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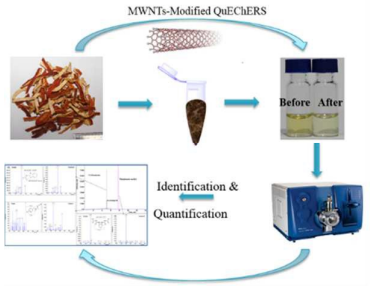

Graphic abstract

Novelty statement:

Carbon nanotubes-based QuEChERS extraction and enhanced product ion scan-assisted confirmation was developed for multi-pesticide residue analysis in dried tangerine peels.

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Carbon Nanotubes-Based QuEChERS Extraction and Enhanced Product Ion Scan-Assisted Confirmation of Multi-Pesticide Residue in Dried Tangerine Peel

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Abstract: A sorbent package consisting of a combination of multiwalled carbon nanotubes (MWNTs) and a primary secondary amine (PSA) has been used for a modified quick, easy, effective, rugged, and safe (QuEChERS) extraction of 104 pesticides from dried tangerine peel samples. MWNTs and graphitized carbon black (GCB) have been compared in terms of purification efficiency and recovery; the best results were achieved with MWNTs. The pesticides were quantified on a liquid chromatography tandem mass spectrometry (LC-MS/MS) system in scheduled multiple reaction monitoring mode, and identified on the basis of product ion abundance ratios as well as characteristic fragments in enhanced product ion spectra. Calibration curves for most of the analytes showed correlation coefficients better than 0.9916. Detection limits ranged from 0.2 to 40 $\mu g \cdot kg^{-1}$. Good precision was achieved with relative standard deviations of less than 20%. Results of accuracy in spiked samples were in the range 71.1-117.6%, except for pesticides such as thiabendazole, teflubenzuron, hexaflumuron, and methomyl. The proposed method has been applied to 57 dried tangerine peel samples from the Chinese market; 16 pesticides were detected, including carbendazim, chlorpyrifos, isoprothiolane and methidathion, at levels that exceeded the recommended maximum residue limits in some samples. The newly established method has advantages of good recovery and a rapid clean-up procedure, showing enhanced product ion scan-assisted confirmation to be a useful tool for obtaining reliable results.

Keywords: multiwalled carbon nanotubes; QuEChERS; LC-MS/MS; enhanced product ion spectra; pesticide residue;

1 Introduction

Dried tangerine peel is popular as a dietary supplement in Asian countries, such as Korea, China, and Japan[1], and is used as an ingredient in spices, condiments, snack food, and tea in many countries. To ensure yields and quality, pesticides have become the most common measure to control insects and diseases. However, deviations from good agricultural practice in the use of pesticides readily leads to pesticide residues, which may pose a potential threat to human health. To ensure that these residues are kept below tolerated levels, the European Commission has stipulated maximum residue levels (MRLs) for some pesticides as low as 0.01 $mg \cdot kg^{-1}$ in the regulation (EC) No. 396/2005 [2, 3], yet only a few methods are available for evaluation of the broadly contaminated multiclass pesticides in dried tangerine peel[4]. In this context, the development of reliable and accurate methods is necessary for monitoring the levels of pesticide residues.

Sample preparation plays a key role in separating trace pesticides from matrices and maintenance of chromatographic systems. Many preparation techniques, such as solid-phase extraction (SPE)[5, 6], gel-permeation chromatography (GPC)[7], matrix solid-phase dispersion extraction (MSPD)[8], supercritical fluid extraction (SFE)[9], solid-phase microextraction (SPME)[10], and liquid-liquid extraction (LLE) [11], have been reported for the determination of pesticides. Some of the aforementioned methods involve large amounts of organic solvents (LLE), special equipment (GPC and SFE), are labor-intensive (MSPD), or incur high costs (SPE), which has limited their use in relation to complicated matrices. The quick, easy, cheap, effective, rugged, and safe (QuEChERS) method, a combination of extraction and purification, has been widely accepted by the international community by virtue of providing high recovery, super efficiency, and good reproducibility. In the QuEChERS procedure, a primary secondary amine (PSA) is most commonly used as a polar adsorbent to remove organic acids, fatty acids, sugars, and polar pigments[12, 13]. Depending on the nature of the chemicals and matrices, graphitized carbon black (GCB) [14, 15] has also been proposed to eliminate non-polar co-

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extracts, especially plant pigments. To achieve good performance in the preparation step, the pursuit of novel sorbents is ongoing. Multi-walled carbon nanotubes (MWNTs), as novel carbon nanomaterials, consist of multiple layers of carbon atoms rolled up into nanoscale tubes[16]. Owing to their extremely large surface area, electron-rich nature, and hydrophobicity, MWNTs possess excellent adsorptive capabilities and can serve as a perfect extraction material. MWNTs as sorbents have been employed in SPE[17-20], MSPD[21, 22], and dispersive solid-phase extraction (dSPE) [23]. In most such previous studies, MWNTs have been used alone as a sorbent in a QuEChERS step [24, 25]. Only a few papers have dealt with evaluation of a combination of MWNTs with other sorbents in matrix effect reduction [26]. To the best of our knowledge, the removal of interfering species by MWNTs and GCB has not hitherto been compared. In addition, there has been no report on the use of MWNTs for pesticide determination in dried tangerine peel by the QuEChERS method.

For the quantification of multi-pesticide residues, especially by high-throughput analytical approaches, the primary focus has been on gas chromatography-mass spectrometry (GC-MS) and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) [27]. LC-MS/MS is preferred over GC-MS in terms of a wider scope of targets, increased sensitivity, and better selectivity [27]. Among available LC-MS/MS techniques for the identification and quantification of unknown chemicals, the hybrid-quadrupole linear ion trap tandem MS (QqLIT-MS/MS) system has recently been suggested for application in drug discovery, the screening of active compounds, and the detection of contaminants [28-32]. This system allows scheduled multiple reaction monitoring simultaneously with information-dependent acquisition-triggered enhanced product ion scan (scheduled MRM-IDA-EPI). In scan mode, quantitative data from MRM chromatography and data on fragments identified from the EPI mass spectrum can be acquired within the same cycle.

In the present study, a sorbent package consisting of MWNTs and PSA has been examined in combination with the QuEChERS method for the extraction and purification of 104 pesticides. QqLIT-MS/MS has been used to quantify the levels of pesticide residues in scheduled MRM mode. The pesticides have been identified on the basis of product ion abundance ratios as well as characteristic fragments from EPI spectra.

2 Experimental

2.1 Standards

All pesticide standards were provided by the Agro-Environmental Protection Institute (Tianjin, China). On the basis of their solubility, individual stock standard solutions of pesticides were prepared in methanol, acetone, or n-hexane, each at a concentration of 100 $\mu g \cdot mL^{-1}$. Intermediate solutions of each pesticide at a concentration of 50 $ng \cdot mL^{-1}$ were prepared in a mixture of acetonitrile/water (50:50, *v/v*) to optimize the MS/MS conditions. Prior to simultaneous analysis, a stock multi-standard solution containing 500 $ng \cdot mL^{-1}$ of each pesticide was prepared in methanol, stored at -20 ºC, and used within 1 month. To avoid degradation of pesticides, standard working solutions at various concentrations were prepared daily by appropriate dilution of the stock multi-standard solution in blank matrix extract or a mixed solvent of acetonitrile/water (3:2, *v/v*).

2.2 Reagents and chemicals

Chromatography grade acetonitrile and methanol were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Cleanert PSA (40-60 μm) and Cleanert GCB (200-400 mesh, specific surface area 100 $m^2 \cdot g^{-1}$) were purchased from Bonna-Agela Technologies (Tianjin, China). Multi-walled carbon nanotubes (outer diameter 10-20 nm, length 10-30 μm, specific surface area > 200 $m^2 \cdot g^{-1}$) were procured from Beijing Dk Nano technology Co., Ltd. (Beijing, China). Other reagents and chemicals were of analytical grade. Formic acid was supplied by Xilong Chemical (Guangdong, China). Anhydrous magnesium sulfate (anh $MgSO_4$) and sodium chloride (NaCl) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.3 Apparatus and analytical conditions

The ultrafast liquid chromatography (UFLC) system consisted of an LC-20A pump, an SIL-20AC autosampler, a DGU-20 A3 degasser, and a CTO-20A column oven (Shimadzu, Japan). Multiple pesticides were separated on an AQUITY UPLC BEH Shield RP18 column (100 × 2.1 mm, 1.7 μm) from Waters (Massachusetts, USA) by using a binary mobile phase composed of 0.1% (*v/v*) formic acid in acetonitrile (A) and 0.1% (*v/v*) formic acid in water (B) with the following gradient elution program: 0 min, 90% B; 3.5 min, 55% B; 5 min, 50% B; 15 min, 25% B; 16 min 1% B; 18 min 1% B; 19 min 90% B; 22 min 90% B, at a flow rate of 0.3 $mL \cdot min^{-1}$. The injection volume was 10 μL.

An Applied Biosystems Sciex QTRAP® 5500 MS/MS spectrometer equipped with a version of 1.6 Analyst software (AB SCIEX, Massachusetts, USA) was employed in the analysis. Pesticides were protonated by an electrospray ionization (ESI) source in positive mode. High purity (99.999%) nitrogen was used as curtain gas (CUR) and collision gas (CAD), while compressed air was used as nebulizer gas (GS1) and heating gas (GS2). The ionization source-dependent parameters were set as follows: ion spray voltage, 5500 V; source temperature (TEM) 550 °C; CUR, 35 psi; CAD, medium; GS1 and GS2, each 50 psi. Scheduled MRM mode was selected, with 120 s as the MRM detection window and 0.8 s as the target scan time.

Fragment-rich EPI spectra were collected through information-dependent acquisition (IDA) experiments, whereby a full scan of the precursor ion for each pesticide was triggered in conjunction with scheduled MRM mode. The IDA criteria included selecting the two most intense precursor ions after dynamic background subtraction of the survey scan, and never excluded the former target ions. Mass tolerance for precursor ions was 250 mDa. EPI spectra were acquired by intensity

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exceeding 500 counts per second (cps) over a mass range of m/z 50-600 for product ions at a scan rate of 10000 $Da \cdot s^{-1}$. The collision energy (CE) and CAD were set at 35 V and high, respectively.

2.4 Sample preparation

A total of 57 batches of dried tangerine peel samples were collected from markets and drugstores across China. Samples were homogenized, ground, and passed through a 65-mesh sieve before analysis. An accurately weighed powder sample (1.0 g) was immersed in 2.5 mL of purified water for 1 min and then extracted by vigorously shaking for 1 min after the addition of acetonitrile (10 mL). NaCl (0.5 g) and anh MgSO4 (2.0 g) were added to the mixture with continuous shaking for 1 min to facilitate transfer of the targets into acetonitrile and removal of water from the acetonitrile. The mixture was then centrifuged for 5 min at 4000 rpm. An aliquot of extract (1 mL) was cleaned up by a mixture of 25 mg PSA, 10 mg MWNTs, and 150 mg anh $MgSO_4$, and the supernatant was concentrated to dryness under a nitrogen flow at 40 ºC. The residue was redissolved in 1 mL of acetonitrile/water (3:2, *v/v*) and the solution was filtered through a 0.22 μm membrane for later use. To investigate the potential benefits of using MWNTs as an alternative absorbent to GCB in the QuEChERS method, the fortified sample was extracted and purified according to the above procedure but using GCB in place of MWNTs.

3 Results and Discussion

3.1 Optimization of MS/MS conditions

The pesticides investigated in dried tangerine peel in this study were selected according to registered pesticides on the China pesticide information network, as well as compounds required to be regulated, specially monitored, prohibited, or limited according to the U.S. Environmental Protection Agency (U.S. EPA), European Union (EU), and China authority. To ensure production output and the quality, almost 90 kinds of pesticides were registered for tangerines in Ministry of Agriculture of the People's Republic of China. As one of the main tangerine cultivation countries, China has widely export the fruits to surrounding nations such as Southeast Asia and Russia. Considering the risk of co-occurrence of those multi-pesticides, a total of 132 pesticides composed of a variety of structural classes, such as organophosphorus, carbamates, triazoles, benzinidazoles, anilines, pyridines, and strobilurins, are in current use or intensively monitored in raw materials from tangerines. MS conditions play an important role in the sensitivity of trace substance detection. In this study, the MS/MS parameters were optimized to efficiently produce the characteristic fragment ions by tuning with each standard using a syringe pump. As a result, 28 pesticides were excluded from the analysis because no precursor was found (cartap, chlorothalonil, cyermethin, etc.), there were no fragments or only one for analysis (formothion, parathion-methyl, benomyl, etc.), no peak was detected (propamocarb), or the intensity was too low (bifenthrin) under LC-MS/MS conditions. For accurate quantification and confirmation of the remaining analytes, two ion transitions per pesticide were monitored, and the optimal MS/MS parameters included declustering potential (DP), collision energy (CE), and collision cell exit potential (CXP), together with scan time, as listed in Table 1.

3.2 Optimization of LC conditions

By application of the optimal gradient program, it took only 22 min to simultaneously determine the 104 pesticides in a single run. Methanol and acetonitrile, the most common components of reversed mobile phases, generally provide different sensitivity and selectivity for the compounds in LC analysis. In our investigation, the majority of the peaks showed superior chromatographic resolution and higher detected signal in acetonitrile compared to those in methanol (Figure 1). In addition, the peak shapes for pesticides such as methamidophos, thiabendazole, and isoprothiolane were markedly improved with acetonitrile as the mobile phase. The volatile formic acid not only contributed to promoting protonation of the targeted compounds, but also suppressed the ionization of residual silanols in the stationary phase and thus improved the peak shapes. Using acetonitrile containing 0.1% formic acid (*v/v*), the intensities of most of the pesticide peaks are greatly enhanced (Figure 1). In some cases, there was a deterioration in the detected intensity, for instance with methomyl. All things considered, acetonitrile containing 0.1% formic acid was chosen.

3.3 MWNTs-based QuEChERS procedure

In spite of the superior chromatographic resolution and sensitivity achieved with the LC-QqLIT-MS/MS system, the instrument is readily contaminated by interfering components from the complicated matrix, and this leads to a decrease in detection performance together with high maintenance costs. Sample pre-treatment including QuEChERS may be essential. Acetonitrile was chosen as the extraction solvent on account of the good solubility of analytes of a wide polarity range therein and its compatibility with LC-MS/MS. The extraction efficiencies were closely related to the volume of solvent. Hence, a fortified powder sample (1.0 g) was infiltrated with deionized water and extracted with 5 mL, 10 mL, or 20 mL acetonitrile in the initial extraction process. The results (Fig. 2A) showed that an increase in the amount of acetonitrile generated an incremental extraction ratio in the range 70-120%. However, excess solvent (20 mL) not only increased the likelihood of co-extracts that could be detrimental to the subsequent analysis, but also brought about the consumption of a hazardous substance. Hence, 10 mL of acetonitrile was used in the extraction step.

To evaluate the capacity for removing impurities in dried tangerine peel, sorbents based on PSA, MWNTs, and GCB were investigated separately or in combination in a preliminary analysis. Dried tangerine peel contains abundant essential oils, organic acids, flavonoids, sugars, and carotenoids (natural pigments) [33-36]. To decrease the interfering effects of these, combinations of PSA with either MWNTs or GCB were found

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to provide better clean-up performances. In order to achieve satisfactory recoveries, different compositions of sorbents provided in Table 2 were tested for three times. The mean recoveries of pesticides after the clean-up of MWNTs group and CNT group were compared by a paring *t*-test, the difference ($P< 0.05$) indicated a statistical significance between the two groups. The effects of MWNTs on representative compounds are shown in Fig. 2B; multi-class pesticides showed acceptable recoveries using composition (4). The results indicated that MWNTs as an alternative gave good recoveries, and 10 mg led to more than 90% of the pesticides showing the required recoveries between 70% and 120%, possibly due to the merits of superior large surface area and the π-π interaction between carotenoids and MWNTs. Excess MWNTs, however, led to a great loss of recovery for analytes with a planar structure, such as azoxystrobin, thiabendazole, and carbendazim. Finally, the composition of 25 mg PSA, 10 mg MWNTs, and 150 mg anh $MgSO_4$ was chosen as the optimal one for clean-up.

Table 1 The optimized MS/MS parameters for each pesticide in scheduled multiple reaction monitoring mode by LC-QqQLIT-MS/MS

Pesticides	Structure	Molecular	Retention/min	*Q* transition	CE1/V	*q* transition	CE2/V	DP/V	CXP/V
Carbendazim	BEZs	C9H9N3O2	5.0	192/160	22	192/132	41	105	9
Thiabendazole	BEZs	C10H7N3S	5.2	202/175.1	34	202/131	43	130	9
Thiophanate-methyl	BEZs	C12H14N4O4S2	7.7	343/151	24	343/311	14	117	12
Chlorfluazuron	BPUs	C20H9Cl3F5N3O3	17.6	540/383	24	540/158.2	23	84	10
Flufenoxuron	BPUs	C21H11ClF6N2O3	16.7	489.2/158.1	22	489.2/141.1	33	91	13
Hexaflumuron	BPUs	C16H8Cl2F6N2O3	17.4	461/141.1	30	461/158.1	22	90	12
Teflubenzuron	BPUs	C14H6Cl2F4N2O2	14.5	381.2/158.1	23	381.2/141.1	30	84	13
Triflumuron	BPUs	C15H10ClF3N2O3	13.1	359.3/156.2	22	359.3/138.8	22	103	10
Aldicarb	CAMs	C7H14N2O2S	7.1	213.1/88.9	21	213.1/116	15	124	10
Aldoxycarb	CAMs	C7H14N2O4S	5.5	223.1/86	17	223.1/88.9	12	63	9
Bendiocarb	CAMs	C11H13NO4	8.0	224.1/167.1	11	224.1/108.9	21	87	8
Carbaryl	CAMs	C12H11NO2	8.5	202.1/145	17	202.1/127.1	42	74	12
Carbofuran	CAMs	C12H15NO3	7.9	222.1/165	16	222.1/122.9	28	71	8
Carbosulfan	CAMs	C20H32N2O3S	18.6	381.2/118	23	381.2/160	19	98	12
Fenobucarb	CAMs	C12H17NO2	9.7	208.1/94.9	18	208.1/152	9	118	12
Furathiocarb	CAMs	C18H26N2O5S	14.6	383.1/195	26	383.1/252	17	130	11
Indoxacarb	CAMs	C22H17ClF3N3O7	14.2	528/149.8	31	528/217.9	30	100	10
Isoprocarb	CAMs	C11H15NO2	8.8	194.1/95	19	194.1/137.1	13	95	
Methiocarb	CAMs	C11H15NO2S	9.9	226/121.1	23	226/169.2	12	101	12
Methomyl	CAMs	C5H10N2O3S	5.6	163/87.9	13	163/106	14	100	12
Metolcarb	CAMs	C9H11NO2	7.6	166/108.8	13	166/90.9	32	117	13
Oxamyl	CAMs	C7H13N3O3S	5.4	220/72	20	220/90	12	83	12
Pirimicarb	CAMs	C11H18N4O2	5.4	239.1/71.9	25	239.1/182.1	20	81	8
Propoxur	CAMs	C11H15NO3	7.8	210.1/111.1	17	210.1/168.1	11	67	12
Thiodicarb	CAMs	C10H18N4O4S3	7.6	355/87.9	25	355/107.9	19	104	12
Diethofencarb	CAMs	C14H21NO4	9.9	268.1/226	13	268.1/180.1	23	90	12
Acephate	OPPs	C4H10N3PS	1.4	184/143.1	11	184/125	23	78	10
Azinphos ethyl	OPPs	C12H16N3O3PS2	11.8	346.1/131.9	21	346.1/260.9	11	51	12
Azinphos-Methyl	OPPs	C10H12N3O3PS2	10.1	318.2/132	20	318.2/77	45	74	12
Chlorfenvinfos	OPPs	C12H14Cl3O4P	11.6	359/155	17	359/127.1	24	91	13
Clorpyrifos	OPPs	C9H11Cl3NO3PS	15.8	350.1/197.9	24	350.1/96.9	40	72	9
Clorpyrifos-methyl	OPPs	C7H7Cl3NO3PS	13.7	322.2/125.1	26	322.2/289.8	20	70	13
Coumaphos	OPPs	C14H16ClO5PS	13.4	363.2/226.9	33	363.2/306.9	23	130	12
Demeton	OPPs	C16H38O6P2S4	8.7	259.1/89	18	259.1/60.9	43	53	10
Diazinon	OPPs	C12H21N2O3PS	12.2	305.1/168.9	27	305.1/153.2	27	110	10
Dichlofenthion	OPPs	C10H13Cl2O3PS	15.7	315.1/258.8	20	315.1/287	14	56	13
Dichlorvos	OPPs	C4H7Cl2O4P	7.5	220.9/109	20	220.9/145	16	120	10
Dicrotophos	OPPs	C8H16NO5P	5.6	238/112	16	238/192.9	13	70	11
Dimethoate	OPPs	C5H12NO3PS2	6.6	230/199	12	230/171	18	70	10

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Table 1 (*continued*)

Pesticides	Structure	Molecular	Retention/min	*Q* transition	CE1/V	*q* transition	CE2/V	DP/V	CXP/V
Disulfoton	OPPs	C8H19O2PS3	13.7	275.1/89	21	275.1/61.2	21	54	12
Ethion	OPPs	C9H22O4P2S4	16.1	385.1/198.9	12	385.1/1171.1	21	90	10
Ethoprophos	OPPs	C8H19O2PS2	10.0	243/131	26	243/215	15	85	10
Fensulfothion	OPPs	C11H17O4PS2	8.5	309/252.9	24	309/280.9	18	120	12
Etrimfos	OPPs	C10H17N2O4PS	12.4	293.1/265	21	293.1/124.7	32	110	13
Fenamiphos	OPPs	C13H22NO3PS	9.8	304.2/216.8	30	304.2/233.9	22	120	12
Fenitrothion	OPPs	C9H12NO5PS	11.6	278.2/125	28	278.2/246.2	23	89	8
Fensulfothion	OPPs	C11H17O4PS2	8.5	309/252.9	24	309/280.9	18	120	12
Fenthion	OPPs	C10H15O3PS2	12.8	279.1/168.8	23	279.1/246.9	17	111	12
Fonophos	OPPs	C10H15OPS2	13.2	246.9/109	24	246.9/136.9	14	80	10
Isazophos	OPPs	C9H17ClN3O3PS	11.7	314/119.9	36	314/119.9	22	81	10
Isocarbophos	OPPs	C11H16NO4PS	9.6	290.3/231	18	290.3/121	36	70	10
Isofenphos-Methyl	OPPs	C14H22NO4PS	13.0	332.1/231	17	332.1/273	7	70	12
Malaoxon	OPPs	C10H19O7PS	7.6	315.1/99	32	315.1/127.1	17	75	13
Malathion	OPPs	C10H19O6PS2	11.1	331.1/126.9	17	331.1/284.8	9	110	10
Methacrifos	OPPs	C7H13O5PS	9.8	241/208.9	12	241/124.9	20	100	6
Methamidophos	OPPs	C2H8NO2PS	1.3	142/93.9	17	142/124.9	16	80	12
Methidathion	OPPs	C6H11N2O4PS3	9.8	303/145	12	303/85	27	104	12
Mevinphos	OPPs	C7H13O6P	6.6	225.1/126.9	19	225.1/193.1	8	85	12
Monocrotophos	OPPs	C7H14NO5P	5.5	224/192.9	11	224/126.9	18	95	11
OMethoate	OPPs	C5H12NO4PS	5.0	214/183	14	214/154.9	20	92	6
Parathion	OPPs	C10H14NO5PS	12.8	292.2/235.9	18	292.2/264	13	60	11
Phenthoate	OPPs	C12H17O4PS2	12.8	321.1/247	14	321.1/163	14	71	11
Phorate	OPPs	C7H17O2PS3	13.4	261.1/74.9	15	261.1/198.9	11	64	7
Phorate sulfone	OPPs	C7H17O4PS3	9.5	293/171	15	293/247	9	102	11
Phorate sulfoxide	OPPs	C7H17O4PS2	8.0	277/199.1	13	277/142.9	27	85	11
Phosalone	OPPs	C12H15ClNO4PS2	13.8	368.2/182	22	368.2/322.1	14	108	12
Phosfolan	OPPs	C7H14NO3PS2	6.6	256.1/140	32	256.1/228	18	82	12
Phosmet	OPPs	C11H12NO4PS2	10.3	318.1/160.2	17	318.1/133.1	48	108	10
PhosphaMidon	OPPs	C10H19ClNO5P	6.9	300.1/174	17	300.1/127	27	120	13
Phoxim	OPPs	C12H15N2O3PS	13.7	299.1/76.9	40	299.1/129.1	16	81	12
Pirimiphos ethyl	OPPs	C13H24N3O3PS	13.6	334.1/198.1	30	334.1/182.1	30	89	9
Pirimiphos-methyl	OPPs	C11H20N3O3PS	11.6	306.1/164.1	30	306.1/108	38	110	8
Profenofos	OPPs	C11H15BrClO3PS	13.7	373.1/302.9	24	373.1/344.7	18	134	12
PropetaMphos	OPPs	C10H20NO4PS	11.5	282.1/137.9	23	282.1/156	18	122	6
Quinalphos	OPPs	C12H15N2O3PS	12.2	299.1/162.9	31	299.1/146.9	32	82	10
Sulfotep	OPPs	C8H20O5P2S2	13.2	323.1/170.9	20	323.1/295	13	118	9
Terbufos	OPPs	C9H21O2PS3	15.1	289/102.9	10	289/232.9	8	84	13
Tetrachlorvinphos	OPPs	C10H9Cl4O4P	11.0	365.1/127.1	16	365.1/239	29	124	11
Triazophos	OPPs	C12H16N3O3PS	11.4	314.1/162	23	314.1/286	16	97	9
Chlorophos	OPPs	C4H8Cl3O4P	5.9	257.1/109.1	22	257.1/220.8	15	111	10
Ditalimfos	OPPs	C12H14NO4PS	11.4	300.1/148.1	23	300.1/244	17	101	11
Iprobenfos	OPPs	C13H21O3PS	10.6	289.1/90.9	26	289.1/205	15	100	11
Pyrazophos	OPPs	C14H20N3O5PS	12.7	374.2/222	28	374.2/194.1	43	76	11
Tolclofos-methyl	OPPs	C9H11Cl2O3PS	13.6	301.1/269	20	301.1/124.9	22	107	11
Bitertanol	TIZs	C20H23N3O2	10.9	338.3/99	19	338.3/269	12	93	10
Difenoconazole	TIZs	C19H17Cl2N3O3	12.1	406/251	29	406/337	24	91	11
Diniconazole	TIZs	C15H17Cl2N3O	11.6	326.1/70	26	326.1/158.9	34	97	12
Flusilazole	TIZs	C16H15F2N3Si	10.6	316.1/165.1	34	316.1/247	23	127	13
Flutriafol	TIZs	C16H13F2N3O	8.0	302.1/70	21	302.1/123	35	85	12

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Table 1 (*continued*)

Pesticides	Structure	Molecular	Retention/min	*Q* transition	CE1/V	*q* transition	CE2/V	DP/V	CXP/V
Hexaconazole	TIZs	C14H17Cl2N3O	10.7	314/70	22	314/159	38	120	10
Myclobutanil	TIZs	C15H17ClN4	9.9	289/70	21	289/125	41	141	9
Penconazole	TIZs	C13H15Cl2N3	10.7	284.1/69.9	23	284.1/158.9	40	105	9
Propiconazole	TIZs	C15H17Cl2N3O2	11.0	342.1/158.9	35	342.1/69	24	95	13
Tebuconazole	TIZs	C16H22ClN3O	10.4	308.2/70	23	308.2/124.9	46	141	8
Triadimefon	TIZs	C14H16ClN3O2	10.2	294/197	19	294/68.9	26	114	9
Triadimenol	TIZs	C14H18ClN3O2	9.1	296/70	15	296/99.1	20	64	12
Acetamiprid	Neonicotinoids	C10H11ClN4	6.5	223/126	25	223/56.1	22	110	11
Imidacloprid	Neonicotinoids	C9H10ClN5O2	6.4	256.1/209	25	256.1/175	27	96	9
Pymetrozine	Pyridines	C10H11N5O	1.3	218/105	30	218/78.9	47	89	13
Tebufenozide	Others	C22H28N2O2	12.1	353.2/132.9	9	353.2/297.1	21	98	11
Azoxystrobin	Others	C22H17N3O5	10.2	404.1/372	21	404.1/372	32	108	10
Isoprothiolane	Others	C12H18O4S2	11.0	291/188.9	27	291/230.9	14	81	11
Metalaxyl	Anilines	C15H21NO4	8.0	280/220	17	280/192.1	24	118	11
Triflumizole	Imidazoles	C15H15ClF3N3O	9.9	346.1/278	14	346.1/73	21	64	11
Fenpropathrin	PYHs	C22H23NO3	15.8	350.1/125.1	20	350.1/97	41	130	10

Note: BEZs, Benzinidazoles; BPUs, Benzoylureas; CAMs, Carbamates; OPPs, organophosphorus; PYHs, pyrethroids; Q, quantification ion; q, qualification ion; DP, declustering potential, CE, collision energy and CXP, collision

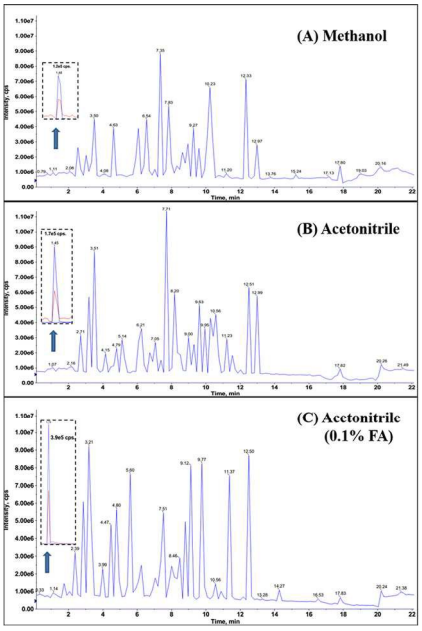

Fig.1 Total ions chromatograms of a 10 μL injection of a 10 ng·ml^{-1} mixture of 104 pesticides acquired with the organic mobile phase of (A) methanol, (B) acetonitrile and (C) acetonitrile containing 0.1% formic acid. The insets indicated the improvement of the peak shape and intensity for phorate as a function of organic mobile phase.

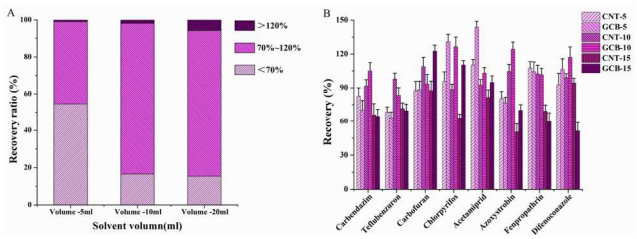

Fig.2 (A) The extraction efficiency affected by the volume of acetonitrile and (B) comparison of extract recovery between graphitized carbon black (GCB) and multi-wall carbon nanotubes (MWNTs) in the clean-up step by QuEChERS method for dried tangerine peel.

Table 2 Different sorbent packages tested in present study

Absorbents	Group Number					
	1	2	3	4	5	6
anh $MgSO_4$ (mg)	150	150	150	150	150	150
PSA (mg)	25	25	25	25	25	25
GCB (mg)	5	-	10	-	15	-
MWNTs (mg)	-	5	-	10	-	15

Note: anh $MgSO_4$, Anhydrous magnesium sulfate; PSA, primary secondary amine; GCB, graphitized carbon black; MWNTs, multi-walled carbon nanotubes.

3.4 Method validation

3.4.1 Matrix effect

According to guidance from SANCO/12495/2011[37], the potential for matrix effects (ME) to occur should be assessed by LC-MS/MS, since the co-eluted substances are prone to be protonated in competition with analytes in the ESI source, thereby causing signal suppression in the detection of analytes. To estimate the ME, serial concentrations (0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 100, 200 ng·mL^{-1}) of standards were prepared in blank extract and in solvent, respectively, and the ME was calculated by comparison between the slopes of calibration curves in the extract ($k_{extract}$) and in the solvent ($k_{solvent}$), as expressed by the following equation: ME=$k_{extract}/k_{solvent}$. If the value was between 0.8 and 1.2, the ME was considered as negligible, whereas a value greater than 1.20 was regarded as a signal enhancement and a value less than 0.8 was regarded as signal suppression. From Fig. 3, it is evident that 36 of the 104 pesticides showed an obvious suppression effect in the quantitative results, whereas the signals of trizaphos and thiodicarb were enhanced because of the matrix. A dramatic

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suppression effect on benzinidazoles (-O-CO-NH-) and benzoylureas (-NH-CO-NH-) was observed, probably because the presence of organic amines from the dried tangerine peel extract made these pesticides difficult to ionize. In order to obtain accurate quantitative results, one of most effective ways to negate the ME is calibration by standard addition, referred to as matrix-matched calibration, as recommended by SANCO/12495/2011.

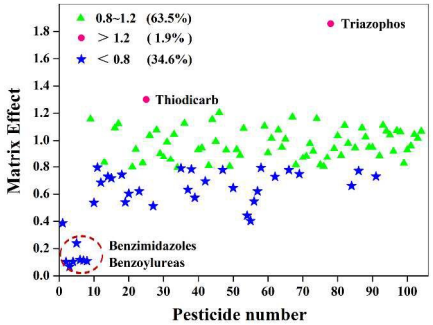

Fig.3 The matrix effect evaluated by the comparison of the slopes of each pesticide in extract and in solvent.

3.4.2 Linearity and limits

Calibration curves for the 104 pesticides were constructed over concentration levels ranging from 0.2 to 200 ng·mL^{-1} in blank extract from dried tangerine peel. According to individual degrees of ionization, the different linearities and ranges were generated by plotting the areas from quantitative transitions versus the concentrations, which are summarized in Table S1; good linearity was obtained for most of the analytes, with correlation coefficients (r) better than 0.9916. The limits of quantification (LOQs) and limits of detection (LODs) were investigated by gradually diluting the matrix-matched calibration with blank extract in triplicate; the values were estimated as the concentrations at signal-to-noise ratios approaching 10:1 and 3:1, respectively. The LOQs and LODs in the method were in the ranges 0.5-100 μg·kg^{-1} and 0.2-40 μg·kg^{-1}, respectively, which met the detection requirements of trace pesticides in dried tangerine peel referring to MRLs (0.01-15 mg·kg^{-1}) stipulated for tangerine by the European Commission.

3.4.3 Selectivity, accuracy, and precision

The selectivity of the method was assessed by comparison of the chromatograms of the mixture of 104 standards dissolved in solvent and blank extract of dried tangerine peel. Fig. 4 shows that no interference from the matrix was observed at the retention time of the analytes, indicating that excellent selectivity was achieved owing to the sample preparation, UFLC separation, and scheduled MRM detection with selecting double-ionization transitions.

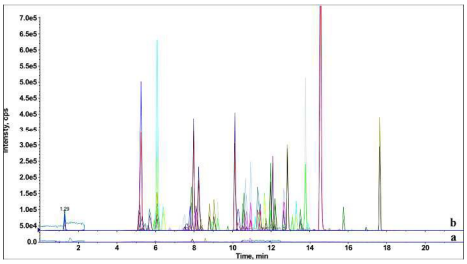

Fig.4 Scheduled MRM chromatograms of (a) 104 standard mixtures at 10 ng·ml^{-1} in solvent and (b) blank matrix extract of dried tangerine peel.

To further validate the robustness of the MWNTs-based QuEChERS method in relation to dried tangerine peel, method recovery was performed with fortified samples at four concentration levels of 10 μg·kg^{-1}, 50 μg·kg^{-1}, 100 μg·kg^{-1}, and 500 μg·kg^{-1} in triplicate, covering the range of EU[2] and Chinese [38] MRLs for pesticides. The results obtained are included in Table 2; the average recoveries at each concentration were in the ranges 68.0-117.2% with RSDs of 1.4-18.1%, 69.8-117.6% with RSDs of 1.5-16.9%, 74.7-110.7% with RSDs of 1.6-15.2%, and 70.8-116.4% with RSDs of 2.2-17.2%. On the whole, the majority of recoveries fell in the range 71.1-117.6% (90.6% on average), except for some pesticides such as thiabendazole, teflubenzuron, hexaflumuron, and methomyl (63.9-69.8%), which could nevertheless also be accepted.

The intra-day precision was assessed by repeatedly injecting a spiked sample solution at 10 ng·mL^{-1} six times, and inter-day precision was measured at the same concentration on three successive days. The RSD values for all the experiments were within 18.9%, thus meeting the EU criterion (RSDs of 20% for precision), and demonstrated good repeatability by the described method.

3.5 Results of real samples and EPI spectra confirmation

To demonstrate the feasibility of the proposed method in routine analysis, it was applied to 57 dried tangerine peel samples, which were randomly selected from drugstores or medicine markets across China. After the optimized MWNTs-based QuEChERS treatment, all samples were analyzed on the system of UFLC coupled with scheduled MRM scan along with synchronous triggering of the acquisition of fragment-rich EPI spectra. The spiked samples, standard solution, and blank matrix were prepared in advance to provide references for the subsequent identification. The analytes were identified by retention time, characteristic ion transitions of the most intense product ion (Q) and secondary ion (q), together with the product ion abundance ratios (Q/q) matching those of standards within 20% relative deviation. To decrease the false-positive results, most of the pesticides were confirmed by comparison of their characteristic fragment ions in a positive sample with those of standards. For example, carbendazim produced prominent fragments at m/z 159.8 ([M+H-CH3OH]$^+$) and m/z 131.8 ([M+H-CH3OH-CO]$^+$) as well as other low intensity ions; the same fragments were observed in a contaminated sample, which revealed the usefulness of the current method with synchronous EPI spectra. For the relevant pesticides, the

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contamination levels in real samples were quantified. The results of the determination are summarized in Table 3, and typical chromatograms and mass spectra are presented in Fig. 5.

Table 3 Pesticides levels in contaminated samples (n=57)

Pesticide	Number of incurred samples/ratio	Detected Range/mg·kg^{-1}	Beyond number	WHO Class**	MRL***/ mg·kg^{-1}
Carbendazim	26(45.6%)	0.019-4.71*	4(28.1%)	U	0.2
Thiophanate-methyl	42(73.7%)	<LOQ-3.99*	-	U	6
Chlorpyrifos	15(26.3%)	0.066-0.863	7(12.3%)	II	0.3
Carbofuran	20(35.1%)	<LOQ-0.030	-	Ib	0.5
Malathion	32(57.9%)	<LOQ-0.074	-	III	2
Methidathion	25(43.9%)	0.033-0.635	25(43.9%)	Ib	0.02
Acetamiprid	23(40.4%)	<LOQ-0.039	-	-	0.9
Imidacloprid	16(28.1%)	<LOQ-0.096	-	II	1
Difenoconazole	20(35.1%)	0.028-0.080	-	II	0.1
Fenpropathrin	2(3.51%)	0.035-0.048	-	II	2
Triazolone	1(1.75%)	0.066	-	II	0.1
Isoprothiolane	3(5.26%)	0.025-0.046	3(5.26%)	II	0.01
Iprobenfos	2(3.51%)	<LOQ-0.025		II	-
Phenthoate	5(8.77%)	0.027-0.081	-	II	-
Isocarbophos	3(5.26%)	0.032-0.093	-	-	-
Isoprocarb	2(3.51%)	<LOQ	-	II	-

Note: *, the detected level exceeded the linearity range was diluted with matrix extract; **, (Ib = Highly hazardous; II = Moderately hazardous; III = slightly hazardous; U = Unlikely to present acute hazard in normal use); ***, Maximum Residue Limit (MRL), Part A of Annex I to Reg. 396/2005; LOQ, the limit of quantification.

A total of 16 of the 104 compounds were detected in the real dried tangerine peel samples. Considering that peel constitutes a raw material derived from tangerines, and there is no specific MRL for pesticides in the former, the MRL values refer to tangerine. Most of the pesticides found in positive samples were below the MRLs, except for carbendazim (28.1%), chlorpyrifos (12.3%), methidathion (43.9%) and isoprothiolane (5.26%). In China, the compounds such as chlorpyrifos, methidathion, thiophanate-methyl et al. are authorized for use in tangerine. However, they must be applied on the basis of agronomic prescription. The frequent occurrence of thiophanate-methyl (73.7%), malathion (57.9%) and methidathion (43.9%) residues revealed their overdose and abuse in tangerine cultivation. Especially for methidathion, which may be classified as highly hazardous[39], greater attention needs to be paid to the development of detection methods as well as good agricultural practice in tangerine cultivation. The results obtained in the present study are similar to those reported by Golge et al. [40] and Bakırcı et al. [41], showing that tangerine and its peel are easily contaminated by the insecticide chlopyrifos. The levels of some compounds found in this study are inconsistent with those reported by Blasco et al. [42], such as carbendazim and imidacloprid at 51.9% and 9.6%, respectively. Probably because the non-systemic insecticide works on the surface of the plant, the occurrence of methidathion residue in fresh tangerine fruits is 32.6%, as compared to 43.9% in dried tangerine peels. Other pesticides, such as acetamiprid, carbofuran, difenoconazole and imidacloprid were found at high frequencies of 40.4%, 35.1%, 35.1 and 28.1%, respectively, but none of their residue levels were beyond their MRLs in fresh tangerine. In view of the wide application of the dried tangerine peels but high contamination with multi-pesticides, it reminds us that the MRL standards in dried tangerine peels are urgently demanded to be established for the human health. Unlike the fresh fruit, the dried peel is processed by washing, peeling, drying and storing. Hence, the standards of pesticide residues in dried tangerine peel should take the procession into consideration as well as dietary habits of human.

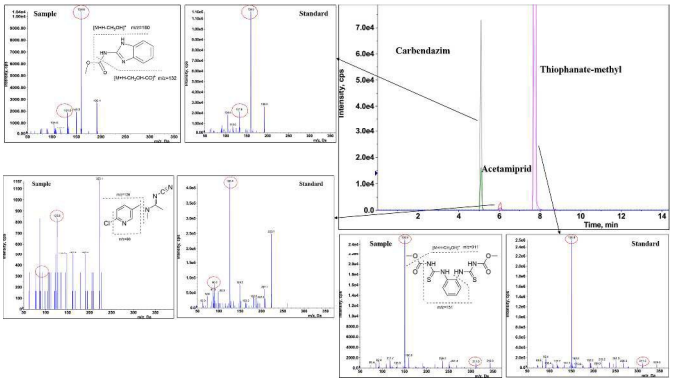

Fig.5 Typical selective ion chromatograms and EPI spectra for confirmation of pesticides in positive dried tangerine peel.

4 Conclusions

In summary, a combination of MWNTs and PSA has been developed as an excellent sorbent for use in a QuEChERS method and validated as a rapid, efficient, and high-recovery pre-treatment measure for multiclass pesticide residues in dried tangerine peel. MWNTs proved to be a good option, not only because of their superior adsorption capacity, but also because of their abundant source, making them inexpensive. By optimizing the volume of acetonitrile and the absorbent package (MWNTs/PSA/MgSO4), the clean-up procedure led to decreased interference from co-extracts and improved matrix effects (63.5% for ME within 0.8-1.2) and recovery (90.6% on average). LC-MS/MS has been demonstrated as a simple, sensitive, and high-throughput analytical method for the simultaneous screening of 104 pesticides in real samples, and methodology validation results meet the criteria of SANCO/12495/2011. Applying the proposed method to 57 dried tangerine peel samples, 16 pesticides were identified, and the residue levels of carbendazim, chlorpyrifos, methidathion and isoprothiolane in some dried tangerine peel samples exceeded their MRLs. In addition, EPI spectra provided a useful tool to confirm the positive results. Therefore, we have developed a MWNTs-based QuEChERS pre-treatment and EPI spectra synchronous LC-MS/MS analytical method that offers a significant improvement in analytical performance in relation to complex matrices for routine pesticide screening.

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