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Simultaneous Determinations of Methoxylated Polybrominated Diphenyl Ethers and Polybrominated Diphenyl Ethers in Dairy Products from China by GC-MS

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Abstract: In this paper, the method of simultaneous determinations of methoxylated polybrominated diphenyl ethers and polybrominated diphenyl ethers in dairy products from China was investigated. PBDEs are widely used brominated flame retardant, which are increasingly reported in the environment. MeO-PBDEs are structural analogs to PBDEs, and reported as natural products and novel pollutants present in the environment. A new isotopic dilution GC-MS method was firstly developed to simultaneously determine fourteen PBDEs and eight MeO-PBDEs in dairy products (full cream milk powder, skim milk powder, pure milk and acidophilus milk) in this study. Solvent extraction, gel permeation chromatography (GPC) and silica gel column cleanup were used, some important steps and crucial parameters were modified and intensified compared with other literatures. Besides, the conditions of GC and MS were also optimized. The limits of quantitation values of 1-50, 4-20 μg kg^{-1} in full cream milk powders, skim milk powders, pure milks and acidophilus milks were calculated for PBDEs and MeO-PBDEs. In addition, good repeatability and accuracy of the whole method were achieved. Moreover, Eighteen kinds of commercial full cream milk powder samples, sixteen skim milk powder samples, twenty pure milk samples and fifteen acidophilus milk samples were collected from local supermarket in Wuhan, Hubei province. These samples were analyzed to determine whether these samples were contaminated by PBDEs and MeO-PBDEs. The method was successfully applied to determine methoxylated polybrominated diphenyl ethers and polybrominated diphenyl ethers in these dairy products. Using the established methods, PBDEs and MeO-PBDEs were not detected in all samples.

Keywords: MeO-PBDEs, PBDEs, Dairy Products, Isotopic Dilution, GC-MS

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are one class of halogenated organic brominated flame retardants (BFRs), and have been used industrially in large volumes for flame protection purposes in various commercial products such as electronic equipment and textiles. The commercial PBDEs products predominantly consist of so-called penta-, octa- and decabromodiphenyl ether products. Because of their physical, chemical and bio-accumulative characteristics, such as environmental persistence and high lipophilicity, these products have been widely distributed in the environment, for example air, dust, fish and human milk, where they are found to persist for a long time [1–8].

Methoxylated polybrominated diphenyl ethers (MeO-PBDEs) are structural analogs to PBDEs. Firstly, to the best of our knowledge, MeO-PBDEs are not produced commercially. Secondly, some studies have revealed the presence of MeO-PBDEs in relatively high concentrations in marine environments, indicating a natural origin [9–11]. Thirdly, the presence of MeO-PBDEs in Arctic biota has been suggested to be caused by metabolic processes of precursor PBDEs, bioaccumulation of PBDE degradation products

and/or naturally produced marine products [12]. A recent study also indicated that MeO-PBDEs occurred as metabolites of PBDEs [13]. In total, MeO-PBDEs have been considered anthropogenic compounds and reported as natural products present in the marine environment. In marine wildlife MeO-PBDEs were first analyzed in the aquatic Baltic environment, e.g., seal and fish from the Baltic Sea in year 1997 [14]. In recent years, MeO-PBDEs have been paid special attention mainly in biotic samples all over the world, and were found in eggs of white-tailed sea eagles breeding in different regions of Sweden [15], in fish and shellfish samples from the Mediterranean Sea [16], in mullet (Mugil cephalus) and sea bass (Dicentrarchus labrax) from Bizerte Lagoon, Tunisia [17], in mollusk and fish from the Bohai Sea and the Donghai Sea, China [18], in blue mussels from the Baltic Sea, Sweden [19], in Japanese common squid (Todarodes pacificus) from Korean offshore waters [20]. MeO-PBDEs are major contributors to the persistent organobromine load in sub-Arctic and Arctic marine mammals, covering a time period of more than 20 years [21].

As the PBDEs and MeO-PBDEs can be introduced into food chian by widespread environment pollution. Following concerns about contamination status of PBDEs and MeO-PBDEs in the dairy products, have led to the rising concern about the possible adverse health effects to humans. Toxicity studies indicate that the liver, thyroid gland and possibly also developing reproductive organs are particular targets of PBDEs toxicity [22, 23]. Evidence is emerging that PBDEs may be developmental neurotoxicants, as behavioural, neurochemical and hormonal deficits have been found following perinatal exposure [24–29]. PBDEs are capable to induce cell death of cerebellar granule cells in culture [30]. Madia et al reports PBDE-99 can induce apoptosis in astrocytoma cells assessed by the TUNEL method and by Hoechst 33258 staining, via a p53 dependent mechanism [31]. Our study indicates PBDE-209 can inhibit the proliferation of Hep G2 cells by inducing apoptosis through ROS generation [32]. A few researches about toxicity of MeO-PBDEs indicate the kind of compounds have effects on steroidogenic genes, aromatase activity and steroid hormones in vitro and may have the potential to affect steroidogenesis and reproduction in whole organisms [33, 34]. To satisfy the requirements of further accurate risk assessments for these chemicals, especially MeO-PBDEs, it is expected that the trend in generating MeO-PBDEs and PBDEs data will be encouraged to grow in dairy products samples.

Human exposure to PBDEs and MeO-PBDEs may occur via household dust, outdoor air, drinking water and food. PBDEs and MeO-PBDEs could be bio-accumulated in the food chain, in general, dietary intake was considered the most important route of human exposure. Dairy products have made and will always make important contributions to the human diet. They provide high quality protein and are good sources of vitamins and minerals, which satisfy the nutrient requirements of individuals. Therefore, it's necessary to quantify the content of PBDEs and MeO-PBDEs in dairy products. To our knowledge, there is little information about the degree of MeO-PBDEs and PBDEs contamination in dairy products in China. In addition, especially there are only few literatures about analysis method and contamination of PBDEs in human milk. The analysis of PBDEs and MeO-PBDEs in dairy products samples is difficult because they are usually present at μg kg^{-1} levels and the matrices involved are generally complex. Beyond that, dairy products usually contain a large number of proteins, the proteins have stronger adsorption to the PBDEs. Therefore, highly selective and sensitive techniques including sample preparation, cleanup, instrument and quantitative method are required.

The present work describes a simultaneous determinations of fourteen PBDEs and eight MeO-PBDEs in dairy products by isotopic dilution GC-MS which is always more reliable. Four common dairy products were choosen as samples, including full cream milk powder, skim milk powder, pure milk and acidophilus milk. In addition, the contamination of MeO-PBDEs and PBDEs in these dairy products from the Hubei province of China was investigated.

2. Experimental

2.1. Instrumentation and Reagents

A 7890B-5977A GC-MS with electron impact ionization (EI) was purchased from Agilent (USA).

Acetone was supplied from JT Baker (Phillipsburg, USA). ethyl acetate, cyclohexane, iso-octane, were supplied from CNW (Germany). Activated silica gel column (5 mm i.d., Wako gel S-1) was from Wako Pure Chemical Industries Ltd., (Osaka, Japan). Gel permeation chromatography (GPC) (Bio-Beads S-X3) was supplied from Bio-Rad Laboratories (CA, USA). Florisil (60-100 mesh) was from Sigma-aldrich (USA).

A standard solution of native MeO-PBDEs containing the congeners 5-MeO-BDE47, 6-MeO-BDE47, 4′-MeO-BDE49, 2′-MeO-BDE68, 5′-MeO-BDE99, 5′-MeO-BDE100, 4′-MeO-BDE101 and 4′-MeO-BDE103, at a concentration of 5 μg mL^{-1} in nonane and toluene (92:8) was supplied by Wellington Laboratories. A standard solution of native PBDEs containing congeners BDE17, BDE28, BDE47, BDE66, BDE71, BDE85, BDE99, BDE100, BDE138, BDE153, BDE154, BDE183 BDE190 and BDE209, at 5 μg mL^{-1} of each congener in iso-octane and toluene (97.5:2.5), was also obtained from AccuStandard, Inc. (USA). For quantification by isotope dilution and internal standard, a standard mixture of $^{13}C_{12}$-labelled PBDEs 28, 47, 99, 100, 154, 153 and 183 (MBDE-MXFS), supplied by Wellington Laboratories, at 2 μg mL^{-1} of each congener in toluene and nonane (74.8:25.2) was used as surrogate internal standard. In addition, a standard mixture of $^{13}C_{12}$-BDE77 and 138 (MBDE-MXFR) at a concentration of 2 μg mL^{-1} in toluene and nonane (92.8:7.2) (Wellington Laboratories) was used as syringe standard for recovery determination. Mixed series working standards of MeO-PBDEs and PBDEs containing MBDE-MXFS and MBDE-MXFR were prepared by serial dilutions of these stock solutions with iso-octane. The detailed information of

these standard substances was presented in Table 1.

2.2. Dairy Products Samples

Eighteen kinds of commercial full cream milk powder samples, sixteen skim milk powder samples, twenty pure milk samples and fifteen acidophilus milk samples were collected from local supermarket from Wuhan of Hubei province in March, 2015. All the samples were stored at 4 °C before extraction.

2.3. Determination of MeO-PBDEs and PBDEs

Analysis of MeO-PBDEs and PBDEs in dairy were prepared by using an literature method modified [38, 39]. Accurately weighed samples 2 g （liquid milk were freeze-dried）and mixed with 10 volumes of Florisil spiked with IS (1 mL of the concentrations of $^{13}C_{12}$-labelled PBDEs 28, 47, 99, 100, 154, 153 and 183 were 100 ng mL-1). The mixtures were wetpacked with ethyl acetate/cyclohexane (1:1, v/v) into a glass column (25.0 mm i.d. 30.0 cm). The the crude extracts were concentrated almost to dryness at 40℃ in a water bath by a K-D vacuum rotary concentrator, and 2.0 mL of ethyl acetate/cyclohexane (1:1, v/v) was added to dissolve the residue.

The resulting mixture was filtrated with a 0.22 um membrane and subjected to gel permeation chromatography (GPC) for lipid removal. The GPC column (30.0 mm i.d. 40.0 cm) was packed with about 60 g (dry weight) of 200–400 mesh S-X3 Bio-Beads that had been soaked in ethyl acetate/cyclohexane mixture (1:1, v/v) overnight, and the actual length of filling column was about 32.0 cm. The column was conditioned with ethyl acetate/cyclohexane (1:1, v/v), and the flow rate was 1.25 mL min^{-1}. The first lipid fraction (F1, 100 mL) from the GPC was discarded. The next fraction containing MeO-PBDEs and PBDEs (F2, 100 mL) was concentrated almost to dryness at 40℃ in a water bath by a K-D vacuum rotary concentrator, and 5.0 mL of iso-octane was added to dissolve the residue. Then the residue was transferred to a glass tube, and concentrated almost to dryness by a gentle stream of nitrogen. 1 mL of iso-octane was added to dissolve the residues.

The residues were concentrated and further purified by passage over 1 g of activated silica gel column , with elution with 15 mL ethyl acetate/cyclohexane (1:1, v/v). The eluate was reduced almost to dryness by a gentle stream of nitrogen. 0.95 mL of iso-octane followed by 0.05 mL syringe standard solution (the concentrations of $^{13}C_{12}$-BDE77 and 138 were 2 μg mL^{-1}) were added to dissolve the residue and transferred to an injection vial prior to GC-MS analysis. Sample blanks were taken through all aspects of the experimental procedure.

2.4. GC-MS Conditions

An Agilent 7890B-5977A GC-MS with electron impact ionization (EI) was used to the simultaneous determinations of MeO-PBDEs and PBDEs. An DB-5MS column (15 m×0.25 mm (id), 0.25 μm film thickness; Agilent, USA), was used to separate eight MeO-PBDEs, fourteen PBDEs, seven labeled PBDEs (surrogate internal standard) and other two labeled PBDEs (syringe standard).

The oven temperature was initially set at 150°C for 1 min, then increased to 325°C at a rate of 5°C min^{-1}, and held at 325°C for 5 min. The total runtime was 41 min. Helium was used as carrier gas at a constant flow rate of 1 mL min^{-1}. 4 microliters of samples and standards were injected in splitless injection mode at an injector temperature of 325°C.

MS operating conditions were the following: electron ionization mode using automatic gain control (AGC) with electron energy of 70 eV and an emission current of 250 μA. The transfer line and ion source temperatures were kept at 320°C and 230°C, respectively. The electron multiplier voltage was set to 370 V. Filament delay was set 4 min. The mass spectrometric date was acquired in SIM mode with selected ions for each analyt (Table 1).

2.5. Quantification and Quality Control

Our laboratory has established a quality assurance system as per ISO/IEC 17025: 2005 for strict controls over personnel, conditions of instruments, experimental situation, etc. Eight MeO-PBDEs and fourteen PBDEs were quantified by isotope dilution or internal standard using MBDE-MXFS, and the quantification of real samples was dealt with by isotope dilution or internal standard in this study. On the one hand, isotope dilution was used for calibration for MeO-PBDEs and PBDEs that have a labeled analog, for example, for BDE28, on the other hand, internal standard was applied to determination of MeO-PBDEs and PBDEs for which a labeled analog was not used in the study. The detailed information of quantitation reference for eight MeO-PBDEs and fourteen PBDEs, was given in Table 2.

In order to ensure the accuracy of the results and the applicability of the method in the study, in the case of repeated analyses of the spiking and real samples, the guideline ranges for the deviation of the experimentally determined recovery corrected mean mass fraction from the spiking value must meet the requirements of the European Union document 2002/657/EC: Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. In addition, the recovery efficiency of all MBDE-MXFS by MBDE-MXFR shall be 60% or better.

3. Results

3.1. GC-MS Parameters for MeO-PBDEs and PBDEs

According to GC-MS conditions in the second section, the information of retention times (RT), start time and end time of retention window, quantitation reference for MeO-PBDEs, PBDEs, MBDE-MXFS and MBDE-MXFR on DB-5MS were presented in Table 2.

Quantitative determination by GC-MS (EI+) was in the selected ion monitoring (SIM) mode. The ions monitored for PBDEs and MeO-PBDEs, including labeled PBDEs were presented in Table 1.

Table 1. *The information and the ions monitored of fourteen PBDEs, eight MeO-PBDEs, MBDE-MXFS and MBDE-MXFR.*

Br No.	Abbreviation	Molar mass	Precursor ions, $[M]^+$ and $[M+2]^+$, or $[M-2]^+$ (m/z)	Product ions, $[M-2Br]^+$ and $[(M+2)-2Br]^+$, or $[(M-2)-2Br]^+$ (m/z)	The ions monitored (m/z)
PBDEs					
3	BDE17	407	406, $[M+2]^+$	246, 248	406,408,246,248
3	BDE28	407	406, $[M+2]^+$	246, 248	406,408,246,248
4	BDE71	486	486, $[M-2]^+$	326, 328	484,486,326,328
4	BDE47	486	486, $[M-2]^+$	326, 328	484,486,326,328
4	BDE66	486	486, $[M-2]^+$	326, 328	484,486,326,328
5	BDE100	565	564, $[M+2]^+$	404, 406	564,566,404,406
5	BDE99	565	564, $[M+2]^+$	404, 406	564,566,404,406
5	BDE85	565	564, $[M+2]^+$	404, 406	564,566,404,406
6	BDE154	644	644, $[M-2]^+$	484, 486	642,644,484,486
6	BDE153	644	644, $[M-2]^+$	484, 486	642,644,484,486
6	BDE138	644	644, $[M-2]^+$	484, 486	642,644,484,486
7	BDE183	723	722, $[M+2]^+$	562, 564	722,724,562,564
7	BDE190	723	722, $[M+2]^+$	562, 564	722,724,562,564
10	BDE209	960	961, $[M-2]^+$	799,801	799,801,959
MeO-PBDEs					
4	2′-MeO-BDE68	516	516, $[M-2]^+$	420, 422, $^*[M-CH_3Br]^+$	514,516,420,422
4	6-MeO-BDE47	516	516, $[M-2]^+$	356, 420, 422, $^*[M-CH_3Br]^+$	514,516,420,422,356
4	5-MeO-BDE47	516	516, $[M-2]^+$	356, 358	514,516,356,358
4	4′-MeO-BDE49	516	516, $[M-2]^+$	356, 358	514,516,356,358
5	5′-MeO-BDE100	595	596, $[M-2]^+$	434, 436	594,596,434,436
5	4′-MeO-BDE103	595	596, $[M-2]^+$	434, 436	594,596,434,436
5	5′-MeO-BDE99	595	596, $[M-2]^+$	434, 436	594,596,434,436
5	4′-MeO-BDE101	595	596, $[M-2]^+$	434, 436	594,596,434,436
MBDE-MXFS					
3	$^{13}C_{12}$-BDE-28	419	418, $[M+2]^+$	258, 260	418,420,258,260
4	$^{13}C_{12}$-BDE-47	500	498, $[M+2]^+$	338, 340	498,500,338,340
5	$^{13}C_{12}$-BDE-100	577	576, $[M+2]^+$	416, 418	576,578,416,418
5	$^{13}C_{12}$-BDE-99	577	576, $[M+2]^+$	416, 418	576,578,416,418
6	$^{13}C_{12}$-BDE-154	656	656, $[M-2]^+$	494, 496	654,656,496,498
6	$^{13}C_{12}$-BDE-153	656	656, $[M+2]^+$	494, 496	656,658,494,496
7	$^{13}C_{12}$-BDE-183	735	734, $[M+2]^+$	574, 576	734,736,574,576
MBDE-MXFR					
4	$^{13}C_{12}$-BDE-77	500	498, $[M+2]^+$	338, 340	498,500,336,338
6	$^{13}C_{12}$-BDE-138	656	656, $[M+2]^+$	494, 496	656,658,496,498

The ion of underline was indicated for quantitative analysis.

Table 2. *Retention times (RT), start time and end time of retention window, quantitation reference for MeO-PBDEs, PBDEs, MBDE-MXFS and MBDE-MXFR on DB-5MS, and LOQ of MeO-PBDEs and PBDEs in full cream milk powders, skim milk powders, pure milks and acidophilus milks.*

Compounds	Quantitation reference	Retention times (RT)	Start time and end time of Retention Window	LOQ	
				full cream milk powders and skim milk powders (μg kg^{-1})	pure milks and acidophilus milks (μg kg^{-1})
Compounds using $^{13}C_{12}$-BDE-77 as Labeled injection internal standard					
BDE17	$^{13}C_{12}$-BDE-28	11.48	4～12	1	1
BDE28	$^{13}C_{12}$-BDE-28	12.18	12～13	1	1
BDE71	$^{13}C_{12}$-BDE-47	15.66	13～16	2	2
BDE47	$^{13}C_{12}$-BDE-47	16.15	16～16.5	2	2
BDE66	$^{13}C_{12}$-BDE-47	16.82	16.5～17.0	2	2
BDE100	$^{13}C_{12}$-BDE-100	19.05	18.6～19.15	4	4
BDE99	$^{13}C_{12}$-BDE-99	19.95	19.8～20.2	4	4
BDE85	$^{13}C_{12}$-BDE-99	21.44	21.4～22.1	4	4
Compounds using $^{13}C_{12}$-BDE-138 as Labeled injection internal standard					
BDE154	$^{13}C_{12}$-BDE-154	22.19	22.1～22.5	4	4
BDE153	$^{13}C_{12}$-BDE-153	23.41	23.2～24.7	4	4
BDE138	$^{13}C_{12}$-BDE-153	24.92	24.7～26.0	10	10

Compounds	Quantitation reference	Retention times (RT)	Start time and end time of Retention Window	LOQ	
				full cream milk powders and skim milk powders (μg kg^{-1})	pure milks and acidophilus milks (μg kg^{-1})
BDE183	$^{13}C_{12}$-BDE-183	26.57	26.0～28.0	10	10
BDE190	$^{13}C_{12}$-BDE-183	28.55	28.0～30.0	20	20
BDE209	$^{13}C_{12}$-BDE-183	38.35	30.0～41	50	50
Compounds using $^{13}C_{12}$-BDE-77 as Labeled injection internal standard					
2′-MeO-BDE68	$^{13}C_{12}$-BDE-100	17.70	17.0～18.1	4	4
6-MeO-BDE47	$^{13}C_{12}$-BDE-100	18.25	18.1～18.6	4	4
5-MeO-BDE47	$^{13}C_{12}$-BDE-100	19.20	19.15～19.4	4	4
4′-MeO-BDE49	$^{13}C_{12}$-BDE-100	19.42	19.4～19.8	10	10
Compounds using $^{13}C_{12}$-BDE-138 as Labeled injection internal standard					
5′-MeO-BDE100	$^{13}C_{12}$-BDE-154	21.17	20.2～21.4	10	10
4′-MeO-BDE103	$^{13}C_{12}$-BDE-154	21.44	21.4～22.1	10	10
5′-MeO-BDE99	$^{13}C_{12}$-BDE-154	22.50	22.5～22.81	10	10
4′-MeO-BDE101	$^{13}C_{12}$-BDE-154	22.78	22.81～23.2	20	20
Labeled compounds					
$^{13}C_{12}$-BDE-28	$^{13}C_{12}$-BDE-77	12.18	12～13	—	—
$^{13}C_{12}$-BDE-47	$^{13}C_{12}$-BDE-77	16.15	16～16.5	—	—
$^{13}C_{12}$-BDE-100	$^{13}C_{12}$-BDE-77	19.05	18.6～19.15	—	—
$^{13}C_{12}$-BDE-99	$^{13}C_{12}$-BDE-77	19.95	19.8～20.2	—	—
$^{13}C_{12}$-BDE-154	$^{13}C_{12}$-BDE-138	22.19	22.1～22.5	—	—
$^{13}C_{12}$-BDE-153	$^{13}C_{12}$-BDE-138	23.41	23.2～24.7	—	—
$^{13}C_{12}$-BDE-183	$^{13}C_{12}$-BDE-138	26.57	26.0～28.0	—	—
Labeled injection internal standards					
$^{13}C_{12}$-BDE-77	$^{13}C_{12}$-BDE-77	17.73	17.0～18.1	—	—
$^{13}C_{12}$-BDE-138	$^{13}C_{12}$-BDE-138	24.91	24.7～26.0	—	—

The transverse line was indicated no data.

Table 3. *Seven mixed series working standards of MeO-PBDEs, PBDEs, MBDE-MXFS and MBDE-MXFR.*

Br No.	Compound	Solutions (Calibration standard solutions were prepared by dilution of standard stock solutions in iso-octane)						
		1	2	3	4	5	6	7
		ng mL^{-1}						
PBDEs		PBDEs standard stock solutions, Mixture of Commonly PBDE Congeners, 5 μg mL^{-1} in iso-octane and toluene (97.5:2.5)						
3	BDE17	5	10	25	50	100	150	250
3	BDE28	5	10	25	50	100	150	250
4	BDE71	5	10	25	50	100	150	250
4	BDE47	5	10	25	50	100	150	250
4	BDE66	5	10	25	50	100	150	250
5	BDE100	5	10	25	50	100	150	250
5	BDE99	5	10	25	50	100	150	250
5	BDE85	5	10	25	50	100	150	250
6	BDE154	5	10	25	50	100	150	250
6	BDE153	5	10	25	50	100	150	250
6	BDE138	5	10	25	50	100	150	250
7	BDE183	5	10	25	50	100	150	250
7	BDE190	5	10	25	50	100	150	250
10	BDE209	5	10	25	50	100	150	250
MeO-PBDEs		MeO-PBDEs standard stock solutions, 5 μg mL^{-1} in nonane and toluene (92:8)						
4	2′-MeO-BDE68	5	10	25	50	100	150	250
4	6-MeO-BDE47	5	10	25	50	100	150	250
4	5-MeO-BDE47	5	10	25	50	100	150	250
4	4′-MeO-BDE49	5	10	25	50	100	150	250
5	5′-MeO-BDE100	5	10	25	50	100	150	250
5	4′-MeO-BDE103	5	10	25	50	100	150	250
5	5′-MeO-BDE99	5	10	25	50	100	150	250
5	4′-MeO-BDE101	5	10	25	50	100	150	250
MBDE-MXFS		Surrogate internal standard for EI+, 2 μg mL^{-1} of each congener in toluene and nonane (74.8:25.2)						
3	$^{13}C_{12}$-BDE-28	100	100	100	100	100	100	100

Br No.	Compound	Solutions (Calibration standard solutions were prepared by dilution of standard stock solutions in iso-octane)						
		1	2	3	4	5	6	7
		ng mL^{-1}						
4	$^{13}C_{12}$-BDE-47	100	100	100	100	100	100	100
5	$^{13}C_{12}$-BDE-100	100	100	100	100	100	100	100
5	$^{13}C_{12}$-BDE-99	100	100	100	100	100	100	100
6	$^{13}C_{12}$-BDE-154	100	100	100	100	100	100	100
6	$^{13}C_{12}$-BDE-153	100	100	100	100	100	100	100
7	$^{13}C_{12}$-BDE-183	100	100	100	100	100	100	100
MBDE-MXFR		Syringe standards, 2 μg mL^{-1} in toluene and nonane (92.8:7.2)						
4	$^{13}C_{12}$-BDE-77	100	100	100	100	100	100	100
6	$^{13}C_{12}$-BDE-138	100	100	100	100	100	100	100

Table 4. *Average recoveries and standard deviation of MeO-PBDEs and PBDEs in full cream milk powders, skim milk powders, pure milks and acidophilus milks (%R±SD, n=6) (μg kg^{-1} dry weight).*

Br No.	Compound	Quantitation reference	Recoveries from full cream milk powders			Recoveries from skim milk powders			Recoveries from pure milks			Recoveries from acidophilus milks		
			2.5	10	25	2.5	10	25	2.5	10	25	2.5	10	25
Compounds using $^{13}C_{12}$-BDE-77 as Labeled injection internal standard														
3	BDE17	$^{13}C_{12}$-BDE-28	81±7	93±5	95±6	71±10	83±8	88±12	88±5	91±5	102±6	91±13	99±8	103±7
3	BDE28	$^{13}C_{12}$-BDE-28	86±7	95±5	92±8	89±13	92±7	91±8	72±6	98±7	97±9	72±6	100±8	101±4
4	BDE71	$^{13}C_{12}$-BDE-47	75±4	88±10	92±5	75±11	90±11	91±10	77±6	101±9	101±7	88±11	98±7	96±6
4	BDE47	$^{13}C_{12}$-BDE-47	87±5	85±5	92±7	84±5	82±8	91±10	87±7	99±6	101±7	81±7	100±5	101±2
4	BDE66	$^{13}C_{12}$-BDE-47	78±9	86±5	93±12	75±5	90±10	90±10	84±9	101±9	95±9	88±10	95±6	97±5
5	BDE100	$^{13}C_{12}$-BDE-100	81±13	83±5	95±5	91±13	86±9	96±4	89±8	103±8	103±8	87±8	99±8	98±6
5	BDE99	$^{13}C_{12}$-BDE-99	81±14	85±7	88±9	81±10	86±7	93±10	90±12	96±7	97±5	93±10	93±10	102±5
5	BDE85	$^{13}C_{12}$-BDE-99	78±16	85±6	82±6	78±5	91±9	92±8	87±5	93±6	104±5	86±7	86±5	97±7
Compounds using $^{13}C_{12}$-BDE-138 as Labeled injection internal standard														
6	BDE154	$^{13}C_{12}$-BDE-154	74±5	82±6	85±7	80±6	85±8	88±12	87±9	96±8	96±9	86±4	90±9	101±7
6	BDE153	$^{13}C_{12}$-BDE-153	75±7	82±4	87±8	73±7	82±5	93±6	88±9	90±7	92±4	82±8	89±7	98±8
6	BDE138	$^{13}C_{12}$-BDE-153	72±8	81±6	90±7	71±6	85±10	82±4	85±7	87±6	94±7	83±11	90±9	90±10
7	BDE183	$^{13}C_{12}$-BDE-183	83±8	83±3	85±5	78±9	87±12	85±4	85±4	94±11	91±8	75±8	91±10	91±6
7	BDE190	$^{13}C_{12}$-BDE-183	—	80±14	84±5	—	82±14	84±8	—	91±9	90±5	—	88±9	87±6
Compounds using $^{13}C_{12}$-BDE-77 as Labeled injection internal standard														
4	2′-MeO-BDE68	$^{13}C_{12}$-BDE-100	82±12	92±7	102±10	77±9	92±3	96±12	82±6	103±6	102±6	100±9	102±8	102±6
4	6-MeO-BDE47	$^{13}C_{12}$-BDE-100	76±7	93±13	96±8	75±8	100±6	100±9	100±6	98±8	98±8	103±7	103±9	103±7
4	5-MeO-BDE47	$^{13}C_{12}$-BDE-100	78±8	99±8	97±4	75±5	92±10	94±12	98±9	99±8	101±9	92±11	98±7	100±6
4	4′-MeO-BDE49	$^{13}C_{12}$-BDE-100	79±8	86±9	91±6	89±4	95±8	95±7	95±7	92±6	100±4	93±7	94±7	100±5
Compounds using $^{13}C_{12}$-BDE-138 as Labeled injection internal standard														
5	5′-MeO-BDE100	$^{13}C_{12}$-BDE-154	79±12	86±6	91±4	76±11	86±5	85±5	95±4	97±5	102±8	88±8	99±7	100±7
5	4′-MeO-BDE103	$^{13}C_{12}$-BDE-154	78±7	85±4	87±4	72±6	87±10	89±10	86±5	97±6	100±7	89±12	104±7	98±12
5	5′-MeO-BDE99	$^{13}C_{12}$-BDE-154	80±7	84±7	88±5	80±13	85±10	89±6	88±10	101±7	100±6	86±8	94±6	103±5
5	4′-MeO-BDE101	$^{13}C_{12}$-BDE-154	—	82±12	88±5	—	81±8	87±5	—	97±7	95±6	—	92±6	94±4

The transverse line was indicated no data because the spiked amount was below LOQ.

3.2. Calibration Curves, Limits of Quantitation and Recovery Rates

Seven mixed series working standards of MeO-PBDEs and PBDEs, including MBDE-MXFS and MBDE-MXFR were prepared according to Table 3. Linear calibration curves for MeO-PBDEs and PBDEs by isotopic dilution and internal standard method were obtained with a γ2 correlation coefficient of more than 0.99. The linearity was checked by calculating the standard deviation of the average of response factors (peak area ratios divided by the corresponding analyte concentration ratios of all standards), which was <15% assuming a linear response.

The limit of quantitation (LOQ), defined as the concentration of analyte which yielded a peak-to-peak signal-to-noise ratio of at least 10:1, was calculated by running a series of 10 negative extracts. The detailed information for LOQ of full cream milk powder, skim milk powder, pure milk and acidophilus milk was presented in Table 2.

Spike recoveries of MeO-PBDEs and PBDEs in these productions were studied. Table 4 provided further information. Average recoveries and standard deviation (SD) of all concentrations analyzed triple samples met the requirements of the European Union document 2002/657/EC: Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. In addition, the recovery efficiency of all MBDE-MXFS by

MBDE-MXFR was more than 80%.

3.3. Analysis of Dairy Products

GC-MS chromatogram of a mixed standard solution of MeO-PBDEs and PBDEs (the concentration of BDE17, BDE28, BDE47, BDE66, BDE71, BDE85, BDE99, BDE100, BDE138, BDE153, BDE154, BDE183 BDE190, and BDE209 were 100 ng mL^{-1}, respectively, the concentration of 5-MeO-BDE47, 6-MeO-BDE47, 4′-MeO-BDE49, 2′-MeO-BDE68, 5′-MeO-BDE99, 5′-MeO-BDE100, 4′-MeO-BDE101 and 4′-MeO-BDE103 were 100 ng mL^{-1}, respectively, the concentrations of $^{13}C_{12}$-labelled PBDEs 28, 47, 99, 100, 154, 153 and 183 as surrogate internal standard were 100 ng mL^{-1}, respectively, and the concentration of $^{13}C_{12}$-labelled PBDEs 77 and 138 as syringe standard were 100 ng mL^{-1}, respectively), was shown in Figure 1. Because PBDEs, MeO-PBDEs and isotope internal standards have similar structure and property, it causes some of those substances have same chromatographic behavior，which is difficult to separate.

Using the established methods, 18 full cream milk powder samples, 16 skim milk powder samples, 20 pure milk samples and 15 acidophilus milk samples were tested. MeO-PBDEs or PBDEs were not found in all dairy products. GC-MS chromatogram of the spiked pure milk sample (the spiked level was 100 μg kg^{-1}) was given in Figure 2. GC-MS chromatogram of the blank pure milk sample was given in Figure 3.

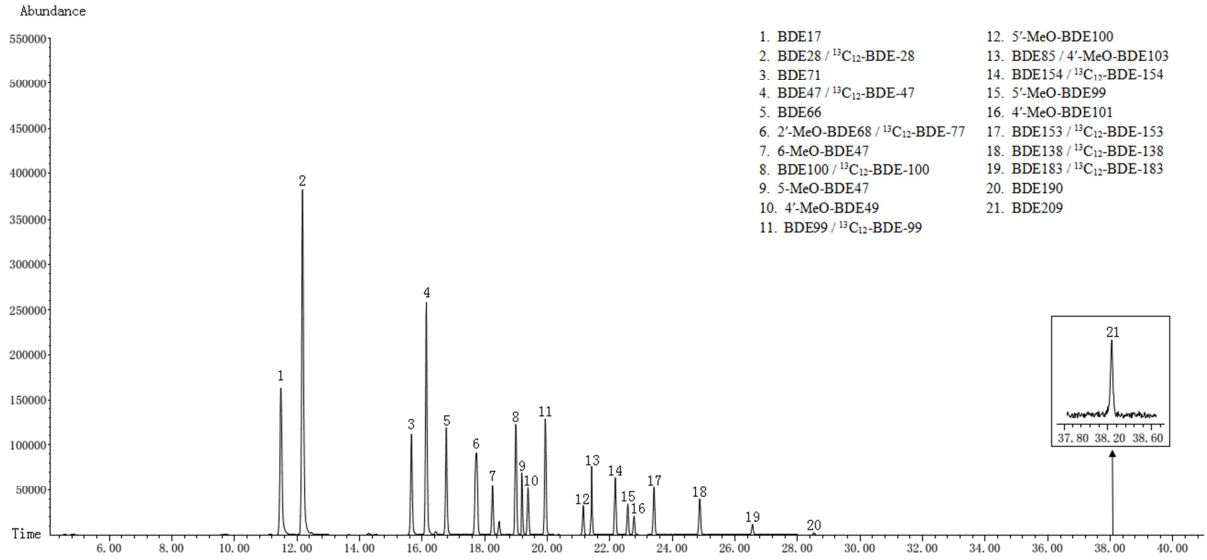

Figure 1. *GC-MS chromatogram of a mixed standard solution of MeO-PBDEs and PBDEs (the concentration of BDE17, BDE28, BDE47, BDE66, BDE71, BDE85, BDE99, BDE100, BDE138, BDE153, BDE154, BDE183, BDE190 and BDE209 were 100 ng mL^{-1}, respectively, the concentration of 5-MeO-BDE47, 6-MeO-BDE47, 4′-MeO-BDE49, 2′-MeO-BDE68, 5′-MeO-BDE99, 5′-MeO-BDE100, 4′-MeO-BDE101 and 4′-MeO-BDE103 were 100 ng mL^{-1}, respectively, the concentrations of $^{13}C_{12}$-labelled PBDEs 28, 47, 99, 100, 154, 153 and 183 were 100 ng mL^{-1}, respectively, and the concentration of $^{13}C_{12}$-labelled PBDEs 77 and 138 were 100 ng mL^{-1}, respectively).*

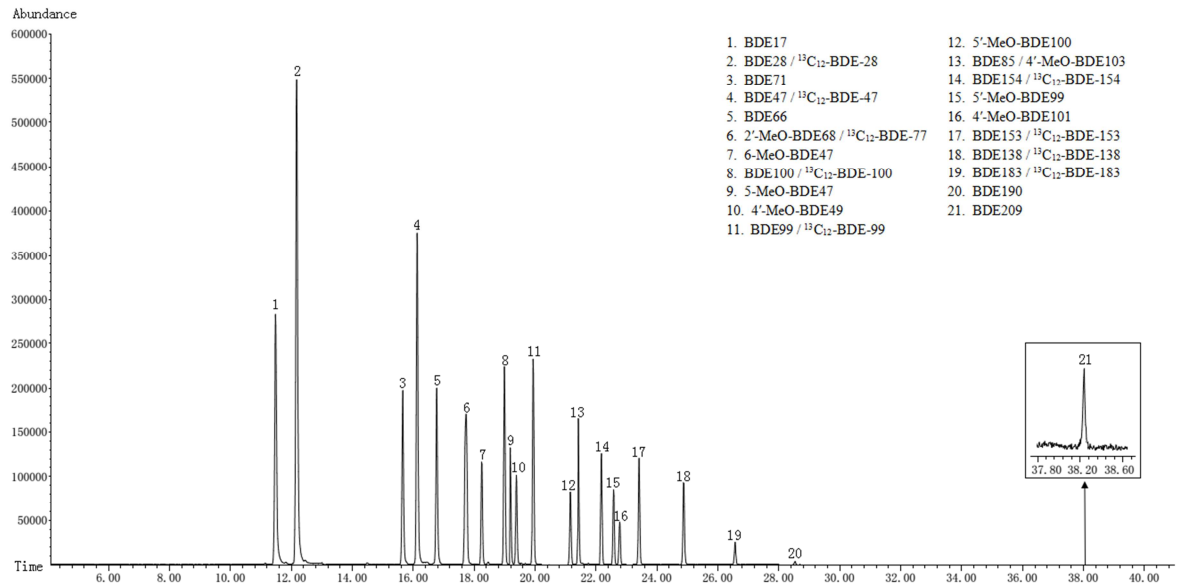

Figure 2. *GC-MS chromatogram of a blank pure milk extract fortified with MeO-PBDEs and PBDEs at 100 μg kg^{-1}.*

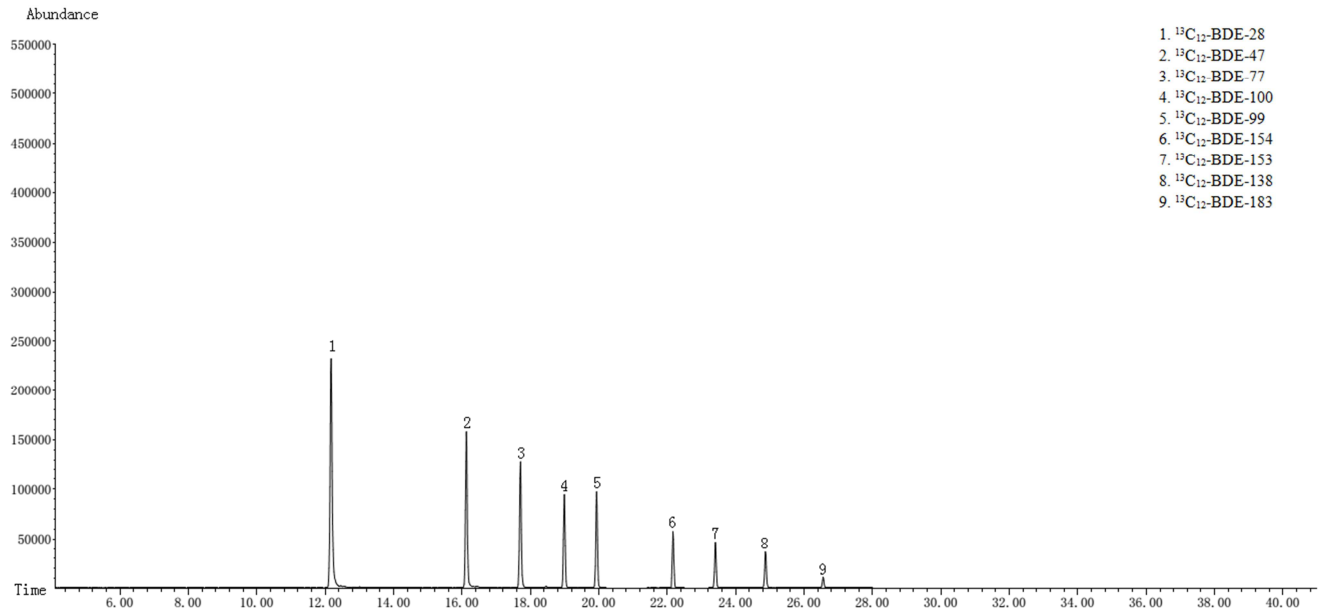

Figure 3. *GC-MS chromatogram of a blank pure milk extract.*

4. Discussion

The method to simultaneously determine eight MeO-PBDEs and fourteen PBDEs in full cream milk powders, skim milk powders, pure milks and acidophilus milks, was principally described by two methods [38, 39] as indicated above, but some important steps and crucial parameters were modified and intensified in this study. The main aspects regarding sample extraction and cleanup for sample preparations were intensified during the development of the method used.

Firstly, for sample extraction and cleanup, in contrast to the described methods [38, 39], the elution ethyl acetate was environmentally friendly reagent. As it is known, dichloromethane has strong toxicity to human and environment, and it also a potential carcinogen. Ethyl acetate was nontoxic, using it can be avoided the damage to the operating personnel.

Next, in comparison, the silica gel chromatography can only be used once. By eluting repeatedly, the Gel permeation chromatography can be used over and over again. On the other hand, GPC cleanup for lipid removal was a very important step for determination of MeO-PBDEs and PBDEs in dairy products, which was used in some literatures, but several crucial parameters in experiments were missing such as the flow rate, lipid fraction volume (F1) and fraction volume containing MeO-PBDEs and PBDEs (F2) by GPC. In contrast to the described methods (38), similarly, some details in the present study were studied to ensure the experimental feasibility. Obviously, the flow rate in GPC cleanup is a very important factor for fraction volume containing MeO-PBDEs and PBDEs collected. The high flow rate is disadvantageous for MeO-PBDEs and PBDEs separation and lipid removal, and low flow rate is time- and solvent-consuming. The flow rate of GPC in this study was optimized and 1.25 mL min^{-1} was chosen to ensure MeO-PBDEs and PBDEs separation and lipid removal. Using the flow rate of 1.25 mL min^{-1}, lipid fraction volume (F1) and fraction volume containing MeO-PBDEs and PBDEs (F2) collected from GPC were studied according to the below method of description. 0.4 mL of the mixed working standard (the concentration of BDE17, BDE28, BDE47, BDE66, BDE71, BDE85, BDE99, BDE100, BDE138, BDE153, BDE154, BDE183, BDE190 and BDE209 were 100 ng mL^{-1}, respectively, the concentration of 5-MeO-BDE47, 6-MeO-BDE47, 4′-MeO-BDE49, 2′-MeO-BDE68, 5′-MeO-BDE99, 5′-MeO-BDE100, 4′-MeO-BDE101 and 4′-MeO-BDE103 were 100 ng mL^{-1}, respectively, the concentrations of $^{13}C_{12}$-labelled PBDEs 28, 47, 99, 100, 154, 153 and 183 were 100 ng mL^{-1}, respectively, and the concentration of $^{13}C_{12}$-labelled PBDEs 77 and 138 were 100 ng mL^{-1}, respectively) was subjected to GPC, and the eight ordinal fractions (f1, f2, f3, f4, f5, f6, f7 and f8), 25 mL viz. 30 min of time-consuming per fraction, were collected from GPC at flow rate of 1.25 mL min^{-1}. The next experiments were conducted according to the above method. The results showed that native MeO-PBDEs and PBDEs were only detected in the fraction f5, and the recoveries of all these compounds were excellent. Similarly, the blank samples of full cream milk powders, skim milk powders, pure milks and acidophilus milks spiked at the same level as above mixed working standard were prepared prior to sample extractions, and the sample extractions were also carried out. The sample cleanup of GPC and following experiments were conducted according to the above method for the mixed working standard. It was also interesting that native MeO-PBDEs and PBDEs and labeled MeO-PBDEs and PBDEs were only detected in the fraction f5, and the recoveries of all these compounds ranged from 75.5% to 105.0%. Moreover, beginning with fraction f5, the fractions

were clean and contained little lipid, which indicated the lipid removal was successful. Therefore, the first lipid fraction (F1, 100 mL) from the GPC was the sum of f1, f2, f3 and f4 and discarded, and the next fraction containing MeO-PBDEs and PBDEs (F2, 100 mL) was the sum of f5, f6, f7 and f8 and collected before following experiments in the present study.

Finally, GC and MS conditions were also very important factors for determinations of MeO-PBDEs and PBDEs, including $^{13}C_{12}$-labelled PBDEs. For MS acquisition, qualitative and quantitative analysis were performed using SIM. Table 1 provided further information optimized for these parameters, especially about quantitation reference for eight MeO-PBDEs and fourteen PBDEs, ensuring the operability of quantitation analysis. GC conditions were also optimized, including temperature programmed conditions and separation column. Temperature programmed conditions were optimized to ensure excellent separation efficiency of MeO-PBDEs, PBDEs and $^{13}C_{12}$-labelled PBDEs. Different separation columns were selected and compared to gain a more suitable separation effect, ensuring excellent experimental results.

For analysis of PBDEs and MeO-PBDEs in full cream milk powders, skim milk powders, pure milks and acidophilus milks, it was reported that PBDEs and MeO-PBDEs was present in soil and sediment at relatively high levels [37]. Six MeO-PBDEs were detected in a soil and sediment sample ranging from less than detection to 43.7 pg g-1 dry weight per MeO-PBDE, and thirty-two PBDEs in the same soil and sediment ranging from less than detection to 1249 pg g-1 dry weight per PBDE [37].

On the basis of those reported results , MeO-PBDEs and PBDEs was possible to be found in dairy products, which was considered to be migrated from contaminated water, soil and sediment. So it is reasonable to conclude that the MeO-PBDEs and PBDEs contamination in dairy products from China should be thoroughly investigated. Although all dairy products in this study have been fortunately proved to be MeO-PBDEs and PBDEs negative, considering the direct links between the safety of dairy products and environmental pollution, contaminations and causes of MeO-PBDEs as novel pollutants and PBDEs in dairy products from China still need to be investigated in the future.

5. Conclusion

In this paper, A new isotopic dilution GC-MS method was firstly developed to simultaneously determine fourteen PBDEs and eight MeO-PBDEs in dairy products (full cream milk powder, skim milk powder, pure milk and acidophilus milk). Solvent extraction, gel permeation chromatography (GPC) and silica gel column cleanup were used, some important steps and crucial parameters were modified and intensified compared with other literatures. Besides, the conditions of GC and MS were also optimized. The limits of quantitation values of 1-50, 4-20 μg kg^{-1} in full cream milk powders, skim milk powders, pure milks and acidophilus milks were calculated for PBDEs and MeO-PBDEs. In addition, good repeatability and accuracy of the whole method were achieved. Therefore, the method was successfully applied to determine methoxylated polybrominated diphenyl ethers and polybrominated diphenyl ethers in dairy products.

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