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Multiresidue method for determination of 90 pesticides in fresh fruits and vegetables using solid-phase extraction and gas chromatography-mass spectrometry☆

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Abstract

A multiresidue method for analysis of 90 pesticides with different physico-chemical properties in fruits and vegetables was developed. The method involves a rapid and small-scale extraction procedure with acetone using vortex mixing. Solid-phase extraction (SPE) on a highly cross-linked polystyrene divinylbenzene column (LiChrolut EN) was used for clean-up and pre-concentration of the pesticides from the water-diluted acetone extracts. For most fruit and vegetable samples this partial clean-up was sufficient, but some of them with more co-extracting substances need further clean-up (cereals, spinach, carrots, etc.). Diethylaminopropyl (DEA) modified silica was used for efficient removal of interferences caused by various organic acids, sugars, etc. The pesticide residues were determined by gas chromatography with a mass selective detector (GC-MS). The majority of pesticide recoveries for various fruits and vegetables were >80% in the concentration range from 0.01 to 0.50 mg/kg, except for the most polar pesticides (methamidophos, acephate, omethoate) which cannot be determined by this method. The limit of quantitation for most of the pesticides was 0.01 mg/kg with majority of relative standard deviations (R.S.D.s) below 10%.

Keywords: Multiresidue methods; Vegetables; Fruits; Food analysis; Pesticides

1. Introduction

Concern over pesticide residues in fruit and vegetables has lead to the development of many multiresidue methods as the most cost-effective approach to residue analysis. Regulatory authorities provide assurance that any pesticide remaining in or on the food is within safe limits through monitoring programmes of random sampling and analysis of raw and processed food on the market, as well as by targeting known problems. In response to this requirement, a number of methods have been developed and applied routinely for the control of pesticide residues in food [1–20].

Due to the low detection levels required by regulatory bodies and the complex nature of the matrices in

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which the target compounds are present, efficient sample preparation and trace-level detection and identification are important aspects in an analytical method. Multiresidue method development is difficult, due to the fact that compounds of different polarities, solubilities, volatilities and pK_a values have to be simultaneously extracted and analysed. Several multiresidue methods for determination of organophosphorus, organochlorine and organonitrogen pesticides in crops using gas chromatography for separation of individual compounds, followed by detection with selective and sensitive detectors (ECD, NPD, FPD, AED or MS) have been proposed [1–8]. Mass spectrometry is a very sensitive and selective technique for both multiresidue determination and trace-level identification of a wide range of pesticides [3,9,10,18].

A number of solvents have been used for multiresidue extractions and the most common include acetone [1,2,5–7], ethyl acetate [3,4] and acetonitrile [8–11]. The presence of matrix interferences in extracts can adversely affect analyte quantification and identification. Clean-up is necessary in order to reduce the detection limits of methods and/or to avoid interferences from the matrix. Extensive clean-up of extracts may result in the partial loss of some compounds as well as increased labour and cost demands, but inadequate clean-up can lead to adverse effects related to the quality of the generated data, such as masking of the residue peaks by co-eluting matrix components, occurrence of false positives and inaccurate quantitation. Sample clean-up techniques include gel permeation chromatography on Bio Beads SX3 [4], liquid–liquid partitioning using various solvents or columns filled with diatomaceous earth [12], solid-phase extraction (SPE), adsorption chromatography (on silica, Florisil, active carbon, alumina, silica gel/charcoal), membrane technologies, matrix solid-phase dispersion (MSPD) [13,14], automated MSPD [15], etc.

Solid-phase extraction is being increasingly used in food analysis, mainly for sample clean-up. Solid-phase extraction columns containing a normal (polar)-phase or reversed (nonpolar)-phase support not only offer the potential of simplifying the purification of the initial extract and reducing the amount of solvent consumed, but also the possibility of automation and high sample throughput. In an on-line clean-up configuration the SPE clean-up is inserted as a part of the chromatographic system, mostly using HPLC [16] because of the compatibility of mobile phases or GC [17].

Many of the published methods for pesticide determination in fresh fruits and vegetables use a combination of two or more commercially available SPE columns for clean-up in the normal-phase (NP) mode. Weak anion-exchange sorbents such as primary secondary amine (PSA), aminopropyl (NH_2), or diethylaminopropyl (DEA) modified silica are often used for clean-up of food samples together with strong anion-exchange sorbents (SAX, QMA). An improved variation of the methodology of Luke and co-workers which has been referred to as Luke II method [18], uses a pre-partition clean-up with a C_{18} SPE column, an acetone–methylene chloride partition clean-up step and post-partition clean-up using strong and weak anion-exchange SPE catridges (QMA and aminopropyl). Other SPE clean-up approaches include the combination of GCB (graphitised carbon black) and PSA columns [11,19], the combination of C_{18}, GCB and aminopropyl [10] and the combination of GCB, PSA and SAX columns [20]. Because of difficulties with elution of certain planar or aromatic pesticides from GCB, only PSA was used for very efficient clean-up of acetonitrile extracts [9].

There are fewer applications using reversed-phase (RP) SPE for pre-concentration/clean-up of pesticide residues from fruit and vegetable samples. Using RP-SPE nonpolar to moderately polar analytes are extracted from polar solutions (e.g. aqueous) onto nonpolar sorbents, which include silica modified with octadecyl, octyl, cyclohexyl or phenyl groups, modified or nonmodified poly(styrene-divinylbenzene) (PS-DVB) resins and GCB [21,22]. Most of the published methods have been developed for analysis of pesticides in wine [23–25]. For solid food samples the first step is the extraction of pesticides using water-miscible solvents, but dilution with water is required to facilitate the retention of pesticides onto RP sorbent such as C_{18} [26–29], GCB [28,30,31] or polymeric sorbents [31–33].

The aim of our work was the development of a sensitive, versatile and selective multiresidue method for the quantitative determination of pesticides from several compound classes using MS detection. The work focused on SPE/clean-up in the multiresidue analysis of various pesticides in fruits and vegetables.

2. Experimental

2.1. Reagents

Pesticide standards were purchased from Dr. Ehrenstorfer (Ausberg, Germany) and most of them were of >99% certified purity. Concentrations of standard solutions were corrected for the certified purity of the standards if below 99%. Residue analysis grade methanol, acetone and ethyl acetate were purchased from J.T. Baker (Deventer, Holland). Ultrapure water was prepared with a Nanopure water purification system (Barnstead Int., Dubuque, IA, USA). Individual stock standard solutions of pesticides were prepared by dissolving 20–50 mg of each compound in 25 ml of acetone and were stored in glass-stoppered flasks at −20 °C. Mixed compound calibration solutions in acetone were prepared from the stock solutions with concentrations disregarding their GC sensitivities (Table 1) and were used as spiking solutions as well. Matrix-matched standards were prepared at the same concentration as that of calibration solutions by adding appropriate amounts of standards to the control matrix extracts. Malathion-D_6 (Dr. Ehrenstorfer, Ausberg, Germany) was used as a surrogate standard (8 ng/μl) and was added to the homogenised sample prior to extraction. Pentachlorobenzene (Dr. Ehrenstorfer, Ausberg, Germany) was used as an internal standard (20.9 ng/μl) to compensate for sample and injection volume changes and was added to the vial prior to GC analysis.

2.2. Materials for solid-phase extraction

A highly cross-linked PS-DVB sorbent material (LiChrolut EN) with a surface area 1200 m^2/g, particle size 40–120 μm, was obtained from Merck (Darmstadt, Germany). DEA columns containing 500 mg of sorbent with a particle size of 120 μm were supplied by Varian (Middelburg, The Netherlands). Other sorbents tested were graphitised carbon black ENVI-Carb obtained from Supelco (Bellefonte, PA, USA), C_{18}, PSA and NH_2 from Varian (Middelburg, The Netherlands), porous graphitic carbon Hypercarb (Thermo Hypersil, Keystone, USA) and ENV+ (IST, Mid Glamorgan, UK).

2.3. Gas chromatography-mass spectrometry

An Agilent Technology (AT, Palo Alto, CA) 6890 gas chromatograph with an HP5973 mass selective detector was employed. The gas chromatograph was equipped with an HP 6890 autosampler and split/splitless injector with electronic pressure control. A DB-35MS, 30 m × 0.25 mm i.d. capillary column with a 0.25 μm film (AT, Palo Alto, CA) was used in combination with the following oven temperature programme: initial temperature 55 °C, held for 2 min, 25 °C/min ramp to 130 °C, then 1 °C/min to 200 °C, followed by 3 °C/min to 250 °C and finally 20 °C/min to 299 °C, held for 16 min. The carrier gas (helium) flow rate was in constant flow mode at 1.0 ml/min. Splitless injection of a 2 μl volume was carried out at 240 °C with the purge valve on at 2 min. The liner used was amino deactivated single gooseneck from Restek (Bellefonte, USA).

The mass spectrometer was operated in electron ionisation mode with a transfer line temperature of 280 °C, ion source 230 °C and selected ion monitoring (SIM) mode. Dwell time was adjusted so that the number of cycles per second was 1.4 throughout the chromatographic run, providing a sufficient number of chromatographic points for all compounds.

2.4. Sample preparation

The portion of the sample to which the maximum residue limit (MRL) applies, was homogenised using a Robot Coupe Blixer 3 Plus blender (Vincennes Cedex, France). A subsample (10 g) was weighed into 40 ml Teflon centrifuge tube (Cole Palmer, Vernon Hills, IL, USA) and spiked with 100 μl of surrogate standard in acetone and extracted with 20 ml acetone by vortexing (Minishaker MS2, IKA, Staufen, Germany) at full speed for 2 min. The extract was centrifuged (Model LC-321,Tehnica, Železniki, Slovenia) at 3000 rpm for 5 min and the supernatant was transferred to a graduated cylinder (25 ml) to measure its volume.

2.5. Solid-phase extraction

Solid-phase extraction was carried out using glass columns packed with 400 mg of highly cross-linked styrene-divinylbenzene copolymer LiChrolut EN. An SPE vacuum manifold (J.T. Baker, Deventer, Holland)

Table 1
Quantiation (target) ions, identification (confirmation) ions, calibration levels, water solubilities, *n*-octanol–water partition coefficient ($\log K_{ow}$), dissociation constant (pK_a) of the selected pesticides [39]

Pesticide	Quantitation ion	Identification ion (*m*/*z*)	STD 1 (mg/kg)	STD 2 (mg/kg)	STD 3 (mg/kg)	Water solubility (mg/l)	$\log K_{ow}$	pK_a
Acephate	136	94, 183	0.02	0.21	0.54	790000	−0.89	
Atrazine	215	200, 173	0.02	0.21	0.54	33	2.5	1.7 (weak base)
Azinphos-ethyl	132	160, 77	0.02	0.21	0.53	4–5	3.18	
Azinphos-methyl	160	132, 77	0.02	0.21	0.52	28	2.96	
Azoxystrobin	344	388, 403	0.02	0.21	0.52	6	2.5	
Biphenyl	154	152, 76	0.02	0.21	0.52	Not soluble	–	
Bitertanol	170	168, 112	0.02	0.21	0.54	2.7 (I), 1.1 (II)	4.1 (I), 4.15 (II)	
Bromophos-methyl	331	125, 109	0.02	0.20	0.51	–	–	
Bromopropylate	341	183, 343	0.02	0.20	0.51	<0.5	5.4	
Captan	79	264, 149	0.02	0.20	0.50	3.3	2.8	
Carbaryl	144	115, 201	0.02	0.21	0.51	120	1.59	
Carbofuran	164	149, 221	0.02	0.21	0.52	320 (20 °C), 351 (25 °C)	1.52	
Chlorfenvinfos	267	323, 295	0.02	0.22	0.54	145	3.85	
Chlorpyrifos-ethyl	197	314, 125, 210	0.02	0.23	0.57	1.4 (25 °C)	4.7	
Chlorpyrifos-methyl	286	125, 109	0.02	0.21	0.54	2.6 (20 °C)	4.24	
Chlorprofam	127	171, 213	0.02	0.20	0.50	89	–	
Coumaphos	362	364, 226	0.02	0.20	0.51	1.5 (20 °C)	4.13	
Cyfluthrin	163	206, 226	0.02	0.20	0.50	0.0021–0.0032	5.9–6.0	
λ-Cyhalothrin	181	197, 199	0.02	0.21	0.54	0.005 (pH 6.5, 20 °C)	7 (20 °C)	>9 (hydrolysis)
Cypermethrin	181	163, 209	0.02	0.20	0.50	0.004 (pH 7)	6.6	
Cyprodinil	224	210, 226	0.02	0.20	0.50	20 (pH 5.0, 25 °C), 13 (pH 7.0, 20 °C), 15 (pH 9.0, 20 °C)	3.9 (pH 5.0, 25 °C), 4.0 (pH 7.0, 20 °C), 4.0 (pH 9.0, 20 °C)	4.4 (weak base)
o,*p*′-DDT	235	165, 237	0.02	0.19	0.48	Pract. insoluble	–	
p,*p*′-DDT	235	165, 237	0.02	0.21	0.54	Pract. insoluble	–	
Deltamethrin	181	253, 209	0.02	0.20	0.50	<0.0002 (25 °C)	4.6 (25 °C)	
Diazinon	179	137, 304	0.02	0.21	0.51	60 (20 °C)	3.30	2.6
Dichlorvos	109	185, 220, 79	0.02	0.20	0.50	ca. 18000 (20 °C)	1.9, 1.42	
Difenconazole	323	265, 325	0.02	0.21	0.52	15	4.20	
Dichlobenil	171	173, 136	0.02	0.21	0.53	14.6 (20 °C)	2.70	
Dichlofluanid	123	224, 167	0.02	0.21	0.52	1.3 (20 °C)	3.7 (21 °C)	
Dicofol	251	139, 253	0.02	0.21	0.53	0.8 (25 °C)	4.30	
Dimethoate	87	93, 229	0.02	0.20	0.51	23800 (pH 7, 20 °C)	0.704	
Endosulfan I	195	339, 159	0.02	0.22	0.55	0.32 (22 °C)	4.74 (pH 5)	
Endosulfan II	195	339, 237	0.02	0.21	0.53	0.33 (22 °C)	4.79 (pH 5)	
Endosulfan sulphate	272	387, 422	0.02	0.20	0.51	–	–	
Ethion	231	384, 153	0.02	0.21	0.53	2 (25 °C)	–	
Fenarimol	139	251, 330	0.02	0.20	0.49	13.7 (pH 7, 25 °C)	3.69 (pH 7, 25 °C)	
Fenchlorphos	285	287, 125, 109	0.02	0.21	0.51	–	–	

Fenitrothion	277	125, 109	0.02	0.20	0.49	14 (30 °C)	3.43 (20 °C)	
Fenthion	278	125, 109	0.02	0.21	0.52	4.2 (20 °C)	4.84	
Fludioxonil	248	154, 182	0.02	0.20	0.50	1.8 (25 °C)	4.12 (25 °C)	$pK_{a_1} < 0$, $pK_{a_1} \approx 14.1$
Folpet	260	262, 295	0.02	0.22	0.54	0.8 (room temperature)	3.11	
Fonofos	246	109, 137	0.02	0.21	0.52	13	–	
Heptenophos	124	109, 250	0.02	0.21	0.54	2200 (20 °C)	2.32	
Imazalil	173	215, 217	0.02	0.23	0.57	180 (pH 7.6, 20 °C)	3.82 (pH 9.2)	6.53 (weak base)
Iprodione	314	316, 187	0.02	0.21	0.54	13 (20 °C)	3.0 (pH 3 in 5)	
Krezoxim-methyl	116	206, 131	0.02	0.21	0.53	2 (20 °C)	3.4 (pH 7, 25 °C)	Not within 2–12
Lindane	181	183, 109	0.02	0.20	0.49	8.52 (25 °C)	3.5	
Malaoxon	127	268, 109	0.02	0.20	0.50	–	–	
Malathion	173	127, 125	0.02	0.21	0.54	145 (25 °C)	2.75	
Mecarbam	329	296, 131	0.02	0.20	0.49	<1000 (room temperature)	–	
Metalaxyl	206	249, 279	0.02	0.19	0.48	8400 (22 °C)	1.75 (25 °C)	$\ll 0$
Methidathion	145	85, 302	0.02	0.21	0.54	200 (25 °C)	2.2	
Methamidophos	94	141, 79	0.02	0.21	0.54	>200000 (20 °C)	−0.8 (20 °C)	
Metolachlor	238	162, 240	0.02	0.20	0.50	488 (25 °C)	2.9 (25 °C)	
Mevinphos	93	137, 179	0.02	0.20	0.51	Miscible	0.127	
Monocrotophos	127	192, 223	0.02	0.20	0.50	100% (20 °C)	−0.22 (calculated)	
Myclobutanil	179	288, 206	0.02	0.21	0.52	142 (25 °C)	2.94 (pH 7–8, 25 °C)	
Omethoate	156	110, 213	0.02	0.20	0.51	Miscible	−0.74 (20 °C)	
Paraoxon-ethyl	275	109, 247	0.02	0.20	0.51	–	–	
Paraoxon-methyl	247	109, 200	0.02	0.21	0.52	–	–	
Parathion-ethyl	291	109, 125, 139	0.02	0.20	0.50	11 (20 °C)	3.83	
Parathion-methyl	263	125, 109	0.02	0.20	0.49	55 (20 °C)	3.0	
Penconazole	248	159, 250	0.02	0.21	0.51	70 (20 °C)	3.72 (pH 5.7, 25 °C)	1.51 (very weak base)
Permethrin	183	163, 165	0.02	0.22	0.55	0.006 (pH 7, 20 °C)	6.1 (20 °C)	
o-Phenylphenol	170	141, 115	0.02	0.20	0.51	700 (25 °C)	–	
Phorate	75	231, 260	0.02	0.20	0.50	50 (25 °C)	3.92	
Phosalone	182	121, 367	0.02	0.21	0.53	3.05 (25 °C)	4.01 (20 °C)	
Phosphamidon	264	127, 109	0.02	0.21	0.52	Miscible	0.79	
Phosmet	160	317, 77	0.02	0.21	0.52	25 (25 °C)	2.95	
Pirimicarb	166	238, 72	0.02	0.20	0.49	3000 (20 °C)	1.7 (nonionic)	4.44 (20 °C) (weak base)
Pirimiphos-methyl	276	305, 290	0.02	0.20	0.50	11 (pH 5, 20 °C), 10 (pH 7, 20 °C), 9.7 (pH 9, 20 °C)	4.2 (20 °C, nonionic)	4.30
Procymidone	283	285, 96	0.02	0.21	0.53	4.5 (25 °C)	3.14 (26 °C)	
Prometryn	241	184, 226	0.02	0.20	0.49	33 (25 °C)	3.1 (25 °C, nonionic)	4.1 (weak base)
Propargite	135	350, 173	0.02	0.22	0.54	632 (25 °C)	3.73	>12
Propetamphos	138	194, 236	0.02	0.21	0.54	110 (24 °C)	3.82	13.67 (23 °C)
Propham	171	173, 136	0.02	0.20	0.49	250 (20 °C)	–	
Propiconazole	259	173, 261	0.02	0.20	0.50	100 (20 °C)	3.72 (pH 6.6, 25 °C)	1.09 (very weak base)
Propizamide	173	255, 109	0.02	0.20	0.49	15 (25 °C)	3.1–3.2	
Pyrazophos	221	232, 373	0.02	0.20	0.50	4.2 (25 °C)	3.8	
Pyridafenthion	340	199, 188	0.02	0.21	0.52	100 ppm (20 °C)	3.2	

Table 1 (*Continued*)

Pesticide	Quantitation ion	Identification ion (*m*/*z*)	STD 1 (mg/kg)	STD 2 (mg/kg)	STD 3 (mg/kg)	Water solubility (mg/l)	$\log K_{ow}$	pK_a
Pyrimethanil	198	200, 183	0.02	0.21	0.53	121 (pH 6.1, 25 °C)	2.84 (pH 6.1, 25 °C)	3.52 (20 °C) (weak base)
Quinalphos	146	157, 298	0.02	0.20	0.49	17.8 (22–23 °C)	4.44 (23 °C)	
Quintozene	237	295, 249	0.02	0.19	0.48	0.1 (20 °C)	5.1	
Tebuconazole	250	125, 252	0.02	0.20	0.49	36 (pH 5–9, 20 °C)	3.7 (20 °C)	
Tecnazene	261	203, 215, 213	0.02	0.21	0.52	0.44 (20 °C)	–	
Tetrachlorvinphos	329	331, 109, 79	0.02	0.21	0.54	11 (20 °C)	–	
Tetradifon	356	159, 227	0.02	0.21	0.53	0.078 (20 °C)	4.61	
Thiabendazol	201	174, 129	0.02	0.22	0.55	160 (pH 4, 20 °C), 30 (pH 7 in 10, 20 °C)	2.39 (pH 7)	$pK_{a_1} = 4.73$, $pK_{a_1} = 12.0$
Tolclophos-methyl	265	125, 109	0.02	0.21	0.53	1.1 (25 °C)	4.56 (25 °C)	
Tolylfluanid	137	238, 181	0.02	0.21	0.52	0.9 (20 °C)	3.90 (20 °C)	
Triadimefon	208	210, 57	0.02	0.21	0.54	64 (20 °C)	3.11	
Triazophos	161	257, 208	0.02	0.19	0.48	39 (pH 7, 20 °C)	3.34	
Trifluralin	306	264, 335	0.02	0.20	0.51	0.184 (pH 5), 0.221 (pH 7), 0.189 (pH 9)	4.83 (20 °C)	
Vinclozolin	198	285, 187	0.02	0.22	0.54	2.6 (20 °C)	3 (pH 7)	
Malathion-D_6	131	99						
PKB	250	252						

was used for simultaneous extraction of 12 samples. The extraction columns were washed with 6 ml of ethyl acetate and conditioned by passing 6 ml of methanol followed by 8 ml of deionised water through the column. The sorbent was never allowed to dry during the conditioning and sample loading steps. Then the extraction columns were fitted with detachable 70-ml polypropylene reservoirs (J.T. Baker, Deventer, Holland) to contain the diluted sample extract. Exactly one-half of the extract (equivalent to 5 g sample) was transferred to the reservoir, which was partially filled with deionised water and deionised water was added to the top. Sample loading was performed under vacuum (no difference in recoveries were observed using flow rates between 5 and 15 ml/min). After the passage of the sample, the column was dried by vacuum aspiration under increased vacuum until the sorbent changed colour from brown to orange (approximately 30 min). The pesticides were eluted with 2 ml of ethyl acetate with 1% triethylamine and with three 2-ml aliquots of ethyl acetate–acetone 90:10. The eluates were collected in 15 ml tubes under gravity flow only.

After all the elution solvent had passed through the extraction column, the residual solvent was forcibly removed from the column. The eluate was evaporated to less than 1 ml under nitrogen using a Turbo Vap LV evaporator (Zymark, Hopkinton, MA, USA) with a water bath at 35 °C, and solvent exchange to acetone was performed by adding two 2-ml portions of acetone and evaporating to low volume after each addition. The extract was quantitatively transferred to a 2 ml GC vial, and concentrated to approximately 350 μl by a gentle stream of nitrogen (Messer Griesheim, Ruše, Slovenia). Fifty microliters of internal standard solution were added to the vial and 2 μl were injected into the GC-MS. The concentration of the sample represented by the extract was 5 g/400 μl.

2.6. Sample clean-up

If necessary, sample clean-up was performed using a weak anion-exchange DEA column. A layer (ca. 1 cm) of anhydrous magnesium sulphate was added to a DEA column to remove traces of water from the eluate. The column was then washed with 6 ml of ethyl acetate and attached below the LiChrolut EN column before elution. The tandem columns were placed on an SPE vacuum manifold and were then eluted with 2 ml of ethyl acetate with 1% triethylamine and three 2 ml aliquots of ethyl acetate–acetone 90:10 into a 15 ml tube under gravity flow only. The procedure was then the same as described in Section 2.5.

2.7. Recovery studies

For recovery studies subsamples of known blanks (10 g) were spiked prior to extraction by addition of 200 μl of composite pesticide standard solutions in acetone to give 0.02, 0.20 or 0.50 mg/kg of each compound. They were then prepared according to the proposed procedure described in Sections 2.5 and 2.6.

2.8. Preparation of calibration standards

Calibration standards in a blank matrix of the commodity being analysed were prepared by adding 100 μl of respective spiking solution, 50 μl of surrogate standard (malathion-D_6) and 50 μl of internal standard (pentachlorobenzene) to 200 μl of blank extract, to produce a final concentration of 0.02, 0.20 and 0.50 mg/kg and 0.16 mg/kg for malathion-D_6. Calibration standards in solvent were prepared in the same manner, replacing blank extract with 200 μl of acetone.

3. Results and discussion

In multiresidue monitoring the most important issues are the selectivity and sensitivity of the method, confirmation of positives, accuracy of quantitation, timely analysis, and cost in resources. Due to the wide range of polarities, ionisation properties, water solubilities and volatilities of modern pesticides (Table 1), compromises often have to be made regarding these issues.

3.1. Pesticide extraction from samples

Acetone was selected as the solvent for extraction of pesticides because of its effectiveness for polar and nonpolar pesticides from a diverse range of matrices. Its other advantages include low toxicity and cost, miscibility with water and ease of evaporation.

Ten grams of sample homogenised using a Robot Coupe blender were taken for analysis in order to

achieve a representative sample. Vortexing proved to be efficient for extraction of pesticides from well homogenised fruit and vegetables, and cross-contamination was minimised because the sample is in contact with only inert PTFE.

3.2. Optimisation of solid-phase extraction

The efficiency of SPE depends on the type and quantity of sorbent, sample volume, flow rate and its pH, as well as the content of organic modifier, elutropic strength and volume of the elution solvent. In the initial experiments several sorbents were tested for extraction of the investigated pesticides from water to optimise the SPE conditions. Among the sorbents tested were graphitised carbon black (ENVI-Carb), porous graphitic carbon (Hypercarb), C_{18} and two different types of PS-DVB copolymers (LiChrolut EN and ENV+). The best results, representing a compromise between good recoveries for polar and nonpolar pesticides, were obtained for highly cross-linked, porous ethylvinylbenzene-divinyl benzene copolymer LiChrolut EN.

For further optimisation the percentage of organic modifier (acetone) in water, sample volume (200, 100, and 70 ml), sorbent mass (400 and 1000 mg), elution solvent strength (ethyl acetate, ethyl acetate with various percentages of methanol or acetone, hexane–acetone) and elution method (by vacuum or gravity) were varied to assess optimal conditions.

The most critical variable governing the recovery and reproducibility of SPE was the percentage of acetone in the sample. As expected, direct application of the acetone–water (2:1) extract to the top of the SPE column was precluded. This problem was eliminated by lowering the percentage of acetone down to 14% by addition of water, enabling good recoveries for both polar and nonpolar pesticides. In other studies using a similar approach, best recoveries were obtained for samples containing between 15 and 20% of acetone after dilution [26,27]. The best conditions for extracting of pesticides of low and medium polarity were achieved by dilution to an acetone/water ratio 3:7 and addition of sodium chloride for “salting out” effect [29]. Dilution of the extract to a 4% acetone [32,33] or 5% acetonitrile [30,31] content was necessary to effect adsorption of pesticides onto the PS-DVB or GCB sorbent, respectively. The chosen content of organic solvent in the sample solution determines the polarity range of pesticides that can be recovered as well as the amount of co-extractives present.

Pesticides were eluted from the air-dried column with 2 ml of ethyl acetate containing 1% triethylamine and 6 ml of ethyl acetate–acetone (90:10) by gravity flow only. The reservoir was rinsed with the elution solvent to recover any nonpolar pesticides that might have been adsorbed onto the walls. Triethylamine improved the extraction recovery of basic pesticides. The most profound effect was observed for imazalil where the recoveries increased from less than 50% to above 80%.

Under the conditions described in Section 2, recoveries were good for moderately polar to nonpolar pesticides, but less than desired for the most polar ones. Increasing the amount of sorbent used to extract the sample was shown to improve the recovery of polar pesticides. Although very polar pesticides can be isolated from 100 ml of deionised water using 1000 mg of PS-DVB with good recoveries (107% for acephate, 79% for methamidophos and 100% for omethoate), even small amounts of acetone in samples caused recoveries to drop below acceptable values.

To improve the recoveries of polar pesticides an aliquot of aqueous acetone extract of lettuce was concentrated to 5 ± 0.5 ml to remove most of the acetone. The resulting aqueous extract was then applied to the pre-conditioned LiChrolut EN column (Method A) or was diluted prior to percolation to 100 ml with deionised water (Method B). Recoveries of the most polar pesticides improved, except for methamidophos and acephate which remained $<10\%$. Average recoveries were better for Method B (omethoate 64%, monocrotophos 87%) than for Method A where no dilution was performed (omethoate 23%, monocrotophos 79%). However, the increased recoveries of polar pesticides were accompanied by a loss of selectivity as the extracts were much dirtier.

The recoveries of most of the pesticides (except the most polar) were better when the sample solution used for SPE was prepared by diluting the aqueous acetone crop extract directly with water, hence bypassing the time consuming acetone removal step. As the recoveries of the very polar compounds acephate, methamidophos, omethoate and monocrotophos were low, they were excluded from further method optimisation.

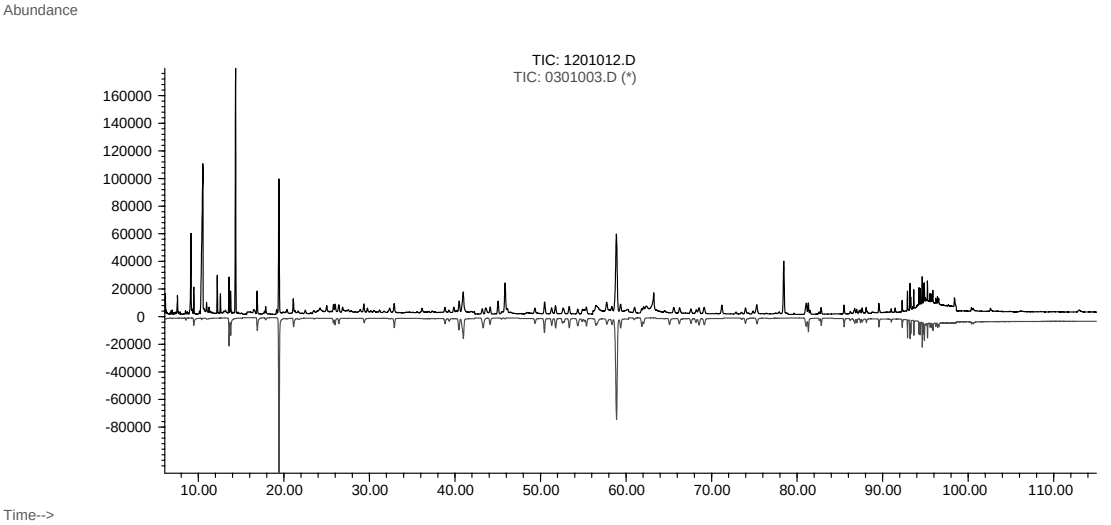

Fig. 1. Comparison of SIM chromatograms of matrix-matched standard in blank apple extract (upper) and standard in acetone (lower) at 0.02 mg/kg level.

3.3. Clean-up of the sample extract

The concentrated sample extracts may contain a high content of co-extractives which can damage the capillary GC column, as well as resulting in a matrix enhancement effect [34]. For most fruit and vegetable samples the final extracts using the proposed SPE method were clean enough for direct GC-MS analysis. Fig. 1 illustrates a comparison of SIM chromatograms obtained for blank apple sample fortified at 0.02 mg/kg extracted under the proposed method (without DEA clean-up) and the corresponding standard solution in acetone. There were no significant interfering peaks present in the elution region of the pesticides.

Samples that contain more fats, sugars or pigments needed further clean-up (cereals, spinach, carrots, etc.), because of the relatively large amount of sample injected (5 g/400 μl). Previous experiments showed that weak anion exchangers (WAX) such as NH_2 and PSA remove many co-extractives interfering with GC determination of pesticides and are also very efficient in lowering the matrix effect [9,10,35]. When an organic solvent extract of a food sample is eluted through a WAX column, the sample matrix co-extractants are retained on the SPE column while the pesticides are eluted. After preliminary tests, DEA columns were chosen for further experiments because of better recoveries of folpet. It has been reported that folpet could not be completely recovered from NH_2 columns [10]. Although PSA or NH_2 sorbents were more effective in removing the pigments, it was shown that pigments had little effect on MS chromatograms or matrix effects [35]. The experiments showed losses of acephate and folpet using the PSA, but no significant losses for the DEA columns, when the standard solution wad added to the extracts just prior to the SPE clean-up.

Co-extractives that accumulate in the injector and at the beginning of the column may change the retention time of certain analytes toward longer retention times. The influence of the DEA clean-up is also clearly visible from SIM profiles obtained for a matrix-matched standard of carrots with and without DEA clean-up and from the peak shape and retention time of thiabendazole (Figs. 2 and 3). Many major interference peaks were either substantially reduced in ion intensity or eliminated altogether. The major interference peaks are saturated and unsaturated fatty acids which were effectively removed. Some more difficult matrices still show some extraneous peaks even after sample clean-up (terpenes in oranges, fatty acid esters and benzopyranones in carrots, sulphur compounds in onions, etc.), but most of them does not compromise the detection and quantitation of analytes.

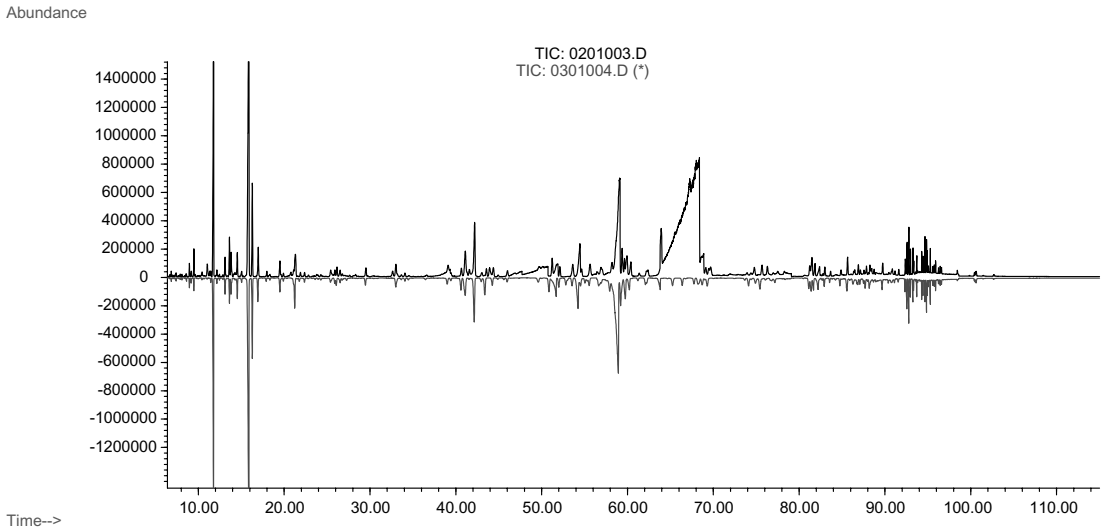

Fig. 2. GC-MS (SIM) chromatograms of carrot extract without (upper) and with DEA clean-up (lower) at 0.20 mg/kg level (interference peak in the middle of the chromatogram not removed by DEA is methyl ester of octadecadienoic acid).

3.4. Recovery study

Satisfactory recoveries (>70%), with the great majority above 80%, were obtained from the three commodities spiked in triplicate at 0.02, 0.20 and 0.50 mg/kg, as shown by the data in Table 2. The precision determined under repeatability conditions was good, with the vast majority of relative standard deviations (R.S.D.s) below 10%, except for dicofol, where the higher values mainly reflect the various degrees of degradation of the compound during sample preparation and/or GC analysis.

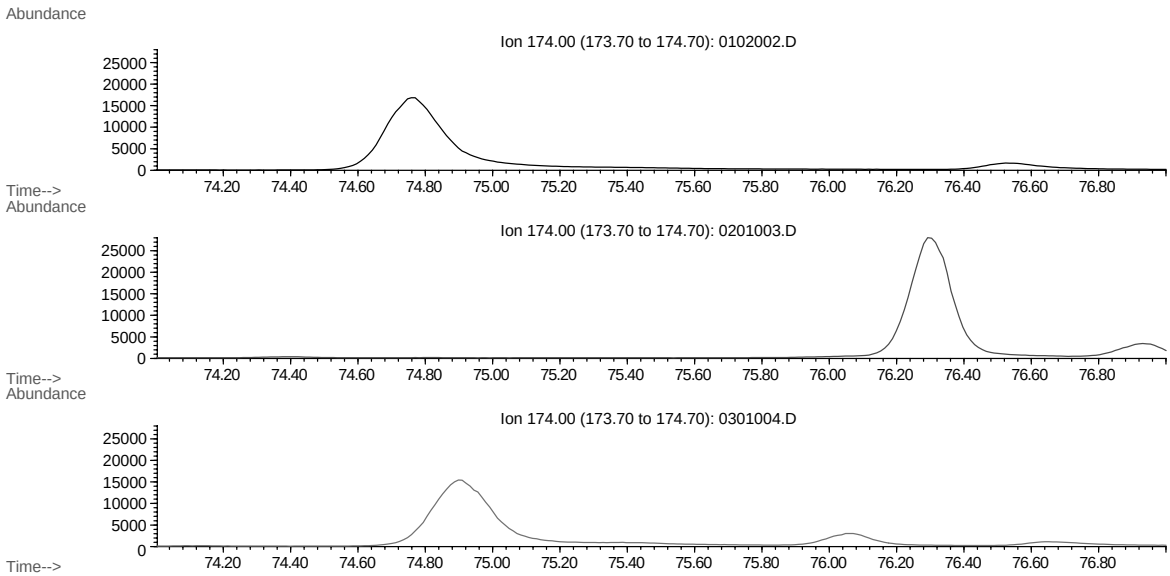

Fig. 3. Retention time shift for thiabendazol in carrot extract (from top to bottom: standard solution in acetone at 0.20 mg/kg level, matrix-matched standard solution at the same level without clean-up and after clean-up with DEA).

Table 2
Average recoveries and relative standard deviations (R.S.D.s, %) from three representative commodities (apples, green beans and oranges) fortified at (0.01, only apples), 0.02, 0.20, 0.50 mg/kg levels

Pesticide	Apples ($n = 5$)				Green beans ($n = 3$)			Oranges ($n = 3$)		
	0.01 (mg/kg)	0.02 (mg/kg)	0.20 (mg/kg)	0.50 (mg/kg)	0.02 (mg/kg)	0.20 (mg/kg)	0.50 (mg/kg)	0.02 (mg/kg)	0.20 (mg/kg)	0.50 (mg/kg)
Dichlorvos	83 ± 2	80 ± 6	78 ± 5	78 ± 5	72 ± 8	78 ± 7	74 ± 9	79 ± 5	75 ± 2	76 ± 1
Biphenyl	79 ± 3	81 ± 3	76 ± 6	74 ± 6	73 ± 3	81 ± 5	75 ± 4	71 ± 3	71 ± 5	68 ± 4
Dichlobenil	82 ± 1	82 ± 3	80 ± 4	81 ± 4	75 ± 3	84 ± 4	78 ± 5	76 ± 3	80 ± 3	77 ± 2
Propham	96 ± 2	87 ± 3	87 ± 3	88 ± 3	83 ± 4	87 ± 3	84 ± 2	86 ± 3	92 ± 5	89 ± 4
Mevinphos	93 ± 3	88 ± 6	87 ± 4	88 ± 4	82 ± 7	86 ± 5	84 ± 6	90 ± 4	91 ± 8	88 ± 4
o-Phenylphenol	98 ± 3	95 ± 4	88 ± 3	88 ± 3	85 ± 5	87 ± 3	88 ± 4	87 ± 6	96 ± 5	94 ± 3
Trifluralin	87 ± 4	86 ± 5	84 ± 3	87 ± 4	84 ± 2	83 ± 3	85 ± 3	85 ± 3	94 ± 6	89 ± 1
Tecnazene	84 ± 6	84 ± 2	83 ± 2	83 ± 2	80 ± 2	79 ± 5	81 ± 4	80 ± 3	86 ± 1	84 ± 1
Heptenophos	92 ± 4	91 ± 2	89 ± 3	89 ± 3	83 ± 3	86 ± 4	84 ± 2	72 ± 9	91 ± 6	92 ± 1
Chlorprofam	97 ± 3	94 ± 2	91 ± 3	90 ± 3	86 ± 2	83 ± 5	88 ± 3	95 ± 8	94 ± 2	92 ± 1
Phorate	71 ± 3	63 ± 3	73 ± 3	78 ± 5	61 ± 8	67 ± 9	73 ± 6	77 ± 3	84 ± 9	82 ± 7
Propizamide	95 ± 6	95 ± 2	92 ± 3	90 ± 4	90 ± 5	81 ± 4	90 ± 3	92 ± 1	101 ± 5	100 ± 3
Quintozene	84 ± 6	87 ± 1	85 ± 2	83 ± 3	84 ± 3	75 ± 6	83 ± 4	82 ± 4	90 ± 5	89 ± 5
Atrazine	96 ± 2	96 ± 2	94 ± 3	92 ± 3	82 ± 5	84 ± 6	90 ± 5	86 ± 2	106 ± 7	99 ± 2
Lindane	92 ± 6	93 ± 3	90 ± 2	89 ± 4	83 ± 6	80 ± 4	85 ± 4	78 ± 9	95 ± 7	96 ± 5
Propetamphos	92 ± 6	92 ± 3	92 ± 7	90 ± 5	86 ± 5	79 ± 4	90 ± 2	94 ± 8	98 ± 2	99 ± 5
Fonofos	90 ± 4	89 ± 2	88 ± 3	88 ± 3	81 ± 4	83 ± 3	84 ± 2	89 ± 2	95 ± 6	94 ± 4
Diazinon	92 ± 4	93 ± 4	89 ± 3	91 ± 3	88 ± 2	87 ± 3	88 ± 4	91 ± 5	96 ± 4	96 ± 4
Pyrimethanil	95 ± 3	96 ± 2	92 ± 3	91 ± 5	87 ± 6	85 ± 5	92 ± 4	94 ± 3	100 ± 5	98 ± 1
Dimethoate	105 ± 3	98 ± 4	93 ± 4	90 ± 4	92 ± 7	80 ± 8	89 ± 3	104 ± 5	101 ± 5	90 ± 7
Carbofuran	98 ± 5	95 ± 3	92 ± 2	91 ± 4	89 ± 4	82 ± 5	90 ± 4	97 ± 3	104 ± 9	101 ± 4
Paraoxon-methyl	110 ± 6	100 ± 5	91 ± 4	84 ± 3	92 ± 6	75 ± 6	85 ± 5	108 ± 8	106 ± 7	69 ± 9
Vinclozolin	99 ± 11	96 ± 6	92 ± 3	89 ± 4	85 ± 5	84 ± 5	88 ± 4	89 ± 3	96 ± 4	98 ± 2
Pirimicarb	97 ± 4	95 ± 2	93 ± 2	91 ± 3	87 ± 1	87 ± 3	90 ± 2	92 ± 7	100 ± 7	101 ± 1
Chlorpyrifos-methyl	92 ± 4	90 ± 2	88 ± 3	87 ± 4	82 ± 2	81 ± 4	85 ± 4	91 ± 4	92 ± 8	96 ± 5
Fenchlorphos	89 ± 5	90 ± 2	87 ± 3	87 ± 4	83 ± 4	81 ± 3	84 ± 4	88 ± 6	90 ± 7	95 ± 1
Phosphamidon	105 ± 5	98 ± 4	95 ± 3	90 ± 4	91 ± 6	83 ± 5	92 ± 5	104 ± 9	108 ± 11	92 ± 9
Parathion-methyl	98 ± 5	92 ± 4	88 ± 4	87 ± 5	87 ± 5	75 ± 6	85 ± 5	95 ± 12	98 ± 11	95 ± 8
Tolclophos-methyl	94 ± 4	91 ± 2	90 ± 3	89 ± 4	86 ± 2	83 ± 4	87 ± 3	89 ± 7	97 ± 9	97 ± 2
Prometryn	97 ± 5	94 ± 3	94 ± 4	91 ± 5	88 ± 9	83 ± 7	93 ± 6	95 ± 13	99 ± 6	99 ± 2
Malaoxon	111 ± 3	103 ± 5	93 ± 4	88 ± 4	95 ± 8	82 ± 6	90 ± 7	130 ± 9	114 ± 7	84 ± 5
Metalaxyl	91 ± 3	101 ± 1	95 ± 3	92 ± 5	91 ± 4	86 ± 3	93 ± 5	91 ± 1	100 ± 4	100 ± 2
Paraoxon-ethyl	108 ± 11	95 ± 6	90 ± 5	89 ± 5	93 ± 6	82 ± 5	90 ± 5	105 ± 8	105 ± 9	97 ± 11
Pirimiphos-methyl	94 ± 3	94 ± 3	91 ± 3	90 ± 4	86 ± 4	85 ± 4	89 ± 5	93 ± 6	98 ± 5	99 ± 2
Metolachlor	98 ± 4	94 ± 2	92 ± 3	91 ± 4	86 ± 3	86 ± 5	91 ± 4	98 ± 9	100 ± 7	104 ± 4
Carbaryl	103 ± 3	98 ± 2	94 ± 3	90 ± 5	95 ± 7	82 ± 8	91 ± 6	99 ± 6	104 ± 9	103 ± 4
Triadimefon	105 ± 5	95 ± 3	94 ± 4	93 ± 4	87 ± 6	85 ± 5	92 ± 6	94 ± 3	99 ± 4	101 ± 2
Fenitrothion	96 ± 7	94 ± 4	89 ± 4	88 ± 5	88 ± 2	79 ± 3	88 ± 4	100 ± 6	101 ± 7	96 ± 3
Chlorpyrifos-ethyl	100 ± 7	93 ± 2	89 ± 2	89 ± 4	92 ± 2	82 ± 4	89 ± 3	96 ± 8	97 ± 6	99 ± 4
Dichlofluanid	93 ± 7	94 ± 5	90 ± 5	91 ± 3	89 ± 11	81 ± 9	88 ± 7	103 ± 12	92 ± 9	101 ± 7
Parathion-ethyl	100 ± 9	93 ± 5	89 ± 3	89 ± 4	90 ± 3	76 ± 4	89 ± 4	100 ± 3	101 ± 7	99 ± 2
Malathion	99 ± 6	94 ± 2	91 ± 4	91 ± 4	92 ± 4	81 ± 5	90 ± 4	95 ± 8	98 ± 7	96 ± 4
Bromophos-methyl	92 ± 6	90 ± 3	88 ± 3	87 ± 5	86 ± 2	77 ± 3	85 ± 3	89 ± 7	94 ± 9	94 ± 5
Fenthion	82 ± 2	78 ± 1	83 ± 3	84 ± 4	71 ± 3	74 ± 1	82 ± 2	87 ± 8	93 ± 9	92 ± 4
Cyprodinil	96 ± 5	96 ± 2	95 ± 3	92 ± 5	90 ± 4	87 ± 4	92 ± 3	103 ± 6	90 ± 5	94 ± 6
Chlorfenvinfos	100 ± 6	96 ± 2	94 ± 3	91 ± 5	95 ± 5	88 ± 3	95 ± 4	95 ± 3	98 ± 3	100 ± 1
Penconazole	100 ± 6	98 ± 2	96 ± 3	93 ± 5	88 ± 3	84 ± 2	93 ± 4	92 ± 7	101 ± 7	101 ± 2
Endosulfan I	114 ± 10	102 ± 4	108 ± 6	70 ± 8	91 ± 5	85 ± 4	90 ± 6	84 ± 8	94 ± 10	96 ± 6
Tolylfluanid	101 ± 6	96 ± 4	91 ± 4	90 ± 4	90 ± 12	80 ± 8	88 ± 9	112 ± 12	93 ± 9	100 ± 8

Table 2 (*Continued*)

Pesticide	Apples ($n = 5$)				Green beans ($n = 3$)			Oranges ($n = 3$)		
	0.01 (mg/kg)	0.02 (mg/kg)	0.20 (mg/kg)	0.50 (mg/kg)	0.02 (mg/kg)	0.20 (mg/kg)	0.50 (mg/kg)	0.02 (mg/kg)	0.20 (mg/kg)	0.50 (mg/kg)
Procymidone	101 ± 4	96 ± 2	94 ± 3	91 ± 5	89 ± 3	85 ± 1	91 ± 4	96 ± 6	97 ± 5	99 ± 8
Quinalphos	99 ± 4	95 ± 3	93 ± 3	90 ± 4	94 ± 2	83 ± 2	92 ± 4	95 ± 3	99 ± 8	105 ± 2
Mecarbam	110 ± 7	99 ± 3	92 ± 4	91 ± 4	91 ± 4	83 ± 5	91 ± 4	100 ± 8	100 ± 6	102 ± 3
Captan	106 ± 9	94 ± 3	91 ± 4	81 ± 5	93 ± 14	68 ± 11	82 ± 9	93 ± 8	107 ± 8	95 ± 10
Thiabendazol	82 ± 9	99 ± 5	98 ± 8	96 ± 4	83 ± 9	79 ± 7	92 ± 8	99 ± 15	87 ± 6	104 ± 10
Folpet	101 ± 10	95 ± 7	86 ± 5	74 ± 8	91 ± 12	64 ± 8	81 ± 9	107 ± 9	104 ± 12	101 ± 10
Tetrachlorvinphos	106 ± 5	99 ± 3	94 ± 3	88 ± 5	93 ± 4	81 ± 4	91 ± 3	103 ± 4	104 ± 8	95 ± 5
Methidathion	103 ± 4	98 ± 2	94 ± 4	90 ± 5	93 ± 3	80 ± 4	89 ± 5	103 ± 9	105 ± 10	94 ± 5
Imazalil	107 ± 15	93 ± 7	98 ± 9	96 ± 6	74 ± 8	66 ± 6	79 ± 7	104 ± 10	90 ± 3	98 ± 5
Myclobutanil	102 ± 8	98 ± 4	97 ± 4	95 ± 5	93 ± 2	84 ± 1	91 ± 4	95 ± 4	95 ± 4	102 ± 8
Endosulfan II	97 ± 4	94 ± 2	95 ± 3	91 ± 5	97 ± 4	81 ± 5	90 ± 5	98 ± 7	97 ± 2	102 ± 2
o,p′-DDT	90 ± 6	92 ± 5	91 ± 3	87 ± 5	86 ± 6	76 ± 4	85 ± 5	89 ± 12	91 ± 10	85 ± 11
Fludioxonil	101 ± 7	97 ± 3	97 ± 5	91 ± 5	98 ± 4	82 ± 3	91 ± 5	98 ± 4	92 ± 3	97 ± 2
Krezoxim-methyl	101 ± 6	96 ± 4	95 ± 3	91 ± 5	93 ± 3	81 ± 2	93 ± 4	91 ± 8	95 ± 4	101 ± 2
Ethion	101 ± 8	95 ± 4	94 ± 3	89 ± 4	92 ± 2	80 ± 1	90 ± 3	94 ± 7	98 ± 9	99 ± 5
p,p′-DDT	92 ± 6	94 ± 6	90 ± 4	87 ± 5	89 ± 7	75 ± 5	84 ± 6	88 ± 10	91 ± 11	85 ± 9
Propiconazole	110 ± 3	100 ± 3	96 ± 4	95 ± 6	98 ± 4	81 ± 3	91 ± 4	101 ± 3	94 ± 3	101 ± 7
Endosulfan sulphate	101 ± 7	98 ± 4	96 ± 4	92 ± 6	94 ± 4	84 ±5	89 ± 6	100 ± 3	98 ± 10	99 ± 6
Tebuconazole	104 ± 7	97 ± 4	97 ± 6	96 ± 6	87 ± 4	84 ± 5	88 ± 2	98 ± 2	93 ± 1	102 ± 3
Propargite	99 ± 6	96 ± 2	97 ± 2	95 ± 4	91 ± 5	95 ± 4	92 ± 3	89 ± 3	100 ± 3	97 ± 5
Triazophos	109 ± 6	97 ± 2	95 ± 5	93 ± 5	94 ± 3	85 ± 2	91 ± 3	92 ± 5	97 ± 4	99 ± 2
Bromopropylate	99 ± 7	95 ± 3	96 ± 4	91 ± 6	96 ± 3	86 ± 2	90 ± 3	102 ± 5	93 ± 5	99 ± 1
Iprodione	100 ± 6	96 ± 1	96 ± 6	94 ± 5	96 ± 7	90 ± 6	89 ± 5	105 ± 9	93 ± 6	102 ± 2
p,p′-Dicofol	102 ± 8	108 ± 6	95 ± 5	91 ± 3	105 ± 14	61 ± 11	81 ± 10	147 ± 12	78 ± 11	80 ± 5
Pyridafenthion	100 ± 7	95 ± 2	96 ± 5	93 ± 5	97 ± 5	87 ± 4	89 ± 6	102 ± 3	97 ± 4	101 ± 3
λ-Cyhalothrin	86 ± 5	82 ± 7	79 ± 8	89 ± 7	77 ± 8	71 ± 6	74 ± 8	87 ± 10	72 ± 9	72 ± 7
Phosmet	101 ± 3	97 ± 2	97 ± 4	97 ± 6	100 ± 4	90 ± 3	88 ± 5	102 ± 8	100 ± 6	101 ± 6
Tetradifon	97 ± 6	93 ± 2	95 ± 5	92 ± 6	96 ± 3	89 ± 2	89 ± 1	100 ± 8	91 ± 8	101 ± 8
Phosalone	100 ± 7	94 ± 2	96 ± 5	94 ± 5	97 ± 2	89 ± 1	90 ± 2	100 ± 6	72 ± 11	97 ± 5
Pyrazophos	101 ± 7	95 ± 1	97 ± 6	96 ± 4	100 ± 1	91 ± 2	91 ± 3	103 ± 5	99 ± 7	99 ± 4
Fenarimol	92 ± 8	95 ± 2	99 ± 6	97 ± 5	97 ± 2	89 ± 3	91 ± 4	96 ± 6	92 ± 2	104 ± 3
Bitertanol	99 ± 3	97 ± 3	98 ± 6	98 ± 5	91 ± 5	90 ± 6	86 ± 7	97 ± 3	93 ± 2	105 ± 3
Permethrin	86 ± 6	82 ± 6	83 ± 9	87 ± 4	82 ± 9	76 ± 5	76 ± 4	82 ± 10	78 ± 5	71 ± 5
Azinphos-methyl	100 ± 8	94 ± 5	95 ± 3	97 ± 8	101 ± 6	88 ± 4	88 ± 4	62 ± 19	37 ± 10	29 ± 19
Azinphos-ethyl	99 ± 7	93 ± 2	97 ± 4	96 ± 4	101 ± 8	91 ± 6	90 ± 5	85 ± 7	121 ± 10	104 ± 6
Cyfluthrin	97 ± 7	81 ± 3	89 ± 8	92 ± 4	93 ± 7	80 ± 7	82 ± 6	100 ± 12	95 ± 12	82 ± 10
Coumaphos	95 ± 8	94 ± 2	96 ± 6	93 ± 4	102 ± 2	90 ± 3	89 ± 4	98 ± 4	92 ± 2	101 ± 1
Cypermethrin	106 ± 5	84 ± 5	90 ± 8	90 ± 4	95 ± 6	80 ± 5	80 ± 3	97 ± 9	84 ± 7	82 ± 8
Deltamethrin	88 ± 10	83 ± 7	78 ± 10	89 ± 6	90 ± 8	72 ± 6	71 ± 7	110 ± 11	87 ± 10	91 ± 8
Difenconazole	99 ± 8	98 ± 4	96 ± 8	94 ± 7	111 ± 5	90 ± 6	89 ± 3	107 ± 5	99 ± 3	112 ± 2
Malathion-D_6	98 ± 4	91 ± 3	92 ± 5	90 ± 4	91 ± 6	81 ± 5	89 ± 4	115 ± 4	97 ± 7	95 ± 3

In order to lower the lowest calibrated levels (LCLs) [36] because of more stringent EU regulations for baby food the blank apple sample was also fortified at 0.01 mg/kg concentration level. The average recoveries were in most cases above 80% and are also reported in Table 2.

Limit of quantitation as the lowest validated spike level with acceptable precision and sensitivity for all ions chosen for quantitation and identification was 0.01 mg/kg for all pesticides, except for captan, dicofol, malaoxon, paraoxon-methyl and paraoxon-ethyl whose LOQ was 0.02 mg/kg.

3.5. Quantitation

A three-level calibration using matrix-matched standards together with bracketing calibration [36] and internal standardisation with pentaclorobenzene as the internal standard was used for quantitation. Because increasing the number of ions monitored decreased the sensitivity of the assay, the run time was prolonged in order to achieve enough sensitivity for a high number of pesticides. The emphasis was therefore on separation rather than fast analysis, thus making the identification easier because of the less cross-reactions between co-eluted pesticides and matrix components.

Residue data were not adjusted for recovery of surrogate standard (malathion-D_6) according to the guidelines and its recovery served only to assure that the performance of the method for each sample was within acceptable limits for routine analysis (60–140%) [36].

For positive identification both retention time and the presence of all three ions (Table 1) in the correct ratio was necessary [36]. Where no interference was observed, the relative SIM responses of each of the ions monitored for the analyte should correspond to those obtained from a standard (within ±20%). At the same time GC amenable degradation products of carbofuran (*m*/*z* 164, 149), captan (*m*/*z* 79, 151), folpet (*m*/*z* 147, 76), dichlofluanid (*m*/*z* 200, 92), dicofol (*m*/*z* 250, 139) and iprodion (*m*/*z* 187, 244) were monitored to help with the identification. Their presence was taken as indicative of parent compounds in the case where a partial or complete degradation occurred during the sample preparation or GC analysis.

Uncertainty of measurement was estimated using data obtained from method validation and quality assurance procedures taking into account the within-laboratory reproducibility, homogeneity and method bias from recovery which is usually significant but not corrected for [37,38]. Combined uncertainties calculated using coverage factor 2 were between 29 and 112% (higher values correspond to captan, folpet and DDT) with great majority between 30 and 60%.

4. Conclusions

Acetone was used for extraction of 90 pesticides of different physico-chemical properties from fruits and vegetables. Solid-phase extraction on a PS-DVB column was used for simultaneous isolation of the investigated pesticides and clean-up of the water-diluted acetone extract. Advantages of this SPE method include simultaneous pre-concentration of many pesticides, partial sample clean-up and water removal (the use of drying agents is not necessary, except for a small amount on top of the DEA sorbent), its applicability to various fruits and vegetables and the use of only small volumes of solvent per sample (30 ml acetone and 14 ml ethyl acetate, 6 ml methanol). The additional clean-up on DEA columns does not significantly influence the recoveries of pesticides and improves chromatographic performance by minimising matrix effects. Using MSD quantification (through selective ion monitoring) and confirmation are achieved simultaneously.

The main drawback of this method is its inability to detect very polar pesticides ($\log K_{ow} < 0$). Acephate, methamidophos, omethoate and monocrotophos were not recovered and probably remained in the water during SPE column loading, because these are highly polar water-soluble compounds, which do not adsorb onto RP SPE materials in the presence of organic solvents.

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