Simultaneous determination of 45 pesticides in fruit and vegetable using an improved QuEChERS method and on-line gel permeation chromatography–gas chromatography/mass spectrometer

Dasheng Lu [a], Xinlei Qiu [a], Chao Feng [a], Yu'e Jin [a], Yuanjie Lin [a], Libei Xiong [a], Yimin Wen [a], Dongli Wang [b], Guoquan Wang [a,*]

[a] Shanghai Municipal Center for Disease Control and Prevention, 1380 Zhongshan West Road, Shanghai 200336, China
[b] Contam Environmental Consulting, Albany, CA 94706, USA

ARTICLE INFO

ABSTRACT

In this study, a method was developed to determine 45 selected pesticides (of different chemical families) in fruit and vegetable (including apple, spinach and cucumber). Samples were extracted using an improved QuEChERS method with salting out and phase separation in two steps. The target pesticides in concentrated extracts were analyzed by an on-line gel permeation chromatography–gas chromatography/mass spectrometer (online-GPC–GC/MS). Online GPC effectively removed matrix interferences and greatly improved the method sensitivity, recoveries and automation. Method limits of quantification were 10 ng/g for uniconazole and metalaxyl, and 5 ng/g for other 43 target analytes. In three fruit and vegetable matrices each spiked with 45 pesticides (0.01 μg/g), mean recoveries ranged from 80 to 118% for most of the tested pesticides except for profenofos (77% in apple) and chlorpyrifos (68% in apple and 75% in cucumber), with relative standard deviations (RSDs) of less than 14%. The results of the proficiency testing showed that the method is very successful in measuring the certified pesticides with less than 1.3 of the absolute value of Z-score. This method has been applied for routinely monitoring pesticides in fresh fruit and vegetable.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

QuEChERS stands for "quick, easy, cheap, effective, rugged and safe" sample preparation methods for the analysis of multiple pesticide residues in fruit, vegetable and other types of food matrices with satisfactory results [1,2]. It was recommended to be used in international standard methods such as AOAC Official Method 2007.01 and European Committee for Standardization (CEN) Standard Method EN 15662 [3]. In contrast with P.A. Mill's method, Luke's method, German DFG-S-19, and Canadian PMRA method [4] of analyzing pesticides in fruit and vegetable and other studies of analyzing pesticides in plant tissues [5,6], the QuEChERS method has many advantages: minimal solvent consumption, faster sample preparation, and analysis of a broader scope of pesticides. However, QuEChERS method, is not an ideal method to eliminate matrix interferences, even if an extra dispersive solid phase extraction (D-SPE) step was added [1,7]. Moreover, in these conventional QuEChERS methods, samples were extracted with salting out and phase separation in one step.

Many studies have reported to use gas chromatography/tandem mass spectrometer (MS/MS) to measure pesticides in fruit and vegetable [8,9], whereas in our laboratory, only GC/MS is available, which is less selective than MS/MS, and therefore, requires exhaustive clean-up steps. Gel permeation chromatography (GPC) has the capability to remove interferences (e.g. grease, pigment, alkaloid, polymer, big molecular compounds, etc.) [10], and was used for deep sample clean-up [11] in German standard method of DFG-S-19, and Japanese standard method "Analytical Methods for Residual Compositional Substances of Agricultural Chemicals, Feed Additives, and Veterinary Drugs in Food" [12]. GPC cleanup system has been connected to a GC–MS system online, which provides a faster and more labor-saving system for screening residual pesticides in foods [13], in comparison with offline operation, which consumes more time and solvent [14].

This study aimed to improve the existing QuEChERS method for sample pretreatment, and to apply online GPC gas chromatography/mass spectrometer (online-GPC–GC/MS) to analyze 45 selected pesticides in spinach, apple and cucumber. These target pesticides were in different chemical families including organochlorine, organophosphate, synthesized pyrethroids, and carbamates. We focused on QuEChERS method modification and

* Corresponding author. Tel.: +86 021 62758710.
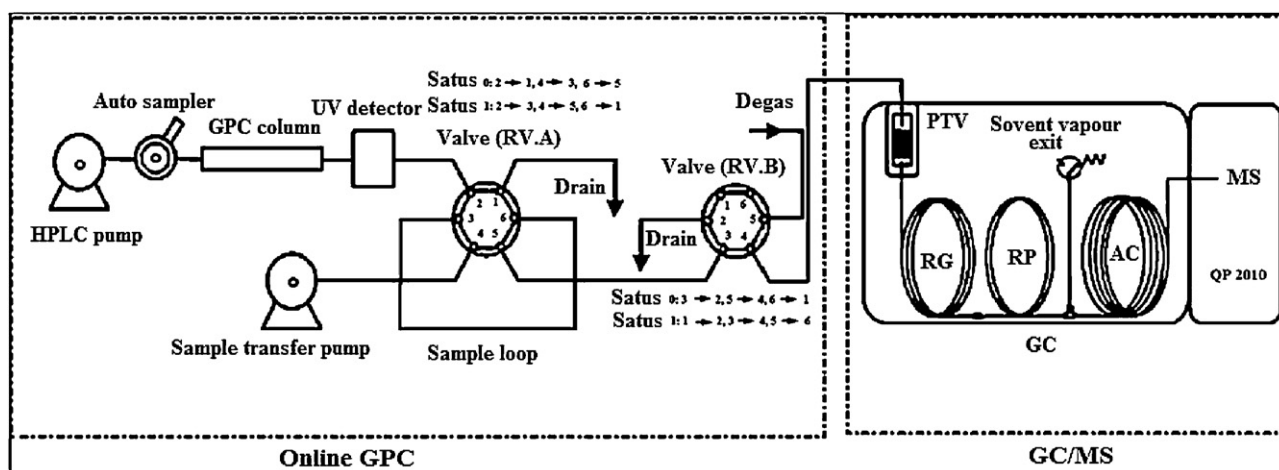E-mail address: gqwang@scdc.sh.cn (G. Wang).

Fig. 1. The schematic flow diagram of online-GPC–GC/MS system.

online GPC cleanup, and improvement of chromatography behavior and method accuracy.

2. Experimental

2.1. Chemicals and materials

All standard chemicals (purities > 95%) including an internal standard (heptachlor epoxide, purity > 98%) were purchased from ChemService Inc. (West Chester, PA, USA) and Dr. Ehrenstorfer Laboratories (Augsburg, Germany). Stock standard solutions were prepared in acetone at 5 mg/mL each. A working standard solution containing all target analytes at 1 μg/mL each was also prepared in acetone. All standard solutions were stored at −18 °C for use. Sodium chloride, sodium acetate and anhydrous magnesium sulfate (anh. MgSO$_4$) were from Chemical Reagents Co., Ltd. of Chinese Medicine and Drug Group (Shanghai, China), and were baked for 4 h at 400 °C muffle furnace before use. Acetonitrile, cyclohexane and acetone (pesticide or HPLC grade) were from Merck (Darmstadt, Germany). Standard reference materials were from Performance Assessment Scheme (FAPAS®, UK).

The D-SPE sorbents including primary secondary amine (PSA), octadecylsilyl silica (C18) and graphite carbon black (GCB, 40 μm) were from Varian Inc. (Harbor City, CA, USA). Millipore Fluoropore PTFE (F) membranes (SLFG013NL, 13-mm diameter, 0.45-pm pore size) were purchased from Millipore (Billerica, MA, USA).

The apple, spinach, and cucumber were screened, and those samples with target pesticides non-detected, were used as alternative matrices and spiked at 0.01 μg/g and 0.025 μg/g to determine method recoveries. A set of six calibration standard solutions was also prepared in extraction solutions of alternative matrices with analytes at levels of 5–50 ng/mL and the internal standard at 10 ng/mL. The matrix selection and preparation met the requirements of method validation and quality control procedures for pesticide residue analysis in food (Document No. SANCO/10684/2009) [15].

2.2. QuEChERS method for sample preparation

Edible parts of apple, spinach, and cucumber were washed by gently rubbing, and then homogenized in a food processor. Ten grams of homogenized samples were weighed in a 40-mL centrifuge tube. One gram of sodium acetate (NaAc) was added and extracted with 10 mL 1% acetic acid (HAc) in acetonitrile (MeCN)

through hand shaking for 1 min, and then 1 g NaCl was added and shaken for one more minute and retained for phase separation. After the MeCN phase was separated from the sample, 4 g anh. MgSO$_4$ was added, and the extraction tube was capped immediately and then shaken vigorously for 1 min. At last the sample was centrifuged at 4000 revolutions per minute (rpm) for 5 min. One milliliter of the supernatant was transferred into a 2 mL centrifuge tube containing D-SPE sorbents including 20 mg GCB, 50 mg C18, 50 mg PSA, and 125 mg anh. MgSO$_4$. After shaken for 1.0 min, the samples were centrifuged at 4000 rpm for 5 min. The supernatant was carefully transferred out and filtrated with Fluoropore PTFE (F) membrane. Five μg internal standard was added into 0.5 mL supernatant for the instrumental analysis.

2.3. Online GPC–GC/MS

The online GPC–GC/MS system was from Shimadzu (Japan) and a schematic flow diagram is shown in Fig. 1. GPC was equipped with an automatic sample injector (SIL-10ADvp), two pumps (LC-10ADvp), a Shodex CLNpak EV-200AC GPC chromatographic column (2 mm (ID) × 150 mm (L)), a column oven (CTO-10ASv), an ultraviolet detector (SPD-10 Avp), two flow channel selection valves-RV.A and RV.B (SCL-10Avp), a 200 μL sample loop and a data processor (C-R8A plus) for processing the data from the ultraviolet detector. The GPC system was coupled with Shimadzu QP-2010 GC/MS. The GC system was equipped with a large volume PTV injector (PTV-2010 injector), a retention gap (RG, 5 m × 0.53 mm (ID) deactivated capillary column), a 5 m retention pre-column (RP, Rtx-5 ms, ID 0.25 mm), a 25 m separation column (AC, 0.25 μm thickness, Restek Corporation, Bellefonte, PA, USA) and a solvent vapor exit (SVE). GC/MS data acquisition was triggered by a contact closure start signal from the HPLC controller.

Acetone and cyclohexane (3/7, v/v) were used as GPC mobile phases at a flow rate of 0.1 mL/min. The oven temperature was set at 40 °C and the injection volume was 10 μL. RV.A, RV.B and degas of GPC system were controlled by the software from the vendor (Table 1).

200 μL of pesticide portion of GPC eluent (PPGE) containing all target pesticides were totally transferred into GC PTV injector in splitless mode with 7 min sampling time, while corresponding PPGE's residues in the GPC pipeline was purged by degas (Table 1). GC system used helium as carrier gas in a constant pressure mode (120 Mpa) to maintain a total flow rate of 30 mL/min and a column flow rate of 1.75 mL/min. Injector temperature ramped from 120 °C

D. Lu et al. / J. Chromatogr. B 895–896 (2012) 17–24

19

Table 1
GPC conditions.

Steps	Time (min)	Functions	Value of status	Description
0	0.01	RV.A	0	Initialize and drain macromolecule eluents
1	0.01	RV.B	0	Initialize and drain macromolecule eluents
2	4.794	RV.A	1	Collect pesticide portion of GPC eluent (PPGE)
3	5.394	Event	1	Trigger GC/MS run
4	6.794	RV.A	0	Terminate PPGE collection
5	6.994	RV.B	1	Transfer PPGE from sample loop to GC injector
6	9.494	Degas	2	Transfer PPGE residues in pipeline using GC carrier gas
7	9.514	Degas	0	Terminate GC carrier gas for pipeline purge
8	9.714	RV.B	0	Initialize RV.B
9	10	Stop		

(hold for 4.5 min) to 250 °C (hold for 34 min) at a rate of 80 °C/min. Through the GC inlet liner, PPGE was introduced to retention gap (RG) (Fig. 1) at the temperature (82 °C in this method) below the solvent boiling point. The pesticides were distributed throughout the sample layer to form a flooded zone. The solvent started to evaporate at the rear end of the flooded zone and was released to an activated carbon absorption tube through opening solvent vapor exit (SVE). The pesticides would be trapped or spread out by a liquid layer of RG and finally refocused by both liquid layer of RG and the stationary phase of retention pre-column (RP). After the last drop of solvent was evaporated, this procedure would be terminated by SVE closure and it totally lasted for 6.5 min. At the same time, the GC oven temperature began to increase and the pesticides were transferred to the GC separation column (AC) (Fig. 1). The oven temperature ramped to 250 °C (held for 34 min) at a rate of 8 °C/min from an initial temperature of 82 °C (held for 5 min).

MS was operated in EI mode with a detector voltage of 1.05 kV and standard electron energy of 70 eV. Temperatures of ion source and transfer line were 200 °C and 250 °C, respectively. MS data were acquired in both full scan (50–450 m/z) mode for identification and selected ion monitoring (SIM) mode for quantification with 8 min of solvent delay. The GC retention time and mass spectrometric parameters in SIM mode are shown in Table 2.

2.4. Quality control and quality assurance

Before daily instrumental analysis, 10 ng/mL of calibration standard solution in acetone was analyzed to confirm acceptable chromatographic resolution and mass spectral sensitivity. This standard was also analyzed after substantive changes in the analytical system. Standard reference materials were analyzed to examine the method performance every six months. A typical sample batch included one reagent blank, six calibration standards, one method blank, 20 unknown samples, and three alternative matrices spiked with two levels of 0.01 μg/g and 0.025 μg/g each. In each batch, three unknown samples were randomly selected for duplicate analysis and less than 15% relative differences were required. The reagent blank was free of analytes and the results of QC samples were acceptable, and therefore, the batch of samples analyzed could be approved. Limits of method detection were estimated as three times the standard deviations of seven replicate analysis of a calibration standard at 5 ng/ml. Qualification of the target compounds was based on the identification of the retention time, quantification ion, and the appropriate ratio (in ±20% deviation from the theoretical value) of the qualification ions relative to the quantification ion based on the spectra of the standards. Target compounds were quantified based on the integration of the extracted ion chromatograms of the quantification ions relative to the integration of the extracted ion chromatogram of the internal standard.

3. Results and discussion

3.1. GPC optimization

Optimal GPC conditions should completely introduce the clean pesticide fraction into GC/MS system. These conditions included mobile phase preparation through mixing, filtering, degassing, sample preparation (such as filtration to remove particles and reducing fat content to less than 2.5% in the final analytical solution), stable mobile phase delivering, and highly precise column oven temperature controlling. In this study, all these GPC parameters were optimized to generate a clean fraction containing all target pesticides, and this fraction was totally delivered to the GC/MS system for precise and accurate analysis. After several times' experiments, we selected acetone and cyclohexane (3/7, v/v) as the optimal GPC mobile phases at a flow rate of 0.1 mL/min. This GPC system was equipped with a micro-column (2 mm id), which was calibrated for three times by running a calibration mixture containing two pesticides [fluvalinate (MW = 502.9) and chinomethionate (MW = 234.3)] at 5 μg/mL each before each batch running. Both pesticides were selected because the molecular weights (MW) of almost all target pesticides fall in the MW range of fluvalinate and chinomethionate. GPC calibration is very important to judge the column separation efficiency and thus to set proper collection time of PPGE. All target pesticides in PPGE were separated from interferences through the micro-column and monitored by an ultraviolet (UV) detector. Sufficient conditioning time (at least 1 h) of GPC micro-column could generate reproducible results. The peaks of calibration standards were sharp and symmetrical (Fig. 2). The retention time gap between these two markers was less than 2 min in view of the exact 2 min collection time of PPGE (Fig. 2). If the micro-column was degraded after running multiple batches of samples and such criteria could not be maintained, it was replaced by a new one. By carefully examining GPC retention times of two calibration markers (Fig. 2), step 2 and step 4 of GPC was set at 4.794 min [retention time (4.734 min) of fluvalinate plus the flow time between UV detector and RV.A (0.06 min)] and 6.794 min, respectively (the time of step 2 plus 2 min of pesticide fraction collection time) (Table 1). The retention time (6.092 min)
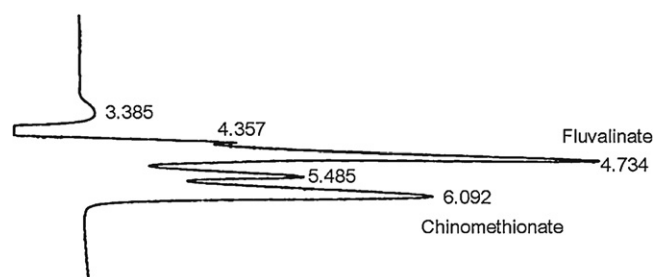
Fig. 2. GPC of two calibration markers (fluvalinate and chinomethionate at 5 μg/mL) obtained under the optimal GPC working conditions.

Table 2
GC/MS parameters, method recoveries (Rec.%) and relative standard deviations (RSD%, $n = 5$) in apple, spinach and cucumber spiked at 0.01 μg/g and 0.025 μg/g. Q, quantitation ions; C, confirmation ions; A, recoveries of 0.01 μg/g; B, recoveries of 0.025 μg/g; M, RSDs of 0.01 μg/g; N, RSDs of 0.025 μg/g.

| Compounds	RT (min)	Ions (m/z) monitored			Apple		Spinach		Cucumber	
		Q	C		Rec. (%) A/B	RSD (%) M/N	Rec. (%) A/B	RSD (%) M/N	Rec. (%) A/B	RSD (%) M/N
Propoxur	17.65	152	110	137	106/90	2/5	97/99	4/2	102/100	3/1
Dicloran	19.417	206	176	160	83/92	6/4	81/89	3/1	86/90	5/1
Isazofos	20.7	285	313	161	91/97	3/3	90/98	5/1	100/99	6/2
Iprobenfos	20.933	204	91	246	93/98	3/2	84/95	6/2	90/101	4/1
Chlorpyrifos-methyl	21.65	271	286	125	95/98	10/1	94/96	8/5	99/102	7/3
Uniconazole	21.658	241	285	213	88/90	4/2	90/90	4/6	92/87	6/1
Metalaxyl	22	249	279	206	104/100	3/1	90/97	4/3	99/100	4/4
Aldrin	22.719	263	293	255	100/90	5/3	105/100	5/2	110/96	4/1
Kelthane	22.994	139	215	251	102/100	5/2	113/92	5/3	109	6/2
Heptachlor	23.884	272	237	185	100/99	7/3	107/98	7/6	99/97	12/2
Methidathion	24.475	145	302	125	85/90	8/4	86/87	3/2	90/93	2/3
Profenofos	25.217	339	374	208	77/87	9/7	80/90	6/1	83/89	7/5
Dieldrin	25.351	345	277	263	101/98	6/3	113/98	4/2	106/97	3/2
Buprofezin	25.533	305	249	172	114/98	3/3	111/97	7/3	118/102	1/3
Endrin	25.908	345	263	281	107/100	4/5	110/99	8/3	108/97	10/1
Ethion	26.417	384	241	203	89/98	4/5	87/90	10/3	85/97	6/2
Oxadixyl	26.55	278	233	163	102/98	6/2	105/108	12/3	98/100	10/3
Pyridaphenthion	28.35	188	199	340	95/98	11/3	98/97	10/2	89/101	13/2
Tetradifon	29.183	356	159	227	87/87	13/2	90/98	10/4	87/96	12/3
Hexachlorobenzene	18.838	284	142	249	108/100	4/5	82/99	3/4	98/100	5/1
α-BCH	18.961	219	183	181	96/99	7/6	113/97	4/2	110/102	6/2
Dimethoate	19.456	229	125	93	111/100	10/7	115/96	7/2	118/98	12/2
β-BCH	19.908	183	181	219	93/98	6/2	100/97	14/3	112/100	5/1
γ-BCH	19.908	183	181	219	98/97	6/1	105/94	4/1	112/98	5/1
Terbufos	19.95	231	288	153	93/98	2/2	89/92	4/5	94/98	5/1
δ-BCH	20.723	181	219	254	103/96	3/2	110/98	8/7	114/101	5/5
Pirimiphos-methyl	22.318	305	290	233	86/87	5/1	87/90	3/2	88/99	4/1
Sumithion	22.347	277	125	260	99/97	2/2	92/98	1/2	89/98	5/3
Bayten	22.829	278	279	151	85/95	5/3	89/97	3/1	88/98	3/2
Chlorpyrifos	22.846	314	197	258	68/87	2/4	80/98	4/2	75/99	7/2
Endosulfan I	24.671	339	241	279	104/97	5/1	107/100	11/6	107/102	5/2
p,p′-DDE	25.247	318	246	210	110/97	3/3	115/114	4/2	113/98	2/2
Fludioxonil	25.499	248	182	154	89/96	11/5	98/99	9/3	89/93	5/3
Endosulfan II	26.172	195	241	339	102/100	5/2	101/97	10/2	91/98	4/2
p,p′-DDD	26.329	235	237	320	108/106	5/3	114/100	4/3	111/92	4/1
o,p′-DDT	26.373	235	246	165	103/98	5/2	115/106	4/2	115/94	4/1
p,p′-DDT	27.209	235	282	165	105/97	3/5	109/105	8/3	119/89	2/4
Bifenthrin	28.344	166	181	422	108/100	5/4	118/99	3/1	111/98	6/3
Fenpropathrin	28.551	181	349	265	104/105	4/3	103/99	6/2	114/98	4/2
Cyhalothrin-1	29.399	197	181	449	107/98	3/2	104/95	1/2	112/100	4/1
Cyhalothrin-2	29.653	181	449	197	103/98	7/1	95/97	5/3	101/101	11/2
Fenarimol	30.046	330	139	151	93/101	14/3	85/89	3/2	88/99	2/2
Permethrin-1	30.7	183	255	163	102/98	6/4	106/98	6/3	115/97	6/1
Permethrin-2	30.894	183	163	255	100/101	5/4	115/96	3/1	108/92	6/4
Cypermethrin-1	32.221	163	181	209	101/100	4/2	116/99	5/2	108/98	7/3
Cypermethrin-2	32.395	163	209	181	97/102	6/3	107/97	7/2	112/93	7/1
Cypermethrin-3	32.547	163	181	209	103/100	4/2	97/102	6/4	113/99	4/3
Cypermethrin-4	32.616	163	181	209	104/99	7/5	116/94	8/1	110/104	7/2
Fenvalerate-1	34.452	167	125	225	102/90	3/2	85/98	5/2	109/98	4/5
Fenvalerate-2	34.803	167	125	225	104/98	2/1	109/102	9/3	107/99	10/1
Deltamethrin-1	35.55	181	253	172	100/89	2/1	111/109	4/4	110/102	4/2
Deltamethrin-2	36.141	172	253	181	105/100	4/3	109/100	5/5	106/100	5/1
Heptachlor-epoxide	23.764	353	263	237	–	–	–	–	–	–

of chinomethionate was earlier than 6.794 min, and thus all target pesticides were within PPGE's collection time.

3.2. QuEChERS method optimization

3.2.1. Two step extraction

Previous studies used NaCl and anh. $MgSO_4$ for salting out and phase separation in one step [1,2,4,13]. Following this QuEChERS procedure, we observed lower recoveries for dimethoate ($78 \pm 10\%$, $n = 3$), propoxur (80 ± 5, $n = 3$) and dicloran ($77 \pm 4\%$, $n = 3$), due to their high polarity and water solubility. The physical and chemical properties of analytes such as pH dependence [3,16] and planarity in chemical structure [14,17], sample matrix contents such as basic matrix [3] and high fat content matrix [18], and the choice

of dehydrated agents [19] in the QuEChERS method may affect recoveries of pesticides. We found that the practical operation of QuEChERS method influenced method recoveries and reproducibility. Simultaneous addition of NaCl and anh. $MgSO_4$ caused $MgSO_4$ agglomeration, which could hardly be dispersed by hand shaking. The agglomeration formation might prevent the inside $MgSO_4$ from water absorption, thus reducing the efficiency of water removal of $MgSO_4$. This resulted in incomplete partition of the target pesticides into acetonitrile and lower clean-up efficiency of D-SPE. However, addition of both agents for salting out and phase separation in two steps improved recoveries of the target pesticides. NaCl was firstly added to achieve a primary phase separation, with only approximate 9% water remaining in the upper acetonitrile layer [19]. Subsequently, addition of anh. $MgSO_4$ into acetonitrile layer with
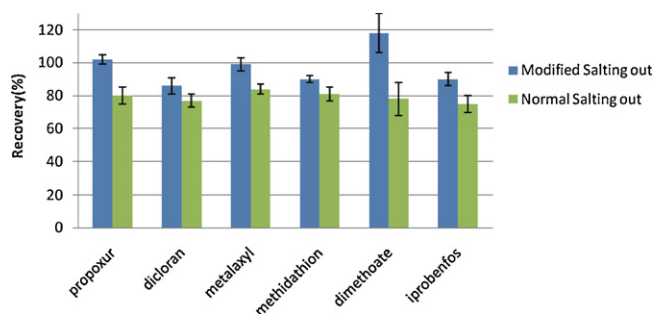
Fig. 3. Recoveries (*n* = 3) of propoxur, dicloran, metalaxyl, methidathion, dimethoate and iprobenfos in cucumber sample spiked at 0.01 μg/g and processed by modified salting out and normal salting out procedures.

lower water content prevented anh. MgSO$_4$ from agglomeration. By using the modified QuEChERS method, recoveries of the target pesticides ranged from 80 to 120% for the non-polar pesticides (such as organochlorine and pyrethroids), with less than ±8% deviation from those using the conventional QuEChERS method. Recoveries of propoxur, dicloran, metalaxyl, methidathion, dimethoate and iprobenfos (high water solubility) had improved 9–40% (Fig. 3).

3.2.2. Acetate buffer

Compared with those from a non-buffered QuEChERS method, the acetate buffered QuEChERS method improved the recovery of Kelthane to ca. 100% from 60%, while the recoveries of other target pesticides were found of no apparent differences. Buffer solution improved stabilities and recoveries of certain pH-dependent pesticides (e.g. chlorothalonil, captan, folpet, tolylfluanid, dichlofluanid and carbaryl) [3,16].

3.2.3. D-SPE cleaning up

Cleanup efficiency of three sorbents (20–100 mg PSA, 20–100 mg C18 and 10–50 mg GCB) was examined using three matrices spiked at 0.01 μg/g each. Take cucumber sample as an example, we found that 50 mg PSA could greatly lowered chromatographic background in a retention time interval (28.25–29.25 min), as shown in a total ion current chromatogram (TIC) in Fig. 4. PSA could efficiently remove interferences from polar organic acids, polar pigments, sugars and fatty acids [1,3,20]. Strong interaction between PSA and acid functional groups of matrices removed most fatty acids and other organic acids in extracts of fruit and vegetable.

GCB could remove sterols, pigments, and planar chemicals in extracts, however, could not eliminate fatty acids' interferences [20]. We observed that use of GCB sorbents eliminated matrix interferences from fruits and vegetables. With GCB added, a lower background chromatogram was observed and thus all target pesticides could be determined. GCB strongly retained planar pesticides
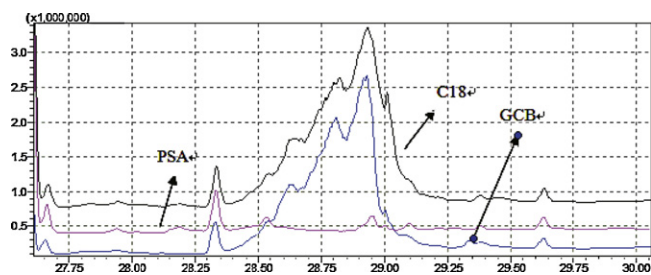
Fig. 4. Cleanup effectiveness of three D-SPE sorbents [PSA (red), C18 (black) and GCB (blue)]. A strong background interference was observed in TIC (27.30–30.00 min) of the cucumber sample spiked at 0.01 μg/g. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

such as thiabendazole, chlorothalonil, coumaphos, hexachlorobenzene, terbufos and other planar matrix compounds [3,17]. To optimize the quantity of GCB for removing matrix interferences, while maintaining a good recovery for a planar pesticide, hexachlorobenzene (HCB), GCB was added with 10, 20, 30, 40 and 50 mg. The results showed that 20–50 mg of GCB could generate a transparent and colorless solution. However, 20 mg GCB generated recoveries of 70–120% in cucumber, spinach and apple spiked at 0.01 μg/g. With the GCB amount increased, HCB recoveries decreased. 50 mg GCB only gave a 34% recovery for HCB. Therefore, 20 mg GCB was used in the QuEChERS method.

It was reported that C18 sorbent could remove fat content [18], and ineffectively reduce chromatographic background [20]. We did observe that TIC background (28.25–29.25 min) of cucumber sample was fairly high (Fig. 4). Therefore, background removal capabilities of C18 and GCB were not as high as PSA. However, non-volatile co-extracts could be built up in GC injector and this could delay the target pesticides into the GC column [1]. Therefore, C18 and GCB sorbents were used to remove non-volatile co-extracts and reduce their buildup in the GC injector.

3.3. Comparison of QuEChERS-GPC–GC/MS chromatogram with QuEChERS-GC/MS

The cucumber sample spiked at 0.01 μg/g was processed using the optimized QuEChERS method. The final solution was analyzed by both GC/MS and online GPC–GC/MS (we called QuEChERS-GC/MS and QuEChERS-GPC–GC/MS, respectively) in full scan mode. An automate switch mode was set to avoid ion saturation on EI filament and electron multiplier to prolong their lifetime. Once ion oversaturation is monitored in MS ionization system, EI filament will automatically off. Fig. 5 shows a TIC in three selected retention time intervals by using QuEChERS-GPC–GC/MS and QuEChERS-GC/MS, respectively. Compared with TIC (Fig. 5B) from QuEChERS-GC/MS, TIC from QuEChERS-GPC–GC/MS (Fig. 5A) had fairly lower background and no observable filament off (Fig. 5A). It showed that GPC was efficient to reduce matrix interferences. The measurement of spinach and apple samples showed results close to those in cucumber sample (data not shown here).

When cucumber sample was analyzed in SIM mode, the results from QuEChERS-GPC–GC/MS were much better than those from QuEChERS-GC/MS. As shown in Fig. 6A and C, a lower baseline, better peak shape and higher peak intensity were observed, especially for *o,p′*-DDT and fenvalerate. However, from QuEChERS-GC/MS, higher backgrounds were observed for both compounds (Fig. 6B and D). The MS similarity indices (SIs) are well defined in the study by Wan et al. [21] and were automatically generated in the software of MS solution (version 2.53, Shimadzu, Kyoto, Japan) by searching the target analytes from their mass spectra and from browsing mass spectral libraries. A SI quantitatively expresses the difference between the spectrum of an unknown and a spectrum registered in a library or obtained with a standard solution and is often used to identify unknown compounds. In this study, we used SIs to evaluate the cleanup efficiency, since co-eluted ions from sample matrices affect SIs. The differences between the respective intensities of the spectral peaks at a certain mass number are determined. The smaller differences mean the greater similarity (SI is equal to 100 when the spectra are perfectly identical) and the higher degree of cleanup for samples. Of 45 target pesticides, SIs of fifteen were calculated, since background interferences were often observed for their analysis. The SIs of these 15 pesticides in the cucumber sample showed that QuEChERS-GPC–GC/MS method was much better than QuEChERS-GC/MS (Fig. 7). It demonstrated higher capabilities of GPC to remove complex matrices interferences.
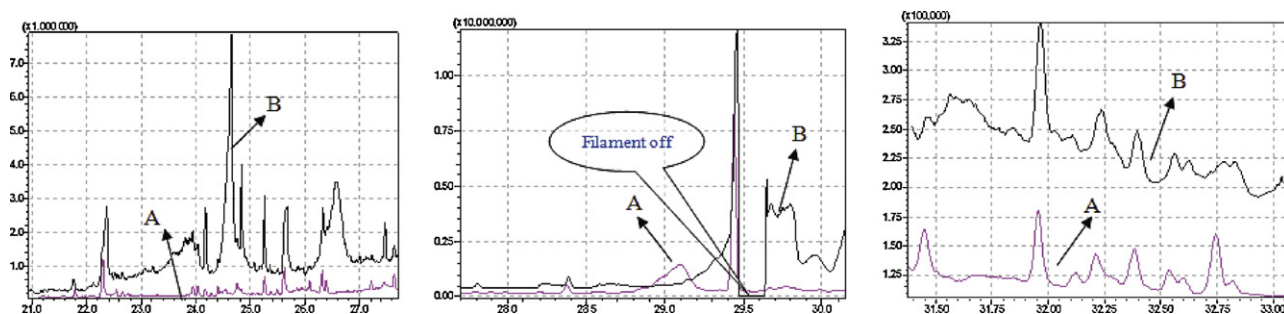
Fig. 5. TICs in three retention time intervals of cucumber sample spiked at 0.01 μg/g and analyzed by QuEChERS-GPC–GC/MS (A, red) and QuEChERS-GC/MS (B, black).

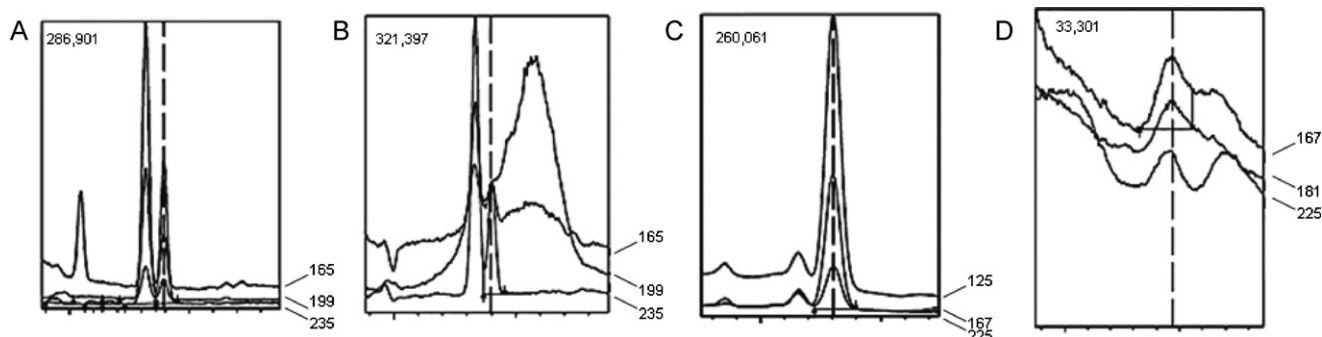**Fig. 6.** Extracted ion chromatogram of o,p'-DDT (A, B) and fenvalerate (C, D) in cucumber sample spiked at 0.01 μg/g and analyzed by QuEChERS-GPC–GC/MS (A, C) and QuEChERS-GC/MS (B, D).

3.4. Method validation and application

3.4.1. Instrumental method

Compared with the study [13], a PTV injector, a retention gap and a pre-column were connected ahead of the analytical column. This setting reduced instrumental contamination and maintenance frequency. Linearity of all 45 target pesticides was good over a range of 5–50 ng/mL. Correlation coefficients were greater than 0.997. The limits of method detection (LODs) were 1.2 ng/g for uniconazole and 0.96 ng/g for metalaxyl, and 0.54 ng/g for others. The method limits of quantification (LOQs), defined on a signal-to-noise ratio of 10:1, were 10 ng/g for uniconazole and metalaxyl, and 5 ng/g for others. LODs and LOQs in this study were fairly lower than those LODs (3–79 ng/g), and LOQs (11–262 ng/g) in a earlier

study [13]. Due to fairly high GPC cleanup effectiveness, and 10 times higher injection volume of online GPC–GC/MS than a regular split/splitless GC injector, the LOQs were much lower than those from the reported GC/MS method using regular split/splitless injection techniques [3,15].

3.4.2. Method trueness and precision

Homogenized spinach, cucumber and apple were spiked with each target pesticides at 0.01 μg/g and 0.025 μg/g, and were analyzed for five times to evaluate method precision and trueness. The spiked concentration of 0.01 μg/g were close or identical to the limits required by "Positive List System for Agricultural Chemical Residues in Foods" of Japanese government, which are the most restrictive regulations on food pesticide residues. By
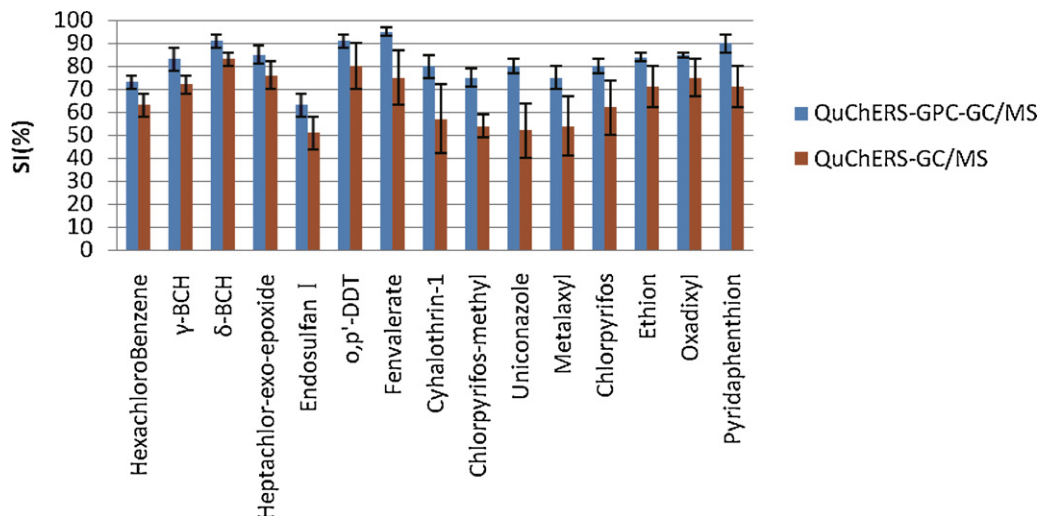

Fig. 7. Average SIs (n = 3) of 15 selected pesticides in cucumber spiked at 0.01 μg/g and analyzed by QuEChERS-GPC–GC/MS and QuEChERS-GC/MS.

Table 3
The results of FAPAS interlaboratory test.

Test round	Target analytes	Results (μg/kg)	Rec. (%)	Z-score	Number of possible pesticide residues
1967	Diazinon	97	97	−1.2	66
	Mepanipyrim	61	96	−1.0	
	Tolylfluanid	170	75	−0.9	
1968	Parathion	391	102	1.3	66
	Procymidone	48	116	0.5	
19108	Aldicarb sulfone	–	–	–	143
	Carbendazim	–	–	–	
	Chlorfenvinphos	27.3	101	−0.8	
	Endosulfan sulfate	298.6	98	0.1	
	Fenhexamid	98.4	79	−0.6	

using QuEChERS-GC/MS method, recoveries of all target pesticides ranged from 61% to 132% with relative standard deviations (RSDs) of 1–28%. Using QuEChERS-GPC–GC/MS method, recoveries were 77% for profenofos in apple, 68% for chlorpyrifos in apple, 75% for chlorpyrifos in cucumber, and 80–118% for others with RSDs of 1–14% in three matrices (Table 2). At the level of 0.025 μg/g, recoveries ranged from 87% to 108% with RSDs of 7%. The spiked levels of 0.01 μg/g and 0.025 μg/g in our study were 10 and 4 times lower than 0.1 μg/g in the previous study [13], while the better or comparable recoveries and RSDs are achieved in our study. These results showed that online GPC has a great capacity to remove matrix interferences and improve the method trueness and precision. QuEChERS-GPC–GC/MS methods are feasible to determine all target pesticides in fruits and vegetables.

3.4.3. FAPAS interlaboratory test

Our laboratory had participated in FAPAS Proficiency Test in three rounds: Pesticides in Pear Purée (round 1967, laboratory number is 45), Pesticides in Tomato Purée (round 1968, laboratory number is 59) and Pesticides in Leek Purée (round 19108, laboratory number is 21). Good results with Z-score ranged from −0.9 to 1.3 were obtained (Table 3). It confirmed that the method is very successful in measuring the target pesticides. The results of both aldicarb sulfone and carbendazim in round 19108 were not shown since the analysis of both compounds should be performed by liquid chromatographic techniques. Due to its high pH dependence, tolyfluanid was determined not so efficiently in a reported study [3]. However, our method measured tolyfluanid with satisfied results in Round 1976 (absolute Z-score less than 1 and a recovery of 75%) (Table 3).

3.4.4. Applicability of the proposed method

Since 2005, the method has been developed and applied in routinely monitoring target pesticides in fruits and vegetables. We analyzed 200 samples yearly, with 0.1% samples positively detected. The internal standard's recoveries were 80–120%. Intra-year method recoveries were 70–130% with RSDs of less than 20%.

4. Conclusions

The QuEChERS method and online-GPC–GC/MS method were developed and well validated to determine pesticide residues in spinach, apple and cucumber. The QuEChERS method was improved with NaCl salting out and anh. $MgSO_4$ phase separation in two steps. To our knowledge, the modification of QuEChERS method in this study has not been reported in other published studies. Online GPC greatly improved sample cleanup efficiency and method automation. The combination of QuEChERS method and online-GPC–GC/MS method generated satisfactory method

recoveries and repeatability for target analytes. The result of analysis of standard reference materials confirmed that the QuEChERS-GPC–GC/MS method is a very practicable and robust method to measure pesticides with different chemical properties in spinach, apple and cucumber.

The number (forty five) of target pesticides in our study are less than the earlier study, which analyzed 97 pesticides in seven commodities [13], but these analytes included many pesticides in different chemical families. This study confirmed that QuEChERS-GPC–GC/MS methods are feasible to determine all these target pesticides in fruits and vegetables. The method is being expanded to more than 150 pesticides including organochlorine, organophosphate, synthesized pyrethroids, carbamates, miscellaneous herbicides, fungicides and acaricides in a wider array of commodities, and the method will be reported in other studies.

References

[1] M. Anastassiades, S.J. Lehotay, D. Stajnbaher, F.J. Schenck, J. AOAC Int. 86 (2003) 412.
[2] A. Wilkowska, M. Biziuk, Food Chem. 125 (2011) 803.
[3] S.J. Lehotay, K. Son, H. Kwon, U. Koesukwiwat, W. Fu, K. Mastovska, E. Hoh, N. Leepipatpiboon, J. Chromatogr. A 1217 (2010) 2548.
[4] C. Díez, W.A. Traag, P. Zommer, P. Marinero, J. Atienza, J. Chromatogr. A 1131 (2006) 11.
[5] B.G. Loganathan, K. Senthilkumar, K. Seaford, K.S. Sajwan, N. Hanari, N. Yamashita, Arch. Environ. Contam. Toxicol. 54 (2008) 422.
[6] K.S. Sajwan, K. Senthilkumar, S. Kelly, B.G. Loganathan, Bull. Environ. Contam. Toxicol. 82 (2009) 444.
[7] S.J. Lehotay, M. Anastassiades, R.E. Majors, LCGC Asia Pacific 13 (2010) 30.
[8] J.L. Martínez Vidal, F.J. Arrebola, M. Mateu-Sánchez, J. Chromatogr. A 959 (2002) 203.
[9] S. Walorczyk, B. Gnusowski, J. Chromatogr. A 1128 (2006) 236.
[10] J.L. Fernández Moreno, F.J. Arrebola Liébanas, A. Garrido Frenich, J.L. Martínez Vidal, J. Chromatogr. A 1111 (2006) 97.
[11] Z. Huang, Y. Li, B. Chen, S. Yao, J. Chromatogr. B 853 (2007) 154.
[12] Syoku-An No.0124001, Director Notice of Japanese Ministry of Health, Labour and Welfare, 2006.
[13] L.B. Liu, Y. Hashi, Y.P. Qin, H.X. Zhou, J.M. Lin, J. Chromatogr. B 845 (2007) 61.
[14] T. Cajka, J. Hajslova, O. Lacina, K. Mastovska, S.J. Lehotay, J. Chromatogr. A 1186 (2008) 281.
[15] European Commission, Method validation and quality control procedures for pesticide residues analysis in food and feed, Document No. SANCO/10684/2009, Brussels, 1 January 2010. http://ec.europa.eu/food/plant/protection/resources/qualcontrol_en.pdf.
[16] S.J. Lehotay, A. Kok, M. Hiemstra, P. Bodegraven, J. AOAC Int. 88 (2005) 595.

[17] T.D. Nguyen, E.M. Han, M.S. Seo, S.R. Kim, M.Y. Yun, D.M. Lee, G.H. Lee, Anal. Chim. Acta 619 (2008) 67.
[18] P. Georgakopoulos, R. Zachari, M. Mataragas, P. Athana-sopoulos, E.H. Drosinos, P.N. Skandamis, Food Chem. 128 (2011) 536.
[19] F.J. Schenck, P. Callery, P.M. Gannett, J.R. Daft, S.J. Lehotay, J. AOAC Int. 85 (2002) 1177.
[20] F.J. Schenck, S.J. Lehotay, V. Vega, J. Sep. Sci. 25 (2002) 883.
[21] K.X. Wan, I. Vidavsky, M.L. Gross, J. Am. Soc. Mass Spectrom. 13 (2002) 85.