

Simultaneous Determination of 69 Pesticide Residues in Coffee by Gas Chromatography–Mass Spectrometry

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Abstract A method using gel permeation chromatography (GPC) combined with solid-phase extraction (SPE) cleanup followed by gas chromatography–mass spectrometry (GC-MS) has been established for quantitative determination of 69 pesticide residues in coffee. Based on an appraisal of the characteristics of GC-MS, validation experiments were conducted for 69 pesticides. In the method, 2.0 g samples were mixed with 5 ml water and 1 g sodium chloride and extracted with 5 ml of ethyl acetate by blender homogenization, centrifugation, and filtration. Evaporation was conducted and the sample was injected into a 250 mm × 10 mm S-X3 GPC column, with ethyl acetate–*n*-hexane (1:2 v/v) as the mobile phase at a flow rate of 3 ml/min. The 4–15 min fraction was collected for the SPE cleanup, which was Envi-Carb SPE cartridge coupled with NH₂-LC SPE cartridge with acetone–ethyl acetate (2:5 v/v) as the eluted solvent. The eluents were collected and then evaporated to dryness, which was redissolved in 0.5 ml ethyl acetate for GC-MS analysis. For the 69 pesticides determined by GC-MS, the portions collected from GPC were concentrated to 0.5 ml and exchanged with 5 ml *n*-hexane. In the linear range of each pesticide, the correlation coefficient was $R^2 \geq 0.99$. At the low, medium, and high fortification levels of 0.05–1.0 mg/kg, recoveries fell within 60–120%. The relative standard deviation was between 1.3% and 22.3%

for all 69 pesticides. The limits of detection for the method were 10 µg/kg to 150 µg/kg, depending on each pesticide.

Keywords Pesticides · Multi-residue Analysis · Coffee · GPC · SPE · GC-MS

Introduction

Coffee belongs to Rubiaceae family along with more than 70 other species, but two of them are of significant economic importance viz., arabica (*Coffea arabica*) and robusta (*Coffea canephora*). Coffee contains several beneficial antioxidants and is one of the richest sources of chlorogenic acid, for many consumers this will be their major dietary source (Clifford 1999). However, coffee drinking can also represent a significant potential source of human exposure to pesticides and other hazardous chemicals, which are unavoidably or improperly used for protection against pests and putrescence during plant cultivation and product-manufacturing processes (Hillocks et al. 1999). Even though coffee beans are toasted, there are still pesticide residues after this process. During recent years, there has been an increasing public concern and scientific investigation related to the presence and control of pesticide residues in plant products to assess the potential health hazards more thoroughly. Because of high consumption rate and significant health risk of detrimental residues in coffees, people from both producer and consumer countries pay more attention to coffee safety. Based on the reasons, many countries establish the limited standard of pesticide residues in the coffee. For example, the government of China formulates a standard on toasted coffee in order to limit the content of DDT and HCH residues (NY/T 605-2006). And the American government

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set down the maximum residue levels of 19 pesticides in the coffee listed in 40CFR180.

Generally, analysis of most pesticide residues is carried out in a sequence of several steps, e.g., target extraction from sample matrix, then cleanup and pre-concentration, followed by chromatographic separation and determination. For the sample pretreatment, gel permeation chromatography (GPC) and solid-phase extraction (SPE) that serve as efficient methods have been widely applied for pesticide residue analysis. The capillary gas chromatography–mass spectrometry (GC-MS) has become very popular in pesticide residue analysis. It can quantify and confirm the results by its selected ion monitoring (SIM) spectra. Nowadays, these methods have been widely developed to analyze multi-residues in fresh vegetables, fruit, and tea (Jansson 2000; Huang et al. 2007; Blasco et al. 2002; Yang et al. 2009). For example, Ferrer et al. developed a multi-residue methodology using liquid chromatography–time-of-flight mass spectrometry for the quantitative (routine) analysis of 15 pesticide residues in different types of fruit and vegetables: pepper, broccoli, tomato, orange, lemon, apple, and melon (Ferrer et al. 2005). Sannino et al. described the determination of 24 new pesticide residues in apple puree, concentrated lemon juice and tomato puree. Residual pesticides were extracted by a miniaturized extraction–partition procedure requiring small amounts of non-chlorinated solvents. The extracts were analyzed by liquid chromatography–electrospray ionization tandem mass spectrometry without any further cleanup step (Sannino et al. 2004). Sandra et al. used the stir bar sorptive extraction for the enrichment of pesticides from vegetables, fruits, and baby food samples. The 300 pesticide residues were analyzed with the retention time locked gas chromatography–mass spectroscopy method (RTL-capillary GC-MS) (Sandra et al. 2003). Adou et al. used accelerated solvent extraction and capillary gas chromatography with electron capture and flame photometric detections for simultaneously determination of different pesticides in fruits and vegetables (Adou et al. 2001). Fillion et al. adopted C18 and a carbon cartridge coupled to an amino propyl SPE cartridge for cleanup, gas chromatography with mass-selective detection, and liquid chromatography with post-column reaction and fluorescence detection for the determination of 251 pesticide and degradation product residues in fruit and vegetable samples (Fillion et al. 2000).

After a detailed analysis of these publications, we established a new method for the determination of 69 pesticide residues in coffees by gas chromatography–mass spectrometry (GC-MS). The samples were extracted with ethyl acetate, cleaned up with GPC and SPE; the pesticide varieties for determination have been added in the new method. The analytical procedures have been simplified

and modes of detection have been changed so that the accuracy and reliability of the method can be improved. And the determination of multipesticide residues in coffees has been not reported in previous works.

Materials and Methods

Apparatus

An Agilent 6890N gas chromatograph connected to Agilent 5973N mass-selective detector equipped with Agilent 7683 autosampler (Agilent Technologies, USA). For GPC system (Gilson, France), a Bio-Beads S-X3 (250 mm×10 mm i.d.×5 μm; Nacalai Tesque, Japan) cleanup column was used. Further, a homogenizer (Qilin, China), a rotary evaporator (Eyela, Japan), a centrifuge (Westfalia Separator AG, F.R. Germany) and a nitrogen evaporator (Organomation Associates, USA) were used.

Reagents and Materials

Acetonitrile (HPLC grade), toluene, toluene–acetone, *n*-hexane, acetone, and ethyl acetate (P.R. grade) were purchased from Sigma Company (Poole, UK). Sodium sulfate, anhydrous (analytical reagent grade), heated at 650°C for 4 h and kept in a desiccator. Pesticide standards were purchased from Supelco (Bellefonte, Pennsylvania, USA) and RDH (Riedel-de-Haen, Seelze, Germany), and were of purity >90% (w/w). Stock standard solutions: accurately weigh 5–10 mg of individual pesticide standards (accurate to 0.1 mg) into a 10 ml volumetric flask, dissolve and dilute to volume with toluene, toluene–acetone combination, depending upon each individual compound's solubility. Mixed standard solution: concentration of mixed standard solutions is decided by sensitivity of each compound for the instrument. Depending upon mixed standard solution concentration and stock standard solution concentration, pipette an adequate amount of individual stock standard solution into a 100-ml volumetric flask and pesticides diluting with toluene. Mixed standard solutions should be stored in dark below 4°C and can be used for 1 month.

Coffee was purchased from a market. They were ground into powders, filtrated through a 20-mesh sieve, and placed in clean vessels for use.

Sample Processing

Extraction

Coffee samples were pulverized and homogenized throughout the 150 mesh (0.100 mm) sieve. Then, 2 g of homogenized sample was accurately weighed into a

Table 1 Parameters for determination of 69 pesticide residues in coffee by GC-MS

Peak no.	Pesticide selected	Retention time(min)	Quantifying ion	Qualifying ion 1	Qualifying ion 2	Qualifying ion 3
1	EPTC	8.81	128	189(30)	132(35)	
2	Butylate	9.69	156	146(110)	217(30)	
3	Dichlobenil	10.11	171	173(70)	136(15)	
4	Vernolate	10.34	128	146(18)	203(10)	
5	Nitrapyrin	11.06	194	196(98)	198(25)	
6	Tecnazene	13.42	203	261(74)	215(84)	
7	Heptanophos	13.73	124	215(18)	250(15)	
8	Hexachlorobenzene	14.25	284	286(80)	282(50)	
9	Propachlor	14.63	120	176(45)	211(10)	
10	Trifluralin	14.98	306	264(70)	335(6)	
11	Chlorpropham	15.23	213	171(60)	153(25)	
12	Sulfotep	15.27	322	202(44)	238(27)	
13	Demeton-S	16.32	88	171(20)	115(15)	
14	Terbumeton	16.61	210	169(65)	225(30)	
15	Fonofos	16.72	246	137(140)	174(15)	
16	Profluralin	16.82	318	304(48)	347(15)	
17	Propazine	17.11	214	229(65)	172(50)	
18	Disulfoton	17.13	88	274(23)	125(16)	
19	Chlorbufam	17.26	223	164(65)	153(53)	
20	Terbutylazine	17.44	214	229(33)	173(35)	
21	Secbumeton	17.60	196	210(39)	225(40)	
22	Heptachlor	17.73	272	237(40)	337(26)	
23	Monocrotophos	18.12	127	192(20)	97(16)	
24	Cyanophos	18.19	243	180(8)	148(4)	
25	Fluchloralin	18.37	264	326(210)	306(186)	
26	Chlorpyrifos-methyl	18.49	286	288(70)	197(6)	
27	Alachlor	19.13	188	237(35)	269(15)	
28	Pirimiphos-methyl	19.23	290	305(75)	276(86)	
29	Simetryn	19.32	213	170(28)	198(16)	
30	Metribuzin	19.52	198	199(20)	144(12)	
31	Thiobencarb	19.56	100	257(25)	259(10)	
32	Aspon	19.65	211	253(50)	378(12)	
33	Pirimiphos-ethyl	20.30	333	318(95)	304(70)	
34	Butralin	20.78	266	224(18)	295(12)	
35	Isopropalin	20.95	280	238(40)	222(5)	
36	Chlorthiamid	21.34	205	170(168)	172(82)	
37	Isofenphos	21.63	213	255(44)	185(45)	
38	Diphenamid	21.68	167	239(30)	165(42)	
39	Chlorfenvinphos	21.80	323	267(140)	269(92)	
40	Penconazole	21.95	248	250(32)	161(50)	
41	Tolyfluanide	22.09	238	240(70)	137(210)	
42	4,4'-DDE	22.26	318	316(80)	246(140)	248(70)
43	Tribufos	22.52	169	202(60)	226(32)	
44	Iodofenphos	22.71	377	379(37)	259(10)	
45	Tetrachlorvinphos	22.80	329	331(95)	333(30)	
46	Procymidone	22.88	283	285(70)	255(15)	
47	Profenofos	23.01	339	374(40)	297(38)	
48	Triadimenol	23.30	112	168(80)	128(16)	
49	Chlorfenson	23.46	302	177(102)	175(280)	

Table 1 (continued)

Peak no.	Pesticide selected	Retention time(min)	Quantifying ion	Qualifying ion 1	Qualifying ion 2	Qualifying ion 3
50	2,4'-DDD	23.65	235	237(66)	165(40)	199(15)
51	Methoprotetryne	23.83	256	213(24)	271(18)	
52	Chloropropylate	24.03	251	141(20)	253(65)	
53	Flamprop-methyl	24.16	105	77(25)	276(10)	
54	Oxyfluorfen	24.32	252	361(35)	300(36)	
55	2,4'-DDT	24.55	235	237(64)	165(38)	199(14)
56	Flamprop-isopropyl	24.67	105	276(20)	363(3)	
57	Fluorodifen	24.85	190	328(36)	162(35)	
58	Myclobutanil	25.07	179	288(15)	150(45)	
59	Benalaxyl	25.11	148	206(35)	325(16)	
60	Bifenthrin	25.49	165	166(109)	181(43)	
61	Bromopropylate	25.95	341	183(35)	339(40)	
62	Benzoylprop-ethyl	26.01	292	365(35)	260(36)	
63	EPN	26.42	157	323(15)	169(53)	
64	Hexazinone	26.54	171	128(12)	252(3)	
65	Nitralin	27.06	316	274(60)	300(15)	
66	Phosalone	27.17	182	367(30)	154(20)	
67	Fenarimol	27.45	139	219(70)	330(41)	
68	Azinphos-Ethyl	27.75	160	132(102)	77(50)	
69	Coumaphos	28.95	362	226(55)	210(10)	364(40)

100-ml centrifuge tube and mixed with 5 ml water and 1 g sodium chloride. The mixture was vortexed for 30 s and allowed to stand for 30 min. After triple extraction each with 5 ml of ethyl acetate for 3 min followed by centrifugation at 3,000 rpm for 5 min, the organic phase was combined and dried with 1 g of anhydrous sodium sulfate, then filtrated and reduced to about 5 ml with nitrogen stream at 45°C. The residual extract was diluted to 10 ml with ethyl acetate for subsequent purification in GPC.

GPC Procedure

The diluted extract was reconstituted and further purified using a Gilson GPC system (Gilson, France) equipped with a cosmosil packed column (250 mm×10 mm i.d.×5 µm; Nacalai Tesque, Japan). The reconstituted solution was injected into the GPC column; Mobile phase, ethyl acetate–*n*-hexane (1:2 v/v); flow rate, 3 ml/min; injection volume, 5 ml; detection wavelength, 254 nm. The eluent was just collected between 4–15 min, and concentrated to about 1 ml for the SPE cleanup.

SPE Procedure

The SPE columns used in the experiment were Supelco Envi-Carb SPE cartridge (3 ml and 250 mg; Supelco, USA)

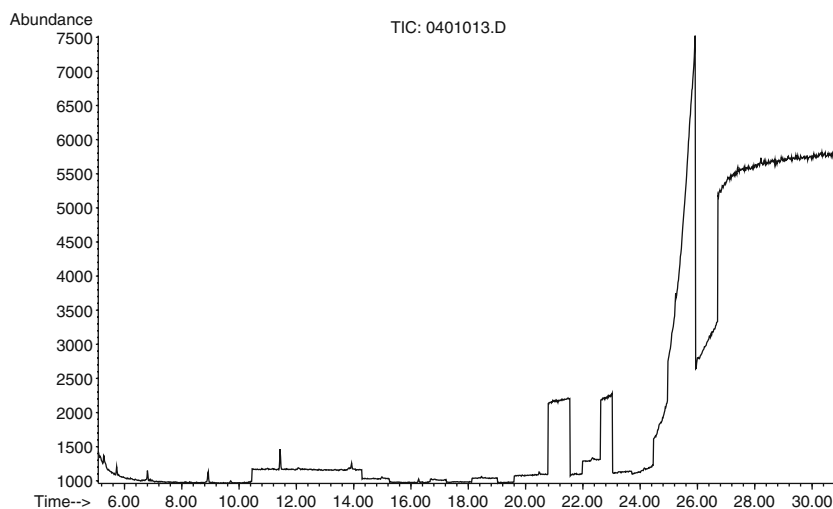
coupled with NH₂-LC SPE cartridge (3 ml and 250 mg; Supelco, USA). A 1-ml volume of the concentrated eluent was introduced into the SPE column, which was preconditioned with 10 ml acetone–ethyl acetate (2:5 v/v), while the retained pesticides on the column were eluted with 10 ml acetone–ethyl acetate (2:5 v/v). The eluents were collected and then evaporated to dryness with nitrogen stream at 45°C. Finally, the residue was redissolved in 0.5 ml ethyl acetate for GC-MS analysis.

Equipment Parameters

Chromatographic conditions: the chromatographic column was a DB-1701, 30 m×0.25 mm i.d.×0.25 µm, and the carrier gas was nitrogen (purity >99.995%). It was operated in continuous flow mode at a flow rate of 1.0 ml/min. The injector temperature was 270 °C and splitless sampling was used (1 µl). Temperature programming conditions were set as follows: the initial temperature, 40°C hold 1 min, at 30°C/min to 130°C, at 6°C/min to 250°C, at 30°C/min to 300°C hold 5 min.

Mass analyzer conditions: the ion source temperature, tetrapolar temperature, and transfer line temperature were 230°C, 150°C, and 280°C, respectively. EI was chosen as the ionization mode, and the mass scanning range was 50–550 amu. The ionization voltage was 70 eV, and the detector was in selected ion monitoring mode. Each

Fig. 1 SIM chromatogram of a typical blank coffee sample. Coffee samples free of pesticides were used for the preparation of a blank matrix. The typical chromatogram of a blank coffee sample is shown



compound selects one target ion and two qualifying ions. Retention times, target ion and qualifying ions of each compound are listed in Table 1.

Method Evaluation

The qualitative analysis of mass spectrum was based on more than three ions, and the relative abundance of the ions should alter within 20%. It was quantified with the external standard method. The blank value must be deducted from the results. When using GC-MS analysis, samples were confirmed to contain a pesticide if: (1) the observed retention times of peaks of sample solutions were the same as the peaks for the standards in blank matrix extracts; (2) the selected ions or monitored ions appeared that would be signal to noise ratio of 3 for limits of detection (LOD) and at least 5 for LOD; and (3) the observed abundance ratio of ions was identical to that of the standard ions. When confirmation by GC-MS SIM failed, samples were re-injected and confirmation was undertaken using scan mode (with sufficient sensitivity),

using chlorbufam, monocrotophos, chlorthiamid, etc. External standard quantification was adopted for GC-MS.

Results and Discussions

Selection of the Extracting Solvent

The selection of the extracting solvent in sample pretreatment process with a proper polarity to match the analyte was beneficial to improve process efficiency and minimize potential interferences from coffee. A comparison was made on the three extracting solvents of acetonitrile, acetone, and ethyl acetate. The test found that these three solvents gave very good extracting efficiency, but the recoveries for some pesticides would be affected when acetonitrile and acetone were adopted as the extracting solvent. As ethyl acetate was used as extracting solvent, the recoveries for most pesticides could be accepted, which would be submitted for GPC cleanup. Therefore, ethyl acetate has been adopted as the extracting solvent in this method.

Fig. 2 SIM chromatogram of typical blank coffee spiked with 2.5 $\mu\text{g/ml}$ of target analyte. Chromatograms of blank matrix extract spiked with the target pesticides at a concentration of 2.5 $\mu\text{g/kg}$ was obtained with the SIM method. There were 69 pesticides in the figure, and the numbers in Fig. 2 represented peak no. of pesticides, respectively

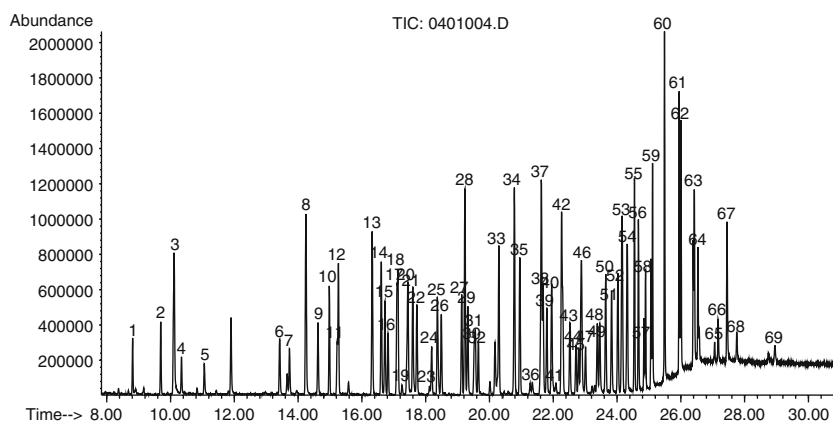


Table 2 Validation parameter1: regression equation, linear range, correlation coefficient and LOD of GC-MS quantitation for 69 pesticides in coffee

Peak no.	Pesticide selected	Regression equation	Correlation coefficient (R^2)	Linear range ^a (µg/ml)	LOD (µg/kg)
1	EPTC	$Y=1.29 \times 10^5 X - 1.64 \times 10^3$	0.9967	0.010–0.250	50
2	Butylate	$Y=1.29 \times 10^5 X - 1.37 \times 10^3$	0.9985	0.010–0.250	50
3	Dichlobenil	$Y=4.19 \times 10^6 X - 3.59 \times 10^4$	0.9951	0.010–0.250	10
4	Vernolate	$Y=7.21 \times 10^5 X - 3.66 \times 10^4$	0.9990	0.010–0.250	50
5	Nitrapyrin	$Y=4.13 \times 10^5 X - 8.77 \times 10^3$	0.9992	0.025–0.500	75
6	Tecnazene	$Y=2.50 \times 10^4 X - 2.30 \times 10^2$	0.9982	0.010–0.250	50
7	Heptanophos	$Y=8.74 \times 10^5 X - 7.79 \times 10^4$	0.9996	0.100–2.500	100
8	Hexachlorobenzene	$Y=8.14 \times 10^5 X - 2.02 \times 10^4$	0.9991	0.010–0.250	25
9	Propachlor	$Y=3.70 \times 10^5 X - 1.43 \times 10^4$	0.9988	0.025–0.500	75
10	Trifluralin	$Y=2.83 \times 10^5 X - 8.02 \times 10^2$	0.9958	0.010–0.250	25
11	Chlorpropham	$Y=2.24 \times 10^5 X - 1.89 \times 10^4$	0.9965	0.025–0.500	75
12	Sulfotep	$Y=1.01 \times 10^5 X - 2.22 \times 10^3$	0.9993	0.025–0.500	25
13	Demeton-S	$Y=2.77 \times 10^5 X - 4.29 \times 10^3$	0.9973	0.025–0.500	25
14	Terbumeton	$Y=4.71 \times 10^5 X - 6.59 \times 10^3$	0.9990	0.010–0.250	50
15	Fonofos	$Y=1.56 \times 10^5 X - 8.17 \times 10^3$	0.9974	0.050–1.000	25
16	Profluralin	$Y=5.83 \times 10^4 X - 7.09 \times 10^2$	0.9990	0.025–0.500	100
17	Propazine	$Y=3.93 \times 10^5 X - 7.89 \times 10^3$	0.9982	0.025–0.500	25
18	Disulfoton	$Y=2.30 \times 10^5 X - 1.23 \times 10^3$	0.9979	0.010–0.250	25
19	Chlorbufam	$Y=4.25 \times 10^5 X - 8.89 \times 10^3$	0.9926	0.025–0.500	100
20	Terbutylazine	$Y=1.73 \times 10^5 X - 6.27 \times 10^3$	0.9990	0.050–1.000	25
21	Secbumeton	$Y=2.24 \times 10^5 X - 2.31 \times 10^3$	0.9896	0.010–0.250	25
22	Heptachlor	$Y=5.72 \times 10^5 X - 4.28 \times 10^4$	0.9976	0.100–2.500	75
23	Monocrotophos	$Y=6.78 \times 10^5 X - 5.64 \times 10^3$	0.9918	0.010–0.250	75
24	Cyanophos	$Y=8.65 \times 10^5 X - 7.33 \times 10^3$	0.9943	0.010–0.250	25
25	Fluchloralin	$Y=2.70 \times 10^4 X - 5.28 \times 10^2$	0.9981	0.025–0.500	50
26	Chlorpyrifos-methyl	$Y=2.56 \times 10^5 X - 7.12 \times 10^3$	0.9993	0.025–0.500	25
27	Alachlor	$Y=2.42 \times 10^5 X - 5.30 \times 10^3$	0.9990	0.025–0.500	75
28	Pirimiphos-methyl	$Y=2.97 \times 10^5 X - 5.85 \times 10^3$	0.9928	0.025–0.500	25
29	Simetryn	$Y=5.60 \times 10^5 X - 9.76 \times 10^4$	0.9981	0.100–2.500	100
30	Metribuzin	$Y=1.79 \times 10^5 X - 6.88 \times 10^3$	0.9991	0.050–1.000	75
31	Thiobencarb	$Y=9.36 \times 10^5 X - 9.27 \times 10^3$	0.9992	0.010–0.250	50
32	Aspon	$Y=5.62 \times 10^5 X - 1.55 \times 10^3$	0.9991	0.025–0.500	50
33	Pirimiphos-ethyl	$Y=1.57 \times 10^5 X - 1.52 \times 10^4$	0.9990	0.100–2.500	75
34	Butralin	$Y=1.87 \times 10^5 X - 1.22 \times 10^3$	0.9980	0.010–0.250	50
35	Isopropalin	$Y=2.41 \times 10^5 X - 9.64 \times 10^3$	0.9972	0.050–1.000	50
36	Chlorthiamid	$Y=4.22 \times 10^5 X - 3.97 \times 10^4$	0.9932	0.100–2.500	150
37	Isofenphos	$Y=3.86 \times 10^5 X - 1.69 \times 10^4$	0.9952	0.050–1.000	50
38	Diphenamid	$Y=6.35 \times 10^5 X - 8.81 \times 10^3$	0.9985	0.010–0.250	25
39	Chlorfenvinphos	$Y=7.76 \times 10^5 X - 1.19 \times 10^4$	0.9976	0.025–0.500	75
40	Penconazole	$Y=1.54 \times 10^5 X - 2.30 \times 10^3$	0.9991	0.025–0.500	75
41	Tolyfluanide	$Y=7.29 \times 10^5 X - 6.38 \times 10^3$	0.9911	0.100–2.500	150
42	4,4'-DDE	$Y=2.36 \times 10^5 X - 3.86 \times 10^2$	0.9992	0.025–0.500	25
43	Tribufos	$Y=1.54 \times 10^5 X - 2.34 \times 10^3$	0.9987	0.025–0.500	25
44	Iodofenphos	$Y=1.21 \times 10^5 X - 3.14 \times 10^3$	0.9978	0.025–0.500	50
45	Tetrachlorvinphos	$Y=4.38 \times 10^5 X - 3.79 \times 10^3$	0.9991	0.010–0.250	75
46	Procymidone	$Y=2.00 \times 10^5 X - 6.79 \times 10^3$	0.9990	0.050–1.000	25
47	Profenofos	$Y=7.66 \times 10^5 X - 2.43 \times 10^3$	0.9917	0.050–1.000	75
48	Triadimenol	$Y=2.15 \times 10^4 X - 1.22 \times 10^2$	0.9952	0.010–0.250	50

Table 2 (continued)

Peak no.	Pesticide selected	Regression equation	Correlation coefficient (R^2)	Linear range ^a ($\mu\text{g/ml}$)	LOD ($\mu\text{g/kg}$)
49	Chlorfenson	$Y=7.12 \times 10^4 X - 3.52 \times 10^3$	0.9979	0.050–1.000	50
50	2,4'-DDD	$Y=4.14 \times 10^5 X - 8.26 \times 10^3$	0.9991	0.025–0.500	25
51	Methoprotetryne	$Y=8.89 \times 10^4 X - 1.02 \times 10^2$	0.9994	0.025–0.500	75
52	Chloropropylate	$Y=2.35 \times 10^5 X - 6.00 \times 10^3$	0.9993	0.025–0.500	50
53	Flamprop-methyl	$Y=1.43 \times 10^6 X - 1.90 \times 10^4$	0.9988	0.010–0.250	25
54	Oxyfluorfen	$Y=9.23 \times 10^4 X - 2.15 \times 10^4$	0.9990	0.100–1.000	150
55	2,4'-DDT	$Y=1.15 \times 10^6 X - 7.64 \times 10^4$	0.9995	0.050–1.000	25
56	Flamprop-isopropyl	$Y=1.51 \times 10^6 X - 5.56 \times 10^4$	0.9992	0.050–1.000	25
57	Fluorodifen	$Y=6.21 \times 10^5 X - 8.77 \times 10^3$	0.9977	0.010–0.250	25
58	Myclobutanil	$Y=1.64 \times 10^5 X - 4.57 \times 10^2$	0.9997	0.010–0.250	10
59	Benalaxyl	$Y=2.78 \times 10^5 X - 2.50 \times 10^3$	0.9976	0.010–0.250	25
60	Bifenthrin	$Y=1.20 \times 10^5 X - 5.12 \times 10^3$	0.9995	0.050–1.000	10
61	Bromopropylate	$Y=1.70 \times 10^5 X - 6.25 \times 10^3$	0.9997	0.050–1.000	25
62	Benzoylprop-ethyl	$Y=8.04 \times 10^4 X - 4.93 \times 10^2$	0.9992	0.010–0.250	10
63	EPN	$Y=1.83 \times 10^5 X - 1.19 \times 10^3$	0.9997	0.010–0.250	50
64	Hexazinone	$Y=6.64 \times 10^5 X - 9.93 \times 10^3$	0.9996	0.025–0.500	25
65	Nitralin	$Y=1.95 \times 10^5 X - 5.29 \times 10^3$	0.9971	0.250–0.500	150
66	Phosalone	$Y=2.16 \times 10^5 X - 4.31 \times 10^4$	0.9986	0.100–2.500	100
67	Fenarimol	$Y=2.63 \times 10^5 X - 7.08 \times 10^3$	0.9992	0.025–0.500	50
68	Azinphos-Ethyl	$Y=4.11 \times 10^5 X - 3.28 \times 10^3$	0.9953	0.010–0.250	50
69	Coumaphos	$Y=7.33 \times 10^5 X - 9.16 \times 10^4$	0.9981	0.100–2.500	150

^a Range covering coffee samples with five different spiked levels ($n=5$)

Selection of Cleanup Conditions

The GPC and SPE technology were applied to clean up the coffee samples in this method. In the GPC system, with Bio-Beads S-X3 (250 mm \times 10 mm) chosen as the cleanup column and ethyl acetate–*n*-hexane (1:2 *v/v*) as the mobile phase, an appraisal was conducted to the GPC behavior of 69 pesticides in our laboratory. In order to optimize the collection window for the pesticide fraction from GPC system, the extracts obtained were injected into the GPC column at 3 ml/min. Ethyl acetate–*n*-hexane (1:2 *v/v*) was found to provide the most efficient mobile phase among the seven solvent systems studied [*n*-hexane, ethyl acetate, acetonitrile, ethyl acetate–*n*-hexane (1:1; 1:2, *v/v*) and acetonitrile–*n*-hexane (1:1; 1:2, *v/v*)]. Molecular masses of most synthetic pesticides were between 200 and 400, whereas that of most lipids ranged from 600 to 1,500. Hence, the larger lipid molecules that are too long to pass through the pores of polymer beads are not retained, and they are the first to be eluted from the column. The fat fraction was eluted between 2 and 3.5 min. On the other hand, the pesticides were detected between 5 and 14 min. Therefore, a pesticide collection window between 4 and 15 min was selected (1 min around the emergence of the pesticide fraction) in order to ensure their complete

collection. In addition, less perturbation phenol and pigment fraction (between 3.5 and 5.5 min) was detected over the chromatographic separation of the pesticides. The fat, pigment, and sugar of coffee needed further clean up in the SPE step.

In the SPE step, the SPE columns chosen were Supelco Envi TM-Carb SPE cartridge (3 ml and 250 mg; Supelco, USA) coupled with NH_2 -LC SPE cartridge (3 ml and 250 mg; Supelco, USA). The efficiency of SPE depends on the type and quantity of sorbent, sample volume and flow rate, as well as the elutropic strength and volume of the elution solvent. In the initial experiments several sorbents were tested for the investigated pesticides from coffee samples to optimize the SPE conditions. Among the sorbents tested were graphitized carbon black (Envi-Carb), porous graphitic carbon (Hypercarb), C18 and NH_2 -LC. The best results, representing a compromise between good recoveries for polar and nonpolar pesticides, were obtained for Envi-Carb cartridge coupled with NH_2 -LC cartridge. For further optimization, elution solvent strength (ethyl acetate, ethyl acetate with various percentages of methanol or acetone, hexane–acetone) was varied to assess optimal conditions. The most critical variable governing the recovery and reproducibility of SPE was the percentage of acetone in the elution solvent system. In our study, best

Table 3 Validation parameter 2: average recovery and precision of GC-MS quantitation for 69 pesticides in coffee

Peak no.	Pesticide selected	Spiking level 1 (0.05mg/kg)		Spiking level 2 (0.1mg/kg)		Spiking level 3(1.0mg/kg)	
		Average recovery%	RSD%	Average recovery%	RSD%	Average recovery%	RSD%
1	EPTC	95	7.6	92	3.6	84	5.4
2	Butylate	112	5.2	102	3.2	97	1.9
3	Dichlobenil	75	4.7	93	4.4	87	2.3
4	Vernolate	65	5.8	89	5.6	78	6.2
5	Nitrapyrin	–	–	80	18.9	71	14.2
6	Tecnazene	78	5.2	81	3.9	88	2.2
7	Heptanophos	–	–	90	4.2	94	1.6
8	Hexachlorobenzene	63	6.4	103	2.6	85	7.3
9	Propachlor	–	–	101	4.2	92	4.2
10	Trifluralin	71	6.4	89	4.3	93	1.5
11	Chlorpropham	–	–	93	6.5	79	10.5
12	Sulfotep	69	5.8	97	6.6	85	6.7
13	Demeton-S	64	7.3	106	3.9	92	6.8
14	Terbumeton	80	4.7	81	1.5	93	5.9
15	Fonofos	74	5.8	84	6.7	89	4.9
16	Profluralin	–	–	93	6.7	86	2.8
17	Propazine	85	4.1	107	1.8	85	2.9
18	Disulfoton	70	4.7	90	3.6	94	2.8
19	Chlorbufam	–	–	81	5.8	72	2.7
20	Terbutylazine	87	5.8	96	3.7	83	1.3
21	Secbumeton	76	11.9	83	9.1	80	7.8
22	Heptachlor	–	–	106	1.6	92	5.9
23	Monocrotophos	–	–	124	6.6	82	7.8
24	Cyanophos	77	3.5	89	4.2	76	5.0
25	Fluchloralin	76	13.5	76	10.9	87	6.6
26	Chlorpyrifos-methyl	80	5.2	88	6.0	77	4.1
27	Alachlor	–	–	87	11.6	92	5.0
28	Pirimiphos-methyl	82	5.8	71	6.1	89	2.5
29	Simetryn	–	–	83	3.1	92	2.0
30	Metribuzin	–	–	75	14.2	94	7.0
31	Thiobencarb	89	4.7	93	2.5	95	9.0
32	Aspon	71	9.4	90	2.4	93	6.0
33	Pirimiphos-ethyl	–	–	93	3.3	83	3.9
34	Butralin	70	5.8	92	6.1	76	4.4
35	Isopropalin	78	8.8	86	1.5	79	7.2
36	Chlorthiamid	–	–	–	–	73	6.6
37	Isofenphos	76	8.8	97	2.4	104	5.6
38	Diphenamid	109	14.7	90	6.1	88	3.8
39	Chlorfenvinphos	–	–	91	5.0	109	6.8
40	Penconazole	–	–	86	8.9	83	4.2
41	Tolyfluanide	–	–	–	–	77	9.8
42	4,4'-DDE	90	4.1	90	5.1	91	3.1
43	Tribufos	69	5.2	95	4.3	98	7.7
44	Iodofenphos	60	11.7	94	2.1	78	4.4
45	Tetrachlorvinphos	–	–	75	3.1	86	7.0
46	Procymidone	106	8.8	91	2.0	114	5.2
47	Profenofos	–	–	107	1.2	109	6.9

Table 3 (continued)

Peak no.	Pesticide selected	Spiking level 1 (0.05mg/kg)		Spiking level 2 (0.1mg/kg)		Spiking level 3(1.0mg/kg)	
		Average recovery%	RSD%	Average recovery%	RSD%	Average recovery%	RSD%
48	Triadimenol	61	16.4	77	7.2	107	5.5
49	Chlorfenson	83	4.7	77	2.6	88	7.6
50	2,4'-DDD	73	22.3	82	10.6	91	9.1
51	Methoprotryne	74	1.1	96	1.6	73	4.1
52	Chloropropylate	67	6.4	71	4.1	86	5.7
53	Flamprop-methyl	86	4.1	97	3.6	88	3.6
54	Oxyfluorfen	–	–	–	–	94	6.1
55	2,4'-DDT	84	3.5	87	6.3	91	6.1
56	Flamprop-isopropyl	84	7.6	99	1.6	72	3.6
57	Fluorodifen	94	23.5	108	12.1	115	16.6
58	Myclobutanil	73	21.1	82	9.1	114	12.5
59	Benalaxyl	69	14.7	104	6.3	84	3.0
60	Bifenthrin	111	19.4	103	10.6	75	3.2
61	Bromopropylate	91	5.8	94	6.4	115	8.1
62	Benzoylprop-ethyl	77	7.6	88	8.6	96	1.6
63	EPN	68	12.9	86	11.0	78	6.1
64	Hexazinone	67	17.0	91	8.2	92	2.8
65	Nitralin	–	–	–	–	87	3.4
66	Phosalone	–	–	92	6.0	119	3.9
67	Fenarimol	76	10.5	102	2.0	108	6.0
68	Azinphos-ethyl	100	4.7	96	7.2	81	2.8
69	Coumaphos	–	–	–	–	84	2.9

Covering coffee samples with three spiked levels ($n=5$)

recoveries were obtained when the elution solvent was acetone–ethyl acetate (2:5 v/v).

Selection of GC-MS Conditions

The GC conditions were optimized to enable separation of the 69 pesticides with a single GC column (DB-1701, 30 m×0.25 mm i.d.×0.25 μ m). Different temperature regimes, flow rates and column identities were tested and verified in order to resolve the analytes of the standard mixture during an acceptable run time.

Column Temperature

In this study, different initial oven temperatures and different ramping programs were assayed. There was a clear decrease in the total run time when initial temperature increased from 40 to 80°C, but the peak width of the more volatile pesticide residues decreased significantly. Based on this result, 40°C was adopted as initial oven temperature. Higher program rates (30°C/min) provided an increase in speed analysis without peak separation decrease.

Flow Rate

In this study, three different constant gas flow rates (0.8, 1, 2 ml/min) were tested being evaluated the chromatographic separation and the S/N ratio of each pesticide. The best results were obtained with 1 ml/min; thus, this flow speed was chosen.

MS Conditions

We initially carried out a scanning test of the standard solution of each pesticide to be analyzed to describe its scanning mass spectrogram and retention time. Once retention times of each analyte were determined in full scan mode, the next step involved the determination of the optimal SIM mode conditions, in order to obtain for each pesticide a correct identification, a chromatographic peak with enough number of points (four to 15 points along the peak), and a good signal intensity. The relevant considerations in SIM mode parameters included a careful choice of the number of ions selected for each pesticide as well as for each time window, and the dwell time (i.e., the time spent

monitoring a single ion). We selected one target ion and two or three qualitative ions for each compound (Table 1)

Validation Procedure

Coffee samples free of pesticides were used for the preparation of a blank matrix. The typical chromatogram of a blank coffee sample is shown in Fig. 1. No matrix interference GC peaks were detected in the SIM chromatograms for targeted pesticides obtained in analyses of blank coffee samples, demonstrating that the method has good selectivity. The selection of the extracting solvent in sample pretreatment process with a proper polarity to match the analyte of interest was beneficial to improve process efficiency and minimize potential interferences from coffee, and little matrix effect on MS detection of low level coffee samples was found under the optimized extraction and chromatographic conditions (Fig. 2).

We undertook quantitative analysis using an external standard. The calibration curve was obtained by analyzing blank coffee samples spiked with the pesticides at five different levels. The signals from the chromatograms of ten blank coffee samples were evaluated as recommended (2002/657/EC) to estimate the lower LOD (Table 2).

We validated analytical results by establishing the precision and recovery of the analysis on quality control samples. Precision was represented by an estimate of the variability of measurements and the reproducibility of the test method. We tested recovery of each pesticide at different concentrations to assess the accuracy of the method. Every spiking level was assessed in five repetitions. The precision of the method was described as the value of relative standard deviation (RSD), and the recovery of the assay was calculated from the percentage of the mean calculated concentration to the nominal spiking value. The concentration of the different spiking levels, recovery data and RSD values obtained are shown in Table 3. Recoveries ($n=5$) were calculated as follows: $\% \text{Recovery} = [(c_F - c_U) / c_S] \times 100$, where c_F = concentration of pesticides measured in the fortified sample, c_U = concentration of pesticides measured in the unfortified sample (set to zero), and c_S = concentration of pesticides added to the fortified sample. Recoveries of the analytes ranged from 60% to 119%. Repeatability of peak areas for all pesticides expressed as RSD was in the range of 1.3–22.3%.

The proposed method was also applied to commercial coffee samples. Six samples were obtained from equal lots of coffee imported to China during 2007 and 2008, and processed in triplicate according to the proposed method. In all samples, no pesticides were detected at low levels.

Conclusions

A multi-residue analysis method based on GPC-SPE-GC-MS for sensitive identification and determination of 69 pesticides in coffee has been demonstrated in this work. The liquid–liquid extraction using a mixture of ethyl acetate was optimized as the typical solvent for extracting multi-class pesticides from coffee samples. With further cleanup by GPC and SPE, the pretreatment provides high extraction efficiency and low matrix effects, thus allowing adaptation of this sensitive and selective method for routine multi-residue analysis of pesticides in coffee matrices. The method described here was found to have good practicality for routine residue analysis of pesticides in coffee matrices.

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