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Development of an analytical method for pesticide residues in berries with dispersive solid phase extraction using multiwalled carbon nanotubes and primary secondary amine sorbents†

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A dispersive solid phase extraction using primary secondary amine and multi-walled carbon nanotubes as a mixed sorbent material was developed for the multiple pesticide residue analysis of berry samples. Gas chromatography-mass spectrometry analysis in selected ion monitoring mode was used for the determination of multiple pesticide residue levels in berry samples. In this study, strawberry, raspberry, blueberry and blackberry were selected as representative matrices. In the sample preparation process, the chosen extraction solvent was acetonitrile and inorganic salt was added to partition pesticide residues into the acetonitrile phase. The cleanup step was carried out by dispersive solid phase extraction based on a mixture of primary secondary amine and multi-walled carbon nanotubes to remove interferences in the extracts. After further optimization of the sample preparation and determination steps, the percentage recovery was in the range of 71% to 123% with an intra-day precision of less than 13% and inter-day precision of less than 20% in most cases. The proposed method was used successfully for the quantitative determination of pesticide in commercial berry samples.

Introduction

Berry fruits provide many important nutrients, such as minerals, vitamins, fiber and folic acid. Especially, their biological action is often most attributed to their polyphenolic content.[1] They are an important part of the human diet and very intensively chemically protected.[2] In 2014, berry production (including blueberries, cranberries, currants, gooseberries, raspberries and strawberries) was 2 509 242 tons in Northern America, 2 922 875 tons in Europe, and 3 986 400 tons in Asia.[3] However, many diseases and insects attack berry fruits, which severely reduces their production and quality, shortens their shelf-life and causes serious loss to producers. Therefore, pesticides are always applied by growers since they minimize the loss caused by diseases and insects. However, the excessive use of pesticides affects environmental safety and causes potential threat to human health.

The consumption of berry fruits by humans represents an important potential source of exposure to pesticides and other harmful chemical substances, such as organophosphorus, organochlorine, pyrethroid and carbamate. This is inevitable for pests and disease control during plant cultivation, processing and manufacturing.[4] Many international organizations and countries have set maximum residue limits (MRLs) for berry fruits, which vary by country and region. This is important in many fields, such as risk assessment, import and export trade, and supervision enforcement. For example, the US has set the MRL for malathion in strawberry as 8 mg kg^{-1}; Codex, Australia and China set it as 1 mg kg^{-1}; but the EU has set it as 0.02 mg kg^{-1} and in Japan and Korea it is as low as 0.5 mg kg^{-1}. Therefore, the trace measurement of pesticide residues in berry fruits has great significance in both human health and international trade.

Many types of methods have been developed for the analysis of pesticide residue according to the nature of the sample, analyte concentration, and measurement technique. In most cases, the analysis follows a few steps, including extracting the analyte from grounded samples, removing the interference substances, and detection by chromatography or mass spectrometry. The most important procedure is probably the removal of interference substances from the extraction. In this procedure, solid phase extraction (SPE) is the most widely used cleanup approach,[4–8] although many other cleanup methods have also been developed such as gel permeation

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chromatography,[9,10] solid phase microextraction,[11,12] microwave-assisted extraction,[13,14] matrix solid phase dispersion.[15,16] Some of these approaches consume large quantities of organic solvents and require a few complex operations, which are expensive and time consuming.

In 2003, Anastassiades and Lehotay *et al.* introduced the QuEChERS (quick, easy, cheap, effective, rugged and safe) method,[17,18] and in recent years, many studies have been focused on its application for pesticides analysis.[19–22] In the extraction step of the QuEChERS method, only several milliliters of the organic solvent acetonitrile are used for one sample without any chlorinated solvent. The percentage recoveries of the QuEChERs method are high for most pesticides with various polarities and volatilities. Also, it requires very little experimental apparatus and improves the safety of operators in many ways.[23] Based on its comprehensive advantages, the QuEChERS method is widely applied for the analysis of pesticides with different polarities. In the cleanup procedure of the QuEChERS method, the dispersive solid phase extraction (D-SPE) technique is carried out to promote the laboratory efficiency and analytical quality of conventional sample preparation processes. The main sorbent used in the D-SPE process is a weak anion exchange material, primary secondary amine (PSA), which can absorb some matrix interferences such as various polar pigments, polar organic acids, fatty acids and some sugars.[24–26] In some studies, a mixture of several types of sorbents showed better cleanup performances and recoveries.[27–29]

In 1991, carbon nanotubes (CNTs) were first reported, which had great possibilities.[30] Their applications are many and varied, including electronic materials, field emitters, nanoprobes, sensors (gas, enzymatic, *etc.*), and electrodes. In the field of analytical chemistry, multi-walled carbon nanotubes (MWCNTs) are highly applied as absorbent fillers in the SPE method.[31–34] In our previous work, MWCNTs exhibited a good cleanup performance as D-SPE sorbents in pesticide residue analysis for vegetable and fruit samples.[35,36] For tea samples, they could be mixed with other sorbents to enhance the cleanup performance of the D-SPE process.[37]

In this study, to enhance the cleanup performance in the QuEChERS method, a mixture of MWCNTs and PSA is employed in the D-SPE procedure. To evaluate the proposed method, 41 pesticides with different log *P* and chemical constituents were analyzed and gas chromatography-mass spectrometry (GC-MS) was used to detect the concentrations of pesticide residue. It is expected that the modified QuEChERS method can be used for the quantitative analysis of pesticide in commercial berry samples.

Experimental

Materials

Analytical pesticide standards were purchased from Restek Corporation (USA). Ten mg L^{-1} of mixed standards was prepared with acetonitrile and stored at 4 °C. Triphenyl phosphate (TPP, Sigma-Aldrich Co. LLC, USA) was used as the internal standard (IS). Chromatographic grade acetonitrile was purchased from Avantor Performance Materials (USA). Analytical reagent magnesium sulfate ($MgSO_4$) and anhydrous sodium chloride (NaCl) were purchased from Fisher Scientific (USA). PSA was purchased from Agilent Technologies (USA). MWCNTs with average external diameters of 10–20 nm were provided by Tianjin Agela Co. Ltd. (China). The blank berry samples were purchased in a supermarket, in which none of the 41 pesticide residues were detected.

Table 1 MS characteristics for the identification and quantitation of 41 compounds using GC-MS: retention time, quantification and identification ions

Compound	Retention time (min)	Quantification ion	Identification ion 1	Identification ion 2
Acetochlor	15.53	146	234	162
Atrazine	12.17	200	215	58
Azinphos-methyl	24.67	160	132	77
Bifenthrin	21.98	181	166	165
Boscalid	31.47	140	342	344
Carbaryl	13.14	144	115	116
Carfentrazone-ethyl	26.39	312	330	340
Chlorpyrifos	13.88	314	258	286
Coumaphos	31.99	362	226	364
Cyfluthrin	31.31	163	165	206
Cypermethrin	31.54	163	181	165
Cyprodinil	14.41	224	225	210
Deltamethrin	33.27	253	181	209
Diazion	11.99	304	179	137
Dichlofluanid	13.68	123	224	167
α-Endosulfan	21.60	241	265	339
β-Endosulfan	24.59	241	265	339
Fenhexamid	19.24	97	177	301
Fenitrothion	17.05	125	109	277
Fenpropathrin	22.51	181	125	265
Fenthion	13.84	278	169	153
Fludioxonil	23.39	248	189	154
Folpet	15.08	260	104	297
Iprodione	21.28	314	187	244
Kresoxim-methyl	24.25	116	131	206
Malathion	13.61	173	158	143
Metalaxyl	13.19	206	249	160
Methiocarb	13.47	168	153	225
Metolachlor	17.89	238	240	162
Myclobutanil	16.41	179	288	150
Napropamide	22.52	128	271	171
Parathion-methyl	15.59	109	125	263
Permethrin-*cis*	30.52	183	163	165
Permethrin-*trans*	31.47	183	163	165
Phenothrin	28.83	123	183	350
Phosalone	24.61	182	367	154
Pirimiphos-methyl	13.43	290	276	305
Propargite	20.00	135	350	173
Propiconazole-*cis*	26.47	259	261	263
Propiconazole-*trans*	26.61	259	261	263
Vinclozolin	12.94	212	198	285
TPP	20.10	326	325	—

GC-MS analytical conditions

An Agilent GC6890/MSD5973 equipped with an HP-5 column (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used to separate the compounds. The inlet temperature and pressure were set as 200 °C and 10.0 psi, respectively. The inlet "pulse" pressure was set as 40 psi for 0.2 min, and the injection volume was 1 μL. The column temperature was first held at 50 °C for 1.0 min, followed by a 20 °C min^{-1} ramp to 180 °C, then increased to 240 °C at 10 °C min^{-1}, and finally kept at 240 °C for 25 min. Ultra-high purity helium was used as the carrier gas with a constant flow rate of 1.3 mL min^{-1}. The total running time was 40 min.

To obtain a maximum signal for each analyte, enough dwell time and adequate acquisition points were needed for each chromatographic peak. In the GC-MS acquisition method, the pesticides were divided into groups, as many as possible according to their retention times. One quantitation and two qualitative ions were monitored for each pesticide by GC-MS. IS (TPP) was used to reduce the possibility of variation in peak areas and retention times, and thus improved the method reliability. Table 1 summarizes the retention time and monitored ions and for each compound.

Sample preparation

The strawberry, raspberry, blueberry and blackberry samples were uniformly comminuted with a homogenizer. For the additive recovery experiment, 10.0 ± 0.1 g of ground sample was added to the standard solutions at concentrations of 20 and 200 μg kg^{-1}. Before extraction, the spiked samples were left to stand for 30 min.

In the process of extraction, 10.0 ± 0.1 g of ground sample was introduced into a 50 mL centrifuge tube, and then 10.0 mL of acetonitrile was added. The centrifuge tube was shaken by a vortex mixer for 60 s. 4 g of $MgSO_4$ and 1 g of NaCl were introduced into the mixture, and then the tube was placed in an ice-water bath immediately until it cooled down to room temperature. Subsequently, to prevent salt agglomeration, the centrifuge tube was again shaken by a vortex mixer for 60 s, and then centrifuged at 3800 rpm (crf: 3802 × *g*) for 5 min. The acetonitrile extraction was used for cleanup.

Sample cleanup

After centrifugation, 1 mL of the acetonitrile supernatant was added into a 2.0 mL micro-centrifuge tube containing 5 mg of

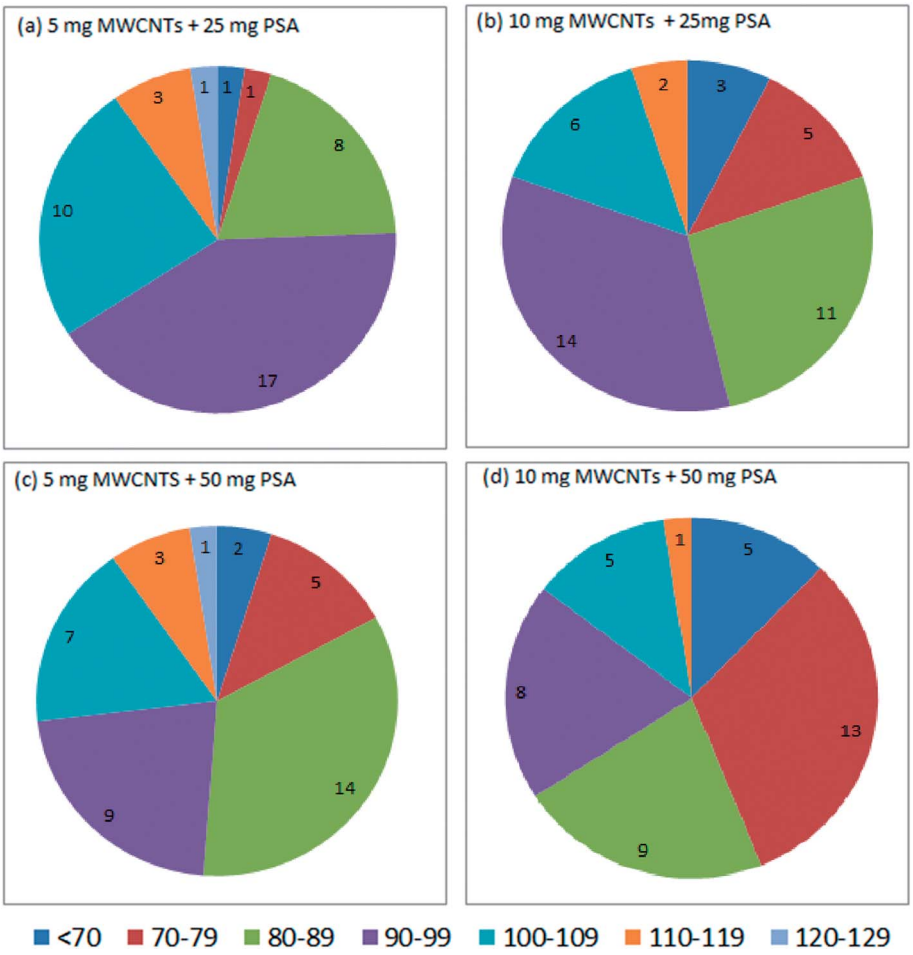

Fig. 1 Number of the 41 tested pesticides in different recovery ranges using different amounts of MWCNTS and PSA. The optimized conditions were 5 mg of MWCNTs and 25 mg of PSA, for 1 mL of extract.

MWCNTs, 25 mg of PSA and 150 mg of $MgSO_4$. After shaking vigorously for 1 min, the tube was centrifuged for 3 min at 10 000 rpm (crf: 6944 × *g*) with a microcentrifuge. The supernatant was filtered through a 13 mm 0.22 μm nylon syringe filter. Finally, 0.5 mL of the extract was placed into a vial. 5 μL of 10 mg L^{-1} TPP was added as the IS to carry out the

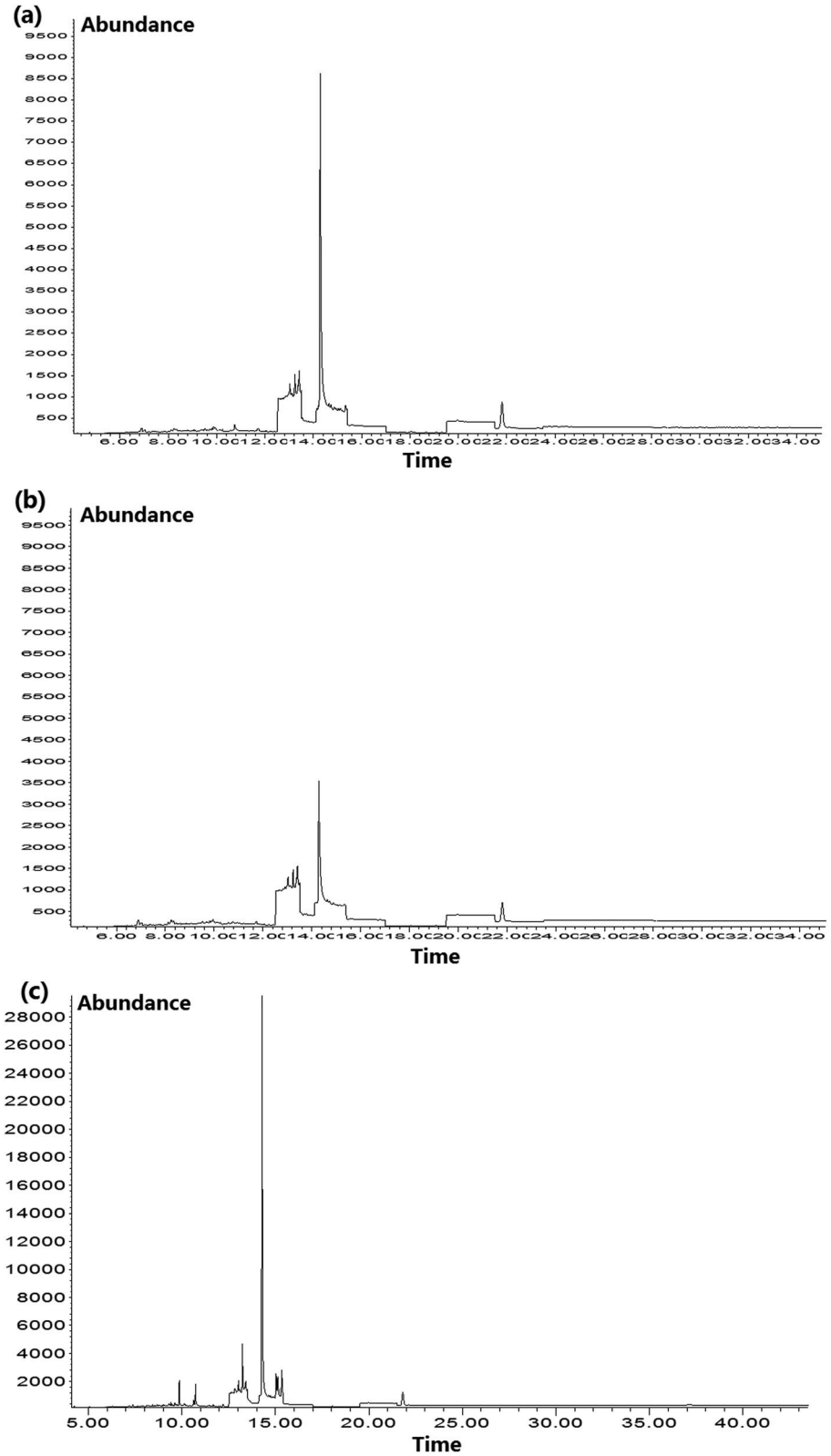

Fig. 2 SIM chromatograms for (a) blank blueberry extract with PSA cleanup; (b) blank blueberry extract with MWCNTs and PSA cleanup and (c) blank blueberry extract without cleanup.

chromatographic analysis. The equivalent of the final extracts was 1000 g mL^{-1}.

Method performances

Strawberry, raspberry, blueberry and blackberry were selected as representative berries for validation purposes. Linearity, recoveries, relative standard deviations and limit of quantification (LOQ) were evaluated to validate the proposed method. Recoveries were calculated according to the following equation:

$$\text{Recovery} = \frac{(\text{Relative A})_{\text{spike}}}{(\text{Relative A})_{\text{std}}}$$

where, Relative A is the relative peak area (analyte/IS). The concentrations of the spiked sample and matrix-matched standard were the same.

To avoid the matrix effect, matrix-matched calibration of strawberry, raspberry, blueberry and blackberry was carried out to evaluate linearity. Each representative sample was spiked at fortification concentrations of 20 and 200 μg kg^{-1}. For intra-day precision, five repeated spiked experiments were carried out in the recovery tests according to the FAO Guidelines.[38] The LOQs were estimated when the concentration of the target gave a signal to noise ratio (S/N) of 10, which was calculated by the Agilent GC/MSD ChemStation Software.

Results and discussion

Optimization of the D-SPE process

Berries are a type of fruit with different colors because they contain many natural plant pigments such as anthocyanins, tannins and flavonoids mainly in their seeds and skins.[39] These matrix compounds may affect the results of pesticide residue analysis, thus it is necessary to remove them before chromatographic analysis. In the cleanup procedure, the amount of cleanup agent affects the cleanup performance and recovery of the extraction.

In the conventional QuEChERS method, PSA serves as the sorbent in the D-SPE process. As a weak anion exchanger, PSA has strong interaction with many polar organic acids such as pigments. In certain conditions, it is mixed with other types of sorbents to increase interference removal. In our previous study, MWCNTs successfully served as an alternative cleanup agent to PSA in the QuEChERS method, which exhibited a better cleanup performance than PSA.[35] In this study, MWCNTs were mixed with PSA in the D-SPE process to increase the interference substance removal.

To obtain a good cleanup performance and high recoveries, the amount of PSA and MWCNTs was optimized. In our preliminary experiment, four different amounts of the mixture were tested for strawberry samples, which are shown in Fig. 1. As the amount and rate changed, the range of recoveries changed. Lower recoveries of some pesticides were obtained as the amount of sorbent increased. For example, when 50 mg of PSA and 10 mg of MWCNTs were added, the recovery of fenhexamid decreased to 50%. When the dosage of the sorbents was reduced to 5 and 25 mg for MWCNTs and PSA, respectively, the recovery reached 94%, which is acceptable. However, the recovery of dichlofluanid remained lower than 70%, even when less sorbent was used. It is known that dichlofluanid is degraded in the QuEChERS method[40] and a buffered extraction method may be used to improve its recoveries in berries.

In addition, Fig. 2(a and b) show the SIM chromatograms of the blueberry samples after cleanup with different sorbents. Fig. 2c shows the SIM chromatogram of the blank blueberry sample without any cleanup. It can be seen that after cleanup with the mixture of PSA and MWCNTs, less interference appeared in the chromatogram of the blank strawberry samples. After cleanup with PSA and MWCNTs, the interference

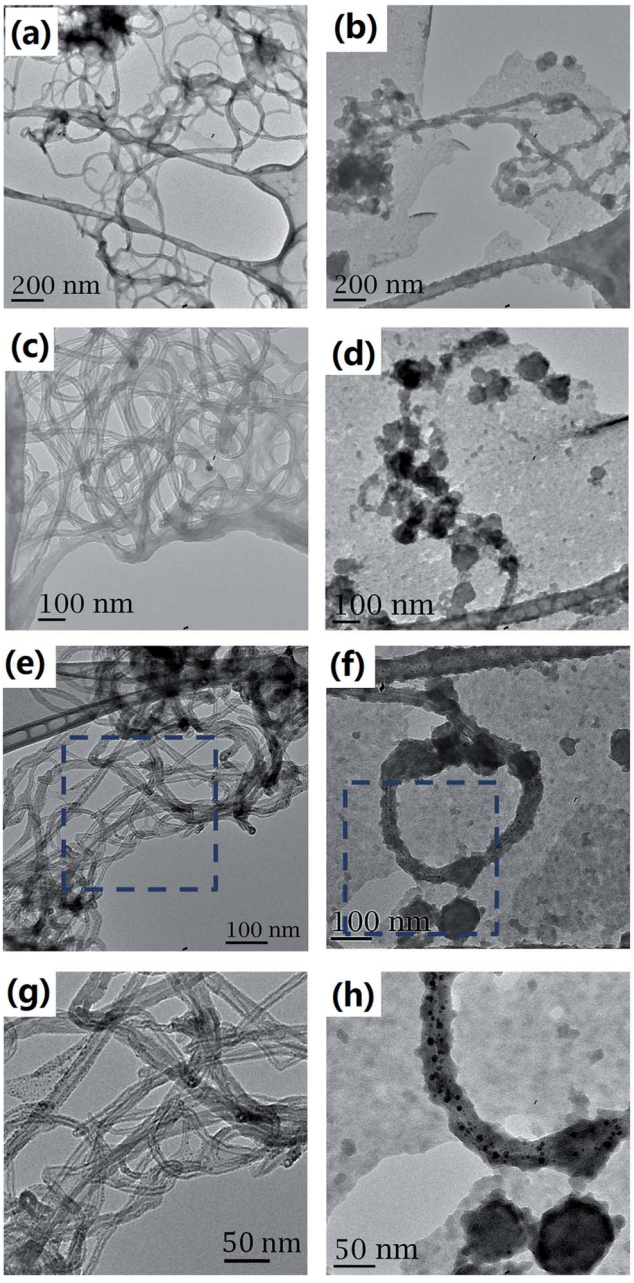

Fig. 3 TEM images of (a, c, e and g) MWCNTs before the D-SPE cleanup process and (b, d, f and h) MWCNTs after the D-SPE cleanup process.

peaks decreased, and the baseline became much lower than only PSA cleanup or without cleanup. Therefore, the optimum cleanup sorbents and dosages in the D-SPE cleanup were chosen as a mixture of 25 mg of PSA and 5 mg of MWCNTs. Under these conditions, we could obtain better recoveries and cleanup performances for most pesticides.

Interaction between MWCNTs and interference substances

As far as we know, there is no report about the theory of the interaction between MWCNTs and interference substances from the matrices. A JEOL 2010 transmission electron microscope (TEM) was used to observe the microstructures of the nanotubes. Fig. 3 shows the TEM images of the MWCNTs before and after adsorption.

In Fig. 3a, c, e and g, it can be seen that the raw MWCNTs are well dispersed with diameters of around 10–20 nm. The hollow cylindrical structures of the MWCNTs can be seen clearly without any other interference substances around them. In our study, it is found that many of the interference substances are removed by the MWCNTs after the D-SPE cleanup procedure, which enhances the cleanup performance of the QuEChERS method. As shown in Fig. 3b, d, f and h, some large interference substances (diameter: 100 nm) appear on the surface of nanotubes after the D-SPE process. Thus, the interaction between the MWCNTs and matrix substances probably occurs on the surface of the MWCNTs.

In Fig. 3h, which is a magnified region in Fig. 3f, it is shown that some small matrix substances (diameter: 1–5 nm) appear in the hollow cylindrical structures of the nanotubes. They

Table 2 Matrix effect (ME), calibration curve coefficients (R^2), LOQs (μg kg^{-1}, S/N ratio of 10) for 41 pesticides in strawberry, raspberry, blueberry and blackberry

	Strawberry			Raspberry			Blueberry			Blackberry		
Compound	ME	R^2	LOQ	ME	R^2	LOQ	ME	R^2	LOQ	ME	R^2	LOQ
Acetochlor	1.55	0.995	10	1.64	0.993	10	1.48	0.996	10	1.53	0.996	10
Atrazine	1.35	0.992	5	1.43	0.995	5	1.29	0.997	10	1.45	0.995	5
Azinphos-methyl	1.89	0.991	20	1.79	0.993	20	1.92	0.989	20	1.83	0.996	20
Bifenthrin	1.42	0.999	1	1.53	0.996	2	1.41	0.995	1	1.54	0.994	3
Boscalid	2.01	0.996	2	1.92	0.993	5	1.88	0.996	3	1.78	0.995	5
Carbaryl	0.92	0.992	5	1.12	0.998	5	1.03	0.993	5	1.23	0.993	10
Carfentrazone-ethyl	1.21	0.997	5	1.32	0.995	10	1.42	0.996	5	1.28	0.995	10
Chlorpyrifos	2.12	0.997	2	2.01	0.997	3	1.98	0.993	3	1.87	0.994	2
Coumaphos	1.89	0.993	20	1.68	0.996	20	1.67	0.993	20	1.82	0.988	20
Cyfluthrin	1.31	0.992	10	1.43	0.995	10	1.72	0.995	5	1.21	0.994	5
Cypermethrin	1.88	0.995	5	1.67	0.996	5	1.65	0.997	5	1.76	0.996	5
Cyprodinil	1.31	0.994	10	1.64	0.995	10	1.11	0.994	10	1.64	0.993	10
Deltamethrin	1.44	0.996	10	1.65	0.992	10	1.88	0.994	10	1.67	0.993	10
Diazion	0.98	0.991	10	0.99	0.995	10	0.71	0.994	15	0.89	0.992	15
Dichlofluanid	1.32	0.997	15	1.53	0.99	20	1.72	0.996	15	1.24	0.996	20
α-Endosulfan	2.12	0.993	10	1.56	0.996	10	1.88	0.995	10	2.01	0.995	10
β-Endosulfan	1.88	0.994	10	1.43	0.994	10	1.78	0.997	10	1.89	0.998	10
Fenhexamid	0.89	0.992	10	0.78	0.997	5	0.70	0.996	5	0.98	0.999	10
Fenitrothion	2.31	0.997	5	2.64	0.997	5	2.54	0.999	5	1.99	0.996	5
Fenpropathrin	1.67	0.993	10	1.56	0.987	10	1.86	0.993	15	1.53	0.996	10
Fenthion	2.42	0.996	2	2.65	0.996	3	1.78	0.995	3	1.86	0.995	3
Fludioxonil	1.52	0.998	2	1.32	0.995	2	1.45	0.996	1	1.53	0.996	1
Folpet	0.72	0.995	20	0.85	0.994	15	0.67	0.994	15	0.85	0.996	20
Iprodione	0.87	0.997	10	0.78	0.991	20	0.65	0.994	20	0.77	0.992	10
Kresoxim-methyl	1.45	0.996	5	1.65	0.994	5	1.43	0.997	5	1.76	0.999	5
Malathion	2.43	0.990	10	2.66	0.998	15	2.54	0.994	10	2.89	0.996	10
Metalaxyl	2.31	0.994	2	1.89	0.995	2	2.64	0.995	2	2.53	0.996	3
Methiocarb	0.98	0.995	5	1.09	0.992	5	1.21	0.997	2	0.89	0.994	2
Metolachlor	2.32	0.998	2	2.43	0.997	2	1.98	0.995	2	1.33	0.995	2
Myclobutanil	1.34	0.993	15	1.54	0.993	15	1.74	0.996	15	1.35	0.993	15
Napropamide	0.93	0.994	10	0.91	0.997	10	1.21	0.994	10	0.88	0.996	10
Parathion-methyl	1.21	0.999	3	1.43	0.996	3	1.32	0.997	3	1.11	0.995	3
Permethrin-*cis*	1.43	0.997	5	1.54	0.996	5	1.87	0.991	10	2.01	0.998	10
Permethrin-*trans*	1.67	0.994	5	1.88	0.995	5	1.56	0.99	10	2.76	0.992	10
Phenothrin	1.43	0.999	1	1.76	0.997	1	1.21	0.999	2	2.21	0.997	1
Phosalone	2.55	0.996	10	2.86	0.994	15	1.65	0.994	20	2.31	0.995	15
Pirimiphos-methyl	0.94	0.998	1	1.21	0.999	1	1.23	0.993	1	1.02	0.997	1
Propargite	1.81	0.993	3	1.43	0.993	5	1.32	0.998	5	1.22	0.998	5
Propiconazole-*cis*	1.21	0.996	5	1.65	0.996	10	1.65	0.996	5	1.49	0.995	5
Propiconazole-*trans*	1.43	0.997	5	1.49	0.996	5	1.47	0.997	5	1.52	0.996	10
Vinclozolin	1.75	0.993	10	1.12	0.994	10	1.54	0.996	10	1.21	0.994	10

Table 3 Recovery and relative standard deviations (RSD) in different berry matrices[a]

	Strawberry						Raspberry						Blueberry						Blackberry					
	Recovery (%)		Intra-day precision (n = 5) (RSD, %)		Inter-day precision (*n* = 25) (RSD, %)		Recovery (%)		Intra-day precision (n = 5) (RSD, %)		Inter-day precision (n = 25) (RSD, %)		Recovery (%)		Intra-day precision (*n* = 5) (RSD, %)		Inter-day precision (*n* = 25) (RSD, %)		Recovery (%)		Intra-day precision (n = 5) (RSD, %)		Inter-day precision (n = 25) (RSD, %)	
Compound	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2
Acetochlor	81	97	5	12	8	13	102	89	12	5	18	8	77	85	6	7	9	10	83	80	6	8	8	10
Atrazine	81	95	10	9	17	11	83	89	7	6	12	9	105	84	3	6	8	10	76	81	2	5	8	9
Azinphos-methyl	123	100	6	7	9	12	111	94	8	9	15	13	95	100	8	7	9	10	99	107	8	7	11	9
Bifenthrin	97	94	2	3	7	8	97	105	5	5	8	7	103	115	8	3	9	7	101	96	3	8	7	10
Boscalid	85	71	9	12	11	15	104	74	6	10	9	14	84	85	9	3	12	5	92	79	7	6	10	8
Carbaryl	97	97	7	12	10	14	95	99	5	5	9	10	110	104	8	12	13	14	109	116	10	10	15	11
Carfentrazone-ethyl	91	83	7	5	10	6	78	79	4	12	8	19	98	91	12	3	16	5	98	88	7	7	14	14
Chlorpyrifos	100	98	10	9	15	15	81	92	11	13	15	16	89	90	8	4	15	6	95	84	6	7	10	12
Coumaphos	118	80	11	8	16	14	114	86	4	8	6	14	88	89	6	7	11	9	99	103	6	8	11	15
Cyfluthrin	88	87	8	4	16	9	85	83	7	7	8	13	77	85	5	7	17	10	103	76	5	3	9	8
Cypermethrin	103	87	1	7	6	9	86	86	8	8	9	10	82	92	9	6	17	10	101	82	7	4	10	8
Cyprodinil	98	91	7	6	17	9	94	100	10	4	12	9	123	107	7	9	15	15	110	97	9	9	13	13
Deltamethrin	95	76	4	8	6	11	79	70	10	4	13	8	109	102	8	7	15	15	81	82	7	7	13	17
Diazion	101	100	5	6	9	14	91	96	12	9	16	11	94	107	8	5	18	10	99	91	9	6	16	15
Dichlofluanid	**60**	**67**	**13**	**14**	**16**	**18**	**51**	**56**	**17**	**14**	**22**	**18**	74	71	6	8	16	11	76	71	9	6	17	10
α-Endosulfan	84	95	9	9	17	14	72	87	12	7	14	13	103	92	4	2	7	6	72	81	9	4	15	7
β-Endosulfan	93	101	8	8	13	12	82	74	6	13	9	17	105	94	8	9	9	10	103	73	9	11	14	13
Fenhexamid	101	111	4	6	7	9	103	110	7	9	10	11	104	85	3	5	8	7	101	104	8	9	12	15
Fenitrothion	103	95	6	7	9	11	85	87	8	2	14	4	96	88	7	6	10	8	97	86	4	2	6	7
Fenpropathrin	96	99	3	2	7	5	96	100	8	11	15	19	102	90	1	5	6	9	90	100	9	11	13	14
Fenthion	98	96	6	10	9	13	87	92	8	11	14	14	91	90	14	9	18	11	108	88	3	7	7	17
Fludioxonil	83	86	9	4	13	8	88	80	3	5	6	16	97	88	8	4	11	6	98	84	7	2	9	10
Folpet	92	90	5	9	9	11	96	103	11	7	17	14	105	87	5	10	7	15	103	100	6	4	9	8
Iprodione	103	106	2	1	5	7	80	112	7	1	9	3	99	105	9	7	10	17	115	104	9	11	11	17
Kresoxim-methyl	99	96	13	3	18	8	73	89	7	5	10	8	92	95	5	3	10	7	100	86	8	3	12	6
Malathion	102	106	6	5	9	9	82	85	6	8	11	9	103	104	9	6	15	9	105	110	8	12	10	15
Metalaxyl	100	98	6	9	10	12	81	114	10	7	15	9	121	111	13	8	18	11	104	122	9	8	14	16
Methiocarb	97	99	6	12	11	16	79	101	7	4	15	10	106	100	7	10	15	15	101	109	11	3	15	5
Metolachlor	101	95	7	4	15	9	90	86	5	3	9	6	100	93	5	1	10	5	91	87	5	1	10	7
Myclobutanil	80	106	7	7	13	12	95	104	6	3	10	9	86	93	6	8	9	14	83	111	11	12	15	14
Napropamide	99	85	11	6	14	10	91	82	6	5	13	6	105	92	2	3	8	5	105	83	11	3	14	6
Parathion-methyl	99	90	13	6	18	10	87	85	11	3	17	8	89	90	11	5	15	8	91	84	5	2	9	8
Permethrin-*cis*	111	98	7	5	10	10	83	95	13	5	15	6	83	91	8	12	16	17	92	101	12	4	17	5
Permethrin-*trans*	107	109	8	6	11	9	110	90	9	7	11	8	116	103	2	6	6	16	93	110	7	3	18	8
Phenothrin	93	88	6	4	8	7	87	80	9	5	15	5	91	89	6	3	6	5	85	87	6	2	7	6
Phosalone	114	103	4	3	6	8	89	97	5	7	10	8	115	96	7	11	9	17	84	101	10	3	11	10

Table 3 (*Contd.*)

Compound	Strawberry						Raspberry						Blueberry						Blackberry					
	Recovery (%)		Intra-day precision (n = 5) (RSD, %)		Inter-day precision (n = 25) (RSD, %)		Recovery (%)		Intra-day precision (n = 5) (RSD, %)		Inter-day precision (n = 25) (RSD, %)		Recovery (%)		Intra-day precision (n = 5) (RSD, %)		Inter-day precision (n = 25) (RSD, %)		Recovery (%)		Intra-day precision (n = 5) (RSD, %)		Inter-day precision (n = 25) (RSD, %)	
	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2
Pirimiphos-methyl	97	99	8	11	9	16	99	92	4	4	6	8	103	98	8	13	10	17	104	89	8	2	16	7
Propargite	96	97	1	4	3	6	92	108	7	1	8	8	91	95	1	12	6	16	90	95	6	8	15	15
Propiconazole-*cis*	81	92	4	7	7	9	93	84	12	6	15	6	103	87	6	8	9	10	75	88	12	7	17	17
Propiconazole-*trans*	76	89	3	5	6	8	93	87	8	2	8	9	103	97	7	6	7	15	111	88	8	1	16	6
Vinclozolin	98	100	6	7	8	10	84	92	9	5	13	8	92	95	11	7	14	9	97	88	1	4	6	9

[a] Spiked levels (L1 and L2) of each compound were 20 and 200 μg kg^{-1}.

could be probably absorbed by the MWCNTs, and after the absorption interaction, the diameter of the MWCNTs increases to 30–40 nm. Therefore, for small matrix substances, their interaction with MWCNTs is probably based on their absorption on the hollow cylindrical structures.

Due to their special physical structure, CNTs have large surface areas, which give them excellent adsorption ability for a wide range of substances, thus the interferences from the extract could be absorbed on the surface of the nanotubes. On the other hand, nanotubes have hollow cylindrical structures, and some interference compounds with small chemical structures could thread the carbon layer into the cylinder. As the hollow cylinders become filled with an increasing amount of these small interference substances, the nanotubes become wider and their diameters increase to 30–40 nm from 10–20 nm. When the absorption reaches saturation, the nanotubes would not absorb these interference substances any more. Thus, the interaction between the MWCNTs and these interference substances is probably based on two types of interactions: adsorption on the surface of the MWCNTs, and the absorptive action of the nanotubes.

Method validation

For the recovery test, the four types of blank berry samples were spiked with the standard working solutions. Matrix-matched calibration standards were used for the quantification of these pesticides. Method validation was carried out to confirm the practicability of the proposed method. The method performance was determined according the following aspects.

Matrix effect. To study the matrix effect on analysis, the slopes obtained using the matrix-matched calibration were compared with solvent-based standards. The slope ratios matrix/solvent were calculated as the matrix effect for each pesticide. As shown in Table 2, the matrix effect much depended on the matrix in most cases. Therefore, it was necessary to employ matrix-matched calibration for quantitation purposes for each type of matrix or sample.

Linearity. To counteract the matrix effects, a matrix-matched calibration was used in the sample analysis.[41] Linearity was studied with five concentrations of 0.02, 0.05, 0.1, 0.2 and 0.5 mg L^{-1} for all analytes using the matrix-matched standard calibration in the blank strawberry, blueberry, raspberry, and blackberry samples. Linear calibration was carried out by fitting the curve with concentration levels of analyte *versus* relative peak area (analyte/IS). The coefficient of determination (R^2) was calculated as the linearity value for each analyte. Good linearities were obtained for major analytes since the determination coefficients were no lower than 0.990. The results are presented in Table 2.

LOQs. In most cases, the LOQ values greatly depend on the matrix. To validate the sensitivity of the proposed method, LOQs were calculated for various matrices. In this study, LOQs were determined when a signal-to-noise ratio of 10 was obtained with a certain concentration level. Table 2 presents the LOQ values for each analyte, which ranged from 1 to 20 μg kg^{-1} in the four types of matrices.

Recovery test. Residue free berry samples were used as the blank samples in the recovery test. The residue free samples were spiked at two concentration levels of 20 and 200 μg kg^{-1}. To evaluate the method accuracy, a fortification test was carried out by applying the proposed method to the spiked berry samples. Table 3 summarizes the average recoveries, and the intra-day and inter-day precisions (relative standard deviations, RSD, %). The recoveries ranged from 71% to 123% (80–123% for strawberry, 79–114% for raspberry, 71–121% for blueberry and 71–122% for blackberry). The intra-day precisions were no more than 13% and the inter-day precisions were no more than 20% for most of the analytes and matrices. In the case of dichlofluanid, low recoveries (<70%) and high RSDs (13–22%) were obtained in the strawberry and raspberry samples. It might be possible to employ a buffered extraction method for dichlofluanid analysis in strawberry and raspberry samples. Therefore, most of the recoveries for these analytes ranged from 70% to 120%. However, some recoveries (60–70% or 120–130%) might be also by satisfactory for pesticide multiresidue analysis.[42] According to the Method Validation Data from the EU Reference Laboratories for Residues of Pesticides,[43] when PSA or GCB were used in the QuEChERS (citrate) method, the recoveries for the 41 pesticides were 58–140% for strawberry, 66–126% for raspberry, 52–129% for blueberry and 71–125% for blackberry, which are consistent with the present results. More detailed validation data from the EU Reference Laboratories are shown in the ESI.†

Sample analysis

The analysis method was developed and validated, which showed sensitive analysis of 41 pesticide residues for four types of spiked berry samples. Validation parameters such as linearity, LOQs, recovery, and intra-day and inter-day precisions were determined to confirm the accuracy and sensitivity of the proposed method.

The method developed was used to measure pesticide residues in eight berry samples (2 samples for each type of berry) from supermarkets in Houston, USA. These samples were prepared following the above procedure. After GC-MS analysis, malathion in one strawberry sample and bifenthrin in one blueberry sample were detected at residue levels of 0.3 and 0.05 mg kg^{-1}, respectively. The MRLs established by the US for malathion in strawberry and bifenthrin in blueberry are 8 and 1.8 mg kg^{-1}, respectively, thus the detected levels were much lower than the MRL values. This successful application proves the practicability of the proposed method since it was easily applied to for the routine detection of trace pesticide residues in strawberry, blueberry, blackberry and raspberry samples.

Conclusions

In this work, a modified QuEChERS method was employed to determine pesticide multiresidue in strawberry, blueberry, raspberry and blackberry samples using GC-MS. A mixture of MWCNTs and PSA was used in the cleanup step, and the amount of sorbent was optimized to obtain a better cleanup performance and higher recoveries. It was shown that MWCNTs could increase the removal of potentially interfering substances from the berry matrix extracts. Validation parameters such as linearity, LOQs, recovery, and intra-day and inter-day precisions were studied to show the accuracy and sensitivity of the proposed method. As expected, the proposed method could be applied successfully to quantitatively monitor residues in commercial berry samples.

Conflicts of interest

The authors declare that no competing interests exist.

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