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Multi-Residue Determination of Pesticides in Vegetables by Gas Chromatography/Ion Trap Mass Spectrometry

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Abstract To monitor possible contamination of edible vegetables by common pesticides, an analytical method using gas chromatography combined with ion trap spectrometry (GC-IT/MS) was developed to measure simultaneously up to 39 pesticide residues, belonging to organophosphors, organochlorines, pyrethroids or carbamates classes, left on four kinds of popular vegetables. The procedure entails addition of acetone, dichloromethane, and sodium chloride to a small amount of vegetable, then the mixture was shaken intensively and centrifuged for phase separation. An aliquot of the organic layer was cleanup using solid-phase extraction (SPE) cartridges filled with graphitized carbon black (GCB) in combination with acidic aluminum oxide. Gas chromatography with ion trap mass spectrometer was then used for qualitative and quantitative determination of the pesticides. The GCB combination with acidic aluminum oxide was found more suitable than florisil, aluminum oxide and silicon dioxide for sample cleanup with recoveries above 70% for most pesticides in removing the majority of co-extracted matrices. Variation coefficients of the repeatability typically smaller than 20% have been achieved for a wide range of the investigated pesticides. A set of critical instrument parameters for the GC-IT/MS Varian system in the MS mode was established. Based on optimization work conducted in this study, the 39 pesticides were separated successively with the limits of detection between 0.02 and 0.1 mg/kg.

Keywords Multi-residue pesticide analysis · Vegetables · Gas chromatography with ion trap spectrometry (GC-IT/MS)

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Great productivity gains can be achieved in agriculture, by using the adequate pesticides. Indeed, they are needed to meet the world's demand on foodstuffs and no other alternative can compete to be used in such a large scale. As a result, consumers are exposed to pesticides, usually in minute quantities, in several food groups including crops, fruits and vegetables and so on. The preservation of human health from exposure to pesticide residues in foodstuffs remains a major objective in China.

Pesticides constitute a very important group of chemical compounds that have to be controlled due to their high toxicity and their widespread use in agricultural practice for field and post-harvest protection. As a consequence, governments and international organization have established maximum residue limits (MRLs) in food to ensure that they are not present at levels that may pose a health risk to the public (Albero et al. 2005).

Developing faster, more cost-effective and environment-friendly procedures of multi-residue analytical methods for simultaneous determination them is very important to monitor the pesticide residues in food. Classic method for extraction and purifying pesticide residues in fruits and vegetable is often a time-consuming, labour-intensive, and expensive process due to the complexity of the many analytes and matrices involved. The procedures include liquid–liquid extraction (LLE), solid-phase extraction (SPE), accelerated solvent extraction (ASE), gel permeation chromatography (GPC), microwave-assisted extraction (MAE), matrix solid-phase dispersion (MSPE) and supercritical fluid extraction (SFE) (Fenoll et al. 2007). A large variety of chromatographic methods have been used for the final determination of the pesticide residues in food. Gas chromatography with different selective detectors as electron-capture (ECD), nitrogen-phosphorus (NPD), and flame photometry (FPD) was used. Liquid chromatograph (LC) with diode-array and fluorescence detection was effective in thermally labile and nonvolatile compounds determination (Albero et al. 2004). But in combination with mass spectral detection chromatographic methods are still the first choice for many applications, by reason of their flexibility, selectivity, wide analytical scope, qualitative and quantitative utility and sensitivity (Fillion et al. 1995, 2000; Lee et al. 1991; Liao et al. 1991).

To our knowledge, little reference is available on the multi-residue analysis using GC-IT/MS concerning 39 pesticides in our selected vegetables. The principal aim of this work was to develop a rapid-multi-residue method for the simultaneous analysis of 39 pesticides in four kinds of vegetables (cucumber, cabbage, cole and capsicum) most widely consumed in China. The paper describes an extraction, using a low volume of organic solvent and SPE cleanup procedure as an alterative to liquid–liquid partition. The residues in selected four kinds of vegetables were final determined and confirmed by GC-IT/MS. GC with IT/MS detection provides high confidence in identification of target analytes, based on a selected parent ion and a whole mass spectrum of its daughter ions, high sensitivity and selectivity, as well as low cost and easy in operation and maintenance. These characteristics have made GC-MS analysis by ion-trap system a very competitive and widely used technique.

Materials and Methods

Pesticides standards were obtained from Ministry of Agricultural of the People's Republic of China, and all compounds were of 99% purity. Table 1 lists the 39 pesticides. Acetone, dichloromethane, methanol and *n*-hexane, of residue analysis grade, were purchased from Dikma Limited (China). Anhydrous sodium sulfate and sodium chloride in power form and analytical grade were also obtained from Dikma Limited (China). The solid-phase extraction sorbents as florisil, graphitized carbon black (GCB), acidic aluminum oxide, and silicon dioxide in power form with a particle size of 40 μm were obtained from Dikma Limited (China). The selected vegetables were

Table 1 Chromatographic and mass spectral data obtained by the GC-MS methods of all tested pesticides as well as selected ions for quantification and qualification

Pesticide	M_R	Retention time (min)	Target ion (m/z)	Qualifier ion (m/z)
α-BHC	288	21.662	219	109, 183, 219
β-BHC	288	22.462	219	109, 183, 219
γ-BHC	288	23.452	219	109, 183, 219
δ-BHC	288	23.823	219	109, 183, 219
O,P′-DDT	352	34.913	235	165, 199, 235, 352
P,P′-DDT	352	35.704	235	165, 199, 235, 352
P,P′-DDE	316	33.745	318	176, 206, 318
P,P′-DDD	318	34.672	235	165, 199, 235
α-Endosulfan	404	33.243	339	205, 267, 339
β-Endosulfan	404	34.513	339	165, 199, 235
Dichlorvos	220	10.453	109	109, 185, 221
Methamidophos	141	9.904	94	94, 141, 142
Omethoate	213	17.851	156	110, 156, 214
Monocrotophos	223	20.034	127	67, 127, 192
Phorate	260	21.323	260	121, 231, 260
Dimethoate	229	21.792	230	87, 212, 230
Phosphamidon	299	26.268	264	127, 264, 300
Diazinon	304	24.333	304	137, 179, 304
Parathion-methyl	263	26.973	263	247, 263
Fenitrothion	277	28.604	277	125, 260, 277
Fenthion	278	29.803	278	125, 278
Parathion	291	30.013	291	109, 275, 291
Chlorpyrifos	349	30.083	314	286, 314, 352
Phosfolan	255	30.953	168	92, 168, 255
Isofenfos-methyl	317	34.484	199	121, 199, 231, 273
Methidathion	302	32.522	145	85, 125, 145
Profenofos	372	33.482	339	297, 339, 375
Triasophos	313	34.742	257	161, 257, 314
Optunal	288	30.174	136	136, 231, 289
Methomyl	162	7.641	105	88, 105, 106
Isoprocarb	193	16.854	121	121, 136, 194
Propuxur	207	18.704	110	110, 152, 210
BPMC	209	18.782	121	121, 150, 208
Carbofuran	221	22.092	164	164, 222
Pirimicarb	238	25.603	166	166, 239
3-OH carbofuran	237	26.393	180	137, 180, 220
β-Cypermethrin	416	42.443	163	91, 163, 181, 208
Fenvalerate	419	44.692/45.414	125	125, 167, 225
Deltamethrin	502	47.514	253	172, 181, 253

Table 2 Mean recoveries (%) at three fortified levels and LODs for pesticides determined by the multi-residue method

Compound	Cucumber	CV (%)	Cabbage	CV (%)	Cole	CV (%)	Capsicum	CV (%)	LOD (mg/kg)
α-BHC	91.5	3.61	77.4	4.49	99.9	13.3	80.4	9.6	0.05
β-BHC	82.1	6.16	85.4	19.88	93.9	4.3	76.6	7.9	0.03
γ-BHC	91.0	6.66	81.5	3.31	89.1	5.1	79.9	5.1	0.03
δ-BHC	92.7	7.74	84.9	12.77	91.9	14.4	73.3	4.5	0.03
O,P′-DDT	96.8	7.5	88.5	19.09	120.8	4.4	75.7	19.2	0.03
P,P′-DDT	109.0	8.52	84.7	13.74	125.7	5.1	69.2	18.5	0.03
P,P′-DDE	91.9	5.18	94.0	13.17	118.5	18.9	82.8	4.3	0.04
P,P′-DDD	80.5	3.34	87.5	10.39	114.6	5.7	83.1	7.3	0.04
α-Endosulfan	82.0	0.19	89.4	7.89	112.8	16.2	70.0	7.9	0.03
β-Endosulfan	93.7	9.93	80.4	10.02	82.5	9.3	78.6	14.5	0.05
Dichlorvos	95.1	12.54	85.0	17.13	107.5	4.5	65.0	5.6	0.05
Methamidophos	68.4	13.19	123.3	6.61	127.4	6.5	91.8	9.9	0.08
Omethoate	66.5	10.72	124.3	12.32	136.1	7.0	83.5	8.5	0.1
Monocrotophos	75.3	11.93	125.7	9.34	126.4	12.3	73.3	8.0	0.1
Phorate	96.0	4.57	58.6	10.89	81.0	7.9	75.9	12.0	0.04
Dimethoate	88.1	11.41	95.1	14.72	93.8	14.9	93.8	14.6	0.08
Phosphamidon	86.0	6.45	98.6	2.31	124.7	11.3	92.3	10.0	0.1
Diazinon	93.0	4.95	78.7	2.61	115.1	7.9	83.4	12.5	0.03
Parathion-methyl	69.9	6.81	74.5	7.70	91.4	5.6	64.3	12.3	0.05
Fenitrothion	76.8	7.24	81.7	3.50	96.2	9.9	73.7	9.5	0.04
Fenthion	78.7	8.12	82.2	8.86	105.3	10.	77.9	9.6	0.04
Parathion	84.0	12.86	83.4	4.79	107.1	16.5	77.9	4.9	0.05
Chlorpyrifos	74.2	9.60	76.7	5.05	94.0	37	67.0	8.9	0.03
Phosfolan	64.0	4.46	120.1	10.83	120.4	7.1	85.2	3.3	0.1
Isofenfos-methyl	86.2	6.34	80.6	10.30	105.1	9.3	92.0	9.0	0.02
Methidathion	84.3	14.07	86.4	19.34	110.4	5.5	72.8	5.7	0.05
Profenofos	80.5	14.28	74.5	13.91	89.1	8.4	72.5	9.9	0.05
Triasophos	28.2	16.15	39.8	18.35	34.2	14.1	41.3	7.8	0.1
Optuanl	86.5	14.51	84.2	4.18	100.5	8.1	79.1	6.3	0.05
Methomyl	94.1	5.87	111.1	11.51	109.1	7.7	114.7	9.6	0.05
Isoprocarb	93.2	5.80	79.9	11.02	112.9	9.0	83.5	4.6	0.03
Propuxur	96.3	3.44	88.8	8.90	123.2	10.2	89.1	3.9	0.02
BPMC	91.3	3.31	70.0	12.09	106.5	6.1	91.6	5.8	0.02
Carbofuran	85.2	5.26	87.8	11.51	120.4	6.8	80.7	6.2	0.05
Pirimicarb	90.2	5.99	84.9	6.60	116.8	4.5	83.4	10.8	0.02
3-OH carbofuran	80.4	6.81	103.3	2.02	124.6	11.7	100.3	3.9	0.1
β-Cypermethrin	91.8	15.46	98.1	19.55	95.6	5.8	87.9	15.0	0.1
Fenvalerate	94.9	13.02	81.9	18.40	100.2	15.0	92.6	12.9	0.1
Deltamethrin	81.3	12.25	76.5	6.11	85.9	4.7	77.4	8.5	0.1

cucumber, cabbage, cole and capsicum, which were purchased from supermarket in Beijing.

A Varian ion trap Saturn 2100 with MS*n*-option and multi CI-option was coupled with a Varian 3800 gas chromatograph. DB-5 ms capillary column of 30 m, 0.25 mm I.D. and 0.25 μm film thickness was employed through the studies. MS transfer line temperature 280°C, ion trap temperature 200°C. Carrier gas (He) at the constant flow rate of 1 mL min^{-1}. The temperature program of the injector was held at 80°C for 1 min, then raised to 150°C at the rate of 10°C min^{-1}, then to 220°C at 30°C min^{-1}, to 280°C at 40°C min^{-1}, with the final hold time of 10 min. The ion trap was operated in full scan mode detection ions between 50 m/z and 650 m/z.

Stock solutions (1 mg mL^{-1}) of each pesticide standard were prepared by dissolving 0.1 g of the pesticide in

100 mL methanol. Work solution was prepared from the stock solution by serial dilutions. Stock standard and working solutions were stored at 4°C.

A 50 g aliquot of chopped and homogenized sample was weighed in a 250-mL polypropylene container; sodium chloride (15 g), acetone (200 mL) and dichloromethane (150 mL) were added. The mixture was vigorously shaken for 40 min, and filtered through a 12-cm Büchner funnel; the solid residues were treated with an additional acetone (30 mL) and dichloromethane (30 mL). The filtrate was transferred into a 500-mL separatory funnel, and the phase was allowed to separate by salting out process. The lower aqueous layer was discarded. Anhydrous sodium sulfate (10 g) was added to the separatory funnel and the funnel was shaken for 30 s in order to remove the residual water. The dried extract was filtered through coarse filter paper, and evaporated at 45°C to dryness on the rotary evaporator. The residue obtained was dissolved in the mixture of acetone and ethyl acetate (5 mL, v/v, 1/1), and 2 mL for further cleanup. Extraction purification was performed employing cartridges (6 mL) filled with 500 mg different sorbents (florisil, GCB, silicon dioxide, or GCB in combination with acidic aluminum oxide). Anhydrous sodium sulfate (ca. 2 cm layer) was always added to top of the cartridges, and the cartridges were preconditioned with *n*-hexane (2 mL). The cartridges were loaded with acetone/ethyl acetate extraction (2 mL). This eluate (2 mL) was discarded. Analytes were eluted with acetone/ethyl acetate (30 mL, 1/1, v/v) and concentrated in a rotary evaporator (40°C) and completely dried under a nitrogen purge. The residues were re-constituted in ethyl acetate (1 mL) for GC IT/MS analysis.

Untreated vegetable samples (50 g) were fortified with known amounts of each pesticide at three levels (0.1, 0.5 and 2.0 mg/kg) and processed according to the above procedure. Every recovery was done on three replicates.

Results and Discussion

The described method comprised an extraction of the residual pesticides in vegetables with the mixture of acetone and dichloromethane considering the polarity of 39 pesticides and solubility ranges of the different pesticide families in organic solvent. Water and water-solubility impurity were removed from the raw extract by salting out with sodium chloride. As next step, the crude extract is clean-up by SPE cartridges. Among the solid-phase materials tested were florisil, GCB, and GCB in combination with acidic aluminum oxide, and the best results were obtained using GCB in combination with acidic aluminum oxide. Clean up on this SPE cartridge eliminated most interfering peaks and allowed good recoveries at low fortification levels.

For the identification of the target compounds, we tested the 39 pesticides based on the calibration standard at 0.1 mg/kg. The results of threefold measurement and all chromatographic and mass spectral data are shown in Table 1.

The GC-MS response for 39 pesticides was linear in the concentration assayed (0.05–2.0 mg/kg) with determination coefficients >0.996 for all pesticides tested. Quantification was based on the abundance of each ion monitored by GC-MS.

Four uncontaminated vegetable samples (cucumber, cabbage, cole and capsicum) were spiked with known amounts of each pesticide (0.1, 0.5 and 2.0 mg/kg) and processed as described. Average recovery data and variation coefficients (CV) obtained are shown in Table 2. Recoveries of >90% of the pesticides were between 70% and 110%, whereas triasophos gave very poor recoveries of <50%. The limit of detection (LOD) was determined as the lowest concentration giving a response of 3 times the average of the baseline noise from four non-fortified samples. In this study, LODs for all compounds range between 0.02 and 0.1 mg/kg.

A simple, rapid and sensitive analytical method for the simultaneous determination of 39 pesticides in four vegetables has been developed. As a result of this study the procedure has been successfully applied in our laboratory for the routine screening of vegetable samples. In addition to removing the use of chlorinated solvents and reducing the volumes of solvents required in general, the SPE procedure allowed increased sample throughput compared with liquid–liquid extraction procedures. Gas chromatography with ion trap mass spectrometer has provided a good way for qualitative and quantitative utility and sensitivity.

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