19th International Conference on Environmental Science and Technology

Kos, Greece, 3 to 6 September 2025

Determination of Polybrominated diphenyl ethers (PBDEs) in Fish Feed by QuEChERS and GCxGC-TOF MS

EFTHIMIOU C.[1], SYKALIA D.[1], TSOUTSI C.[1], HELA D., ALBANIS T.[1], KONSTANTINOU I.[1*]

[1]Department of Chemistry, University of Ioannina, 45110 Ioannina, Greece

*corresponding author:
e-mail: iokonst@uoi.gr

Abstract Polybrominated diphenyl ethers (PBDEs) are organic pollutants widely used as flame retardants in various products. Due to their stability and lipophilic nature, PBDEs tend to bioaccumulate in aquatic organisms, raising concerns about their presence in aquaculture products. In this study, a sensitive and reliable analytical method was developed for the determination of PBDEs in fish feed using a modified QuEChERS extraction followed by comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOF MS). Method optimization and validation were carried out using spiked samples. The procedure yielded satisfactory recoveries. Limits of detection ranged between 0.03 and 0.40 ng/g, while linearity was excellent. The results confirm the method's robustness and suitability for future application in routine analysis of PBDEs in fish feed and related matrices. This work contributes to improving food safety monitoring in aquaculture by enabling the trace-level determination of flame retardants in complex sample matrices.

Keywords: *PBDEs; Flame retardants; Fish feed; QuEChERS; GC×GC-TOF MS*

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are a group of synthetic organic compounds that are widely used as flame retardants in an extensive variety of products, such as electronics, textiles, filling materials and plastics. Despite their advantages in fire protection, their use has been associated with serious environmental and toxicological impacts. Due to their stability, low bioavailability and strong lipophilic nature, PBDEs exhibit significant resistance to biodegradation and a tendency to bioaccumulate and bioconcentrate in organisms [1]. In recent years, an increasing concern has been raised about their presence in aquaculture products, particularly in commercial fish feed, as these compounds can be introduced through contaminated raw materials, along with secondary contamination sources. Through fish feed, PBDEs can enter the food chain and pose potential risks to consumer health [1].

The determination of PBDEs in complex samples is a challenge for analytical techniques, mainly due to the complexity of the matrix and the low concentrations of the contaminants. The key step is the selection of an appropriate methodology for the extraction of the analytes from the sample. Among the most widespread techniques are the *Quick, Easy, Cheap, Efficient, Rugged and Safe* (QuEChERS) extraction approach, *Dispersive Liquid-Liquid Microextraction* method (DLLME), Matrix Solid Phase Dispersion (MSPD) and, in some cases, *Solid Phase Extraction* (SPE) as a cleanup procedure [1,2]. Each technique offers different advantages in terms of selectivity, recovery and sample cleanup, and the selection is based on both the nature of the matrix and the properties of the analytes. The final extract is then subjected to analysis with highly sensitive and precise chromatographic-mass spectrometric techniques.

The most commonly used technique for the quantitative and qualitative determination of PBDEs is gas chromatography coupled to mass spectrometry (GC-MS), which offers selectivity and sensitivity for most homologous compounds [3,4]. For samples of particularly complex composition, such as fish feed containing fats, proteins, carbohydrates and vitamins two-dimensional gas chromatography (GC×GC) combined with time-of-flight mass spectrometry (GC×GC-TOF MS) is increasingly chosen [3]. This approach offers enhanced separation power and identification capabilities even at trace levels, making it particularly effective in the identification and quantification of PBDEs in complex environmental or food samples.

2. Methodology

2.1 Extraction methodology

PBDEs were extracted from fish feed samples using a modified QuEChERS procedure. In particular, 10mL of ultra-pure water and a respective volume of surrogate standard was added in 50 mL polypropylene tube containing 2g of homogenised fish feed sample and was left for 15 min to dry. The addition of extraction solvent

(10mL acetonitrile) and salts (2g $MgSO_4$, 1g NaCl) was followed. After shaking vigorously for 1 min, the material was centrifuged at 4000 rpm for 5 min. To purify the organic layer, 2 mL was transferred from the supernatant to a fresh tube with 100 mg PSA and 500 mg $MgSO_4$ (d-SPE). The extract was evaporated under nitrogen at 30 °C after shaking and centrifugation, then reconstituted in 200µL of n-hexane. The final extract was filtered (PTFE, 0.22µm) and kept at -20 °C in a GC vial until analysis.

2.2. GC × GC analysis

Analysis of PBDEs was performed using a Pegasus 4D (Leco Corp., St. Joseph, MI) consisting of an Agilent 6890 GC and a ToF mass spectrometer with electron ionization. Chromatographic conditions were optimized to ensure complete separation of the target compounds from co-extracted matrix components of the samples. Once optimized, samples were injected into hot splitless mode (1 µL, 250 ◦C) in a Rxi-5MS × Rxi-17Sil-MS column setup (30 m × 0.25 mm × 0.25 µm and 1.27 m × 0.25 mm × 0.25 µm, respectively). The temperature offset of the first-dimension oven was as follows: 60 ◦C (2.5 min) to 190 ◦C at a rate of 20 ◦C/min and then to 300 ◦C (2 min) at 3 ◦C/min. The second-dimension oven was programmed to follow the first oven (+5◦C) up to 300 ◦C where it was kept stable until the end of analysis. Helium was used as carrier gas. A nitrogen quad-jet dual-stage cryogenic modulator was used for sample focusing and injection in the second-dimension column. The temperature of the modulator was set 20 ◦C above that of the secondary oven. Finally, hot and cold pulses were optimized as follows: two 2.4 s hot pulses with 0.6 s cold pulses between stages. The transfer line temperature was set at 280 ◦C.

Table 1. Absolute retention times and reference ions (m/z) for the target PBDE congeners analyzed.

Compound	Absolute Retention Time (min)	Target ions (m/z)
PBDE 28	21.789	139,**248**,406
PBDE 47	27.520	324,483,**485**
PBDE 66	28.330	324,483,**485**
PBDE 77 (SS**)	29.735	326,483,**485**
PBDE 100	32.145	297,**406**,564
PBDE 99	33.541	202,**404**,564
PBDE 85	35.875	202,**404**,564
PBDE 154	37.157	242,**484**,644
PBDE 153	38.957	242,**484**,644
PBDE 138	41.391	242,**484**,644

* Quantification ions in bold; **SS: Surrogate standard

Table 2. Analytical characteristics of the extraction method

Compound	LOD (ng/g)	LOQ (ng/g)	Mean R% (±RSD%)
PBDE 28	0.30	1.00	105.8 (4.1)
PBDE 47	0.40	1.30	100.9 (9.7)
PBDE 66	0.07	0.25	98.1 (1.9)
PBDE 100	0.30	1.00	102.2 (6.4)
PBDE 99	0.03	0.10	74.3 (2.1)
PBDE 85	0.03	0.10	92.6 (2.6)
PBDE 154	0.07	0.25	90.1 (5.6)
PBDE 153	0.40	1.30	72.6 (1.2)
PBDE 138	0.30	1.00	82.9 (1.3)

3. Results

Method optimization and validation were performed using spiked fish feed samples to evaluate extraction efficiency and analytical performance. Recoveries for selected PBDE congeners ranged from 72.6% for PBDE 153 to 106.8% for PBDE 28, with relative standard deviations (RSDs) below 15%, indicating good method precision. The method showed excellent linearity (R^2 > 0.99) across the tested concentration range. Limits of quantification (LOQs) varied from 0.25 ng/g (for PBDE 99 & 100) to 1.30 ng/g (for PBDE 153 and 147). Matrix effects were minimized through optimized cleanup, to be in the range ±35%. These results confirm that the developed QuEChERS-GC×GC-TOF MS method is suitable for trace analysis of PBDEs in complex feed matrices.

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Acknowledgments: The research project is implemented in the framework of H.F.R.I call "Basic research Financing (Horizontal support of all Sciences)" under the National Recovery and Resilience Plan "Greece 2.0" funded by the European Union – Next Generation EU (H.F.R.I. Project Number: 16849).