

Dispersive Cleanup of Acetonitrile Extracts of Tea Samples by Mixed Multiwalled Carbon Nanotubes, Primary Secondary Amine, and Graphitized Carbon Black Sorbents

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ABSTRACT: A method for analysis of 37 pesticide residues in tea samples was developed and validated and was based on reversed-dispersive solid-phase extraction (r-DSPE) cleanup in acetonitrile solution, followed by liquid chromatography–electrospray tandem mass spectrometry determination. Green tea, oolong tea, and puer tea were selected as matrixes and represent the majority of tea types. Acetonitrile was used as the extraction solvent, with sodium chloride and magnesium sulfate enhancing partitioning of analytes into the organic phase. The extract was then cleaned up by r-DSPE using a mixture of multiwalled carbon nanotubes, primary secondary amine, and graphitized carbon black as sorbents to absorb interferences. Further optimization of sample preparation and determination allowed recoveries of between 70% and 111% for all 37 pesticides with relative standard deviations lower than 14% at two concentration levels of 10 and 100 $\mu\text{g kg}^{-1}$. Limits of quantification ranged from 5 to 20 $\mu\text{g kg}^{-1}$ for all pesticides. The developed method was successfully applied to the determination of pesticide residues in market tea samples.

KEYWORDS: multiwalled carbon nanotubes, reversed-dispersive solid-phase extraction, tea, pesticide multiresidue analysis

INTRODUCTION

The consumption of tea has been a habit since 4700 years ago in China, India, and many other countries. Many reports have been published concerning the effects of tea and its major constituents on human health.^{1,2} Tea farming is affected by a great multitude of diseases, weeds, and pests, especially mites, leaf-eating beetles, and caterpillars.³ To ensure high-quality tea crop production, pesticides are considered to be indispensable to minimize those problems. However, unsafe pesticide residues in tea may cause potential health risk to tea consumers or force unnecessary pressure on the environment. Similar to other raw agricultural commodities, many regulations such as maximum residue limits (MRLs) have been established for teas by several international organizations and countries.^{3–5} As a result, the monitoring of pesticide residues in tea at trace levels can be an important task in public health safety and trade.

Tea leaves are known as complex matrixes as they may contain complex components, including pigments, alkaloids, and polyphenols, etc. The analysis of pesticide multiresidues in tea is usually difficult owing to matrix interference and complicated extraction procedures. Recently, numerous extraction and cleanup methods have been published on the determination of pesticide residues in tea using solid-phase extraction,^{3,4,6–8} gel permeation chromatography,^{6,9,10} solid-phase microextraction,^{10,11} accelerated solvent extraction,⁹ stir bar sorptive extraction–thermal desorption,^{12,13} dispersive liquid–liquid microextraction,¹⁴ microwave-assisted steam distillation,⁹ and so on. Some of the methods use a lot of solvent and several cleanup procedures, which may be tedious and costly.

Since the QuEChERS (quick, easy, cheap, effective, rugged, and safe) method was introduced by Anastassades and Lehoaty et al. in 2003,^{15,16} this technique has been widely used as a pesticide multiresidue method in vegetables, fruits,

and many other matrixes due to its advantages of high extraction efficiency, smaller volume of organic solvent, simplicity of operation, and low cost per sample. It involves miniaturized extraction with acetonitrile, liquid–liquid partitioning, and a cleanup step which was carried out by mixing the acetonitrile extract with loose sorbents. This cleanup technique is based on reversed-dispersive solid-phase extraction (r-DSPE) to absorb the interfering substances in the matrixes, rather than the analytes. The QuEChERS approach takes advantage of high recoveries in pesticides with a wide range of polarity and volatility, high sample throughput, nonsophisticated equipment, smaller volume of organic solvent, and low cost per sample. The excellent results provided by QuEChERS sample preparation combined GC–MS and LC–MS have helped lead to the great popularity of QuEChERS concepts.¹⁷ Mostly, a small amount of primary secondary amine (PSA) and/or graphitized carbon black (GCB) and/or C18 was used as the r-DSPE sorbent, as each of these materials could remove various polar organic acids, sterols, pigments, and nonpolar interfering substances.¹⁸ However, in the case of complex tea samples, the original QuEChERS method with r-DSPE (PSA/GCB/C18) may not achieve effective cleanup like SPE. A new material mixed with PSA and GCB as the r-DSPE sorbent was tested in this study with the purpose of achieving better cleanup performance for tea samples with r-DSPE cleanup.

As new carbon-based nanomaterials, carbon nanotubes (CNTs) are novel and interesting carbonaceous materials first reported by Iijima in 1991.¹⁹ CNTs have high surface area, mechanical strength, and chemical stability. According to the

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carbon atom layers in the wall of the nanotubes, CNTs can be divided into single-walled carbon nanotubes (SWCNTs) and multiwalled carbon nanotubes (MWCNTs). The potential of MWCNTs as sorbents of SPE to extract pesticides from water samples,^{20–25} garlic,²⁶ fruit juices,²⁷ and pork meat²⁸ has been investigated in recent years. Some other applications of MWCNTs such as a matrix solid-phase dispersion extraction material^{29,30} and a dispersive solid-phase extraction sorbent for water³¹ were also reported. There are no previous reports of using MWCNTs mixed with other sorbents with the QuEChERS method for the determination of pesticides in tea samples.

In this paper, a modified QuEChERS method using MWCNTs, PSA, and GCB as r-DSPE sorbents for tea samples is reported. The performance of the proposed method was studied by extraction of 37 representative pesticides in tea samples and using liquid chromatography–electrospray tandem mass spectrometry (LC–MS/MS) as the determination method. The method was successfully applied in market survey samples.

MATERIALS AND METHODS

Chemicals and Reagents. Analytical standards of the pesticides in the study were provided by the Institute of the Control of Agrochemicals, Ministry of Agriculture, People's Republic of China. The purities of the standard pesticides were from 95% to 99%. Stock solutions of 10 mg L⁻¹ for mixture pesticides were prepared in acetonitrile and stored at -20 °C. The working solutions were prepared daily. HPLC-grade acetonitrile was obtained from Fisher Chemicals (Fair Lawn, NJ). Analytical-reagent-grade anhydrous sodium chloride (NaCl) and magnesium sulfate (MgSO₄) were obtained from Sinopharm Chemical Reagent Co. (Beijing, China).

MWCNTs with average external diameters of 10–20 nm, GCB, and PSA were provided by Tianjing Agela Co. Ltd. (China). MWCNTs were dried for 2 h at 120 °C to remove the absorbed water and then kept in desiccators for storage.

Instrumentation and LC–MS/MS Analytical Conditions. Chromatographic separation was carried out on an Agilent Technologies 6410 high-performance liquid chromatograph (Agilent, Santa Clara, CA) equipped with an Eclipse Plus C18 column (2.1 × 50 mm, 3.5 μm; Agilent). Separation of the analytes from the C18 column was performed at a flow rate of 0.2 mL min⁻¹. The column was kept at 25 °C. The sample volume injected was maintained at 5 μL. Gradient ultraperformance liquid chromatography (UPLC) elution was performed with acetonitrile (LC grade) as mobile phase A and 0.1% formic acid in ultrapure water as mobile phase B. Table 1 summarizes the gradient LC conditions in positive mode.

Analysis of these compounds was carried out on a triple-quadrupole mass spectrometer (Agilent) using the multiple reaction monitoring (MRM) mode and positive electrospray ionization (ESI) mode. The capillary current was 9 nA, and the pressure of the nebulizing gas was 35.0 psi. The drying gas had a temperature of 350 °C and a flow rate of 8.00 L min⁻¹. The mass scan ranged from 50 to 500, and then the

triple-quadrupole mass spectrometer was operated in MRM after selected ion monitoring had been performed. Table 2 shows MRM data acquisition parameters of LC–MS/MS for the 37 pesticides selected.

Sample Preparation. Green tea, oolong tea, and puer tea were obtained from a local supermarket. The samples were homogenized for 10 s at room temperature. A tea sample (5 g, weighed to a precision of 0.01 g) was introduced into a 50 mL Teflon centrifuge tube. A 5 mL volume of water was added, and the samples were allowed to stand for 5 min before extraction. After that, 10 mL of acetonitrile was added, and the tube was shaken vigorously for 1 min with a vortex mixer, ensuring that the solvent interacted well with the entire sample. Anhydrous NaCl (1 g) and anhydrous MgSO₄ (4 g) were added to the mixture, and the shaking step was repeated for 1 min. After the tube was cooled in an ice–water bath immediately for 5 min, the tube was centrifuged for 10 min at 3800 rpm.

Sample Cleanup. After centrifugation, 1 mL of the clarified supernatant was introduced into a 2.0 mL microcentrifuge tube containing 15 mg of MWCNTs, 25 mg of PSA, 5 mg of GCB, and 150 mg of MgSO₄. The mixture was shaken vigorously for 1 min and centrifuged for 3 min at 10000 rpm with a microcentrifuge. Finally the acetonitrile layer was filtered through a 0.22 μm filter membrane, and 0.5 mL of the extract was placed into an LC vial to carry out the chromatographic analysis.

Method Performances. Three kinds of teas were selected for validation purposes: green tea (unfermented), oolong tea (semi-fermented), and puer tea (fermented). The validation data were carried out for each type of tea. The following parameters were determined during validation of the analytical method: linearity, matrix effect, LOQ (limit of quantification), LOD (limit of detection), accuracy, and precision. Linearity was studied using matrix-matched calibration by analyzing samples of green tea, oolong tea, and puer tea. The accuracy and precision of the method were tested via recovery and reproducibility experiments which were carried out for each tea sample in five replicates each at two fortification levels (10 and 100 μg kg⁻¹). The LODs were determined as the concentration of analyte giving a signal-to-noise ratio (S/N) of 3 for the target ion (the less intense transition); LOQs were determined as the concentration of analytes giving a signal-to-noise ratio (S/N) of 10 for the target ion (the less intense transition).

RESULTS AND DISCUSSION

Liquid Chromatography–Tandem Mass Spectrometry. For the purpose of finding the retention times and the best resolution between the analyte peaks, preliminary experiments were carried out to systematically vary the strength of the mobile phase and fragmentor voltage in full scan mode using compound standard solutions.

At the initial development, the LC condition was set up using CH₃CN/H₂O (0.1% HCOOH), 50:50 (v/v). However, this attempt did not contribute to good peak shapes or an ideal separation among the analytes, and quite long retention times for some pesticides with low polarities were presented. With respect to separation efficiency (peak shape) and sensitivity, gradient elution with aqueous acetonitrile–formic acid was more desirable than the other options, so acetonitrile–formic acid was chosen as the mobile phase.

A tandem mass detector has high selectivity and sensitivity and provides an effective solution. The mass spectrometric parameter option was initially performed by full scan and daughter scan for the compounds. The [M + H]⁺ ion was chosen as the precursor ion for all analytes. The characteristic ion transition, fragmentor voltage, and collision energy for each compound during MRM acquisition are listed in Table 2. Product ion mass spectra for the pesticides were obtained in electrospray ionization using collision-induced dissociation. The collision energy was optimized for two selective ion

Table 1. Gradient LC Conditions^a

time (min)	mobile phase A concn (%)	mobile phase B concn (%)
0	30	70
3	60	40
6	70	30
15	99	1
16	30	70
26	30	70

^aAcetonitrile (LC grade) as mobile phase A and 0.1% formic acid in ultrapure water as mobile phase B.

Table 2. MRM Transitions and Other LC–MS/MS Parameters

no.	pesticide	t_R (min)	confirmation transition ^a	quantification transition ^a	fragmentor voltage (V)
1	carbendazim	1.00	192 → 132 (25)	192 → 160 (20)	90
2	thiamethoxam	1.36	292 → 181 (20)	292 → 211 (10)	80
3	imidacloprid	1.73	256 → 175 (10)	256 → 209 (10)	80
4	acetamiprid	2.00	223 → 90 (20)	223 → 126 (15)	80
5	thiacloprid	2.66	253 → 126 (20)	253 → 186 (10)	90
6	prometryn	5.24	242 → 200 (20)	242 → 158 (20)	120
7	atrazine	6.17	216 → 132 (20)	216 → 174 (15)	120
8	flutriafol	6.68	302 → 123 (20)	302 → 70 (15)	120
9	carboxin	6.78	236 → 87 (20)	236 → 143 (20)	120
10	metalaxy-M	6.82	280 → 192 (15)	280 → 220 (15)	120
11	RH-5849	7.60	319 → 263 (10)	319 → 197 (10)	120
12	clomazone	8.01	240 → 89 (30)	240 → 125 (20)	120
13	paclobutrazol	8.36	294 → 125 (25)	294 → 70 (20)	120
14	prochloraz	8.57	376 → 266 (10)	376 → 308 (10)	80
15	methidathion	8.73	303 → 85 (10)	303 → 145 (5)	80
16	azoxystrobin	9.22	404 → 344 (15)	404 → 372 (10)	85
17	triadimefene	9.28	294 → 69 (15)	294 → 197 (10)	85
18	tebuconazole	9.40	308 → 125 (30)	308 → 70 (30)	85
19	metolachlor	9.87	284 → 176 (15)	284 → 252 (10)	120
20	acetochlor	10.01	270 → 148 (10)	270 → 224 (10)	120
21	diconazole	10.04	326 → 159 (30)	326 → 70 (25)	120
22	propiconazole	10.07	342 → 69 (20)	342 → 159 (20)	120
23	fluorochloridone	10.10	312 → 89 (25)	312 → 292 (25)	100
24	chlorfenvinphos	10.40	359 → 155 (10)	359 → 99 (25)	120
25	difenoconazole	10.73	406 → 337 (15)	406 → 251 (20)	160
26	anilofos	11.06	368 → 125 (10)	368 → 199 (5)	100
27	diazinon	11.24	305 → 97 (25)	305 → 153 (20)	160
28	phoxim	11.84	299 → 129 (10)	299 → 77 (20)	80
29	haloxyfop-P-methyl	11.99	376 → 288 (20)	376 → 316 (15)	120
30	fenoxaprop-P-ethyl	12.33	362 → 244 (20)	362 → 288 (20)	120
31	quizalofop-P-ethyl	12.40	373 → 271 (25)	373 → 299 (15)	120
32	clethodim	12.71	360 → 268 (10)	360 → 164 (20)	120
33	butachlor	13.43	312 → 162 (20)	312 → 238 (10)	80
34	fluazifop-P-butyl	13.52	384 → 328 (15)	384 → 282 (20)	120
35	oxadiazon	13.58	345 → 177 (20)	345 → 220 (20)	100
36	tralkoxydim	13.60	330 → 138 (20)	330 → 284 (5)	100
37	chlorpyrifos	13.70	350 → 97 (15)	350 → 198 (20)	100

^aThe collision energy (eV) is given in parentheses.

transitions for every pesticide. Both pairs of MRM transitions were used for confirmation analysis, which meets the requirements of the European Union (EU) decision,³² and the most sensitive transitions were selected for quantification analysis. Dwell times for different transitions were optimized to achieve higher sensitivities as well.

In total, the ionization of 37 pesticides in the positive mode electrospray ion source was investigated. Conditions for interfacing the LC system to the MS were evaluated. The developed method was also highly selective with the monitoring of a specific MRM of each analyte, which was essential to reduce the risk of false-positive results.

Extraction Procedure Optimization. Taking into account that according to the QuEChERS method samples with a water content between 25% and 80% require the addition of water to achieve the same amount of sample and water, the addition of water was considered.¹⁶ A certain amount of water was added to achieve sufficient participation of the target analytes among the matrix, water, and an organic solvent such as acetonitrile. The QuEChERS method recommends the addition of water in some cases (depending on the humidity of the sample) to allow

the extraction solvent access to the sample and to increase the extraction efficiency. Addition of water to the dry samples before extraction has been reported.^{33–36}

Due to the low content of water in tea, it might be necessary to add a small amount of water to the tea samples before the analytes are extracted by acetonitrile. To evaluate the effect of water, different amounts of water were investigated before the same extraction procedure. The amount of water was progressively increased from 0 to 10 mL (i.e., 0, 2, 3, 5, 8, and 10 mL). For comparison, after water addition, tea samples (5 g) spiked at a level of 100 $\mu\text{g kg}^{-1}$ were extracted. As the amount of water increased, most recoveries of the analytes were at the acceptable range, 70–120%,³⁷ for the tea samples, except for three pesticides. As shown in Figure 1, the recoveries for tebuconazole and prochloraz were no more than 70% when 0 or 2 mL of water was added, but with more than 3 mL of water added, they became higher. As for carbendazim, 3 mL of water was not enough to attain an acceptable recovery, and 5 mL or more of water should be added. In addition, although good recoveries were also achieved with addition of 8 or 10 mL of water, more chromatography interferences and more obvious

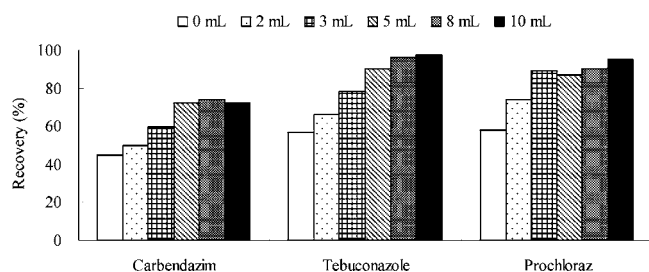


Figure 1. Effects of water addition before extraction on recoveries of carbendazim, tebuconazole, and prochloraz at a spiked level of $100 \mu\text{g kg}^{-1}$ for a green tea sample.

matrix effects appeared when more than 5 mL of water was used. Therefore, 5 mL of water (for 5 g tea samples) added before extraction was employed as the optimum amount in the study.

Optimization of the r-DSPE Procedure. As it is known that a tea sample is a complex matrix, it is difficult to purify, so one important influence on the analysis results may be attributed to the matrix cleanup efficiency. The original QuEChERS method involved extraction with acetonitrile, partitioning between acetonitrile and the aqueous phase after addition of sodium chloride and magnesium sulfate, and an r-DSPE cleanup procedure with a small quantity of SPE sorbents (PSA/GCB/C18). During the process of sample preparation, it was found that the differences among different dispersive sorbents had a significant influence on the purification and recovery of pesticide extracts.

PSA sorbent, a weak anion exchanger which strongly interacts with various polar organic acids, was used as the sorbent in most cases (50 mg mL^{-1}). Because of the high pigment content in tea samples, we attempted purification using GCB mixed with PSA because sterols and pigments such as chlorophyll could be absorbed by GCB. At this stage, different amounts of GCB (i.e., 5, 8, 10, and 15 mg) were used to purify the tea samples. However, with more than 5 mg of GCB mixed with 25 mg of PSA, the recoveries became lower than 70% for carbendazim, thiacloprid, carboxin, and chlorpyrifos, and the recoveries became much lower when 10 mg of GCB without PSA was used as the r-DSPE sorbent. Using less GCB and more PSA or MWCNTs may work better. As a result, only 5 mg of GCB mixed with PSA was chosen.

In this study, we attempted to mix MWCNTs with PSA and GCB to improve the performance of r-DSPE cleanup. To evaluate the effect of this parameter, different amounts of MWCNTs and PSA were investigated with the same amount of GCB (5 mg). As the amount of the sorbents decreased, most recoveries of the analytes increased from 40–80% to 70–110%, and Table 3 shows the number of tested pesticides in different ranges of recoveries.

For example, different amounts of MWCNTs and PSA affected the recoveries for carbendazim, chlorpyrifos, and butachlor as shown in Figure 2. When 25 mg of PSA, 15 mg of MWCNTs, and 5 mg of GCB were used, the recoveries ranged from 80% to 100%, which were acceptable. Moreover, as shown in Figure 3, the final tea sample processed by MWCNTs (15 mg) mixed with PSA (25 mg) and GCB (5 mg) had a lighter color than that processed by only PSA (25 mg) and GCB (5 mg). MWCNTs mixed with PSA and GCB displayed a better cleanup performance in removal of pigment in tea samples. Figure 4 shows an LC–MS/MS chromatogram of the

Table 3. Number of 37 Tested Pesticides in Different Ranges of Recoveries Using Different Amounts of MWCNTs and PSA

recovery range (%)	50 mg of PSA + 20 mg of MWCNTs	50 mg of PSA + 15 mg of MWCNTs	25 mg of PSA + 20 mg of MWCNTs	25 mg of PSA + 15 mg of MWCNTs
40–60	3	3	1	
60–70	21	3	5	
70–80	13	6	6	6
80–90		18	17	14
90–100		7	8	16
100–110				1

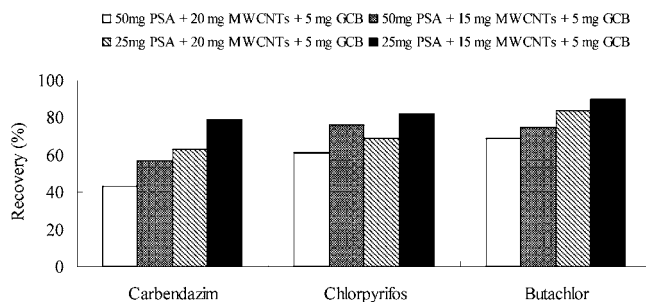


Figure 2. Effects of r-DSPE sorbents on the recoveries of carbendazim, chlorpyrifos, and butachlor at a spiked level of $100 \mu\text{g kg}^{-1}$ for a green tea sample.

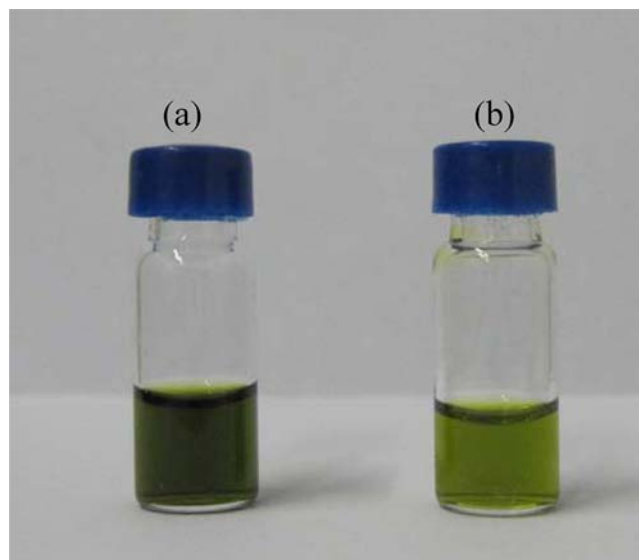


Figure 3. Photography of cleanup performance by different r-DSPE sorbents: (a) extract for green tea with PSA and GCB cleanup; (b) extract for green tea with MWCNT, PSA, and GCB cleanup.

blank tea samples after cleanup procedures using MWCNTs with PSA and GCB and only PSA and GCB, the standard solution, and the spiked sample after three kinds of sorbent cleanups, respectively. There were fewer interference appearances in the chromatogram of samples with the MWCNT cleanup step than those with only PSA and GCB cleanup. Although it was reported that the recoveries might still be acceptable even if only PSA and GCB were used,³⁸ the r-DSPE cleanup efficiency for MWCNTs mixed with PSA and GCB was higher than that for the PSA and GCB cleanup step, and few interference peaks around the peaks of each pesticide were observed in our study. As a result, 25 mg of PSA, 15 mg of

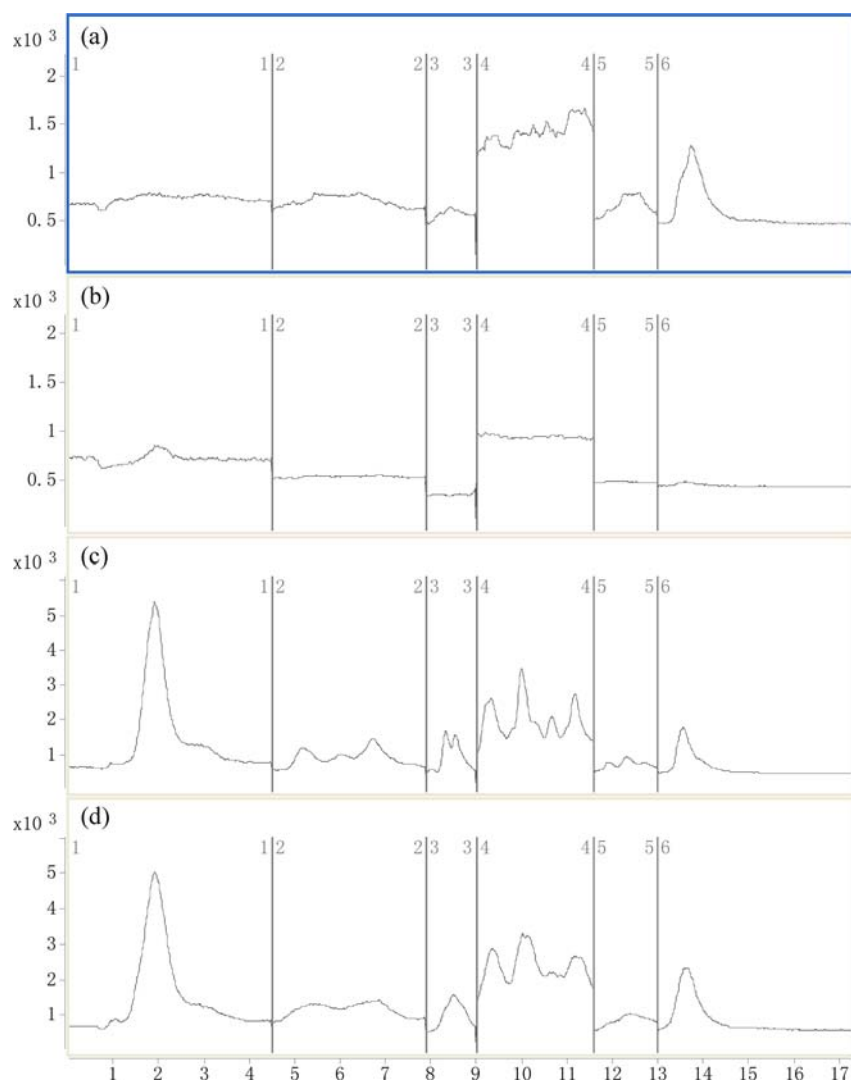


Figure 4. Chromatograms for a green tea extract after cleanup with different r-DSPE sorbents: (a) Total ion current (TIC) chromatogram for a typical blank sample with PSA and GCB cleanup; (b) TIC chromatogram for a typical blank sample with MWCNT, PSA, and GCB cleanup; (c) chromatogram for the standard solution at $10 \mu\text{g kg}^{-1}$; (d) TIC chromatogram for a typical blank sample spiked at $10 \mu\text{g kg}^{-1}$ of the target analytes with MWCNT, PSA, and GCB cleanup.

MWCNTs, and 5 mg of GCB (1 mL of extract) were chosen as the optimum amounts for the r-DSPE cleanup in further studies since acceptable recoveries and good cleanup performances were obtained.

Method Validation. Calibration curves were constructed from calibration solutions in extracts of three kinds of teas, green tea, oolong tea, and puer tea, at five different concentrations in the range of $5\text{--}500 \mu\text{g L}^{-1}$ for all pesticides. The quantitative results of a detection method greatly depend on its calibration. As shown in Table 4, good linearity was found for most pesticides with coefficients of determination (R^2) better than 0.995.

Matrix effects may be different due to the various kind of teas, e.g., green, oolong, and puer tea. To evaluate the impact of the matrix on the analytes, the slopes obtained in the calibration with matrix-matched standards were compared with those obtained with solvent-based standards. The slope ratio matrix/solvent was calculated for each of the 37 studied pesticides in the three kinds of teas. The results are summarized in Table 4. Minimal matrix effects occurred on most compounds studied (<0.9) in the three kinds of teas, and no matrix enhancement

effect was observed. As a result, compensation of the matrix effects by matrix-matched calibration is required and could be more efficient.

Table 4 also summarizes LOQ and LOD values for the pesticides studied. The described method was tested for simultaneous extraction and determination of 37 pesticides in three kinds of teas, which manifested varying levels of LOD and LOQ, because LOQs and LODs were matrix dependent. The LODs (S/N ratio of 3) and LOQs (S/N ratio of 10) for 37 pesticides in the three teas ranged from 1 to $5 \mu\text{g kg}^{-1}$ and from 5 to $20 \mu\text{g kg}^{-1}$, respectively.

Recovery and repeatability of the method were established to evaluate the method performance. The repeatability and the accuracy of the method were studied by carrying out five consecutive extractions ($n = 5$) of spiked matrixes at two concentration levels (10 and $100 \mu\text{g kg}^{-1}$). All the recoveries were determined by analyzing 37 pesticides in green tea, oolong tea, and puer tea. Table 5 shows detailed recovery and repeatability data for all pesticides analyzed in the three teas. The recoveries of all pesticides were in the range of 71–111% (71–111% for green tea, 70–106% for oolong tea, and 73–

Table 4. Matrix Effects, Calibration Curve Coefficients (R^2), LOQs ($\mu\text{g kg}^{-1}$, S/N Ratio of 10), and LODs ($\mu\text{g kg}^{-1}$, S/N Ratio of 3) for 37 Pesticides in Green Tea, Oolong Tea, and Puer Tea

compd	green tea				oolong tea				puer tea			
	matrix effect	R^2	LOQ	LOD	matrix effect	R^2	LOQ	LOD	matrix effect	R^2	LOQ	LOD
carbendazim	0.71	0.9992	10	3	0.81	0.9997	10	3	0.73	0.9992	15	5
thiamethoxam	0.75	0.9999	10	3	0.78	0.9998	10	3	0.72	0.9999	10	3
imidacloprid	0.84	0.9970	10	3	0.86	0.9996	10	3	0.82	0.9995	10	3
acetamiprid	0.74	0.9940	5	2	0.76	0.9936	5	2	0.75	0.9949	5	2
thiacloprid	0.91	0.9940	5	2	0.86	0.9949	10	3	0.81	0.9938	5	2
prometryn	0.95	0.9996	5	2	1.00	0.9999	5	2	1.05	1.0000	5	2
atrazine	0.78	0.9996	5	2	0.80	0.9999	5	2	0.88	0.9999	5	2
flutriafol	0.82	0.9997	5	2	0.76	0.9997	5	1	0.80	0.9997	5	2
carboxin	0.60	0.9987	5	2	0.57	0.9998	5	1	0.62	0.9998	5	2
metalaxy-M	0.77	0.9998	5	2	0.76	0.9996	5	1	0.87	0.9996	5	2
RH-5849	0.78	0.9995	5	1	0.73	1.0000	5	1	0.76	1.0000	5	2
clomazone	0.60	0.9993	5	1	0.57	0.9999	5	2	0.63	0.9999	5	2
paclobutrazol	0.74	0.9987	5	1	0.78	0.9997	5	2	0.81	0.9949	5	2
prochloraz	0.85	0.9997	5	2	0.81	0.9998	5	2	0.88	0.9999	5	2
methidathion	0.53	0.9999	5	2	0.40	0.9996	5	2	0.46	0.9999	5	2
azoxystrobin	0.71	0.9978	5	2	0.82	0.9940	5	2	0.71	0.9977	5	1
triadimefone	0.79	0.9997	5	2	0.87	0.9996	5	2	0.83	0.9998	5	2
tebuconazole	0.87	0.9995	5	2	0.96	0.9996	5	2	0.83	0.9999	5	2
metolachlor	0.85	0.9998	5	2	0.94	0.9997	5	2	0.99	0.9992	5	2
acetochor	0.75	1.0000	5	1	0.82	0.9923	10	3	0.78	0.9999	10	3
diniconazole	0.78	0.9998	5	2	0.92	0.9998	5	2	0.83	0.9942	5	2
propiconazole	0.86	0.9997	5	2	0.94	0.9999	5	2	0.85	0.9983	5	2
fluorochloridone	0.35	0.9989	10	3	0.44	0.9998	20	5	0.26	0.9999	20	5
chlorfenvinphos	0.69	0.9999	5	2	0.77	0.9997	10	3	0.91	0.9996	10	3
difenoconazole	0.85	0.9997	5	2	0.89	0.9945	5	2	1.04	0.9999	5	2
anilofos	0.93	0.9998	5	2	0.92	0.9997	5	2	0.99	0.9998	5	2
diazinon	0.81	0.9996	5	2	0.80	0.9969	5	2	0.81	0.9999	5	2
phoxim	0.77	0.9998	5	2	0.70	0.9999	5	2	0.78	0.9998	5	1
haloxyfop-P-methyl	0.81	0.9996	5	2	0.90	0.9988	5	2	0.89	0.9997	5	2
fenoxaprop-P-ethyl	0.83	0.9967	5	1	0.90	0.9999	5	2	0.98	0.9945	5	2
quizalofop-P-ethyl	0.81	0.9997	5	2	0.88	0.9999	5	2	0.92	0.9997	5	2
clethodim	0.79	0.9986	5	2	0.89	0.9978	5	1	0.96	0.9958	5	2
butachlor	0.91	0.9999	5	1	1.10	0.9998	5	2	1.07	0.9966	10	3
fluazifop-P-butyl	0.82	0.9997	5	2	1.03	0.9999	5	2	1.07	0.9948	5	2
oxadiazon	0.66	0.9998	5	2	0.62	0.9998	10	3	0.76	0.9999	10	3
tralkoxydim	0.80	0.9996	5	2	0.97	0.9966	5	2	0.92	0.9979	5	2
chlorthrifos	0.68	0.9997	5	2	0.58	0.9998	10	3	0.78	0.9999	10	3

109% for puer tea). Relative standard deviations (RSDs) were below 14% for all cases. As shown in Table 5, in some cases, for example, the value for the $10 \mu\text{g kg}^{-1}$ spiked carbendazim in puer tea is “<LOQ”. The LOQ value for carbendazim in puer tea was higher than $10 \mu\text{g kg}^{-1}$, and it did not meet the requirement to quantify the lower residue concentration levels ($10 \mu\text{g kg}^{-1}$) of the analyses. However, the LOQs in green tea and oolong tea were $10 \mu\text{g kg}^{-1}$, and those did meet the requirement. Therefore, the values for the $10 \mu\text{g kg}^{-1}$ spiked level were <LOQ in some cases. In general, the validation data for all pesticides were in accordance with the EU guidelines for pesticide residue analysis,³⁷ reflecting good method performance.

Method Application. The effectiveness of this method in measuring trace levels of the 37 pesticides was monitored by analyzing 10 tea samples (green tea, oolong tea, and puer tea) from local markets and supermarkets in Beijing. The samples were made in six different areas of China: Zhejiang, Sichuan, Fujian, Yunnan, Anhui, and Henan provinces. The developed QuEChERS method with a MWCNT, PSA, and GCB cleanup

step was applied to the real samples. They were treated with the sample preparation method described in the sections “Sample Preparation” and “Sample Cleanup” and then analyzed by LC–MS. All of the residue data were lower than the LOQ values.

A modified QuEChERS method coupled with LC–MS/MS was developed in the present study for the simultaneous determination of 37 pesticides in 3 kinds of teas: green, oolong, and puer tea. The cleanup step was optimized as the typical r-DSPE method for extracting multipesticides from tea samples. It was demonstrated for the first time that MWCNTs could be mixed with PSA and GCB as r-DSPE materials for the cleanup of extract. Satisfactory precision, accuracy, and selectivity were also acquired with recoveries ranging from 70% to 111% and RSD values below 14%. The original QuEChERS preparation method was employed. In conclusion, MWCNTs proved to be a new type of r-DSPE sorbent material. These findings are important for pesticide analysis at trace levels for sample cleanup. This method is expected to be widely applied for monitoring of pesticides at trace levels in the future for various agricultural commodities.

Table 5. Average Recoveries (%) and RSDs at Two Spiked Levels in Green Tea, Oolong Tea, and Puer Tea

pesticide	average recovery, % (RSD, %) (n = 5)					
	green tea		Oolong tea		puer tea	
	10 $\mu\text{g kg}^{-1}$	100 $\mu\text{g kg}^{-1}$	10 $\mu\text{g kg}^{-1}$	100 $\mu\text{g kg}^{-1}$	10 $\mu\text{g kg}^{-1}$	100 $\mu\text{g kg}^{-1}$
carbendazim	76(9)	72(8)	72(12)	71(4)	<LOQ	78(9)
thiamethoxam	77(8)	72(4)	80(7)	74(7)	85(7)	73(8)
imidacloprid	98(14)	73(6)	88(6)	75(4)	88(8)	82(4)
acetamiprid	105(14)	72(2)	100(5)	89(3)	83(7)	88(6)
thiacloprid	73(14)	70(3)	83(6)	79(8)	91(8)	77(7)
prometryn	81(5)	79(4)	91(5)	92(3)	101(6)	84(5)
atrazine	81(7)	80(8)	87(8)	96(3)	104(10)	86(6)
flutriafol	76(7)	80(4)	95(3)	89(3)	99(5)	90(5)
carboxin	74(12)	74(6)	70(8)	84(5)	79(8)	74(4)
metalaxy-M	81(13)	85(6)	104(14)	97(2)	91(5)	79(4)
RH-5849	99(2)	105(5)	109(5)	93(4)	105(6)	88(7)
clomazone	93(5)	76(14)	90(4)	106(2)	109(17)	76(6)
paclobutrazol	86(6)	75(6)	90(6)	97(1)	108(3)	87(3)
prochloraz	85(2)	74(6)	83(3)	85(4)	99(9)	77(4)
methidathion	79(9)	82(3)	91(11)	94(2)	100(4)	72(5)
azoxystrobin	78(12)	87(2)	92(3)	102(3)	77(6)	79(5)
triadimefene	92(4)	86(6)	98(6)	100(3)	109(9)	90(10)
tebuconazole	86(4)	81(4)	91(5)	91(3)	102(7)	85(6)
metolachlor	93(9)	82(2)	94(5)	104(4)	100(9)	83(5)
acetochlor	86(13)	79(7)	105(13)	103(3)	91(7)	77(4)
diniconazole	82(8)	79(6)	86(3)	92(3)	102(7)	84(4)
propiconazole	90(9)	81(2)	97(2)	92(4)	107(8)	86(4)
fluorochloridone	92(10)	87(13)	<LOQ	96(6)	<LOQ	80(11)
chlorfenvinphos	86(9)	75(8)	92(4)	98(6)	99(8)	86(3)
difenoconazole	74(10)	79(3)	95(3)	92(5)	98(8)	87(8)
anilofos	79(8)	75(4)	96(4)	91(3)	98(3)	86(5)
diazinon	104(4)	77(3)	91(3)	99(3)	104(9)	93(4)
phoxim	72(13)	72(10)	84(7)	102(4)	90(8)	89(4)
haloxyfop-P-methyl	83(7)	80(5)	98(3)	106(4)	97(8)	93(7)
fenoxaprop-P-ethyl	76(4)	78(1)	105(7)	96(1)	98(7)	92(6)
quizalofop-P-ethyl	88(10)	71(2)	100(5)	92(3)	107(5)	91(7)
clethodim	74(12)	74(2)	70(7)	80(4)	81(5)	78(11)
butachlor	105(12)	71(8)	99(7)	92(3)	92(7)	85(5)
fluzifop-P-butyl	83(2)	76(4)	101(3)	106(4)	88(13)	101(8)
oxadiazon	88(10)	111(5)	93(7)	99(2)	101(8)	90(4)
tralkoxydim	71(7)	71(4)	77(4)	80(2)	88(4)	84(5)
chlorpyrifos	90(13)	74(13)	101(12)	104(4)	105(9)	104(11)

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ABBREVIATIONS USED

r-DSPE, reversed-dispersive solid-phase extraction; LC-MS/MS, liquid chromatography-electrospray tandem mass spectrometry; MWCNTs, multiwalled carbon nanotubes; PSA, primary secondary amine; GCB, graphitized carbon black; LOQ, limit of quantification; MRL, maximum residue limit; QuEChERS, quick, easy, cheap, effective, rugged, and safe; CNTs, carbon nanotubes; MRM, multiple reaction monitoring; LOD, limit of detection; S/N, signal-to-noise ratio; RSD, relative standard deviation; TIC, total ion current

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