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Multiresidue analysis of 30 organochlorine pesticides in milk and milk powder by gel permeation chromatography-solid phase extraction-gas chromatography-tandem mass spectrometry

Guocan Zheng,*[1] Chao Han,†[1] Yi Liu,* Jing Wang,* Meiwen Zhu,* Chengjun Wang,‡ and Yan Shen‡[2]
*Chongqing Entry-Exit Inspection and Quarantine Bureau of P.R.C, Chongqing 400020, China
†Wenzhou Entry-Exit Inspection and Quarantine Bureau of P.R.C, Wenzhou 325027, China
‡College of Chemistry and Materials Engineering, Wenzhou University, Wenzhou 325035, China

ABSTRACT

A method for simultaneous determination of the 30 organochlorine pesticides (OCP) in milk and milk powder samples has been developed. Prior to the gas chromatography-tandem mass spectrometric analysis, the residual OCP in samples were extracted with *n*-hexane and acetone mixture (1/1, vol/vol) and cleaned up by gel permeation chromatography and solid phase extraction. Selected reaction monitoring mode was used for gas chromatography-tandem mass spectrometric data acquisition to identify and quantify the OCP. To avoid the matrix effects, matrix-matched calibration solutions ranging from 2 to 50 ng/mL were used to record the calibration curve. Limits of quantification of all OCP were 0.8 μg/kg. With the exception of endrin, limits of quantification are significantly lower than maximum residue limits set by the European Union and China. The average recoveries were in the range of 70.1 to 114.7% at 3 spiked concentration levels (0.8, 2.0, and 10.0 μg/kg) with residual standard deviation lower than 12.9%. The developed method was successfully applied to analyze the OCP in commercial milk products.

Key words: milk, milk powder, organochlorine pesticide, gas chromatography-tandem mass spectrometry

INTRODUCTION

Since the 1960s, organochlorine pesticides (**OCP**) have been widely used for the control of disease vectors and agricultural pests. As one important class of the persistent organic pollutants, OCP are nonpolar lipophilic compounds with high resistance to degradation and can be bio-accumulated (Smith and Gangolli, 2002). Several studies have demonstrated that the OCP has led to the adverse effects on the human procreation, development, and immunological systems (Langer et al., 2003; Cooper et al., 2004; Dalvie et al., 2004; Soto and Sonnenschein, 2010). Although these pesticides have been banned in most countries for many years, the large production, uncontrolled use in past decades, and inherent volatility and persistence of these pesticides have led to a universal environmental contamination of OCP. The OCP have been found in all kinds of environmental samples all over the world (Tanabe, 2002; Falandysz et al., 2004; Zhou et al., 2011; Li et al., 2014), including the regions where they have never been used or produced, such as the polar regions (Baek et al., 2011; Cabrerizo et al., 2012).

Cow's milk is considered a nearly complete food, because it is a good source of protein, fat, and major minerals. Milk and milk-derived products, such as milk powder, are the main constituent of the daily diet, especially for vulnerable groups such as infants, school-age children, and the elderly. Meanwhile, milk is vulnerable to pesticide contamination because it is a good solvent for fat-soluble substances. High-level pesticide contamination of milk may occur as a result of residue accumulation in the fat-rich tissues of cows, due to the long exposure from feeding on contaminated feedstocks or from drinking contaminated water, or the direct use of pesticides on dairy cattle for ectoparasitic control (Mezcua et al., 2007; Kampire et al., 2011). Among them, OCP are an important concern. Therefore, it is very important to monitor the OCP residues in milk and milk powder for evaluating the human exposure to these contaminants. Furthermore, to protect the health and safety of the consumers, legal directives to control OCP residue levels in foods through the maximum residue limits (**MRL**) have been established in many countries. In the European Union (**EU**) and China, the MRL of OCP in milk and milk powder range from 0.8 to 50.0 μg/kg [DG SANCO, 2014; GB 2763 (P. R. China, 2014)].

Many methods have been developed to determine OCP in different kinds of samples, such as soil (Rashid

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[1]Both authors contributed equally to this work.
[2]Corresponding author: shenyan@wzu.edu.cn

et al., 2010), edible vegetable oils (Li et al., 2014), honey (Zacharis et al., 2012), shrimp (Norazlina et al., 2013), edible marine biota (Santhi et al., 2012), human blood (Wittsiepe et al., 2014), infant formulas (Mezcua et al., 2007), breast milk (Zhou et al., 2011), and so on. To meet the requirements of the low MRL of OCP in foods, more powerful and sensitive analytical method has to be developed. Gas chromatography-tandem mass spectrometry (**GC-MS/MS**) has become one of the most extensively used analytical methods for the identification and quantification of residues in food because of its higher selectivity, sensitivity, and the necessity for confirmation (Albero et al., 2013; Lu et al., 2013; Walorczyk et al., 2013). Conversely, the sample pretreatment for effective clean-up of the extracts from complex matrix, such as food before determination, is another concern. The lipids, which are often co-extracted along with the analytes of interest, is the main problem associated with the analysis of milk samples, as they may jam in the injector and at the top of the column of GC (Mezcua et al., 2007). Because of the robustness, rapidity, convenience, and fat clean-up efficiency, gel permeation chromatography (**GPC**) and solid-phase extraction (**SPE**) are quite suitable techniques for the pretreatment of the complex samples before multiresidue analysis with high throughput and sensitivity (Dubois et al., 2011; Song et al., 2014).

The aim of this work was to develop a multiresidue analysis method for the simultaneous determination of 30 OCP in milk and milk powder based on GC-MS/MS combined with a GPC-SPE 2-step clean up. To the best of our knowledge, this is the first time that 2 clean-up procedures of GPC and SPE are used together for effective clean-up and preconcentration of OCP multiresidues in milk and milk powder samples.

MATERIALS AND METHODS

Chemicals, Reagents, and Equipment

All reagents and solvents were analytical grade unless otherwise specified. All OCP including α-benzene hexachloride (**BHC**), β-BHC, δ-BHC, ν-BHC, *o*,*p*′-dichlorodiphenyltrichloroethane (**DDT**), *p*,*p*′-DDT, methoxychlor, *o*,*p*′-dichlorodiphenyldichloroethylene (**DDE**), *p*,*p*′-DDE, *o*,*p*′-dichlorodiphenyldichloroethane (**DDD**), *p*,*p*′-DDD, heptachlor, heptachlorepoxide, aldrin, dieldrin, endrin, endrin-aldehyde, endrin-ketone, α-chlordane (*cis*), ν-chlordane (*trans*), oxychlordane, α-endosulfan, β-endosulfan, endosulfan sulfate, hexachlorobenzene, tecnazene, quintozene, pentachloroaniline, pentachlorothioanisole, and mirex were purchased from Dr. Ehrenstorfer GmBH (Augsburg, Germany). Chemical structures of the analytes were shown in Figure 1.

The water used was purified with a Milli-Q water purification system from Millipore (Bedford, MA). The HPLC-grade methanol, *n*-hexane, cyclohexane, and ethyl acetate were obtained from CNW (Shanghai, China), acetone were obtained from Tedia (Fairfield, OH).

Preparation of Standards

Individual standard stock solutions (500 mg/L) were prepared in *n*-hexane and stored at 4°C in the dark. Multiple component work standard solution (10 mg/L) was prepared by diluting each above stock solution with *n*-hexane. This working standard solution was used for spiking blank milk and milk powder samples and for preparation of matrix matched calibration solutions.

Sample Preparation

An accurately weighed 5.0-g sample (milk or milk powder) was placed into a 50-mL polypropylene centrifuge tube (10 mL of water was first added in milk powder sample) and then 5.0 g of sodium chloride and 10 mL of *n*-hexane and acetone mixture (1/1, vol/vol) were added. The tube was capped and shaken vigorously for 30 s, and then vortex mixed for 1 min. This extraction solution was centrifuged at 8,875 × *g* for 10 min at 8°C, and the organic layer was transferred into a volumetric flask. The residues were reextracted once with about 10 mL of *n*-hexane and acetone mixture (1/1, vol/vol) as per the previous procedure, and the organic layer was combined and then evaporated to near dryness under vacuum at 40°C. The dried extract was reconstituted in 10 mL of cyclohexane and ethyl acetate mixture (1/1, vol/vol). The extracts were then forwarded to the GPC and SPE procedure. Blanks were periodically run during the analysis to confirm the absence of contamination.

GPC

To remove lipid compounds and other potential interfering substances, clean up was performed using a 2-step clean-up technique of GPC followed by SPE. The GPC columns (400 × 25 mm i.d.) were packed with 5 g (dry weight) of 200–400 mesh Bio-Beads S-X3 (Bio-Rad Laboratories, Hercules, CA) that had been soaked in the cyclohexane and ethyl acetate mixture (1/1, vol/vol) overnight; this mixture also constituted the mobile phase. Once the GPC columns were conditioned, 10 mL of sample extracts were injected into the GPC system. The elution was carried out with a