CEST 2025

19^{th} *International Conference on Environmental Science and Technology*

Kos, Greece, 3 to 6 September 2025

Green and simple analysis of brominated and organophosphate flame retardants in Fish Samples Using QuEChERS Extraction and GC-MS

SYKALIA D.[1], TRANTOPOULOS E.[1], TSOUTSI C.[1], KONSTANTINOU I.[1,*], ALBANIS T[1].

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

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

Abstract The persistence of PBDEs (Polybrominated Diphenyl Ethers) and the increasing risk of OPFRs (Organophosphate Flame Retardants) to aquatic ecosystems and human health require detailed studies on their prevalence in fish, an essential component of the food web. The present work describes the development of a QuEChERS extraction method followed by gas chromatography-mass spectrometry (GC-MS) for the extraction and analysis of PBDEs and OPRFRs in fish samples. Several parameters of the extraction process were evaluated with the aim of enhancing recovery and minimizing interferences, and, thereby, a simple and efficient protocol is given. The validation results showed efficient recoveries and reproducibility. This work focuses on the development and application of QuEChERS-GC-MS as an easy method with environmentally friendly aspect for the analysis of PBDEs and OPFRs in fish.

Keywords: PBDEs, OPFRs, QuEChERS, GC-MS, Fish Samples

1. Introduction

Worries about pollution in the Mediterranean Sea have escalated in recent years. The use of flame retardants has become common in many consumer products. Among these, polybrominated diphenyl ethers (PBDEs) and organophosphate flame retardants (OPFRs) have attracted much attention due to their resistance and potential to cause negative impact on human health and ecosystem. PBDEs, are widely used in textiles, electronic equipment and furniture and are classified as POPs based on their bioaccumulation and endocrine-disrupting potential (Bellanger et al., 2015). Human exposure primarily occurs through dietary intake, especially from fish and animal-derived feed, which contributes to the accumulation of these chemicals (Pietron et al., 2023).

Meanwhile, as replacements for polybrominated diphenyl ethers (PBDEs), OPFRs have gained popularity due to their cost-effectiveness and compatibility with polymers. Their non-covalent embedding into materials raises concerns regarding their release into the environment, which could result in environmental pollution. Some OPFRs may have hazardous effects such as neurotoxicity and carcinogenicity (Zhang et al., 2018). The bioaccumulation of OPFRs in aquatic organisms, particularly fish, poses additional risks to human health through dietary exposure (Bekele et al., 2021).

The QuEChERS extraction (Quick, Easy, Cheap, Effective, Rugged, and Safe) gained ground as routine analysis for the determination of various organic contaminants in complex matrices such as environmental and food matrices. Its widespread use is due to its simplicity and effectiveness in achieving high recovery rates with low solvent consumption and handling time. When combined with chromatographic-mass spectrometric instrumentation, it allows the sensitive and selective analysis of trace-level contaminants. Moreover, the method's greenness with low environmental impact makes it even more suitable. Based on the above, this work focuses on optimization and validation of QuEChERS extraction procedure combined with GC-MS for the determination of PBDEs and OPRFRs in fish samples towards effective monitoring of these major environmental pollutants.

2. Sample preparation and extraction

Fish samples (*Sparus aurata*) were collected from Greek aquaculture in the open commerce. The samples were transferred to the laboratory in portable freezers. Following collection, fish samples were filleted and the muscle tissue was homogenized using a stainless-steel blender. All samples utilized for optimization and validation had been previously examined, confirming their absence of the target compounds.

At the optimization step, different clean-up approaches were tested: (a) 300 mg $MgSO_4$ and 50 mg PSA, (b) 300 mg $MgSO_4$, 50 mg PSA and 150 mg C_{18} and (c) 300 mg $MgSO_4$ and 200 mg EMR-Lipid. The second approach containing C_{18} demonstrated the best recoveries, as shown in Figure 1.

For the extraction process followed after optimization, 2 grams of fish sample were placed into a 50 mL

polypropylene tube followed by the addition of appropriate volume of the surrogate standard solution and standard solution of the selected flame retardants. After adding 10 mL of ACN, the tube was vortexed followed by the addition of the extraction salts (2 g $MgSO_4$/1 g NaCl). Afterwards, the sample was centrifuged at 4000 rpm for 5 minutes and 2 mL of the organic layer supernatant was recovered. Next, a 15-mL polypropylene centrifuge tube containing 300 mg $MgSO_4$, 50 mg PSA, and 150 mg C18 filled with the aliquot of the organic layer (2 mL). The tube was centrifuged for five minutes at 4000 rpm. The last aliquot was gathered and dried by evaporation until dryness and reconstituted in 200 μL of n-hexane. Before the analysis the sample was filtered with a polytetrafuoroethyene filter (PTFE, 0.22 μm).
Furthermore, the ‘’greenness’’ of the method was evaluated by the green analytical procedure index (GAPI) and it was classified as an excellent green method.

3. GC-MS analysis

A SHIMADZU QP-2020 GC-MS system (Shimadzu Corporation, Kyoto, Japan) equipped with an AOC-20i autosampler was used. The column was a MEGA–5MS plus column (5% phenyl composition and 95% methyl polysiloxane) (30 m × 0.25 mm, i.d. 0.25 μm). The ion source temperature was 220 °C and the injector temperature was 250 °C. Helium was used as the carrier gas. The total program time was 37.9 min and SIM mode was used.

4. Results and discussion

The validation was performed in accordance with the EU criteria (Pihlström et al., 2021). The method's linearity was assessed from the LOQ value of each analyte up to 500 ng g^{-1}, represented by the determination coefficient (R^2). The recoveries were assessed using fortified fish samples at three concentration levels (10, 50, and 100 ng g^{-1}). Precision was assessed by fortifying five duplicates on a single day (%RSDr (intra-day)) and within laboratory reproducibility (%RSDwR (inter-day)) was assessed using five replicate samples across five consecutive days. The recoveries ranged from 93.06% to 109.72% with PBDE100 exhibited the lowest and PBDE154 the highest recovery. The limits of quantification ranged from 1 ng g^{-1} to 10 ng g^{-1}, with TEHP presenting the highest and TBP the lowest value. The relative standard deviations (RSDs) values were below 12.9% for all compounds. The GAPI index tool has been successfully applied to assess the holistic greenness of the proposed method providing low environmental impact and high safety, achieving a total score of 76 on a scale from 0 to 100, as shown in Fig. 2.

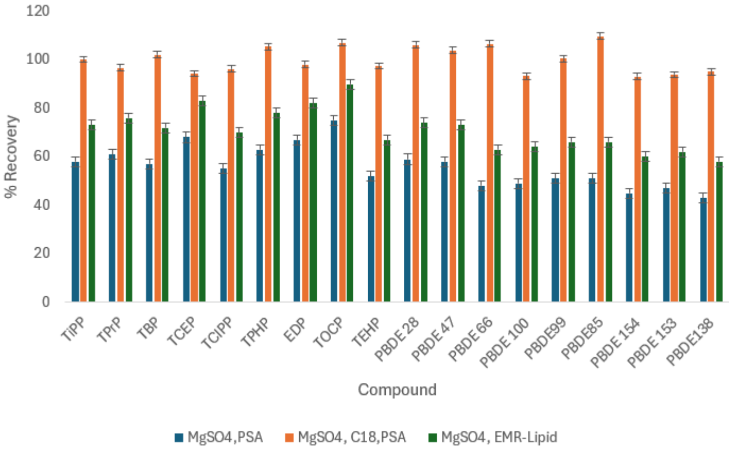

Figure 1. Recovery for all selected compounds after three clean-up procedures

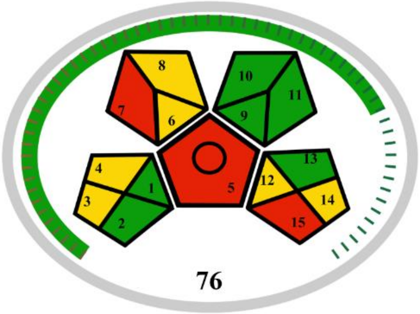

Figure 2. GAPI score of the method

References

Bekele, T. G., Zhao, H., & Wang, Q. (2021). Tissue distribution and bioaccumulation of organophosphate esters in wild marine fish from Laizhou Bay, North China: Implications of human exposure via fish consumption. *Journal of Hazardous Materials*, ***401***, 123410.

Bellanger, M., Demeneix, B., Grandjean, P., Zoeller, R. T., & Trasande, L. (2015). Neurobehavioral Deficits, Diseases, and Associated Costs of Exposure to Endocrine-Disrupting Chemicals in the European Union. *The Journal of Clinical Endocrinology & Metabolism*, *100*(4), 1256–1266.

Pietron, W. J., Malagocki, P., & Warenik-Bany, M. (2023). Feed as a source of polybrominated diphenyl ethers (PBDEs). *Environmental Research*, *231*, 116257.

Pihlström, T., Fernández-Alba, A. R., Ferrer Amate, C., Erecius Poulsen, M., Lippold, R., Carrasco Cabrera, L., Pelosi, P., Valverde, A., Unterluggauer, H., Mol, H., Jezussek, M., Malato, O., Štěpán, R., & Lambert, M. (2021). *SANTE 11312/2021 v2*.

Zhang, Y., Zheng, X., Wei, L., Sun, R., Guo, H., Liu, X., Liu, S., Li, Y., & Mai, B. (2018). The distribution and accumulation of phosphate flame retardants (PFRs) in water environment. *Science of The Total Environment*, ***630***, 164–170.

Acknowledgements: 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).