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Simultaneous determination of pesticide residues and antioxidants in blended oil using a liquid-liquid extraction combined with dispersive solid phase extraction method

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ABSTRACT

This article developed a method to detect two antioxidants (butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT)) and twelve pesticides (dichlorvos, pirimicarb, prothiofos, fenitrothion, ethoprophos, malathion, beta-Cypermethrin, profenofos, diazinon, propoxur, procymidon, captan) in blended oil samples after their extraction simultaneously. The establishment of the method was based on two-step process of screening and optimization experiment design. With a Plackett-Burman (PB) design, significant parameters were found by screening experiment and single factor experiment accompanied by a central composite design (CCD) experiment were used to make important parameters optimal. The coefficients of determination (r^2) was between 0.9931 and 0.9996 while the limits of quantification (LOQs) and limits of detection (LODs) were found in scope of 0.002–0.04 mg·kg^{-1} and 0.0006–0.0012 mg·kg^{-1}. Recovery values of analytes were above 74%, at the same time the relative standard deviations (RSDs) under 10% at the concentrations ranging from 0.05 mg·kg^{-1} to 0.20 mg·kg^{-1}. To sum up, this method with shorter time and fewer consumption of reagents could be employed in various occasions to detect antioxidants and pesticides.

1. Introduction

In our daily diet, edible oil as an important supplement contains plenty of triglycerides, fatty acid and fat-soluble vitamins (Gunstone, 2011). Blended oil is composed of various vegetable oils (such as peanut oil, soybean oil, sunflower seed oil, cottonseed oil and rapeseed oil, etc) in a certain proportion, and it is widely used in food processing and cooking in China. It has been reported that the consumption of edible oils is 2235 million tons in China in 2007 (Li et al., 2014), and has increased to 3406 million tons in which blended oil make a contribution of 30% in 2015.

In order to improve crops yield, there are increasing utilization of pesticides, for instance: organophosphates, carbamates, fungicide and synthetic pyrethriods, which could accumulate in the plant and cause the severe contamination in the seeds. It is now clear that these pesticides could be extracted into the oils through distillation during the production of blended oil (Nguyen, Lee, & Lee, 2010). Meanwhile in order to prevent automatic oxidation of lipid so as to increase the oil shelf life, synthetic antioxidant were added into oil such as butylated hydroxyanisole (BHA) and Butylated hydroxytoluene (BHT), due to their high stability, effectiveness, availability and low cost (Wang et al., 2014). Therefore, monitoring the residual levels of the pesticides and antioxidants simultaneously in edible blended oils makes a great necessity (Deme et al., 2014).

Due to its complex matrix, sample preparation becomes much important due to the co-extraction of pesticides and antioxidants as well as other non-polar compounds in the oil without clean-up treatment, which would cause the interference to the targeted analytes using GC–MS and also detrimental effects on the instruments (García-Reyes, Ferrer, Gómez-Ramos, Fernández-Alba, & Molina-Díaz, 2007; Gilbert-López, García-Reyes, Fernández-Alba, & Molina-Díaz, 2010; Gilbert-López, García-Reyes, & Molina-Díaz, 2009; Lehotay, Maštovská, & Yun, 2005). There are a few methods developed for sample extraction and purification methods, such as liquid–liquid extraction (LLE) (Nguyen et al., 2010; Xiu-Qin, Chao, Yan-Yan, Min-Li, & Xiao-Gang, 2009), solid-phase extraction (SPE) (Amvrazi & Albanis, 2006; Tuzimski & Rejczak, 2016), matrix solid-phase dispersion (MSPD) (Li et al., 2014), dispersive solid-phase extraction (d-SPE) (Deme et al., 2014; Tuzimski & Rejczak, 2016), dispersive liquid–liquid micro-extraction (DLMME)

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(Farajzadeh, Khoshmaram, & Nabil, 2014), and Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) (Rajski, Lozano, Uclés, Ferrer, & Fernández-Alba, 2013). Besides, determination methods of pesticide residues and antioxidants mainly include high performance liquid chromatography (HPLC) (Amlashi, Hadjmohammadi, & Nazari, 2014; Wang et al., 2014), gas chromatography (GC) (Farajzadeh et al., 2014; Yang, Lin, & Choong, 2002), liquid chromatography-mass spectrometry (LC-MS) (Gilbert-López et al., 2010; Lacina et al., 2012; Xiu-Qin et al., 2009), gas chromatography–mass spectrometry (GC–MS) (Guo, Xie, Yan, Wan, & Wu, 2006; Su et al., 2011), enzyme immunoassay (Qian et al., 2009), biosensor technology (Chouteau, Dzyadevych, Durrieu, & Chovelon, 2005) and spectrophotometry (Santalad, Srijaranai, Burakham, Sakai, & Deming, 2008). Particularly, the current research mainly focus on the methods of purification of the sample. The Gel Permeation Chromatography (GPC) method is the advanced purification method in the residue analysis for sample with high fat content. However, this method has disadvantages such as consumption of large amount of organic solvent, high running cost and long analyzing time. The SPE method is the most commonly used, but the operation is complicated, large amount of organic solvent and longer analysis time are required. Therefore, LEE-dSPE (Liquid Liquid Extraction-dispersive Solid Phase Extraction) method was employed to extract the analytes in edible oil due to lower consumption of organic solvent used and shorter running time. For LEE-dSPE, different authors had used different parameters. Majority of studies have been focused on the optimization of the parameters of extraction and purification. Lentza-Rizos, Avramides, & Visi (2001) found that acetonitrile alone was the most appropriate extraction solvent. Nguyen et al. (2010) found that extraction by acetonitrile after oil dissolved by n-hexane would be better for 95 pesticides. However, no reports have been available concerning the effect of various parameters on the final recovery. In this work, the importance of these parameters was first studied by PB design which haven't been seen in previous research on LEE-dSPE.

As discussed above, four types of pesticides are widely used including organophosphates, carbamates, fungicide and synthetic pyrethriods. In the current study, 12 pesticides from the above types were selected, among which 10 selected pesticides have been defined MRLs (maximum residue limits) in edible oils in the Chinese standard (http://bz.cfsa.net.cn/staticPages/34A684DB-3809-46C8-83B5-F5A09B059607.html), with the exception of prothiofos and propoxur which have similar structure to profenofos and pirimicarb. Meanwhile two most common antioxidants together with these 12 pesticides in blended oil were monitored to optimize the parameters for LEE-dSPE process. Finally, a reliable and rapid method was developed and validated for simultaneous detection of 12 pesticides and 2 antioxidants in blended oil.

2. Materials and methods

2.1. Reagents and materials

Butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), dichlorvos, pirimicarb, prothiofos, fenitrothion, ethoprophos, malathion, beta-Cypermethrin, profenofos, diazinon, propoxur, procymidon and captan were gifted by Sigma-Aldrich (Shanghai, China). Stock standard solutions were prepared in acetonitrile at 1000 μg/ml and working standard solution of analytes was obtained by diluting stock standard solution, during storage at −20 °C. The solvents, acetonitrile (MeCN), was of guaranteed reagent and acquired from Tedia (Nanjing, China). Anhydrous $MgSO_4$, primary secondary amine (PSA) BONDER SILICA, ENVI-Carb™ graphitized carbon black (GCB) and Discovery® DSC-18 was obtained from Sigma-Aldrich (Shanghai, China). Acetic acid (HoAc) was obtained from Sigma–Aldrich (Shanghai, China). The edible blended oil samples were adopted from a local supermarket, during storage at 4 °C.

2.2. Instrumentation

A agilent GC-7890A gas chromatograph, equipped with a MS-5975c mass spectrometer owning a agilent J&W HP-5MS Ultra Inert (30 m × 0.25 mm, 0.25 μm) (Agilent Technologies Inc, California, USA) was used to separate and detect all analytes. Helium was treated as the carrier gas with inlet in splitless mode at a constant pressure of 19.6 psi. Purge flow to split vent was set as 30 ml/min at 0.75 min. The oven temperature program started at 70 °C for 1 min and ramped to 150 °C at 25 °C/min, increased to 200 °C at 3 °C/min, then ramped to 280 °C at 8 °C/min where was kept for 10 min. Respectively, the source, quad and transfer line temperature of mass spectrometer were set at 230 °C, 150 °C and 280 °C. As shown in Fig. 1a, the total ion chromatogram of all analytes was obtained by GC–MS. And for propoxur, two peaks at different retention times stand for this matter, its recovery rate would be calculated apart from each other.

2.3. Sample preparation

2.3.1. Extraction

A centrifuge tube of 50 ml was used to include 5 g edible blended oil almost, followed by extraction with 10 ml of MeCN for 6 min using a vortex apparatus. The centrifugation of tube cost 10 min at 8000 rpm at room temperature. The upper MeCN layer was removed by a pipetting straw and stored at −20 °C for 4 h to make precipitation of fat. With N-EVAP nitrogen evaporator equipment, 5 ml of the supernatant was enriched to dryness and the residues were redissolve in 1 ml of MeCN.

2.3.2. Clean-up

An aliquot of 1 ml of MeCN extract was pipetted into a 2 ml centrifuge tube. After addition of 80 mg PSA and 20 mg GCB, the tube was vigorously mingled for 2 min with a vortex mixer. Subsequently, with the help of a micro-centrifuge the tube was centrifuged at 12,000 rpm for 5 min at room temperature. Finally, supernatant was transferred into sampler vial for analyses.

2.4. Experimental design

Design of experimental (DOE) was used to establish an optimized and improved method through the combination of mathematical and statistical means. The statistical experimental design which was adopted to improve and set up analytical method had huge advantages in improving the efficiency of extraction and reducing experimental variability. Meanwhile fewer consumption of time, reagent and workload was another advantage (Garbi, Sakkas, Fiamegos, Stalikas, & Albanis, 2010).

In DOE, screening design was the most powerful design for selecting the key factors without large projects. Plackett–Burman (PB) design with good precision was the most common screening design to confirm the critical factors in numerous factors by a less experimental frequency. In general, the number of experiment was 2^n or 4 multiples in PB design instead of 2 compared with full factorial design (Rahman, Zidan, Habib, & Khan, 2010). 12 experiments were established in PB screening design with Design-Expert version 8.0.6. The model of linear equation Eq. (1) was as follows:

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_4X_4 + a_5X_5 + \ldots\ldots + a_nX_n \quad (1)$$