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Analytical Methods

Development of a matrix solid-phase dispersion-sonication extraction method for the determination of fungicides residues in ginseng extract

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ABSTRACT

A rapid method based on matrix solid-phase dispersion (MSPD) was developed for the determination of pentachloronitrobenzene, pentachloroaniline, methylpentachloro-phenylsulphide and procymidone in ginseng extract using gas chromatography. The optimal conditions selected for MSPD extraction were as follows: after blending 5 mL of aqueous ginseng extract (10%, w/v) with Florisil (10 g), the mixture was passed into a small chromatographic column and extracted twice with 10 mL ethyl acetate–hexane solvent mixture (70:30, v/v) for 15 min in an ultrasonic bath at room temperature. The analytical performance of this method showed MSPD to be efficient, fast, simple and had little or no matrix effect. The method detection limits varied from 0.1 to 0.4 μg/L. Mean recoveries were found in the range of 83.5–97.4% and had a good linear relationship ($r^2 \geqslant 0.9987$) with relative standard deviations less than 10%. The proposed method has proved to be a feasible one for the analysis of fungicide residues in ginseng extract.

1. Introduction

Any of several plants of the genus *Panax*, especially *Panax pseudoginseng* of eastern Asia or *Panax quinquefolius* of North America, having small greenish flowers grouped in umbels, palmately compound leaves, and forked roots believed to have medicinal properties. Ginseng, the root of Panax Ginseng C.A. Meyer, is one of the most common herbal medicines in China (Attele, Wu, & Yuan, 1999; Gillis, 1997). It has been shown to exhibit both anti-stress and antioxidant activity and to exert various benefits relating to stress and the immune system (Kim, Kim, Kim, Hu, & Rhe, 1970; Simsek, Karadeniz, & Karaca, 2007; Takahashi, Tokuyama, & Kaneto, 1992).

This plant of ginseng requires a longer growing time, and is highly susceptible to phyto-pathogen. Therefore, the ginseng employed often pesticides, such as pentachloronitrobenzene and procymidone, in the growing process. Being the longer half-life of these pesticides, and chronic adverse as well as bioaccumulation and genotoxic effect in biotic fat, the determination of pesticides residue is very important in ginseng products and its extracts (Ling, Teng, & Cartwright, 1999; Manirakiza, Covaci, & Schepens, 2000).

Scientific knowledge about chemical contamination of food has grown considerably in recent years. Until 15 years ago, this science was considered relatively young. Since then, this area of science has continued to develop, in particular becoming an established part of regulatory reviews of food safety across the world.

Furthermore, improper use of pesticides not only pollutes the cultivating soil, and ground water, but also leads to accumulation of pesticides in the plants. Therefore, from a healthcare perspective, safety issues considering the balance between adverse effects and therapeutic benefit must be dealt with, the need to extract and analyse residual contaminants in ginseng and its extract is an apparent problem (Ling et al., 1999; Nerdìn, Battle, Sartaguda, & Pedrochì, 2002).

Procymidone, a dicarboximide fungicide, has been used in ginseng cultivation and residue is distinctively present in ginseng extract (Li, Kim, & Lee, 2007). Procymidone is found to act as androgen receptor and shows xenoendocrine disrupting properties in rats (Gray, 1998; Gray et al., 1999) and monkeys (Ostby et al., 1999). It was stated that it was very likely that humans would adversely be affected if the human foetus were exposed to sufficient levels during critical stages of neonatal life. Pentachloronitrobenzene, sometimes referred to as quintozene, is a commonly applied fungicidal treatment for control of a garden rot that affects many plants during cool and wet months. It has been widely used to protect ginseng crops. Pentachloroaniline and methylpentachlorophenylsulphide are two main metabolism of pentachloronitrobenzene, and have been found residues in some vegetables (Lehotay & Ibrahim, 1995). Due to the toxicity, the use of these fungicides is strictly restricted or forbidden. Maximum Residue Limits (MRLs) of procymidone and pentachloronitrobenzene had been established in many countries for the ginseng, were not more than

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0.1 mg kg^{-1}, but no limits for pentachloroaniline and methylpentachloro-phenylsulphide in ginseng extract yet. Moreover, the analytical methods for the determination of pentachloroaniline and methylpentachloro-phenylsulphide residues in ginseng and its extracts have not been proposed.

Generally, pesticide analyses are carried out by gas chromatography (GC) or liquid chromatography (LC). These analytical techniques generally require critical steps such as liquid–liquid solvent extraction and series of cleanup procedures for sample preparation. Proper sampling largely determines the validity of an analytical sample for residue analysis. Although many suitable methods for the extraction of pesticides prior to the chromatographic separation have been developed, such as liquid–liquid extraction (LLE) (Fernández, Rodríguez, Picó, & Mañes, 2001; Navarro, Barba, Navarro, Vela, & Oliva, 2000; Sala, Busto, & Guasch, 1997; Strandberg & Ronald, 2001), solid-phase extraction (SPE) (Jiménez, Bernal, Nozal, Toribio, & Arias, 2001; Nozal, Bernal, Jiménez, Martín, & Bernal, 2005; Wong et al., 2003; Young, Phillips, Iraneta, & Krol, 2001), solid-phase microextraction (SPME) (Carlo, Maurizio, Nicoletta, & Francesco, 2004; Hwang & Lee, 2000; Zuin, Lopes, Yariwake, & Augusto, 2004), stir bar sorptive extraction (SBSE) (Jiménez et al., 2001; Nozal et al., 2005; Wong et al., 2003; Young et al., 2001) and micro-porous membrane liquid–liquid extraction (MMLLE) (Hyötyläinen, Tuutijärvi, Kuosmanen, & Riekkola, 2002). Most routine methods used in pesticide residue analysis are often time and solvent consuming due to the steps involved in sample preparation before chromatographic analysis. The ginseng extract matrices had the complex nature in which the pesticide residues and other compounds such as saponins were present, and required a large sample (10–25 g). One alternative that simplifies the preparation of samples is matrix solid-phase dispersion (MSPD). This technique, based on the dispersion of the sample on an adsorbent, such as Florisil, C18, alumina, or silica, integrates sampling, extraction, and pre-concentration into a simple, single-step procedure. The dispersion of solid samples is previously done in a mortar, and then the mixture is transferred to the extraction columns (Kadenczki et al., 1992; Kristenson, Ramos, & Brinkman, 2006). In the case of liquid samples, the dispersion of the matrix in the adsorbent can be done directly in the extraction columns (Tadeo & Sánchez, 2003; Albero, Sánchez, & Tadeo, 2003b). MSPD has many advantages over the traditional techniques, such as the use of smaller amounts of organic solvent, lower solvent cost and reduced toxic organic solvent. At present, MSPD has been successfully applied to the extraction of a wide range of drugs, pesticides, naturally occurring constituents, and other compounds from a wide variety of complex plant and animal samples (Barker, 2007; Bogialli & Di Corcia, 2007; Kristenson et al., 2006).

To the best of our knowledge, only a method using a Keele injector for GC–ECD or GC–MS analysis of procymidone residues in ginseng without performing solvent extraction and clean up procedures was reported (Li et al., 2007). In addition, in spite of the great number of MSPD publications, well-described and validated MSPD methods for the extraction of the most used fungicides applied to ginseng are scarce. The aim of this work was to develop a rapid MSPD method at low levels for the determination of procymidone, pentachloronitrobenzene, pentachloroaniline and methylpentachloro-phenylsulphide residues in ginseng extract.

2. Materials and methods

2.1. Chemicals and materials

Pentachloronitrobenzene, pentachloroaniline, methylpentachloro-phenylsulphide and procymidone standards (98.85–99.2% purity) were purchased from Riedel-de Häen (Steinheim, Germany). Stock solutions (500 μg/mL) of each fungicide were prepared by dissolving 50 mg of the fungicide in 100 mL acetone and stored at 4 °C. Standard solutions containing 1.0, 0.5, 0.1, 0.05 and 0.01 μg/mL of each fungicide in acetone were used as chromatographic standards.

Ethyl acetate, acetone, dichloromethane and *n*-hexane (pesticide grade) were obtained from Sinopharm (Shanghai, China).

Florisil 60–100 mesh, heated at 650 °C for 4 h before use, was purchased from Fluka (Buchs, Switzerland). Anhydrous sodium sulphate, diatomaceous earth and aluminium oxide 90 standardised were from Merck (Darmstadt, Germany).

Glass columns (10 cm × 20 mm I.D.) were purchased from Pobel (Madrid, Spain) and Whatman No. 1 filter paper circles of 2 cm diameter placed at the bottom end, were from Whatman (Maidstone, UK).

An ultrasonic water bath (KQ3200B, KunShan, China) was used in the extraction procedure. The generator of this apparatus has an output of 150 W and a frequency of 40 kHz. A 12-port vacuum manifold from Supelco (Bellefonte, PA, USA) was employed.

2.2. Samples

Various commercial brands of ginseng extract were purchased in China market.

2.3. Sample preparation

The sample preparation is based on MSPD which a previously published method for the determination of insecticide, fungicide and herbicide residues in fruit juices (Albero, Sánchez, Donoso, & Tadeo, 2004; Albero, Sánchez, & Tadeo, 2003a; Albero et al., 2003b; Tadeo & Sánchez, 2003).

A 5 mL (1 g ginseng extract dissolved in 10 mL water) of aqueous ginseng extract was blended with Florisil (10 g) and anhydrous Na_2SO_4 (200 mg) in a mortar, and the mixture was passed into a small chromatographic column which Whatman No. 1 filters were previously placed at the bottom end. An additional amount of Florisil (1 g) was added to enhance the dispersion of the aqueous ginseng extract sample in the matrix. The samples were fortified when required with 0.5 mL of the fungicide standard mixture in acetone, while 0.5 mL of acetone was added in non-fortified samples. The use of acetone was to improve the sample distribution throughout the column. Then the columns were placed in a tube rack and closed with one-way stopcocks. Aqueous ginseng extract samples were extracted twice with 10 mL ethyl acetate–hexane (70:30, v/v) for 15 min in an ultrasonic bath (KQ3200B, KunShan, China) at room temperature, and water level in the bath was adjusted to solvent level inside the columns. After extracted in an ultrasonic bath, columns were placed in a vacuum manifold and the eluting solution was collected in 25 mL graduated glass tubes and then concentrated to 1 mL with a gentle stream of nitrogen. The concentrated extracts were diluted to 10 mL with acetone for the highest fortification level (0.5 μg/mL) and to 5 mL or 2 mL for the other two levels (0.1 and 0.05 μg/mL, respectively). And extracts of commercial ginseng extract samples were diluted to 2 mL.

2.4. GC–ECD analysis

An Agilent 6890 gas chromatograph (Little Falls, DE, USA) equipped with an automatic split-splitless injector Model HP 7683 and a ^{63}Ni micro-electron capture detector (μECD) was used. A fused silica capillary column, HP-5, with 5% phenyl methyl siloxane as nonpolar stationary phase (30 m × 0.32 mm I.D., 0.25 μm film thickness) supplied by Agilent (Little Falls, DE, USA) was employed. Operating conditions were as follows: injection port and detector temperatures were 280 and 300 °C, respectively, with