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Analysis and occurrences of methoxylated polybrominated diphenyl ethers and polybrominated diphenyl ethers in channel catfish, crayfish, fish feeds and fishmeal from China

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Abstract: 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. Concentrations of thirteen PBDEs and eight MeO-PBDEs in a large number of channel catfish, crayfish, fish feeds and fishmeal collected from Hubei province of China were investigated in this study. A fast isotopic dilution GC-MS method was firstly developed to simultaneously determine thirteen PBDEs and eight MeO-PBDEs in channel catfish, crayfish, fish feeds and fishmeal in this study, and especially for the first time MeO-PBDEs and PBDEs in crayfish, fish feeds and fishmeal. Pressurized liquid extraction and multi-layer silica gel column chromatography cleanup were used, and 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 0.25-5, 1-5 μg kg^{-1} wet weight in channel catfish and crayfish were calculated for PBDEs and MeO-PBDEs, respectively; so did 1-20, 4-20 μg kg^{-1} wet weight in fish feeds and fishmeal. In addition, good repeatability and accuracy of the whole method were achieved. The established methods were therefore suitable for the simultaneous determinations of thirteen PBDEs and eight MeO-PBDEs in these samples at trace contamination levels. Using the established methods, PBDEs or MeO-PBDEs emerged in 2 of 80 channel catfish, 1 of 80 crayfish, 6 of 60 fish feeds and 4 of 40 fishmeal, and in low-μg kg^{-1} wet weight for these samples.

Keywords: Methoxylated Polybrominated Diphenyl Ethers (MeO-PBDEs), Channel Catfish, Crayfish, Fish Feeds, Fishmeal

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. They have been widely distributed in the air, dust, fish and human milk due to their physical, chemical and bio-accumulative characteristics, such as environmental persistence and high lipophilicity [1–8].

Methoxylated polybrominated diphenyl ethers (MeO-PBDEs) are structural analogs to PBDEs, which have been considered synthetic 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 [9]. 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 [10], in fish and shellfish samples from the Mediterranean Sea [11], in mullet (Mugil cephalus) and sea bass (Dicentrarchus labrax) from Bizerte Lagoon, Tunisia [12], in mollusk and fish from the Bohai Sea and the Donghai Sea, China [13], in blue mussels from the Baltic Sea, Sweden [14], in fish from local supermarket in Catalonia, Spain [15], and in Japanese common squid (Todarodes pacificus) from Korean offshore waters [16]. 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 [17].

Following concerns about contamination status of PBDEs and MeO-PBDEs in the environments, have led to the rising concern about the possible adverse health effects to humans. Toxicity studies indicate that the liver, thyroid gland and possibly developmental reproductive organs are particular targets of PBDEs toxicity [18, 19]. Evidence is emerging that PBDEs may be developmental neurotoxicants, as behavioural, neurochemical and hormonal deficits have been found following perinatal exposure [20–25]. PBDEs are capable to induce cell death of cerebellar granule cells in culture [26]. 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 [27]. Our study indicates PBDE-209 and PBDE-47 can inhibit the proliferation of Hep G2 cells by inducing apoptosis through ROS or NO generation [28, 29]. 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 [30, 31]. 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 environmental and biotic samples.

The Hubei province of China has an old farm and aquaculture production tradition. Especially there are a plenty of channel catfish and crayfish from Hubei exported to other countries. These productions are favorite food for people and their safety has been highly concerned by our previous papers [32, 33]. Moreover, fish feeds are one kind of farm products, their contaminations have also been concerned in our previous papers [33, 34]. To our knowledge, there is little information about the degree of MeO-PBDEs and PBDEs contamination in channel catfish, crayfish, fish feeds and fishmeal from the region in China. In addition, there are only few literatures about simultaneous analysis method and contamination of MeO-PBDEs and PBDEs in fish [11–13, 16–17], especially no literature about analysis method and contamination in crayfish, fish feeds and fishmeal. The analysis of PBDEs and MeO-PBDEs in biological samples is difficult because they are usually present at μg kg^{-1} levels and the matrices involved are generally complex. Therefore, highly selective and sensitive techniques including sample preparation, cleanup, instrument and quantitative method are required.

Since the two kinds of compounds usually exit in a same environment compartment, the present work describes a simultaneous determinations of thirteen PBDEs and eight MeO-PBDEs in channel catfish, crayfish, fish feeds and fishmeal by isotopic dilution GC-MS which is always more reliable and especially for the first time for simultaneous determinations of MeO-PBDEs and PBDEs in crayfish, fish feeds and fishmeal. In addition, the contamination of MeO-PBDEs and PBDEs in these samples matrix from the Hubei province of China was investigated, and the contamination of MeO-PBDEs was more concerned.

2. Material and Methods

2.1. Chemicals and Reagents

Acetone was supplied from JT Baker (Phillipsburg, USA). Cyclohexane, dichloromethane (DCM) and iso-octane were supplied from CNW (Germany). Anhydrous sodium sulfate (Na_2SO_4) (p.a.) was from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Florisil was from Sigma–Aldrich Laborchemikalien GmbH (Seelze, Germany). Silica gel 60 (0.063–0.200 mm, 70–230 mesh ASTM) was from Merck (Darmstadt, Germany).

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 and BDE190, 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.

Table 1. *The information and the ions monitored of thirteen 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)	No. of MS Scan Functions
PBDEs						
3	BDE17	407	406, $[M+2]^+$	246, 248	406,408,<u>246</u>,248	2
3	BDE28	407	406, $[M+2]^+$	246, 248	406,408,<u>246</u>,248	4
4	BDE71	486	486, $[M-2]^+$	326, 328	484,486,<u>326</u>,328	5
4	BDE47	486	486, $[M-2]^+$	326, 328	484,486,<u>326</u>,328	6
4	BDE66	486	486, $[M-2]^+$	326, 328	484,486,<u>326</u>,328	8
5	BDE100	565	564, $[M+2]^+$	404, 406	564,566,<u>404</u>,406	13
5	BDE99	565	564, $[M+2]^+$	404, 406	564,566,<u>404</u>,406	17
5	BDE85	565	564, $[M+2]^+$	404, 406	564,566,<u>404</u>,406	20
6	BDE154	644	644, $[M-2]^+$	484, 486	642,644,<u>484</u>,486	22
6	BDE153	644	644, $[M-2]^+$	484, 486	642,644,<u>484</u>,486	26
6	BDE138	644	644, $[M-2]^+$	484, 486	642,644,<u>484</u>,486	28
7	BDE183	723	722, $[M+2]^+$	562, 564	722,724,562,<u>564</u>	30
7	BDE190	723	722, $[M+2]^+$	562, 564	722,724,562,<u>564</u>	31
MeO-PBDEs						
4	2′-MeO-BDE68	516	516, $[M-2]^+$	420, 422, $^*[M-CH_3Br]^+$	514,516,<u>420</u>,422	9
4	6-MeO-BDE47	516	516, $[M-2]^+$	356, 420, 422, $^*[M-CH_3Br]^+$	514,<u>516</u>,420,422,356	11
4	5-MeO-BDE47	516	516, $[M-2]^+$	356, 358	514,516,<u>356</u>,358	14
4	4′-MeO-BDE49	516	516, $[M-2]^+$	356, 358	514,<u>516</u>,356,358	15
5	5′-MeO-BDE100	595	596, $[M-2]^+$	434, 436	594,596,<u>434</u>,436	18
5	4′-MeO-BDE103	595	596, $[M-2]^+$	434, 436	<u>594</u>,596,434,436	19
5	5′-MeO-BDE99	595	596, $[M-2]^+$	434, 436	594,596,434,<u>436</u>	23
5	4′-MeO-BDE101	595	596, $[M-2]^+$	434, 436	<u>594</u>,596,434,436	24
MBDE-MXFS						
3	$^{13}C_{12}$-BDE-28	419	418, $[M+2]^+$	258, 260	418,420,<u>258</u>,260	3
4	$^{13}C_{12}$-BDE-47	500	498, $[M+2]^+$	338, 340	498,500,<u>338</u>,340	7
5	$^{13}C_{12}$-BDE-100	577	576, $[M+2]^+$	416, 418	576,578,<u>416</u>,418	12
5	$^{13}C_{12}$-BDE-99	577	576, $[M+2]^+$	416, 418	576,578,<u>416</u>,418	16
6	$^{13}C_{12}$-BDE-154	656	656, $[M-2]^+$	494, 496	654,656,<u>496</u>,498	21
6	$^{13}C_{12}$-BDE-153	656	656, $[M+2]^+$	494, 496	656,658,494,<u>496</u>	25
7	$^{13}C_{12}$-BDE-183	735	734, $[M+2]^+$	574, 576	734,736,<u>574</u>,576	29
MBDE-MXFR						
4	$^{13}C_{12}$-BDE-77	500	498, $[M+2]^+$	338, 340	<u>498</u>,500,336,338	10
6	$^{13}C_{12}$-BDE-138	656	656, $[M+2]^+$	494, 496	656,658,<u>496</u>,498	27

The ion of underline was indicated for quantitative analysis.

2.2. Channel Catfish, Crayfish, Fish Feeds and Fishmeal Samples

Eighty commercial channel catfish and crayfish, and sixty fish feeds and forty fishmeal samples collected from local markets in the Hubei province of China, were used in the present study from August to November, 2013. Muscle tissues of channel catfish and crayfish were collected and homogenized. Fish feeds and fishmeal were filtered through a US 20 mesh screen to ensure the samples uniformity.

2.3. Determination of MeO-PBDEs and PBDEs

Analysis of MeO-PBDEs and PBDEs in channel catfish, crayfish, fish feeds and fishmeal samples was prepared by using a literature method and an ISO method modified [15, 35]. The ASE 200 (DIONEX, USA) was used for the pressurized liquid extraction (PLE) experiments. 10 g of muscle tissue of channel catfish and crayfish, and 2.5 g of fish feeds and fishmeal were homogenized with Florisil at a proportion of 1/2 (w/w) after adding 1 mL of surrogate internal standard solution (the concentrations of $^{13}C_{12}$-labelled PBDEs 28, 47, 99, 100, 154, 153 and 183 were 100 ng mL^{-1}) were placed in an extraction cell. DCM-acetone (1:1, v/v) was used as extraction solvent. The system pressure was set at 1500 psi and the temperature at 100°C (100% flush volume) with a heat-up time of 6 min. Three cycles of extraction were performed during 5 min in static mode and the purge time was set at 90 s.

The above total extracts of four kinds of samples were firstly concentrated approximately 1 mL using iso-octane as the keeper at 40°C 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 about 2 mL by a gentle stream of nitrogen. The resulting mixture was filtrated with a 0.22 μm membrane for next cleanup. Cleanup procedure was performed according to the ISO method modified [35]. The extract was subjected to multi-layer silica column chromatography for the removals of acid compounds, basic compounds, sulfur and sulfur-containing molecules and small amounts of water. The silica column (22 mm i.d.×20 cm) was packed in the following sequence: 2 g of silica, 5 g

of 34% NaOH silica, 2 g of silica, 10 g 44% H_2SO_4 silica, 2 g of silica, 5 g of 10% $AgNO_3$ silica and 10 g of Na_2SO_4. The column was firstly conditioned with 50 mL of DCM, and then 50 mL of cyclohexane. The above extract was transferred to the column. For elution, 50 mL of cyclohexane was used, and followed by 50 mL of cyclohexane: DCM (1:1). The flow rate was 2.5 mL min^{-1}. The 100 mL eluates were combined and concentrated approximately 1 mL using iso-octane as the keeper at 40°C 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. 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

A PE Clarus 600 GC-MS with electron impact ionization (EI) was used to the simultaneous determinations of MeO-PBDEs and PBDEs. An Elite-5MS column (30 m×0.25 mm (id), 0.25 μm film thickness; perkinelmer, USA), was used to separate eight MeO-PBDEs, thirteen PBDEs, seven labeled PBDEs (surrogate internal standard) and other two labeled PBDEs (syringe standard). The oven temperature was programmed from 100°C (held for 1min) to 200°C at 20°C min^{-1} and then to 280°C at 2.5°C min^{-1} and finally to 320°C at 5°C min^{-1} (held for 10 min). The total runtime was 56 min. Helium was used as carrier gas at a constant flow rate of 1 mL min^{-1}. 2.5 microliter of samples and standards were injected in splitless injection mode at an injector temperature of 275°C. The information of retention times (RT), start time and end time of retention window, No. of MS scan functions and quantitation reference for MeO-PBDEs, PBDEs, MBDE-MXFS and MBDE-MXFR on Elite-5MS were presented in table 2.

Table 2. *Retention times (RT), start time and end time of retention window, No. of MS scan functions, quantitation reference for MeO-PBDEs, PBDEs, MBDE-MXFS and MBDE-MXFR on Elite-5MS, and LOQ of MeO-PBDEs and PBDEs in channel catfish, crayfish, fish feeds and fishmeal*

Br No.	Compounds	Quantitation reference	Retention times (RT)	Start time and end time of Retention Window	No. of MS Scan Functions	LOQ	
						Channel catfish and crayfish (μg kg^{-1})	Fish feeds and fishmeal (μg kg^{-1})
Compounds using $^{13}C_{12}$-BDE-77 as labeled injection internal standard							
3	BDE17	$^{13}C_{12}$-BDE-28	13.84	13.44～14.38	2	0.25	1
3	BDE28	$^{13}C_{12}$-BDE-28	14.66	14.24～15.25	4	0.25	1
4	BDE71	$^{13}C_{12}$-BDE-47	19.31	18.93～19.90	5	0.5	2
4	BDE47	$^{13}C_{12}$-BDE-47	20.02	19.66～20.72	6	0.5	2
4	BDE66	$^{13}C_{12}$-BDE-47	21.03	20.64～21.73	8	0.5	2
5	BDE100	$^{13}C_{12}$-BDE-100	24.65	24.27～25.30	13	1	4
5	BDE99	$^{13}C_{12}$-BDE-99	26.28	25.96～26.90	17	1	4
5	BDE85	$^{13}C_{12}$-BDE-99	28.95	28.61～29.73	20	1	4
Compounds using $^{13}C_{12}$-BDE-138 as labeled injection internal standard							
6	BDE154	$^{13}C_{12}$-BDE-154	30.27	29.86～30.91	22	1	4
6	BDE153	$^{13}C_{12}$-BDE-153	32.57	32.23～33.22	26	1	4
6	BDE138	$^{13}C_{12}$-BDE-153	35.40	35.02～36.05	28	2.5	10
7	BDE183	$^{13}C_{12}$-BDE-183	38.56	38.22～39.18	30	2.5	10
7	BDE190	$^{13}C_{12}$-BDE-183	41.84	41.46～42.42	31	5	20
Compounds using $^{13}C_{12}$-BDE-77 as labeled injection internal standard							
4	2′-MeO-BDE68	$^{13}C_{12}$-BDE-100	22.44	22.00～23.06	9	1	4
4	6-MeO-BDE47	$^{13}C_{12}$-BDE-100	23.25	22.81～23.87	11	1	4
4	5-MeO-BDE47	$^{13}C_{12}$-BDE-100	24.84	24.28～25.57	14	1	4
4	4′-MeO-BDE49	$^{13}C_{12}$-BDE-100	25.15	24.66～25.95	15	2.5	10
Compounds using $^{13}C_{12}$-BDE-138 as labeled injection internal standard							
5	5′-MeO-BDE100	$^{13}C_{12}$-BDE-154	28.32	27.88～29.00	18	2.5	10
5	4′-MeO-BDE103	$^{13}C_{12}$-BDE-154	28.75	28.21～29.55	19	2.5	10
5	5′-MeO-BDE99	$^{13}C_{12}$-BDE-154	30.89	30.48～31.56	23	2.5	10
5	4′-MeO-BDE101	$^{13}C_{12}$-BDE-154	31.25	30.75～32.09	24	5	20
Labeled compounds							
3	$^{13}C_{12}$-BDE-28	$^{13}C_{12}$-BDE-77	14.64	14.21～15.28	3	—	—
4	$^{13}C_{12}$-BDE-47	$^{13}C_{12}$-BDE-77	20.03	19.68～20.72	7	—	—
5	$^{13}C_{12}$-BDE-100	$^{13}C_{12}$-BDE-77	24.64	24.21～25.27	12	—	—
5	$^{13}C_{12}$-BDE-99	$^{13}C_{12}$-BDE-77	26.26	25.93～26.99	16	—	—
6	$^{13}C_{12}$-BDE-154	$^{13}C_{12}$-BDE-138	30.25	29.82～30.90	21	—	—
6	$^{13}C_{12}$-BDE-153	$^{13}C_{12}$-BDE-138	32.55	32.20～33.20	25	—	—
7	$^{13}C_{12}$-BDE-183	$^{13}C_{12}$-BDE-138	38.52	38.18～39.20	29	—	—
Labeled injection internal standards							
4	$^{13}C_{12}$-BDE-77	$^{13}C_{12}$-BDE-77	22.54	22.17～23.22	10	—	—
6	$^{13}C_{12}$-BDE-138	$^{13}C_{12}$-BDE-138	35.38	35.00～36.10	27	—	—

The transverse line was indicated no data.

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 250°C, respectively. The electron multiplier voltage was set to 370 V.

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.

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 thirteen 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. Isotope dilution quantitation, internal standard quantitation and labeled compound recovery were used according to an EPA method [36]. 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 thirteen 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 and Discussion

3.1. Method Development

The analysis method in the study was principally described by two literature methods as indicated above, but some important steps and crucial parameters were modified and intensified compared with literatures in this study. The main aspects regarding sample extraction and sample cleanup were intensified during the development of the method.

Firstly, for sample extraction, PBDEs and MeO-PBDEs only fish samples were analyzed, and five to 10 g of the sample (wet weight) were homogenized with hydromatrix and extracted by PLE [15]. In contrast to the described method [15], the extraction in detail in the present study was reported to ensure the experimental feasibility. Moreover, new sample species such as crayfish, fish feeds and fishmeal were applied in this study, and for the first time analysis method of PBDEs and MeO-PBDEs in crayfish, fish feeds and fishmeal by isotopic dilution GC-MS was established in this paper. Because crayfish contained comparative lipids as channel catfish, 10 g of the same mass for crayfish was chosen. Three factors, lipid contents of fish feeds and fishmeal, feasibility of filtration with a 0.22 μm membrane and 2.0 mL of sample injection volume subjected to multi-layer silica column chromatography during the sample preparation and cleanup, were carefully taken into consideration, and 2.5 g of fish feeds and fishmeal were chosen.

Next, multi-layer silica cleanup for the removals of acid compounds, basic compounds, sulfur and sulfur-containing molecules and small amounts of water was a very important step for determination of PBDEs in biotic samples [35]. It was also considered that elution solvents and their compositions were most important factor for MeO-PBDEs in the study. Elution solvents and their compositions were modified in contrast to the ISO method [35]. For elution, 50 mL of cyclohexane was firstly used to ensure that all impurities could be well adsorbed in the silica, and cyclohexane eluent need be collected because a small amount of MeO-PBDEs and PBDEs were also eluted down. Then MeO-PBDEs and PBDEs were completely eluted by 50 mL of mixed solvent of cyclohexane and DCM. The volume ratio of cyclohexane and DCM were optimized according to the below method of description, involving four groups of experiments.

1 mL of the mixed working standard (the concentration of BDE17, BDE28, BDE47, BDE66, BDE71, BDE85, BDE99, BDE100, BDE138, BDE153, BDE154, BDE183 and BDE190 were 100 ng mL^{-1}, 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}, the concentrations of $^{13}C_{12}$-labelled PBDEs 28, 47, 99, 100, 154, 153 and 183 were 100 ng mL^{-1}, and the concentration of $^{13}C_{12}$-labelled PBDEs 77 and 138 were 100 ng mL^{-1}) was subjected to four multi-layer silica columns, respectively. Four 50 mL of cyclohexane was firstly used to elute in four multi-layer silica columns，then 50 mL of cyclohexane: DCM (8:2), 50 mL of cyclohexane: DCM (7:3), 50 mL of cyclohexane: DCM (6:4) and 50 mL of cyclohexane: DCM (5:5), were respectively used to elute in four multi-layer silica columns. The flow rate is 2.5 mL min^{-1}. The next experiments were conducted according to the section 2. The results showed the recoveries of all these compounds were most excellent by elution of 50 mL of cyclohexane: DCM (5:5). Similarly, the blank samples of channel catfish, crayfish, fish feeds and fishmeal spiked at the same level as above mixed working standard were prepared prior to

sample extractions, and the sample extractions were carried out according to the section 2. The sample cleanup of multi-layer silica column and following experiments were conducted according to the above method for the mixed working standard. It was also interesting that the recoveries of all these compounds were also most excellent by elution of 50 mL of cyclohexane: DCM (5:5). Moreover, the fractions collected were clean and contained little impurity, which indicated the impurity removal was successful. Therefore, the cleanup procedure that 50 mL of cyclohexane was used, and followed by 50 mL of cyclohexane: DCM (1:1) was chosen.

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 thirteen 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 experiment results.

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 0.9991. 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.

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
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
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

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 channel catfish, crayfish, fish feeds and fishmeal 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%.

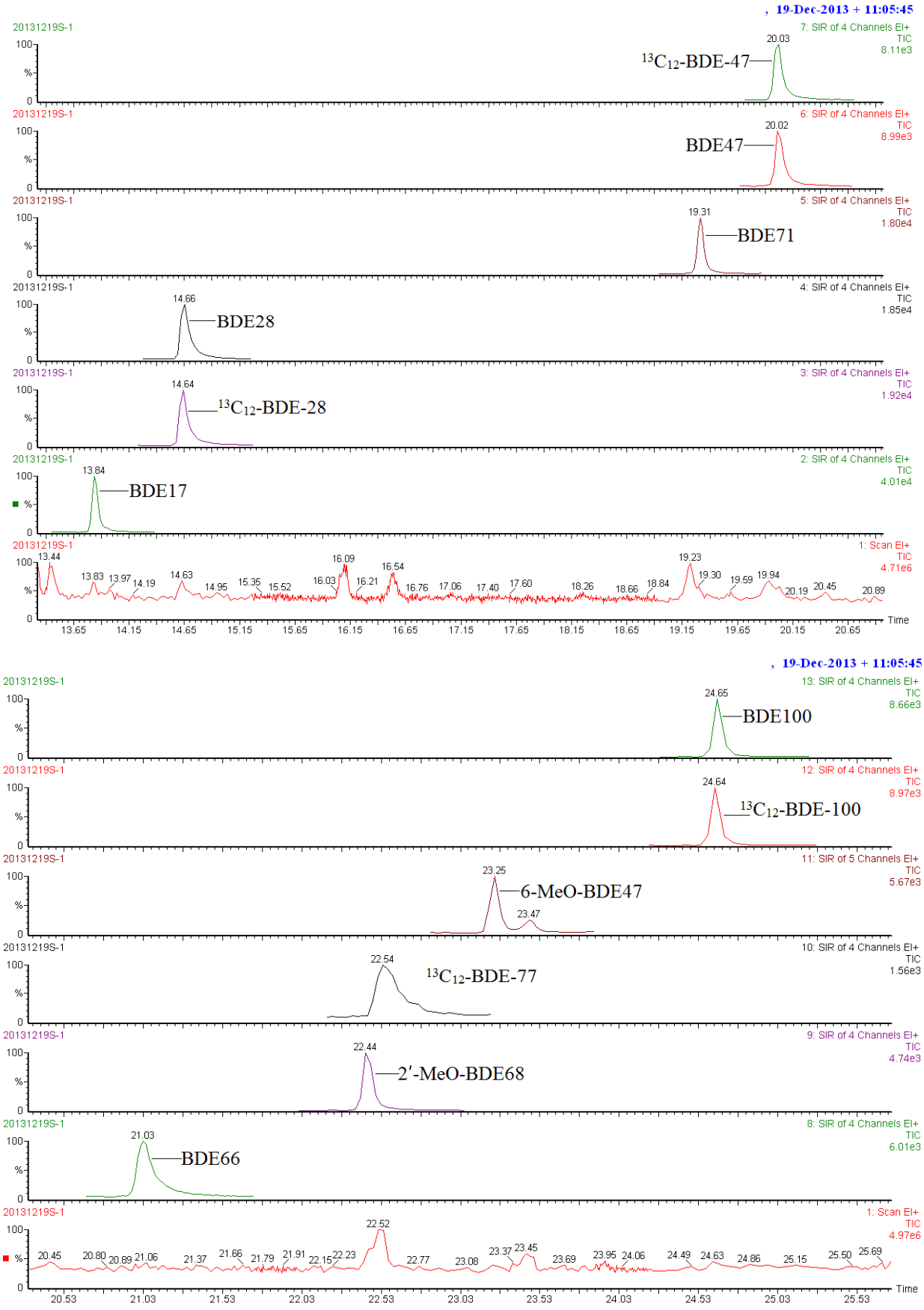
Table 4. *Average recoveries and standard deviation of MeO-PBDEs and PBDEs in channel catfish, crayfish, fish feeds and fishmeal (%R±SD, n=3) (μg kg^{-1} wet weight)*

Br No.	Compound	Quantitation reference	Recoveries from spiked channel catfish			Recoveries from spiked crayfish			Recoveries from spiked fish feeds			Recoveries from spiked fishmeal		
			2.5	10	25	2.5	10	25	10	40	100	10	40	100
Compounds using $^{13}C_{12}$-BDE-77 as labeled injection internal standard														
3	BDE17	$^{13}C_{12}$-BDE-28	92±7	92±5	96±6	84±10	94±8	96±12	92±5	103±5	102±6	96±13	99±8	104±7
3	BDE28	$^{13}C_{12}$-BDE-28	87±7	96±5	94±8	89±13	90±7	92±8	96±5	101±9	98±9	90±6	100±8	100±7
4	BDE71	$^{13}C_{12}$-BDE-47	74±6	89±10	95±5	76±11	90±11	94±10	84±6	101±9	101±7	88±11	98±7	99±6
4	BDE47	$^{13}C_{12}$-BDE-47	87±5	91±7	96±10	82±4	84±8	91±10	93±7	99±6	101±7	90±7	100±5	101±7
4	BDE66	$^{13}C_{12}$-BDE-47	78±9	86±5	93±12	75±5	90±10	90±10	92±10	101±9	100±9	90±10	95±6	98±5
5	BDE100	$^{13}C_{12}$-BDE-100	82±12	83±6	95±5	83±11	86±9	96±5	91±8	103±8	103±8	92±8	99±8	100±6
5	BDE99	$^{13}C_{12}$-BDE-99	81±14	85±7	94±9	82±10	86±7	93±10	90±11	96±7	97±5	93±10	93±10	102±5
5	BDE85	$^{13}C_{12}$-BDE-99	78±16	85±6	87±5	77±6	86±7	92±8	87±5	95±7	101±6	86±7	86±7	97±7
Compounds using $^{13}C_{12}$-BDE-138 as labeled injection internal standard														
6	BDE154	$^{13}C_{12}$-BDE-154	74±7	84±6	89±7	78±5	85±8	88±12	87±8	96±10	96±11	86±4	93±8	101±7
6	BDE153	$^{13}C_{12}$-BDE-153	75±8	82±4	90±9	73±8	84±5	90±5	88±9	90±7	96±10	85±10	93±7	98±8
6	BDE138	$^{13}C_{12}$-BDE-153	75±8	82±4	85±7	73±6	85±10	84±5	85±7	87±6	94±7	85±12	90±9	90±9
7	BDE183	$^{13}C_{12}$-BDE-183	83±8	83±3	85±5	78±9	87±11	85±4	85±4	97±11	91±7	84±8	91±10	91±8
7	BDE190	$^{13}C_{12}$-BDE-183	—	80±13	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	80±10	96±8	102±9	77±9	96±3	96±13	99±6	103±6	101±5	100±9	102±8	102±6
4	6-MeO-BDE47	$^{13}C_{12}$-BDE-100	77±7	93±13	96±8	75±8	100±7	100±9	100±5	100±8	98±7	103±7	103±7	103±7
4	5-MeO-BDE47	$^{13}C_{12}$-BDE-100	78±11	100±8	96±5	75±5	94±10	94±10	99±9	99±8	101±9	92±11	98±6	100±6
4	4′-MeO-BDE49	$^{13}C_{12}$-BDE-100	79±9	85±8	92±6	86±5	95±8	95±7	95±7	94±6	100±4	93±7	95±7	100±6
Compounds using $^{13}C_{12}$-BDE-138 as labeled injection internal standard														
5	5′-MeO-BDE100	$^{13}C_{12}$-BDE-154	78±12	86±6	91±4	76±11	86±6	86±5	95±3	97±5	102±9	91±8	99±7	100±7
5	4′-MeO-BDE103	$^{13}C_{12}$-BDE-154	76±7	85±7	85±4	77±6	86±10	89±10	86±5	97±7	100±6	89±10	104±7	98±12
5	5′-MeO-BDE99	$^{13}C_{12}$-BDE-154	80±8	85±7	88±5	79±13	85±10	89±6	88±10	101±7	100±6	86±8	94±6	103±6
5	4′-MeO-BDE101	$^{13}C_{12}$-BDE-154	—	81±12	87±5	—	82±8	87±5	—	97±7	95±6	—	92±6	94±6

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

3.3. Analysis of Channel Catfish, Crayfish, Fish Feeds and Fishmeal

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 and BDE190 were 100 ng mL^{-1}, 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}, 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}, and the concentration of $^{13}C_{12}$-labelled PBDEs 77 and 138 as syringe standard were 100 ng mL^{-1}), was shown in Fig.1.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6: SIR of 4 Channels EI+
TIC
8.99e3
20.02
BDE47
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TIC
1.80e4
19.31
BDE71
4: SIR of 4 Channels EI+
TIC
1.85e4
14.66
BDE28
3: SIR of 4 Channels EI+
TIC
1.92e4
14.64
$^{13}C_{12}$-BDE-28
2: SIR of 4 Channels EI+
TIC
4.01e4
13.84
BDE17
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TIC
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, 19-Dec-2013 + 11:05:45
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TIC
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BDE100
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TIC
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11: SIR of 5 Channels EI+
TIC
5.67e3
23.25
6-MeO-BDE47
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TIC
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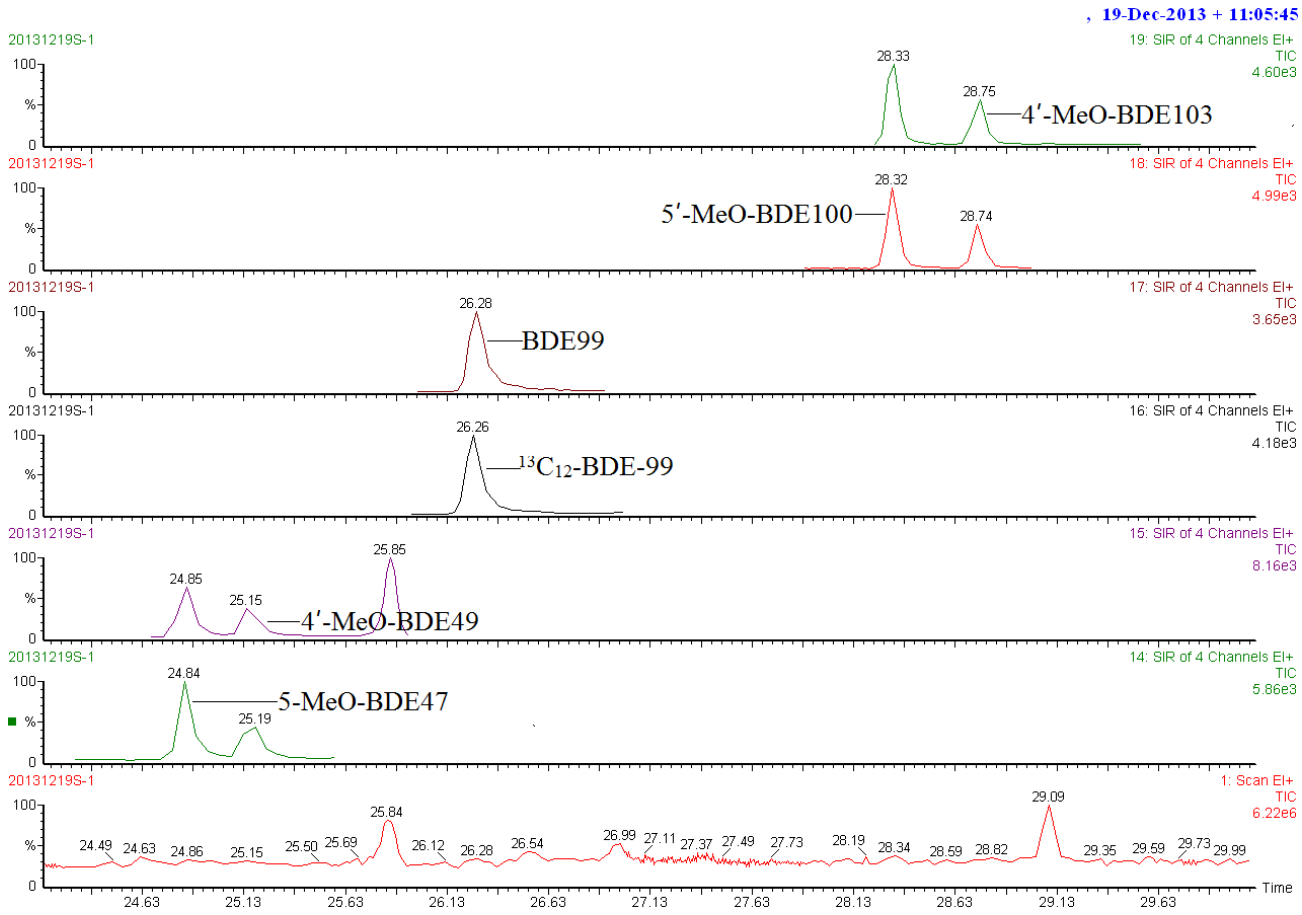
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28.74
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BDE99
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26.26
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25.15
25.85
4′-MeO-BDE49
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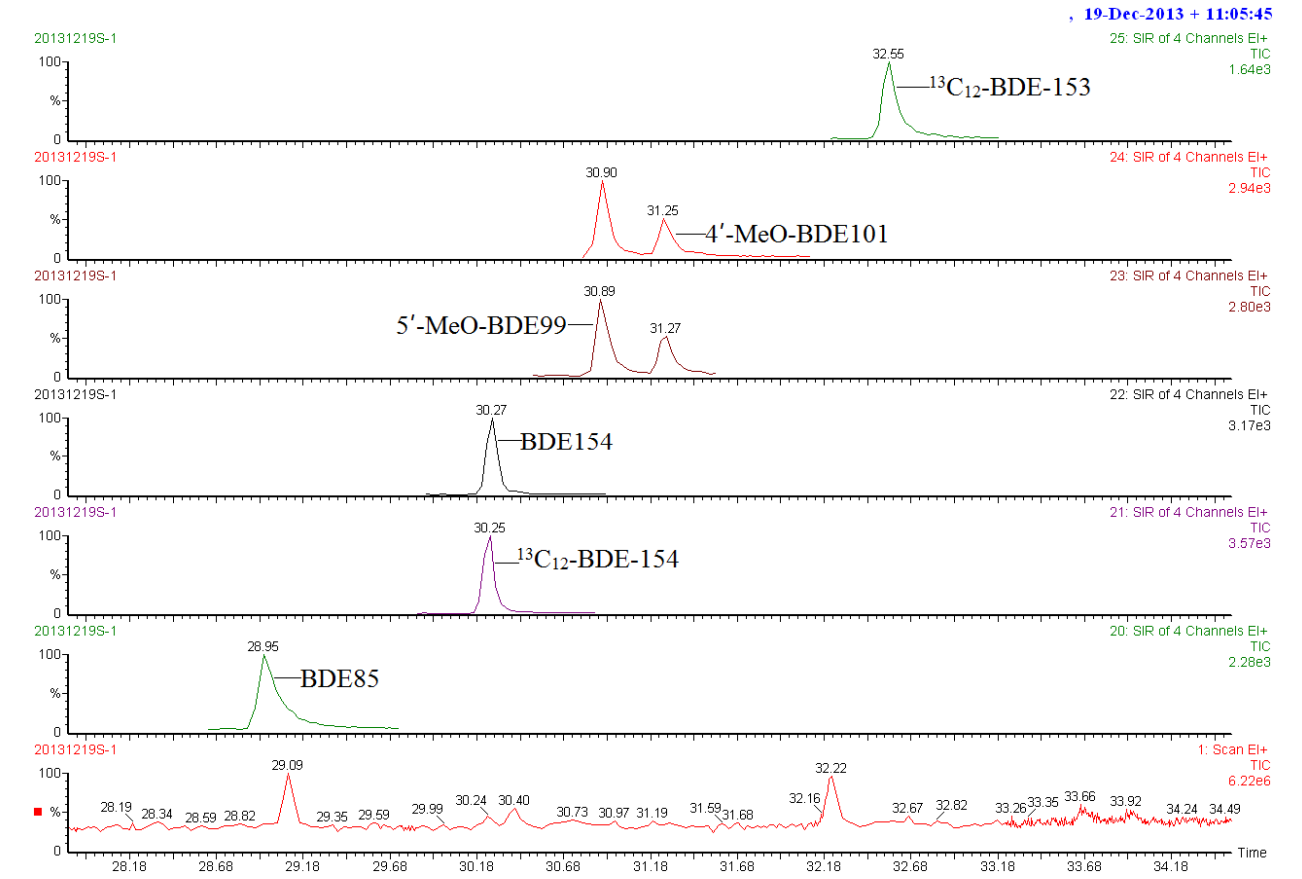
Time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Time

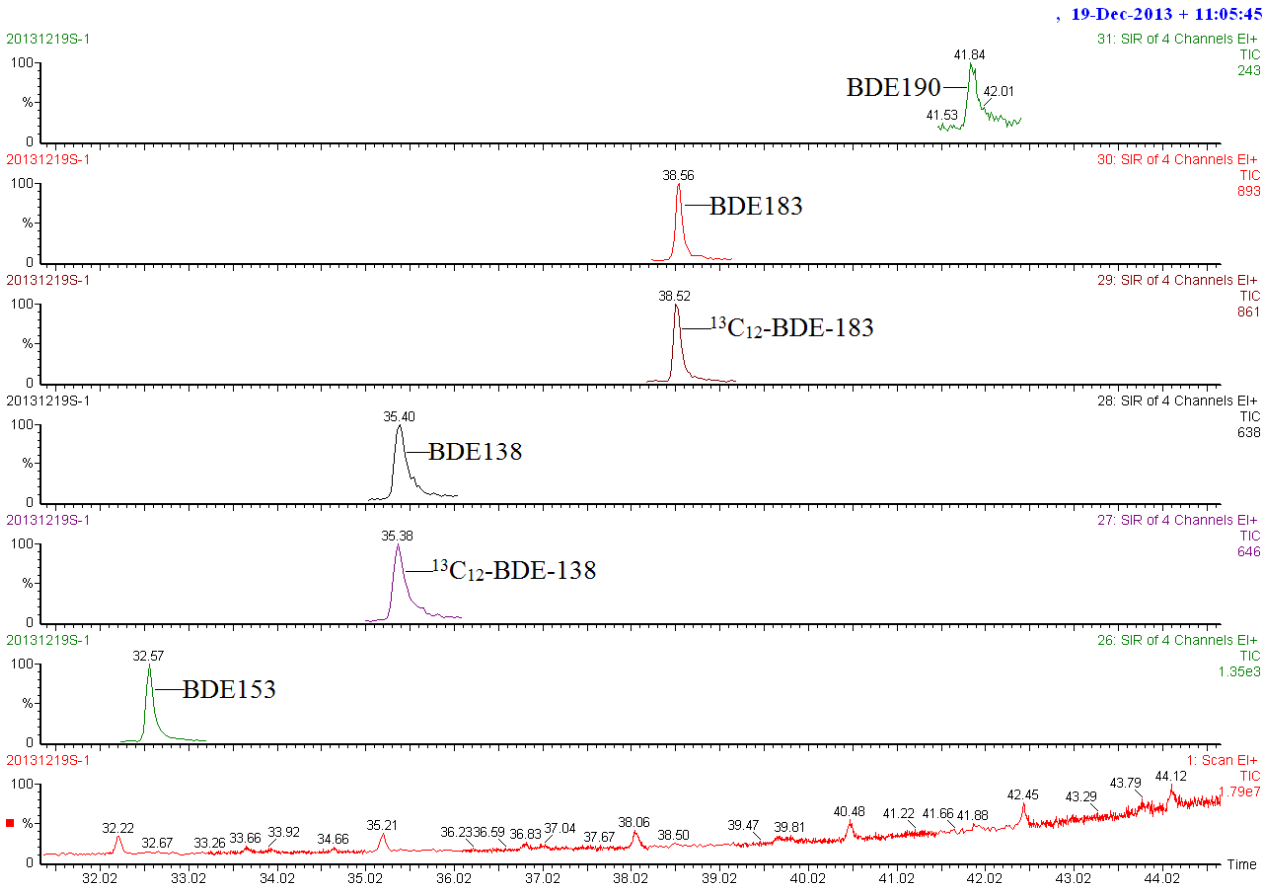

Figure 1. *GC-MS chromatogram of a mixed standard solution of MeO-PBDEs, PBDEs and $^{13}C_{12}$-labelled PBDEs (the concentrations of eight MeO-PBDEs, thirteen PBDEs and nine $^{13}C_{12}$-labelled PBDEs are all 100 ng mL^{-1})*

By the established methods, a large number of channel catfish, crayfish, fish feeds and fishmeal were tested. PBDEs or MeO-PBDEs emerged in 2 of 80 channel catfish, 1 of 80 crayfish, 6 of 60 fish feeds and 4 of 40 fishmeal. Especially, PBDEs or MeO-PBDEs were tested in fish feeds and fishmeal with a positive rate as high as 10%. Table 5 provided further information.

Table 5. *Contents of MeO-PBDEs and PBDEs in channel catfish, crayfish, fish feeds and fishmeal (μg kg^{-1} wet weight)*

Br No.	Compound	Channel catfish		Crayfish	Fish feeds						Fishmeal			
		#1	#2	#1	#1	#2	#3	#4	#5	#6	#1	#2	#3	#4
3	BDE28	ND	ND	ND	ND	ND	ND	ND	4.14	ND	ND	ND	ND	ND
4	BDE47	2.73	2.54	4.04	6.11	4.39	ND	ND	5.75	9.38	6.03	ND	ND	11.64
5	BDE100	4.98	3.41	ND	12.17	ND	ND	ND	9.84	8.69	ND	ND	ND	8.03
5	BDE99	4.25	ND	ND	9.78	ND	ND	ND	11.47	8.71	12.38	11.12	8.14	ND
6	BDE154	ND	2.95	ND	ND	ND	ND	10.07	ND	ND	ND	ND	16.37	ND
6	BDE153	ND	3.77	ND	ND	ND	ND	12.31	ND	ND	ND	ND	10.05	ND
7	BDE183	ND	ND	ND	ND	ND	ND	23.65	ND	ND	ND	ND	ND	ND
4	2′-MeO-BDE68	ND	ND	ND	14.32	9.11	ND	ND	ND	ND	10.79	ND	ND	ND
4	6-MeO-BDE47	ND	ND	ND	ND	15.45	ND	ND	ND	ND	8.97	ND	ND	ND
4	4′-MeO-BDE49	ND	ND	ND	27.78	ND	19.33	ND	ND	ND	ND	22.31	ND	ND

ND, lower than LOQ

It was reported that PBDEs and MeO-PBDEs was together present in fish samples at relatively high levels [11–13, 15–16]. Considering that MeO-PBDEs were a novel class of pollutants, contamination of MeO-PBDEs in these reports was more concerned than that of PBDEs in our opinion. Concentrations of 6-MeO-BDE47 and 2'-MeO-BDE68 in fish and shellfish samples from the Mediterranean Sea ranged from less than detection to 12.6 ng g^{-1} lipid weight and from less than detection to 2.15 ng g^{-1} lipid weight, respectively [11]. MeO-PBDEs were also detected in mullet (Mugil cephalus) and sea bass (Dicentrarchus labrax) from Bizerte Lagoon, Tunisia ranging from 6.46 to 798 ng g^{-1} lipid weight [12]. Moreover, MeO-PBDEs were also detected in fish from the Bohai Sea and the Donghai Sea, China ranging from less than detection to 3.2 ng g^{-1} dry weight [13]. Recent reports showed that the

concentrations of MeO-PBDEs ranged from less than detection to 368 pg g^{-1} lipid weight [15], and concentrations of 6-MeO-BDE47 and 2'-MeO-BDE68 in Japanese common squid collected from East Sea/Japan Sea and Yellow Sea were 40.3 and 99.7 ng g^{-1} lipid weight and 12.9 and 79.4 ng g^{-1} lipid weight, respectively [16].

On the basis of those reported results, it is reasonable the MeO-PBDEs and PBDEs contamination in channel catfish, crayfish, fish feeds and fishmeal from China should be highly investigated. Although channel catfish and crayfish in this study have been fortunately proved to be MeO-PBDEs and PBDEs less positive and in low-μg kg^{-1} wet weight, PBDEs or MeO-PBDEs were tested in 6 of 60 fish feeds and 4 of 40 fishmeal with a positive rate as high as 10% possibly due of complexity of fish feeds and fishmeal production and especially the use of polluted raw material. Considering the direct links between fish feeds and fishmeal safety and safety of channel catfish and crayfish, contaminations and causes of MeO-PBDEs as novel pollutants and PBDEs in channel catfish, crayfish, fish feeds and fishmeal and other aquatic and agricultural products from China still need to be investigated in the future.

Acknowledgements

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