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Development of automated online gel permeation chromatography–gas chromatograph mass spectrometry for measuring multiresidual pesticides in agricultural products

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Abstract

An automated online gel permeation chromatography–gas chromatograph mass spectrometer (GPC–GC/MS) was developed for the rapid determination of residual pesticides in agricultural products. Pesticides were extracted from homogenized food samples with acetonitrile and decontaminated via the matrix solid-phase dispersion (MSPD) technique, using a primary secondary amine as sorbent prior to GPC–GC/MS analysis. A slightly modified preparation method and automated GPC step proved useful in minimizing matrix interference. To evaluate the performance of the system, 97 target pesticides were spiked at a concentration of 0.1 mg/kg into a range of food types, including potato, cabbage, carrot, apple, orange, cucumber, and rice. A low flow rate of 0.1 mL/min in GPC resulted in a 40-fold reduction in solvent consumption compared with conventional GPC column applications. The combination of MSPD technique and GPC–GC/MS for the analysis of the 97 pesticides can be accomplished within 90 min. Most pesticides were recovered in the range of 70–120%, with relative standard deviation generally less than 10%. The results demonstrate that the method can be successfully applied with acceptable recoveries to a broad range of target pesticides within a diverse range of food types.

Keywords: Residual pesticides; Agricultural products; GPC–GC/MS

1. Introduction

In recent years, with increasing public awareness of food safety, to develop some rapidly and accurately analytical methods are required to be developed for the determination of residual pesticides in agricultural products. Generally, the complex matrix of agricultural products adversely affects analysis precision, and it is necessary to remove the matrix interference by sample pre-treatment, such as extraction and clean-up steps [1,2]. In order to improve the quantitative analysis, many efforts have been made to develop extraction methods and clean-up protocols [3]. However, the off-line sample preparation often suffers from time consuming, high cost and poor reproducibility. Therefore, an automatic sample pre-treatment is desirable and popularly studied. As one of the powerful analytical methods, gel permeation chromatography (GPC) is a recently developed and popular post-extraction cleanup method. GPC is highly effective in removing high molecular-weight interferences, such as lipids, proteins and pigments prior to analysis by gas chromatography (GC), gas chromatograph mass spectrometry (GC/MS), high-performance liquid chromatograph (HPLC), and liquid chromatograph mass spectrometry (LC/MS). The use of GPC greatly reduces the downtime of instrument, extends column life and increases the analytical precision and accuracy [4–6]. In addition, GPC has indicated the potential for automated analysis with LC or GC; several groups have successfully introduced an automated GPC clean-up technique for the determination of pesticides [7–9]. However, considering the entire analytical process, much effort is still required to reduce analysis time and cost, such as reducing the consumption of solvent. Recently, the use of

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an online gel permeation chromatography–gas chromatograph mass spectrometry (GPC–GC/MS) for the analysis of residual pesticides has been simply introduced by Hashi et al. [10].

In the present study, on the basis of our previous work [10], an automated online GPC–GC/MS for measuring residual pesticides in agricultural products is proposed. This system can determine 97 pesticides in 90 min. A diverse range of pesticides, mainly including organophosphorus, organochlorine, organonitrogen, carbamate, and thiocarbamate substances were selected as test targets. The precision and accuracy of the method were validated by seven different agricultural products spiked with 0.1 mg/kg of residue. The matrix solid-phase dispersion (MSPD) technique with slight modification was employed for the clean-up of samples. This technique is suitable for the clean-up of homogeneously dispersed samples and can be applied to GC or GC/MS analysis [11–19]. After extraction, the samples were injected into the automated online GPC–GC/MS system. The combination of the MSPD technique and online GPC–GC/MS system enables us to accomplish a high throughput of pesticide analysis at low cost and satisfactory recovery.

2. Experimental

2.1. Chemicals and solvents

All chemicals used were of analytical reagent or chromatographic grade. Acetone, acetonitrile, and cyclohexane were obtained from Tedia Company (Fairfield, OH, USA). The HPLC grade-water was obtained by purification of de-ionized water through a Milli-Q system (Bedford, USA) with a 0.22-μm fiber filter.

Analytical grade sodium chloride was obtained from Beijing Chemical Factory (Beijing, China). Anhydrous magnesium sulfate was obtained from Kanto Chemical (Tokyo, Japan). Primary secondary amine (PSA) sorbent [Bond Elut PSA] was obtained from Varian (Harbor, USA).

Fluvalinate and chinomethionate, used as markers, were both of analytical standard and obtained from Sigma-Aldrich (USA).

Two groups of pesticides stock standard solutions (for pesticide residue analysis) were obtained from Kanto Chemical (Tokyo, Japan). One group contained 50 pesticides and another 47 pesticides, with both being dissolved in acetone. The concentrations of the two group stock standard solutions were 10 μg/mL, and they were stored at −25 °C. Prior to each experiment, the solutions containing 97 pesticides for analysis were prepared by mixing the two group stock standard solutions and diluting by acetone into the desired concentration.

2.2. Apparatus

Fig. 1 shows a schematic diagram of the gel permeation chromatography–gas chromatograph mass spectrometry system. The GPC consists of two LC-10ADvp pumps, a SIL-10ADvp auto-sampler, a Shodex CLNpak EV-200AC column (2 mm i.d. × 150 mm) and CTO-10ASvp column oven, a SPD-10Avp UV detector, two FCV-12AH flow channel selection valves (RV.A, RV.B) and a SCL-10Avp system controller. GC/MS machine is a Shimadzu GC/MS-QP2010 instrumentation equipped with a PTV-2010 large-volume injection device. GC/MS data analysis was triggered by a contact closure start signal from the HPLC controller. Data acquisition was performed using a C-R8A plus data processor. All these parts are the products of Shimadzu (Kyoto, Japan), except the Shodex CLNpak EV-200AC column (Shoko Co., Tokyo, Japan). Acetone/cyclohexane mixing solvent (3/7, v/v) was used as the mobile phase of GPC, and the flow rate was set at 0.1 mL/min. The mobile phase was degassed using DGU-14A degasser (Shimadzu), and the GPC column was kept at 40 °C in the column oven.

2.3. Sample preparation

All samples were purchased at local markets in Beijing. Samples were extensively crushed to achieve good sample homogeneity. After crushing, in the case of rice, an extra step of filtering through a sifter (0.45 mm aperture) was required. Once homogenized, samples were stored at −25 °C until GPC–GC/MS analysis.

To prepare each sample, 10 g of a previously homogenized food material was transferred into a suitable glass vessel (for rice,

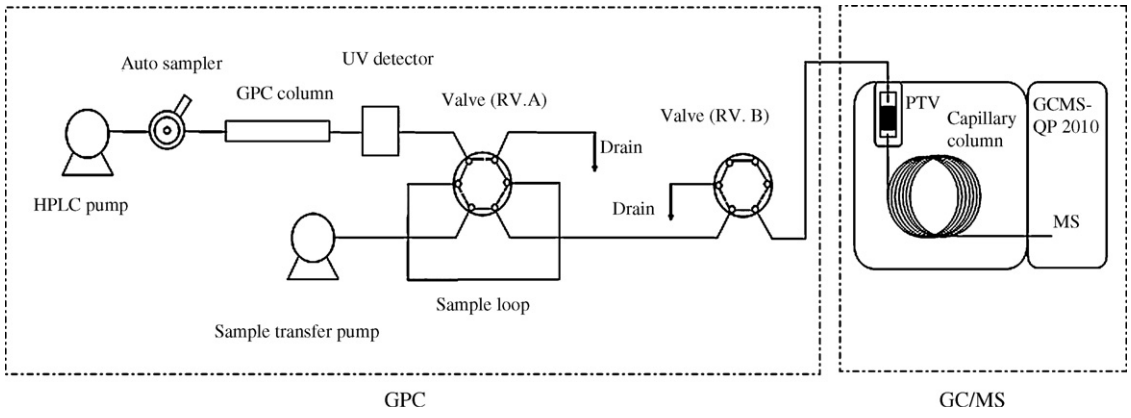

Fig. 1. Schematic flow diagram of the GPC–GC/MS system.

a further 10 mL deionized water was added). Then, 10 mL acetonitrile was added to each sample using an adjustable-volume solvent dispenser. The glass vessels were capped before vortex mixing for 1 min at maximum speed. Once the initial sample mixing was completed, 1 g NaCl and 4 g anhydrous $MgSO_4$ were added and mixed immediately on a Vortex mixer for 1 min. It is important to note that this step must be taken immediately after the initial mixing step to prevent the formation of $MgSO_4$ conglomerates. To separate phases, samples were centrifuged for 10 min at 1570 × *g*. Using an adjustable repeating pipette, 1.0 mL aliquot of upper acetonitrile layer was transferred into a 1.5 mL flip-top microcentrifuge vial containing 150 mg anhydrous $MgSO_4$ and 50 mg PSA sorbent. The vial was tightly capped and shaken on a vortex mixer for 1 min before extraction. Then the mixed extraction solution was centrifuged for 5 min to separate solids from solution. The solution was then transferred into an autosampler for GPC–GC/MS analysis. For spiked samples, standard pesticides were spiked into the samples before adding acetonitrile for extraction. The other steps are the same as those described above.

2.4. Procedure

Before the GC/MS determination, the GPC procedure was carried out on the GPC–GC/MS system. As shown in Fig. 1,

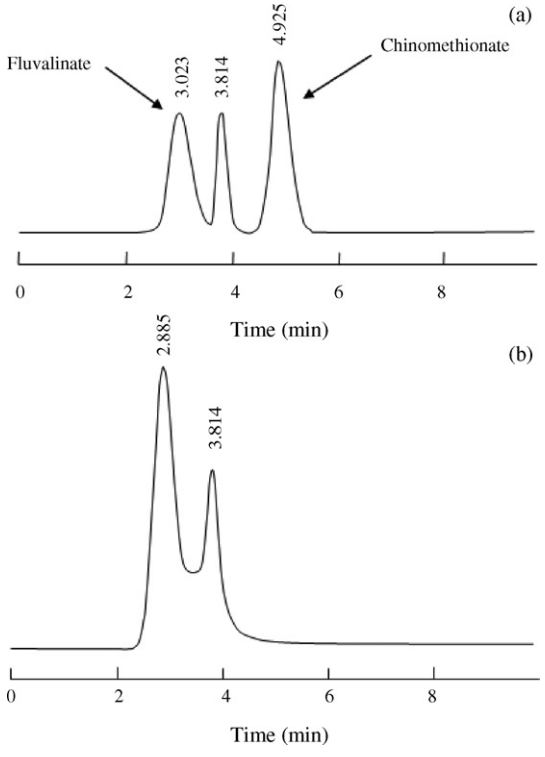

Fig. 2. GPC chromatograms obtained at a UV wavelength of 210 nm. (a) GPC chromatogram of two markers, fluvalinate (MW = 502.9) as the upper molecular weight marker and chinomethionate (MW = 234.3) as the lower molecular marker at 1000 ng/mL. (b) GPC chromatogram of rice sample spiked with a mixture of 97 standard pesticides at 2350.1 mg/kg. Acetone/cyclohexane mixed solvent (3/7, v/v) was used as the mobile phase of GPC and the flow rate was set at 0.1 mL/min. The GPC column was kept at 40 °C in the column oven.

Table 1
Retention times and quantification ions for all analyzed pesticides

	Pesticide	Retention time (min)	Quantification ion (*m*/*z*)
1	Methamidophos	10.333	94
2	DDVP	10.507	109
3	EPTC	11.968	128
4	Butylate	12.974	156
5	Acephate	13.031	136
6	Isoprocarb	14.497	121
7	BPMC	15.474	121
8	Ethoprophos	15.880	158
9	Chlorpropham	16.235	213
10	Bendiocarb	16.404	151
11	Cadusafos	16.606	158
12	α-BHC	16.710	219
13	Thiometon	16.806	88
14	β-BHC	17.401	219
15	Dimethipin	17.442	118
16	γ-BHC	17.579	219
17	Terbufos	17.786	231
18	Diazinon	18.035	304
19	δ-BHC	18.149	219
20	Etrimfos	18.326	292
21	Tefluthrin	18.439	177
22	Ethiofencarb	18.475	107
23	Pirimicarb	18.590	166
24	Benfuresate	18.955	121
25	Methyl-Parathion	19.238	263
26	Tolclofos-methyl	19.381	265
27	NAC	19.416	144
28	Pirimiphos-methyl	19.756	305
29	MEP	19.860	277
30	Methiocarb	19.959	168
31	Dichlofluanid	20.019	224
32	Esprocarb	20.080	222
33	Malathion	20.165	173
34	Metolachlor	20.226	162
35	Chlorpyrifos	20.298	197
36	Thiobencarb	20.382	100
37	(Z)-Dimethylvinphos	20.429	295
38	Diethofencarb	20.456	267
39	Parathion	20.605	291
40	Isofenphos Oxon	20.678	229
41	DCBP	20.832	139
42	Fosthiazate	20.886	195
43	Pendimethalin	21.096	252
44	α-CVP	21.244	267
45	Isophenphos	21.353	213
46	Captan	21.401	117
47	PAP	21.562	274
48	Quinalphos	21.678	146
49	Tricyclazole	21.747	189
50	Triadimenol	21.815	112
51	Chinomethionat	21.901	234
52	Pyrifenox	21.951	262
53	Paclobutrazol	22.087	236
54	β-CVP	22.466	267
55	Flutolanil	22.492	173
56	Prothiofos	22.509	309
57	Pretilachlor	22.571	238
58	*p*,*p*'-DDE	22.662	318
59	Myclobutanil	22.800	179
60	Flusilazole	22.890	233
61	Cyprocpnazole	23.197	222
62	Chlorobenzilate	23.459	251
63	MPP	23.520	278

Table 1 (*Continued*)

	Pesticide	Retention time (min)	Quantification ion (*m/z*)
64	Fensulfothion	23.565	293
65	*p,p*'-DDD	23.676	235
66	Mepronil	24.064	119
67	EDDP	24.358	310
68	Propiconazole	24.418	259
69	Lenacil	24.486	153
70	Thenylchlor	24.750	127
71	Tebuconazole	24.888	250
72	Difolatan	24.985	107
73	Acetamiprid	25.472	152
74	Iprodione	25.532	314
75	EPN	25.696	157
76	Tebufenpyrad	26.095	318
77	Phosalone	26.462	182
78	Pyriproxyfen	26.693	136
79	Mefenacet	26.750	192
80	Cyhalothrin	26.974	181
81	Fenarimol	27.052	251
82	Acrinathrin	27.254	181
83	Pyraclofos	27.486	360
84	Bitertanol	27.804	170
85	Permethrin	27.909	183
86	Pyridaben	28.109	147
87	Cyfluthrin	28.800	163
88	Halfenprox	29.062	263
89	Cypermethrin	29.133	163
90	Flucythrinate	29.471	199
91	Silafluofen	29.574	286
92	Pyrimidufen	29.872	184
93	Fenvalerate	30.073	225
94	Fluvalinate	30.343	250
95	Difenoconazole	30.673	323
96	Deltamethrin	30.988	253
97	Imibenconazole	32.001	125

acetonitrile extracts of samples were injected onto the GPC column. Acetone/cyclohexane mixed solvent (3/7, v/v) was used as the sample delivery solvent. Sample clean-up and transfer were achieved by changing the flow line on the flow channel selection valves (RV.A, RV.B). The LC-10ADvp pump transferred the target pesticides to the sample loop and then delivered them to the PTV injector. In this system, a semi-micro GPC column was employed to reduce mobile phase consumption and lower operating costs. In this experiment, the injection volume was 10 μL and the volume of the sample loop was set to 200 μL.

For the GC/MS determination, the temperature of the PTV injector was set at 120 °C for the initial 5 min of sampling time, and then increased to 250 °C at 100 °C/min. An RTX-5 ms column ((5% phenyl) methylpolysiloxane; 0.25 mm i.d. × 30 m with a film thickness of 0.25 μm; Restek Corporation, Bellefonte, PA, USA) was used for the separation of the pesticides. The temperature of the capillary column was set at 82 °C for the initial 5 min and increased to 300 °C at 8 °C/min. Helium was used as the carrier gas. The quadrupole mass spectrometer was operated in the electron impact ion (EI) mode with a source temperature of 230 °C and electron energy of 70 eV. Chromatograms were acquired in 'scan' mode, scanning from *m/z* 86 to *m/z* 500.

Table 2
Regression data for representative pesticides

Pesticide	$y = ax^2 + bx + c$			*r*
	a	*b*	*c*	
EPTC	4.3355	7593.205	60394.5	0.9998
Butylate	2.731533	3955.23	47570.17	0.9997
α-BHC	1.55405	2477.382	13524.25	0.9998
δ-BHC	1.156636	1558.815	10873.18	0.9997
NAC	13.95821	11939.0	−65252.46	0.9995
Diethofencarb	2.132611	933.7883	10793.56	0.9991
MPP	11.30101	4272.468	−29395.44	0.9989
Chlorpyrifos	1.481919	2362.142	−7932.403	0.9998
Tebufenpyrad	3.301733	2073.86	15727.67	0.9993
Pyridaben	22.14579	8767.302	76838.93	0.9990

y and *x* are the peak area and concentration (ng/ml) of the pesticides, respectively. Correlation coefficients are expressed as *r*.

3. Results and discussion

3.1. GPC conditions

In optimizing the transfer of solvent between the GPC and GC/MS systems, the GPC mobile phase flow rate was reduced compared to that in conventional GPC. In this application, a flow rate of 0.1 mL/min was used with a 2 mm i.d. GPC column; this resulted in a 40-fold reduction in solvent consumption compared to conventional GPC column applications. To investigate the fraction time of the pesticides, two marker molecules were selected. Fluvalinate (MW = 502.9) was used as the upper molecular weight marker and Chinomethionate (MW = 234.3) was used as the lower molecular weight marker, corresponding to a retention time of between 3.023 and 4.925 min (the GPC chromatograms of the two marker molecules and the rice sample spiked with 97 pesticides standard are shown in Fig. 2). In this study, GPC eluent from 2.9 to 4.9 min was fractionated by the sample loop.

Table 3
Estimated limits of detection (LOD) and quantitation (LOQ) calculated as the concentrations that produced a signal equal to 3-times and 10-times the background noise level, respectively, in GPC–GC/MS

Pesticide	LOD (μg/kg)			LOQ (μg/kg)		
	Potato	Apple	Rice	Potato	Apple	Rice
EPTC	9	18	13	31	59	44
Butylate	38	79	30	127	262	101
α-BHC	15	14	16	51	48	52
δ-BHC	17	18	26	56	57	87
NAC	3	21	18	11	69	60
Diethofencarb	26	24	15	85	75	49
MPP	29	8	8	97	27	28
Chlorpyrifos	27	22	18	88	72	61
Tebufenpyrad	10	16	14	36	53	47
Pyridaben	7	14	17	23	46	55

3.2. Analysis time

By combining automated GPC clean-up with GC/MS analysis, the total analysis time for 97 target pesticides was only 50 min. As 40 min is sufficient for sample preparation, the combination of the MSPD technique and GPC–GC/MS instrument enables us to complete the analysis of 97 residual pesticides within 90 min per sample. The retention times and ions used for quantification of the pesticides are shown in Table 1.

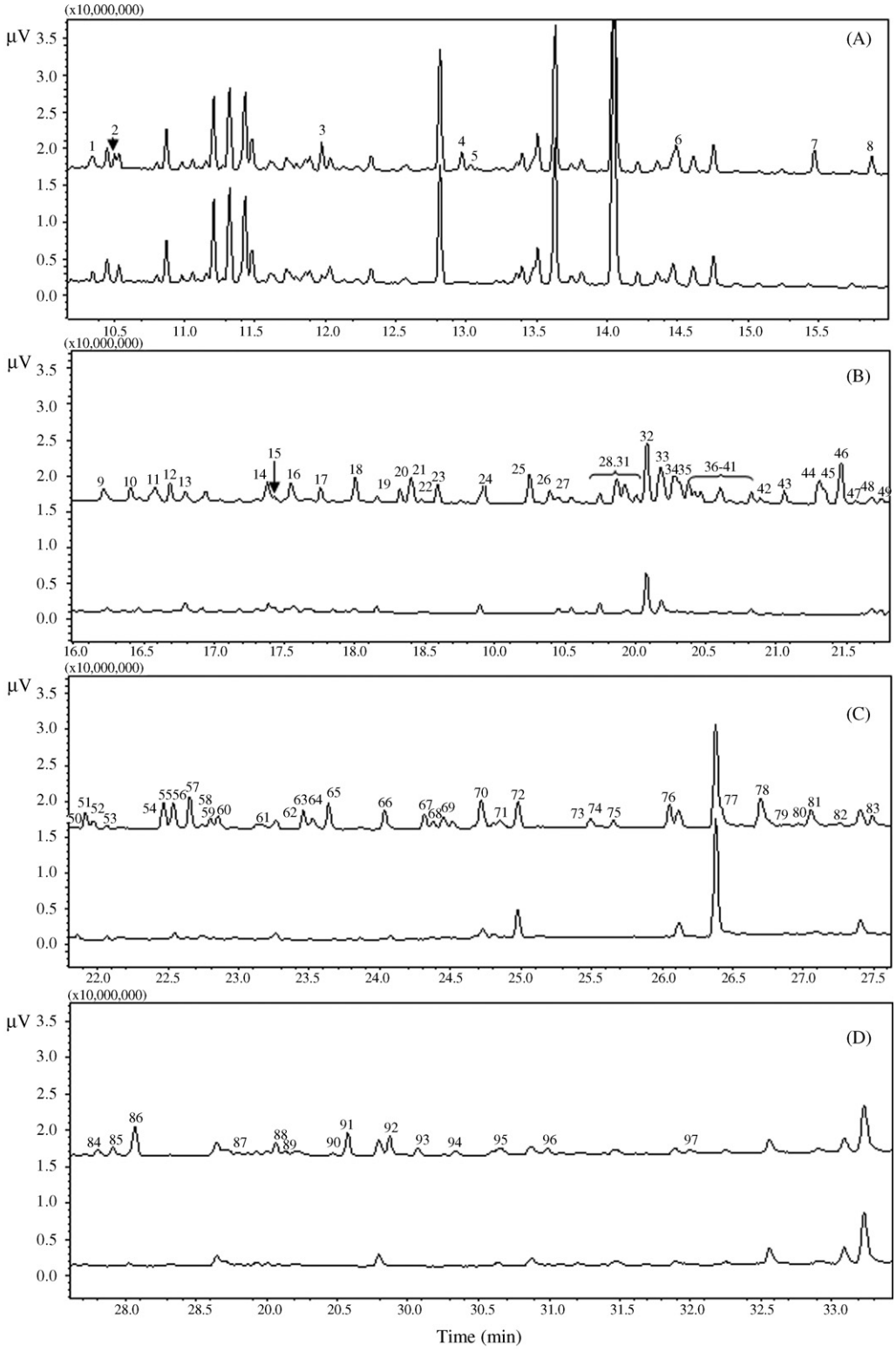

Fig. 3. Total ion chromatograms (TIC) of potato spiked with 97 pesticides at 0.1 mg/kg (upper chromatogram) and unspiked potato (lower chromatogram). Multiple chromatograms (A, B, C and D) are used to show all the pesticides. The order of peaks corresponds to the order of pesticides listed in Table 1.

Table 4
Comparison of recovery (%) achieved by GPC–GC/MS and GC/MS

	Pesticide	Potato			Cabbage			Carrot			Cucumber			Apple			Orange			Rice		
		GPC–GC/MS		*GC/MS*	GPC–GC/MS		GC/MS	GPC–GC/MS		GC/MS	GPC–GC/MS		GC/MS	GPC–GC/MS		GC/MS	GPC–GC/MS		GC/MS	GPC–GC/MS		GC/MS
		Mean (%)	R.S.D. (%)	Mean (%)	Mean (%)	R.S.D. (%)	Mean (%)	Mean (%)	R.S.D. (%)	Mean (%)	Mean (%)	R.S.D. (%)	Mean (%)	Mean (%)	R.S.D. (%)	Mean (%)	Mean (%)	R.S.D. (%)	Mean (%)	Mean (%)	R.S.D. (%)	Mean (%)
1	Acephate	73	0.3	194	74	1.1	205	70	1.3	220	59	1.3	75	57	3.5	116	55	2.9	108	60	5.7	108
2	Acetamiprid	71	10.7	89	91	2.8	100	99	4.4	108	88	4.1	106	86	9.8	88	99	11.6	101	92	3.6	198
3	Acrinathrin	115	5.7	85	135	5.4	113	188	2.8	88	110	4.5	104	134	1.4	116	121	2.3	120	111	7.6	114
4	Bendiocarb	86	0.3	71	87	2.1	94	92	3.0	75	100	1.3	97	87	3.5	107	106	3.5	165	101	1.9	111
5	Benfuresate	94	1.0	75	94	0.9	77	102	0.8	77	108	1.3	71	96	1.8	78	111	2.4	114	97	3.2	87
6	α-BHC	97	1.5	76	97	1.0	79	102	2.4	79	113	3.8	73	104	2.4	56	114	5.0	107	118	2.5	69
7	β-BHC	107	0.3	80	106	3.2	83	112	4.1	83	103	1.1	90	120	1.7	99	137	3.5	113	104	1.3	89
8	γ-BHC	99	0.9	76	101	2.6	77	106	1.6	76	112	2.4	84	108	2.0	63	116	4.5	114	105	2.9	72
9	δ-BHC	105	1.1	66	106	2.7	82	108	0.7	75	110	1.2	68	115	1.2	53	111	4.3	76	110	2.0	56
10	Bitertanol	125	2.2	95	119	2.4	91	100	7.5	114	118	1.1	189	109	1.3	115	111	7.8	130	117	2.5	199
11	BPMC	98	1.7	79	97	1.3	86	100	1.4	74	104	1.4	93	109	1.3	79	119	1.4	119	111	1.9	83
12	Butylate	110	1.2	68	111	3.4	69	110	2.7	69	100	1.9	64	90	0.5	57	112	4.0	93	106	5.5	60
13	Cadusafos	104	0.7	74	102	2.3	77	108	2.8	80	110	2.1	92	118	3.3	81	112	6.7	97	116	1.6	84
14	Captan	91	3.0	69	66	1.4	ND	77	2.4	51	110	5.1	37	101	0.4	61	145	6.3	86	26	5.6	25
15	Chinomethionat	56	1.8	49	55	2.1	49	55	2.3	48	16	5.3	43	19	10.6	44	35	4.2	53	22	9.9	41
16	Chlorobenzilate	114	1.5	86	111	3.0	84	117	1.8	92	115	5.0	129	127	4.3	116	115	3.8	148	148	4.6	143
17	Chlorpropham	112	1.9	72	113	1.2	77	115	3.5	76	118	1.7	77	109	1.3	72	117	0.8	110	117	1.7	77
18	Chlorpyrifos	97	0.8	70	95	3.3	71	100	4.0	73	113	0.9	88	114	1.3	82	111	5.3	120	116	3.2	98
19	α-CVP	103	4.1	91	104	3.5	95	107	1.7	94	115	2.2	119	112	2.2	116	105	4.2	160	115	2.8	117
20	β-CVP	104	0.5	91	100	2.7	95	106	2.2	94	116	2.2	119	112	2.2	119	105	4.2	153	117	3.6	130
21	Cyfluthrin	92	7.2	67	101	2.6	79	124	7.0	75	101	0.9	105	96	4.9	110	115	7.4	95	112	1.3	116
22	Cyhalothrin	112	2.5	86	121	2.9	112	118	2.5	110	115	3.5	113	99	0.5	107	154	1.3	118	150	1.4	132
23	Cypermethrin	106	4.6	55	104	3.6	85	103	1.8	79	112	4.8	91	124	2.7	79	111	2.5	91	117	2.8	98
24	Cyproconazole	106	4.3	83	105	4.3	84	117	4.1	91	100	3.3	125	113	1.1	109	102	4.8	139	155	4.3	134
25	DCBP	104	6.0	78	108	4.7	82	120	1.7	80	191	6.9	88	181	0.8	81	159	8.1	114	197	7.8	95
26	*p,p*'-DDD	105	0.3	82	104	1.9	87	107	2.0	89	110	1.8	103	119	1.1	85	113	5.2	118	120	7.1	114
27	*p,p*'-DDE	100	4.0	82	97	1.6	82	95	0.8	81	113	0.3	85	109	2.6	80	120	5.3	115	113	4.8	87
28	DDVP	88	0.8	72	88	1.7	78	91	1.3	73	98	2.1	74	85	1.1	67	98	4.3	99	105	0.8	64
29	Deltamethrin	109	3.3	78	95	6.1	81	111	4.3	108	64	2.5	62	62	0.7	92	63	1.6	82	44	0.6	31
30	Diazinon	106	0.7	76	104	3.4	78	109	2.2	79	121	0.7	86	111	1.4	83	119	3.8	116	120	3.2	86
31	Dichlofluanid	72	5.6	63	62	2.0	58	50	3.9	61	37	0.2	47	61	8.4	58	56	7.6	81	20	5.5	44
32	Diethofencarb	117	3.1	86	106	4.9	84	115	2.1	89	114	2.1	102	99	2.7	102	115	3.1	152	132	0.9	128
33	Difenoconazole	123	4.1	95	115	8.9	87	137	8.1	117	163	3.0	143	113	1.8	111	112	5.0	142	112	4.8	144
34	Difolatan	90	4.2	*ND*	58	7.0	ND	112	5.3	ND	65	5.6	45	68	5.6	96	87	3.8	53	19	5.5	46
35	Dimethipin	77	1.3	84	79	3.5	89	79	1.7	73	72	3.7	85	54	1.5	85	71	7.4	112	72	2.3	73
36	(Z)-Dimethylvinphos	97	1.6	94	94	2.1	99	102	2.0	97	112	4.7	112	104	0.6	116	115	3.4	164	113	2.4	116
37	EDDP	107	0.9	132	107	1.3	143	118	2.8	128	94	2.3	143	119	2.0	136	116	1.8	180	115	2.3	169
38	EPN	110	0.9	95	112	2.9	97	138	4.7	113	94	4.8	158	116	1.5	103	97	6.3	113	112	4.8	183
39	EPTC	99	1.0	69	97	1.5	69	101	1.7	70	120	0.2	60	111	1.1	50	118	2.9	84	116	1.3	60
40	Esprocarb	99	1.3	77	98	1.9	79	103	1.4	82	117	0.7	91	111	3.0	85	117	1.2	116	116	2.3	94
41	Ethiofencarb	94	5.1	105	92	2.5	121	91	3.3	98	72	7.4	113	95	6.4	115	88	6.8	177	77	3.7	112
42	Ethoprophos	99	0.5	78	100	1.9	78	103	3.0	82	112	1.4	84	113	2.0	85	114	0.8	118	107	1.8	79
43	Etrimfos	103	0.6	74	101	2.3	75	107	3.4	78	119	1.5	103	119	1.2	93	110	6.1	132	114	2.5	95
44	Fenarimol	100	1.3	85	99	3.0	85	111	3.0	95	108	1.7	132	118	1.2	110	112	5.9	141	112	4.3	143
45	Fensulfothion	99	1.5	89	102	1.8	97	119	1.4	122	110	5.7	136	101	1.6	110	78	6.2	110	111	6.0	140
46	Fenvalerate	101	6.5	74	101	2.2	105	104	2.1	89	113	3.4	81	115	2.0	113	119	1.0	111	112	2.0	115

47	Flucythrinate	114	2.8	93	120	2.8	111	120	3.8	110	153	5.8	116	110	4.2	119	118	5.1	129	117	6.3	116
48	Flusilazole	93	1.2	80	92	2.5	85	97	2.1	87	116	5.0	115	98	4.7	107	117	1.8	120	111	3.3	117
49	Flutolanil	113	2.6	85	110	1.8	88	110	5.4	104	116	6.4	120	106	0.6	115	114	6.8	139	157	1.0	130
50	Fluvalinate	142	5.9	82	155	0.5	133	152	1.6	75	145	4.0	112	135	2.1	127	151	3.7	113	162	4.8	129
51	Fosthiazate	108	0.7	127	105	3.6	127	118	3.9	129	103	3.3	110	106	7.3	147	105	5.9	193	90	4.2	92
52	Halfenprox	94	1.1	92	97	1.6	99	103	3.7	115	158	1.0	120	106	1.5	120	117	2.0	130	120	2.6	139
53	Imibenconazole	157	8.1	88	156	9.2	99	180	5.9	113	119	3.2	110	110	2.6	75	115	6.3	107	105	5.1	104
54	Iprodione	103	0.8	138	104	2.6	156	112	1.7	91	104	1.1	111	120	0.7	117	106	4.3	97	119	1.5	136
55	Isofenphos Oxon	120	2.8	118	117	2.8	112	88	6.0	110	102	3.2	191	116	3.8	167	105	4.0	178	139	1.4	195
56	Isophenphos	116	1.5	80	114	2.7	83	120	1.2	88	118	2.5	118	115	0.8	112	118	2.1	135	144	1.7	118
57	Isoprocarb	95	0.6	80	96	2.1	87	96	2.1	69	112	1.3	88	100	0.8	81	113	3.5	114	115	0.7	83
58	Lenacil	109	0.6	91	107	2.1	92	119	2.4	104	111	7.4	124	112	2.4	118	117	2.9	134	168	2.9	143
59	Malathion	97	0.8	83	95	1.3	91	104	2.1	90	116	3.1	113	110	1.4	106	98	4.3	139	119	0.7	112
60	Mefenacet	104	1.3	93	108	1.7	95	116	3.4	107	96	3.2	125	93	3.2	115	96	7.6	131	99	4.7	144
61	MEP	100	2.1	81	102	1.5	74	112	3.7	94	113	2.5	120	119	0.6	119	87	6.4	156	114	6.4	125
62	Mepronil	109	2.0	85	110	2.3	85	119	1.6	93	112	0.8	120	119	2.2	116	116	2.1	129	153	3.6	134
63	Methamidophos	68	1.2	71	72	2.0	84	71	0.8	68	61	9.3	109	57	5.9	110	56	3.6	111	48	5.0	103
64	Methiocarb	112	2.1	90	114	3.0	126	118	2.1	80	115	1.5	134	103	2.2	113	115	2.8	185	117	2.2	109
65	Methyl-Parathion	104	1.4	85	120	3.5	82	115	4.6	92	119	1.4	108	118	2.7	115	84	2.1	151	114	2.0	120
66	Metolachlor	102	0.3	80	99	2.5	80	107	2.2	86	107	1.4	118	116	0.6	100	106	8.3	139	118	2.4	117
67	MPP	63	1.1	76	57	1.8	81	56	1.7	83	149	1.3	95	108	2.5	89	118	2.2	112	97	5.6	88
68	Myclobutanil	91	2.1	84	92	1.6	84	98	2.2	93	91	1.8	110	110	1.9	111	93	6.2	112	118	0.5	128
69	NAC	99	0.9	78	106	2.6	124	109	5.2	78	113	1.5	115	98	0.6	120	110	6.9	146	111	3.7	111
70	Paclobutrazol	106	0.1	84	102	2.9	83	112	4.1	92	106	6.2	118	115	0.9	110	108	3.7	150	120	7.8	81
71	PAP	98	0.8	78	95	2.2	80	106	3.2	89	119	1.3	111	118	2.8	103	102	5.6	135	112	6.2	114
72	Parathion	114	1.9	83	108	3.8	77	120	3.2	96	119	1.1	136	84	1.6	118	101	5.6	158	117	4.1	135
73	Pendimethalin	110	0.5	85	108	3.0	79	120	2.7	96	93	3.5	129	119	1.0	114	102	6.5	154	117	2.2	138
74	Permethrin	113	0.4	92	111	2.6	95	115	2.6	98	117	0.5	108	111	3.4	112	116	5.4	109	114	3.6	119
75	Phosalone	111	0.5	87	116	2.3	95	120	2.6	103	105	4.1	131	94	2.0	115	146	4.4	97	113	3.0	151
76	Pirimicarb	92	0.5	76	89	1.8	78	97	2.6	80	107	2.1	82	97	0.6	79	107	6.5	114	110	2.7	89
77	Pirimiphos-methyl	103	0.5	76	102	3.0	82	108	1.4	84	111	2.2	103	118	1.2	98	114	4.8	131	118	2.1	112
78	Pretilachlor	114	0.5	88	113	1.8	83	118	7.8	101	117	1.8	134	119	0.2	120	120	0.2	164	118	3.4	146
79	Propiconazole	97	0.6	85	98	4.2	86	105	2.9	92	94	1.2	143	94	2.5	113	94	3.9	115	110	6.2	136
80	Prothiofos	103	0.8	80	101	1.2	83	86	4.0	85	109	1.6	104	120	0.3	97	111	6.4	130	119	5.3	114
81	Pyraclofos	108	1.7	141	107	1.4	157	191	5.5	161	104	0.3	164	109	3.8	151	108	5.4	185	108	3.4	199
82	Pyridaben	120	1.1	89	121	1.4	96	83	1.6	103	118	2.0	116	118	2.5	117	118	1.9	113	117	5.4	130
83	Pyrifenox	97	0.9	79	94	2.3	81	102	2.7	85	98	3.1	119	85	0.6	100	99	7.1	138	117	2.0	130
84	Pyrimidufen	82	1.3	94	85	2.8	101	86	7.0	107	114	2.3	120	111	3.3	125	113	3.2	138	120	2.2	132
85	Pyriproxyfen	99	0.8	87	103	1.2	89	106	2.2	93	109	1.1	119	90	3.2	117	110	5.4	120	116	0.9	130
86	Quinalphos	98	0.7	80	98	2.1	84	104	2.0	89	118	1.7	114	111	3.3	93	102	6.8	117	115	7.0	102
87	Silafluofen	88	0.1	90	119	1.1	92	118	1.9	92	176	2.6	119	137	2.1	115	143	2.9	120	147	1.5	128
88	Tebuconazole	109	2.7	82	102	0.5	84	117	3.0	93	135	4.2	132	112	1.8	111	106	4.5	133	113	7.8	136
89	Tebufenpyrad	112	1.8	89	108	2.2	91	119	2.1	92	114	2.7	114	114	1.0	111	115	3.4	129	118	2.1	130
90	Tefluthrin	118	1.8	76	116	2.9	79	117	3.1	80	186	2.9	86	207	2.2	78	168	4.0	114	190	3.0	79
91	Terbufos	120	0.9	75	122	2.4	78	120	1.3	81	156	0.5	111	109	1.3	101	116	1.8	129	112	1.2	108
92	Thenylchlor	100	0.4	88	101	1.7	85	109	3.5	98	110	2.5	117	112	1.6	95	113	5.4	119	115	6.1	129
93	Thiobencarb	96	0.6	77	97	1.1	78	101	2.3	81	118	1.9	95	111	1.9	75	115	1.3	105	116	1.2	86
94	Thiometon	103	1.2	74	107	1.8	75	107	2.6	79	150	2.8	93	100	3.1	84	104	2.5	120	110	4.1	88
95	Tolclofos-methyl	97	0.9	76	97	1.5	80	101	2.5	82	117	1.8	91	106	2.2	84	118	1.6	118	106	2.9	88
96	Triadimenol	102	1.4	79	97	3.5	82	109	5.1	91	110	2.2	86	112	3.7	114	112	7.8	116	117	2.1	117
97	Tricyclazole	94	3.2	94	100	2.4	100	104	4.6	111	105	5.4	110	111	1.9	84	101	7.0	92	109	7.5	108

Mean: average recovery; R.S.D.: relative standard deviation; ND: not detected.

3.3. Calibration curves for the standard mixture

To assess the method as a quantitative tool, three standard solutions of all the pesticides (50, 100, and 500 ng/mL) were determined by GPC–GC/MS. Each calibration point was obtained based on three duplicate injections of standard sample. In our experience, some pesticides are weakly absorbed to the inner surface of the capillary column. To adequately fit the calibration curve for all pesticides, a second-order calibration curve was employed. The curve between the peak area (y) and the concentration (x, ng/ml) was then investigated. The regression equations for representative pesticides, including organophosphorus, organochlorine, organonitrogen, carbamate, and thiocarbamate substances are provided in Table 2.

3.4. GPC–GC/MS analysis of pesticides in agricultural products

Ninety-seven target pesticides were spiked at a concentration level of 0.1 mg/kg into several kinds of agricultural products, including potato, cabbage, carrot, apple, orange, cucumber, and rice. Total ion chromatograms (TIC) of the spiked potato sample (adding standard pesticides 0.1 mg/kg) and the unspiked potato sample are shown in Fig. 3.

Table 3 provides estimated limits of detection (LOD) and quantitation (LOQ), calculated as the concentration that produced a signal that was 3-times and 10-times the background noise level, respectively, for three analyzed matrices: potato, apple, and rice.

Recovery of the pesticides (0.1 mg/kg) spiked into potato, cabbage, carrot, apple, orange, cucumber, and rice was investigated via GPC–GC/MS. To calculate the recovery, the spiked sample from each agricultural product was prepared three times and the unspiked samples were also investigated. Average recovery results (background of unspiked samples were deducted from the spiked recovery) and the relative standard deviation (R.S.D.) are listed in Table 4. Most pesticides were recovered within an acceptable recovery range from 70 to 120%. Several exceptional recoveries (>150%) probably arose from interferences remaining in the matrix (e.g., the conjugation effect of some endogenous compounds may contribute to high recoveries), whereas some pesticides, such as acephate, captan, dichlofluanid, and methamidophos, showed low recoveries (<70%) in some matrices. As acephate and methamidophos are highly water-soluble pesticides, they would probably move into the water layer during the extraction step. Other unstable compounds such as captan and dichlofluanid may have been decomposed during the extraction step or the analysis process. More pesticides in vegetables and fruits showed acceptable recoveries than those in rice because of the more complicated matrix of rice. It is apparent from Table 4 that R.S.D. values were generally <10%. The low R.S.D. values indicate the high reproducibility of analyses of this newly developed GPC–GC/MS system.

3.5. Comparison of the GPC–GC/MS and GC/MS systems

The recovery test was also investigated using a conventional GC/MS system. The results are shown in Table 4. Comparing the average recovery data, it is easy to find much better recovery results with GPC–GC/MS than with GC/MS method. For example, in GPC–GC/MS, 83 of 97 pesticides showed acceptable recovery for orange, but in GC/MS, only 54 pesticides showed acceptable recovery. The inherent characteristics of GPC have proved highly advantageous in sample pre-treatment to minimize matrix interferences associated with limited solvent extraction protocols.

4. Conclusion

An automated GPC–GC/MS analysis system for the determination of multiple pesticides was developed. Recovery results in the presented pesticides analysis demonstrated that this newly developed system is superior to the conventional GC/MS. The method is accurate and rapid to measure a diverse range of pesticides in agricultural products and it is possible to be of use as a routine tool in monitoring pesticide residues.

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