19th International Conference on Environmental Science and Technology

Kos, Greece, 3 to 6 September 2025

Application of QuEChERS-GCxGC-TOF MS method for the determination of flame retardants in aquaculture fish samples.

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

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

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

Abstract Polybrominated diphenyl ethers (PBDEs) and organophosphate flame retardants (OPFRs) are two groups of chemical compounds commonly found in the aquatic environment, mainly because of their widespread use in consumer and industrial products. These substances can accumulate in fish, especially in fatty tissues, raising concerns about their possible transfer to humans through diet. In this study, an analytical methodology was developed and validated to detect and quantify PBDEs and OPFRs in fish samples using a modified QuEChERS extraction followed by two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC-TOF MS) analysis. The method showed good performance in terms of recovery, precision, and detection limits. The two-dimensional chromatography allowed for better separation of isomer compounds, which are often difficult to be analyzed using conventional methods. This approach provides a reliable and sensitive tool for monitoring flame retardants in fish and can contribute to assessing both environmental contamination and potential risks for consumers.

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

1. Introduction

The aquatic ecosystem is one of the main recipients of pollutants such as Polybrominated diphenyl ethers (PBDEs) and organophosphate flame retardants (OPFRs), due to the direct or indirect discharge of waste from industrial, agricultural and municipal activities [1]. These compounds, due to their lipophilicity, tend to adsorb to suspended particles and sediments, but also to accumulate fatty tissues. Multiple studies have confirmed the presence of PBDEs and OPFRs in various fish species, reinforcing the concern for the dietary exposure of consumers. Bioaccumulation in fatty tissues can lead to significant concentration, even when environmental levels are low. The assessment of the presence of these compounds in fish samples is therefore crucial, not only for environmental impact but also for the protection of public health through food safety [2].

Effective extraction of PBDEs and OPFRs is a critical step for their successful analysis in lipid-rich tissues such as fish. Various extraction techniques have been developed to address the challenges posed by complex biological matrices. Among the most commonly applied are the QuEChERS method, Dispersive Liquid-Liquid Microextraction (DLLME), and Matrix Solid Phase Dispersion (MSPD), each offering specific benefits regarding analyte selectivity, recovery efficiency, and minimization of matrix effects [3]. The selection of the appropriate technique depends largely on the matrix and the physicochemical characteristics of the target compounds. After extraction and cleanup, advanced analytical instruments combining chromatographic separation and mass spectrometric detection are employed to accurately detect and quantify contaminants at even low concentration levels.

The analysis of PBDEs and OPFRs in biological samples, such as fish tissue, requires the application of sensitive and selective analytical techniques, capable of detecting contaminants at trace levels within complex matrices. Gas chromatography coupled with mass spectrometry (GC-MS) is widely used for such analyses. However, due to the complexity of the sample and the coexistence of numerous confounding compounds, one-dimensional chromatography is often not sufficient for complete separation from matrix constituents. Two-dimensional gas chromatography-time-of-flight mass spectrometry (GC×GC-TOF MS) technology offers significant advantages, as it ensures higher separation power and sensitivity [2]. The use of this technology enables the detection and quantification of PBDEs and OPFRs in fish samples, providing a comprehensive assessment of exposure and enhancing environmental monitoring procedures [2,4].

2. Materials & Methods

2.1 Sample Collection and Preparation

Fish samples (sparus aurata) were filleted, homogenized and stored at –20 °C, before extraction.

2.2 Extraction Procedure

A modified QuEChERS protocol was used. Briefly, 2.0 g of fish tissue was placed in a 50 mL Falcon-type tube, an appropriate volume of surrogate standard solution was added and left to dry for 15 min. 10 mL of acetonitrile were added to the tube and vortexed, followed by the addition of extraction salts (2 g $MgSO_4$/1 g NaCl). The mixture was centrifuged at 4000 rpm for 5 min and 2 mL of the upper phase were transferred to a d-SPE tube containing 50 mg PSA, 150 mg C18 and 300 mg $MgSO_4$. After cleanup, the extract was evaporated under nitrogen at 25 °C, reconstituted in 200 µL n-hexane and filtered (PTFE 0,22µm) into GC-vial to be stored until analysis.

2.3 Instrumental Analysis (GC×GC-TOF MS)

Analysis was performed using a Pegasus 4D GC×GC-TOF MS system (Leco Corp., USA) with a dual-column setup: Rxi-5MS and Rti-17SilMS. Spitless injection mode was achieved (1 µL, 250 °C), with helium as the carrier gas. The temperature program was optimized for full separation. The modulator was cryogenic, with hot/cold pulse settings at 2.4 s and 0.6 s, respectively. Mass spectra were acquired under EI conditions (70 eV), and data were processed with ChromaTOF software, using internal standard calibration.

3. Results

The use of a modified QuEChERS extraction method combined with GC×GC-TOF MS provided a robust and sensitive approach for the determination of both PBDEs and OPFRs in fish tissue. The sample preparation protocol demonstrated satisfactory recoveries (<120%) as well as RSDs <16.9%). The application of GC×GC-TOF MS significantly enhanced compound separation and identification (LODs 0.03-2.5 ng/g). This was especially important for isomeric PBDEs and structurally similar OPFRs. The developed method provides a basis for large-scale monitoring studies of flame retardants in aquatic food products. Future work should focus on applying the method to real samples from different aquatic environments, assessing their implications for food safety and human exposure risk assessment. A study on the occurrence of flame retardants in aquaculture and open-fish species (*Sparus aurata*) is under progress.

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

Compound	R_t (min)	Target ions (m/z)
Triethyl phosphate	7.241	99,**127**,155
Tributhyl phosphate	11.342	99,125,**155**
Tris(2-chloroethyl)phosphate	12.801	63,205,**249**
Tris(2-chloroisopropyl) phosphate	13.291	99,**125**,201
2-Ethylhexyl Diphenyl Phosphate	25.362	250,**251**,362
Tri-o-cresyl phosphate	29.011	**165**,179,368
PBDE 28	21.691	139,**248**,406
PBDE 47	27.427	324,483,**485**
PBDE 66	28.232	324,483,**485**
PBDE 100	32.043	297,**406**,564
PBDE 99	33.346	202,**404**,564
PBDE 85	35.778	202,**404**,564
PBDE 154	37.058	242,**484**,644
PBDE 153	38.8598	242,**484**,644
PBDE 138	41.294	242,**484**,644

*Ions used for quantification are highlighted in **bold.**

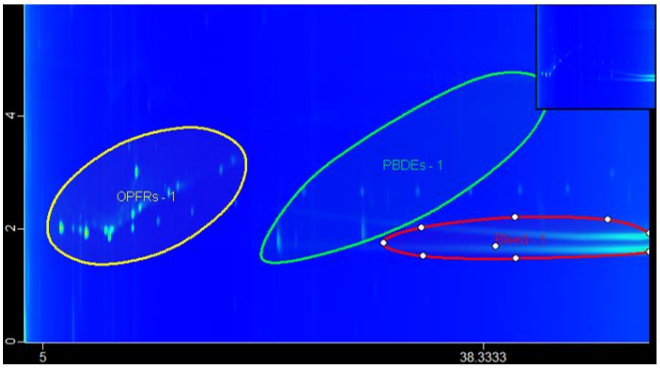

Figure 1. 2D-GC TOF MS Chromatogram of PBDEs and OPFRs in spiked fish sample.

References

1. Dang, T.T.M.; Li, L.-M.; Huang, B.-W.; Huang, C.-E.; Wu, C.-H.; Cheruiyot, N.K.; Chang-Chien, G.-P. Flame Retardant Contamination and Ecological Risk Analysis in Estuarine and Marine Sediments around an Industrial Port. *Mar. Pollut. Bull.* **2025**, *214*, 117783, doi:10.1016/j.marpolbul.2025.117783.
2. Escobar-Arnanz, J.; Pena-Abaurrea, M.; Santos, F.J.; Ramos, L. Non-Target Analysis of Organohalogenated Contaminants in Deep-Sea Fishes from the Mediterranean Sea by Comprehensive Two-Dimensional Gas Chromatography–Time-of-Flight Mass Spectrometry. *Sci. Total Environ.* **2023**, *877*, 162873, doi:10.1016/j.scitotenv.2023.162873.
3. Santini, S.; Baini, M.; Martellini, T.; Bissoli, M.; Galli, M.; Concato, M.; Fossi, M.C.; Cincinelli, A. Novel Ultrasound Assisted Extraction and D-SPE Clean-up for the Analysis of Multiple Legacy and Emerging Organic Contaminants in Edible Fish. *Food Chem.* **2024**, *443*, 138582, doi:10.1016/j.foodchem.2024.138582.
4. Pena-Abaurrea, M.; Covaci, A.; Ramos, L. Comprehensive Two-Dimensional Gas Chromatography–Time-of-Flight Mass Spectrometry for the Identification of Organobrominated Compounds in Bluefin Tuna. *J. Chromatogr. A* **2011**, *1218*, 6995–7002, doi:10.1016/j.chroma.2011.08.031.

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).