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Preparation and characterization of amino functionalized nano-composite material and its application for multi-residue analysis of pesticides in cabbage by gas chromatography–triple quadrupole mass spectrometry

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ABSTRACT

In this paper, a novel and recyclable amino-functionalized nano-composite material (NCM) using tetraethylenepentamine (TEPA) as a coupling agent was synthesized. The properties of the TEPA-NCM were characterized by transmission electron microscopy (TEM), Fourier transform infrared analysis (FTIR), thermogravimetric analysis (TGA) and elemental analysis (EA). An effective dispersive solid-phase extraction (dSPE) procedure using the TEPA-NCM was developed, and comparative studies were carried out among Carbon/NH_2 SPE, primary secondary amine (PSA) dSPE and TEPA-NCM dSPE. The results showed that TEPA-NCM dSPE was faster, easier and more effective to clean and enrich than the Carbon/NH_2 cartridges, and the TEPA-NCM was much more effective to remove the pigments in vegetable samples than the PSA materials. The TEPA-NCM could be reused at least five times without much sacrifice of the cleanup efficiency. Furthermore, a gas chromatography–triple quadrupole mass spectrometry (GC–QqQ-MS/MS) method was established for the simultaneous determination of 29 pesticides (such as organochlorine and organophosphorus pesticides) in vegetables by dSPE using acetonitrile as an extraction solvent and TEPA-NCM as an adsorbent instead of PSA. The recoveries were in the range of 75–114% for all analytes except for trans-chlordane. The RSDs were in the range of 2–17%. The linearities were in the range of 0.4–100.0 μg/kg with determination coefficients (r^2) higher than 0.986 for all compounds. The limits of detection (LODs) for all pesticides were less than 0.29 μg/kg and the limits of quantification (LOQs) were between 0.17 and 0.95 μg/kg. The developed method was applied to fifteen real vegetable samples, and it was confirmed that the TEPA-NCM was one of a kind of highly effective dSPE materials used for the pesticides analyses.

1. Introduction

In recent years, scientific and public concern in pesticide residues, *i.e.*, organochlorine pesticides (OCPs) and organophosphorus (OPPs) pesticides in agricultural products and related commodities have been increased with each passing day [1,2]. The physicochemical characteristics of these pesticides together with their indiscriminate use in the past have led to their occurrence in the environment, biota and foodstuffs, as well as in human beings [3]. OCPs and OPPs are known of inducing or aggravating certain health problems in humans such as cancer or the disruption of hormonal functions [4]. Therefore, due to the monitoring concerns about food safety, many countries have established legal directives and monitoring programs to control the use of pesticides on agricultural crops, and find out whether the residues are compliant with the statutory maximum residue levels (MRLs) [2]. In consequence, the rapid multi-residue determination of wide range of pesticides in agricultural crops is an urgent requirement.

The analysis of pesticides in food samples usually involves the extraction of the analytes from the matrix, the subsequent cleanup of the extracts and the final chromatographic analysis. One of the main problems in trace analysis in complex matrices is the suppression/enhancement matrix effect which can seriously affect the quantification [3]. And so, many cleanup technologies have been developed such as Soxhlet extraction [5,6], supercritical fluid extraction (SFE) [7,8], pressurized liquid extraction (PLE) [9–11], microwave-assisted extraction (MAE) [12], ultrasonic extraction (UE) [13], and solid-phase extraction (SPE) [1,2,7,14–16]. Although Soxhlet extraction is relatively inexpensive and easy to yield a satisfactory recovery, it usually requires several hours and large volumes of organic solvents. SFE, PLE, MAE, etc., do not require as much solvent or extraction time, but these methods are expensive and involve other complex parameters [14]. Relatively, SPE is a conventional and efficient technique to clean up the matrix due

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to its low cost, short processing time, and minimal solvent usage [14]. Recently, based on the SPE technique, more popular cleanup methods, *i.e.*, dispersive solid-phase extraction (dSPE)/QuEChERS (quick, easy, cheap, effective, rugged and safe) method [17–25], and matrix solid phase dispersion (MSPD) [26] have been developed. In SPE, dSPE and MSPD procedures, the choice of appropriate adsorbent is a critical factor to obtain high recoveries. Nowadays, many adsorbents such as octadecyl-bonded silica (C18), graphitized carbon black (GCB), polymeric adsorbents, primary secondary amine (PSA) and aminopropyl (NH_2) or diethylaminopropyl (DEA) modified silica have been developed and used for sample clean-up in food analyses [23,26,27]. Among these adsorbents, weak anion exchangers such as NH_2 and PSA can remove many co-extractives interfering with gas chromatography (GC) determination of pesticides and are also very efficient in decreasing the matrix effect [28–30]. In a recent paper, DEA modified silica was used for efficient removal of interferences caused by various pigments, organic acids, sugars, etc. [27].

Not only NH_2/PSA SPE is a well-known technique for cleanup of OCPs and OPPs from different matrices [27], but it also shows weak ability of removal of various pigments in our experiments, we chose tetraethylenepentamine (TEPA) as a functional group for the modifications of co-poly (methyl methacrylate-glycidylmethacrylate) polymer particles to form TEPA nano-composite materials (TEPA-NCM). The amino-functionalized nano-size composite materials are expected to possess much stronger adsorbability than NH_2/PSA materials for the removal of various natural pigments, organic acids and sugars. By using these materials for cleanup and enrichment of the studied pesticides, we expect a short sample preparation time and an easy cleanup/enrichment procedure.

Besides efficient sample preparation, due to the low detection levels required by regulatory bodies and the complex nature of the matrices in which the target compounds are present, the trace-level detection and identification are also important aspects in an analytical method. However, multiresidue method development is difficult, due to the fact that compounds of different polarities, solubilities, volatilities and pK_a values have to be simultaneously extracted and analyzed [27]. Several multiresidue methods for the determination of organophosphorus, organochlorine and organonitrogen pesticides using gas chromatography for separation of individual compounds, followed by detection with selective and sensitive detectors (ECD, NPD, FPD and MS) have been proposed [31–38]. Among these detectors, mass spectrometry is a sensitive and selective technique for both multiresidue determination and trace-level identification of a wide range of pesticides [39–43]. However, due to the fact that many of the modern pesticides are neither amenable to GC nor detectable at sufficiently low levels, a more sensitive and selective analytical technique is needed to qualify and quantify pesticide residues. Instead of these detectors and single mass spectrometry, GC coupled with triple quadrupole mass spectrometry is used for the rapid, sensitive and selective determination of trace-level analytes [23,26,44,45]. And the remarkable advantage of the triple quadrupoles, in comparison with previously used ion traps, is the possibility of operating in multiple reaction monitoring mode (MRM) which is a faster scan mode than product ion scan available on the ion traps [2,3].

In this study, a novel amino-functionalized nano-size composite material using TEPA as a coupling agent, named as TEPA-NCM *infra*, had been prepared. The TEPA-NCM properties were characterized by transmission electron microscopy (TEM), Fourier transform infrared analysis (FTIR), thermogravimetric analysis (TGA) and elementary analyzer (EA). An effective dSPE procedure using the TEPA-NCM was developed, and a comparison with the Carbon/NH_2 and the PSA materials was performed. Furthermore, a gas chromatography–triple quadrupole mass spectrometry (GC–QqQ-MS/MS) method was established and validated for the simultaneous determination of 29 pesticides in cabbage by dSPE using acetonitrile as an extraction solvent and TEPA-NCM as an adsorbent instead of PSA.

2. Experimental

2.1. Reagents and materials

Methyl methacrylate (MMA), glycidylmethacrylate (GMA), tetraethylenepentamine (TEPA), polyvinyl alcohol (PVA 217), benzoyl peroxide (BPO), sodium chloride, disodium hydrogen citrate sesquehydrate, trisodium citrate dihydrate and anhydrous magnesium sulphate of analytical grade were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Dinitramine, allidochlor, oxadiazon, diazinon, thiometon, etridiazole, propyzamide, terbufos, quintozene, ethalfluralin, dichlofenthion, trichloronat, chlorpyrifos, pirimiphos-methyl, linuron, fenchlorphos, cyanazine, isocarbofos, vinclozolin, prothiophos, profenofos, phenthoate, iodofenphos, pendimethalin, trans-chlordane, procymidone, chlorbenside, methidathion and carboxin were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Acetonitrile, acetone, and dichloromethane of HPLC grade were purchased from TEDIA Company (Fairfield, USA). Carbon/NH_2 SPE and Primary secondary amine (PSA) were purchased from Agilent Technologies (Palo Alto, USA). The cabbage samples were acquired on the local market (Ningbo, China).

2.2. Equipment

The characterizations of TEPA-NCM were carried out by using transmission electron microscopy (TEM) (Hitachi H-7650) (Hitachi, Japan), Thermo Nicolet (NEXUS-470) FTIR spectrometer (Thermo Nicolet, USA), and elementary analyzer (EA) (ThermoFisher Flash-1112) (ThermoFisher, USA). A vortex mixer Hualida WH-866 (Taicang, China), Ultra Turraxmixer T25 (IKA-Werke, Germany) and bench top centrifuge capable of producing $5000 \times g$ Heraeus Legend RT (Hanau, Germany) were used during extraction. GC–QqQ-MS/MS analysis were performed with an Agilent 7890A GC system equipped with a 7693 autosampler, a split/splitless injector with electronic pressure control and an Agilent 7000B triple quadrupole mass spectrometer (mass range from m/z 10 to 1050) (Palo Alto, CA, USA).

The instrument data system also held an electron ionization (EI)-MS/MS library specially created for the target analytes under our experimental conditions. Other EI-MS/MS libraries were also available. The mass spectrometer scale was weekly calibrated with perfluorotributylamine. Agilent MassHunter Date Acquisition Software (Ver. B.04.00) was used for instrument control and date acquisition, MassHunter Workstation Software (Ver. B.03.01) was used for data analysis.

2.3. Preparation of TEPA-NCM

2.0 g polyglycol was dissolved into 200 mL hot water, followed by adding 4 mL (0.04 mol) methyl methacrylate (MMA) and 8 mL (0.052 mol) glycidylmethacrylate (GMA). Then 1.0 g benzoyl peroxide (BPO) dissolved in 20.0 mL ethanol was added dropwisely under vigorously stirring. The mixture was continuously reacted at 80 °C for 3 h, yielding M-co-poly (MMA-GMA) polymer. The resulting M-co-poly (MMA-GMA) was isolated and washed with water and ethanol to make it free from redundant MMA and GMA.

1.25 g of the M-co-poly (MMA-GMA) was dispersed into 50 mL methanol in a 100 mL flask. 15 mL (0.08 mol) of the TEPA was added dropwise under stirring. The flask was then fitted with a water condenser and heated at 80 °C for 8 h. The final amino-functionalized polymer, named TEPA-NCM, was isolated and washed with water

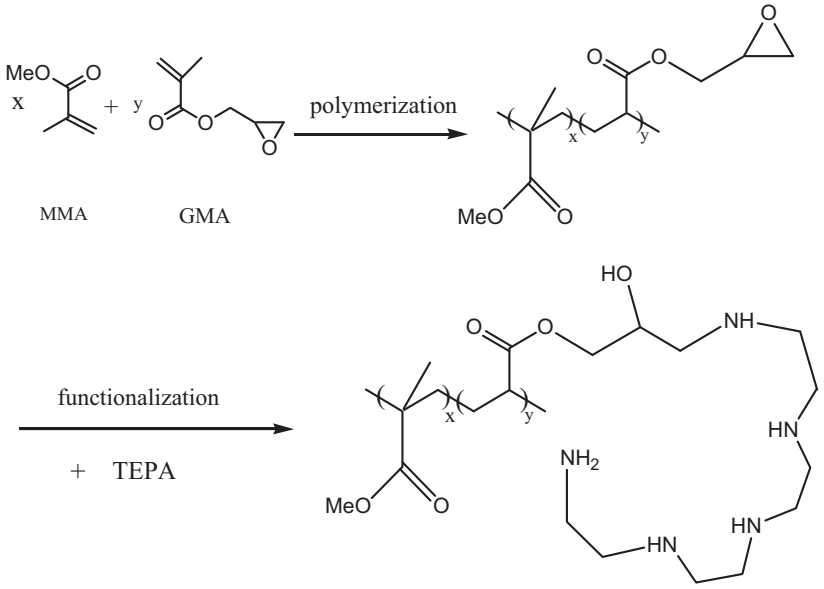

Fig. 1. The preparation procedure of the TEPA-NCM.

and methanol to pH value at about 7.0 to remove the redundant diamines. The TEPA-NCM was dried in a vacuum oven at 60 °C and stored in a sealed bottle for further use. The preparation procedure of the TEPA-NCM was illustrated in Fig. 1.

2.4. Characterization of TEPA-NCM

The morphology and dimensions of the synthesized TEPA-NCM were examined by a Hitachi H-7650 transmission electron microscopy at 80 kV. FTIR spectra were recorded on a NEXUS-470 Thermo Nicolet FTIR spectrometer. Nitrogen content of TEPA-NCM was analyzed by a ThermoFisher Flash-1112 elementary analyzer.

2.5. Sample preparation

In order to evaluate the effectiveness of the TEPA-NCM for the cleanup properties for pesticides, comparative studies were carried out among Carbon/NH_2 SPE cartridges, dSPE with PSA and dSPE with TEPA-NCM, and the extraction procedures were performed as follows.

2.5.1. Cleanup and enrichment via Carbon/NH_2-SPE (Approach I)

10.0 g sample was weighed into a polypropylene centrifuge tube (50.0 mL), and then 20.0 mL acetonitrile was added. The contents were homogenized for 2.0 min using an Ultra Turraxmixer. Subsequently, 3.0 g sodium chloride were added, and the mixture was immediately hand-shaken for 2.0 min, and then centrifuged at 6800 rpm for 3.0 min. Afterwards, 10.0 mL aliquot of the supernatant was concentrated to dryness with a nitrogen stream. For Carbon/NH_2-SPE purification, the column was conditioned sequentially with 5.0 mL acetone and 5.0 mL acetone/dichloromethane (1:1, v/v) solution. The dry residues were redissolved with 3.0 mL acetone/dichloromethane (1:1, v/v) and loaded onto the Carbon/NH_2 cartridge. The sample tube was washed by another 1.0 mL acetone/dichloromethane (1:1, v/v) and the eluents were loaded onto the Carbon/NH_2 cartridge. The Carbon/NH_2 cartridge was eluted with 12.0 mL acetone/dichloromethane (1:1, v/v) at a flow rate of 1.0 mL/min. The final eluate was concentrated with a nitrogen stream until the last drop of solution visibly disappeared. The residues were redissolved in 1.0 mL acetone, vortexed, and transferred into an autosampler vial for GC–MS/MS analysis.

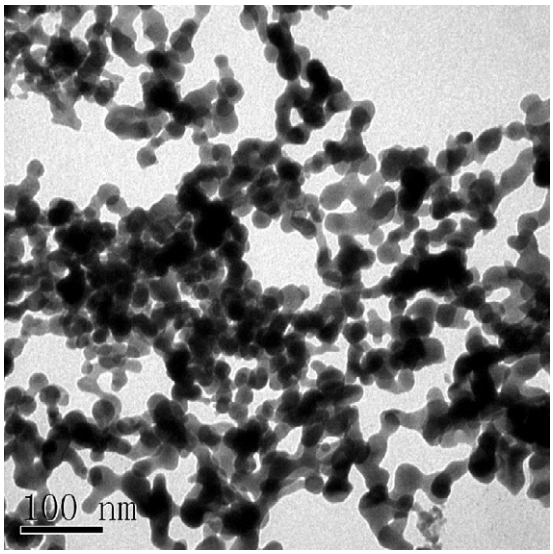

Fig. 2. TEM image of TEPA-NCM.

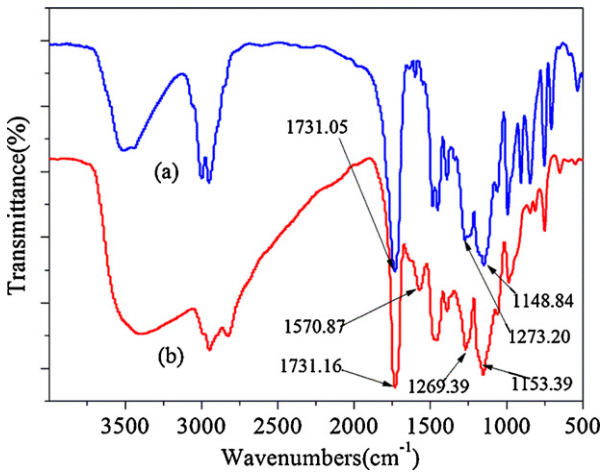

Fig. 3. FTIR adsorption spectra of (a) M-co-poly (MMA-GMA) and (b) TEPA-NCM.

2.5.2. Cleanup and enrichment via dSPE with PSA (Approach II)

10.0 g sample was weighed into a polypropylene centrifuge tube (50.0 mL), 20.0 mL acetonitrile was added. The contents were homogenized for 2.0 min using an Ultra Turraxmixer. Subsequently, 0.5 g disodium hydrogencitrate sesquehydrate, 1.0 g trisodium citrate dihydrate, 4.0 g anhydrous magnesium sulphate, and 1.0 g sodium chloride were added, and the mixture was immediately hand-shaken for 2.0 min, then centrifuged at 6800 rpm for 3.0 min.

Afterwards, 12.5 mL aliquot of the supernatant was transferred to a polypropylene centrifuge tube (15.0 mL) containing 0.9 g anhydrous magnesium sulphate and 0.1 g PSA. The tube was vortexed for 1.0 min and centrifuged at 6800 rpm for 5.0 min. 10.0 mL aliquot of the supernatant was concentrated to dryness with a nitrogen stream and redissolved in 1.0 mL of acetone prior to its injection into the GC–MS/MS system.

2.5.3. Cleanup and enrichment via dSPE with TEPA-NCM (Approach III)

The overall procedure was similar to that of *Approach II*. The PSA was replaced by the TEPA-NCM.

2.6. Method validation

2.6.1. Standard preparation

Individual stock standard solutions were prepared by exact weighing and dissolution in acetone (concentrations in the range of 100–500 mg/L); these solutions were stored under refrigeration ($T \leq 4\,^{\circ}C$). The stock mixture solution of the standards at a concentration of 10.0 mg/L was prepared by appropriate dilution of the stock solutions with acetone. Calibration standards in acetone with concentration in the range of 2.0–500.0 μg/L (equivalent to 0.4–100.0 μg/kg) were also prepared before use for the calibration curves. The calibration curves made by peak area vs. concentration (μg/L) were used to calibrate the GC–MS/MS system and spike samples in recovery experiments.

2.6.2. Spiked samples

Spiked recoveries were performed at concentrations of 2.0, 10.0, 20.0 and 80 μg/kg for 29 pesticides in the samples. For each spiked sample, stock mixture solution of the standards was added to 10.0 g comminuted cabbage, which was free from the target compounds. The spiked samples prepared were stored at 4 °C for about 12 h to let the OPPs permeate uniformly into the smashed cabbage tissues. Five recoveries at each level were run along with both a reagent and a sample blank.

2.7. GC–MS/MS analysis

Capillary GC analysis was performed on a DB-5ms capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). Two microlitres of the final extract were injected into the chromatographic system. The temperature of the injector was set at 260 °C. The time when injector works as splitless and after splitless time the split valve is opened. The initial temperature of the column oven was 70 °C (hold for 2.0 min). This temperature was increased at a rate of 25 °C/min up to 150 °C; next, the temperature was increased up to 200 °C at a rate of 3 °C/min; then, the temperature was increased up to 280 °C (hold for 2.0 min) at a rate of 30 °C/min. Helium (99.999%) at constant pressure mode of 13.4 Torr was used as carrier gas; nitrogen (99.999%) at a pressure in the range of 7.31–7.35 mTorr was used as collision gas. The running time was of 26.29 min, divided into seven segments. The QqQ mass spectrometer was operated in EI at 70 eV in the multiple reaction monitoring (MRM) mode. The transfer line, manifold and ionization source temperatures were set at 280, 40 and 280 °C, respectively. A filament multiplier delay of 3.75 min was fixed in order to prevent instrument damages. The electron multiplier voltage was set at 1640 V (+200 V offset above the auto-tuning process). Precursor and product ions, collision energies and other parameters used were shown in Table 1.

3. Results and discussion

3.1. Characterization of TEPA-NCM

The TEM image of the TEPA-NCM (shown in Fig. 2) revealed that the TEPA-NCM synthesized in this study was multidispersed with an average diameter of approximately 10–20 nm in size.

The IR spectra of M-co-poly (MMA-GMA) and TEPA-NCM were shown in Fig. 3. In the IR spectra of M-co-poly(MMA-GMA), the characteristic absorptions of C=O groups at $\sim$1731 cm^{-1}, C–O–C groups at $\sim$1273 cm^{-1} and $\sim$1148 cm^{-1} appeared, as shown in Fig. 3(a). After further amino-functionalization, the characteristic peaks of –NH– and $-NH_2-$ groups at $\sim$1570 cm^{-1} and $\sim$3425 cm^{-1} appeared, as shown in Fig. 3(b). This revealed that the epoxyl- of co-poly (MMA-GMA) had been functionalized successfully with the amino groups via ring-opening reaction, and the nitrogen percentage of TEPA-NCM obtained from EA was 10.9%.

3.2. Optimization of GC–MS/MS conditions

Coupled with the QqQ analyzers, the chromatographic separation is not a critical stage in the development of a multiresidue method because of the possibility of monitoring co-eluted compounds in MRM. In order to get optimization of triple quadrupole MS/MS conditions, relevant consideration included the choice of precursor ions, product ions, and optimization of collision energies for best response were required for each target compound in order to conduct analyses. After obtaining the full scan spectra, the precursor ion for each analyte was selected, then subjected to collision energy voltages (potential on second quadrupole) to generate MS/MS product ions, and in this work, collision energies (CEs) from 2 to 35 eV were applied, and the results were shown in Table 1. The final purpose was to develop a MRM method with two or three transitions per compound.

Moreover, the sensitivity and peak shapes were highly related with scan time, dwell time, scan rate and the number of monitored transitions [2,3]. In order to obtain good sensitivity and well-shaped chromatographic peaks, dwell time was adjusted so that the number of cycles per second was 3.3 throughout the chromatographic run, providing a sufficient number of chromatographic points for all compounds, and scan time were listed in Table 1. While the scan time is fixed, the signal and thus the sensitivity should decrease with the increasing of MRM transitions measured in a particular time window. Obviously, it would be impracticable that two or three MRM transitions of each analyte have its own retention time-window in multi-residue analysis. Therefore, after through examination of the distribution of peaks on the chromatogram which was divided into 7 retention time-windows, where no more than 13 MRM transitions were entered into any of them. The final MS/MS conditions used in this study were detailed in Table 1, and the multiple reaction monitoring (MRM) of 29 coeluting pesticides were shown in Fig. 4.

3.3. Comparison of cleanup procedure via three different approaches

Although MS/MS detection was employed in this work, sample preparation represented a critical part of the method due to high complexity of vegetables matrix which contained large amounts

of various natural pigments, organic acids, sugars, and other substances readily extractable by organic solvents. The main challenge in developing the cleanup method was the separation of the interested pesticides from the co-extracted matrix.

An overview of the procedure for analysis of 29 pesticides was shown in Fig. 5. The aim of the present study was to investigate the cleanup procedure via three different approaches, kept up the other variables unchanged. Cabbage spiked with 29 pesticides at a concentration of 10.0 μg/kg (equivalent to 50.0 μg/L) was used to compare the cleanup procedures via the three different approaches. The average recoveries and relative standard deviations (RSDs) of the analytes studied were listed in Table 2. The total ion chromatograms (TIC) for the spiked samples for the three different approaches as well as the calibration standard solution of 29 pesticides at a concentration of 50.0 μg/L were shown in Fig. 6. According to the TIC of calibration standard solution at 50.0 μg/L, it can be seen that the analytes were prone to strong matrix enhancement interactions in samples, which could not be avoided well by using *Approach I*, since the recoveries of all the analytes were obviously high, ranged from 110% to 197%. With the use of *Approach II* and *Approach III*, the recoveries of all the analytes were ranged from 68% to 129% and 85% to 118% except for the trans-chlordane (141%) with acceptable RSDs (2–9%) ($n = 5$), which revealed the cleanup property of TEPA-NCM was comparable with that of PSA materials. Additionally, with the use of TEPA-NCM dSPE, the recoveries of all the pesticides were much more satisfactory than PSA/GCB-dSPE of the recoveries ranged from 68% to 131% with RSDs (1–26%) [20]. Moreover, as shown in Fig. 7, the TEPA-NCM was more effec-

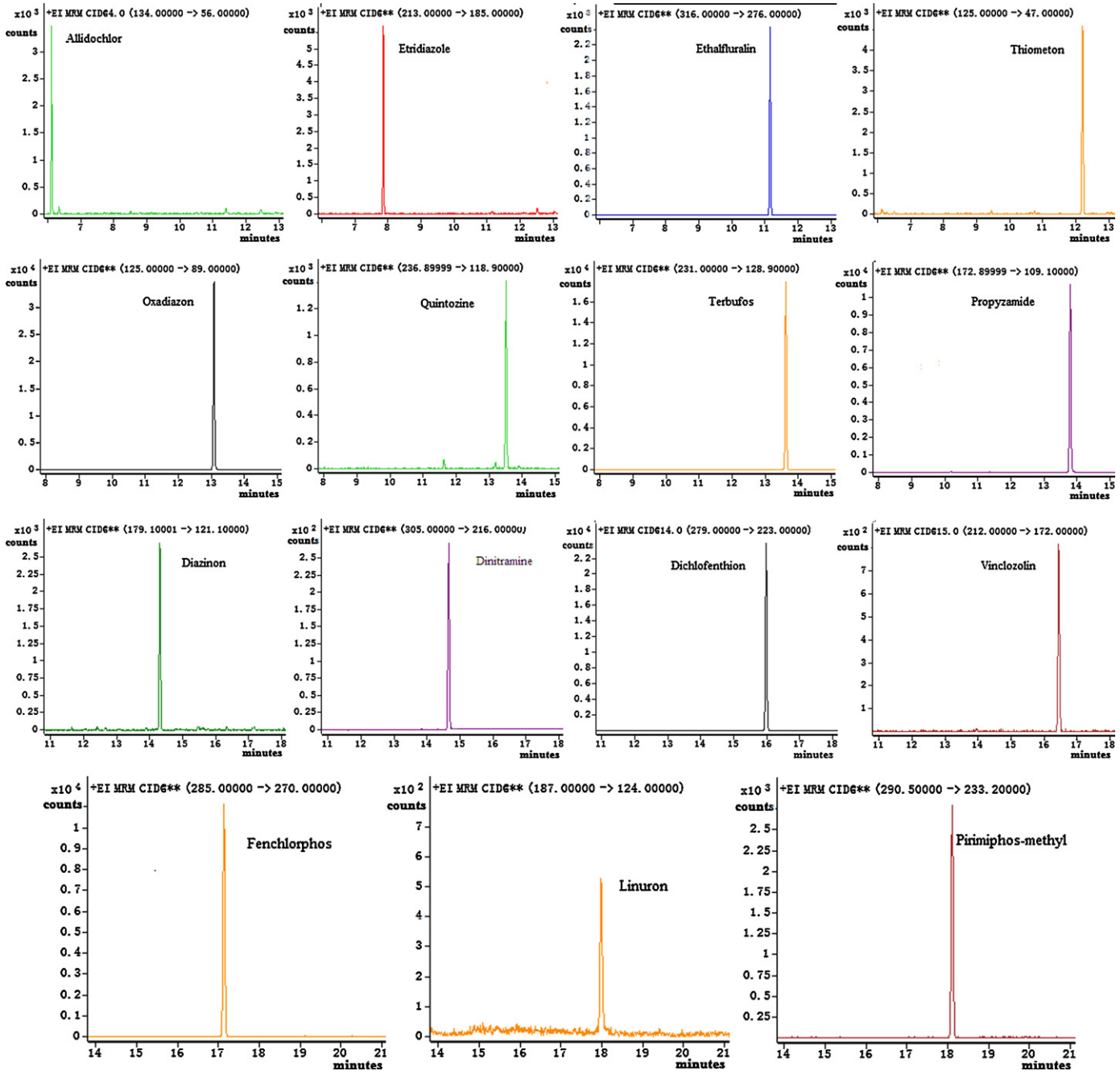

Fig. 4. Chromatograms obtained simultaneously by MRM for 29 of the pesticides with the cleanup procedure via *Approach III*. Each chromatogram is represented monitoring the quantification ion of each pesticide.

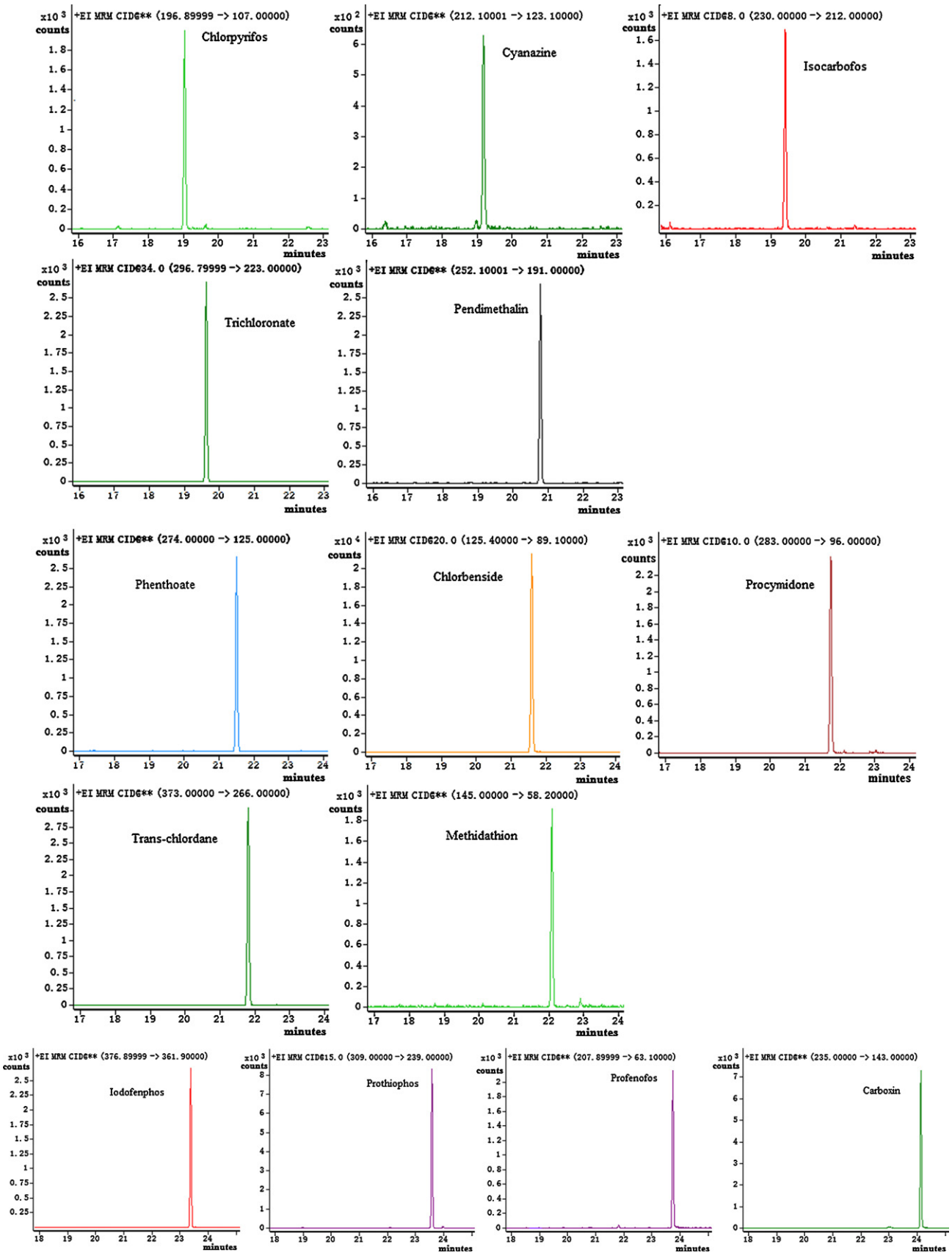

Fig. 4. (*Continued*).

Table 1
Conditions of the optimized GC–MS/MS method.

Compound	Time-window	Retention time (min)	Scan time (s)	Precursor ion (*m/z*)	Product ion, *m/z* (collision energy, eV)
Allidochlor	1	6.176	0.3	134	56 (4)
Etridiazole	1	7.871	0.3	213	185 (10), 142 (18)
Ethalfluralin	1	11.127	0.3	316	276 (4), 202 (24)
Thiometon	1	12.220	0.3	125	47 (20), 62 (8), 79 (10)
Oxadiazon	2	13.087	0.4	125	89 (10), 99 (15)
Quintozine	2	13.618	0.4	237	119 (30), 143 (25), 65 (8)
Terbufos	2	13.772	0.4	231	175 (10), 129 (25)
Propyzamide	2	13.900	0.4	173	145 (15), 109 (20)
Diazinon	3	14.307	0.4	179	137 (18), 164 (18), 121 (35)
Dinitramine	3	14.633	0.4	305	216 (22), 244 (15), 189 (10)
Dichlofenthion	3	15.981	0.4	279	223 (14), 205 (28), 159 (35)
Vinclozolin	3	16.440	0.4	212	172 (15), 145 (25), 109 (40)
Fenchlorphos	4	17.152	0.4	285	270 (12), 240 (25)
Linuron	4	18.087	0.4	187	124 (31), 159 (12)
Pirimiphos-methyl	4	18.207	0.4	291	233 (5), 151 (15),
Chlorpyrifos	5	19.034	0.4	197	169 (15), 107 (40)
Cyanazine	5	19.149	0.4	212	123 (22), 151 (10)
Isocarbofos	5	19.404	0.4	230	196 (5), 136 (28), 212 (8)
Trichloronate	5	19.640	0.4	297	269 (8), 223 (34), 240 (35)
Pendimethalin	5	20.762	0.4	252	208 (2), 191 (4), 162 (10)
Phenthoate	6	21.523	0.4	274	246 (4), 121 (10), 125 (16)
Chlorbenside	6	21.603	0.4	125	89 (20), 99 (20)
Procymidone	6	21.751	0.4	283	255 (8), 67 (30), 96 (10)
Trans-chlordane	6	21.802	0.4	373	266 (25), 264 (25), 337 (4)
Methidathion	6	22.084	0.4	145	85 (15), 58 (10)
Iodofenphos	7	23.380	0.4	377	362 (20), 93 (35)
Prothiophos	7	23.652	0.4	309	281 (8), 239 (15), 205 (40)
Profenofos	7	23.804	0.4	208	63 (40), 99 (25)
Carboxin	7	24.308	0.4	235	143 (6), 162 (10)

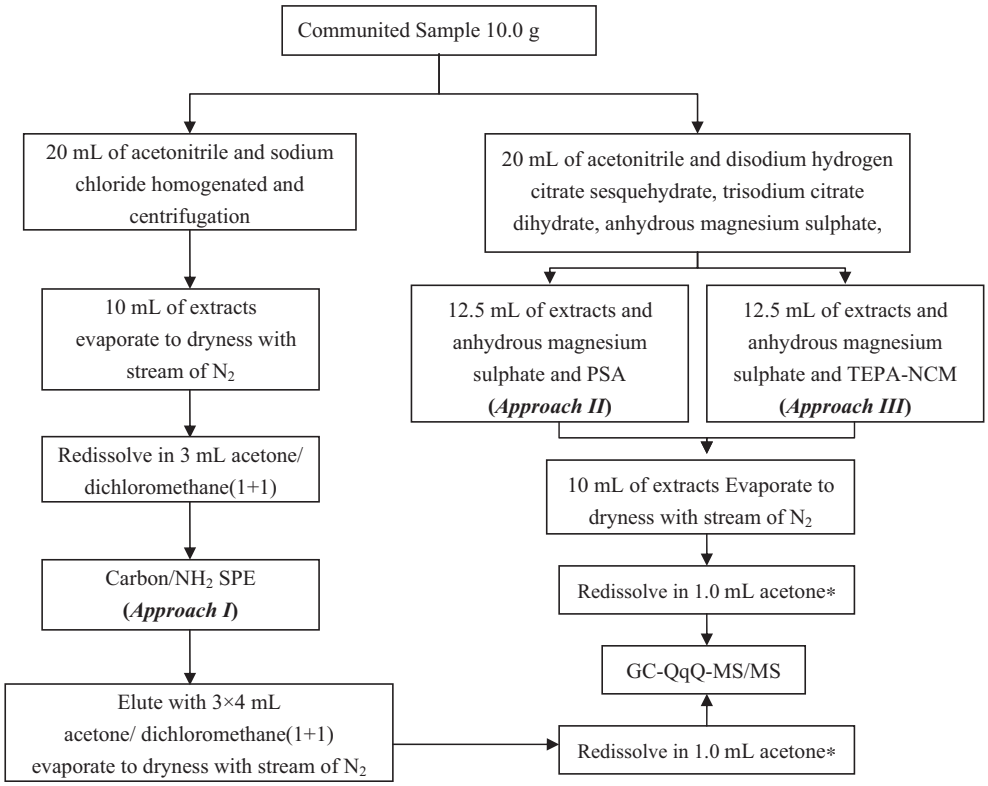

Fig. 5. Overview of the procedure for sample preparation for determination of pesticides residues (* the analytes were enriched 5 times).

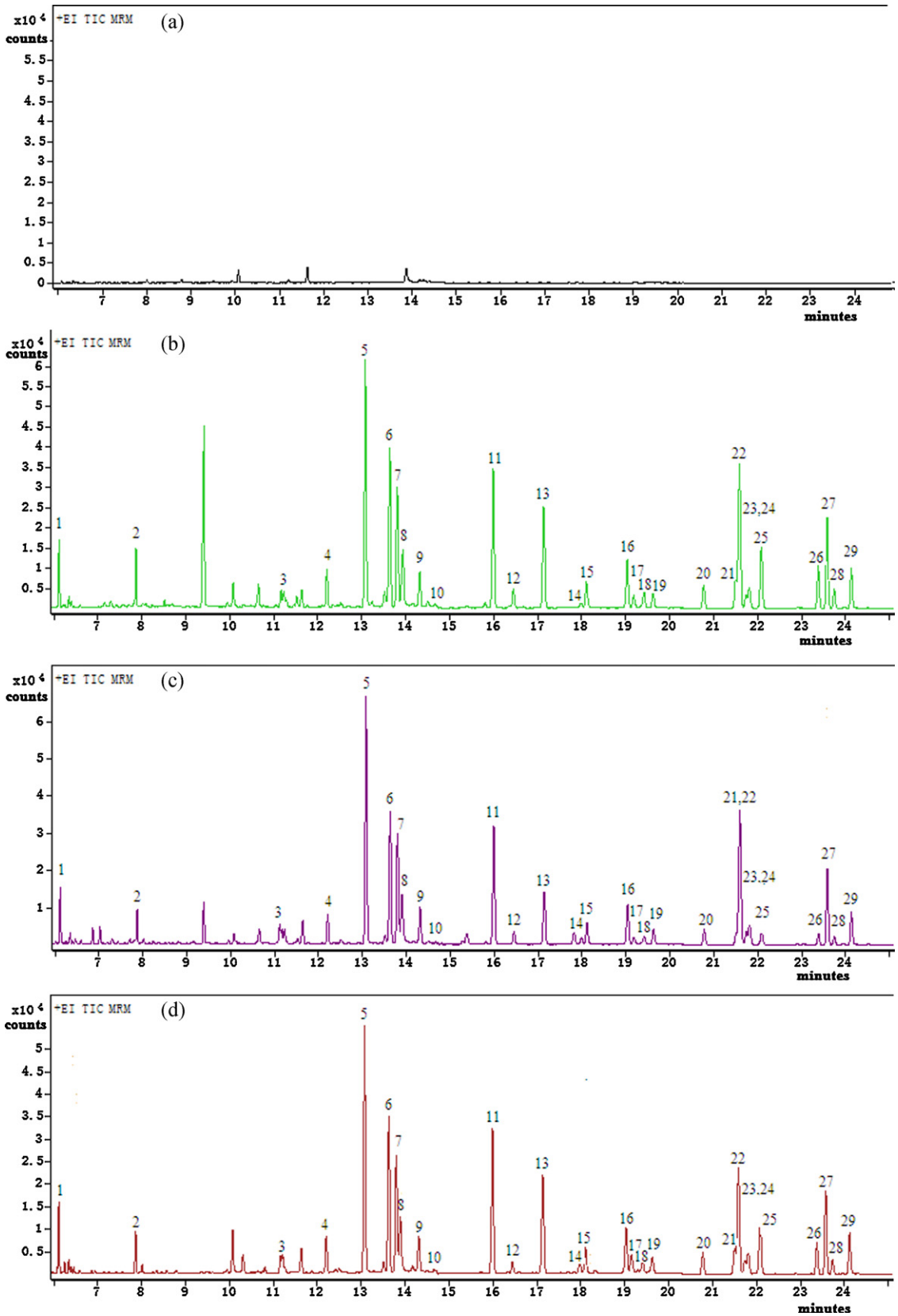

Fig. 6. The total ion chromatogram (TIC) for the cabbage not spiked (a) and spiked with analyte at 10.0 μg/kg for the *Approach I* (b), *Approach II* (c), and *Approach III* (d) as well as the calibration standard solution of multi pesticides at 50 μg/L (e) with peak numbering: allidochlor (1), etridiazole (2), ethalfluralin (3), thiometon (4), oxadiazon (5), quintozene (6), terbufos (7), propyzamide (8), diazinon (9), dinitramine (10), dichlofenthion (11), vinclozolin (12), fenchlorphos (13), linuron (14), pirimiphos-methyl (15), chlorpyrifos (16), cyanazine (17), isocarbofos (18), trichloronate (19), pendimethalin (20), phenthoate (21), chlorbenside (22), procymidone (23), trans-chlordane (24), methidathion (25), iodofenphos (26), prothiophos (27), profenofos (28), and carboxin (29).

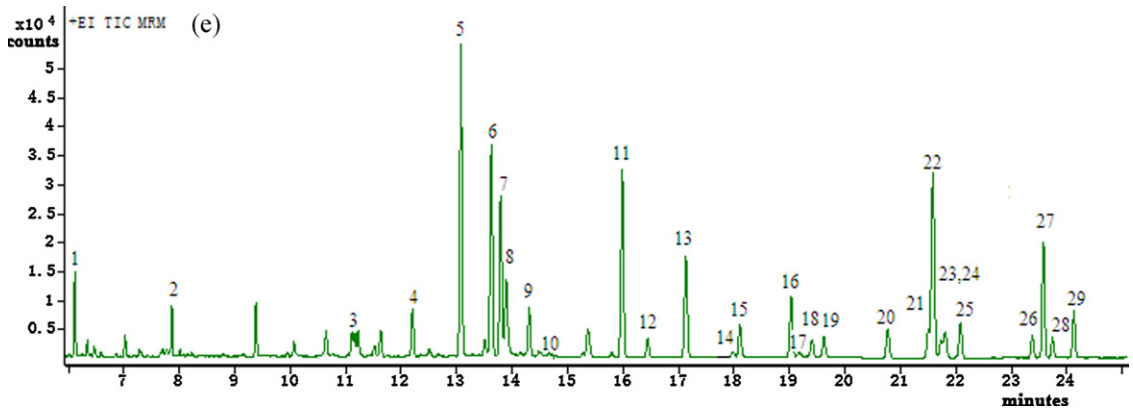

Fig. 6. (*Continued*).

tive to remove the pigments existed in the matrix than the PSA materials.

3.4. Effect of the amount of TEPA-NCM on the cleanup properties

According to the discussion above, it can be seen that the TEPA-NCM was more effective to remove various matrix such as natural pigments, organic acids, sugars, and other substances readily extractable by organic solvents than the PSA. When designing the TEPA-NCM SPE cleanup optimization experiments, the primary consideration was to employ a suitable amount of the TEPA-NCM without affecting the pesticides recoveries. For this purpose, the effectiveness of various amounts of adsorbents on cleanup efficiency and pesticide recoveries was studied with the cabbage extracts spiked with each of the 29 pesticides at 10.0 μg/kg. The spiked extracts were purified by using different amounts of the TEPA-NCM with constant amounts of the rest of adsorbents, and the results were shown in Fig. 8. It can be seen that the dispersive TEPA-NCM adsorbents had an impact on recoveries of the studied pesticides, and it was easy to determine a clear trend in the recovery when increasing the amount of adsorbents from 25 to 150 mg/mL of acetonitrile extract. When using TEPA-NCM adsorbents of 25 mg/mL of acetonitrile extract, the high recovery of the 29 pesticides was in the range of 110–147% which could be resulted from matrix enhancement interactions in samples. With the increasing of the amount of TEPA-NCM adsorbents from 50 to 100 mg/mL of acetonitrile extract, the satisfactory recoveries of the 29 pesticides were consistently in the range of 83–119% except for trans-chlordane, for which the recoveries ranged between 131 and 141%. And so, it can be seen that the least amounts of TEPA-NCM adsorbents of 50 mg/mL of acetonitrile extract could ensure to remove the various matrix such as natural pigments, organic acids, sugars, and other substances readily extractable by organic solvents which resulted in the matrix enhancement interactions in

Table 2
Comparison of cleanup procedure via three different approaches.

Compound	10.0 μg/kg (n = 5)					
	Approach I		*Approach II*		*Approach III*	
	Average recovery (%)	RSD (%)	Average recovery (%)	RSD (%)	Average recovery (%)	RSD (%)
Allidochlor	120	6	121	7	103	3
Etridiazole	145	6	102	2	102	3
Ethalfluralin	113	3	91	8	100	6
Thiometon	110	7	86	8	103	3
Oxadiazon	110	9	123	3	111	8
Quintozine	111	7	97	5	111	4
Terbufos	121	7	117	9	99	7
Propyzamide	123	6	91	6	89	6
Diazinon	110	10	89	8	97	5
Dinitramine	141	3	129	9	88	8
Dichlofenthion	121	4	87	5	101	8
Vinclozolin	144	6	83	5	85	7
Fenchlorphos	150	6	80	7	118	8
Linuron	112	5	102	8	110	9
Pirimiphos-methyl	140	6	88	7	104	5
Chlorpyrifos	120	5	92	8	99	4
Cyanazine	121	8	84	7	100	4
Isocarbofos	145	4	89	5	99	8
Trichloronate	144	4	100	5	102	3
Pendimethalin	131	6	83	5	99	4
Phenthoate	110	9	113	8	87	6
Chlorbenside	144	3	122	7	113	6
Procymidone	140	6	122	8	114	6
Trans-chlordane	197	4	76	4	141	6
Methidathion	130	3	68	7	100	7
Iodofenphos	197	3	87	5	115	4
Prothiophos	123	7	96	5	98	5
Profenofos	124	8	80	6	99	4
Carboxin	123	5	97	6	102	6

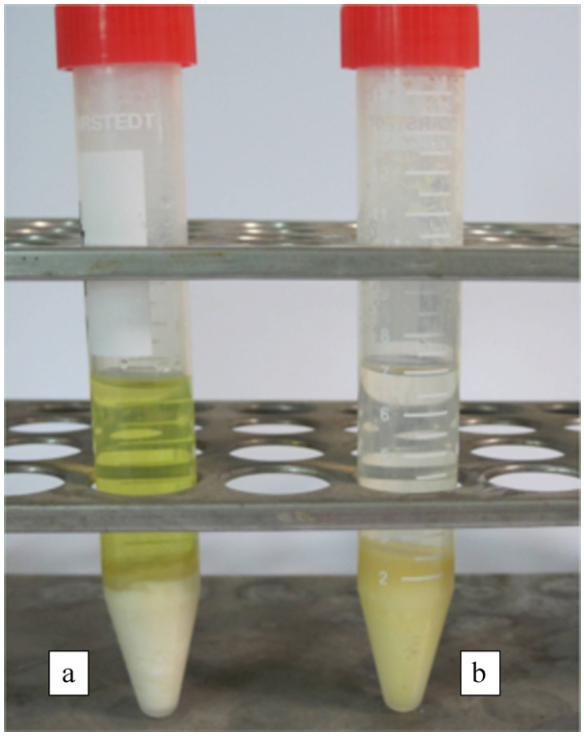

Fig. 7. The cleanup properties of (a) PSA and (b) TEPA-NCM materials for cabbage.

samples. However, quantification using amounts between 100 and 150 mg of TEPA-NCM seemed to be inappropriate for satisfactory reproducibility of recoveries (55–135%). Based on the results of the experiments, it can be seen that combination of the least amounts of TEPA-NCM adsorbents in the proportion of 50 mg per 1 mL acetonitrile extract would ensure efficient and robust cleanup while maintaining quantitative recovery of the target pesticides.

In order to investigate recycling of the TEPA-NCM, all the TEPA-NCM used for cleanup procedures for *Approach III*, were collected and soaked in sodium hydroxide solution at a concentration of 0.2 mol/L for at least half an hour. Then they were washed with water and acetone to pH value at about 7.0 to remove the redundant sodium hydroxide, separated and dried under a vacuum at 60 °C for 12 h. The recoveries of the 29 pesticides by the recycling TEPA-NCM via *Approach III* were shown in Fig. 9. The results showed that TEPA-NCM could be reused at least five times without much sacrifice of the cleanup efficiency.

3.5. Method linear range, accuracy, LOD, LOQ and selectivity

The linearity of the calibration curves made by peak area vs. concentration (μg/L) was studied using calibration standards in solvent at seven concentrations of 2.0, 5.0, 10.0, 50.0, 100.0, 200.0 and 500.0 μg/L (equivalent to 0.4, 1.0, 2.0, 10.0, 20.0, 40.0 and 100.0 μg/kg). The response function was found to be linear with a coefficient of determination (r^2) higher than 0.99 in the tested range listed in Table 3 for all pesticides except for dinitramine, trichloronate, methidathion, for which coefficients of determination was 0.9893, 0.9861 and 0.9882, respectively.

Method accuracy and precision data were obtained for all the pesticides spiked at concentrations of 2.0, 20.0 and 80.0 μg/kg in cabbage. The results were summarized in Table 3. The majority of mean recoveries were in the range of 75–114% at these two spiking levels with associated relative standard deviations (RSDs) in the range of 2–17% except for trans-chlordane. Hence, taking into account a broad range of analyzed pesticides, recovery between 70 and 120% and RSD < 20% were accepted as reasonable criteria for validation of the screening method. Pesticide not satisfied with these criteria was trans-chlordane.

The LODs and LOQs values for the analyzed pesticides were shown in Table 3. It can be seen that the LODs and LOQs calculated as the lowest analyte concentration that yielded a signal-to-noise (S/N) ratio of 3 and 10 were in the range of 0.052–0.29 μg/kg and 0.17–0.95 μg/kg, respectively. Such LOQs were little higher than Martínez Vidal's work [46], and the possible reasons were originated from the measurement of the enriched sample process and the difference of the used instrument's sensitivity. The Martínez Vidal's work was used the Combi PAL (CTC Analytics AG), which could be more effective to enrich than TEPA-NCM dSPE. On the other hand, the Martínez Vidal's work was used a Varian 3800 GC system coupled with the Varian 1200 L triple quadrupole mass spectrometer, and our experiment used an Agilent 7890A GC system coupled with an Agilent 7000B triple quadrupole mass spectrometer. However, the LOQs in this work were much lower in comparison with the other dSPE-GC–QqQ-MS/MS literatures [20,23], and all of them were lower than the maximum residue levels (MRLs) established by European legislation.

Selectivity is assessed to show that the intended analytes are measured and that their quantitation is not affected by the matrix, and the interference is considered insignificant if the peak area of interfering peak at the retention time (RT) of the analyte less than 20% of analyte peak area. In this work, test for selectivity was carried out using blank cabbage processed by the same extraction method (*Approach III*) and analyzed to determine the extent to which substances may contribute to the interferences for analytes.

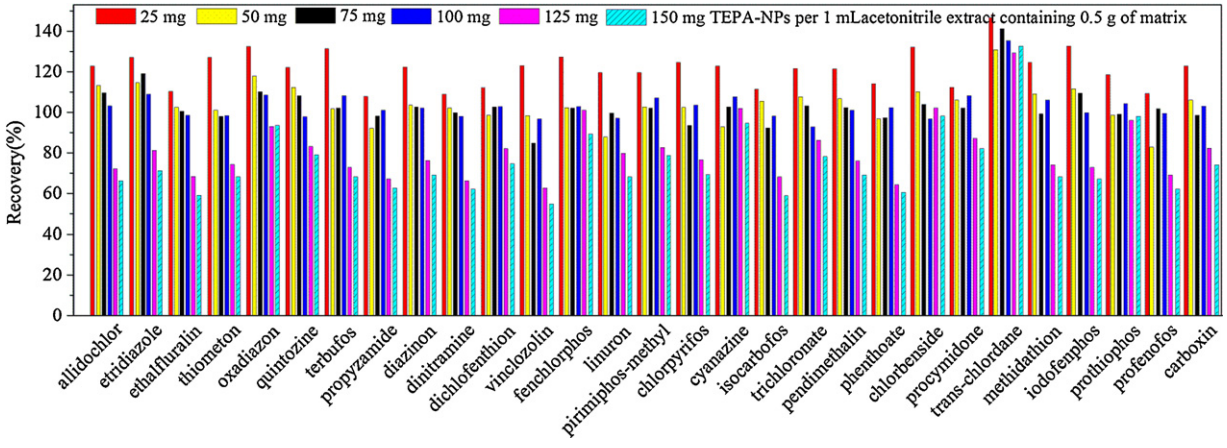

Fig. 8. Effect of the amount of TEPA-NCM on the cleanup properties.

Table 3
Validation parameters ($n = 5$) obtained for the target compounds at three concentration levels in cabbage matrix.

Compound	Linear equation	Linearity range (μg/kg)	2.0 μg/kg		20.0 μg/kg		80.0 μg/kg		LOD (μg/kg)	LOQ (μg/kg)	R^2
			Average recovery (%)	RSD (%)	Average recovery (%)	RSD (%)	Average recovery (%)	RSD (%)			
Allidochlor	$Y = 169.7 \times X - 192.6$	1.0–100.0	94	7	104	8	98	5	0.12	0.41	0.9993
Etridiazole	$Y = 188.8 \times X - 218.9$	1.0–100.0	88	7	101	8	94	7	0.16	0.54	0.9989
Ethalfluralin	$Y = 38.7 \times X - 104.7$	1.0–100.0	96	13	97	13	109	10	0.22	0.72	0.9995
Thiometon	$Y = 772.6 \times X - 1045.5$	1.0–100.0	106	9	103	4	88	6	0.18	0.58	0.9989
Oxadiazon	$Y = 32.9 \times X - 54.4$	0.4–100.0	93	9	109	7	98	12	0.052	0.17	0.9991
Quintozene	$Y = 200.6 \times X - 375.8$	0.4–100.0	110	6	109	8	107	5	0.066	0.22	0.9979
Terbufos	$Y = 318.2 \times X + 57.7$	0.4–100.0	112	7	111	2	112	5	0.098	0.33	0.9996
Propyzamide	$Y = 68.6 \times X - 10.1$	0.4–100.0	85	3	88	8	94	12	0.12	0.40	0.9991
Diazinon	$Y = 69.9 \times X - 303.9$	1.0–100.0	92	6	94	5	100	9	0.15	0.50	0.9998
Dinitramine	$Y = 5.3 \times X - 3.1$	1.0–100.0	79	14	81	11	83	11	0.22	0.72	0.9893
Dichlofenthion	$Y = 104.9 \times X - 10.5$	0.4–100.0	99	6	104	8	98	5	0.068	0.23	0.9986
Vinclozolin	$Y = 23.3 \times X - 23.6$	1.0–100.0	87	13	93	8	96	12	0.20	0.68	0.9990
Fenchlorphos	$Y = 422.0 \times X - 762.6$	0.4–100.0	98	4	113	8	108	4	0.11	0.36	0.9986
Linuron	$Y = 145.5 \times X - 79.7$	1.0–100.0	114	5	101	8	103	5	0.22	0.72	0.9996
Pirimiphos-methyl	$Y = 55.4 \times X - 121.7$	1.0–100.0	100	9	98	9	105	7	0.29	0.95	0.9937
Chlorpyrifos	$Y = 166.2 \times X - 187.6$	1.0–100.0	107	10	95	6	91	7	0.20	0.65	0.9991
Cyanazine	$Y = 151.6 \times X - 355.1$	1.0–100.0	109	4	88	7	86	6	0.21	0.69	0.9986
Isocarbofos	$Y = 17.3 \times X - 73.2$	1.0–100.0	88	10	96	8	97	10	0.24	0.79	0.9993
Trichloronate	$Y = 76.3 \times X - 182.6$	1.0–100.0	79	14	75	10	83	11	0.20	0.65	0.9861
Pendimethalin	$Y = 39.4 \times X - 73.0$	1.0–100.0	95	5	98	9	104	9	0.19	0.63	0.9986
Phenthoate	$Y = 58.6 \times X - 108.2$	0.4–100.0	83	7	88	8	85	5	0.067	0.22	0.9982
Chlorbenside	$Y = 520.2 \times X - 1158.2$	1.0–100.0	89	10	105	6	103	7	0.21	0.71	0.9990
Procymidone	$Y = 75.7 \times X + 583.9$	1.0–100.0	84	7	112	6	106	5	0.19	0.64	0.9991
Trans-chlordane	$Y = 144.2 \times X - 194.2$	1.0–100.0	127	6	144	9	130	10	0.16	0.55	0.9992
Methidathion	$Y = 184.8 \times X - 379.4$	1.0–100.0	80	17	86	14	88	12	0.20	0.68	0.9882
Iodofenphos	$Y = 272.4 \times X - 530.4$	1.0–100.0	96	6	101	8	105	5	0.28	0.93	0.9987
Prothiophos	$Y = 190.0 \times X - 373.3$	0.4–100.0	101	3	105	7	109	11	0.12	0.39	0.9990
Profenofos	$Y = 77.3 \times X - 133.2$	1.0–100.0	96	6	97	9	93	6	0.28	0.93	0.9999
Carboxin	$Y = 495.4 \times X - 1165.3$	1.0–100.0	103	4	104	9	98	4	0.15	0.51	0.9995

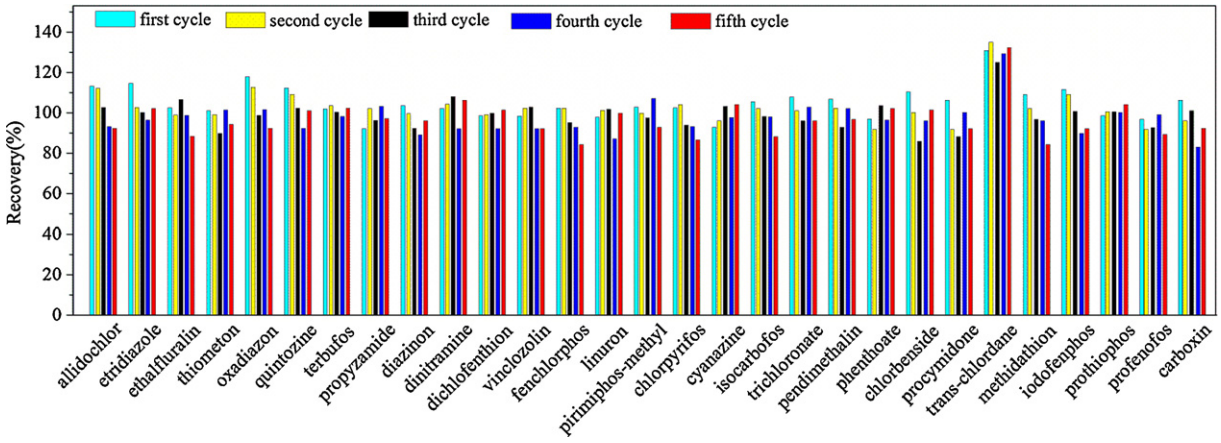

Fig. 9. The recoveries of 29 pesticides with the cleanup procedure via *Approach III* by reusing TEPA-NCM.

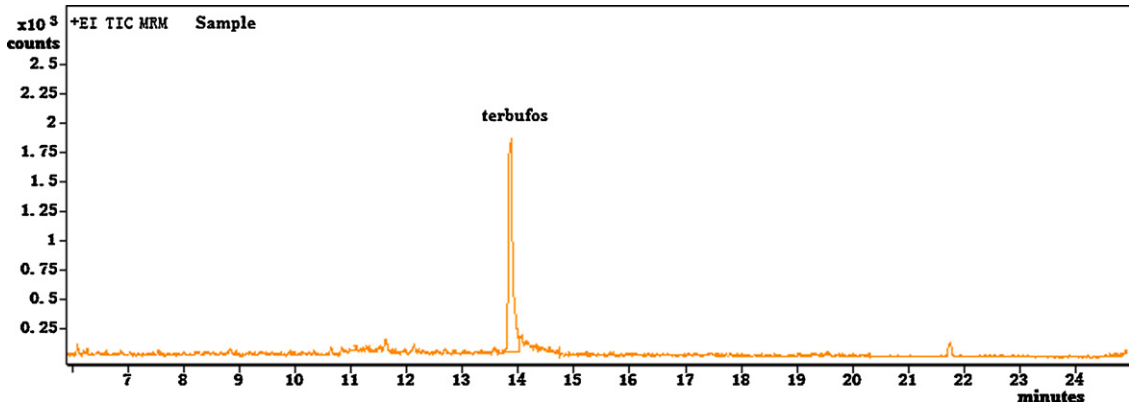

Fig. 10. The total ion chromatogram (TIC) for one of the examined samples.

According to Fig. 6(a) and (d) which demonstrates the selectivity results with the chromatograms of blank cabbage, peak response of 29 pesticides at 10 μg/kg. The area observed in blank cabbage was much less than 20% in the analyte peak area. The dSPE with TEPA-NCM method employed gave a very good selectivity for the analysis of 29 pesticides except for trans-chlordane in the blank cabbage.

3.6. Real samples

In order to apply the proposed method, five kinds of vegetables (three samples for each kind), including cabbage, kidney bean, spinage, lettuce, and eggplant, were analyzed with the developed method (*Approach III*), performing several internal quality controls in order to guarantee that the measurement process was under statistical control. Each batch of samples was processed together with a matrix blank which was obtained from a pesticide-free sample. The matrix blank eliminated the false positive as result of contamination in the extraction process, instrument or chemicals. A reagent blank was obtained by performing the whole process without a sample. This sample eliminated possible false positives produced by contamination in the instrument or solvent used. A blank extract spiked at the third calibration level (10.0 μg/L) permitted to control the extraction efficiency. Calibration curves were prepared daily obtaining determination coefficients >0.99. The results show the presence of terbufos in three of the fifteen collected samples with concentrations below 1.77 μg/kg, and other 28 pesticides were not found above LOQ in the analyzed samples (Fig. 10).

4. Conclusions

A simple and reproducible analytical dSPE-GC–QqQ-MS/MS method for determining residues of OCPs and OPPs in vegetable samples was developed. The dSPE extraction procedure using a novel adsorbent (TEPA-NCM), which was synthesized by amino-functionalized nano-composite material (NCM) used tetraethylenepentamine (TEPA) as a coupling agent. The cleanup properties of TEPA-NCM are comparable with PSA composite materials and more effectively remove the pigments, and the cleanup and enrichment of TEPA-NCM dSPE are faster, easier and more effectively to perform than Carbon/NH_2 SPE. The combination of the least amounts of TEPA-NCM was carefully optimized to maximize recovery of the pesticides while eliminating most of the interfering matrix components. The present TEPA-NCM can be recycled more than five times without much effect on their cleanup properties. Acceptable recoveries for the 29 pesticides were obtained in the range of 75–114% except for trans-chlordane. The results demonstrate that the accuracy, precision and selectivity of the proposed method are satisfactory for analysis of the OPPs and OCPs examined in this study. The present work revealed that the synthesized TEPA-NCM has potential applications in cleanup procedure for the determination of pesticides. Obviously, the proposed method has a potential to be applied to other vegetables matrices too.

Acknowledgements

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