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Multi-walled carbon nanotubes as alternative reversed-dispersive solid phase extraction materials in pesticide multi-residue analysis with QuEChERS method

Pengyue Zhao, Lei Wang, Li Zhou, Fengzu Zhang, Shu Kang, Canping Pan*

Department of Applied Chemistry, College of Science, China Agricultural University, Beijing 100193, China

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ABSTRACT

A multi-residue method based on modified QuEChERS sample preparation with multi-walled carbon nanotubes (MWCNTs) as reversed-dispersive solid phase extraction (r-DSPE) material and gas chromatography–mass spectrometry determination by selected ion monitoring (GC/MS-SIM) mode was validated on 30 representative pesticides residues in vegetables and fruits. The acetonitrile-based QuEChERS (quick, easy, cheap, effective, rugged and safe) sample preparation technique was used to obtain the extracts, and the further cleanup was carried out by applying r-DSPE. It was found that the amount of MWCNTs influenced the cleanup performance and the recoveries. The optimal amount of 10 mg MWCNTs was suitable for cleaning up all selected matrices, as a suitable alternative r-DSPE material to primary secondary amine (PSA). This method was validated on cabbage, spinach, grape and orange spiked at concentration levels of 0.02 and 0.2 mg/kg. The recoveries of 30 pesticides were in the range of 71–110%, with relative standard deviations (RSDs, $n = 5$) lower than 15%. Matrix effects were observed by comparing the slope of matrix-matched standard calibration with that of solvent. Good linearity was achieved at the concentration levels of 0.02–0.5 mg/L. The limits of quantification (LOQs) and the limits of detection (LODs) for 30 pesticides ranged from 0.003 to 0.05 mg/kg and 0.001 to 0.02 mg/kg at the signal-to-noise ratio (S/N) of 10 and 3, respectively. The method was successfully applied to analysis real samples in Beijing. In conclusion, the modified QuEChERS method with MWCNTs cleanup step showed reliable method validation performances and good cleanup effects in this study.

1. Introduction

Organic pesticides have been widely applied to crops or public health to provide protection against pests, diseases, weeds and thus to ensure sufficient and high-quality food production all over the world. Use of chemicals like pesticides, fertilizers and biotechnologies are cost-effective ways. However, violating Good Agricultural Practice use of pesticides may cause potential health risk to human beings or force unnecessary pressure to the environment. Many regulations have been established for agro-products like fruits and vegetables. At the same time, analytical methods for pesticide determination are being introduced as well, which are used for regulatory enforcement, risk assessment, organic food verification, trade and so on. Verifying Good Agricultural Practice use and ensuring the safety of various commodities are extremely important tasks in commodities trading.

Recently, the QuEChERS (quick, easy, cheap, effective, rugged and safe) method which was introduced by Anastassiades et al. in 2003 [1,2], is widely used as pesticide multi-residue methods (MRM) by many governments and organization laboratories, especially in vegetables, fruits and many other matrices. It involves miniaturized extraction with acetonitrile, liquid–liquid partition by salting out with sodium chloride and magnesium sulfate and a cleanup step which is carried out by mixing the acetonitrile extract with loose sorbents before GC or LC injection rather than passing through a traditional solid-phase extraction (SPE) column. The reversed-dispersive solid phase extraction (r-DSPE) intends to absorb the interfering substances in the matrices, rather than the analytes. The main advantages of QuEChERS extraction are high recoveries in pesticides with a wide range of polarity and volatility, high sample throughput, non-sophisticated equipment, smaller volume of organic solvent and low cost per sample. Mostly, primary secondary amine (PSA) sorbent was used as r-DSPE sorbent, which could remove various polar organic acids, polar pigments, some sugars and fatty acids. Modified QuEChERS cleanup steps with graphitized carbon black (GCB) or C18 as sorbents were also reported [3–5], in these cases sterols and pigments such as chlorophyll may be absorbed by GCB and non-polar interfering substances like lipids could be removed by C18 [6]. The main disadvantage of the QuEChERS method is that with r-DSPE it cannot achieve

* Corresponding author. Tel.: +86 10 62731978; fax: +86 10 62733620.
E-mail address: canpingp@cau.edu.cn (C. Pan).

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effective cleanup like SPE, especially for some difficult matrices. This causes more maintenance of chromatography and restricts most of these applications with no concentration factor upon injection solution, which leads to unsatisfying LODs (limits of detection). Moreover, when handling difficult matrices like herbs [7,8], tea [9], leek [10] and soft drink [11], the r-DSPE cleanup performance was not good enough to remove interferences and then it was necessary to cleanup the extract with SPE columns, which is more tedious and costly. In this study, an alternative material to PSA/GCB/C18 as r-DSPE sorbent was tested, with the purpose of achieving better cleanup performance and thus to minimize chromatography maintenance and to meet the MRM analysis for those difficult matrices.

Carbon nanotubes (CNTs) are novel and interesting carbonaceous materials first reported by Iijiama [12] in 1991, which were classified as single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) on the principle of carbon atom layers in the wall of nanotubes. As carbon-based nano-material, it has special physical and chemical characteristics exploited including unique thermal, mechanical, electronic and chemical properties [13–16]. Owing to their extremely large surface area and unique structure, CNTs can have excellent adsorption ability. In recent reports, CNTs was primarily focusing on the use of SPE method, as a kind of sorbents in a packed column, applied to the extraction of pesticides for water samples [17–22]. With the application of MWCNTs for SPE, Du et al. developed a new analytical method to determinate organophosphate pesticides in garlic [23]. Ravelo-Perez et al. investigated that MWCNTs can be used as effective SPE materials for the extraction of pesticides from apple, grape, orange and pineapple fruit juices [24]. Wang et al. developed SPE method using MWCNTs for determination of four benzodiazepine residues in pork meat [25]. Recently, Su et al. [26] used MWCNTs as matrix solid phase dispersion extraction material in butter samples. And Asensio-Ramo et al. extracted some pesticides from water using MWCNTs as dispersive solid-phase extraction (DSPE) sorbent [27]. However, up to now, no report has been published on the use of MWCNTs as a type of r-DSPE materials to absorb the interfering substances in the fruit and vegetable matrices, rather than the analytes. In addition, no study has been reported that MWCNTs as the cleanup material combined with QuEChERS process method to analysis pesticide residues.

In this study, MWCNTs were used as r-DSPE sorbents combined with the QuEChERS preparation method for the extraction of pesticides. Four fruits and vegetables representative matrices were chosen: cabbage, spinach, grape, and orange. Thirty pesticides with different Log *P* and different chemical structural catalogues were selected to validate the method. GC–MS was used to identify and quantify the residue levels of multi-pesticides.

2. Experimental

2.1. Chemicals

The standard compounds in Table 1 were provided by the Institute of the Control of Agrochemicals, Ministry of Agriculture, Peoples' Republic of China. The purities of the standard pesticides were from 95% to 99%. Stock solutions of 10 mg/L for mixture pesticides were prepared in acetonitrile and stored at −20 °C. The working solutions were prepared daily. A working solution of triphenyl phosphate (TPP, Sigma, Milwaukee, WI, USA), used as internal standard (IS), was prepared by an appropriate dilution of stock solution with acetonitrile and stored at −20 °C. HPLC-grade acetonitrile was obtained from Fisher Chemicals (Fair Lawn, New Jersey, USA). Analytical reagent grade anhydrous sodium chloride (NaCl) and magnesium sulfate ($MgSO_4$) were obtained from Sinopharm Chemical Reagent (Beijing, China).

MWCNTs with average external diameters of 10–20 nm, 5 nm i.d. and PSA were provided by Tianjing Agela Co. Ltd. Co. (China). MWCNTs were dried for 2 h at 120 °C to remove the absorbed water and then kept it in desiccators for storage.

2.2. Apparatus

Centrifugation was performed in two different instruments: an Anke TDL-40B centrifuge equipped with a bucket rotor (4 × 100 mL) (Shanghai, China) and a SIGMA 3K15 microcentrifuge equipped with angular rotor (24 × 2.0 mL) (BMH Instruments Co., Ltd., China), and a QL-901 Vortex (Kylin-bell Lab Instruments Co., Ltd, Jiangsu, China) were used for preparing the samples.

A Meiling BCD-245W Refrigerator Freezer (Beijing, China) was used to control the temperature of samples.

2.3. Sample preparation

A thoroughly homogenized sample (10 g) was weighted into a 50 mL Teflon centrifuge tube. 10 mL acetonitrile was added and the tube was shaken vigorously for 1 min with vortex mixer ensuring that the solvent interacted well with the entire sample. Anhydrous NaCl (1 g) and anhydrous $MgSO_4$ (4 g) were added into the mixture and the shaking step was repeated for 1 min. After centrifugation (3800 rpm, 5 min), 1 mL of the clarified supernatant was introduced into a 2.0 mL micro-centrifuge tube containing 10 mg MWCNTs and 150 mg $MgSO_4$. Then the mixture was shaken vigorously for 1 min and centrifuged for 3 min at 10,000 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 a GC vial (containing 10 μL 5 mg/L internal standard , TPP) to carry out the chromatographic analysis.

2.4. GC–MS conditions

An Agilent 6890N Network GC system (Agilent Technologies, USA) with a 7683B Series splitless auto-injector, a 7683 Series Auto sampler and a 9575B inter XL EI/CI MSD was used for analysis of pesticides.

Agilent Technologies Capillary Column HP-5MS analytical column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) was used for GC separation, with helium (99.9999%) as carrier gas at a constant flow rate of 1.2 mL/min. The column temperature was initially at 60 °C (hold 1 min), increased to 130 °C at the rate of 20 °C/min, and then to 250 °C at the rate of 5 °C/min, finally to 300 °C at the rate of 10 °C/min holding for 5 min. The temperature of the injector port was 260 °C and a volume of 1 μL was injected in split less mode. The total running time was 38.5 min.

Based on the pesticides retention time, the GC–MS acquisition method was divided into many time-windows (groups) in order to obtain enough sampling points for each chromatographic peak and adequate dwell times to obtain maximized signal for pesticides that especially gave low response. This method consisted of eleven retention time-windows. The dwell time for each ion was adjusted to ensure that the number of data acquisition points was sufficient for all the compounds monitored and that each peak was of a cycled scan in the constant cycling scan time. Changing the dwell time did not affect the results of integration.

The mass spectrometer was operated in electron ionization (EI) mode at 70 eV, scanning the characteristic fragment ions of each pesticide at 0.5 s per scan. The temperatures of ion source and mass spectrometer transfer line were set at 230 °C and 280 °C, respectively. The instrument data system held an EI-MS library specially created for target analytes under the experimental conditions.

The analysis were determined by GC–MS-SIM with one quantitation and at least two identification ions, in addition to their

Table 1
The different MS characteristics for identification and quantitation of 30 pesticides using GC–MS retention time, quantization and identification ions of 30 pesticides.

No.	Pesticide	Group no.	Retention time (min)	Quantifying ion	Qualifying ion 1	Qualifying ion 2
1	2-Phenylphenol	1	10.54	170	169	141
2	Atrazine	1	14.73	200	215	58
3	Clomazone	1	14.74	204	138	205
4	Propyzamide	2	15.42	173	255	240
5	Diazinon	2	15.73	304	179	137
6	Pirimiphos-methyl	3	18.52	290	276	305
7	Ethofumesate	3	18.52	207	161	286
8	Chlorpyrifos	4	19.18	314	258	286
9	Fenthion	4	19.10	278	169	153
10	Triadimefon	4	19.33	208	210	181
11	Metazachlor	5	20.24	133	209	211
12	Chlorfenvinphos	5	20.71	267	323	269
13	Procymidone	5	21.02	283	96	285
14	Haloxyfop-P-methyl	6	21.57	316	375	288
15	Butachlor	6	21.88	160	176	188
16	Flutriafol	6	21.87	219	164	201
17	Napropamide	6	22.09	128	271	171
18	Oxadiazon	7	22.83	175	258	302
19	Uniconazole	7	22.86	234	236	131
20	Flusilazole	7	23.00	233	206	315
21	Oxyfluorfen	7	23.07	252	361	300
22	RH-5849	8	24.66	105	240	77
23	Diclofop-methyl	9	26.05	253	281	342
24	Diflufenican	9	26.22	266	394	267
25	Epoxiconazole	9	26.47	192	183	138
26	Pyriproxyfen	10	28.68	136	78	96
27	Cyhalofop-butyl	10	28.91	256	357	229
28	Lambda-cyhalothrin	11	29.33	181	197	141
29	Fenoxaprop-P-ethyl	11	29.99	288	361	63
30	Pyridaben	11	30.57	117	147	364
IS	TPP		25.82	326	325	–

relative abundances, the retention time and the assistance of the NIST's pesticides library. Moreover, the internal standard minimized the possible variations in retention time and peak areas improving the reliability of the method. Table 1 summarized the chosen ions and the typical retention time.

2.5. Method performances

Four representative matrices were selected for validation purposes. Orange was selected as representative commodity with high acid content, grape as high water and low or no chlorophyll content and cabbage and spinach as high water and chlorophyll content [28]. Because cabbage contains more sulfur-containing compounds than other representative matrices, it was considered alone as different from spinach. Therefore, four validation data sets were carried out for each type of matrix [29]. The following parameters were determined during validation of the analytical method: linearity, matrix effect, LOQ (limit of quantification), LOD, trueness and precision. Linearity was studied using matrix-matched calibration by analyzing samples of cabbage, spinach, grape and orange. The trueness and precision of the method was tested via recovery and reproducibility experiments which were carried out for each sample matrix in 5 replicates each at two fortification levels (0.02 and 0.20 mg/kg). The LODs were determined as the concentration of analyte giving a signal to noise ratio (S/N) of 3 for the target ion; LOQs were determined as the concentration of analyte giving a signal to noise ratio (S/N) of 10 for the target ion.

3. Results and discussion

3.1. Modified r-DSPE cleanup process

To obtain the high recoveries, the parameter that affects the partition of analytes among the different matrices was optimized such as the amount of the MWCNTs. In this work, the proposed cleanup method was based on r-DSPE cleanup procedure with MWCNTs. The amount of solid phase sorbent as the most important factor has shown to affect the recoveries in the QuEChERS method. Moreover, the cleanup performance compared with PSA was studied.

3.1.1. Optimization of the amount of the MWCNTs

After analytes were extracted by 10 mL acetonitrile followed by partitioning of the analyte molecules in organic solvent in the presences of a salt mixture (salting out effect), the acetonitrile phase was further cleaned up and dried by mixing with the MWCNTs sorbents and anhydrous $MgSO_4$. The MWCNTs cleanup step was designed to retain matrix components and allow the analytes of interest into the acetonitrile phase. During the process of sample preparation, it was found that different amounts of dispersive sorbents had significant influences on the purification and recoveries of the pesticide extracts.

To evaluate the effect of this parameter, different amounts of MWCNTs were investigated in the same r-DSPE procedure. The amount of sorbent material was progressively increased from 5 to 20 mg. The experiment was performed using 1 mL of the acetonitrile extract at the spiked level of 0.2 mg/kg that was placed into 2.0 mL micro-centrifuge tubes containing 150 mg $MgSO_4$ and different amounts of MWCNTs (i.e., 5, 10, 15, 20 mg). As the amount of MWCNTs increased, most recoveries of the analytes were at the acceptable range 70–120% [29] for cabbages, spinaches, grapes and oranges, except for four pesticides. As shown in Fig. 1, by increasing the amount of MWCNTs from 5 mg to 10 mg, the recoveries for uniconazole, epoxiconazole, diflufenican and fenoxaprop-P-ethyl remained at the acceptable level (74–103%). However, the recoveries decreased to 16–68% when the amount of MWCNTs was increased to 15 mg and 20 mg. In addition, although better recoveries were achieved with 5 mg r-DSPE materials, the cleanup

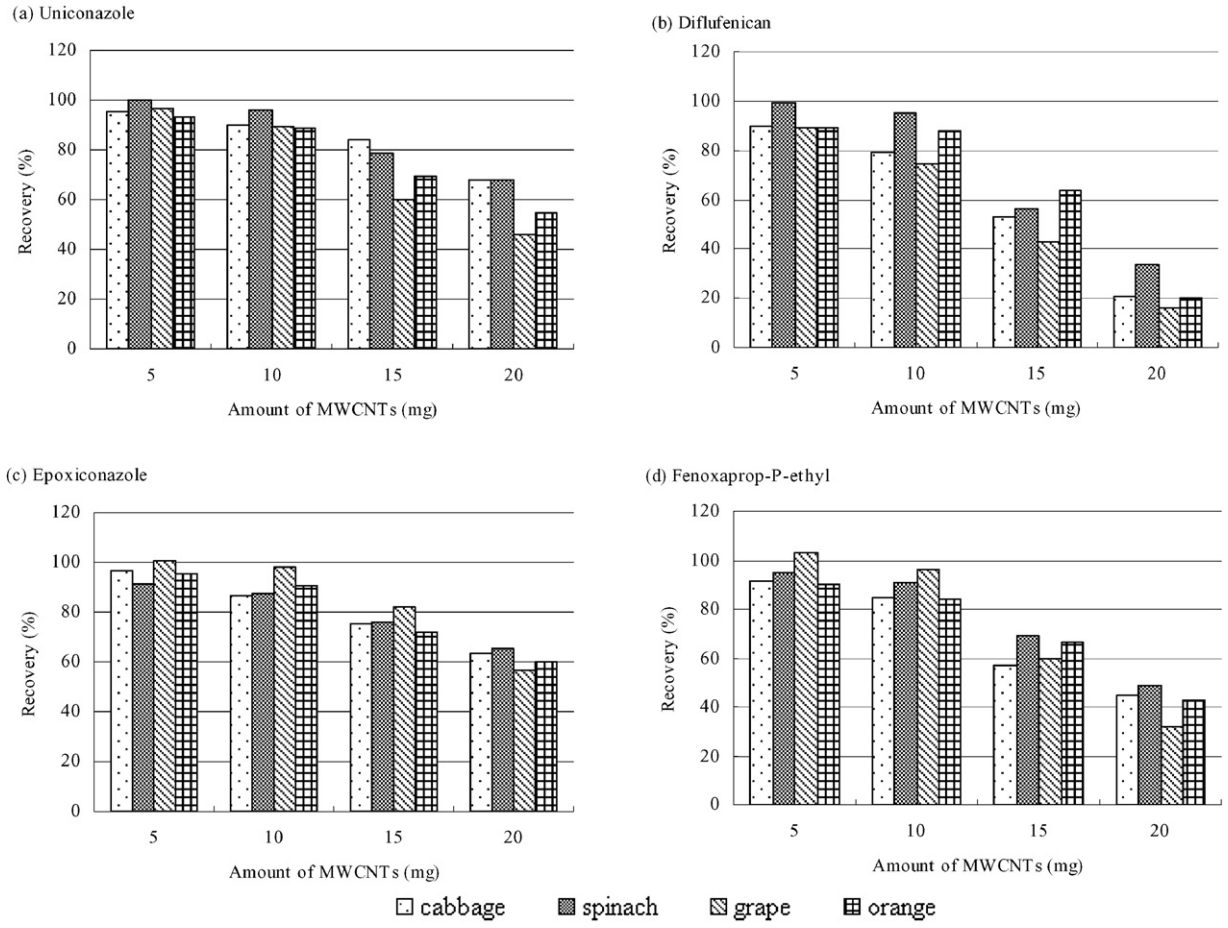

Fig. 1. Effects of amount of MWCNTs on method recoveries.

performance was not as good as 10 mg and there were more chromatography interferences when 5 mg was used. The recoveries were also acceptable at the amount of 10 mg MWCNTs. Consequently, 10 mg (1 mL extract) was used as the optimum amount for the r-DSPE cleanup in the further studies since acceptable recoveries and good cleanup performances were obtained at this amount.

3.1.2. MWCNTs-cleanup procedure compared with PSA

The original QuEChERS method involves extraction with acetonitrile, partition between acetonitrile and aqueous phase after addition of sodium chloride and magnesium sulfate, and then magnesium sulfate and r-DSPE cleanup procedure with a small quantity of SPE adsorbents (mostly, PSA 50 mg/mL). However, sometimes the PSA-cleanup performance is not good enough to remove the interfering substances in the matrices. As shown in Fig. 2, the final cabbage sample processed by MWCNTs looked transparent in color and the PSA-cleanup sample had deeper color. MWCNTs displayed a better cleanup performance than PSA to remove pigment in cabbages and the other three matrices as well. Fig. 3a–c shows a GC–MS-SIM chromatogram of the blank cabbage samples after PSA, MWCNTs r-DSPE cleanup procedure and the spiked cabbage sample after MWCNTs cleanup, respectively. There were fewer interference appearances in the chromatogram of samples with MWCNTs cleanup step than that with PSA cleanup. The MWCNTs r-DSPE cleanup efficiency was higher than the one of PSA cleanup step, and little interference peaks around the peaks of each pesticide were observed (this also occurred to the other three matrices). In our study, we found that the GC–MS contamination with MWCNTs cleanup step was less than that with PSA. Moreover, we have not found that the use of MWCNTs shortened the lifetime of liner. As shown in Table 2, the matrix effects of PSA and MWCNTs were compared in order to check the effectiveness of the clean-up proposed in relation to the described in the original papers published by Anastassiades et al., which was stated in Section 3.2.

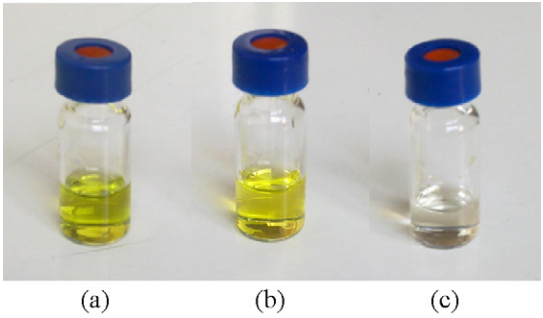

Fig. 2. Photography of cleanup performance by different r-DSPE sorbents: (a) extract for cabbages without r-DSPE cleanup step; (b) extract for cabbages with r-DSPE cleanup by PSA; (c) extract for cabbages with r-DSPE cleanup by MWCNTs.

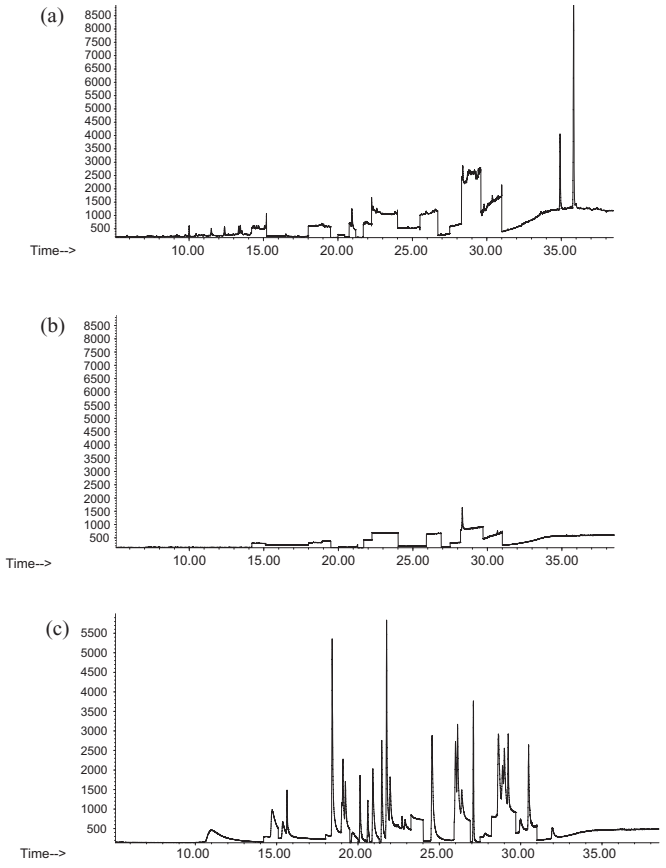

Fig. 3. Chromatogram for cabbage extract after different r-DSPE sorbents: (a) chromatogram for a typical blank cabbage sample with PSA cleanup; (b) chromatogram for a typical blank cabbage sample with MWCNTs cleanup; (c) SIM chromatogram for a typical blank cabbage sample spiked at 0.1 mg/L of the target analytes with MWCNTs cleanup.

The proposed sample pretreatment procedure was combined QuEChERS sample preparation with MWCNTs (10 mg/mL) as a suitable alternative to PSA (50 mg/mL). It was used as a modified QuEChERS cleanup method for the pesticides and applied to residue analysis in four fruit and vegetable representative matrices.

3.2. Matrix effects

The occurrence of matrix effects is regarded as a signal suppression or enhancement of the analyte due to the co-elution of matrix components [30–33], playing an important role in the quality of the quantitative data generated by the method. Matrix enhancement effect may be based on the assumption that the analyte has interacted with the active sites in the GC system, such as silanol groups and metal ions present at the glass surface, which results in losses and distorted peak shapes. Matrix components could competitively interact with active sites in the liner and column, so that the response enhancement from analytes can be maximized. On the other hand, in some cases matrix components can also reduce the signal given by the analytes when they reach the detector. The problem is originated in the interface (source) when the matrix constituents influence the ionization of an analyte, causing ion suppression. Suppression or enhancement of the analyte response can vary considerably from matrix to matrix and differ substantially in pure solvent and in matrix. The matrix effect also depends strongly on the chemical nature of the analyte and the sample preparation procedure utilized. Therefore, it is essential to take into account assessments of the matrix effects and/or the use of matrix-matched calibration standards in order to minimize quantitative errors of pesticide residues.

A comparison between the calibration equations obtained from standards dissolved in normal organic solvents (in our case acetonitrile) and matrix-matched standards should be carried out. This assessment (statistical comparison) can clearly demonstrate if there are strong matrix effects for the compounds under study and if suitable calibration in the sample matrix should be developed. 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, calculating slope ratios matrix/solvent for each of the 30 studied pesticides in the four different matrices. Table 2 summarizes the results. In this work, it was considered that, if the value was in the range of 0.9–1.1, the matrix effect could be ignored; if the value was lower than 0.9, it could show matrix enhancement effect; if the value was higher than 1.1, it could show matrix enhancement effect. For example, napropamide, RH-5849 and diclofop-methyl presented negligible matrix effects in any type of matrix cleaned up with MWCNTs as observed from the slope ratios. This fact is also confirmed in the matrix matched calibration plot for napropamide from Fig. 4. Thus, in this case, solvent-based standards could be used for accurate quantitation of real samples for these pesticides. However, in most cases (MWCNTs cleanup), the signal obtained strongly depends on the matrices, as it can be noticed from the different coefficients (matrix/solvent slope) included in Table 2. In this case, matrix-matched calibration must be used for quantitation purposes for every type of matrix or sample. The nature of the matrix also played an important role in the matrix effects for some specific compounds. As it can be seen in Table 2, after MWCNTs cleanup step, fenthion, metazachlor, procymidone, butachlor and flutriafol presented matrix enhancement effects in vegetable matrices (cabbage and spinach), but matrix suppression effects in fruit matrices (grape and orange), due probably to the different acidities between vegetables and fruits. In fact, the pesticides with phosphate (—PO), hydroxyl (—OH), azoles (—N), amino groups (—R—NH—), imidazole, benzimidazole, carboxyl (—COOH), carbamate (—O—CO—NH—) and urea (—NH—CO—NH—) are the most susceptible type of analytes to matrix effect [7]. In all cases (MWCNTs cleanup), 28% of the compounds presented very low signal suppression (<0.9)relative to solvent calibration and it is noticeable that for 43% matrix enhancement effects was noted in comparison to solvent calibration. That shows that matrix-matched standards must be used as calibration mode to compensate for the matrix effect. In addition, the matrix effects of the original QuEChERS method (PSA cleanup) were also checked to compare with that of the proposed method (MWCNTs cleanup). As shown in Table 2, all the compounds presented stronger matrix effects with PSA cleanup steps for the four matrices, which indicated that the MWCNTs cleanup efficiency was higher than the one of PSA cleanup step.

For the more accurate results, pesticide residue concentrations in non-compliance samples, and validation experiments were calculated using matrix-matched calibration standards, excluding any influence produced by matrix effects, as recommended in EU guidelines [34].

3.3. Validation of method

The optimization of the amount allowed us to meet the residue requirements of recovery within the range of 70–120% and RSD $\leq$ 20% [29] to use MWCNTs as suitable alternative r-DSPE materials to PSA. Method performance characteristics were evaluated and compared in terms of recovery (trueness), detect ability (LODs and LOQs) and linearity (R^2). The results obtained

Table 2
Matrix effect (ME) and determination coefficients (R^2) obtained for the target compounds in cabbage, spinach, grape and orange.

No.	Pesticide	Matrix: solvent (acetonitrile)		Cabbage			Spinach			Grape			Orange		
		Equation	R^2	ME[a]	ME[b]	R^2	ME[a]	ME[b]	R^2	ME[a]	ME[b]	R^2	ME[a]	ME[b]	R^2
1	2-Phenylphenol	$y = 20.534x + 0.2597$	0.999	1.27	1.39	0.999	0.96	1.56	0.999	0.85	1.28	0.999	0.81	1.61	0.999
2	Atrazine	$y = 4.1238x - 0.1193$	0.991	1.85	1.33	0.991	1.67	1.88	0.995	1.72	1.75	0.993	1.76	2.02	0.993
3	Clomazone	$y = 9.2197x - 0.1553$	0.995	2.09	2.60	0.994	1.77	2.38	0.991	1.64	1.60	0.994	1.73	2.59	0.998
4	Propyzamide	$y = 8.3794x - 0.0539$	0.999	1.16	1.47	0.998	1.08	1.95	0.999	0.49	1.48	0.997	0.64	2.17	0.999
5	Diazinon	$y = 4.1669x - 0.1179$	0.992	0.99	1.33	0.992	0.95	1.65	0.992	0.71	1.32	0.992	0.66	1.65	0.992
6	Pirimiphos-methyl	$y = 8.1529x - 0.0728$	0.999	1.45	1.46	0.998	1.45	1.86	0.999	1.19	1.44	0.994	0.97	1.82	0.999
7	Ethofumesate	$y = 12.117x - 0.1354$	0.995	1.22	1.18	0.995	1.47	1.44	0.993	1.07	1.23	0.992	1.14	1.42	0.998
8	Chlorpyrifos	$y = 2.1945x + 0.0553$	0.995	2.11	1.33	0.998	2.27	1.62	0.993	1.42	1.29	0.993	1.21	1.66	0.999
9	Fenthion	$y = 6.4619x - 0.0427$	0.999	2.01	2.52	0.998	2.21	2.20	0.999	0.85	1.53	0.996	0.29	2.19	0.995
10	Triadimefon	$y = 3.6402x + 0.1026$	0.992	1.80	1.83	0.991	1.81	2.28	0.993	1.55	1.63	0.992	1.89	2.42	0.994
11	Metazachlor	$y = 2.652x + 0.0727$	0.999	2.71	2.49	0.998	2.56	2.65	0.997	0.89	1.61	0.994	0.37	2.45	0.993
12	Chlorfenvinphos	$y = 1.6246x + 0.0292$	0.994	1.95	1.95	0.992	2.13	1.98	0.994	1.81	1.85	0.992	2.31	1.89	0.991
13	Procymidone	$y = 4.3306x - 0.0397$	0.996	1.67	1.33	0.994	1.66	1.87	0.996	0.87	1.37	0.994	0.80	1.80	0.991
14	Haloxyfop-P-methyl	$y = 9.1311x - 0.174$	0.995	1.14	1.60	0.992	1.20	2.21	0.999	0.86	1.56	0.993	0.91	2.21	1
15	Butachlor	$y = 0.4402x + 0.0372$	0.999	3.27	1.51	0.997	3.43	2.33	0.999	0.63	1.56	0.993	0.36	2.35	1
16	Flutriafol	$y = 2.5857x + 0.0324$	0.999	2.28	1.83	0.995	2.49	2.81	0.999	0.78	1.93	0.997	0.64	3.47	0.996
17	Napropamide	$y = 7.6664x - 0.1938$	0.994	0.94	1.70	0.997	0.97	3.45	0.993	0.90	2.16	0.994	1.08	5.68	0.992
18	Oxadiazon	$y = 7.6211x + 0.0914$	0.999	1.06	1.23	0.999	1.11	1.43	0.998	0.68	1.27	0.997	0.81	1.41	0.998
19	Uniconazole	$y = 8.9862x - 0.1932$	0.995	0.96	1.73	0.997	1.15	3.38	0.995	1.30	2.02	0.993	1.32	3.94	0.999
20	Flusilazole	$y = 25.934x - 0.2384$	0.998	0.84	1.53	0.998	0.96	2.24	0.997	0.85	1.61	0.995	0.90	2.44	0.998
21	Oxyfluorfen	$y = 3.3251x - 0.0219$	0.996	0.71	1.52	0.996	0.79	3.14	0.996	0.81	1.45	0.995	1.20	3.91	0.997
22	RH-5849	$y = 32.017x - 0.0599$	1	0.94	1.71	0.996	0.90	3.05	0.998	0.95	1.83	0.993	1.10	3.38	0.997
23	Diclofop-methyl	$y = 5.0485x + 0.0023$	0.997	0.98	1.76	0.992	0.97	2.26	0.996	0.90	1.73	0.996	0.90	2.37	0.999
24	Diflufenican	$y = 20.927x - 0.1472$	0.999	1.07	1.77	0.993	0.74	3.22	0.999	0.96	1.99	0.991	1.08	3.45	0.999
25	Epoxiconazole	$y = 4.7153x - 0.1199$	0.993	1.20	2.06	0.994	1.06	8.23	0.991	1.03	3.24	0.995	1.45	7.82	0.992
26	Pyriproxyfen	$y = 33.142x - 0.6678$	0.999	1.01	1.93	0.991	0.70	3.07	0.998	0.84	2.05	0.991	0.90	3.32	1
27	Cyhalofop-butyl	$y = 6.8724x - 0.0777$	0.997	0.79	2.30	0.999	0.69	4.31	0.992	0.83	2.83	0.997	1.03	4.97	0.992
28	Lambda-cyhalothrin	$y = 4.262x - 0.0137$	0.998	1.58	2.13	0.990	1.00	4.69	0.996	1.95	2.35	0.990	1.95	4.05	0.992
29	Fenoxaprop-P-ethyl	$y = 5.0054x - 0.0894$	0.993	1.03	4.58	0.993	0.73	4.11	1	0.81	2.60	0.997	0.94	4.57	0.999
30	Pyridaben	$y = 19.806x - 0.4972$	0.992	1.05	1.98	0.992	0.60	4.09	0.992	1.15	2.69	0.995	1.31	4.91	0.998

[a] ME: matrix effects are expressed as the ratio between the calibration curve slopes of matrix-matched standards (MWCNTs cleanup) and solvent-based standards.
[b] ME: matrix effects are expressed as the ratio between the calibration curve slopes of matrix-matched standards (PSA cleanup) and solvent-based standards.

in the method performance experiments for the determination of the 30 pesticides in cabbage, spinach, grape and orange using the proposed QuEChERS method were summarized in this section.

3.3.1. Linearity

Linearity was studied in the range 0.02–0.5 mg/L for all pesticides with five calibration levels (0.02, 0.05, 0.1, 0.2 and 0.5 mg/L) by matrix-matched standard calibration in blank extracts of cabbage, spinach, grape and orange. Linear calibration graphs were constructed by plotting analyte concentrations versus relative peak area (analyte/IS) of the calibration standards. Linearity values, calculated as determination coefficients (R^2) for each pesticide from the matrix-matched calibration (MWCNTs cleanup) plots are shown in Table 2. The quantitative results of a detection method greatly depend on its calibration. Good linearity was found for most the pesticides with R^2 values better than 0.995, and both pure solvent-based as well as matrix-matched gave R^2 values better than 0.990. It was remarkable considering the complexity of the matrices.

3.3.2. Limits of quantification and limits of detection

The described method was tested for simultaneous extraction and determination of 30 pesticides in four representative matrices, which manifested varying levels of LOD and LOQ. Because LODs and LOQs are matrix dependent, it is recommended to perform matrix-matched calibration for quantitative analysis for unknown samples in complex matrices such as fruit and vegetables. Table 3 showed the LOD and LOQ values for the 30 pesticides studied in cabbage, spinach, grape and orange. The LODs and LOQs for 30 pesticides ranged from 0.001 to 0.02 mg/kg and 0.003 to 0.05 mg/kg, respectively. For pirimiphos-methyl, LOD ranged from 0.001 mg/kg (cabbage) and 0.002 mg/kg (spinach, grape and orange). And diazinon, chlorpyrifos, fenthion and chlorfenvinphos also had lower LODs than the other pesticides in the different matrices. It showed that this method was more sensitive for organophosphorus. On the other hand, LODs that were higher than 0.010 mg/kg appeared for diclofop-methyl and cyhalofop-butyl for all matrices, due probably to the less sensitivity for aryloxyphenoxypropionate by the method.

3.3.3. Recovery study (trueness and precision)

Recovery and repeatability of the method were established to evaluate the method performance. The repeatability and the trueness of the method were studied by carrying out five consecutive extractions ($n = 5$) of spiked matrices at two concentration levels (0.02 and 0.2 mg/kg). All the recoveries were determined from the analyses of 30 pesticides in the matrices, cabbage, spinach, grape and orange, respectively. The values were calculated using matrix-matched calibration standards, as stated in Section 3.3.1. Table 4 shows detailed recovery and repeatability data for all pesticides analyzed in the four matrices. The recoveries of all pesticides were in the range 71–110% (between 71% and 109% for cabbage, 72% and 110% for spinach, 75% and 109% for grape and 72% and 110% for orange). Relative standard deviations (RSDs) were below 15% for all cases. However, for example, the value for the 0.02 mg/kg spiked of 2-phenylphenol in orange is "<LOQ" in Table 4. As explained in Section 3.3.2, LOQs are matrix dependent. LOQ value for 2-phenylphenol was no higher than 0.02 mg/kg in cabbage, spinach and grape, but it was 0.05 mg/kg in orange, which

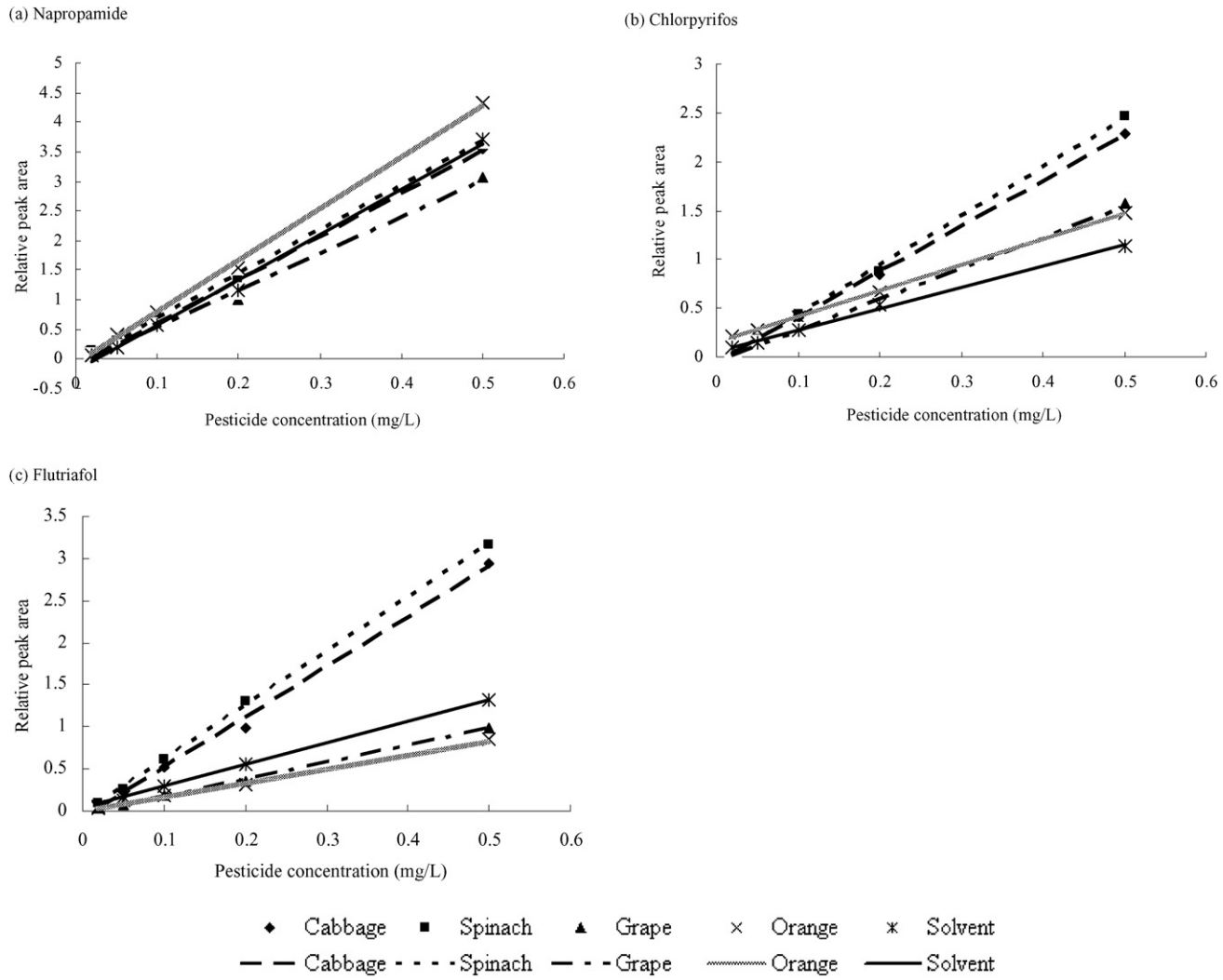

Fig. 4. Matrix-matched calibration plots for napropamide, chlorpyrifos and flutriafol in different fruit and vegetable matrices.

cannot meet the requirement of quantify the lower residue concentration levels (0.02 mg/kg) of the analyses. Therefore, the values for the 0.02 mg/kg 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 [29], reflecting good method performance.

3.4. Comparison of MWCNTs with the other QuEChERS r-DSPE cleanup materials

The efficiency of the presented MWCNTs r-DSPE cleanup method is compared with the other QuEChERS cleanup methods from the viewpoint of spiked level, recovery and separation method.

From the reference data, the recoveries for the 30 pesticides in the four representative matrices were in the range of 61–135% with the other QuEChERS cleanup method such as PSA, PSA/GCB and PSA/C18. The spiked levels were in the range of 0.01–0.5 mg/kg. GC, LC, GC–MS, LC–MS, GC–MS–MS and LC–MS–MS were used to identify and quantify the residue levels of multi-pesticides. The data were obtained in the EU Reference Laboratories for residues of Pesticides [35]. The MWCNTs based r-DSPE cleanup method has comparable recoveries with the other cleanup materials, but requires lower spiked levels. It is well known that GC–MS analysis does not always result in excellent selectivity or sensitivity, and GC–MS–MS or LC–MS–MS may perform better. However, the MWCNTs based r-DSPE cleanup process can achieve the satisfying recoveries with a smaller amount (10 mg/mL), which is adequate for cleanup these "dirty" fruit and vegetable matrices owing to their extremely large surface area and unique structure. The current study reveal that MWCNTs are suitable alternative r-DSPE materials to PSA combined with the original QuEChERS preparation method for the cleanup of fruit and vegetable samples.

3.5. Method application

The developed QuEChERS method with MWCNTs cleanup step was applied to real samples. Ten fruit and vegetable samples (including cabbage, grape, orange and spinach) from local markets and supermarkets in Beijing were treated with the sample preparation method described in Section 2.3 and analyzed by GC–MS. All of the residues were lower than LOQs and traces of pesticides below LOQ.

Table 3
LODs and LOQs of the 30 pesticides in different matrices (mg/kg).

No.	Pesticide	LOD (mg/kg)				LOQ (mg/kg)			
		Cabbage	Spinach	Grape	Orange	Cabbage	Spinach	Grape	Orange
1	2-Phenylphenol	0.006	0.005	0.006	0.015	0.020	0.015	0.020	0.050
2	Atrazine	0.006	0.005	0.006	0.005	0.020	0.015	0.020	0.015
3	Clomazone	0.003	0.006	0.005	0.005	0.010	0.020	0.015	0.015
4	Propyzamide	0.005	0.005	0.006	0.006	0.015	0.015	0.020	0.020
5	Diazinon	0.003	0.003	0.003	0.003	0.010	0.010	0.010	0.010
6	Pirimiphos-methyl	0.001	0.002	0.002	0.002	0.003	0.005	0.005	0.005
7	Ethofumesate	0.006	0.006	0.003	0.006	0.020	0.020	0.010	0.020
8	Chlorpyrifos	0.005	0.005	0.003	0.003	0.015	0.015	0.010	0.010
9	Fenthion	0.002	0.005	0.003	0.005	0.005	0.015	0.010	0.015
10	Triadimefon	0.006	0.006	0.005	0.010	0.020	0.020	0.015	0.030
11	Metazachlor	0.006	0.005	0.015	0.006	0.020	0.015	0.050	0.020
12	Chlorfenvinphos	0.005	0.003	0.006	0.005	0.015	0.010	0.020	0.015
13	Procymidone	0.006	0.006	0.005	0.005	0.020	0.020	0.015	0.015
14	Haloxyfop-P-methyl	0.003	0.005	0.003	0.006	0.010	0.015	0.010	0.020
15	Butachlor	0.005	0.005	0.015	0.010	0.015	0.015	0.050	0.030
16	Flutriafol	0.006	0.006	0.006	0.010	0.020	0.020	0.020	0.030
17	Napropamide	0.005	0.005	0.006	0.003	0.015	0.015	0.020	0.010
18	Oxadiazon	0.006	0.005	0.015	0.006	0.020	0.015	0.050	0.020
19	Uniconazole	0.006	0.006	0.006	0.005	0.020	0.020	0.020	0.015
20	Flusilazole	0.005	0.005	0.003	0.003	0.015	0.015	0.010	0.010
21	Oxyfluorfen	0.005	0.010	0.005	0.005	0.015	0.030	0.015	0.015
22	RH-5849	0.006	0.005	0.003	0.003	0.020	0.015	0.010	0.010
23	Diclofop-methyl	0.015	0.015	0.015	0.015	0.050	0.050	0.050	0.050
24	Diflufenican	0.006	0.005	0.006	0.006	0.020	0.015	0.020	0.020
25	Epoxiconazole	0.005	0.003	0.003	0.005	0.015	0.010	0.010	0.015
26	Pyriproxyfen	0.006	0.003	0.006	0.006	0.020	0.010	0.020	0.020
27	Cyhalofop-butyl	0.015	0.010	0.015	0.015	0.050	0.030	0.050	0.050
28	Lambda-cyhalothrin	0.006	0.003	0.006	0.005	0.020	0.010	0.020	0.015
29	Fenoxaprop-P-ethyl	0.006	0.003	0.006	0.006	0.020	0.010	0.020	0.020
30	Pyridaben	0.005	0.010	0.006	0.006	0.015	0.030	0.020	0.020

Table 4
Average recoveries (%) and relative standard deviations(RSDs) obtained at two spiked levels in different matrices ($n = 5$).

No.	Pesticide	Average recovery % (RSD %)							
		Cabbage		Spinach		Grape		Orange	
		0.02 mg/kg	0.2 mg/kg	0.02 mg/kg	0.2 mg/kg	0.02 mg/kg	0.2 mg/kg	0.02 mg/kg	0.2 mg/kg
1	2-Phenylphenol	94(6)	109(10)	94(10)	91(11)	103(6)	101(9)	<LOQ	81(9)
2	Atrazine	90(10)	83(11)	97(9)	101(6)	106(4)	103(10)	100(7)	88(6)
3	Clomazone	105(8)	99(13)	98(6)	97(5)	106(8)	102(5)	93(8)	81(6)
4	Propyzamide	99(8)	100(11)	90(10)	89(8)	87(9)	104(7)	102(8)	81(10)
5	Diazinon	109(12)	108(14)	88(10)	93(10)	99(5)	98(5)	100(6)	80(7)
6	Pirimiphos-methyl	105(10)	100(13)	97(9)	92(9)	106(8)	91(7)	108(8)	82(6)
7	Ethofumesate	107(13)	84(6)	102(5)	87(10)	107(11)	104(8)	105(13)	85(6)
8	Chlorpyrifos	97(11)	94(7)	94(5)	88(7)	84(10)	98(5)	85(8)	87(8)
9	Fenthion	99(5)	99(7)	99(10)	82(8)	98(11)	83(10)	82(8)	72(12)
10	Triadimefon	93(11)	92(7)	105(14)	97(8)	<LOQ	109(10)	82(8)	82(7)
11	Metazachlor	104(7)	90(10)	110(14)	92(6)	99(5)	92(11)	<LOQ	81(11)
12	Chlorfenvinphos	102(10)	97(13)	72(10)	92(10)	97(9)	90(7)	99(8)	79(5)
13	Procymidone	92(8)	104(10)	102(7)	97(11)	98(13)	97(5)	97(5)	78(6)
14	Haloxyfop-P-methyl	100(6)	102(11)	97(9)	98(8)	102(11)	100(5)	92(5)	80(4)
15	Butachlor	99(6)	97(4)	101(5)	103(9)	<LOQ	82(13)	<LOQ	75(14)
16	Flutriafol	101(11)	84(12)	99(10)	90(8)	<LOQ	89(12)	84(12)	81(6)
17	Napropamide	104(4)	107(5)	100(8)	104(9)	93(11)	99(7)	105(3)	85(4)
18	Oxadiazon	90(12)	101(8)	94(3)	98(10)	107(12)	104(6)	<LOQ	87(7)
19	Uniconazole	80(1)	83(11)	92(4)	92(13)	85(5)	86(9)	84(7)	89(5)
20	Flusilazole	99(1)	102(14)	106(4)	103(8)	96(10)	89(8)	84(8)	91(5)
21	Oxyfluorfen	99(6)	96(12)	<LOQ	97(6)	102(10)	86(8)	91(5)	94(6)
22	RH-5849	91(12)	98(13)	100(7)	105(4)	100(9)	96(4)	92(11)	90(4)
23	Diclofop-methyl	<LOQ	81(7)	<LOQ	86(12)	<LOQ	104(8)	<LOQ	89(5)
24	Diflufenican	73(10)	75(10)	84(12)	87(9)	75(12)	75(6)	86(8)	83(3)
25	Epoxiconazole	71(12)	73(8)	86(14)	87(10)	97(12)	96(7)	81(14)	89(5)
26	Pyriproxyfen	74(10)	85(11)	98(13)	109(9)	104(13)	97(3)	89(9)	84(3)
27	Cyhalofop-butyl	<LOQ	94(10)	<LOQ	92(13)	<LOQ	82(7)	<LOQ	85(3)
28	Lambda-cyhalothrin	86(10)	86(13)	110(10)	96(8)	99(10)	90(4)	109(8)	88(4)
29	Fenoxaprop-P-ethyl	89(13)	77(8)	86(9)	90(9)	98(9)	79(9)	83(5)	87(7)
30	Pyridaben	90(12)	87(13)	<LOQ	106(6)	92(12)	84(7)	86(4)	90(5)

4. Conclusions

In this work, a very quick, easy, effective, rugged, reliable and accurate multi-residue method based on modified QuEChERS method was developed for the determination of pesticides in fruits and vegetables by GC–MS-SIM. It is demonstrated for the first time that MWCNTs can be used as effective r-DSPE materials with QuEChERS method as suitable alternative materials to PSA for the cleanup of extract from different matrices. The validation parameters of the method in terms of analytical range, precision, recovery, trueness and selectivity, etc. showed that the proposed method meets the requirements for pesticide analysis (average recovery values were in the range 71–110% for all selected pesticides with RSD values lower than 15%). MWCNTs proved to be a new type of r-DSPE sorbent materials and are expected to be widely applied for monitoring of pesticides at trace levels in the future for sample cleanup.

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