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A multiresidue method for simultaneous determination of 44 organophosphorous pesticides in *Pogostemon cablin* and related products using modified QuEChERS sample preparation procedure and GC–FPD

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ABSTRACT

In this study, a modified quick, easy, cheap, efficient, rugged and safe (QuEChERS) method coupled with gas chromatography with flame photometric detection (GC–FPD) was developed for the determination of 44 organophosphorous pesticide (OPP) residues in 44 batches of *Pogostemon cablin* and its related products for the first time. The QuEChERS extraction conditions were optimized, and the matrix effect that may influence recoveries was evaluated and minimized by matrix-matched calibration curves. Under the optimized conditions, the calibration curves for all OPPs showed good linearities in the concentration range of 0.04–1.5 μg mL^{-1} with correlation coefficients better than 0.9909. The limits of detection were in the range of 0.004–0.02 μg mL^{-1} and quantification were 0.01–0.04 μg mL^{-1}, below the regulatory maximum residue limits suggested. Mean recoveries ranged between 76.62 and 113.7% (99.34% on average), and relative standard deviation was 3.71% on average. The validated method was applied on 44 real samples including *P. cablin*, and *P. cablin* oil and powder. Two (4.5%) samples were found to be contaminated by chlorpyrifos with levels below the legal limits, which were successfully confirmed by gas chromatography–mass spectrometry (GC–MS). Based on the results, the developed method was proved to be simple, fast, accurate, low cost and environmentally friendly and can be successfully applied in the determination of targeted OPP residues in *P. cablin* and its related products. Moreover, it also attaches great importance to pesticide monitoring programs in food, soil and air in the future.

1. Introduction

Pogostemon cablin (Blanco) Benth., a member of genus *Pogostemon*, Lamiaceae family, is native to subtropical Himalayas, Southeast Asia and the Far East and has been cultivated extensively in Indonesia, Malaysia, China and Brazil [1]. *P. cablin* is not only an important spice, but also a traditional Chinese medicine (TCM) [2]. It has been used in the perfume industry because of its essential oil obtained by steam distillation. The oil is widely applied in perfume for its characteristic pleasant and long-lasting woody, earthy, camphoraceous odor and strong fixative properties [3,4] and is also used as a flavor ingredient in many major food products [5]. What is more, *P. cablin* is a frequently used TCM and has been recorded in the Chinese Pharmacopoeia to remove dampness, relieve summer heat and overcome nausea [6]. Besides, *P. cablin* powder is loved for the functions of anti-inflammatory, repellency against mosquitoes and antifatigue.

Up to now, *P. cablin* and its related products cover a wide range of vocations and produce good social and economic interests. However, *P. cablin* is a perennial herb with a long growth cycle, and the natural oil cannot be synthesized [4]. More importantly, it will encounter various diseases and insect pests during the growth process. So, different pesticides are applied with high frequency to enhance the quality and improve the quantity of raw *P. cablin*. But,

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improper or incorrect use of pesticides may lead to the residue and pollution of pesticides. Besides, pesticides are easily ignored in the environment [7,8] which can contaminate *P. cablin* by means of absorption and translocation in the growth process [9,10]. Since the organochlorine pesticides (OCPs) have been withdrawn from registered use, organophosphorous pesticides (OPPs) are currently the largest and most versatile classes in use [11] due to better diseases and insect pests control and faster degradation in the environment [12,13]. However, some OPPs are particularly unfriendly toward animals and humans [13], which may result in acute or chronic toxicity [14,15]. To the best of our knowledge, few studies about determination of OPPs in *P. cablin* and its related products have been reported previously, so, a simple, efficient and reliable analytical method becomes extremely urgent.

To date, the methods used to determine OPP residues are mainly based on gas chromatography (GC) [11,16], liquid chromatography (LC) [17,18] and sensor [19,20]. Among them, GC–FPD [15,21] is the most frequently used technique because of its high sensitivity, selectivity and relatively low cost. Owing to the low concentrations and complex matrices in the samples, OPPs cannot be directly analyzed with GC or HPLC. Therefore, sample pretreatment steps, particularly the extraction and concentration steps, become very pivotal and the quality of these steps largely determines the success of an analysis [22]. QuEChERS (quick, easy, cheap, effective, rugged and safe) method not only has shown its usefulness in the analysis of residues in complex samples, but also presents some advantages such as its simplicity, minimum steps and effectiveness for cleaning up [16,23]. Also, the method is environmentally friendly because of the less consumption of organic solvents, being in agreement with the new trends of green analytical chemistry [24,25]. QuEChERS has been used for the determination of pesticides in tea [26], blackcurrant [27] and vegetable [28]. However, as far as we know, it has not been used for the analysis of pesticides in *P. cablin* and its related products.

The objective of this work is to optimize and validate a GC–FPD method for the simultaneous analysis of 44 OPPs in *P. cablin* and its related products including *P. cablin* oil and powder using a simple and fast QuEChERS extraction procedure. The positive samples contaminated by chlorpyrifos with levels below the legal limits (MRL 0.05 mg kg^{-1}) were further confirmed by gas chromatography–mass spectrometry (GC–MS). This study is not only to raise concerns to the pesticide residues in *P. cablin* and its related products, but also to attract attention to the security of pesticide employment technology and the contamination level of pesticide in the environment.

2. Materials and methods

2.1. Chemicals and reagents

Forty-four pesticide standards (97.00–99.97% purity) including trichlorfon, etrimfos, ditalimfos, dichlorvos, orthodibrom, demeton, terbufos, methamidophos, mevinphos, methacrifos, acephate, ethoprophos, sulfotep, phorate, diazinon, omethoate, fonofos, disulfoton, moncrotophos, dimethoate, chlorpyrifos-methyl, pirimiphos-methyl, methyl paraoxon, chlorpyrifos, parathion-methyl, phosphamidon, pirimiphos, fenthion, malathion, bromophos, fenitrothion, parathion, quinalphos, isofenphos, isocarbophos, phenthoate, methidathion, profenofos, azinphos-methyl, fenamiphos, fensulfothion, triazophos, phosalone and triazotion were purchased from the Agro-Environment Protection Institute (Tianjin, China). Graphitized carbon black (GCB) and primary secondary amine (PSA) were purchased from Sepax-UCT Inc. (USA). Anhydrous sodium chloride (NaCl) and anhydrous magnesium sulfate ($MgSO_4$) were manufactured by Beijing Chemical Co. Acetonitrile, ethyl acetate and acetone (HPLC grade) were obtained from J.T. Baker (Phillipsburg, NJ, USA). Cyclohexane (HPLC grade) was obtained from the Sinopharm Chemical Reagent Co., Ltd. (Beijing, China).

2.2. Samples

Thirty batches of *P. cablin* samples were taken from Guangdong and Guangxi provinces and the plant materials were collected in 2013. Ten batches of *P. cablin* oil were purchased from different markets in China in 2013, which were originally produced in India, Germany, Indonesia, Portugal and China. Four batches of *P. cablin* powder produced in Guangdong were purchased from different markets in China in 2013. All samples were stored in a refrigerator before analysis.

2.3. GC–FPD and GC–MS conditions

An Agilent 6890N gas chromatograph equipped with an FPD detector, an autosampler 7683 (Agilent Technologies, Wilmington, DE, USA) and an injector was used for determining 44 pesticides. All pesticides were separated through a DB-1701 (30 m × 0.25 mm i.d. with 0.25 μm) capillary column. Injector and detector temperatures were 250 and 260 °C, respectively. The oven temperature was programmed as follows: 70 °C for 1 min, raised to 210 °C (15 °C min^{-1}) for 6 min, raised to 220 °C (1.5 °C min^{-1}) for 2 min, and raised to 260 °C (20 °C min^{-1}) for 8 min. High-purity (over 99.99%) nitrogen was used as the carrier and make-up gas at 1.3 and 3 mL min^{-1}, respectively. An HP Chem Station (Hewlett-Packard, Palo Alto, CA, USA) was used for instrument control and data analysis. Quantification of the pesticides was performed by the external standard method based on the detected and integrated peak area.

A Shimadzu GCMS-TQ8030 (Tokyo, Japan) was used to confirm the target analytes in positive samples. A Rxi-5Sil MS (30 m × 0.25 mm i.d. with 0.25 μm) capillary column was used. The temperature of the injection port was set at 260 °C and 1.0 μL of the sample was injected in splitless mode. The oven temperature was programmed as follows: 60 °C for 3 min, raised to 200 °C (20 °C min^{-1}), raised to 220 °C (5 °C min^{-1}). High-purity (over 99.999%) helium was selected as the carrier gas. The mass conditions were set as follows: ion-source temperature, 230 °C; interface temperature, 280 °C; ionization mode, electron ionization (EI); ionization energy, 70 eV; scan time, 0.5 s/scan. The scan range was from *m/z* 50–500.

2.4. Standard solution preparation

A stock standard solution of each OPP was prepared in acetone at a concentration of 100 μg mL^{-1} and stored at −20 °C in the refrigerator. The standard working solutions were daily obtained by appropriate dilution of the stock standard solution.

2.5. Sample preparation

For *P. cablin* and *P. cablin* powder, subsample flour (1.0 g, through 60-mesh screen) was weighed into a 10 mL Teflon centrifuge tube. For *P. cablin* oil, 250 mg was weighed into a 10 mL Teflon centrifuge tube. Then 5 mL of mixed solvent of acetonitrile: water containing 1% HAc (9:1, v/v) was added and the tube was capped and shaken vigorously for 30 s by hand and followed by vortex mixing for 1 min, ensuring that the solvent interacted well with the entire sample. To induce phase separation and pesticide partitioning, 3.5 g of anhydrous magnesium sulfate and 1.0 g of anhydrous sodium chloride were added, after which the tube was sealed. Then the tube was followed by vortex mixing for 30 s and was centrifuged at 10,000 rpm