Analytical Methods

A multi-residue method for the determination of pesticides in tea using multi-walled carbon nanotubes as a dispersive solid phase extraction absorbent

Xue Hou [a,b,c,]*, ShaoRong Lei [a], ShiTing Qiu [a], LingAn Guo [a], ShengGuo Yi [a], Wei Liu [a]

[a] Center of Analysis and Testing, Sichuan Academy of Agricultural Sciences, Chengdu 610066, PR China
[b] Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences, Chengdu 610041, PR China
[c] University of Chinese Academy of Sciences, Beijing 100039, PR China

ABSTRACT

A modified QuEChERS (quick, easy, cheap, effective, rugged and safe) method using multi-walled carbon nanotubes (MWCNTs) as a dispersive solid phase extraction (*d*-SPE) absorbent was established for analysis of 78 pesticide residues in tea. A 6 mg MWCNT sample was selected as the optimised amount based on the distribution of pesticide recoveries and clean-up efficiency from 6 mL acetonitrile extracts. The matrix effects of the method were evaluated and matrix-matched calibration was recommended. The method was validated employing gas chromatography coupled to tandem mass spectrometry (GC–MS/MS) at the spiked concentration levels of 0.05, 0.1 and 0.15 mg kg^{-1}. For most of the targeted pesticides, the percent recoveries range from 70% to 120%, with relative standard deviations (RSDs) <20%. The linear correlation coefficients (r^2) were higher than 0.99 at concentration levels of 0.025–0.500 mg kg^{-1}. In this study, MWCNTs were proved to be a promising *d*-SPE absorbent with excellent cleanup efficiency, which could be widely applied for the analysis of pesticide residues.

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1. Introduction

Plantation-grown teas are sensitive to a variety of plant diseases and pests, such as leafhoppers, aphids, as well as tea cakes disease and tea tree charcoal (Chen, Cao, & Liu, 2011). Efforts to combat these problems have resulted in extensive usage of hazardous pesticides which have been proven to have negative effects on human health (Dejonckheere, Steurbaut, Drieghe, Verstraeten, & Braeckman, 1996; Kamel & Hoppin, 2004; Sood, Jaggi, Kumar, Ravindranath, & Shankar, 2004). This issue has received great attentions from the public as well as the government. Recently, maximum residues limits (MRLs) for 448 pesticide residues in tea have been established in the EU (Regulation (EC), 2005).

QuEChERS (quick, easy, cheap, effective, rugged and safe) was first introduced by Anastassiades, Maštovská, and Lehotay (2003), Anastassiades, Lehotay, Stajnbaher, and Schenck (2003). Due to high extraction efficiency, swift deployment time and low cost, QuEChERS has been widely applied for the analysis of pesticide residues in various matrixes, including vegetables, fruits and grains. Tea is known as a troublesome matrix; the co-extracts may interfere with the testing process and deteriorate the instrument performance. Mostly, dispersive solid phase (*d*-SPE) absorbents were applied to the analysis of pesticide residues in tea, such as primary secondary amine (PSA), octadecyl (C_{18}) and graphitized carbon black (GCB) (Cajka et al., 2012; Steiniger, Lu, Butler, Phillips, & Fintschenko, 2010; Xu et al., 2011; Zhang et al., 2010).

Carbon nanotubes (CNTs) were first introduced by Iijiama (1991) as a type of carbonaceous material in 1991. CNTs are divided into two classifications: single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) (Ebbesen et al., 1996; Kumar, Ramesh, Lin, & Fey, 2004).

Due to their large surface area and unique structure (Pan et al., 2005), CNTs present amazing absorption capability. As an effective solid-phase extraction adsorbent, CNTs have been primarily used to remove trace chemical compounds in the environment, such as dioxins (Cai, Jiang, & Liu, 2003), polycyclic aromatic hydrocarbons (Wang, Huang, Shu, & Cao, 2007) and pesticides from water (Basheer, Alnedhary, Rao, Valliyaveettil, & Lee, 2006; Wang, Zhao, Min, & Fang, 2007).

Currently, CNTs have been successfully applied to the pretreatment procedure of troublesome matrices containing unknown pesticides. López-Feria, Cárdenasb, and Valcárcel (2009) introduced a solid-phase extraction (SPE) method for multiclass pesticide analysis in olive oils. Du et al. (2008) developed a method using CNTs to analyse organophosphate pesticides in garlic. Ravelo-Péreza,

* Corresponding author at: Center of Analysis and Testing, Sichuan Academy of Agricultural Sciences, Chengdu 610066, PR China. Tel.: +86 28 84504141; fax: +86 28 84791119.

E-mail address: houxue127@163.com (X. Hou).

Hernández-Borges, and Rodríguez-Delgadoa (2008) developed a method involving SPE and direct gas chromatography with nitrogen phosphorus detection analysis. Using this method, eight organophosphorus pesticides in different fruit juices (i.e. apple, grape, orange and pineapple) were detected. Recently, Pan et al. reported a method of pesticide residues determination by liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS), in which MWCNTs were used as an alternative absorbent (Zhao, Wang, Jiang, Zhang, & Pan, 2012). Generally, due to the existence of concentric layers of grapheme, MWCNTs offer better sorption capabilities than SWCNTs (López-Feria, Cárdenasb, & Valcárcel, 2009; Pyrzynska, 2011).

This paper presents a rapid, multi-residue method based on a modified QuEChERS extraction method using MWCNTs as a *d*-SPE extraction absorbent for tea samples. 78 pesticides were simultaneously identified in tea samples by GC/MS/MS.

2. Experiment-materials and methods

2.1. Chemicals and reagents

HPLC-grade acetonitrile, acetone and *n*-hexane were obtained from Tedia (Fairfield, OH, USA). PSA was obtained from Dikma (Beijing, China). Analytical grade anhydrous magnesium sulphate ($MgSO_4$) was heated at 650 °C for 4 h and kept in a desiccator. Prepacked QuEChERS extraction bags with 4 g $MgSO_4$, 1 g sodium chloride (NaCl), 1 g tri-sodium citrate dehydrate (tri-Na) and disodium hydrogencitrate sesquihydrate (di-Na) were purchased from Agilent (Palo Alto, CA, USA). Standard pesticides (Table 1) with purities ranging from 95% to 99% were purchased from Sigma–Aldrich (Madrid, Spain) and Dr. Ehrenstorfer (Augsburg, Germany). 97% pure Chlophrifos-methyl D_6 was obtained from Dr. Ehrenstorfer (Augsburg, Germany) and prepared in acetone for use as an internal standard (IS). Stock solutions of mixture pesticides were prepared in acetone stored in freezer (−18 °C). The working solutions were prepared daily.

MWCNTs with outer diameters of 20–30 nm, 10–30 μm length were purchased from Chengdu Organic Chemicals Co. Ltd. (Chengdu, China), and were dried for 2 h at 130 °C and stored in a desiccator to remove the absorbed water.

2.2. Equipment

A Vortex (IKA, Germany), a centrifuge (Xingke, China), and a nitrogen evaporator (Organomation Associates, USA) were used for preparing the samples.

GC–MS/MS system (Varian, Inc., USA) was employed, consisting of a Varian 450 gas chromatograph (equipped with a CP 8400 autosampler and a 1079 injector) and a 320 triple quadrupole MS. A capillary fused silica column VF-5 MS (60 m × 0.25 mm i.d. × 0.25 μm, Agilent, Inc., USA) was applied in this method. Programmed temperature vaporation (PTV) was employed as the injection mode. The injector temperature program was the following: initial temperature was held at 60 °C for 0.8 min, increased to 260 °C at the rate of 200 °C min^{-1} and was held for 10 min. Aliquots of sample extract (5 μL) were injected. The GC oven was operated with the following temperature program: initial temperature was held at 50 °C for 3 min, increased to 150 °C at the rate of 25 °C min^{-1}, and then to 210 °C at the rate of 3 °C min^{-1}, finally to 280 °C at the rate of 8 °C min^{-1}, and held for 20 min. Helium was used as carrier gas at a constant flow of 1.2 mL min^{-1}.

Mass spectrometry was performed in electron impact (EI) ionisation mode at 70 eV. The ion and transfer line temperatures were set at 230 and 280 °C respectively.

The analyses were carried out with a solvent delay of 6 min. The multiple reaction monitoring (MRM) mode was employed. For each pesticide, one quantitative ion transition and one qualitative ion transition were selected. All the parameters for MRM transitions and collision energy were optimised in order to obtain highest sensitivity and resolution (Table 1).

2.3. Sample preparation

Tea sample was grinded and sieved through prescription sieve (0.425 mm aperture size). A thoroughly homogenised tea powder (2 g) was weighed and transferred into a 50 mL centrifuge tube. 10 mL water was added, and after standing for 30 min, 10 mL acetonitrile and 100 μL of the 2.0 mg L^{-1} IS was added. The solution was then vortexed for 1 min. QuEChERS extraction bag with 4 g anhydrous $MgSO_4$ and 1 g NaCl was added, and the tube was vortexed immediately for 1 min. After centrifugation (6000 rpm, 10 min, −10 °C), 6 mL of the upper acetonitrile layer was transferred into a 10 mL centrifuge tube containing 6 mg MWCNTs, 150 mg PSA and 750 mg anhydrous $MgSO_4$. The tube was vortexed for 1 min and centrifuged for 20 min at 6000 rpm at −10 °C. 1 mL extract was transferred into a GC vial, and reduced to nearly dryness by evaporating with a weak nitrogen stream at 40 °C. Finally, the residue was redissolved with 0.5 mL mixture of *n*-hexane and acetone (9 + 1; v/v) for GC–MS/MS analysis.

2.4. Gravimetric determination of co-extracts

5 mL of crude MeCN extracts and 5 mL of MeCN extracts purified through *d*-SPE procedure were evaporated until dryness with a weak nitrogen stream, and the remaining co-extracts were gravimetrically determined by analytical balance. Average amounts of 3 replicates were evaluated, the error bars (standard deviations) and RSDs of the weights for each group were also showed in Fig. 1.

2.5. Method validation procedure

Blank tea samples were selected for validation purposes. Different validation parameters were evaluated, including recovery, precision, linear range, and limit of quantification (LOQ). Linearity was studied by evaluating the matrix-matched standard calibration curves for all targeted pesticides. Calibration curves were calculated with standards in blank tea samples at the concentrations of 0.025, 0.050, 0.100, 0.200, and 0.500 mg kg^{-1} (corresponding to 0.010, 0.020, 0.040, 0.080, and 0.200 mg L^{-1}). Linear calibration curves were constructed by plotting the targeted pesticide/IS peak areas ratio obtained against the concentration values. The recovery and repeatability of the method were evaluated by carrying out six consecutive extractions (*n* = 6) of spiked tea at the three concentration levels (0.05, 0.1 and 0.15 mg kg^{-1}) with 78 targeted pesticides. The values were calculated using matrix-matched calibration standards.

3. Results and discussion

3.1. Optimisation of the amount of the MWCNTs

The large surface area and unique structure of the MWCNTs provided them with extraordinary absorption capability during this study. The amount of MWCNTs used during sample preparation process may have had a direct influence on the recoveries of targeted pesticides and the cleanup efficiency of co-extracts.

Our results suggest that excessive amounts of MWCNTs may have absorbed targeted pesticides, resulting in lower recoveries. Therefore, different amounts of MWCNTs were tested in the hope