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; Cabrero 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

Received March 31, 2014.

Accepted June 26, 2014.

¹Both authors contributed equally to this work.

²Corresponding 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, heptachlorepoxyde, 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 $8,875 \times g$ 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 $\times$ 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

Figure 1. Chemical structures of 30 organochlorine pesticides analyzed. BHC = benzene hexachloride; DDE = dichlorodiphenyldichloroethylene; DDD = dichlorodiphenyldichloroethane; DDT = dichlorodiphenyltrichloroethane.

mixture of cyclohexane and ethyl acetate (1/1, vol/vol) at a flow rate of 5 mL/min. The elution fraction was collected in 10 to 22 min. The collected solutions were evaporated to approximately 2 mL at 40°C for further clean up with SPE.

SPE

A Florisil cartridges SPE cartridge (1,000 mg, 6 mL, Supelco, Sigma-Aldrich, Bellefonte, PA) was used to clean up sample extracts. The SPE cartridge was

conditioned with *n*-hexane (5 mL). The sample was applied to the conditioned cartridge and allowed to elute by gravity. The column was eluted into a glass culture tube using 8 mL of *n*-hexane and dichloromethane mixture (5/95, vol/vol); the eluate was evaporated to dryness under vacuum at 40°C. The dried extract was reconstituted in 1.0 mL of *n*-hexane, vortex mixed for 60 s, filtered through a 0.22- μ m filter, and weighted for GC-MS/MS analysis.

GC-MS/MS Analysis

A Thermo Fisher Trace 1300 GC coupled with a Thermo Fisher TSQ 8000 triple quadrupole mass spectrometer and Triplus RSH autosampler (Thermo Fisher Scientific, Waltham, MA) was used in the GC-MS/MS system. Analytes were separated on a Thermo Scientific TR-35 MS (30 m $\times$ 0.25 mm $\times$ 0.25 μ m) capillary column. The carrier gas was helium (99.9999% purity) at a flow rate of 1.2 mL/min. The injector temperature was set to 250°C. Injection mode was splitless and injection volume was 1.0 μ L. The GC column temperature was programmed as an initial temperature of 55°C, held for 1 min, increased at a rate of 40°C/min to 140°C and held for 5 min, then increased at a rate of 2°C/min to 210°C, and finally increased at a rate of 10°C/min to 280°C and held for 10 min.

The MS was operated in electron ionization mode at 70 eV of ionization energy. The ion source and transfer line temperatures were set at 250 and 250°C, respectively. The mass spectra and retention time of all 30 OCP analytes were acquired in full scan mode. The GC-MS/MS data acquisition was performed in selected reaction monitoring (SRM) mode, which allowed the unambiguous identification of each OCP pesticide, as well as good signal to noise ratios for MS/MS detection. The SRM conditions were further optimized (Table 1) to obtain maximum sensitivity for each target compound once the fragment ions were chosen.

RESULTS AND DISCUSSION

Optimization of GC-MS/MS Conditions

To identify the optimal precursor ions for further MS/MS analysis, individual OCP standards were tested in full scan mode one after another by recording mass spectra from 50 to 800 *m/z*. Then, different values of collision energies (between 10 and 30 eV) were tested to find the 2 most abundant fragments when the precursor ions were selected. Because parent ions of some compounds (DDD, DDE, DDT, chlordane, tecnazene, hexachlorobenzene, heptachlor, pentachlorothioanisole, endrin-aldehyde, endosulfan sulfate, endrin-ketone,

and mirex) that have only 1 product ion, we selected 2 different parent ions for their SRM monitoring, which is in compliance with the identification requirements of DG SANCO (2009) and consistent with previous reports (Lozano et al., 2012; Chen et al., 2013). To unambiguously identify the trace OCP in real samples, SRM conditions and collision energies were optimized. The optimal conditions and ratios of abundance for each transition are listed in Table 1.

To improve chromatographic separation, different columns, HP-5MS (30 m $\times$ 0.25 mm $\times$ 0.25 μ m, Agilent Technologies, Inc., Santa Clara, CA), TR-35 MS (30 m $\times$ 0.25 mm $\times$ 0.25 μ m, Thermo Fisher Scientific), and DB-17 MS (30 m $\times$ 0.25 mm $\times$ 0.25 μ m, Agilent Technologies Inc.), were compared for the separation of 30 OCP. On the basis of the test result, TR-35 MS column was the optimal one and showed good resolution in real sample analysis. Figure 2 shows the GC-MS/MS total ion chromatogram of a mixed standard solution of 30 OCP pesticides at 4 ng/L. A complete SRM chromatogram of a spiked milk powder sample at 10 μ g/kg is shown in Figure 3. Confirmation of the target compound was identified by retention time and typical SRM transitions, further ascertained by the relative ion abundance ratio between the qualitative ion and the quantitative ion. A clean background was obtained in the spiked sample analysis, demonstrating the absence of chromatographic interference.

Optimization of Extraction Procedure

Several organic solvents, including *n*-hexane, methanol, dichloromethane, ethyl acetate, acetonitrile, and acetone, were investigated as extractants for extracting the OCP from the spiked samples. It is important to note that 10 mL of water was first added to milk powder samples before extraction, from which higher recovery was obtained. On the basis of the test result, *n*-hexane and acetone mixture (1/1, vol/vol) was the best extraction solvent. The efficiencies of extraction of OCP by vortex and ultrasonication were also compared. The higher recoveries for all OCP were achieved by vortex, and the extraction time consumed by vortexing was much less than that by ultrasonic extraction. Thus, the vortex-assisted extraction was used in the following experiments.

Optimization of Clean-Up Methods

The main interfering compounds that have been co-extracted from milk samples are FA and lipophilic substances. The GPC has proven to be a very useful method for removal of large molecular interferent from sample solutions (Zhu et al., 2014). Therefore, the

Table 1. Analytical conditions for MS/MS (Thermo Fisher TSQ 8000 triple quadrupole mass spectrometer, Waltham, MA)

Compound ¹	Retention time (min)	Parent ion (<i>m/z</i>)	Product ion (<i>m/z</i>)	Collision energy (eV)	Ratio of abundance (qualitative/quantify)
Tecnazene	15.9	259	201	15	81
		261	203 ²	15	
Hexachlorobenzene	19.6	284	214	20	96
		286	251 ²	20	
α-BHC	20.5	181	145 ²	15	48
		219	183	15	
Quintozene	23.2	237	143	25	22
		295	237 ²	20	
ν-BHC	24.0	181	145 ²	15	22
		219	183	15	
β-BHC	26.3	181	145 ²	15	67
		219	183	15	
Heptachlor	27.1	272	237 ²	15	63
		274	239	15	
Pentachloroaniline	27.8	265	158 ²	30	74
		265	192	30	
δ-BHC	28.8	181	145 ²	15	85
		219	183	15	
Aldrin	29.9	263	193 ²	32	26
		263	228	26	
Pentachlorothioanisole	31.3	296	263 ²	20	61
		298	265	20	
Oxychlordane	34.1	387	263 ²	15	74
		387	323	15	
Heptachlorepoxyde	35.2	353	253 ²	15	58
		353	289	15	
ν-Chlordane (<i>trans</i>)	37.2	373	266 ²	18	46
		375	268	18	
α-Chlordane (<i>cis</i>)	38.3	373	266 ²	18	41
		375	268	18	
<i>o,p'</i> -DDE	38.4	246	176 ²	25	41
		248	176	20	
α-Endosulfan	38.4	241	206 ²	20	74
		272	237	18	
<i>p,p'</i> -DDE	40.5	246	176 ²	25	52
		248	176	20	
Dieldrin	40.5	277	205	20	19
		263	193 ²	26	
<i>o,p'</i> -DDD	41.4	235	165 ²	20	64
		237	165	20	
Endrin	41.9	263	193 ²	26	40
		281	245	12	
<i>o,p'</i> -DDT	42.6	235	165 ²	20	66
		237	165	20	
<i>p,p'</i> -DDD	43.0	235	165 ²	20	65
		237	165	20	
β-Endosulfan	43.0	241	206 ²	20	97
		272	237	18	
<i>p,p'</i> -DDT	43.9	235	165 ²	20	65
		237	165	20	
Endrin-aldehyde	43.9	345	317 ²	10	41
		347	319	10	
Endosulfan sulfate	44.5	272	237 ²	15	54
		274	239	15	
Methoxychlor	46.1	227	212	15	68
		227	169 ²	20	
Endrin-ketone	46.2	315	279	10	75
		317	281 ²	10	
Mirex	46.6	270	235	15	60
		272	237 ²	15	

¹BHC = benzene hexachloride; DDE = dichlorodiphenyldichloroethylene; DDD = dichlorodiphenyldichloroethane; DDT = dichlorodiphenyltrichloroethane.

²The MS/MS transition was used for quantitation.

Figure 2. Total ion chromatograms of 30 organochlorine pesticide standards (4 ng/mL) by GC-MS/MS. 1. tecnazene; 2. hexachlorobenzene; 3. α -benzene hexachloride (BHC); 4. quintozone; 5. ν -BHC; 6. β -BHC; 7. heptachlor; 8. pentachloroaniline; 9. δ -BHC; 10. aldrin; 11. pentachlorothioanisole; 12. oxychlordane; 13. heptachlorepoxyde; 14. ν -chlordane (*trans*); 15. α -chlordane (*cis*); 16. *o,p'*-dichlorodiphenyldichloroethylene (DDE); 17. α -endosulfan; 18. *p,p'*-DDE; 19. dieldrin; 20. *o,p'*-dichlorodiphenyldichloroethane (DDD); 21. endrin; 22. *o,p'*-dichlorodiphenyltrichloroethane (DDT); 23. *p,p'*-DDD; 24. β -endosulfan; 25. *p,p'*-DDT; 26. endrin-aldehyde; 27. endosulfan sulfate; 28. methoxychlor; 29. endrin-ketone; 30. mirex.

aforementioned extracts were first cleaned up by GPC. Of these 30 OCP compounds, mirex has the highest molecular weight (545.5 m/z), and tecnazene has the lowest molecular weight (260.9 m/z); 5 mL of mixed standard dilution (20 ng/mL for mirex and tecnazene) and the extracts of milk sample were injected into the GPC system, respectively. Then the analytes were eluted out with cyclohexane and ethyl acetate mixture (1/1, vol/vol) at a flow rate of 5 mL/min. Mirex and tecnazene were eluted out at 10 and 22 min, respectively, whereas most of interferents were eluted out in 10 min. The GPC extract was then purified by SPE column (C_{18} , Florisil, and hydrophilic-lipophilic balance) for further clean up and analyte enrichment. The maximum recoveries for the 30 OCP ranged from 72.5 to 108.4%, achieved on a Florisil SPE column eluted with 8 mL of *n*-hexane and dichloromethane mixture (5/95, vol/vol). Thus, the Florisil SPE column was used in the following experiments.

Method Validation

Linearity of Calibration Standards. In pesticide residue analyses, matrix effects are a known problem (Lehotay et al., 2010; Niell et al., 2014). Response enhancement or diminishment effect can occur when co-extractives fill active sites in the chromatographic system or build-up of nonvolatile materials occurs in the GC inlet. Matrix effects could be effectively avoided if a matrix-matched calibration standard was used (Martins et al., 2011; Hou et al., 2014). In the current study, the calibration curve was derived using a matrix-matched calibration solution ranging from 2 to 50 ng/mL. Calibration curves with $1/x$ weighting were plotted for each individual analyte. The correlation coefficient was >0.997 for all OCP. The limits of quantification were calculated as the pesticide concentration that produces a peak signal-to-noise ratio of 10/1, and was 0.8 $\mu\text{g/kg}$ for all OCP. Quantification was performed

based on calibration plots using the peak area of the most intense transition of the analyte. Some MRL of China and EU for milk and milk powder are shown in

Tables 2 and 3. With the exception of endrin, limits of quantification were significantly lower than MRL set by EU and China, indicating that this method is suitable

Figure 3. Selected reaction monitoring chromatograms of a milk powder sample spiked with 10.0 µg/kg of organochlorine pesticides. RT = retention time (min); BHC = benzene hexachloride. Color version available in the online PDF.

Figure 3 (Continued). Selected reaction monitoring chromatograms of a milk powder sample spiked with 10.0 $\mu\text{g/kg}$ of organochlorine pesticides. RT = retention time (min); BHC = benzene hexachloride. Color version available in the online PDF.

Table 2. Mean recoveries and repeatability of the developed method (milk) at 3 concentration levels with maximum residue limits (MRL; n = 6)

Compound ¹	Added 0.8 µg/kg		Added 2.0 µg/kg		Added 10.0 µg/kg		MRL (µg/kg; China, European Union)
	Recovery (%)	Relative SD (%)	Recovery (%)	Relative SD (%)	Recovery (%)	Relative SD (%)	
Tecnazene	77.5	7.2	85.3	6.6	93.0	3.6	—, 50
Hexachlorobenzene	73.9	6.2	76.7	4.5	93.2	3.6	—, 10
α-BHC	71.2	8.5	80.1	6.2	108.6	4.2	20, 4
Quintozone	76.5	7.5	86.9	5.7	90.7	4.8	—
ν-BHC	74.9	8.8	85.1	6.0	94.7	4.9	10, 1
β-BHC	78.5	7.2	83.6	6.1	106.2	4.9	20, 3
Heptachlor	76.6	7.4	85.6	6.2	94.9	3.9	6, 4
Pentachloroaniline	74.0	7.7	82.1	5.4	94.5	4.3	—
δ-BHC	75.3	7.6	85.4	6.3	92.0	4.7	20, 3
Aldrin	72.1	7.4	83.6	6.4	91.7	4.5	6, 6
Pentachlorothioanisole	75.6	7.1	84.6	5.5	109.6	4.3	—
Oxychlordane	77.6	6.9	85.7	5.8	89.8	4.3	—
Heptachlorepoxyde	73.5	8.6	83.6	7.2	95.0	5.3	6, 4
ν-Chlordane (<i>trans</i>)	74.4	6.9	83.2	6.4	95.1	5.2	2, 2
α-Chlordane (<i>cis</i>)	72.7	6.9	86.9	4.8	108.9	4.0	2, 2
<i>o,p'</i> -DDE	78.7	6.7	85.1	5.3	94.1	4.3	20, 40
α-Endosulfan	82.3	8.0	85.7	6.4	87.3	5.6	10, 50
<i>p,p'</i> -DDE	78.2	8.3	83.8	6.0	91.3	5.3	20, 40
Dieldrin	81.3	7.2	90.3	5.6	106.8	3.4	6, 6
<i>o,p'</i> -DDD	83.3	7.9	84.2	7.2	90.5	4.8	20, 40
Endrin	78.4	7.5	85.8	6.5	105.7	3.6	—, 0.8
<i>o,p'</i> -DDT	75.7	5.7	82.5	5.2	95.1	4.6	20, 40
<i>p,p'</i> -DDD	70.1	12.9	77.0	7.2	87.6	5.8	20, 40
β-Endosulfan	77.3	9.1	85.4	5.0	92.2	3.7	10, 50
<i>p,p'</i> -DDT	78.4	8.7	85.0	5.8	109.6	5.3	20, 40
Endrin-aldehyde	78.5	6.4	86.7	5.1	95.7	4.0	—
Endosulfan sulfate	83.9	8.0	89.4	6.6	114.7	5.3	10, —
Methoxychlor	79.7	7.7	85.6	5.9	95.6	4.6	—, 10
Endrin-ketone	80.0	6.8	82.9	5.6	95.1	4.2	—
Mirex	75.1	6.7	82.6	6.0	95.4	4.4	—

¹BHC = benzene hexachloride; DDE = dichlorodiphenyldichloroethylene; DDD = dichlorodiphenyldichloroethane; DDT = dichlorodiphenyltrichloroethane.

for multiresidue analysis of 30 OCP in milk and milk powder.

Assay Specificity. The specificity was evaluated by the analysis of 10 blank samples of different milk and milk powder samples. No interfering peaks from endogenous compounds were found in the retention time of the target analyte for milk and milk powder samples.

Precision and Accuracy. A series of fortified samples were made and used as samples to evaluate the precision and accuracy of the developed method. Blank milk and milk powder samples were spiked with the appropriate amounts of the target compounds at 3 concentration levels, 0.8, 2.0, and 10.0 µg/kg. The intra- and interday precision of fortified samples were detected and the relative standard deviations with values of 4.82 and 4.91% were obtained, respectively. The results showed the method was stable and reproducible. At the same time, accuracy inspection was performed, and average recoveries ranged from 70.1 to 114.7% with relative standard deviations below 12.9% (as shown in Tables 2 and 3), which complied with DG SANCO

(2009) and were indicative of the good accuracy and precision of the method.

Real Samples. Fifty real milk and milk powder samples were purchased from local supermarkets and analyzed following the proposed method. No OCP in all samples were detected. The consumption of these foods does not constitute a health risk for consumers.

CONCLUSIONS

A comprehensive method, GPC-SPE-GC-MS/MS, was developed and validated for the determination of 30 OCP in milk and milk powder samples. The combination of GPC with an SPE clean-up technique could effectively eliminate the interferents from extracts of the milk and milk powder samples. The clean-up efficiency was satisfactory. Based on these results, the developed GPC-SPE clean-up procedure combined with GC-MS/MS can be used for sensitive and accurate determination and confirmation of the 30 OCP in milk and milk powder samples.

Table 3. Mean recoveries and repeatability of the developed method (milk powder) at 3 concentration levels with maximum residue limits (MRL; n = 6)

Compound ¹	Added 0.8 µg/kg		Added 2.0 µg/kg		Added 10.0 µg/kg		MRL (µg/kg; China, European Union)
	Recovery (%)	Relative SD (%)	Recovery (%)	Relative SD (%)	Recovery (%)	Relative SD (%)	
Tecnazene	72.4	8.3	86.0	5.1	97.5	3.2	—, 50
Hexachlorobenzene	77.2	6.7	92.1	4.2	102.4	3.7	—, 10
α-BHC	80.2	6.6	92.1	5.1	113.9	4.5	20, 4
Quintozene	71.5	9.3	81.6	6.4	92.5	5.0	—
ν-BHC	77.7	7.5	86.9	5.6	96.2	3.5	10, 1
β-BHC	82.1	7.0	88.6	5.6	96.1	4.2	20, 3
Heptachlor	73.5	8.2	79.2	6.0	109.1	4.6	6, 4
Pentachloroaniline	72.5	8.5	88.2	6.7	104.6	4.5	—
δ-BHC	79.2	6.5	86.3	5.9	95.1	4.2	20, 3
Aldrin	75.2	8.1	85.4	5.9	93.3	3.8	6, 6
Pentachlorothioanisole	73.9	9.2	85.6	7.2	105.4	4.2	—
Oxychlordane	76.2	7.1	88.9	4.3	90.6	3.5	—
Heptachlorepoxyde	80.7	8.2	88.5	6.4	106.3	3.9	6, 4
ν-Chlordane (<i>trans</i>)	75.5	7.2	84.5	5.4	97.2	3.5	2, 2
α-Chlordane (<i>cis</i>)	73.3	9.2	82.4	7.2	105.9	4.4	2, 2
<i>o,p'</i> -DDE	76.5	6.5	86.2	4.7	102.4	3.4	20, 40
α-Endosulfan	72.5	9.4	91.2	6.2	96.4	5.2	10, 50
<i>p,p'</i> -DDE	80.2	8.5	89.2	7.3	95.8	5.2	20, 40
Dieldrin	79.2	8.1	95.4	5.9	105.2	5.2	6, 6
<i>o,p'</i> -DDD	80.5	8.3	86.4	6.2	95.5	4.5	20, 40
Endrin	79.2	6.6	90.2	5.4	106.6	4.1	—, 0.8
<i>o,p'</i> -DDT	72.5	8.1	85.3	6.6	96.6	3.9	20, 40
<i>p,p'</i> -DDD	76.9	10.1	79.2	8.3	89.6	6.6	20, 40
β-Endosulfan	70.2	9.6	88.3	4.5	94.4	3.9	10, 50
<i>p,p'</i> -DDT	80.2	6.9	90.0	5.5	104.4	3.8	20, 40
Endrin-aldehyde	70.9	11.5	82.5	6.3	99.5	3.9	—
Endosulfan sulfate	82.1	7.5	86.5	6.5	108.7	5.2	10, —
Methoxychlor	71.6	9.2	92.4	6.7	111.6	4.5	—, 10
Endrin-ketone	81.6	7.2	86.9	6.7	96.2	4.5	—
Mirex	74.6	6.9	81.5	5.6	96.2	4.3	—

¹BHC = benzene hexachloride; DDE = dichlorodiphenyldichloroethylene; DDD = dichlorodiphenyldichloroethane; DDT = dichlorodiphenyltrichloroethane.

ACKNOWLEDGMENTS

Financial support from the Science Technology Department of Zhejiang Province (2013C37065), ZJCIQ (ZK201417), NSFC (21207102) and Wenzhou Municipal Science and Technology Bureau project (2013G0007) is gratefully acknowledged.

REFERENCES

- Albero, B., C. Sánchez-Brunete, E. Miguel, R. A. Pérez, and J. L. Tadeo. 2013. Analysis of natural-occurring and synthetic sexual hormones in sludge-amended soils by matrix solid-phase dispersion and isotope dilution gas chromatography-tandem mass spectrometry. *J. Chromatogr. A* 1283:39–45.
- Baek, S. Y., S. D. Choi, and Y. S. Chang. 2011. Three-year atmospheric monitoring of organochlorine pesticides and polychlorinated biphenyls in polar regions and the South Pacific. *Environ. Sci. Technol.* 45:4475–4482.
- Cabrerizo, A., J. Dachs, D. Barcelo, and K. C. Jonest. 2012. Influence of organic matter content and human activities on the occurrence of organic pollutants in Antarctic soils, lichens, grass, and mosses. *Environ. Sci. Technol.* 46:1396–1405.
- Chen, X., Z. Y. Bian, H. W. Hou, F. Yang, S. S. Liu, G. L. Tang, and Q. Y. Hu. 2013. Development and validation of a method for the determination of 159 pesticide residues in tobacco by gas chromatography-tandem mass spectrometry. *J. Agric. Food Chem.* 61:5746–5757.
- Cooper, G. S., S. A. Martin, M. P. Longnecker, D. P. Sandler, and D. R. Germolec. 2004. Associations between plasma DDE levels and immunologic measures in African-American farmers in North Carolina. *Environ. Health Perspect.* 112:1080–1084.
- Dalvie, M. A., J. E. Myers, M. L. Thompson, S. Dyer, T. G. Robins, S. Omar, J. Riebow, J. Molekwa, P. Kruger, and R. Millar. 2004. The hormonal effects of long-term DDT exposure on malaria vector-control workers in Limpopo Province. South Africa. *Environ. Res.* 96:9–19.
- DG SANCO (European Commission Directorate General for Health and Consumers). 2009. Method validation and quality control procedures for pesticide residues analyses in food and feed. 10684. DG SANCO, Brussels, Belgium.
- DG SANCO (European Commission Directorate General for Health and Consumers). 2014. EU Pesticides Database. Accessed June 5, 2014. http://ec.europa.eu/sanco_pesticides/public/index.cfm?event=substance.selection.
- Dubois, M., A. Tarres, T. Goldmann, G. Loeffelmann, A. Donaubaue, and W. Seefelder. 2011. Determination of seven glycidyl esters in edible oils by gel permeation chromatography extraction and liquid chromatography coupled to mass spectrometry detection. *J. Agric. Food Chem.* 59:12291–12301.
- Falandysz, J., B. Wyrzykowska, J. Warzocha, I. Barska, A. Garbacik-Wesolowska, and P. Szefer. 2004. Organochlorine pesticides and PCBs in perch *Perca fluviatilis* from the Odra/Oder river estuary, Baltic Sea. *Food Chem.* 87:17–23.

- Hou, X., S. R. Lei, S. T. Qiu, L. A. Guo, S. G. Yi, and W. Liu. 2014. A multi-residue method for the determination of pesticides in tea using multi-walled carbon nanotubes as a dispersive solid phase extraction absorbent. *Food Chem.* 153:121–129.
- Kampire, E., B. T. Kiremire, S. A. Nyanzi, and M. Kishimba. 2011. Organochlorine pesticide in fresh and pasteurized cow's milk from Kampala markets. *Chemosphere* 84:923–927.
- Langer, P., A. Kocan, M. Tajtakova, J. Petrik, J. Chovancova, B. Drobna, S. Jursa, M. Pavuk, J. Koska, T. Trnovec, E. Sebkova, and I. Klimes. 2003. Possible effects of polychlorinated biphenyls and organochlorinated pesticides on the thyroid after long-term exposure to heavy environmental pollution. *J. Occup. Environ. Med.* 45:526–532.
- Lehotay, S. J., K. A. Son, H. Kwon, U. Koesukwiwat, W. Fu, K. Mastovska, E. Hoh, and N. Leepipatpiboon. 2010. Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables. *J. Chromatogr. A* 1217:2548–2560.
- Li, J., D. H. Liu, T. Wu, W. T. Zhao, Z. Q. Zhou, and P. Wang. 2014. A simplified procedure for the determination of organochlorine pesticides and polychlorobiphenyls in edible vegetable oils. *Food Chem.* 151:47–52.
- Lozano, A., L. Rajska, N. Belmonte-Valles, A. Uclés, S. Uclés, M. Mezcuca, and A. R. Fernández-Alba. 2012. Pesticide analysis in teas and chamomile by liquid chromatography and gas chromatography tandem mass spectrometry using a modified QuEChERS method: Validation and pilot survey in real samples. *J. Chromatogr. A* 1268:109–122.
- Lu, J., J. Wu, P. J. Stoffella, and P. C. Wilson. 2013. Analysis of bisphenol A, nonylphenol, and natural estrogens in vegetables and fruits using gas chromatography-tandem mass spectrometry. *J. Agric. Food Chem.* 61:84–89.
- Martins, J., C. Esteves, T. Simoes, M. Correia, and C. Delerue-Matos. 2011. Determination of 24 pesticide residues in fortified wines by solid-phase microextraction and gas chromatography-tandem mass spectrometry. *J. Agric. Food Chem.* 59:6847–6855.
- Mezcua, M., M. R. Repetti, A. Agüera, C. Ferrer, J. F. García-Reyes, and A. R. Fernández-Alba. 2007. Determination of pesticides in milk-based infant formulas by pressurized liquid extraction followed by gas chromatography tandem mass spectrometry. *Anal. Bioanal. Chem.* 389:1833–1840.
- Niell, S., V. Cesio, J. Hepperle, D. Doerk, L. Kirsch, D. Kolberg, E. Scherbaum, M. Anastasiades, and H. Heinzen. 2014. QuEChERS-based method for the multiresidue analysis of pesticides in beeswax by LC-MS/MS and GC×GC-TOF. *J. Agric. Food Chem.* 62:3675–3683.
- Norazlina, O., B. Jamilah, and M. Kharidah. 2013. Determination of organochlorine pesticides in shrimp by gas chromatography-mass spectrometry using a modified QuEChERS approach. *Food Contr.* 34:318–322.
- P. R. China. 2014. GB 2763–2014, National Food Safety Standard Maximum Residue Limits for Pesticides in Food. Beijing, China.
- Rashid, A., S. Nawaz, H. Barker, I. Ahmad, and M. Ashraf. 2010. Development of a simple extraction and clean-up procedure for determination of organochlorine pesticides in soil using gas chromatography-tandem mass spectrometry. *J. Chromatogr. A* 1217:2933–2939.
- Santhi, V. A., T. Hairin, and A. M. Mustafa. 2012. Simultaneous determination of organochlorine pesticides and bisphenol A in edible marine biota by GC-MS. *Chemosphere* 86:1066–1071.
- Smith, A. G., and S. D. Gangolli. 2002. Organochlorine chemicals in seafood: Occurrence and health concerns. *Food Chem. Toxicol.* 40:767–779.
- Song, W., Y. Q. Zhang, G. J. Li, H. Y. Chen, H. Wang, Q. Zhao, D. He, C. Zhao, and L. Ding. 2014. A fast, simple and green method for the extraction of carbamate pesticides from rice by microwave assisted steam extraction coupled with solid phase extraction. *Food Chem.* 143:192–198.
- Soto, A. M., and C. Sonnenschein. 2010. Environmental causes of cancer: Endocrine disruptors as carcinogens. *Nat. Rev. Endocrinol.* 6:363–370.
- Tanabe, S. 2002. Contamination and toxic effects of persistent endocrine disruptors in marine mammals and birds. *Mar. Pollut. Bull.* 45:69–77.
- Walorczyk, S., D. Drożdżyński, J. Kowalska, D. Remlein-Starosta, A. Ziolkowski, M. Przewoźniak, and B. Gnusowski. 2013. Pesticide residues determination in Polish organic crops in 2007–2010 applying gas chromatography-tandem quadrupole mass spectrometry. *Food Chem.* 139:482–487.
- Wittsiepe, J., M. Nestola, M. Kohne, P. Zinn, and M. Wilhelm. 2014. Determination of polychlorinated biphenyls and organochlorine pesticides in small volumes of human blood by high-throughput on-line SPE-LVI-GC-HRMS. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 945–946:217–224.
- Zacharis, C. K., I. Rotsias, P. G. Zachariadis, and A. Zotos. 2012. Dispersive liquid-liquid microextraction for the determination of organochlorine pesticides residues in honey by gas chromatography-electron capture and ion trap mass spectrometric detection. *Food Chem.* 134:1665–1672.
- Zhou, P., Y. Wu, S. Yin, J. Li, Y. Zhao, L. Zhang, H. Chen, Y. Liu, X. Yang, and X. Li. 2011. National survey of the levels of persistent organochlorine pesticides in the breast milk of mothers in China. *Environ. Pollut.* 159:524–531.
- Zhu, Y., B. Zhao, R. Q. Xiao, W. Yun, Z. J. Xiao, D. W. Tu, and S. Q. Chen. 2014. Simultaneous determination of 14 oil-soluble synthetic dyes in chili products by high performance liquid chromatography with a gel permeation chromatography clean-up procedure. *Food Chem.* 145:956–962.