Journal of Chromatography B, 1031 (2016) 99–108

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Multi-residue determination of 171 pesticides in cowpea using modified QuEChERS method with multi-walled carbon nanotubes as reversed-dispersive solid-phase extraction materials

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ARTICLE INFO

Article history:
Received 27 May 2016
Received in revised form 21 July 2016
Accepted 23 July 2016
Available online 25 July 2016

Keywords:
Cowpea
MWCNTs
r-DSPE
Pesticide residues

ABSTRACT

A rapid and sensitive method for the determination of 171 pesticides in cowpea was developed using multi-walled carbon nanotubes (MWCNTs) as reversed-dispersive solid-phase (r-DSPE) extraction materials. The clean-up performance of MWCNTs was proved to be obviously superior to PSA and GCB. This method was validated on cowpea spiked at 0.01 and 0.1 mg kg^{-1} with five replicates. The mean recoveries for 169 pesticides ranged from 74% to 129% with relative standard deviations (RSDs) (n = 5) lower than 16.4%, except diflufenican and quizalofop-ethyl. Good linearity for all pesticides was obtained with the calibration curve coefficients (R^2) larger than 0.9970. The limit of detection (LODs) and limit of quantification (LOQs) for the 171 pesticides ranged from 0.001 to 0.003 mg kg^{-1} and from 0.002 to 0.009 mg kg^{-1}, respectively. The method was demonstrated to be reliable and sensitive for the routine monitoring of the 171 pesticides in cowpea samples.

1. Introduction

Cowpea (*Vigna unguiculata*) is one of the most important legume crops in China, with a total planting area of 330,000 ha [1]. Unfortunately, cowpea is highly susceptible to weeds, pests and diseases infestation during the growth in the field [2]. To improve the quality and quantity, pesticides are widely used by farmers [3]. However, the extensively use of pesticides often induce pesticide residues in cowpea, thereby causing hazard to human health [4,5]. Some literatures have reported the pesticides residues in cowpea and other vegetables [1,6–8]. Hence, monitoring the pesticide residues in cowpea is of great significance to human health.

QuEChERS (quick, easy, cheap, effective, rugged, and safe) method has attracted the attention of pesticide residue analysis studies worldwide since it was first published by **Anastassiades, Lehotay, Stajnbaher and Schenck** [9]. The original approach consists of extracting with acetonitrile, partitioning between the aqueous and acetonitrile phase through adding magnesium sulfate and sodium chloride, and a d-SPE cleanup step by primary secondary amine (PSA) and anhydrous magnesium sulfate. Generally, PSA was used as the r-DSPE sorbent to remove polar pigments, polar organic acids, fatty acids, and some sugars [10]. However, the clean-up performance is not always satisfactory, especially for pigments [11]. Graphitized carbon black (GCB) is also applied in modified QuEChERS method to remove pigments, but it can adsorb planar pesticides [12,13]. Thus, study of new clean up method is important for pesticide residue analysis.

Carbon nanotubes (CNTs) are interesting and novel carbonaceous materials reported by **Iijima** [14]. It was divided into single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) according to the principle of carbon atom layers in the wall of nanotubes [15]. Due to its huge surface area, MWCNTs has been reported to be applied in the analysis of pesticides to adsorb the interfering substances in the fruit and vegetable [16–19]. Moreover, MWCNTs has also been reported to have a good clean-up performance for complex matrices, e.g. tea, onion, leek, garlic and ginger [11,20,21].

This study emphatically studied the multi-residue method for the determination of 171 pesticides in cowpea with MWCNTs as r-DSPE cleanup sorbents. The 171 pesticides were selected based on the registration and the routine monitoring in cowpea. PSA and GCB were used as comparison.

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http://dx.doi.org/10.1016/j.jchromb.2016.07.043
1570-0232/

2. Materials and methods

2.1. Materials and reagents

Pesticides standards (purity for all standards ≥95.0%) were acquired from the National Institute of Metrology (Beijing, China). Standard stock solutions of 10 mg L^{-1} of mixed pesticides were prepared in acetonitrile and stored at −20 °C. The working solutions were diluted daily. Sodium chloride (NaCl) and anhydrous magnesium sulfate ($MgSO_4$) were of analytical reagent grade and purchased from Sinopharm Chemical Reagent (Beijing, China). Acetonitrile for HPLC grade were obtained from Fisher Chemicals (New Jersey, USA). MWCNTs (5–10 nm), Graphitized Carbon Black (GCB, 40 μm), Primary Secondary Amine (PSA, 40 μm) were purchased from Tianjin Bonna-Agela Technologies Co., Ltd. (Tianjin, China). Cowpea samples were collected from the supermarket and vegetable base of Beijing.

2.2. GC–MS/MS analytical conditions

The analysis was performed using the Thermo Scientific TSQ 8000 EVO triple quadrupole mass spectrometer coupled with a Trace 1300 gas chromatograph and a TriPlus AI 1310 autosampler (Thermo Fisher Scientific, San Jose, CA). The analytical column was TR-Pesticide column, 30 m × 0.25 mm i.d., 0.25 μm film thickness (Thermo Fisher Scientific, Runcorn, UK). The temperature programmed as follows: 80 °C hold for 1 min, ramp to 150 °C at 30 °C min^{-1}, then increase to 210 °C at 3 °C min^{-1}, finally ramp to 290 °C at 10 °C min^{-1} and hold for 12 min. The total run time was 42.33 min. The MS transfer line temperature and ion source temperature were both set at 280 °C. The inlet temperature was set at 250 °C. A volume of 1 μL extraction was injected in splitless mode with a split flow of 50 mL min^{-1} and a splitless time of 1.0 min. Helium gas with a constant flow of 1.2 mL min^{-1} was used as carrier gas. Argon gas with the pressure of 1.5 mTorr was chosen as collision gas. The triple quadrupole operated in the electron ionization mode with the electron energy of 70 eV and emission current of 25 μA. The scheduled selected reaction monitoring mode was used as acquisition mode with the cycle time of 0.2 s. The product ion and collision energy (CE) were optimized for each compound listed in Table 1.

2.3. Sample preparations

An amount of 10 g homogenized cowpea samples were weighed into a 50 mL centrifuge tube. After the addition of 10 mL acetonitrile, the mixture was shaken for 2 min on a VX-III Multi-Tube Vortexer (Beijing Targin Technology, China). Then 1 g of NaCl and 4 g of anhydrous $MgSO_4$ were added and shaken for 1 min. Following, the centrifuge tube was centrifuged for 5 min at 3800 rpm. An aliquot of 1 mL acetonitrile portion was transferred to a new centrifuge tube containing different cleanup sorbents plus 150 mg anhydrous $MgSO_4$. Then the mixture was shaken for 1 min and centrifuged for 3 min at 10000 rpm. Finally, an aliquot of 1 mL supernatant was filtered through a 0.22 μm Nylon syringe filters (Agela Technologies, China) into an autosampler vial for analysis.

2.4. Method performances

The method was validated through the following parameters: linearity, limit of detection (LODs), limit of quantification (LOQs), accuracy and precision, and matrix effect. Linearity was studied by applying matrix-matched calibration through analyzing cowpea samples. The LODs and LOQs for each pesticide were calculated by the lowest concentration that produced a signal-to-noise (S/N) ratio of 3 and 10, respectively. Recovery assays were carried out to study the accuracy and precision of the method with five replicates spiked at two levels (0.01 and 0.1 mg kg^{-1}). Matrix effects were estimated via comparing the calibration curves slopes of matrix and solvent.

3. Results and discussion

3.1. Optimization of clean-up procedure

A good clean-up procedure is indispensable for the pesticide residue analysis in vegetables [17], especially for cowpea which contains plenty of pigments. Generally, PSA (usually 50 mg/mL) is often used in the original QuEChERS method with the purpose of absorbing the polar pigments and some sugars in fruits and vegetables [10]. However, the clean-up effect of PSA was not satisfactory to remove pigments in cowpea matrix, as indicated in Fig. 1. GCB is also proposed by Lehotay et al. for the purification of pigments in vegetables [13], but it can adsorb the planar pesticides such as thiabendazole and hexachlorobenzene [22]. Due to its unique structure, MWCNTs has been used as an alternative absorbent for removing pigments in our previous study [23]. In this study, the comparison for the clean-up efficiency of PSA (25 mg and 50 mg), GCB (5 mg and 10 mg) and MWCNTs (5 mg and 10 mg) was evaluated. As shown in Fig. 1, the clean-up efficiency became better with the increase of the amount of each absorbent. The final cowpea sample purified by PSA had deeper color which was almost the same as no clean-up, and the sample purified by GCB was a little better than PSA. All in all, the clean-up effect of MWCNTs was the best which was almost no color.

Moreover, the clean-up efficiency was also estimated by the recovery assays at 0.1 mg kg^{-1} spiked level. As presented in Table S1, recoveries of 95 pesticides were in the acceptable range (70–120%) when 10 mg GCB was applied in sample purification and 76 pesticides were lower than 70% which was worse than 5 mg GCB and other clean-up sorbents. This might be due to the reason that GCB could adsorb the target compounds when the dosage is too large [11]. As shown in Fig. 2, recoveries of 167 pesticides were in the acceptable range when 5 mg MWCNTs was used which was better than 10 mg MWCNTs and other clean-up sorbents. Taking account of the cleanup performance as previously described, 5 mg MWCNTs was the optimized amounts for the d-SPE cleanup of cowpea extract.

3.2. Matrix effect

Due to the presence of co-elution of the matrix, the ionization of the target compounds may be interfered, reflecting as ion suppression or enhancement [24]. To investigate the matrix effects, the matrix-matched calibration slopes were compared with the solvent-based calibration curves slopes. In this study, the matrix effect could be ignored if the slope ratios of matrix and solvent were between 0.9 and 1.1, while it could be perceived as matrix enhancement effect with the values larger than 1.1, and it would be considered as matrix suppression effect with the values lower than 0.9. The slope ratios of matrix and solvent for the 171 pesticides in cowpea were summarized in Table S2. A total of 91 (53.2%) pesticides were perceived as no matrix effects when 5 mg MWCNTs was used to clean up the cowpea matrix, which was obviously larger than other sorbents. 5 mg GCB and 25 mg PSA were deemed to have stronger enhancement effect with the values of 122 (71.3%) and 100 (58.5%) pesticides larger than 1.1 respectively. As shown in Fig. 3,5 (2.9%) pesticides were regarded as matrix suppression effect when 5 mg MWCNTs was used which was the lowest. This suggested that the cleanup performance of 5 mg MWCNTs was the best which was in accordance with the previously study.