dimethoate	106	4	89	10	90	5	85	7	108	12	43	98	14
Diuron	108	3	85	20	77	6	85	6	98	7	43	96	13
Ethiofencarb[d]	32	28	0	—	3	173	39	22	NC[f]	—	34	29	96
Ethiofencarb sulfone	108	3	97	5	84	2	95	8	119	4	43	110	23
Ethiofencarb sulfoxide[e]	195	4	163	10	144	6	114	9	NC[f]	—	34	168	22
Sum of ethiofencarb, ethiofencarb sulfoxide, and ethiofencarb sulfone	111	2	87	8	77	1	83	9	NC[f]	—	34	101	24
Flazasulfuron	84	19	69	19	69	11	73	8	103	6	43	82	26
Florasulam	91	5	114	2	130	31	72	23	78	17	25	93	28
Fosthiazate	88	2	98	13	111	10	83	17	95	5	25	96	13
Imazalil	87	12	79	4	62	24	65	32	77	5	43	74	21
Imidacloprid	111	3	94	13	71	17	73	10	94	7	43	96	18
Iodosulfuron-methyl	94	11	80	16	117	21	82	11	99	7	25	95	18
Ioxynil	103	3	78	19	86	8	27	5	76	17	43	79	35
Iprovalicarb	104	2	88	4	75	2	71	9	86	9	43	89	13
Isoproturon	120	5	82	26	93	9	94	22	106	10	43	99	13
MCPA	105	11	75	13	81	20	22	66	16	19	43	60	72
Metalaxyl	102	1	88	7	74	7	83	7	94	6	43	93	11
Metamitron	91	21	43	49	75	36	80	4	82	12	43	81	27
Methamidophos	87	7	68	23	93	25	82	16	75	22	43	82	18
Methiocarb	97	1	78	8	65	14	69	10	71	10	43	86	20
Metolachlor	104	3	78	10	72	16	67	6	85	18	43	85	15
Metsulfuron-methyl	103	1	84	16	75	8	48	30	97	3	43	86	26
Monocrotophos	105	6	87	20	75	4	101	3	91	6	43	97	14
Nicosulfuron	114	5	97	15	117	39	40	23	81	8	43	83	36
Omethoate	103	7	81	17	70	7	100	8	90	2	43	97	20
Oxamyl	108	2	94	14	93	5	96	11	81	17	43	105	20
Pirimicarb	108	1	104	13	76	14	81	10	74	6	43	94	15
Desmethylformamido-pirimicarb	90	3	88	8	106	4	91	12	104	10	25	98	11
Desmethyl-pirimicarb	89	2	89	10	97	8	85	7	81	17	25	91	11
Sum of pirimicarb, desmethyl-pirimicarb, and desmethylformamido-pirimicarb	96	2	93	10	93	7	86	10	86	10	25	93	9

Table 7. (*continued*)

Analyte	Tomato		Lemon		Raisins		Wheat flour		Avocado		All matrixes and all levels[c]		
	Mean, %	RSD, %[b]	Mean, %	RSD, %	Mean, %	RSD, %	Mean, %	RSD, %	Mean, %	RSD, %	*n*	Mean, %	RSD, %
Primisulfuron-methyl	NF[g]		79	10	77	21	81	31	103	18	36	82	41
Promecarb	109	4	95	30	105	12	70	25	101	12	43	95	17
Propoxur	110	8	96	15	85	15	82	7	110	20	43	100	15
Tebuconazole	93	8	57	19	72	5	73	16	99	23	43	82	20
Tebufenozid	99	3	81	5	72	5	63	21	88	5	43	85	17
Thiabendazole	111	5	76	16	88	7	85	13	107	17	43	94	16
Thiacloprid	107	5	82	6	74	8	80	11	118	10	43	96	17
Thifensulfuron-methyl	98	11	77	11	72	21	48	16	90	8	43	83	22
Thiodicarb[d]	9	12	32	28	97	7	0	—	NC[f]	—	34	42	80
Methomyl[e]	185	4	129	11	35	173	143	7	196	5	43	138	34
Sum of thiodicarb and methomyl	97	3	81	14	66	44	72	7	NC[f]	—	34	85	16
Thiofanox[d]	66	4	16	21	0	—	62	2	NC[f]	—	34	51	67
Thiofanox sulfone	89	7	104	7	106	8	91	11	120	4	25	104	14
Thiofanox sulfoxide[e]	132	6	216	8	180	10	102	11	195	5	25	167	24
Sum of thiofanox, thiofanox sulfoxide, and thiofanox sulfone	96	3	112	8	95	8	85	8	NC[f]	—	25	100	11
Triasulfuron	101	13	82	16	80	3	93	21	101	3	43	93	18

[a] Fortification level, 0.01 mg/kg; quantified by 3-point calibration with matrix-matched standards; $n = 3$.

[b] RSD = Relative standard deviation.

[c] Levels and typical number of repetitions: 1.0 mg/kg (2×, not added to tomato); 0.1 mg/kg (2×); 0.05 mg/kg (2×); 0.01 mg/kg (3×).

[d] Decomposition in at least some samples during extraction and cleanup.

[e] Formation in at least some samples during extraction and cleanup.

[f] NC = Not calculated because of decomposition of matrix-matched standards.

[g] NF = Not fortified.

Table 8. Recovery data[a] for 37 analytes with recoveries outside the range of 70–120% and/or relative standard deviations of >25% for ≥3 matrixes

	Tomato		Lemon		Raisins		Wheat flour		Avocado		All matrixes and all levels[c]		
Analyte	Mean, %	RSD, %[b]	Mean, %	RSD, %	Mean, %	RSD, %	Mean, %	RSD, %	Mean, %	RSD, %	*n*	Mean, %	RSD, %
2,4-D	104	3	92	26	51	21	11	173	0	—	43	54	82
5-Hydroxythiabendazole	112	3	47	67	135	8	100	14	135	8	25	94	42
Bendiocarb	110	6	63	32	67	25	114	41	139	5	43	119	42
Carbendazim[d]	81	9	89	9	93	39	70	12	99	8	43	95	20
Thiophanate-methyl[e]	16	19	0	—	2	173	8	68	NC[f]	—	34	23	270
Sum of thiophanate-methyl and carbendazim	48	8	44	9	48	38	39	16	Nc[f]	—	34	57	50
Carbofuran	132	2	100	18	77	6	80	6	121	8	43	104	19
3-Hydroxycarbofuran	113	1	161	13	107	1	100	19	I[g]	I[g]	34	111	21
Sum of carbofuran and 3-hydroxycarbofuran	122	1	130	15	92	3	90	13	I[g]	I[g]	34	106	17
Cyprodinil	88	10	43	25	94	49	58	24	56	22	43	66	35
Daminozide	0	NC[f]	69	20	83	40	30	19	40	61	43	31	125
Diflubenzuron	90	5	63	13	62	113	122	66	71	12	43	76	41
Fenhexamide	44	17	58	14	74	6	67	5	94	1	43	84	26
Fenoxycarb	99	5	47	14	56	12	56	2	74	23	43	69	27
Fenpropimorph	92	6	70	9	35	11	50	3	18	58	43	53	51
Fluazifop-P-butyl	76	5	16	23	34	4	48	5	27	28	43	46	50
Fludioxonil	93	4	52	26	59	68	89	26	29	25	43	70	37
Flufenoxuron	87	24	3	173	63	39	40	50	20	26	43	45	71
Furathiocarb	32	2	38	12	48	13	47	2	32	16	43	46	29
Haloxyfop-ethoxyethyl	78	8	30	13	42	29	53	9	44	5	43	55	39
Haloxyfop-methyl	71	7	35	17	49	10	51	9	24	22	43	53	36
Hexaflumuron	81	31	15	70	42	41	54	51	38	31	43	51	60
Indoxacarb	NF[h]	NC[f]	11	112	67	38	41	7	30	54	36	46	59
Isoxaflutole	77	31	80	5	87	21	75	44	61	29	43	98	65
Linuron	101	11	92	27	64	8	87	16	80	27	43	91	17
Mecoprop-P	89	6	81	7	68	28	18	62	24	20	43	62	59
Propamocarb	58	4	36	18	33	13	59	11	45	5	43	55	45
Prosulfuron	108	15	60	68	102	54	78	27	65	14	43	83	30
Pymetrozin	111	5	53	16	53	20	81	14	59	7	43	73	31

Table 8. (*continued*)

	Tomato		Lemon		Raisins		Wheat flour		Avocado		All matrixes and all levels[c]		
Analyte	Mean, %	RSD, %[b]	Mean, %	RSD, %	Mean, %	RSD, %	Mean, %	RSD, %	Mean, %	RSD, %	*n*	Mean, %	RSD, %
Pyrimethanil	99	10	83	16	64	13	56	29	70	19	43	81	21
Quinmerac	95	7	97	3	57	10	2	173	7	13	43	52	81
Quizalofop-ethyl	60	14	23	29	37	12	53	8	24	39	43	48	48
Rimsulfuron	104	17	NC[f]	—	40	33	64	14	0	0	43	99	74
Spiroxamine	95	4	75	9	45	25	51	11	63	15	43	68	23
Teflubenzuron	82	16	0	—	0	—	66	40	18	30	43	49	72
Tribenuron-methyl	NF[h]	NF[h]	NC[f]	—	19	51	62	17	101	12	28	52	77
Triflumuron	82	13	28	42	44	15	60	23	48	15	43	58	41
Triflusulfuron-methyl	109	10	58	108	45	112	94	33	0	0	43	172	109
Vamidothion	99	6	53	10	16	60	87	10	32	35	43	70	44

[a] Fortification level, 0.01 mg/kg; quantified 3-point by calibration with matrix-matched standards; $n = 3$.
[b] RSD = Relative standard deviation.
[c] Levels and typical number of repetitions: 1.0 mg/kg (2×, not added to tomato); 0.1 mg/kg (2×); 0.05 mg/kg (2×); 0.01 mg/kg (3).
[d] Formation in at least some samples during extraction and cleanup.
[e] Decomposition in at least some samples during extraction and cleanup.
[f] NC = Not calculated because of decomposition of matrix-matched standards.
[g] I = Interference
[h] NF = Not fortified.

els in Figure 5 influence the R^2 value to a much lower extent than do the higher concentrations, this measure of calibration quality must be used with caution. The observed deviation from linearity at higher concentrations in both figures is probably caused by the competition of the analytes for the charges supplied during the ionization process. Because of the findings described above, a calibration function for the entire range is not justified. Therefore, quantification for each spiking level was performed with a linear calibration using 3 related matrix standards only, e.g., standard concentrations of 0.005, 0.01, and 0.025 μg/mL for a spiking level of 0.01 mg/kg. When this kind of calibration is used, estimates of even low concentrations are nearly unbiased. The calculation of the $104 \times 4 \times 5 = 2080$ calibration functions finally needed for each analyte, level, and matrix was simplified by standardized injection batches (identical sequence of samples and standards) and export of integration results into an Excel® sheet.

(**b**) *Standards in solvent versus matrix-matched standards*.—Because it is well known (12, 34, 44) that the matrix may either suppress or enhance the analyte response, standards both in solvent and in blank extracts were prepared. Calibration graphs obtained with standards in solvent show the same typical behavior with saturation effects as was observed for matrix-matched standards. However, ionization yields and, consequently, the slopes of the calibration curves differ notably for some matrix/analyte combinations. At present, this pronounced matrix effect cannot be predicted. It extends from intensity suppression to <20% up to an enhancement of 100%. A total of >3000 response ratios (area of matrix-matched standard/area measured with standards in solvent) from standards of 5 matrixes, each at 7 concentration levels, were calculated. The histogram in Figure 6 shows the frequency of the different matrix effects observed.

An unusually high signal enhancement in matrix (>150%) was observed for ethiofencarb sulfoxide, bendiocarb, thiodicarb, primisulfuron, isoxaflutole, hexaflumuron, triflumuron, and mecoprop-P in >10% of all matrix/concentration combinations. At least for thiodicarb and primisulfuron, the different decomposition rates in solvent and matrix may account for this effect, whereas additional ethiofencarb sulfoxide was formed by oxidation of ethiofencarb under matrix conditions. An explanation for the enhanced response of bendiocarb, isoxaflutole, hexaflumuron, triflumuron, and mecoprop-P cannot yet be given.

A signal reduction of ≥60% was measured for 21 analytes in >10% of the standard/matrix pairs. This group of analytes appears to comprise mostly basic and fat-soluble compounds. Indeed, lemon and avocado extracts tend to suppress the analyte response.

(**c**) *Stability of standard solutions*.—Pronounced irregularities were observed in the data sets of desmedipham, phenmedipham, and pyridate and its metabolite, 6-chloro-3-phenylpyridazine-4-ol. Comparison of the response factors within one series of measurements and between several experiments revealed fast decomposition of the analytes at room temperature in the autosampler, and a slower but also pronounced decomposition in the standard stock solutions that were kept frozen but had to be warmed to ambient temperature for spiking experiments and preparation of calibration standards. Therefore, these 4 analytes were omitted from further discussion of matrix effects and recoveries. The fast decomposition of tribenuron-methyl and primisulfuron in methanolic solution was minimized by using water for the preparation of stock solutions and pH adjustment to 8 by the addition of ammonia. When this modified stock solution was used for standard preparation and sample fortification, the response factors determined within each sample set remained almost constant and quantification was possible, except for tribenuron-methyl in lemon. Rimsulfuron also decomposed in the matrix-matched standards of lemon. Therefore, no data for these pesticides in lemon were taken into account.

The oxidation of the sulfur-containing pesticides aldicarb, butocarboxim, clethodim, demeton-S-methyl, ethiofencarb, and thiofanox and the decomposition of furathiocarb (to carbofuran), thiodicarb (to methomyl), and thiophanate-methyl (to carbendazim) in standard solutions are well-known problems. To test the stability of these analytes in standard mixtures, a solution of these pesticides and a separate mixture of their metabolites were prepared and measured on 3 occasions (Day 0, Day 1, and Day 4). The concentration of analytes in these standard solutions, which were prepared in solvent as well as in the blank extracts of avocado, lemon, tomato, raisins, and wheat flour, corresponded to 0.1 mg/kg. Measurement of the change in pesticide concentrations in each mixture of Day 0 kept at 5°C (refrigerator) was followed by comparison of its LC/MS/MS peak intensity with those of standards freshly prepared on Days 1 and 4. These experiments revealed that aldicarb, butocarboxim, and furathiocarb remain stable in all standard solutions for ≥4 days. For the other pesticides, the more pronounced results in matrix-matched standards are presented in Table 6. The data show a significant decomposition of thiodicarb and thiophanate-methyl in all matrixes after ≥4 days. However, calibration was possible because all measurements were made on the day of extraction. In contrast, the pesticides clethodim, ethiofencarb, thiodicarb, thiofanox, and thiophanate-methyl decompose in matrix-matched standards of avocado so fast that calibration of results and calculation of recovery rates were not performed.

Recovery Experiments

According to the analytical procedure given in Figure 1, 11 samples per matrix (2 blanks and 9 fortified) and 7 matrix-matched standards were prepared for each matrix under study.

Typical chromatograms of individual MRM transitions are shown in Figure 7. The upper 6 chromatograms of this figure are for analytes showing the poorest response of all pesticides under investigation. Their peak intensities and S/N values illustrate a sufficient sensitivity of electrospray MS/MS detection at the lowest fortification level. Depending on the matrix, ≤10–15 traces out of the 108 acquired show significant interferences with peaks higher than 10% of the analyte peak. At the retention times of the analytes, chromatograms of blank

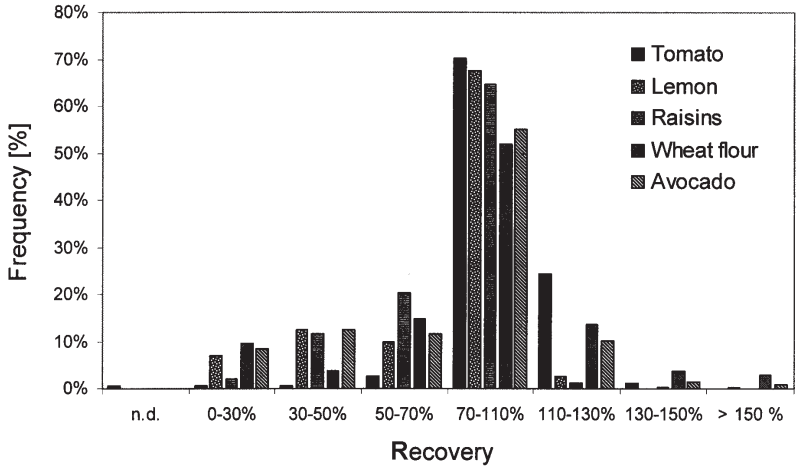

Figure 8. Frequency of observed recoveries of 104 pesticides at 3 spiking levels (0.05 μg/kg, 0.1 μg/kg, and 1 mg/kg).

matrixes used for fortification experiments had no relevant signals. In all cases, interference peaks were well resolved from the analyte peak, and thus an unequivocal assignment resulted. Generally, the individual MRM traces show a remarkably clean baseline throughout the entire chromatogram. For that reason, any use of second transitions (Table 1) was unnecessary for accurate recovery determinations. The only exception was the pesticide daminozide. The most pronounced transition of this analyte is an unspecific loss of a water molecule from the precursor ion. No other significant fragmentation was observed. As a result, daminozide was the only analyte that generally could not be quantified at 0.01 and 0.05 mg/kg levels.

Chromatographic peak integration was performed by using the Analyst software of the API 2000 system. After inspection of integration results and manual correction, if necessary, the resulting peak area reports containing retention time, peak height, peak area, S/N, and analyte name of each expected peak were saved as Excel files. These raw data files were used in a standardized format for all fortification experiments, allowing the stability check of peak retention, check of minimum S/N (>10), and comparison of peak height obtained and a threshold value. Peak heights below trigger values were automatically replaced by a comment. On the basis of these inspected data sets, calibration graphs were constructed, analyte concentrations in fortified samples were calculated, and recoveries were determined without manual treatment of the data.

The recovery data obtained with matrix-matched standards are summarized in Tables 7 and 8 as well as in Figure 8. In the tables, the results obtained at the 0.01 mg/kg level and their respective standard deviations are compiled. All analytes with recoveries between 70 and 120% and relative standard deviations of ≤25% (in ≥3 different matrixes) are listed in Table 7. In contrast, Table 8 contains all analytes showing a recovery outside the range 70–120% and/or a relative standard deviation of >25% for ≥3 matrixes. All recoveries at higher concentration levels are summarized in Figure 8.

Especially at the spiking level of 0.01 mg/kg, there are some analyte/matrix combinations with standard deviations of >25% (e.g., hexaflumuron in all matrixes). Many, but not all, of them belong to the group of analytes with the lowest relative response factors. It has to be pointed out that the entire method covers a wide range of relative response factors between 0.01 and 13.

The decomposition of 9 analytes (aldicarb, butocarboxim, clethodim, demeton-S-methyl, ethiofencarb, furathiocarb, thiodicarb, thiofanox, and thiophanate-methyl) during extraction and cleanup was examined by spiking avocado and lemon with only these analytes at a level of 0.1 mg/kg. The fortified samples were subjected to the normal procedure (Figure 1). Additionally, to avoid misinterpretation caused by incurred pesticides and incurred metabolites or degradation products, the same lemon and avocado samples were analyzed without fortification. All analytes were shown to decompose to their metabolites: (*1*) thiophanate-methyl decomposed to carbendazim; (*2*) clethodim, ethiofencarb, thiofanox, aldicarb, butocarboxim, and demeton-S-methyl were oxidized to the

Table 9. Reasons for partial failure of the method (15 analytes)

No or extremely low ESI response	Poor chromatographic behavior	Fast decomposition
Azadirachtin	Fentin hydroxide	Phenmedipham
Cymoxanil	Fenbutatin oxide	Desmedipham
Fipronil	Cyhexatin	Pyridate
Bromoxynil octanoate[a]		
Ioxynil octanoate[a]		
MCPA 2-ethylhexyl ester[a]		
MCPA 2-ethyl ester[a]		
MCPA 2-butoxyethyl ester[a]		
MCPA 2-thioethyl ester[a]		

[a] Tested here for simultaneous determination with the associated free acid. Esters are part of the residue definition and are detectable by GC methods.

corresponding sulfoxides; (*3*) thiodicarb formed methomyl; and (*4*) furathiocarb was converted to carbofuran. Whereas only 10–15% of aldicarb, butocarboxim, and demeton-S-methyl was oxidized to the corresponding sulfoxide during the entire analytical procedure, nearly complete conversion was observed for thiophanate-methyl and ethiofencarb. However, most of these transformations did not impede correct quantification of residues because residue definitions often require the determination of the sum of the parent compound and metabolites. Therefore, Tables 7 and 8 as well as Figure 8 are based on residue definitions for the sum of aldicarb/aldoxycarb/aldicarb sulfone, butocarboxim/butoxycarboxim/butocarboxim sulfone, carbendazim/3-hydroxycarbendazim, clethodim/clethodim sulfoxide/clethodim sulfone/5-hydroxyclethodim sulfone/clethodim-imin sulfone/clethodim-imin sulfoxide, demeton-S-methyl/oxydemeton-methyl/demeton-S-methyl sulfone, ethiofencarb/ethiofencarb sulfoxide/ethiofencarb sulfone, thiodicarb/methomyl, thiofanox/thiofanox sulfoxide/thiofanox sulfone, and thiophanate-methyl/carbendazim. Despite this significant degradation of analytes in samples, decomposition of these compounds in standards does not influence correct calibration, because matrix-matched standards were proven to be sufficiently stable. Only 5 pesticides in avocado (clethodim, ethiofencarb, thiodicarb, thiofanox, and thiophanate-methyl) and 2 pesticides in lemon (rimsulfuron and tribenuron-methyl) were excluded from calculations because of their instability.

In total, approximately 60% of all pesticide/matrix combinations investigated showed good recovery and precision. Therefore, our investigations have demonstrated that there is a chance to introduce a new multiresidue procedure for many pesticides in very different matrixes that is complementary to established GC-based multiresidue methods. In cases in which pesticide recoveries fall outside an acceptable range, labeled surrogate standards may help to resolve this problem.

Finally, Table 9 lists the analytes that were tested without success. Most often, the reason was insufficient ionization efficiency of the respective pesticide. Fast decomposition in samples and poor chromatographic behavior were further causes of failure.

Conclusions

It has been shown that a modern commercial triple-quadrupole mass spectrometer is suitable to detect approximately 100 analytes simultaneously with a sensitivity sufficient for residue determination at the 0.01 mg/kg level. The use of time-window programming (periods) is not necessary unless the number of analytes to be analyzed within one run is significantly increased or pesticides with very low response have to be determined. Two separate injections of a sample (or time windows) are preferred if switching between positive and negative modes is needed for multiresidue determination. At a flow rate of 200 μL/min, the electrospray ion source of an API 2000 triple-quadrupole mass spectrometer is the better multipurpose ionization device for most pesticides, compared with APCI. Because of the very high selectivity achieved by MS/MS, gradient elution on a small reversed-phase analytical column (50 × 2 mm) is usually sufficient for unambiguous identification. Interfering peaks from other pesticides or crop matrix are rarely observed. For confirmation of results, a second fragmentation of the selected parent ions can be used. The most appropriate calibration of results requires adapted functions for each analyte, level, and matrix. The effort for this calibration can be significantly reduced if standard spreadsheet software is used to process the integration results obtained. Even though not all pesticides demonstrated acceptable recovery and precision, the tested method offers a simple and fast way of screening for many pesticide classes. For the accurate quantification of pesticides with recoveries of <70% or with lower precision, the use of stable isotope-labeled standards may offer a simple alternative and will be tested in the future. An application note (45) and complete method files for API 2000™/3000™/4000™ LC/MS/MS instruments on CD-ROM are available from Applied Biosystems/MDS SCIEX or from the authors. The method is generally applicable and has been successfully tested by using the Waters Quattro Micro API and Quattro Ultima Platinum triple-quadrupole instruments. Documents and all method files for these instruments will soon be available either directly from Waters Corp. or from the authors.

Acknowledgments

We acknowledge the skilled technical assistance of Volker Happel, Nataša Markovic, and Marilyn Menden (Federal Institute of Risk Assessment, Berlin, Germany). Sonja Luederitz performed the thorough investigation of APCI source parameters, which is gratefully acknowledged (Federal Institute of Risk Assessment, Berlin, Germany). Furthermore, we thank Andre Schreiber (Applied Biosystems/MDS SCIEX, Darmstadt, Germany), Gordon Kearney, and Anthony Newton (Micromass Ltd., Manchester, UK), who tested the applicability of the method and produced the parameter files needed for other types of instruments.

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