Analytical Methods

Gas chromatography with flame photometric detection of 31 organophosphorus pesticide residues in *Alpinia oxyphylla* dried fruits

Xiangsheng Zhao [a,b], Weijun Kong [a], Jianhe Wei [a,b], Meihua Yang [a,b,*]

[a] *Institute of Medicinal Plant Development, Chinese Academy of Medicinal Science & Peking Union Medical College, Beijing 100193, China*
[b] *Hainan Branch Institute of Medicinal Plant Development, Chinese Academy of Medicinal Sciences & Peking Union Medical College, Wanning 571533, China*

ABSTRACT

A simple, rapid and effective gas chromatography–flame photometric detection method was established for simultaneous multi-component determination of 31 organophosphorus pesticides (OPPs) residues in *Alpinia oxyphylla*, which is widely consumed as a traditional medicine and food in China. Sample preparation was completed in a single step without any clean-up procedure. All pesticides expressed good linear relationships between 0.004 and 1.0 μg/mL with correlation coefficients higher than 0.9973. The method gave satisfactory recoveries for most pesticides. The limits of detection varied from 1 to 10 ng/mL, and the limits of quantification (LOQs) were between 4 and 30 ng/mL. The proposed method was successfully applied to 55 commercial samples purchased from five different areas. Five pesticide residues were detected in four (7.27%) samples. The positive samples were confirmed by gas chromatography with tandem mass spectrometry (GC–MS/MS).

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1. Introduction

Alpinia oxyphylla (Zingiberaceae) is a perennial herb that is widely cultivated in southern China. The fruit of this herb is used as a traditional Chinese medicine (Chinese Pharmacopeia Commission, 2010), and is also widely consumed as a health food. Many compounds, including sesquiterpenes, diterpenes, flavonoids, and diarylheptanoids, have been isolated from *A. oxyphylla* (Morikawa, Matsuda, Toguchida, Ueda, & Yoshikawa, 2002; Muraoka et al., 2001; Xie, Sun, Wang, & Ito, 2009; Xu, Tan, Zeng, Han, & Peng, 2010; Xu et al., 2009). Some of these compounds showed insecticidal activity (Miyazawa, Nakamura, & Ishikawa, 2000), significant neuroprotective activity (Guan, Bao, Jiang, & An, 2006), and inhibition of nitric oxide production in lipopolysaccharide-activated mouse peritoneal macrophages (Morikawa et al., 2002; Muraoka et al., 2001). *A. oxyphylla* takes 2–3 years to reach maturity, and during this period it can accumulate contaminants from the environment or the pesticides directly applied. Therefore, there is a risk of contamination from agricultural chemicals.

Organophosphorus pesticides (OPPs) are widely used in agriculture because they are inexpensive and effectively control crop pests and diseases. The output of pesticides in China has appeared to increase steadily in recent years, and the amount of OPPs used accounted for more than 70% of the total pesticides used between 1980 and 2000 (Jin, Wang, Shao, & Jin, 2010). While pesticides increase agricultural production, bioaccumulation through the food chain can eventually become a risk to mammals because the majority of those pesticides act as acetylcholinesterase inhibitors (Banks & Lein, 2012). Public health concerns around improper use of pesticides and poisoning have increased in recent years. To date, some countries, regions, and international organizations have established maximum residue limits (MRLs) for foodstuffs and medicinal plant materials. The Codex Alimentarius Commission of the United Nation's Food and Agriculture Organization and the World Health Organization have established international standards for foods and spices (European Pharmacopoeia, 2010; Kosalec, Cvek, & Tomić, 2009; World Health Organization (WHO), 2007). The United States Pharmacopoeia and European Pharmacopoeia (EP) have established MRLs for medicinal plant materials (e.g., 0.1 mg/kg for chlorpyrifos-methyl, 2.0 mg/kg for ethion) (Kosalec et al., 2009; World Health Organization (WHO), 2007). However, to date, a MRL has not been set for *A. oxyphylla* in China, and there are few reports on chemical contamination of this plant. The amount of pesticides residues in dried fruit is relative higher than that in fresh fruit, and more easily detected (Athanasopoulos, Pappas, Kyriakidis, & Thanos, 2005). There is only one report on organochlorine pesticide residues in *A. oxyphylla* (Zhang, Lai, Fu, Liu, & Liu, 2004). Therefore, for consumer health and safety, it is very important to

* Corresponding author at: Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100193, China. Tel.: +86 10 57833277; fax: +86 10 62896288.
   E-mail address: yangmeihua15@hotmail.com (M. Yang).

establish a fast, accurate and sensitive analytical method for the analysis of multiple OPPs in *A. oxyphylla* dried fruits.

Gas chromatography (GC) method is commonly used for OPP analysis and has been coupled with different detection methods, such as flame photometric detection (FPD) (Wong et al., 2007; Bidari, Ganjali, Norouzi, Hosseini, & Assadi, 2011; Wong et al., 2007), nitrogen–phosphorous detection (García de Llasera & Reyes-Reyes, 2009), and mass spectrometry (MS) (Dai, Ren, He, & Huo, 2011; Wong et al., 2007).

Prior to the analysis, the sample preparation a crucial step in the analysis of trace compounds. Commonly used sample preparation methods include liquid–liquid extraction and solid-phase extraction, and these require large volumes of organic solvents. Analysis for solid matrices requires several steps, such as extraction, isolation, clean-up, and enrichment. These steps can greatly influence the reliability of the method. Analytical technology developments have led to new techniques with fewer steps, low-time requirements, and use of lower amounts of solvents. These methods include matrix solid-phase dispersion (MSPD) (García de Llasera & Reyes-Reyes, 2009; Rodrigues, Caldas, & Primel 2010), solid-phase micro-extraction (SPME) (Cai et al., 2006; Bidari et al., 2011), stir-bar sorptive extraction (SBSE) (Ochiai et al., 2005), Quick, Effective, Cheap, Effective, Rugged and Safe method (QuEChERS) (Anastassiades, Lehotay, Stajnbaher, & Schenck, 2003), liquid-phase microextractions (LPME) (Jeannot & Cantwell, 1996), dispersive liquid–liquid microextraction (DLLME) (Ho, Tsoi, & Leung, 2013). The main pitfalls of sorbent-based extraction methods (MSPD, SPME, SBSE) are the lack of flexibility, or the unideal coating selectivity and reproducibility for complex matrices (Wei, Ge, & Lv, 2009). While the solvent-based extraction methods, such as QuEChERS, LPME, DLLME, increased the amount of organic solvents used and the sample-pretreatment procedure.

In the present study, a multi-residue GC–FPD method was developed for the analysis of 31 organophosphorus pesticides (OPPs) residues in 55 commercial dry *A. oxyphylla* fruit samples purchased from five different regions in China (Hainan, Guangdong, Guangxi, Yunnan, and Beijing). This method was simple and fast with using a low volume of organic solvent, and all the sample preparation procedures were completed in one step. No significant interference was encountered in this matrix, and the positive reports were further confirmed by gas chromatography with tandem mass spectrometry (GC–MS/MS). To the best of our knowledge, this is the first report of simultaneous determination of OPPs in *A. oxyphylla* dried fruits.

2. Materials and methods

2.1. Materials and standards

All chemical reagents used were of residue analysis or HPLC grade. Dichloromethane (DCM), *n*-hexane, toluene, and ethyl acetate (EA) for residue analysis were purchased from Sinopharm Chemical Reagent Co., Ltd (Beijing, China), while HPLC grade acetone and acetonitrile (MeCN) were obtained from Sigma–Aldrich (St. Louis, MO, USA).

Opps (>98% purity), including methamidophos, trichlorfon, dichlorvos, acephate, formothion, omethoate, monocrotophos, phorate, demeton, dimethoate, diazinon, disulfoton, phosphamidon, parathion-methyl, chlorpyrifos-methyl, fenitrothion, malathion, fenthion, parathion, chlorpyrifos, isocarbophos, isofenphos-methyl, quinalphos, methidathion, ditalimfos, profenofos, ethion, triazophos, phosmet, azinphos-methyl, and phosalone, were purchased from the Agriculture Environment Quality Supervision, Inspection and Testing Center in Tianjin, China. The stock solution (100 mg/kg) of each pesticide was prepared in acetone and stored at

−20 °C. The working solutions (0.004–1.0 mg/kg) were prepared by serial dilution of the stock solutions with acetone/ethyl acetate (1:1, v/v) and stored in brown bottles at −20 °C.

The dry, ripe *A. oxyphylla* fruits were purchased from pharmacies or markets in Hainan, Guangdong, Guangxi and Yunnan province, and Beijing city, China. These materials were identified by Prof. Weiping Chen (Hainan Branch Institute of Medicinal Plant Development, Chinese Academy of Medicinal Sciences, Wanning, China). The obtained samples were stored at −20 °C in a refrigerator and ground before analysis.

2.2. Apparatus

An Agilent 6890A GC (Agilent Technologies, Palo Alto, CA, USA) was equipped with an Agilent 7683B autosampler and an FPD operated in phosphorus mode. A DB-5 capillary column (30.0 m × 0.25 mm I. D., 0.25 μm film thickness, Agilent Technologies, USA) was used. The injector and detector temperature were both set at 250 °C. The injection volume was 1.0 μL in splitless mode. Nitrogen (⩾99.99%) was used as the carrier gas at 1.0 mL/min and the make-up gas at 3.0 mL/min. Hydrogen and air were used as the detector gases at 75.0 and 100.0 mL/min, respectively. The column temperature program was as follows: the initial temperature was 60 °C, held for 1 min; increased to 180 °C at 20 °C/min, held for 10 min; increased to 200 °C at 3 °C/min, held for 10 min; then increased to 250 °C at 5 °C/min, held for 5 min. The total run time was 49 min.

The GC–MS/MS analysis was performed on an Agilent 7890A GC (Agilent Technologies) coupled with an Agilent 7000A Triple Quad mass detector (Agilent Technologies) operated in the electron ionization mode and an Agilent 7693 autosampler. A 1.0 μL aliquot of each sample was injected at an injector temperature of 260 °C in splitless mode, and separation was performed on an Agilent DB-1701 column (30.0 m × 0.25 mm I. D., 0.25 μm film thickness, Agilent Technologies, USA). The temperatures of the ion-source, quadrupole, and transfer line were set at 250, 150 and 270 °C, respectively. Helium at a constant flow of 1.2 mL/min was used as the carrier gas. The oven temperature program was as follows: initial temperature, 80 °C, held for 1 min; increased at 30 °C/min to 130 °C; increased at 6 °C/min to 210 °C; and increased to 290 °C at 10 °C/min, held for 8 min. The total run time was 32 min.

2.3. Sample preparation

All the samples were crushed and passed through a 40-mesh sieve. An aliquot of 0.5 g homogenized sample powder was accurately weighed and transferred into a 10-mL centrifuge tube for analysis. Four milliliters of acetone/ethyl acetate (1: 1, v/v) were added, followed by vortex mixing for 1 min and centrifugation (1062.5g) for 5 min. The supernatant was transferred to a 25 mL flask. The extract was concentrated to near dryness on a rotary vacuum evaporator (EYELA-N-1100D-WD, Tokyo, Japan) at 30 °C. The residue was re-dissolved in 1 mL of acetone/ethyl acetate (1:1, v/v) and then filtered through a 0.22 μm PTFE membrane (Agela Technology, Tianjin, China). The filtrate was transferred into an autosampler vial for GC–FPD analysis under the above-described conditions.

3. Results and discussion

3.1. Optimization of sample preparation procedure

The influence of extraction solvent, solvent volume and extraction time on the extraction efficiency was firstly investigated. GC–FPD was used to optimize these extraction conditions. The