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A new multi-residue method for analysis of pesticide residues in fruit and vegetables using liquid chromatography with tandem mass spectrometric detection☆

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Abstract

A new multi-residue method for determination of pesticide residues in a wide variety of fruit and vegetables, using the National Food Administration (NFA) ethyl acetate extraction and determination by means of LC–MS/MS, is presented. The method includes pesticides normally detected by LC–UV or LC-fluorescence such as benzimidazoles, carbamates, *N*-methylcarbamates and organophosphorus compounds with an oxidisable sulphide group as well. After extraction with ethyl acetate, the extract is concentrated and an aliquot of the extract is evaporated to dryness and redissolved in methanol before injection on LC–MS/MS. The method has been validated for 57 different pesticides and metabolites. Representative species from different commodity groups were chosen as matrices in order to study the influence from different matrices on recoveries. The fortification levels studied were 0.01–0.5 mg kg^{-1}. Matrix effects were tested for all matrices by means of standard addition to blank extracts. The matrix effect, expressed as signal in solvent compared to signal in matrix, was in general found to be small. The obtained recoveries are, with a few exceptions, in the range 70–100%. The proposed method is quick and straightforward and no additional clean-up steps are needed. The method can be used for the analysis of all 57 pesticides in one single determination step at 0.01 mg kg^{-1}.
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1. Introduction

For the national authorities responsible for monitoring pesticide residues in food and vegetables, multi-residue methods are highly preferable due to the simplicity of detecting several analytes in a single extraction. Multi-residue methods facilitate the demands of more efficient monitoring, when the authorities have to create the necessary conditions for monitoring new pesticides. Multi-residue methods usually used in pesticide analysis are designed for determination with gas chromatographs with different selective detectors. In contrast to older, non-polar pesticides, modern, recently introduced pesticides are often more polar and less volatile. This complicated nature of pesticides gives rise to the development of special methods which are intended for analysis of a certain pesticide or group of pesticides. Several special methods have thus been used in the Swedish monitoring of pesticide residues [1]. The special methods include pesticides normally detected by LC–UV or LC-fluorescence such as benzimidazoles, carbamates and *N*-methylcarbamates. The LC–UV methods often have low sensitivity, while the fluorescence methods normally have better sensitivity. Both techniques lack sufficient selectivity towards endogenous compounds, which absorb at the same wavelength. These methods are often time-consuming and costly, including several steps in sample preparation. Therefore, it was decided to develop a multi-residue method which could replace the special methods and create a foundation for more efficient monitoring.

Liquid chromatography/mass spectrometry (LC–MS) has been used for analysis of these compounds and is rapidly becoming an accepted technique in pesticide residue analysis for regulatory monitoring purposes. Recent reviews in

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pesticide analysis deal with applications of using two ionisation techniques, atmospheric pressure chemical ionisation (APCI) and electrospray (ES) [2]. Previous studies have shown the suitability of both interfaces in pesticide analysis. The techniques have been successfully applied to the determination of carbamates, benzoylureas, phenylureas and triazines. However, the applications are still limited to certain pesticides or groups of pesticides and use different extraction and chromatographic systems [3–5].

In the present work, a multi-residue method for determination of pesticide residues in fruit and vegetables, using the established the National Food Administration (NFA) ethyl acetate extraction [8–10] and determination by means of LC–ESI–MS/MS, is presented. The main task was to develop a multi-class and multi-matrix method which could replace specific methods and include new pesticides which up until now have not been analysed. After extraction with ethyl acetate, the extract is concentrated and an aliquot of the extract is evaporated to dryness and redissolved in methanol before injection on LC–MS/MS. The study has shown that no clean-up steps are needed, which results in a convenient and straightforward sample preparation.

The method includes benzimidazoles, carbamates and *N*-methylcarbamates, which traditionally have been analysed using LC–UV or LC-fluorescence. Another group of pesticides included in the method consists of organophosphorus compounds with an oxidisable sulphide group. Many of them tend to decompose under GC conditions or are more polar and are therefore not amenable to GC. Furthermore, their sulphoxides and other metabolites are often thermally labile and the determination of residues has been associated with several analytical difficulties [6,7]. Since organophosphorus pesticides are of current interest due to the EU directive for baby foods, where the metabolites are included in the application of maximum residue levels (MRL), there is a need for a method that can detect both the parent pesticides and the metabolites simultaneously.

The method has been validated for a total of 57 different pesticides and pesticide metabolites. Representative species from different commodity groups were chosen as matrices in order to illuminate the possible contribution from matrix on recoveries.

The proposed method is quick and straightforward. It can be used for the analysis of all 57 pesticides in one single determination step at 0.01 mg kg^{-1}.

2. Experimental

2.1. Materials and reagents

Pesticide standards of analytical grade were purchased from Riedel-de-Haën (Hannover, Germany) or Ehrensdorfer (Augsburg, Germany). Purity was >95%. Stock solutions were prepared in pesticide-grade acetone or methanol according to their solubility and stability. Four intermediate standard solutions of pesticide mixtures were prepared in acetone, in which the parent compound and its breakdown product (Table 1) were separated into different solutions. The intermediate solutions were prepared at 50 μg ml^{-1} and then further diluted to 3.75 μg/ml in methanol for recovery studies. Pesticides available as solutions in low concentrations were not included in the 50 μg ml^{-1} solution, while they were present in the 3.75 μg ml^{-1} mixture. Working solutions were prepared daily in methanol.

Ethyl acetate and cyclohexane, pesticide grade (Lab-Scan, Dublin, Ireland), and methanol, gradient grade, LiChrosolv (Merck, Darmstadt, Germany) were used for extraction and

Table 1
Overview of breakdown of included pesticides

Pesticide	Breakdown product(s)			
Aldicarb	→	Aldicarb sulphoxide	→	Aldicarb sulphone
Butocarboxim	→	Butocarboxim sulphoxide	→	Butocarboxim sulphone[a]
Carbosulfan	→	Carbofuran	→	Carbofuran-3-OH
Demeton-*S*-methyl	→	Demeton-*S*-methyl sulphoxide	→	Demeton-*S*-methyl sulphone
Disulfoton	→	Disulfoton sulphoxide	→	Disulfoton sulphone
Ethiofencarb	→	Ethiofencarb sulphoxide	→	Ethiofencarb sulphone
Furathiocarb	→	Carbofuran	→	Carbofuran-3-OH
Imidacloprid	→	Several products[a]	→	
Methiocarb	→	Methiocarb sulphoxide	→	Methiocarb sulphone
Oxamyl	→	Oxamyl-oxime		
Phorate	→	Phorate sulphoxide	→	Phorate sulphone
Phorate	→	Phorate-*O*-analogue	→	Phorate-*O*-analogue sulphoxide and sulphone[a]
Terbufos	→	Terbufos-*O*-sulphoxide[a]	→	Terbufos-*O*-sulphone
Thiodicarb	→	Methomyl	→	Methomyl-oxime[b]
Thiophanate methyl	→	Carbendazim		
Thiometon	→	Thiometon sulphoxide	→	Thiometon sulphone
Vamidothion	→	Vamidothion sulphoxide	→	Vamidothion sulphone

[a] Not included.
[b] Searched for but no recovery studies.

sample preparation. For preparation of buffer solution in methanol/water (20 + 80), formic acid 98–100% (Merck), 25% ammonia solution (Riedel-de-Haën) and Milli-Q water were used. After the addition of 1.53 ml formic acid, ammonia was dropped into 800 ml water, to give pH 3.75–3.80, and finally 200 ml methanol was added. When this stock buffer solution, at 50 mM ammonium formate, was diluted five times to 10 mM with methanol/water (20+80) for preparation of the working mobile phase, the pH of the solution rose to the final pH of between 4.0 and 4.2.

Disposable syringe filters, 0.45 μm Acrodisc CR PTFE (Gelman, MI, USA), were used for filtration of the samples.

2.2. Instrumentation

Waters Alliance 2690 (Milford, MA, USA) with a quaternary gradient pump and vacuum degassing was used for liquid chromatography.

Separations were carried out using Genesis C18 (100 mm × 3 mm, 4 μm, Jones Chromatography Ltd, Mid Glamorgan, UK) with a 1 cm long guard column with the same packing material. The mobile phase was filtered through a 0.45 μm membrane filter (HVLP, Millipore, Ireland). Separation was performed using a gradient between methanol and 10 mM ammonium formate, pH 4 (Table 2). The column should be rinsed with acetonitrile/water (80 + 20) between each run, and also stored in the same solution.

Drying gas and nebulising gas for the LC–MS were produced in situ by a nitrogen generator (Aquilo NG 11, Aquilo Gas Separation BV, Etten-Leur, The Netherlands) fed by compressed air at 7 bars. LC–MS was carried out using a Quattro LC (Micromass, Manchester, UK) triple quadrupole mass spectrometer equipped with a standard pneumatically assisted ES ion source, operated in both positive and negative mode. Experimental conditions were as follows: nebulising gas at a flow of about 90 l h^{-1}, drying gas was heated to 400 °C at a flow rate of 600 l h^{-1}, nitrogen was used as both nebulising and desolvation gas, the capillary voltage was switched between +4.0 kV and −3.5 kV for the positive and the negative ions, the source block was held at 120 °C, and the resolution was 15.0 (unit resolution) for both the first and the second quadrupole. The optimised settings for cone voltage (CV) and collision energy (CE) were tested for each compound by flow injection analysis. The CV was varied between 10 and 70 V and the CE between 5 and 50 eV. As first choice the protonated or deprotonated molecular ion was chosen as the precursor ion. In some cases, when the intensity from the molecular ion was too low, the sodium or ammonium adduct, and for dinocap a fragment, was chosen as precursor ion instead. By varying the collision energy for the precursor ion, the product ions for each compound were optimised by selecting the most intense product ion. However, when two compounds gave similar transitions, another product ion was selected, see Table 3 for optimised settings for each pesticide.

Table 2
Settings for the HPLC system

Time (min)	*A* (%)	*B* (%)
0	0	100
15	90	10
20	90	10
23	0	100

Mobile phase A is pure methanol, B is 10 mM ammonium formate pH 4 in water/methanol (80 + 20 (v/v)). The flow rate was 0.3 ml min^{-1} and the injection volume was 5 μl. Next injection after 30 min.

2.3. Sample pre-treatment

Extraction of the pesticides was performed using ethyl acetate in the presence of sodium sulphate. For the basic pesticides, sodium hydroxide was also added for matrices with a pH below 4.5. After concentration and filtration, an aliquot of the extract was evaporated to dryness and dissolved in pure methanol. The extract was filtered prior to analysis by LC–MS/MS. The final sample concentration was 2.5 g ml^{-1}. No further purification was performed.

The extraction method used, up to the change of solvent, was the same as in the Swedish GC multi-residue method for pesticides in fruit and vegetables [8–10].

2.4. Matrices and recovery tests

The proposed method is meant to be a multi-residue method for pesticides in many different matrices. In order to test the performance of the method for a wide range of matrices, we used the same grouping of commodities as used by the European commission, when establishing MRLs. In this system the fruit and vegetables are divided into 14 different groups depending on their properties. From these 14 commodity groups, 11 groups were selected for the method validation. Three groups with species with lower consumption, tree nuts, legume vegetables and mushrooms were excluded. In each remaining group one to four of the most representative species were selected and tested for recovery (Table 4). Recovery tests were done at three levels, 0.01, 0.05 and at 0.5 mg kg^{-1}. At the two lowest levels, four recovery tests were done in each commodity group at each level and for each pesticide. At the 0.5 mg kg^{-1} level only one test was done in each commodity group, using the most representative species concerning the consumption pattern. Recovery tests were done with all 57 pesticides in all listed matrices, regardless of whether all pesticides are normally used for all matrices. A total of 44 recovery tests were done at 0.01 and 0.05 mg kg^{-1} and 11 tests at 0.5 mg kg^{-1} including all 57 pesticides. For some of the pesticides with more basic properties, recoveries were also done after the addition of sodium hydroxide in the extraction step to the matrices with the lowest pH, as described in Section 2.3.

Table 3
Monitored ions together with recovery data

Peak no.	Pesticide																	
										With NaOH			Matrix effect			With NaOH		
		Rt. min	M[a]	MS/MS m/z	CV V	CE eV	Spiking level mg/kg	Mean %	RSD %	n	Mean %	RSD %	n	Mean %	RSD %	n	Mean %	RSD %	n
18	Acetamiprid	10.9	222.1	223>126	30	20	0.01	87	22	24									
							0.05	85	18	24				102	10	16			
							0.5	86	19	12				96	6	12			
23	Aldicarb	12.7	190.1	208>116	10	5	0.01	94	15	44									
							0.05	93	12	44				107	25	44			
							0.5	92	5	11				95	11	11			
5	Aldicarb sulphone	5.9	222.1	223>148	20	10	0.01	94	18	44									
							0.05	93	13	44				111	18	44			
							0.5	98	7	11				96	4	11			
3	Aldicarb sulphoxide	4.6	206.1	207>89	20	13	0.01	83	25	33	70	18	23						
							0.05	86	19	25	73	15	23	105	13	45	99	20	25
							0.5	94	4	7	71	18	7	101	4	7	104	5	7
30	Bendiocarb	14.2	223.1	224>167	20	10	0.01	93	12	44									
							0.05	91	10	44				101	27	44			
							0.5	96	5	11				89	9	11			
21	Butocarboxim	12.5	190.1	213>75	20	15	0.01	92	18	44									
							0.05	95	13	44				96	44	44			
							0.5	92	9	11				84	25	11			
1	Butocarboxim sulphoxide	4.0	206.1	207>75	15	15	0.01	81	18	23	79	15	23						
							0.05	80	20	25	82	8	23	107	20	45	102	19	25
							0.5	87	6	7	79	14	7	102	8	7	105	4	7
4	Butoxycarboxim	5.6	222.1	223>106	20	10	0.01	92	16	44									
							0.05	93	12	44				108	15	44			
							0.5	97	7	11				95	3	11			
32	Carbaryl	14.8	201.1	202>145	20	10	0.01	94	11	45	87	16	23						
							0.05	94	11	44	88	14	23	99	22	45	104	23	25
							0.5	91	10	11	88	11	7	93	17	11	96	17	7
19	Carbendazim	10.9	191.1	192>160	20	20	0.01	87	18	27	98	19	22						
							0.05	90	18	29	94	13	22	91	24	44	95	37	24
							0.5	94	3	7	91	12	7	88	13	7	88	18	7
29	Carbofuran	14.1	221.1	222>165	25	10	0.01	95	11	44									
							0.05	93	10	44				100	27	44			
							0.5	97	4	11				88	11	11			
17	Carbofuran-3-OH	10.5	237.1	238>220	20	10	0.01	94	19	43									
							0.05	92	13	44				116	27	44			
							0.5	99	7	11				96	8	11			

57	Carbosulfan	24.2	380.2	381>118	30	15	0.01	60	57	44				
							0.05	47	80	41	92	29	44	
							0.5	71	32	9	92	15	12	
51	Clofentezine	19.8	302.0	303>138	20	15	0.01	79	32	42				
							0.05	81	29	43	88	24	44	
							0.5	73	15	11	94	21	11	
44	Demeton	16.8	258.1	259>89	10	10	0.01	84	19	44				
							0.05	83	18	41	110	30	44	
							0.5	79	15	12	117	13	12	
31	Demeton-S-methyl	14.4	230.0	231>89	20	10	0.01	88	27	44				
							0.05	90	24	41	114	29	44	
							0.5	83	18	12	106	9	12	
10	Demeton-S-methyl sulphone	7.6	262.0	263>169	30	20	0.01	100	21	44				
							0.05	98	18	41	118	30	44	
							0.5	83	16	12	108	15	12	
8	Demeton-S-methyl sulphoxide	6.9	246.0	247>169	20	10	0.01	75	39	44				
							0.05	68	38	41	106	19	44	
							0.5	54	39	9	109	11	12	
55	Dinocap	21.3	364.2	295>134	40	30	0.01	83	34	40				
							0.05	79	27	40	96	23	42	
							0.5	104	21	11	90	11	11	
52	Disulfoton	20.0	274.0	275>89	10	5	0.01	84	20	44				
							0.05	79	31	41	90	25	44	
							0.5	78	10	12	110	10	12	
38	Disulfoton sulphone	15.6	306.0	307>171	20	5	0.01	94	25	44				
							0.05	89	17	41	111	23	44	
							0.5	94	13	12	106	11	12	
35	Disulfoton sulphoxide	15.4	290.0	291>213	20	10	0.01	93	20	44				
							0.05	89	12	41	106	20	44	
							0.5	81	9	12	99	12	12	
34	Ethiofencarb	15.2	225.1	226>107	20	15	0.01	111	19	42				
							0.05	108	17	42	98	24	43	
							0.5	114	23	11	83	17	11	
12	Ethiofencarb sulphone	9.2	257.1	258>107	20	20	0.01	94	12	44				
							0.05	93	11	44	116	28	44	
							0.5	99	7	11				
13	Ethiofencarb sulphoxide	9.4	241.1	242>107	20	20	0.01	88	22	45				
							0.05	90	21	44	110	21	45	
							0.5	85	21	11	101	10	11	
49	Fenoxycarb	18.8	301.1	302>116	20	10	0.01	85	21	43				
							0.05	85	20	43	86	21	44	
							0.5	83	12	11	82	25	11	

Table 3 (*Continued*)

Peak no.	Pesticide													Matrix effect					
										With NaOH						With NaOH		
		Rt. min	M[a]	MS/MS m/z	CV V	CE eV	Spiking level mg/kg	Mean %	RSD %	n	Mean %	RSD %	n	Mean %	RSD %	n	Mean %	RSD %	n
53	Furathiocarb	20.6	382.2	383>195	25	20	0.01	83	25	44									
							0.05	81	19	41				96	13	44			
							0.5	87	13	12									
56	Hexythiazox	21.4	352.1	353>228	20	15	0.01	80	27	42									
							0.05	86	26	42				99	19	44			
							0.5	94	12	11				91	13	11			
45	Imazalil	17.1	296.1	297>159	30	20	0.01	68	18	22	88	14	21						
							0.05	70	18	22	89	13	22	94	28	41	98	16	23
							0.5	79	17	7	87	11	7	77	22	7	86	25	7
14	Imidacloprid	9.8	255.1	256>209	30	20	0.01	90	14	39	100	12	23						
							0.05	91	14	37	99	13	23	123	23	44	120	19	25
							0.5	89	12	11	96	15	7	115	10	11	116	8	7
40	Isoprocarb	15.8	193.1	194>95	20	20	0.01	91	10	44									
							0.05	91	10	44				103	31	44			
							0.5	92	6	11				90	18	11			
46	Linuron	17.4	248.0	249>160	30	20	0.01	80	21	43									
							0.05	83	15	43				94	28	44			
							0.5	86	12	11				87	15	11			
47	Methiocarb	17.4	225.1	226>169	20	8	0.01	84	18	43									
							0.05	86	14	43				91	31	44			
							0.5	90	10	11				79	23	11			
20	Methiocarb sulphone	11.2	257.1	258>122	25	20	0.01	100	16	44									
							0.05	98	16	44				149	33	42			
							0.5	103	9	11				100	11	11			
15	Methiocarb sulphoxide	10.0	241.1	242>122	25	30	0.01	118	29	43									
							0.05	110	37	42				139	40	43			
							0.5	92	17	11				100	5	11			
9	Methomyl	7.3	162.0	163>106	15	10	0.01	92	12	44									
							0.05	93	11	44				111	19	44			
							0.5	93	10	11				95	8	11			
6	Oxamyl	6.0	219.1	237>72	10	30	0.01	88	18	45	71	39	22						
							0.05	86	31	44	79	28	22	106	8	45	102	20	25
							0.5	92	11	11	67	24	7	103	6	11	105	5	7
2	Oxamyl-oxime	4.3	162.0	163>72	20	10	0.01	95	13	42									
							0.05	93	12	43				123	25	44			
							0.5	91	7	11				104	6	11			

50	Phorate	19.8	260.0	261>75	15	10	0.01	78	23	44									
							0.05	74	22	41				113	25	44			
							0.5	78	5	12				111	14	12			
39	Phorate sulphone	15.7	292.0	293>247	20	5	0.01	93	17	42				118	39	13			
							0.05	n.a.[b]											
							0.5	n.a.											
36	Phorate sulphoxide	15.4	276.0	277>199	20	10	0.01	96	21	44									
							0.05	91	14	35				105	20	44			
							0.5	82	12	12				97	9	12			
43	Phorate-O-analogue	16.4	244.0	245>75	20	20	0.01	101	27	44				133	14	13			
							0.05	n.a.											
							0.5	n.a.											
48	Promecarb	17.6	207.1	208>151	20	10	0.01	85	14	43									
							0.05	86	13	43				92	31	44			
							0.5	87	7	11				77	25	11			
27	Propoxur	14.0	209.1	210>111	20	10	0.01	96	13	45	90	13	23						
							0.05	96	11	44	93	11	23	103	21	45	104	21	25
							0.5	94	6	11	92	12	7	94	15	11	96	14	7
54	Terbufos	20.9	288.0	289>103	10	10	0.01	83	27	44									
							0.05	79	22	41				92	17	44			
							0.5	80	10	12				98	12	12			
25	Terbufos-O-sulphone	13.1	304.1	305>203	20	20	0.01	92	26	40				108	10	13			
							0.05	n.a.											
							0.5	n.a.											
22	Thiabendazole	12.6	201.0	202>175	40	20	0.01	80	21	21	94	10	21						
							0.05	85	19	21	93	11	23	96	30	43	97	31	24
							0.5	84	8	7	93	13	7	86	17	7	81	18	7
33	Thiodicarb	15.1	354.1	355>88	20	20	0.01	80	29	45	86	16	23						
							0.05	81	28	44	85	23	23	108	23	45	110	24	25
							0.5	82	19	11	78	8	7	97	22	11	99	12	7
37	Thiometon	15.6	246.0	247>89	10	10	0.01	92	23	44									
							0.05	86	27	41				105	19	44			
							0.5	83	9	12				112	9	12			
24	Thiometon sulphoxide	12.7	262.0	263>185	30	10	0.01	93	20	44									
							0.05	90	15	41				108	22	44			
							0.5	84	10	12				98	6	12			
26	Thiometon sulphone	13.1	278.0	279>143	30	10	0.01	98	18	44				113	14	12			
							0.05	92	13	41				112	21	44			
							0.5	78	13	12									
28	Thiophanate methyl	14.0	342.0	343>151	20	20	0.01	44	82	43									
							0.05	54	56	44				113	86	38			
							0.5	45	43	11				94	15	11			

Table 3 (*Continued*)

Peak no.	Pesticide													Matrix effect					
										With NaOH						With NaOH		
		Rt. min	M[a]	MS/MS m/z	CV V	CE eV	Spiking level mg/kg	Mean %	RSD %	n	Mean %	RSD %	n	Mean %	RSD %	n	Mean %	RSD %	n
41	Trimethacarb-2,3,5	16.2	193.3	194>137	17	10	0.01	92	11	45	94	12	23						
							0.05	93	11	44	94	12	23	96	18	45	98	13	25
							0.5	92	8	11	93	7	7	87	22	11	87	28	7
42	Trimethacarb-3,4,5	16.2	193.3	194>122	17	30	0.01	92	11	45									
							0.05	93	11	44				100	32	44			
							0.5	88	9	11				87	20	11			
16	Vamidothion	10.4	287.0	288>146	20	10	0.01	90	19	44									
							0.05	83	23	41				107	12	44			
							0.5	80	15	12				101	14	12			
11	Vamidothion sulphone	7.7	319.0	320>178	20	15	0.01	97	23	44									
							0.05	87	22	41				119	20	44			
							0.5	87	18	12				114	19	12			
7	Vamidothion sulphoxide	6.6	303.0	304>201	20	10	0.01	57	49	44									
							0.05	55	48	44				106	13	44			
							0.5	62	34	9				102	10	12			

Dinocap is analysed using ES−, the other pesticides by using ES+, one fragment ion per compound. All recoveries are corrected for matrix effect and for formation of breakdown products during sample preparation. Peak no. refers to Fig. 1.

[a] M is monoisotopic molecular mass

[b] n.a. Not analysed.

Table 4
Matrices included in the method validation

EU number	Commodity group	Species
1.1	Citrus fruits	Grapefruits, lemons, mandarins, oranges
1.3	Pome fruits	Apples, pears
1.4	Stone fruits	Apricots, nectarines, peaches, plums
1.5	Berries and small fruits	Grapes, strawberries
1.6	Various fruits	Bananas, kiwi fruits, mangoes, pineapples
2.1	Root/tuber vegetables	Carrots, sweet potatoes
2.2	Bulb vegetables	Onions
2.3	Fruiting vegetables	Cucumbers, melons, peppers, tomatoes
2.4	Brassica vegetables	Cabbages, cauliflower, brussels sprouts
2.5	Leafy vegetables	Lettuce, spinach
2.7	Stalk/stem vegetables	Celery, leeks

Standards, used for spiking, were divided into four different mixtures. The parent compound and possible breakdown products (Table 1) were separated into different mixtures to be able to detect any breakdown product. Possible breakdown products were always monitored and quantified. If any breakdown product was formed, recovery of the parent pesticide was corrected for this formation.

2.5. Test of matrix effect

Matrix effect, expressed as the signal from the pesticide in matrix compared to the signal in solvent, was tested in all matrices. To an aliquot of blank extract in methanol a pesticide mixture, 3–10% of the extract volume, was added, to a final concentration of 0.05 of each and 0.5 mg kg^{-1} of selected crops. The matrix effect of terbufos-*O*-sulphone, phorate-*O*-analogue and phorate sulphone was studied at the lowest level, 0.01 mg kg^{-1}. The LC–MS/MS signal of the standard additions was compared to the signal of standard in methanol.

3. Results and discussion

3.1. Influence of different mobile phase on signal response

The signal intensity in LC–MS can be strongly influenced by the mobile phase composition. In order to optimise the signal intensity, standard mixtures in methanol were injected directly into the interface, with no column installed, using different mobile phase compositions. Evaluation was done by recording the MS/MS signal for each pesticide.

Six different buffer constituents were tested: 10 mM ammonium acetate at pH 4.8, 10 mM ammonium formate at pH 4.2, at pH 4.0 and at pH 3.8, 10 mM acetic acid at pH 3.6 and 10 mM formic acid at pH 3.1. The mobile phase during this test was composed of 50% buffer constituent in water and 50% methanol.

In general, the lowest signal was achieved in acetic acid and in ammonium acetate, while the highest signals were achieved in ammonium formate at pH 3.8–4.2. Dinocap was the only pesticide tested which gave the highest signal in acetic acid and in ammonium acetate. The signals for dinocap in both buffers were about twice the signal in ammonium formate. In order to select the buffer for the final method, it was examined whether different buffers influenced the response of the pesticides with lowest signal. The best signal response was obtained with pH ranging from 4.0 to 4.2.

The signal of analytes in LC–MS is normally affected by the ion strength, so that the highest signal is achieved at the lowest ion strength. However, when analysing different samples, which themselves can influence the signal by altering the mobile phase composition, it is important to use a buffer with a sufficient buffering capacity to stabilise the system. Therefore, higher ion strength contributes to a more stable system, both for retention and signal. A buffer strength of 10 mM was chosen as a compromise.

The effect of methanol and acetonitrile as modifiers in the mobile phase was also tested. Injections were made on the HPLC column, with gradient elution. The gradient was as described when using methanol as the modifier, while when using acetonitrile, the initial concentration was lowered to 10%. Ammonium formate at pH 4 was used as the buffer constituent in both systems. For all the included pesticides the signal was normally much higher in methanol than in acetonitrile. Similar results have been reported earlier for different groups of pesticides [11,12]. For most compounds the signal in acetonitrile was in the range 10–40% compared to the signal in methanol, but for some pesticides the signal was even lower than 10%. In reality it was not possible to detect all pesticides at a concentration equivalent to 0.01 mg kg^{-1} when using acetonitrile as the modifier. Imazalil gave about the same response in methanol and acetonitrile and for dinocap the signal was about 20% higher in acetonitrile.

Since we intend to set up a system which can detect all studied pesticides at the lowest level, 0.01 mg kg^{-1}, and with the fewest possible modifications, preferably only in one single system, the mobile phase in the final system was composed of a gradient between ammonium formate, pH 4, and methanol.

3.2. Optimisation of the detection system

In a search for the most appropriate conditions to optimise the mass spectrometric system for analysis of pesticide residues, different ionisation techniques were examined. The responses of pesticides using atmospheric pressure chemical ionisation (APCI) and electrospary ionisation (ESI) were compared. A standard mixture of some pesticides was injected using both techniques. The study resulted

in 10–20 times higher response in ESI than in APCI for all tested pesticides. The ESI technique was thus selected for further validation of the method. One possible reason for the results obtained may be that the intensity of the signal depends on different factors, e.g., the system used, the choice of mobile phase and buffer and obviously the compounds studied. In the final method the pesticides are monitored and quantified using one product ion, which was produced by a known precursor ion. However, when confirmation of the results is required, a sufficient number of product ions should be monitored. All pesticides studied, as shown in Fig. 1, were separated with high sensitivity and selectivity. Linearity was examined for all included pesticides and was defined from the standard curve with eight concentration levels over the expected concentration range 0.01–2.0 $\mu g\,ml^{-1}$ (0.004–0.8 $mg\,kg^{-1}$). The method is proved to be linear obtaining correlation coefficients from 0.96 to 1.00 over the whole range.

3.3. Recoveries and formation of breakdown products

In the majority of cases quantitative results for most pesticides and pesticide metabolites were obtained. The mean recoveries lie higher than 70% at each level. The results of thiophanate methyl, on the other hand, appear to differ considerably. Thiophanate methyl is partly found as carbendazim, but the high RSD values make it questionable if the results can be regarded as quantitative. Carbendazim, imazalil and thiabendazole are basic pesticides with pK_a between 4.5 and 6.5, therefore sodium hydroxide was added before extraction in all selected matrices with a lower pH, as described in Section 2.3. As shown in Table 3 the addition of sodium hydroxide resulted in recoveries of between 88 and 98%. Furthermore, some of the sulphoxides, such as aldicarb sulphoxide and butocarboxim sulphoxide, showed better recoveries from acidic matrices after the addition of sodium hydroxide.

The higher RSD values for demeton-*S*-methyl sulphoxide and vamidothion sulphoxide can also be explained by poor recoveries in more acidic fruits. In the group of bulb vegetables, different species of onion also gave poor recoveries, 20–40% of these pesticides, probably due to the low pH of 4.0–4.5 in onion. However, when sodium hydroxide was added, the recoveries in all the above-mentioned commodities improved to 60–98%.

In general, the extent of breakdown varied from not detectable up to 70%. However, an increase of breakdown was observed when extraction was performed in the presence of sodium hydroxide. The main degradation which could be monitored was the formation of the corresponding

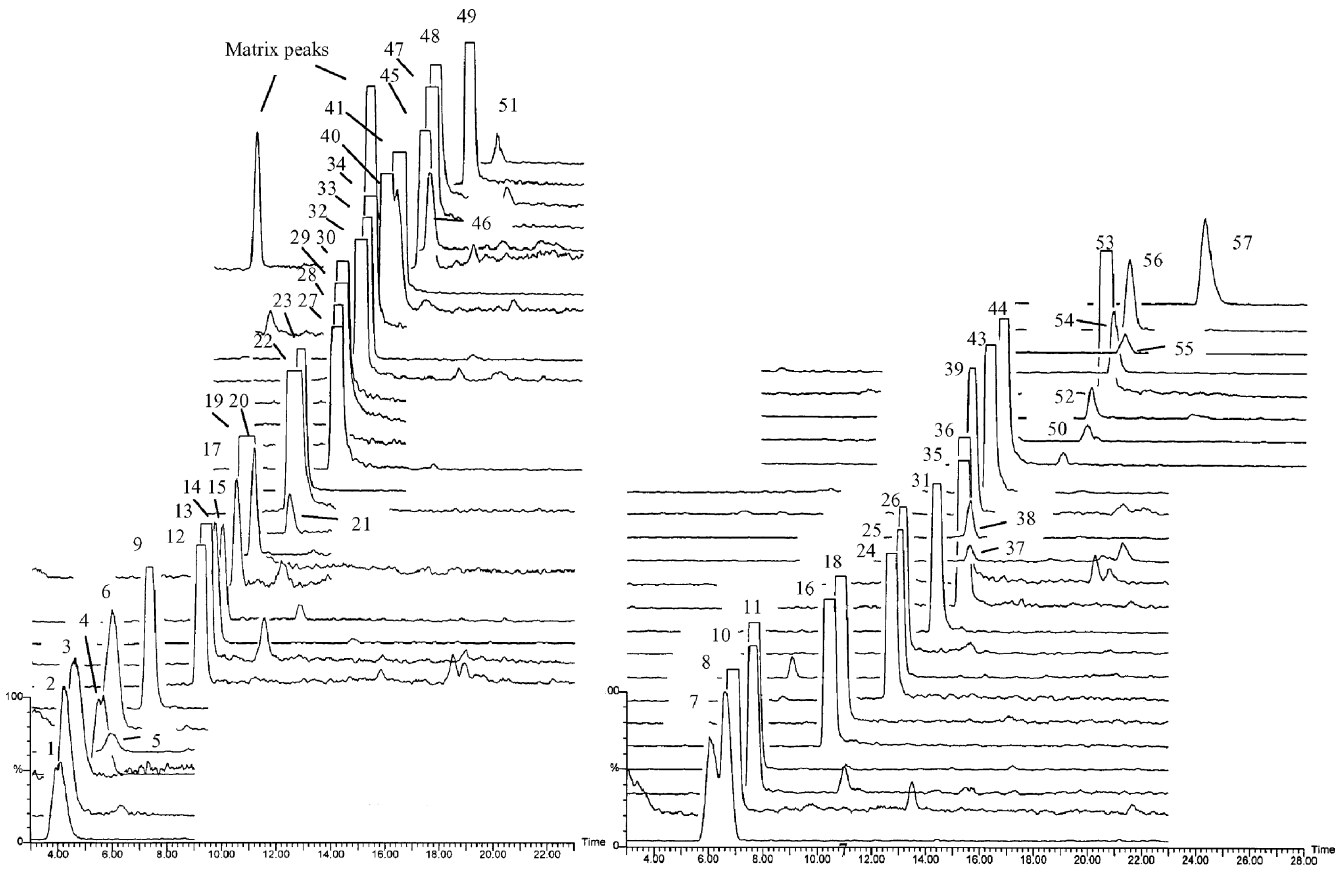

Fig. 1. Chromatogram of orange spiked with studied pesticides at 0.025 $\mu g\,ml^{-1}$ (0.01 $mg\,kg^{-1}$). One MS/MS fragment for each pesticide. See Table 3 for identification of peaks.

sulphoxide from some of the compounds (Table 1), but also in some matrices, the conversion of thiodicarb to methomyl and thiophanate methyl to carbendazim. Carbendazim can also be a product of benomyl. As was seen in a previous study [10], benomyl is completely decomposed to carbendazim during the extraction and was therefore not included in this study. No degradation to sulphone was observed. In all cases where any degradation was observed the recoveries were corrected to the corresponding parent substance.

The low recovery and high RSD of carbosulfan, which is a carbamate pesticide, were mostly caused by degradation. The recoveries varied from 0 to 115% in different crops. The lowest recoveries, or no recoveries at all, were obtained in fruits with a high acid content and in bulb vegetables. Carbosulfan is easily metabolised by hydrolysis and oxidation and its principal metabolite is carbofuran (Table 1). The obtained recoveries are derived either from the intact parent compound or its metabolite or a combination of them.

In some cases carbosulfan was not recovered at all. It should be mentioned that in those cases, where carbosulfan was entirely decomposed, the same behaviour was also detected when standard was added in corresponding blank extract to test the matrix effect. However, after addition of sodium hydroxide, the recoveries ranged from 58 to 84%. Thiodicarb gave acceptable recoveries, 79% on average, in all matrices studied except for kiwi and pepper, in which the recoveries were below 10%. Although furathiocarb is easily metabolised to carbofuran, the recoveries of furathiocarb averaged 82% in all crops and only slight degradation of furathiocarb to carbofuran could be observed.

Compounds such as disulphoton, thiometon, phorate, vamidothion, demeton-*S*-methyl and ethiofencarb were oxidised to their sulphoxide during the recovery test. The extent of breakdown varied from a few percent to 65%. However, no degradation to the corresponding sulphone was detected.

As shown in Table 1 there are further metabolites which have not been included in this study. Either they are very unstable and therefore not easily available or they have been included in previous studies where they not have been detected, such as metabolites of imidacloprid [13,14].

As a consequence, the results obtained in the recovery studies show the importance of using sodium hydroxide for more basic pesticides in acidic matrices and each time verifying a possible breakdown of pesticides as well. This is particularly the case when the decomposition of studied pesticides is highly dependent on the type of matrix.

3.4. Matrix effect and robustness

In every run, a test of the matrix effect has been performed. The pesticides have been injected in both solvent and in matrix and the signals have been compared.

The influence from the matrix can be very variable. The effect, expressed as suppression or enhancement, for one specific combination of pesticide and matrix can vary from one time to another. A pesticide that is affected by 30% suppression on one occasion can be affected by 30% enhancement on the next occasion. This means that it is not possible to test for matrix effect only once and then use this result for future calculations. Furthermore, it is not possible to use the matrix effect for one pesticide in a specific matrix to predict the matrix effect of other pesticides in the same matrix. The matrix effect is very compound-dependent, often probably due to co-eluting matrix components which interact with the pesticide in the ionisation step in the interface. Fig. 2 shows an example of suppression where only two compounds, carbendazim and thiabendazole, of the five compounds in the same injection, are suppressed, while the others are not affected by the matrix at all.

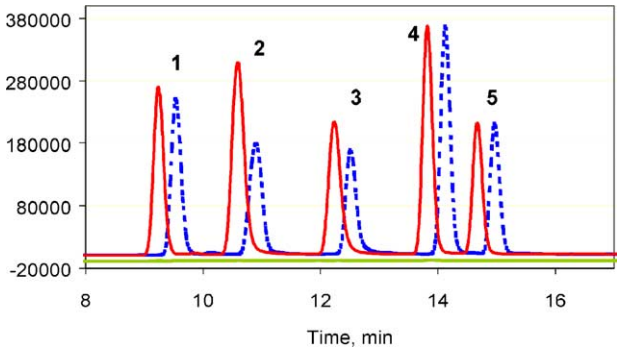

Fig. 2. Example of suppression of some pesticides in one injection. Injection of some pesticides at 0.05 mg kg^{-1} (0.125 μg ml^{-1}) in strawberry, overlaid with the same pesticides in methanol and a blank chromatogram of strawberry. Retention time of pesticides in strawberry (- - -) is manually moved 0.3 min, true retention time is identical. Only peak no. 2 and no. 3 are suppressed. ES + /MS/MS. Peak 1: ethiofencarb-sulphoxide; 2: carbendazim; 3: thiabendazole; 4: propoxur; 5: carbaryl.

More than 2000 tests of matrix effect on the incorporated pesticides have been done using the proposed method. In general the measured matrix effect is quite small, with a mean value of 104% and a relative standard deviation of 23%. Although the mean value is very near to 100%, within these values there is a variation, mainly depending on different matrices, but also to a smaller extent on the compound. In Table 3 the mean results from each pesticide in all matrices are reported. Methiocarb sulphone and methiocarb sulphoxide show a mean value of 149 and 139%, respectively. This high mean value can partly be explained by the somewhat low stability of the compounds in working solutions of pure methanol, and a better stability in matrix. After the first experiments, these standards were diluted in pesticide-free banana extract in methanol (0.25 g ml^{-1}). The stability of these pesticides in working solution, as well as of thiophanate methyl, was improved by dilution in this low concentration matrix instead of in pure methanol.

Although it is possible, and normally also necessary, to correct for matrix effect by matrix-matched standards, it is important for a multi-matrix method that the matrix effect is low and consistent. This is due to the fact that, when analysing many different matrices in one run, it is not convenient, or even possible, to use matrix-matched standards for

all compounds in every different matrix. To be able to estimate the true concentration of a pesticide in a specific matrix, it is then important to know quite well what a detected concentration, in relation to standards in solvent, means in that specific matrix. If not, samples with very low estimated concentrations relative to the MRL also have to be reanalysed and quantified using matrix-matched standards.

Finally, the object of this study, with a number of matrices, was not only to test the influence of different matrices on recovery but also to investigate the robustness of the method. It should be noticed that in none of the matrices tested has the detection of the pesticides been interfered with by matrix peaks.

With the described method, nearly 2500 recovery tests with 57 pesticides and their metabolites in different crops were conducted. The overall recovery was found to be 87%, including all studied concentration levels. Despite the number of different matrices with varying nature which have been tested, the functioning of the instrument was fully adequate. The routine clean-up of the cone has been shown to be sufficient to maintain a tidy performance.

4. Conclusions

The present multi-residue method is simple and gives quantitative results for most pesticides and pesticide metabolites tested at low levels. In general, the overall recoveries lie higher than 70% even at the $0.01\,mg\,kg^{-1}$ level. The higher R.S.D. values for some pesticides can in many cases be explained by poor recoveries in fruits with high acid content. However, acceptable recoveries, >70%, were obtained in most cases when sodium hydroxide was added before extraction. The analysis time has been shortened and the time-consuming clean-up step has been shown to be unnecessary. LC–MS/MS is a sensitive technique and provides confirmation of identity, which is an important feature when low MRLs are introduced for certain commodities.

The use of the established ethyl acetate extraction makes it applicable and efficient for monitoring purposes and a number of different extraction systems can be replaced by using the proposed multi-residue method. Furthermore, the proposed method has the advantage of detecting pesticides and their metabolites in one single extraction and detection system and thus leads to awareness of the behaviour of pesticides and their possible degradation. Although the GC multi-residue method is still the primary choice for new pesticides, the LC–MS/MS is undoubtedly indispensable as a complementary technique for monitoring purposes for future needs.

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