



Original Article

Optimization of a multi-residue method for 101 pesticides in green tea leaves using gas chromatography–tandem mass spectrometry



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ABSTRACT

A method for analysis of 101 pesticide residues in tea leaves was developed and validated for the first time. Pure acetonitrile was used as extraction solvent rather than acetonitrile after matrix hydration based on the amount of co-extracts and recoveries performance. During clean-up procedure, primary-secondary amine/graphitized carbon black (500 mg) was selected, which exhibited outstanding properties in clean-up capabilities and recoveries of pesticides comparing to primary-secondary amine/graphitized carbon black (250 mg), NH₂-Carbon and TPT absorbents. The method was validated employing gas chromatography coupled to tandem mass spectrometry at the spiked concentration levels of 0.050 and 0.100 mg kg⁻¹. For most of the targeted pesticides, the percent recoveries range from 70 to 120%, with relative standard deviations <20%. The linear correlation coefficients (r^2) were higher than 0.99 at concentration levels of 0.025–0.250 mg kg⁻¹. Limits of quantification ranged from 1.1 to 25.3 µg kg⁻¹ for all pesticides. The developed method was successfully applied to the determination of pesticides in tea leaf samples.

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Introduction

Tea, the dried leaves of the plant of *Camellia sinensis* (L.) Kuntze, Theaceae (Yang and Landau, 2000), is an aromatic beverage that consumed worldwide. Due to its properties of antioxidant, antimicrobial, anticarcinogenic and anti-inflammatory (de Mejia et al., 2009), tea has attracted great attention. However, tea farming is sensitive to many kinds of diseases, pests and weeds, which causes the widely use of pesticides. Until now, more than 300 kinds of pesticide residues in tea have been reported (Pang et al., 2011). Pesticides may cause potential health risk to consumers and impose great pressure on the environment (Jaggi et al., 2001), thus many countries have established maximum residue limits (MRL) for many pesticides, such as European Community (EC) no. 42/2000 and (EC) no. 1881/2006 (Li et al., 2013).

Meanwhile, tremendous efforts have been performed in order to develop analytical methods for pesticides determination in tea. Generally, the preparation method of multi-residues analysis is carried out in a sequence of several steps, including extraction with solvent, purification and detection. For the sample clean-up procedure, there are several efficient ways, such as gel permeation chromatography (GPC) (Pang et al., 2006; Huang et al., 2007)

and solid-phase extraction (SPE) (Huang et al., 2009). Apart from these more common approaches, during the analysis of some pesticide residues, head-space solid-phase micro-extraction (SPME) (Schurek et al., 2008) and stir bar sorptive extraction (SBSE) (Li et al., 2012) were also successfully applied. Another famous way for the detection of multi-residues is known as QuEChERS (quick, easy, cheap, effective, rugged and safe), which involves MeCN extraction and purification with dispersed solid-phase extraction (d-SPE) (Anastassiades et al., 2003a,b; Zhang et al., 2010). The main concept of QuEChERS for sample processing strategy is to use different type of absorbents on the basis of the component of matrix, for instance: primary-secondary amine (PSA), C₁₈ silica, Florisil and graphitized carbon black (GCB). Hayward et al. have developed a multi-residues determination method in botanical dietary supplements using PSA/GCB as clean-up materials. Whereas, in this study, several SPE materials were compared depending on clean-up capabilities and recoveries of pesticides (Hayward et al., 2013).

For the detection techniques, at the beginning, gas chromatography (GC) and high performance liquid chromatography (HPLC) with variety of detectors were widely employed to detect pesticide residues. The most commonly used detectors including electron capture detector (ECD) (Xia et al., 2008), flame photometric detector (FPD) (Moinfar and Hosseini, 2009), nitrogen phosphorus detector (NPD) (Oh, 2007) for GC and fluorescence detector (FLD) (Wu et al., 2009), diode array detection (DAD) (Sharma et al.,

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2008) for HPLC. Owing to better selection, sensitivity and higher throughput in the detection of multi-residues, chromatography coupled to MS (Chen et al., 2012; Zhao et al., 2012) and tandem MS (MS/MS) (Zhao et al., 2012; Cajkaa et al., 2012) have been widely used nowadays. These techniques enable the analysis of multi-residues simultaneously with lower LODs and higher accuracy.

Unfortunately, although control of pesticides in tea leaves may increase the safety of tea from the source, for such detection method, little research has been done until now. In this study, we describe for the first time the method of 101 residues in tea leaves based on the application of GC–MS/MS combined with optimized extraction and SPE clean-up procedures. Moreover, the proposed method has been successfully applied to the determination of these pesticides in real samples.

Experimental

Material and chemicals

HPLC-grade MeCN, acetone, toluene and hexane were provided by Tedia (Fairfield, OH, USA). Prepacked QuEChERS extraction bags with 4 g MgSO_4 , 1 g sodium chloride (NaCl), 1 g *tri*-sodium citrate dehydrate (*tri*-Na) and disodium hydrogencitrate sesquihydrate (*di*-Na) were purchased from Agilent (Palo Alto, CA, USA). Standard pesticides (Table 1) with purities ranging from 95 to 99% were supplied by Sigma–Aldrich (Madrid, Spain) and Dr. Ehrenstorfer (Augsburg, Germany). Stock solutions of mixture pesticides were prepared in acetone and stored in freezer (-18°C). The working solutions were prepared daily.

For SPE, PSA/GCB (500 mg) cartridges, PSA/GCB (250 mg) cartridges, and NH_2 -Carbon cartridges were supplied by Agilent (Palo Alto, CA, USA). TPT cartridges were obtained from Agela Technologies (Tianjin, China).

Equipment

A Vortex (IKA, Germany), a vacuum distillation apparatus (IKA, Germany) and a centrifuge (Xingke, China) were used for preparing the samples. GC–MS/MS system (Varian, Inc., USA) was employed, equipped with a Varian 450 gas chromatograph (equipped with a CP 8400 autosampler and a 1079 injector) and a 320 triple quadrupole MS. A VF-5 MS fused silica capillary column of $30\text{ m} \times 0.25\text{ mm}$ I.D. and $0.25\text{ }\mu\text{m}$ was used (Agilent, Inc., USA). Helium (purity $\geq 99.999\%$) was used as a carrier gas at a flow rate of 1.2 mL min^{-1} . Programmed temperature vaporization (PTV) was employed as the injection mode. The injector temperature program was the following: initial temperature was held at 60°C for 1 min, increased to 250°C at the rate of $200^\circ\text{C min}^{-1}$ and was held for 10 min. Aliquots of sample extract ($5\text{ }\mu\text{L}$) were injected. The GC oven was operated with the following temperature program: initial temperature was held at 50°C for 3 min, increased to 150°C at the rate of $25^\circ\text{C min}^{-1}$, and to 220°C at the rate of 5°C min^{-1} , then held for 5 min, finally to 280°C at the rate of 8°C min^{-1} , and held for 6.5 min.

The mass spectrometry was operated with an electron impact (EI) source. The electron energy was 70 eV, and the ion and transfer line temperatures were set at 230°C and 300°C respectively. The solvent delay was set to 6 min. Mass spectrometric confirmation was carried out in the multiple reaction monitoring (MRM) mode using one quantitative ion transition and one qualitative ion transition for each pesticide. All the parameters for MRM transitions and collision energies were optimized in order to obtain highest sensitivity and resolution (Table 1).

Sample preparation and clean-up

Sample preparation

Tea leaves were collected from organic tea plantation located in Sichuan province in China during March 2014, which was identified as *Camellia sinensis* (L.) Kuntze cv. Mengshan 9 by Prof. Yun Wang (Tea Research Institute, Sichuan Academy of Agricultural Sciences). The tea leaf samples were comminuted with dry ice and homogenized, and then 50 g subsamples were kept frozen until spiking or analysis.

Tea leaf subsamples (5 g) were weighed in polypropylene centrifuge tubes (50 ml) and 20 ml MeCN were added. The solution was then vortexed for 1 min. QuEChERS extraction bag with 4 g anhydrous MgSO_4 , 1 g NaCl, 1 g *tri*-sodium citrate dehydrate (*tri*-Na) and disodium hydrogencitrate sesquihydrate (*di*-Na) was added, and the tube was vortexed immediately to prevent coagulation of MgSO_4 for 1 min. After centrifugation (1210 g, 10 min, -10°C), 5 ml of the upper acetonitrile layer was transferred and purified with SPE.

SPE procedure

SPE cartridges were preconditioned with 5 ml MeCN–toluene (3:1, v/v). Then concentrated extract was introduced into the cartridge. For all experiments, MeCN–toluene (3:1, v/v) was used as eluting solvent. The eluents were collected and then concentrated to dryness using vacuum concentration. Finally, the residue was redissolved in 2.5 ml mixture of *n*-hexane and acetone (9 + 1, v/v) for GC–MS/MS analysis.

Gravimetric determination of co-extracts

For sample extraction procedure, three types of 5 ml of crude MeCN extracts obtained by pure MeCN, MeCN after matrix hydration using 5 ml and 10 ml water were evaporated separately until dryness with a weak nitrogen stream, and the residues were gravimetrically determined by analytical balance. Average amounts of five replicates were evaluated (Fig. 1), the error bars (standard deviations) of the weights for each group were also showed.

For clean-up procedure, 5 ml of crude MeCN extracts and 5 ml of MeCN extracts purified through four types of SPE were evaluated according to gravimetric method described above (Fig. 3).

Method performance

The precision and accuracy of the method was tested with spiked tea leaves. Recoveries were determined for five replicates at two spiking concentrations (0.050 and 0.100 mg kg^{-1}).

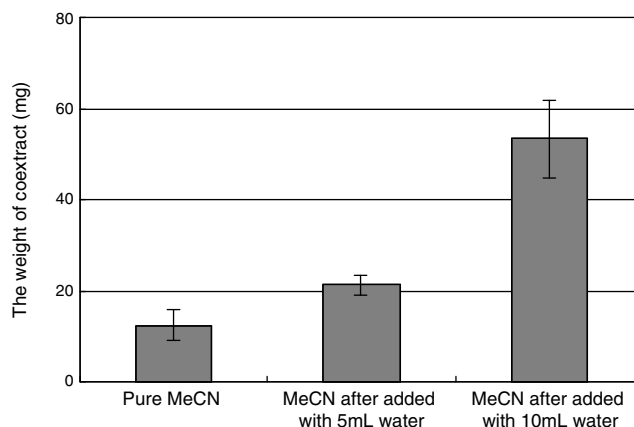


Fig. 1. Amount of co-extracts per 1 ml MeCN extracts determined gravimetrically in tea leaves ($n = 5$).

Table 1

Parameters for 101 pesticide residue analysis by GC/MS–MS.

No.	Pesticides	Retention time (min)	Quantitative ion pairs (<i>m/z</i>)	Collision energy (V)	Qualitative ion pairs (<i>m/z</i>)	Collision energy (V)
1	Isoprocarb	10.74	136 > 121	10	121 > 77	20
2	Omethoate	11.52	156 > 110	10	156 > 79	20
3	Fenobucarb	11.72	121 > 77	20	150 > 121	7
4	Profluralin	11.77	152 > 110	10	109 > 81	10
5	Dipheylamine	11.90	167 > 139	30	141 > 115	17
6	Diallate-I	13.01	234 > 150	20	234 > 192	20
7	Phorate	13.01	260 > 75	10	260 > 231	10
8	Alpha-HCH	13.14	181 > 145	15	217 > 181	12
9	Diallate-II	13.26	234 > 150	20	234 > 192	20
10	Dazomet	13.45	162 > 89	5	89 > 44	10
11	Diemthoate	13.57	125 > 79	10	229 > 87	10
12	Carbofuran	13.79	164 > 149	10	221 > 164	10
13	Chlorbufam	13.83	171 > 127	10	223 > 171	10
14	Beta-HCH	14.00	181 > 145	15	217 > 181	12
15	Gamma-HCH	14.15	181 > 145	15	217 > 181	12
16	Quintozene	14.31	295 > 237	20	293 > 235	20
17	Terbufos	14.35	231 > 175	13	231 > 129	25
18	Propetamphos	14.40	236 > 194	10	222 > 138	10
19	Fonofos	14.44	246 > 137	10	246 > 109	20
20	Pyrimethanil	14.61	198 > 183	10	198 > 118	30
21	Diazinon	14.78	304 > 179	10	304 > 162	10
22	Delta-HCH	14.92	217 > 181	10	181 > 145	15
23	Isazofos	15.19	257 > 162	10	257 > 119	20
24	Tefluthrin	15.20	177 > 127	15	197 > 141	10
25	Fenclozim	15.54	176 > 120	10	120 > 93	20
26	Formothion	15.61	224 > 125	10	170 > 93	10
27	Vinclozolin	16.34	285 > 212	10	285 > 198	20
28	Alachlor	16.59	188 > 160	10	160 > 132	10
29	Fenchlorphos	16.79	285 > 270	25	287 > 272	15
30	Fenitrothion	17.26	277 > 260	10	260 > 125	10
31	Methyl parathion	17.26	263 > 109	10	125 > 79	10
32	Malathion	17.68	173 > 99	15	173 > 127	10
33	Fenthion	17.92	278 > 109	20	278 > 125	20
34	Chlorpyrifos	17.99	314 > 258	10	314 > 286	10
35	Parathion	18.02	291 > 109	15	291 > 137	10
36	Triadimefon	18.11	208 > 181	10	208 > 111	10
37	Isocarbophos	18.27	289 > 136	10	230 > 212	10
38	Chlorthion	18.33	125 > 79	10	297 > 109	10
39	Fosthiazate-I	18.56	195 > 103	10	195 > 139	7
40	Fosthiazate-II	18.65	195 > 103	10	195 > 139	7
41	Isofenphos-methyl	18.93	199 > 121	12	241 > 199	10
42	Quinalphos	19.56	298 > 156	10	298 > 190	10
43	Fipronil	19.66	367 > 213	30	367 > 255	15
44	Chlorbenside	19.72	125 > 89	20	268 > 125	10
45	Methidathion	19.99	145 > 85	10	302 > 14	10
46	Chlorfenson	20.70	175 > 111	10	302 > 175	10
47	Profenofos	21.12	337 > 267	15	339 > 269	15
48	Barban	21.23	222 > 69	10	257 > 222	10
49	<i>p,p'</i> -DDE	21.23	246 > 176	20	316 > 246	10
50	Buprofezin	21.66	305 > 175	10	305 > 190	5
51	<i>p,p'</i> -DDD	22.91	235 > 165	10	235 > 200	15
52	<i>o,p'</i> -DDT	23.03	235 > 165	10	235 > 200	15
53	Ethion	23.25	231 > 129	25	231 > 203	10
54	Triazophos	23.88	257 > 161	10	161 > 134	10
55	Quinoxifen	24.39	272 > 237	15	237 > 208	20
56	Carfentrazone-ethyl	24.59	376 > 330	10	411 > 312	20
57	<i>p,p'</i> -DDT	24.75	235 > 165	10	235 > 200	15
58	Pyraflufen-ethyl	25.46	349 > 307	15	349 > 279	20
59	Propargite	26.11	350 > 201	10	350 > 173	15
60	Bioresmethrin	26.61	123 > 81	10	171 > 128	10
61	Phosmet	27.40	160 > 133	10	317 > 160	10
62	Picolinafen	27.91	376 > 239	15	238 > 145	25
63	Bifenazate	28.02	300 > 196	20	196 > 141	20
64	Bifenthrin	28.07	181 > 166	10	181 > 165	20
65	Fenpropathrin	28.28	181 > 152	20	265 > 210	10
66	Fenamidone	28.34	268 > 180	20	311 > 283	7
67	Phosalone	29.21	182 > 138	10	367 > 182	10
68	Pyriproxyfen	29.54	136 > 96	13	136 > 78	23
69	Cyhalofop-butyl	29.81	256 > 120	10	357 > 256	10
70	Amitraz	29.96	293 > 162	7	293 > 132	17
71	Lambda-Cyhalothrin	30.32	197 > 141	10	208 > 181	10
72	Pyrazophos	30.65	221 > 193	10	232 > 204	10
73	Pyraclifos	30.82	360 > 194	10	360 > 139	10
74	Acrinathrin	30.82	208 > 181	10	181 > 152	10

Table 1 (Continued)

No.	Pesticides	Retention time (min)	Quantitative ion pairs (<i>m/z</i>)	Collision energy (V)	Qualitative ion pairs (<i>m/z</i>)	Collision energy (V)
75	Bitertanol	31.31	170 > 141	23	170 > 115	33
76	Permethrin-I	31.54	183 > 153	15	163 > 127	10
77	Pyridaben	31.68	147 > 117	30	364 > 147	20
78	Permethrin-II	31.78	183 > 153	15	163 > 127	10
79	Coumafos	31.83	362 > 109	20	226 > 198	10
80	Fenbuconazole	32.43	198 > 129	10	129 > 102	10
81	Cyfluthrin-I	32.61	226 > 206	10	226 > 199	10
82	Cyfluthrin-II	32.76	226 > 206	10	226 > 199	10
83	Cyfluthrin-III	32.88	226 > 206	10	226 > 199	10
84	Cyfluthrin-IV	32.88	226 > 206	10	226 > 199	10
85	Cypermethrin-I	33.25	163 > 91	15	181 > 152	15
86	Cypermethrin-II	33.36	163 > 91	15	181 > 152	15
87	Cypermethrin-III	33.43	163 > 91	15	181 > 152	15
88	Flucythrinate-I	33.45	199 > 107	20	199 > 157	15
89	Ethofenprox	33.52	163 > 135	13	163 > 107	20
90	Flucythrinate-II	33.76	199 > 107	20	199 > 157	15
91	Silafluofen	33.79	179 > 151	10	286 > 258	5
92	Fenvalerate-I	34.56	419 > 225	8	419 > 167	12
93	Fenvalerate-II	34.95	419 > 225	8	419 > 167	12
94	Tau-fluvalinate-I	35.00	502 > 250	10	250 > 55	20
95	Tau-fluvalinate-II	35.13	502 > 250	10	250 > 55	20
96	Difenoconazole-I	35.43	323 > 265	15	325 > 267	15
97	Difenoconazole-II	35.43	323 > 265	15	325 > 267	15
98	Deltamethrin	36.05	251 > 172	8	253 > 174	8
99	Indoxacarb	36.06	218 > 203	10	264 > 176	10
100	Azoxystrobin	36.67	344 > 329	10	403 > 344	10
101	Cinidon-ethyl	38.51	358 > 330	20	330 > 302	10

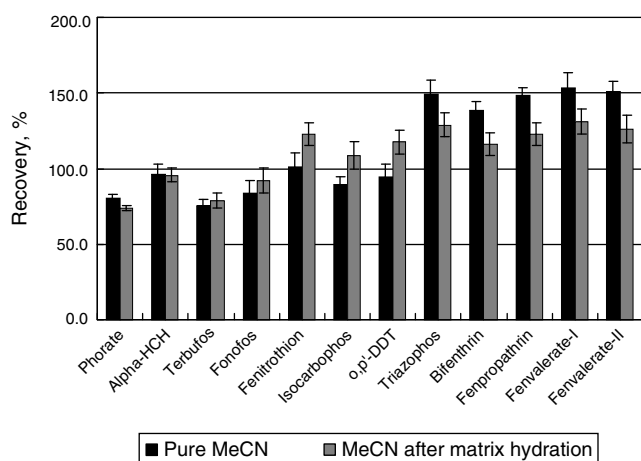


Fig. 2. Comparison of recoveries obtained by analysis of pesticide residues using different extraction procedures (solvent calibration).

Matrix-matched standards at 0.025, 0.050, 0.100, 0.150, 0.250 mg kg⁻¹ were for calibration.

Results and discussion

Extraction efficiency

Generally, when dealing with the extraction of pesticides from tea, either pure MeCN (Pang et al., 2006; Xu et al., 2011) or MeCN (Pang et al., 2011) after matrix hydration could be used. The two methods have its own advantages, using MeCN as extract solvent is recommended for the analysis of high-moisture matrices, and less polar matrix components remains (Sanco, 2010); whereas, for some pesticides, the recovery would be better, if some water could be added to the matrix before extracted with MeCN (Poulsen et al., 2009). In our study, the amount of co-extracts using pure MeCN and MeCN after matrix hydration was compared. As shown in Fig. 1, the addition of extra water into tea leaves resulted in the increase of the weight of co-extracts. Meanwhile, for extracts using pure MeCN, the

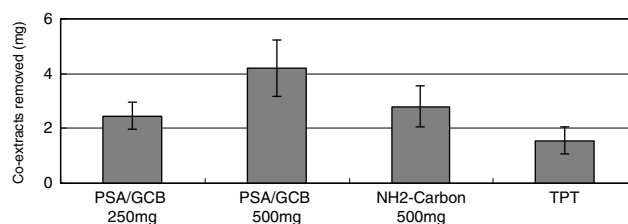


Fig. 3. Clean-up capabilities of different absorbents using SPE of 1 ml MeCN extracts (*n* = 5).

ion intensity was lower when analyzed with GC–MS in scan mode, revealing that pure MeCN is favorable when dealing with matrix effects and interference phenomenon.

Moreover, the recoveries of several pesticides belong to different types were studied (Fig. 2). For organophosphorus and organochlorine pesticides (Phorate, Alpha-HCH, Terbufos, Fonofos, Fenitrothion, Isocarbophos and *o,p'*-DDT), the recoveries were distributed around 100%, and there was little difference exhibited between using pure MeCN and MeCN after matrix hydration. For pyrethroid pesticides, matrix effect was much more severe, leading to high recoveries. However, matrix effect could be compensated by using matrix-matched calibration (Table 2). Thus, considering all the aspects discussed above, pure MeCN was used as the extraction solvent.

Comparison of different SPE cartridges

Tea leaves represent a very complex matrix, which contain a great amount of caffeine, pigments, polyphenols etc. Due to the co-extracts interferences and matrix effects, those components could cause a great trouble on the analysis of multiple residues (Steiniger et al., 2010). Considering the clean-up capabilities as well as the pesticide recoveries, the most commonly used absorbents for tea including PSA/GCB (250 mg), PSA/GCB (500 mg), NH₂-Carbon, and TPT were compared. Fig. 3 summarises the final results of the evaluation experiments in terms of weights of co-extracts removed by the different absorbents. For point of co-extracts removing, both PSA/GCB and NH₂-Carbon exhibited better clean-up capabilities

Table 2Results of the validation study [mean recoveries (%), relative standard deviations (RSDs, %), limits of quantity ($\mu\text{g kg}^{-1}$)].

Pesticides	Spiked 0.050 (mg kg^{-1})		Spiked 0.100 (mg kg^{-1})		LOQs ($\mu\text{g kg}^{-1}$)	Linearity (r^2)
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)		
Isoprocarb	110.3	11.0	106.6	8.1	1.6	0.9946
Omethoate	97.7	10.6	79.6	7.3	3.2	0.9910
Fenobucarb	121.7	7.2	113.4	5.1	4.6	0.9973
Profluralin	112.0	6.9	109.2	6.2	11.5	0.9912
Dipheylamine	106.7	30.8	99.8	13.5	8.3	0.9985
Diallate-I	72.0	12.9	79.6	11.0	12.5	0.9961
Phorate	72.3	25.5	77.6	18.4	12.5	0.9942
a-666	113.3	20.6	86.0	11.4	15.6	0.9987
Diallate-II	73.6	14.4	76.4	13.9	12.5	0.9948
Dazomet	99.7	8.6	65.4	5.4	6.6	0.9933
Diemthoate	130.7	12.1	132.6	5.1	6.3	0.9970
Carbofuran	127.0	7.4	111.4	4.9	7.8	0.9936
Chlorbufam	129.3	7.6	99.6	4.6	12.5	0.9952
b-666	120.7	5.0	98.2	4.8	6.3	0.9960
r-666	128.0	18.7	114.4	3.8	15.6	0.9910
Quintozene	68.7	17.4	69.6	14.8	12.5	0.9919
Terbufos	120.0	14.3	98.4	7.5	8.3	0.9902
Propetamphos	116.0	4.8	105.2	3.7	12.5	0.9949
Fonofos	125.0	13.8	96.4	8.2	15.6	0.9951
Pyrimethanil	91.3	4.3	65.2	6.8	20.8	0.9947
Diazinon	119.3	9.1	116.4	5.1	25.3	0.9974
d-666	126.0	4.4	97.0	5.5	17.9	0.9959
Isazofos	119.3	5.7	109.2	4.3	6.0	0.9904
Tefluthrin	126.0	7.5	103.4	5.2	11.9	0.9924
Fenclorim	113.3	5.1	111.4	3.8	5.0	0.9974
Formothion	68.7	15.7	72.2	14.5	3.6	0.9938
Vinclozolin	131.3	3.6	108.2	4.2	2.5	0.9916
Alachlor	131.0	5.2	103.8	3.6	1.7	0.9960
Fenchlorphos	155.0	6.6	124.6	5.2	2.8	0.9976
Fenitrothion	139.7	3.2	113.2	1.3	5.7	0.9903
Methyl parathion	136.7	3.3	112.2	1.5	5.0	0.9929
Malathion	122.3	3.6	99.0	4	1.8	0.9953
Fenthion	135.3	4.9	108.4	2.1	2.3	0.9915
Chlorpyrifos	143.7	4.9	117.6	2.7	16.7	0.9943
Parathion	142.3	3.5	121.4	1.9	6.3	0.9926
Triadimefon	96.0	4.8	84.6	6.1	4.6	0.9964
Isocarbophos	102.0	12.0	123.2	4.3	7.7	0.9968
Chlorthion	124.0	3.1	100.6	1.1	11.9	0.9909
Fosthiazate	100.7	5.8	85.2	2.1	2.5	0.9983
Fosthiazate	98.0	7.2	85.6	3.0	1.8	0.9934
Isofenphos-methyl	114.0	4.3	95.0	3.6	1.6	0.9973
Quinalphos	141.3	8.6	120.8	5.7	1.1	0.9965
Fipronil	101.0	4.5	93.6	10.2	2.5	0.9989
Chlorbenside	127.0	4.1	107.8	2.6	1.5	0.9913
Methidathion	118.3	4.2	99.4	2.3	1.5	0.9978
Chlorfenson	109.3	4.6	93.0	3.8	1.5	0.9976
Profenofos	104.3	3.9	93.4	3.6	1.4	0.9964
Barban	90.0	6.3	76.8	15.6	1.5	0.9971
p,p'-DDE	113.3	4.8	95.4	4.9	1.5	0.9980
Buprofezin	102.0	4.1	92.0	5.4	2.5	0.9981
p,p'-DDD	100.3	5.3	85.4	6.2	1.5	0.9973
o,p'-DDT	113.0	4.2	94.2	4.9	1.6	0.9985
Ethion	99.3	4.7	85.4	6.1	1.2	0.9990
Triazophos	92.3	5.0	83.4	5.3	1.8	0.9980
Quinoxifen	85.3	5.3	80.6	4.2	1.8	0.9975
Carfentrazone-ethyl	90.3	3.3	87.0	3.7	4.0	0.9943
p,p'-DDT	108.0	5	90.4	5.3	1.5	0.9987
Pyraflufen-ethyl	84.3	4.6	82.8	4.4	9.5	0.9962
Propargite	97.3	8.5	88.0	12	13.2	0.9980
Bioresmethrin	85.0	5.7	55.6	6.8	1.6	0.9966
Phosmet	86.7	9.6	81.0	5.0	2.3	0.9975
Picolinafen	95.7	4.1	90.6	3.6	2.3	0.9963
Bifenazate	77.3	18.5	74.0	7.6	3.1	0.9988
Bifenthrin	99.0	6.9	83.2	6.7	1.5	0.9960
Fenpropathrin	92.7	5.0	81.4	5.4	1.8	0.9984
Fenamidone	82.3	6.4	77.6	5.1	1.2	0.9981
Phosalone	93.0	7.7	78.4	9.6	1.4	0.9979
Pyriproxyfen	84.7	6.6	78.6	5.4	1.5	0.9990
Cyhalofop-butyl	87.3	7.6	77.2	6.0	1.5	0.9981
Amitraz	76.0	10.0	76.4	14.7	4.2	0.9970
Lambda-Cyhalothrin	95.0	9.1	80.8	5.6	1.3	0.9962
Pyrazophos	88.7	8.4	74.8	8.5	1.5	0.9964
Pyraclafos	101	4.5	89.2	8.0	1.3	0.9961
Acrinathrin	88.3	10.2	84.6	39	1.8	0.9991

Table 2 (Continued)

Pesticides	Spiked 0.050 (mg kg ⁻¹)		Spiked 0.100 (mg kg ⁻¹)		LOQs (μg kg ⁻¹)	Linearity (r ²)
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)		
Bitertanol	91.7	5.8	86.6	5.7	1.6	0.9933
Permethrin	98.0	7.6	80.4	8.9	1.6	0.9934
Pyridaben	93.3	7.4	75.6	7.9	2.1	0.9954
Permethrin	96.0	7.0	78.0	7.5	2.9	0.9931
Coumafos	95.7	4.8	86.4	6.5	2.6	0.9983
Fenbuconazole	82.7	9.5	84.6	11.2	3.1	0.9913
Cyfluthrin	89.7	11.9	71.4	13.0	8.9	0.9920
Cyfluthrin	94.3	8.5	74.4	9.1	8.1	0.9928
Cyfluthrin	95.7	12.6	73.8	6.7	13.2	0.9978
Cyfluthrin	124.0	34.3	115.4	7.6	15.6	0.9912
Cypermethrin	95.3	7.7	74.4	11.3	15.6	0.9944
Cypermethrin	109.7	30.3	64.0	10	16.7	0.9938
Cypermethrin	106.3	5.2	88.6	15.3	11.9	0.9976
Flucythrinate	96.7	7.6	75.2	11.1	7.8	0.9932
Ethofenprox	93.3	10.3	71.8	10.4	1.8	0.9995
Flucythrinate	98.7	7.5	75.6	11.7	6.4	0.9929
Silafluofen	98.7	10.8	76.8	7.6	1.4	0.9950
Fenvalerate	94.7	6.4	82.0	9.0	1.7	0.9984
Fenvalerate	102.7	4.7	86.0	8.7	3.3	0.9974
Tau-fluvalinate	80.7	10.6	63.6	14.6	5.8	0.9972
Tau-fluvalinate	92.7	6.4	81.2	11.1	7.5	0.9977
Difenoconazole	76.0	8.8	63.6	14.0	5.0	0.9972
Difenoconazole	78.3	10.3	73.3	12.8	3.9	0.9968
Deltamethrin	76.7	14.8	75.0	18.9	3.1	0.9960
Indoxacarb	78.3	15.8	73.4	17.5	5.8	0.9932
Azoxystrobin	67.3	13.4	64.8	18.2	2.6	0.9988
Cinidon-ethyl	77.3	13.4	72.6	17.7	3.2	0.9987

comparing to TPT. However, as the amount of PSA/GCB doubled, clean-up capability was increased significantly.

It is widely recognized that the absorbents used during SPE procedure could absorb not only co-extracts interferences but also the targeted pesticides. Thus, in order to determine whether the different absorbents could influence the residue analysis efficiency obviously, all the targeted pesticides were added before SPE procedure, and the distribution of recoveries were evaluated. As shown in Fig. 4, the recoveries of most pesticides were located in the range of 70–120% for all the evaluated absorbents, whereas, the recoveries in the range of <70% exhibited the greatest number when TPT was used. This indicated that the pesticide-absorbing phenomenon of TPT was severe. Moreover, for PSA/GCB (500 mg), the number of pesticides in the range of >120% was fewer than PSA/GCB (250 mg) and NH₂-Carbon, revealing that PSA/GCB (500 mg) has greater capability in interference-absorbing.

Moreover, caffeine was evaluated by MS in full-scan mode, which is the main interference in fresh tea leaves. The result showed that the caffeine-removing capability were of little difference for NH₂-Carbon, TPT and PSA/GCB (500 mg) absorbents. Although the

caffeine-removing capability for PSA/GCB (250 mg) was poor, this could be attributed to the absorbent amount. Thus, according to the gravimetric experiment and recovery distribution, PSA/GCB (500 mg) was selected in the procedure of SPE clean-up.

Method validation

Method validation of the newly proposed residue-analyzing method in tea leaves was conducted. Several validation parameters including recovery, precision, linear range, and limit of quantification (LOQ) were determined and summarized in Table 2.

The linearity for each pesticide was evaluated using matrix-matched calibration standards at five concentration levels, i.e. 0.025, 0.050, 0.100, 0.150, 0.250 mg l⁻¹. Good linearity results with the regression coefficients more than 0.99 for all the pesticides were obtained. The LOQ calculated by considering a value ten times of the background noise respectively. For all pesticides, the LOQ ranged from 1.1 to 25.3 μg kg⁻¹.

The recovery and precision of the method for the 101 targeted pesticides were evaluated by carrying out six consecutive extractions (*n* = 6) of spiked tea at the two concentration levels (0.050 and 0.100 mg kg⁻¹). The results were calculated using matrix-matched calibration standards by external calibration method. When spiked levels were 0.050 and 0.100 mg kg⁻¹, the average recoveries for all the targeted pesticides ranged from 67.3 to 130.7 and 63.6 to 132.6 respectively. The relative standard deviations (RSDs) for the two spiked levels were lower than 20%.

Real sample

To prove the effectiveness and suitability, twenty real samples were analyzed by this newly proposed method. The tea samples were pursued in tea plantation and seven pesticides were detected, including Bifenthrin, Chlorpyrifos, Buprofezin, Fipronil, Pyridaben, Triazophos and Cypermethrin. The developed method was proved to be a suitable and stable method for the determination of pesticides in real tea leaf samples.

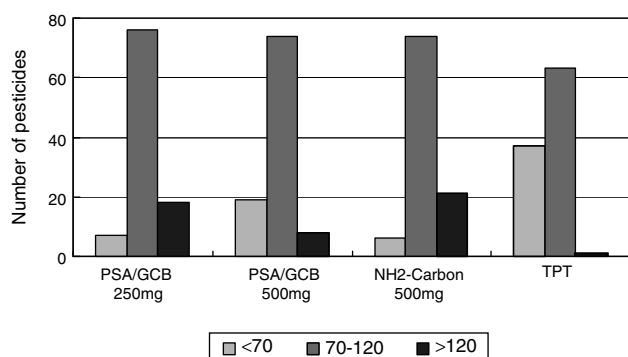


Fig. 4. Distribution of the recoveries (*n* = 3) obtained by using different absorbents during SPE process for 101 targeted pesticides in tea leaves.

Conclusion

In this work, the extraction methods based on pure MeCN and MeCN after matrix hydration were evaluated through gravimetric experiment and comparison the recoveries of representative pesticides. Then, the clean-up capabilities of different absorbents, i.e. PSA/GCB (250 mg), PSA/GCB (500 mg), NH₂-Carbon, and TPT were compared. Finally, the method for multi-pesticides analysis in tea leaves was developed using pure MeCN as the extraction solvent and PSA/GCB (500 mg) in SPE procedure. During GC–MS/MS analysis, MRM mode was used, and quantitative analysis was achieved by external calibration method. The calibration parameters of the method including recovery, precision, linear range, and LOQ were examined, which showed this newly proposed method was suitable for multi-pesticides analysis in tea leaves.

Authors' contributions

XH, STQ performed the laboratory work, data and GC–MS/MS analysis. XH drafted the paper. SRL, LAG helped perform the analysis with constructive discussions, participated in the results discussion and the manuscript final writing.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

Conflicts of interest

The authors declare no conflicts of interest.

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References

- Anastassiades, M., Maštovská, K., Lehotay, S.J., 2003a. Evaluation of analyte protectants to improve gas chromatographic analysis of pesticides. *J. Chromatogr. A* 1015, 163–184.
- Anastassiades, M., Lehotay, S.J., Stajnbaher, D., Schenck, F.J., 2003b. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce. *J. AOAC Int.* 86, 412–431.
- Chen, L., Shang, G.L., Wu, Y., Xu, L., Fu, F., 2012. Study on the residue and degradation of fluorine-containing pesticides in Oolong tea by using gas chromatography–mass spectrometry. *Food Control* 25, 433–440.
- Cajka, T., Sandy, C., Bachanova, V., Drabova, L., Kalachova, K., Pulkrabova, J., Hajšlova, J., 2012. Streamlining sample preparation and gas chromatography–tandem mass spectrometry analysis of multiple pesticide residues in tea. *Anal. Chim. Acta* 743, 51–60.
- de Mejia, E.G., Ramirez-Mares, M.V., Puangphanth, S., 2009. Bioactive components of tea: cancer, inflammation and behavior. *Brain Behav. Immun.* 23, 721–731.
- Hayward, D.G., Wong, J.W., Shi, F., Zhang, K., Lee, N.S., DiBenedetto, A.L., Hengel, M.L., 2013. Multi-residue pesticide analysis of botanical dietary supplements using salt-out acetonitrile extraction, solid-phase extraction cleanup column and gas chromatography–triple quadrupole mass spectrometry. *Anal. Chem.* 85, 4686–4693.
- Huang, Z.Q., Li, Y.J., Chen, B., Yao, S.Z., 2007. Simultaneous determination of 102 pesticide residues in Chinese teas by gas chromatography–mass spectrometry. *J. Chromatogr. B* 853, 154–162.
- Huang, Z., Zhang, Y., Wang, L., Ding, L., Wang, M., Yan, H., 2009. Simultaneous determination of 103 pesticide residues in tea samples by LC–MS/MS. *J. Sep. Sci.* 32, 1294–1301.
- Jaggi, S., Sood, C., Kumar, V., Ravindranath, S.D., Shanker, A., 2001. Leaching of pesticides in tea brew. *J. Agric. Food Chem.* 49, 5479–5483.
- Li, X., Zhang, Z.W., Li, P.W., Zhang, Q., Zhang, W., Ding, X.X., 2013. Determination for major chemical contaminants in tea (*Camellia sinensis*) matrices. A review. *Food Res. Int.* 53, 649–658.
- Li, B., Zeng, F.G., Dong, Q.C., Cao, Y., Fan, H.T., Deng, C.F., 2012. Rapid determination method for 12 pyrethroid pesticide residues in tea by stir bar sorptive extraction–thermal desorption–gas chromatography. *Phys. Proc.* 25, 1776–1780.
- Moinfar, S., Hosseini, M.R.M., 2009. Development of dispersive liquid–liquid microextraction method for the analysis of organophosphorus pesticides in tea. *J. Hazard. Mater.* 169, 907–911.
- Oh, C.H., 2007. Purification method for multi-residual pesticides in green tea. *Nat. Prod. Commun.* 2, 1025–1030.
- Pang, G.F., Fan, C.L., Zhang, F., Li, Y., Chang, Q.Y., Cao, Y.Z., 2011. High-throughput GC–MS and HPLC–MS–MS techniques for the multiclass multiresidue determination of 653 pesticides and chemical pollutants in tea. *J. AOAC Int.* 94, 1253–1296.
- Pang, Y.F., Cao, Y.Z., Zhang, J.J., Fan, C.L., Liu, Y.M., Li, X.M., Jia, G.Q., Li, Z.Y., Shi, Y.Q., Wu, Y.P., Guo, T.T., 2006. Validation study on 660 pesticide residues in animal tissues by gel permeation chromatography cleanup/gas chromatography–mass spectrometry and liquid chromatography–tandem mass spectrometry. *J. Chromatogr. A* 1125, 1–30.
- Poulsen, M.E., Christensen, H.B., Herrmann, S.S., 2009. Proficiency test on incurred and spiked pesticide residues in cereals. *Accredit. Qual. Assur.* 14, 477–485.
- Schurek, J., Portolés, T., Hajšlova, J., Riddellova, K., Hernández, F., 2008. Application of head-space solid-phase microextraction coupled to comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry for the determination of multiple pesticide residues in tea samples. *Anal. Chim. Acta* 611, 163–172.
- Sharma, A., Gupta, M., Shanker, A., 2008. Fenvalerate residue level and dissipation in tea and in its infusion. *Food Addit. Contam.* 25, 97–104.
- Steiniger, D., Lu, G., Butler, J., Phillips, E., Fintschenko, T., 2010. Determination of multiresidue pesticides in green tea by using a modified QuEChERS extraction and ion-trap gas chromatography/mass spectrometry. *J. AOAC Int.* 93, 1169–1179.
- SANCO/10684/2009, 2010. Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed. Commission of the European Communities, 8, pp. 18.
- Wu, C.C., Chu, C., Wang, Y.S., Lur, H.S., 2009. Multiresidue method for high-performance liquid chromatography determination of carbamate pesticides residues in tea samples. *J. Environ. Sci. Heal. B* 44, 58–68.
- Xia, H., Ma, X., Tu, Y., 2008. Comparison of the relative dissipation rates of endosulfan pesticide residues between oolong and green tea. *Food Addit. Contam.* 25, 70–75.
- Xu, X.M., Yu, C., Han, J.L., Li, J.P., El-Sepai, F., Zhu, Y., Huang, B.F., Cai, Z.X., Wu, H.W., Ren, Y.P., 2011. Multi-residue analysis of pesticides in tea by online SEC–GC/MS. *J. Sep. Sci.* 34, 210–216.
- Yang, C.S., Landau, J.M., 2000. Effects of tea consumption on nutrition and health. *J. Nutr.* 13, 2409–2412.
- Zhang, X., Mobley, N., Zhang, J.G., Zheng, X.M., Lu, L., Ragin, O., Smith, C.J., 2010. Analysis of agricultural residues on tea using d-SPE sample preparation with GC–NCI–MS and UHPLC–MS/MS. *J. Agric. Food Chem.* 58, 11553–11560.
- Zhao, P., Wang, L., Jiang, Y., Zhang, F., Pan, C., 2012. Dispersive cleanup of acetonitrile extracts of tea samples by mixed multiwalled carbon nanotubes, primary secondary amine, and graphitized carbon black sorbents. *J. Agric. Food Chem.* 60, 4026–4033.