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Ultra-trace multi-matrix LC–MS/MS determination of organophosphate flame retardants and metabolites in marine waters, sediments, and algae

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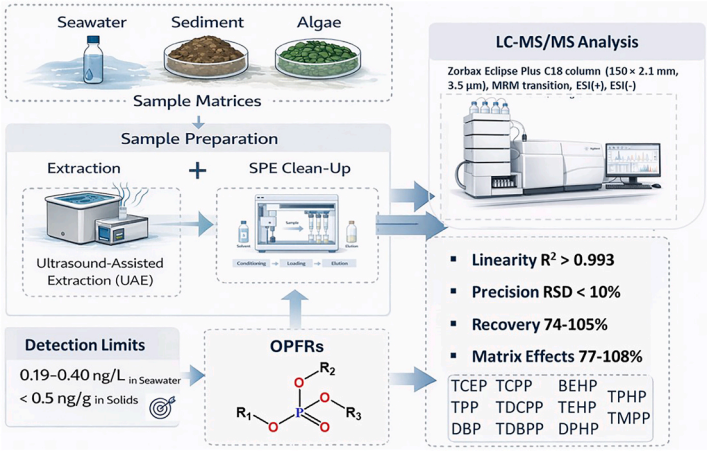
HIGHLIGHTS

- Multi-matrix LC–MS/MS method for 11 OPFRs
- Ultra-trace detection (0.19–0.40 ng/L in seawater)
- Validated in seawater, sediment and algae
- Recoveries 74–105% and precision ≤10%
- Matrix effects controlled across complex matrices

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Organophosphate flame retardants (OPFRs) are emerging contaminants increasingly detected in marine environments due to their extensive industrial use and replacement of brominated flame retardants. Their occurrence in different environmental compartments, combined with the formation of transformation products, raises concerns regarding persistence, transport, and ecological risks. However, the simultaneous determination of OPFRs and their metabolites in complex marine matrices remains analytically challenging due to their diverse physicochemical properties and low environmental concentrations.

Results: A sensitive and selective liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was developed and validated for the simultaneous determination of eight OPFRs and three transformation products in seawater, sediments, and marine algae. The method combines solid-phase extraction for aqueous samples (200 mL) with ultrasound-assisted extraction for solid matrices, followed by chromatographic separation on a C18 column and multiple reaction monitoring (MRM) detection using electrospray ionization. Optimization of chromatographic and ionization parameters enabled the reliable separation and detection of compounds with

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diverse physicochemical characteristics within a single analytical run. The method demonstrated excellent linearity ($R^2 \geq 0.99$), satisfactory recoveries (74–105%), and good precision (relative standard deviation $\leq$ 10%) for all target analytes. Matrix effects, assessed by post-extraction addition, ranged from 77% to 108%, indicating only moderate signal suppression or enhancement depending on matrix type. Method detection limits ranged from 0.19 to 0.40 ng/L in seawater and remained below 0.5 ng/g in sediments and algae, confirming the suitability of the method for ultra-trace analysis.

Significance and novelty: The proposed analytical approach enables the simultaneous assessment of OPFRs and transformation products in both abiotic and biotic marine compartments using a single harmonized workflow. The inclusion of algae alongside seawater and sediments provides a more comprehensive understanding of contaminant distribution and potential bioaccumulation pathways in marine ecosystems. The method represents a robust, sensitive, and reliable analytical tool suitable for environmental assessment, contamination surveillance, and large-scale marine monitoring programs.

1. Introduction

Organophosphate esters (OPEs) represent a structurally diverse class of phosphorus-containing compounds comprising a central phosphate group substituted with halogenated or non-halogenated alkyl or aryl moieties [1,2]. They are widely used as organophosphate flame retardants (OPFRs) and plasticizers in polymers, textiles, electronics, construction materials, lubricants and coatings [1–3]. Because OPFRs are generally incorporated as additive components rather than chemically bound to polymer matrices, they can be released through volatilization, leaching, abrasion and dissolution processes [4]. This intrinsic physicochemical behavior, combined with large production volumes, has led to their widespread detection in multiple environmental compartments worldwide [5]. OPFRs have been frequently detected in aquatic systems, including drinking water, rivers, sediments and marine environments [6–9]. In aquatic environments, OPFRs may undergo partitioning between water, suspended solids and sediments, with potential for trophic transfer [10–12]. Several chlorinated OPFRs, including TCEP, TCPP and TDCPP, have raised increasing environmental and regulatory concern due to their persistence and toxicological effects [13,14].

The progressive global restriction of several brominated flame retardants, particularly polybrominated diphenyl ethers (PBDEs), under the Stockholm Convention on Persistent Organic Pollutants has significantly reduced their production and use due to their persistence, bioaccumulation potential, and toxicity (UNEP, 2023) [15]. Therefore, organophosphate flame retardants (OPFRs) have been increasingly introduced as alternative flame retardants in a wide range of consumer products [16]. Within the European Union, regulatory attention toward OPFRs has increased under the REACH framework, with tris (2-chloroethyl) phosphate (TCEP) classified as a Substance of Very High Concern (SVHC), while triphenyl phosphate (TPHP) was added to the REACH Candidate List in 2024 due to endocrine-disrupting concerns [17]. However, despite regulatory attention, a substantial proportion of OPFRs remain insufficiently characterized in terms of environmental occurrence and fate [18]. Robust analytical data are therefore essential to support exposure assessment. In this context, marine and coastal systems represent particularly sensitive compartments, receiving inputs from wastewater treatment plant effluents, riverine transport and atmospheric deposition, yet they remain less investigated compared to freshwater systems [19,20].

From an analytical perspective, the determination of OPFRs presents several challenges. These compounds span a wide polarity and hydrophobicity range (log Kow $\approx$ 1.6–9.5), include structurally similar congeners, and occur at ultra-trace levels in complex matrices. Matrix-induced ion suppression or enhancement is frequently observed in electrospray ionization (ESI) sources [21,22], complicating accurate quantification. Gas chromatography–mass spectrometry (GC–MS) methods have been widely applied but may require extensive clean-up and are less suited for polar and thermolabile metabolites [23]. Liquid chromatography–tandem mass spectrometry (LC–MS/MS), particularly in multiple reaction monitoring (MRM) mode, provides enhanced selectivity and sensitivity, especially for simultaneous multi-residue determination in complex environmental matrices [21,22]. Furthermore, studies addressing saline and organic-rich marine matrices remain scarce, particularly regarding systematic evaluation of matrix-induced ionization effects and quantitative robustness under harmonized analytical conditions. Most currently available LC–MS/MS methods focus on a single environmental matrix (typically water or sediment) and frequently target parent OPFRs only [23].

Therefore, the methodological novelty of the present work lies not in the individual analytical components themselves, but in their integrated optimization into a unified and transferable multi-matrix workflow for marine systems. Unlike previous approaches, this study combines optimized solid-phase extraction (SPE) for seawater, ultrasound-assisted extraction for solid matrices, and segmented multiple reaction monitoring (MRM) acquisition within a single LC–MS/MS platform to enable the simultaneous determination of both parent OPFRs and metabolites across seawater, sediment and algae. This harmonized design allows direct quantitative comparison between abiotic and biotic marine compartments while minimizing analytical variability associated with matrix-specific methodologies.

The present study addresses these analytical gaps by developing and comprehensively validating a multi-matrix LC–MS/MS method for the simultaneous determination of eight OPFRs and three metabolites in seawater, sediment and algae. Compared with previously reported OPFR methods, particular emphasis was placed on harmonized validation across chemically distinct marine matrices, including systematic evaluation of selectivity, linearity, precision, recovery, matrix effects and ultra-trace detection limits under comparable conditions. By demonstrating reliable ultra-trace quantification across abiotic and biotic marine compartments, this work provides a transferable analytical framework suitable for integrated environmental monitoring.

2. Materials and methods

2.1. Chemicals and standards

Analytical reference standards of high purity were purchased from Sigma-Aldrich (Darmstadt, Germany). The investigated compounds comprised triphenyl phosphate (TPHP), tri(2-ethylhexyl) phosphate (TMPP), tri(2-chloroethyl) phosphate (TCEP), tri(1,3-dibromopropyl) phosphate (TDBPP), tripropyl phosphate (TPP), tri(2-chloroisopropyl) phosphate (TCPP), and tri(1,3-dichloro-2-propyl) phosphate (TDCPP), along with the metabolites dibutyl phosphate (DBP), diphenyl phosphate (DPHP), and bis(2-ethylhexyl) phosphate (BEHP). The main physicochemical characteristics of these analytes are summarized in Table S3. Solvents such as methanol (MeOH), acetonitrile (ACN), and formic acid (FA), together with silica gel, were obtained from Sigma-Aldrich and Merck (both from Darmstadt, Germany). Solid-phase extraction (SPE) was carried out using Strata-X polymeric reverse-phase cartridges (500 mg/6 mL, 33 μm), supplied by Phenomenex (Torrance, CA, USA). Their phisoco-chemical properties are summarized in Table 1.

2.2. Sample collection and preparation

Marine samples (seawater, sediments, and algae) used for method development and optimization were collected from the coastal area of the Black Sea, in the vicinity of Constanța (Romania). Seawater samples were collected in pre-cleaned amber glass bottles from the surface layer (0–50 cm), while sediment samples were collected using a stainless-steel grab sampler and transferred into inert containers. Algal samples were manually collected from the nearshore zone, rinsed with site water to remove debris, and stored in clean polyethylene bags. Immediately after collection, samples were stored at 4 °C and processed within 48 h to minimize degradation. For seawater samples, 200 mL aliquots were subjected to solid-phase extraction (SPE) using an automated Dionex AutoTrace 280 system. Cartridges were conditioned with methanol and ultrapure water before loading. After the sample passage, the cartridges were rinsed with ultrapure water, dried under nitrogen, and the analytes were eluted with acetonitrile. Extracts were concentrated under a gentle nitrogen stream and reconstituted in 1 mL ultrapure water before LC–MS/MS analysis. Sediments and algae were freeze-dried at −110 °C, homogenized, and sieved. For extraction, 0.5–1 g aliquot were subjected to ultrasound-assisted extraction (UAE) with 2 × 10 mL acetonitrile for 15 min at 25 °C, followed by centrifugation (4000 rpm, 10 min). Supernatants were combined, evaporated to dryness at 40 °C under nitrogen, and reconstituted in 1 mL of methanol before analysis.

2.3. Optimization of LC separation and MS detection

The chromatographic conditions were optimized to achieve efficient separation of OPFRs and their metabolites with diverse hydrophobicity (log Kow 1.6–9.5). Three reversed-phase columns were tested: Luna C18 (2) (150 × 2.0 mm, 3 μm), Synergi Polar-RP (150 × 3.0 mm, 4 μm), and Zorbax Eclipse Plus C18 (150 × 2.1 mm, 3.5 μm). The best peak resolution and retention were obtained using the Zorbax Eclipse Plus C18 column, with a total run time of ~35 min. The optimized gradient program employed 0.1% formic acid in ultrapure water (A) and 0.1% formic acid in methanol (B), starting at 60% B, ramping to 100% B within 15 min, held for 10 min, and returned to initial conditions for column re-equilibration. The optimal injection volume was 10 μL, which ensured sufficient sensitivity without peak distortion.

Mass spectrometric conditions were established on an Agilent 6410B triple quadrupole equipped with an electrospray ionization (ESI) source. Positive ionization was applied for triesters (e.g., TCEP, TCPP, TDCPP, TPHP), while negative ionization was more suitable for diesters (e.g., DBP, DPHP, BEHP). Source parameters were optimized as follows: capillary voltage 4000 V, drying gas 8 L/min at 300 °C, and nebulizer pressure 40 psi. For each analyte, multiple reaction monitoring (MRM) transitions were optimized by directly infusing standard solutions. Two transitions were monitored—one for quantification and one for confirmation—using optimized fragmentor voltages and collision energies. Segmented MRM acquisition improved dwell time and sensitivity across the chromatographic run.

2.4. LC–MS/MS Instrumentation and operating conditions

Analyses were performed on an Agilent 6410B triple quadrupole LC–MS/MS system equipped with an electrospray ionization (ESI) source, operating in both positive and negative modes, depending on the analyte type (positive for triesters and negative for diesters). Separation was performed on a Zorbax Eclipse Plus C18 column (150 × 2.1 mm, 3.5 μm, Agilent Technologies) maintained at 40 °C. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in methanol (B), delivered at 0.2 mL/min in gradient mode. The injection volume was set at 10 μL. MRM transitions were optimized for each analyte by direct infusion of standard solutions. Two transitions were monitored per compound: the most intense product ion was used for quantification, while the second served for confirmation. Ion source conditions included: capillary voltage 4000 V, drying gas 8 L/min at 300 °C, and nebulizer pressure 40 psi.

2.5. Method validation

The developed LC–MS/MS method was validated following the principles described in the Eurachem Guide the Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics (3rd edition, 2025), adapted to the requirements of LC–MS/MS determination of trace organic contaminants in marine environmental matrices. Validation parameters included selectivity, linearity, sensitivity, precision, recovery, matrix effects, and robustness to ensure fitness for purpose for marine environmental monitoring applications. Seawater, sediment, and algae were used as representative complex marine matrices to comprehensively assess analytical performance. The following validation parameters were systematically evaluated:

Selectivity was verified by analyzing procedural blanks and matrix blanks to ensure the absence of interfering signals at the retention times and MRM transitions of the target analytes. The method was considered selective when no peaks exceeding a signal-to-noise ratio (S/N) of 3 were observed. Linearity was assessed using seven calibration levels (0.1–200 μg/L). Calibration curves were constructed by plotting peak area ratios (analyte/internal standard) against concentration. All analytes exhibited excellent linearity over the tested range, with correlation coefficients (R^2) greater than 0.99. Sensitivity was determined by establishing instrumental limits of detection (LOD) and quantification (LOQ) based on S/N ratios of 3 and 10, respectively. Method detection limits (MDLs) and method quantification limits (MQLs) were calculated by incorporating extraction recoveries and sample concentration factors, thus reflecting the performance of the entire analytical workflow and ensuring applicability at ultra-trace levels. Precision was evaluated at three levels: instrumental repeatability, intra-day repeatability, and inter-day reproducibility. Results were expressed as relative standard deviation (RSD%), with values below 10% across all matrices, demonstrating stable chromatographic and mass spectrometric performance as well as reproducible extraction efficiency. Accuracy and recovery were

Table 1
Physicochemical properties of the investigated OPFRs grouped by structural type.

Type	Compound	Abbrev.	CAS	MW (g/mol)	LogKow	LogKoc	Solubility (mg/L)	BCF
Chlorinated OPFRs	Tris(2-chloroethyl) phosphate	TCEP	115-96-8	285.5	1.63	2.48	878	0.42
	Tris(1-chloro-2-propyl) phosphate	TCPP	13674-84-5	327.6	2.59	2.21	1600	7.94
	Tris(1,3-dichloro-2-propyl) phosphate	TDCPP	13674-87-8	430.9	3.65	3.96	1.5	–
Alkyl OPFRs	Dibutyl phosphate	DBP	107-66-4	210.2	2.29	2.18	3830	1.92
	Tripropyl phosphate	TPP	513-08-6	224.2	1.87	2.83	827	0.91
	Bis(2-ethylhexyl) phosphate	BEHP	298-07-7	322.4	6.07	4.23	0.06	49.5
	Tris(2-ethylhexyl) phosphate	TEHP	78-42-2	434.6	9.49	6.36	0.0003	3.16
Aryl OPFRs	Diphenyl phosphate	DPHP	838-85-7	250.2	2.88	2.08	82.4	5.44
	Triphenyl phosphate	TPHP	115-86-6	326.3	4.59	3.72	1.03	113.3
	Tricresyl phosphate	TMPP	1330-78-5	368.4	6.34	4.34	0.018	2534
	Tris(2,3-dibromopropyl) phosphate	TDBPP	126-72-7	697.6	3.71	3.4	8	21.4

determined by spiking seawater, sediment, and algae samples at 1 μg/L (n = 3) and processing them through the complete analytical procedure. Mean recoveries ranged between 70 and 120%, confirming the reliability and quantitative performance of both sample preparation and instrumental analysis. Matrix effects (ME) were evaluated using a post-extraction addition approach. ME (%) was calculated as the ratio between the analyte signal in the matrix extract and in the solvent standard at equivalent concentration. Signal suppression or enhancement was compound- and matrix-dependent, generally ranging from 77% to 108%. These effects were quantitatively evaluated using a post-extraction addition approach and, when necessary, compensated through matrix-matched calibration. No isotope-labelled internal standards were employed in this study. The observed matrix effects remained within an acceptable range (77–108%), indicating controlled ionization variability across the investigated matrices. Robustness was assessed by deliberately introducing small variations in critical analytical parameters, including mobile phase composition (±1% organic modifier), flow rate (±0.05 mL/min), column temperature (±1 °C), and minor deviations in extraction conditions (sonication time and SPE cartridge batch). The resulting changes in retention times, peak areas, recoveries, and precision remained within ±10% of optimized conditions, indicating that the method tolerates minor operational fluctuations without significant impact on analytical performance.

2.6. Quality assurance and quality control (QA & QC)

Strict quality assurance and quality control procedures were implemented to ensure analytical reliability and reproducibility throughout the study. All validation experiments were conducted in accordance with internationally accepted guidelines for tracing organic contaminant analysis in environmental matrices. Calibration curves were constructed using at least seven concentration levels covering the expected environmental concentration range. External calibration was primarily applied using peak area versus concentration. In matrices exhibiting measurable ionization effects, matrix-matched calibration was additionally employed when necessary to compensate for signal suppression or enhancement. Correlation coefficients (R^2) exceeded 0.99 for all analytes. The calibration model was constrained through the origin following preliminary comparison between constrained and unconstrained regressions. Blank responses were consistently negligible (S/N < 3 at analyte retention times), while unconstrained calibration models yielded intercepts close to zero and statistically insignificant relative to analytical variability. Residual distributions were further examined to verify linear model suitability across the working range. Procedural blanks (Milli-Q water subjected to the complete extraction protocol), solvent blanks, and instrument blanks were included in each analytical batch to monitor laboratory background, contamination, and carry-over. No analyte signals exceeding the detection limit (S/N < 3) were observed in blanks at the retention times of the target compounds. Instrumental performance was additionally monitored throughout analytical sequences by evaluating retention time consistency, chromatographic peak shape, background signal, and system pressure stability. No evidence of progressive signal suppression, retention time instability, pressure increase, or carry-over was observed during repeated injections of environmental extracts. Because isotope-labelled internal standards were not employed, matrix effects were carefully evaluated using the post-extraction addition approach. Matrix-matched calibration was applied when necessary to compensate for signal suppression or enhancement. The observed matrix effects (77–108%) were within acceptable limits for LC–ESI–MS/MS analysis and were consistent across replicate experiments, indicating controlled ionization variability. Accuracy was assessed by spiking representative matrices (surface water, wastewater, sediment, and algae) at known concentrations (1 μg/L for aqueous samples and 50 ng/g for solids) and processing them through the entire analytical procedure. Recoveries ranged between 74 and 105%, demonstrating satisfactory extraction efficiency and quantitative performance. Precision was evaluated under repeatability (intra-day) and intermediate precision (inter-day, multi-analyst) conditions. Relative standard deviation (RSD%) values were consistently below 10%, confirming stable chromatographic retention, reproducible MRM transitions, and robust sample preparation. Instrumental limits of detection (LOD) and quantification (LOQ) were established using signal-to-noise criteria (S/N = 3 and 10, respectively). Method detection and quantification limits were calculated by incorporating extraction recoveries and concentration factors to reflect the entire analytical workflow. LODs ranged from 0.03 to 0.40 ng/L in seawater, while LOQs for sediment and algae were typically below 0.5 ng/g, confirming suitability for ultra-trace analysis in complex marine matrices. The implemented QA/QC strategy ensured reliable quantification despite the absence of isotope-labelled internal standards, supported by systematic matrix effect evaluation, matrix-matched calibration, and rigorous validation of accuracy and precision.

3. Results and discussion

3.1. Chromatographic and MS optimization

3.1.1. Optimization of chromatographic separation

Chromatographic separation was optimized to achieve baseline resolution and reproducible retention for eleven OPFRs and metabolites spanning a broad polarity range (log K_{ow} 1.63–9.49). Three reversed-phase columns (Luna C18(2), Synergi Polar-RP, and Zorbax Eclipse Plus C18) and three gradient programs were evaluated. Among the tested conditions, the Zorbax Eclipse Plus C18 column combined with gradient program C provided the best chromatographic performance, ensuring complete baseline separation, symmetric peak shapes, and reproducible retention within approximately 20 min (Fig. 1).

Chromatographic performance was further evaluated on the Synergi Polar-RP and Zorbax Eclipse Plus C18 columns under identical gradient conditions (Fig. 2). While Luna C18(2) and Synergi Polar-RP provided acceptable retention, partial co-elution and peak broadening were observed for several analytes. In contrast, the Zorbax Eclipse Plus C18 column achieved complete baseline separation with narrow and reproducible peaks within approximately 20 min, and was therefore selected for subsequent analyses (Fig. 2c).

Following column selection, the gradient program was refined to achieve complete baseline separation while maintaining reproducible retention and minimizing run time. The optimized gradient increased from 60% to 100% B over 20 min, followed by re-equilibration to ensure chromatographic stability across injections. Under these conditions, all eleven analytes were clearly resolved with sharp peaks and stable retention behavior (Fig. 3).

Injection volume optimization was performed by comparing 1, 5, and 10 μL under the optimized LC–MS/MS conditions (Fig. 4). Among the tested volumes, 10 μL provided the best compromise between sensitivity and chromatographic performance, yielding strong and reproducible responses while maintaining sharp peak shapes and stable retention times. This volume was therefore selected for subsequent analyses.

The final chromatographic conditions consisted of a Zorbax Eclipse Plus C18 column, water/methanol mobile phase containing 0.1% formic acid, gradient elution, and a 10 μL injection volume, providing reproducible separation of all target analytes within a 35 min run. These conditions were subsequently applied for method validation and quantitative analysis.

3.1.2. Optimization of MS/MS detection parameters

MS/MS conditions were optimized to maximize sensitivity and selectivity for the eleven target analytes using an Agilent 6410B triple quadrupole instrument equipped with an electrospray ionization (ESI) source. Both positive and negative ionization modes were evaluated. Positive ESI provided the best response for triester OPFRs, whereas

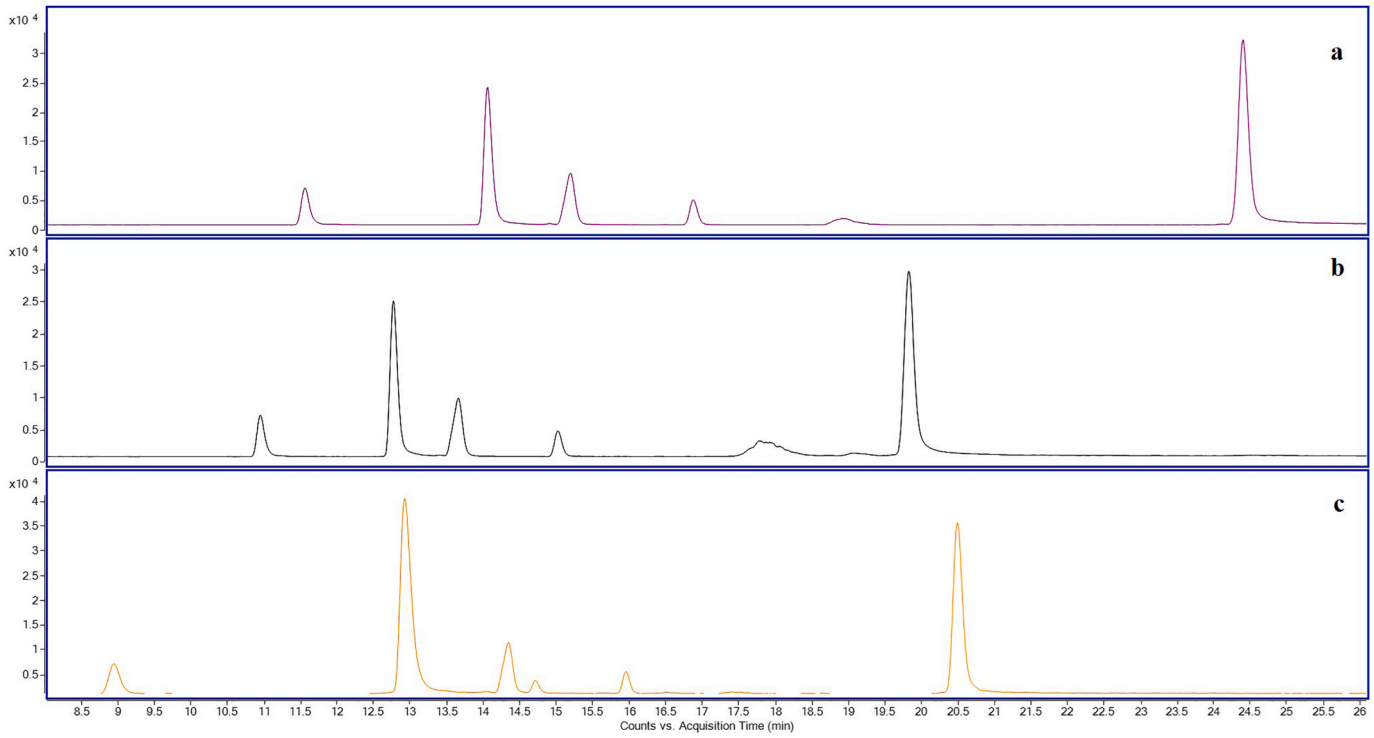

Fig. 1. Elution profiles of the target analytes on the Luna C18(2) column using three different gradient programs: (a) 0–0.50 min, 40% B; 0.51–8.50 min, 90% B; 8.51–14.50 min, 95% B; 14.51–25.50 min, 100% B; 25.51–30.50 min, 100% B; 30.51 min, return to 40% B; 30.52–40.50 min, 40% B. (b) 0–0.50 min, 40% B; 0.51–6.50 min, 90% B; 6.51–11.50 min, 95% B; 11.51–20.50 min, 100% B; 20.51–30.50 min, 100% B; 30.51 min, return to 40% B; 30.52–40.50 min, 40% B. (c) 0–0.50 min, 50% B; 0.51–8.00 min, 90% B; 8.01–12.00 min, 100% B; 12.01–20.00 min, 100% B; 20.01 min, return to 50% B; 20.01–30.00 min, 50% B.

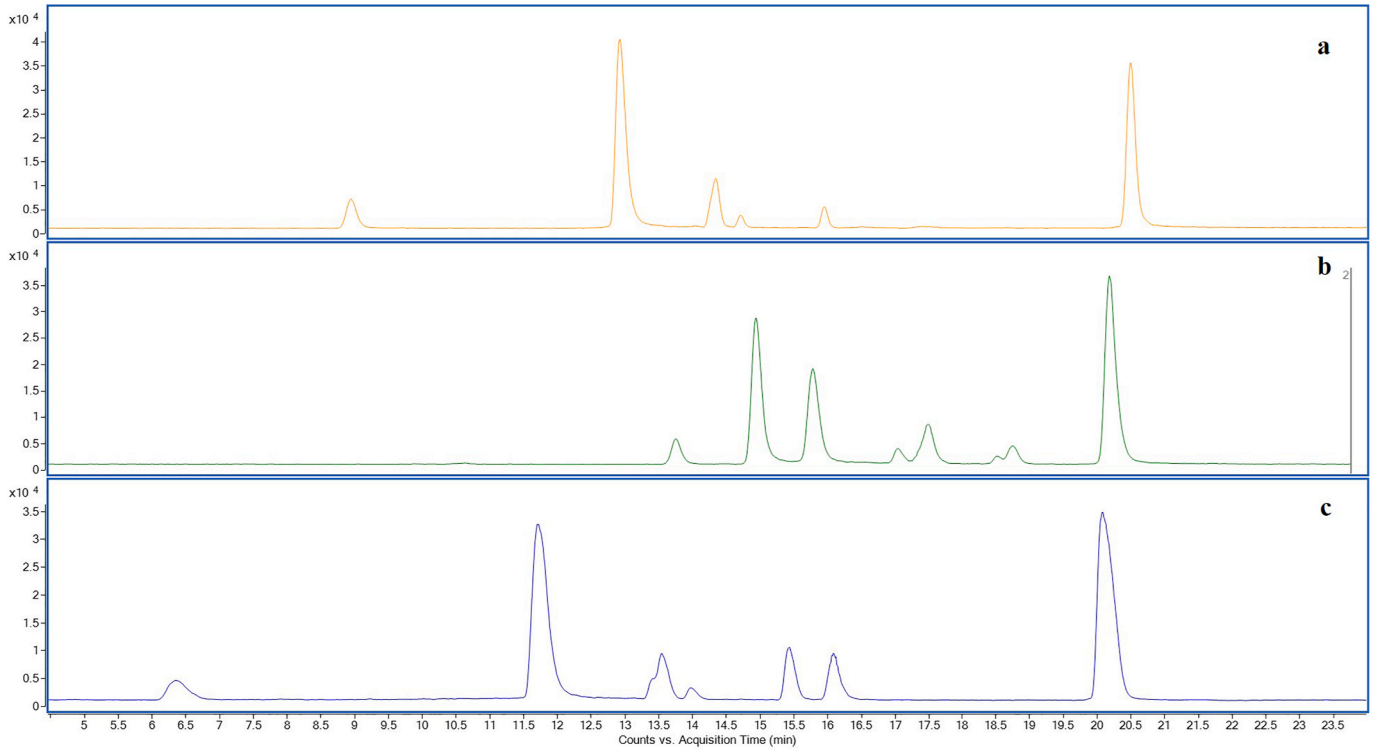

Fig. 2. Chromatograms obtained using the following chromatographic columns: (a) Luna C18(2); (b) Synergi Polar-RP; (c) Zorbax Eclipse Plus C18.

negative ESI improved sensitivity for diester metabolites. For each analyte, precursor ions were identified by direct infusion and fragmented to select two MRM transitions (quantifier and qualifier). Instrumental parameters, including fragmentor voltage, collision energy, and cell accelerator voltage, were optimized individually, and the final settings are summarized in Table 2.

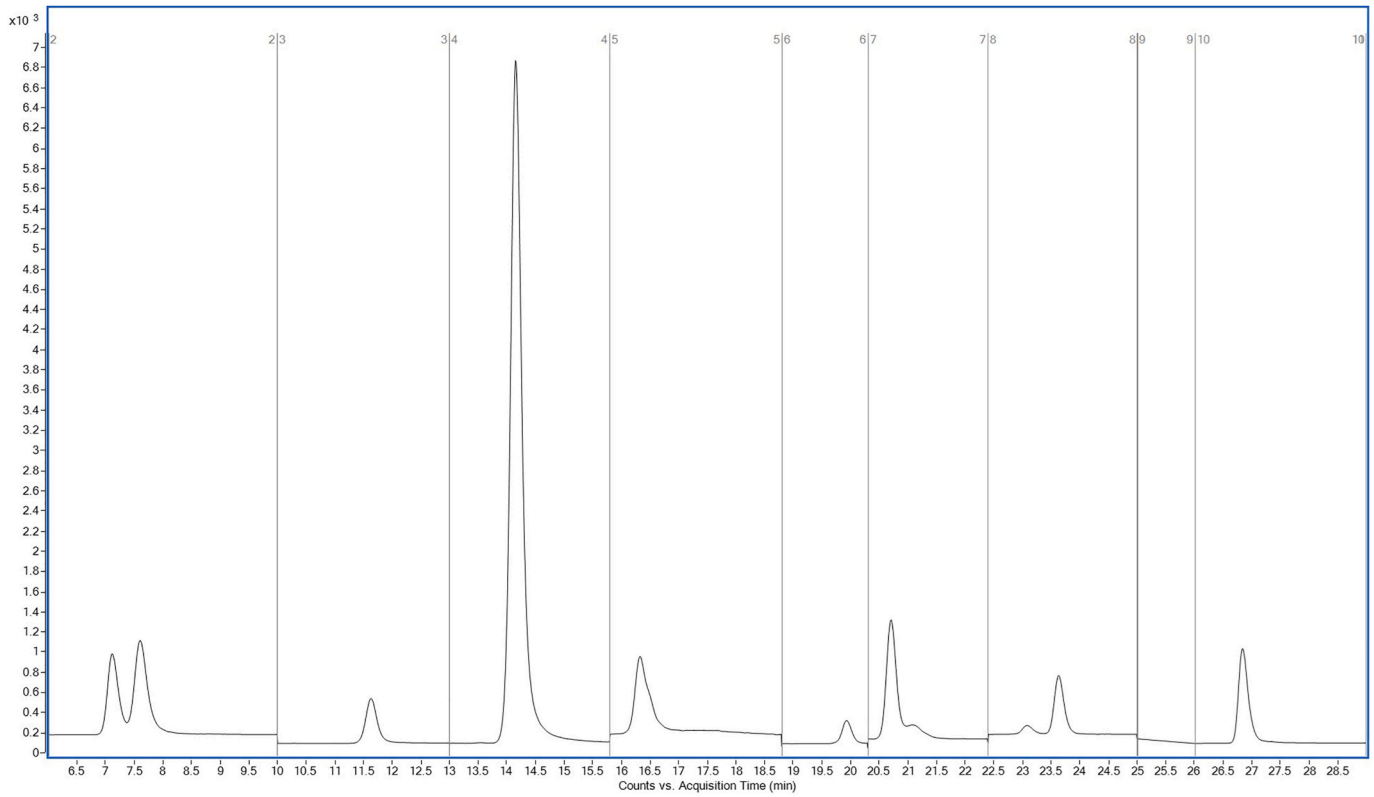

Fig. 3. MRM chromatogram of the eleven target analytes obtained under the final optimized gradient program of the mobile phase.

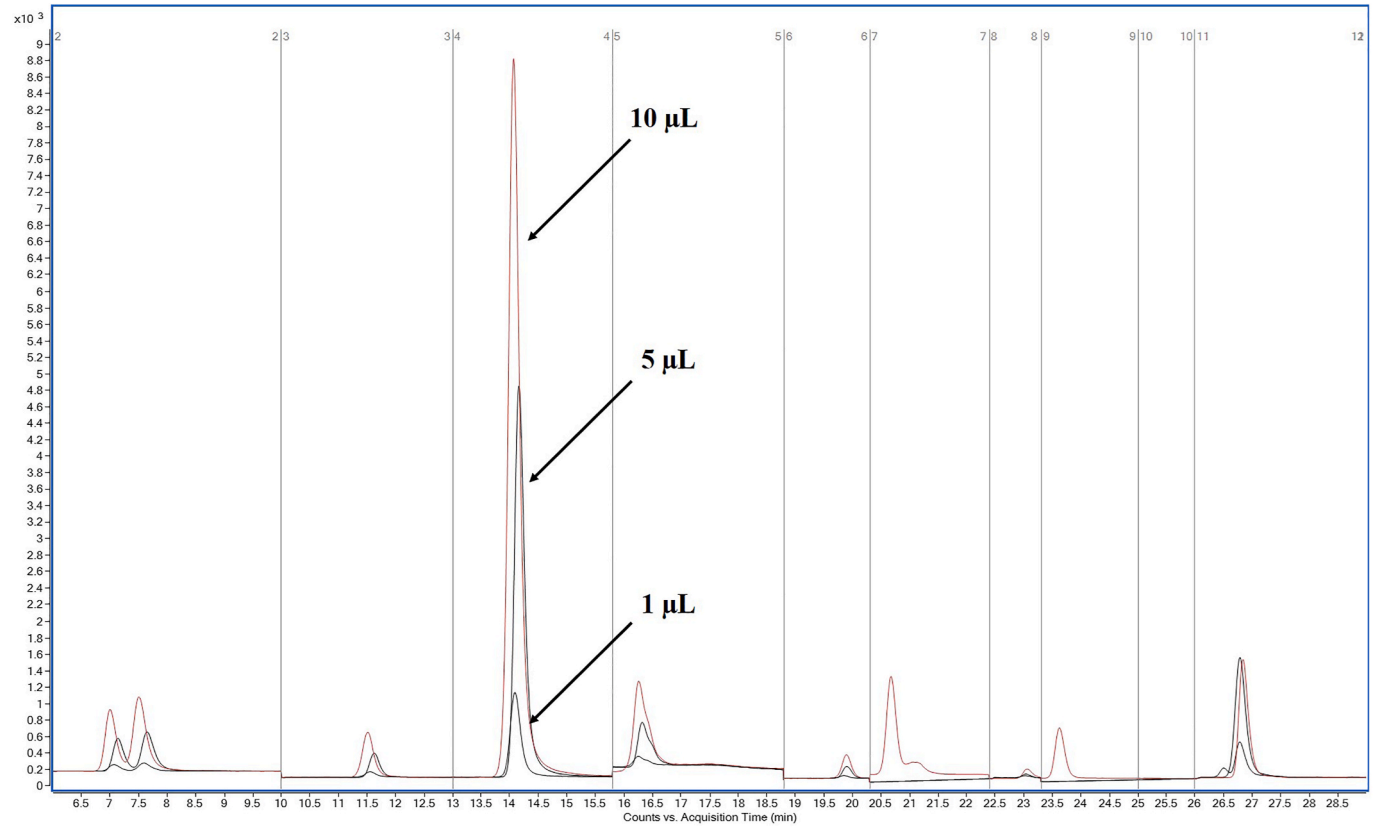

Fig. 4. MRM chromatograms obtained with three different injection volumes (1, 5, and 10 µL).

To enhance sensitivity and reproducibility, segmented MRM acquisition was implemented by dividing the chromatographic run into

Table 2
Optimized MS/MS parameters for OPFRs and metabolites used in the validated method.

Compound	rT (min)	Precursor (*m/z*)	Product ions (*m/z*) Q/q	Fragmentor (V)	CE (V) Q/q	CAV (V)	ESI
DPHP	7.05	249	249/249	145	20/30	0/1	–
DBP	7.56	209	53/79	120	10/25	4/6	–
TCEP	11.48	285	99/63	110	25/36	5	+
TPP	14.01	225	98.9/41	95	15/5	3/4	+
TCPP	16.18	327	75/99	90–100	10/25	2/4	+
TDCPP	19.8	433/431	99.1/99.1	125	30	1	+
TPHP	20.57	327	215/152	165	25/60	4/1	+
BEHP	20.93	321	79.2	190	35	2	+
TDBPP	22.93	699/697	98.9/98.6	135–150	25	5	+
TMPP	23.52	369	91/243	185	45/30	3	+
TEHP	26.75	435	98.9/71	125	15/20	4/3	+

retention-time windows (Table 3), thereby reducing concurrent transitions and increasing dwell times up to 250 ms per transition. This strategy improved signal intensity and signal-to-noise ratios while maintaining reproducible ion ratios for confirmatory analysis. The optimized MS/MS conditions ensured selective and robust quantification of OPFRs and metabolites in marine matrices.

The optimized LC gradient and stationary phase provided clear baseline separation of all eleven OPFRs and their metabolites within a single chromatographic run. As illustrated in Fig. 5, the compounds exhibited sharp and symmetric peaks with consistent retention times, covering a wide polarity range from short chain diesters (e.g., DBP, DPHP) to bulky triesters (e.g., TDCPP, TEHP). This result confirms the robustness of the chromatographic conditions and their suitability for trace-level quantification in complex marine matrices.

The systematic optimization of both LC separation and MS/MS detection resulted in complete baseline resolution, enhanced sensitivity, and highly reproducible retention times across all target compounds. By combining optimized chromatographic conditions with segmented MRM acquisition, the method achieved robust performance suitable for trace-level detection in complex marine matrices. These optimized parameters were subsequently applied in the validation stage to assess accuracy, precision, detection limits, and matrix effects.

3.2. Optimization of extraction procedures for seawater, sediment, and algae samples

3.2.1. Solid-phase extraction (SPE) optimization for seawater samples

Solid-phase extraction (SPE) was optimized to ensure efficient and reproducible preconcentration of OPFRs from saline aqueous matrices. Considering the broad polarity range of the target analytes (log K_{ow} 1.6–9.5) and the presence of both triester and diester compounds, sorbent chemistry and elution solvent were systematically evaluated. Three reversed-phase sorbents were compared: Strata-C18-U, C18 Hydra, and the polymeric Strata-X phase. Unlike silica-based C18 sorbents, Strata-X provides a broader interaction profile, potentially enhancing retention of both aromatic OPFRs and moderately polar diester metabolites. Cartridges were conditioned with organic solvent (ACN or MeOH) followed by ultrapure water, and optimization was performed using 200 mL ultrapure water spiked with a mixed OPFR standard (100 μg/L). After sample loading and washing, cartridges were dried under nitrogen and analytes were eluted with methanol or acetonitrile. Extracts were evaporated to dryness, reconstituted, and analyzed by LC–MS/MS. Recovery results (Table 4) demonstrated a marked influence of both sorbent type and elution solvent. Methanol yielded moderate and compound-dependent recoveries (54–92%), whereas polymeric Strata-X consistently outperformed silica-based C18 sorbents, particularly for more polar analytes. The higher recoveries observed for Strata-X suggest improved retention of analytes spanning a broad polarity range.

Acetonitrile consistently improved extraction efficiency compared with methanol across all tested sorbents. When combined with Strata-X cartridges, recoveries exceeded 90% for all analytes (up to 98%), including highly hydrophobic compounds such as TEHP and BEHP (Table 4). In contrast, Strata-C18 and C18 Hydra showed lower and more variable recoveries (65–91%), particularly for several hydrophobic OPFRs. The improved performance of the Strata-X/ACN combination likely reflects the broader retention capability of the polymeric sorbent and the stronger elution efficiency of ACN across analytes with different polarity. Based on recovery and reproducibility, this protocol was selected for subsequent validation and application to environmental seawater samples.

Table 3
LC–MS/MS acquisition segments and divert valve program.

Segment	Time range (min)	Divert valve position	Data acquisition
S1	0 – 6	Waste	No
S2	6 – 10	MS	Yes
S3	10 – 13	MS	Yes
S4	13 – 15.8	MS	Yes
S5	15.8 – 18.8	MS	Yes
S6	18.8 – 20.3	MS	Yes
S7	20.3 – 22.4	MS	Yes
S8	22.4 – 25	MS	Yes
S9	25 – 26	Waste	No
S10	26 – 29	MS	Yes
S11	>29	Waste	No

3.2.2. Ultrasound-assisted extraction (UAE) optimization for sediment samples

Ultrasound-assisted extraction (UAE) was selected as a rapid and solvent-efficient approach for recovering OPFRs from sediment matrices. Considering the broad polarity range of the target analytes, solvent selection was evaluated to maximize extraction efficiency across compounds with different physicochemical properties. Freeze-dried and homogenized sediment (0.5 g) spiked with a mixed OPFR standard (100 μg/L) was extracted using five solvents: methanol (MeOH), acetonitrile (ACN), hexane, hexane/ACN (1:1, v/v), and hexane/acetone (1:1, v/v). Following ultrasonication, centrifugation, repeated extraction, evaporation, and filtration, extracts were analyzed by LC–MS/MS. Recovery data (Table 5) demonstrated marked solvent-dependent variability. Methanol and hexane generally produced lower recoveries (<65% for most analytes), whereas binary solvent mixtures provided intermediate performance. ACN yielded the highest and most consistent recoveries across the analyte set, indicating efficient extraction of both moderately polar and hydrophobic OPFRs from sediment matrices.

Binary solvent systems (hexane/ACN and hexane/acetone) showed intermediate extraction performance (61–83%), whereas ACN provided the highest and most consistent recoveries across the analyte set (>85% for most compounds), reaching 119.6% for TCPP (Table 5). The improved performance of ACN likely reflects its ability to efficiently extract compounds spanning a broad polarity range. The elevated recovery observed for TCPP was attributed to matrix-induced signal enhancement rather than extraction over-efficiency. Based on recovery

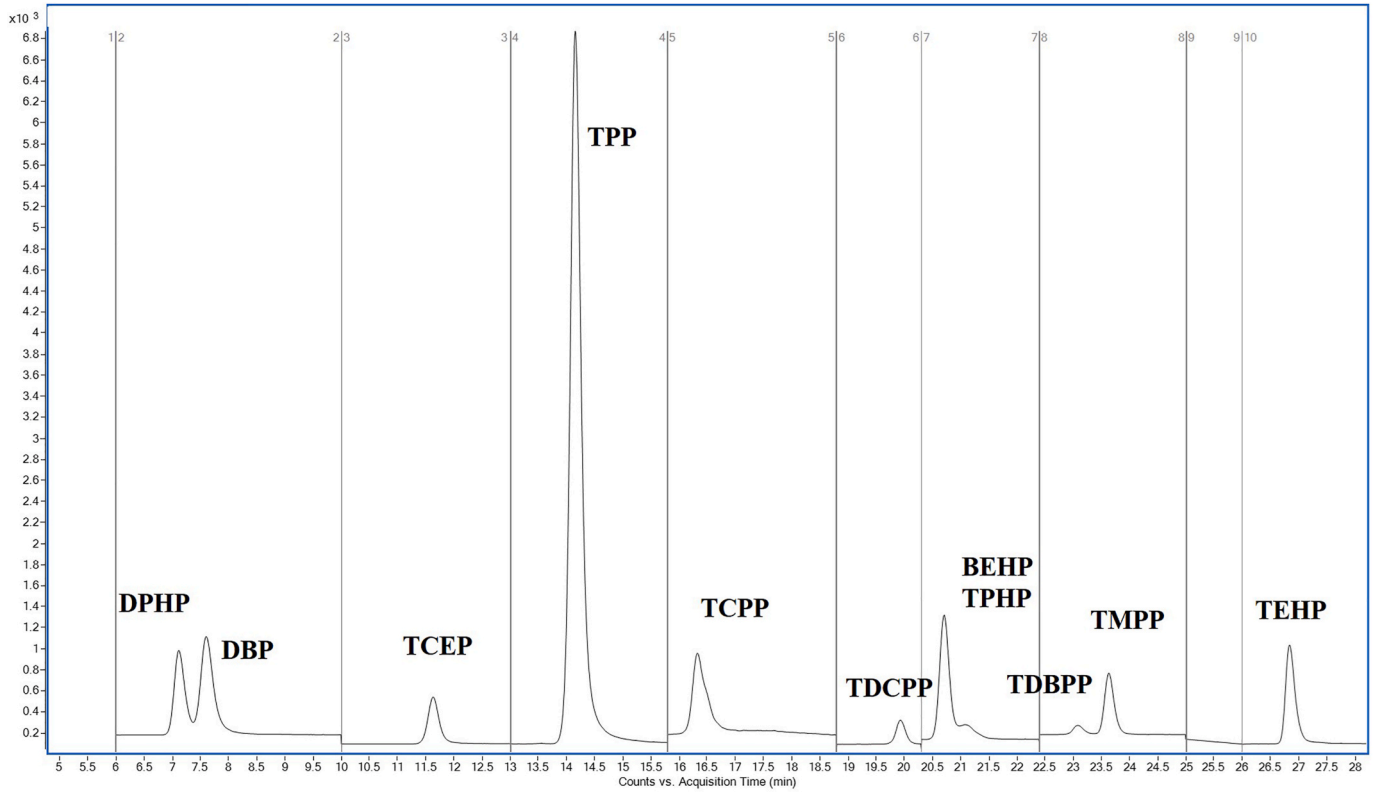

Fig. 5. MRM chromatogram for a standard solution mixture of 25 μg/L OPFRs.

Table 4
Summary of recovery efficiencies for OPFRs in seawater samples using different SPE sorbents and elution solvents.

SPE sorbent/ Elution solvent	Recovery range (%)	Mean recovery (%)	Compounds with recovery >80%	Compounds with recovery <60%
Strata X – MeOH	54–92	72	3	1
Strata C18 – MeOH	46–85	61	1	6
C18 Hydra – MeOH	38–77	53	0	8
Strata X – ACN	91–98	94	11	0
Strata C18 – ACN	77–92	82	8	0
C18 Hydra – ACN	64–96	78	7	0

Table 5
Performance of extraction solvents for UAE recovery of OPFRs from sediments.

Extraction solvent	Recovery range (%)	Mean recovery (%)	Compounds with recovery >80%	Compounds with recovery <60%
MeOH	49–66	55	0	10
ACN	82–120	91	11	0
Hexane	51–88	61	1	6
Hexane/ ACN	61–83	69	1	0
Hexane/ Acetone	41–55	48	0	11

and reproducibility, ACN was selected as the optimal UAE solvent for sediment samples and subsequently applied to algae to ensure methodological consistency across solid marine matrices.

Although no dedicated clean-up sorbent was applied following UAE, the investigated marine sediments consisted predominantly of sandy material with relatively low organic matter content, resulting in lower matrix complexity than fine-grained river sediments or sludge-rich samples. Algal matrices, despite their higher organic content and natural pigments, were subjected to freeze-drying, homogenization, centrifugation, and 0.45 μm filtration prior to analysis to reduce particulate burden. In addition, ACN extraction, evaporation/reconstitution, and membrane filtration minimized matrix transfer to the LC–MS/MS system. Instrumental robustness was monitored throughout analytical sequences by evaluating retention time stability, peak shape, system pressure, procedural blanks, carry-over, and reproducibility of matrix effects and recoveries. No progressive signal suppression, retention time drift, abnormal pressure increase, or analyte carry-over was observed, indicating satisfactory LC–MS/MS robustness under routine analytical conditions.

3.3. Method performance

The analytical performance of the developed LC–MS/MS method was systematically evaluated in seawater, sediment, and algae to verify its suitability for ultra-trace quantification in complex marine matrices. Method validation was performed in accordance with internationally accepted criteria for trace organic contaminants, with an emphasis on quantitative reliability across matrices that differ in salinity, organic content, and physicochemical complexity. The applicability of the optimized extraction protocols was confirmed through repeated blank and spiked experiments. Procedural blanks subjected to the complete analytical workflow showed no detectable analyte signals above the instrumental detection limit (S/N < 3), confirming the absence of significant laboratory background contamination or carry-over. Method performance was assessed in terms of selectivity, linearity, sensitivity (LOD and LOQ), accuracy, precision, recovery, matrix effects, and

robustness. Inter-matrix comparability was evaluated by applying identical chromatographic conditions, MRM transitions, validation criteria, and performance metrics across seawater, sediment, and algae, allowing direct assessment of extraction efficiency, matrix effects, and quantitative reproducibility between marine compartments. Particular attention was given to matrix-induced ionization variability and extraction efficiency, as these factors represent the primary sources of systematic error in LC–ESI–MS/MS analysis of OPFRs. The validation results demonstrate that the proposed workflow provides consistent quantitative performance across all investigated matrices, with controlled matrix effects, satisfactory recoveries, and reproducible signal responses. Collectively, these metrics confirm the robustness and transferability of the method for multi-compartment environmental monitoring of OPFRs in marine systems.

3.3.1. Linearity

Linearity was evaluated over seven concentration levels (0.1–200 μg/L), covering more than three orders of magnitude and encompassing the expected environmental concentration range after sample preconcentration. Calibration curves were constructed using external standardization, and regression analysis was performed using a zero-intercept model after comparison with unconstrained regression. Blank responses were consistently negligible (S/N < 3), while unconstrained models yielded intercepts close to zero and statistically insignificant relative to analytical variability. Residual analysis showed no systematic concentration-dependent bias, supporting the suitability of a proportional detector response across the investigated range. Constraining the calibration through the origin minimized uncertainty at ultra-trace concentration levels while preserving excellent linearity ($R^2 \geq 0.993$) for all analytes (Table 6).

For most compounds, R^2 values exceeded 0.998, confirming proportional detector response over the full dynamic range. Slightly lower coefficients were observed for TCPP ($R^2 = 0.9932$) and TMPP ($R^2 = 0.9962$), but these values remain within acceptable criteria for quantitative trace analysis. The slopes of the regression lines varied considerably among analytes, reflecting compound-dependent ionization efficiencies and fragmentation behavior under ESI–MRM conditions. Aromatic triesters such as TPP (slope = 920.334) and TEHP (185.084) showed higher detector responses, whereas bulkier or less efficiently ionized compounds such as TDBPP (10.7346) and BEHP (16.4293) exhibited lower sensitivities. These differences underline the necessity of individual compound calibration rather than reliance on surrogate response factors. Residual analysis confirmed homoscedastic behavior across the calibration range, indicating stable detector response without systematic deviation at low or high concentrations. Overall, the calibration performance demonstrates that the developed LC–MS/MS method provides reliable quantitative response for OPFR determination over a broad dynamic range suitable for environmental monitoring applications.

Table 6
Calibration characteristics of the LC–MS/MS method for OPFR determination.

Compound	Calibration model	Slope (b)	Coefficient of determination (R^2)
DPHP	Linear	92.7806	0.9997
DBP	Linear	75.3601	0.9999
TCEP	Linear	42.5791	0.9997
TPP	Linear	920.334	0.9997
TCPP	Linear	113.055	0.9932
TDCPP	Linear	23.1225	0.9995
TPHP	Linear	89.1888	0.9985
BEHP	Linear	16.4293	0.9988
TDBPP	Linear	10.7346	0.9989
TMPP	Linear	51.6309	0.9962
TEHP	Linear	185.084	0.9995

3.3.2. Precision

Method precision was evaluated at three levels: instrumental repeatability, intra-day repeatability (method repeatability), and intermediate precision (inter-day, multi-analyst reproducibility). Instrumental repeatability was assessed by six consecutive injections of a single extract following one complete sample preparation step (SPE for aqueous samples; UAE for solids). Method repeatability was determined by processing six independent sub-samples from the same homogenized matrix through the entire analytical workflow. Intermediate precision was evaluated by preparing and analyzing six sub-samples on different days and by different analysts. The corresponding relative standard deviation (RSD%) values obtained at a spiked concentration of 1 μg/L are presented in Table 7.

Instrumental repeatability was consistently below 1% (0.42–0.78%), demonstrating excellent stability of chromatographic retention, ion transmission, and MRM response. Intra-day precision ranged between 3.2 and 5.9% across matrices, reflecting the combined variability of extraction and instrumental analysis.

Intermediate precision values were generally below 10% (6.9–9.8%), indicating robust reproducibility under routine operating conditions. Slightly higher inter-day variability was observed for TCPP, TDBPP, and TPHP in sediment and algae, likely associated with matrix heterogeneity and extraction-related variability rather than instrumental instability, as confirmed by the low injection repeatability values. Nevertheless, all RSD values remained within widely accepted criteria for quantitative trace analysis in complex environmental matrices. Overall, the precision data demonstrate that the developed LC–MS/MS method provides highly reproducible quantification across aqueous and solid marine matrices, with minimal contribution of instrumental drift and controlled matrix-induced variability.

Table 7
Precision of the LC–MS/MS method expressed as RSD (%) for OPFRs at 1 μg/L.

Compound	Matrix	Instrument repeatability	Intra-day precision	Inter-day precision
DPHP	Seawater	0.62	3.23	7.35
	Sediment	0.73	5.22	8.44
	Algae	0.66	4.17	7.82
DBP	Seawater	0.53	3.51	7.49
	Sediment	0.66	4.03	8.13
	Algae	0.55	3.82	7.51
TCEP	Seawater	0.64	3.48	8.21
	Sediment	0.78	4.96	8.39
	Algae	0.69	4.37	8.26
TPP	Seawater	0.42	4.12	8.46
	Sediment	0.52	5.46	8.78
	Algae	0.47	4.66	8.53
TCPP	Seawater	0.57	4.58	8.72
	Sediment	0.63	5.82	9.02
	Algae	0.61	5.29	8.89
TDCPP	Seawater	0.44	3.74	6.95
	Sediment	0.51	4.33	8.44
	Algae	0.48	3.88	7.53
TPHP	Seawater	0.61	4.23	8.27
	Sediment	0.66	5.86	9.35
	Algae	0.63	4.65	8.64
BEHP	Seawater	0.52	3.44	7.57
	Sediment	0.58	5.02	8.96
	Algae	0.55	4.26	7.85
TDBPP	Seawater	0.49	4.62	8.82
	Sediment	0.53	5.73	9.82
	Algae	0.52	5.31	9.36
TMPP	Seawater	0.43	3.87	7.53
	Sediment	0.49	5.12	9.03
	Algae	0.47	4.98	8.74
TEHP	Seawater	0.48	4.12	8.41
	Sediment	0.56	5.38	9.27
	Algae	0.52	4.67	8.85

3.3.3. Selectivity

Selectivity was evaluated to verify the capability of the LC–MS/MS method to unequivocally distinguish target OPFRs from potential co-eluting matrix components in complex marine samples. Attention was given to structurally related compounds and to the high organic background typically associated with sediment and algal extracts. Method selectivity was assessed by processing blank samples through the complete sample preparation protocol (SPE for aqueous matrices and UAE for solids). Triplicate blank extracts were analyzed under identical chromatographic and MRM conditions as real samples. The acceptance criterion required the absence of detectable signals at the retention times and monitored MRM transitions of the target analytes, or signals not exceeding a signal-to-noise ratio (S/N) of 3. No interfering peaks were observed at the retention times corresponding to the eleven OPFRs in any blank extract. In instances where minor background responses were detected, signal intensities remained below the instrumental detection limit and did not affect compound identification or quantification. Furthermore, analyte confirmation was based on retention time matching and the presence of two MRM transitions per compound, ensuring reliable identification in accordance with commonly accepted confirmatory criteria for tandem mass spectrometry. These results demonstrate that the developed LC–MS/MS method provides high selectivity for OPFR determination in seawater, sediment, and algae matrices. Representative chromatograms illustrating blank and spiked samples are shown in Fig. 6.

3.3.4. Matrix effects

Matrix effects (ME) represent a critical source of systematic error in quantitative LC–MS/MS analysis, particularly when electrospray ionization (ESI) is employed. Because ion formation occurs in the liquid phase prior to droplet desolvation and gas-phase transfer, co-eluting matrix constituents may alter ionization efficiency, leading to signal suppression or enhancement [23,24]. Accurate evaluation of matrix effects is therefore essential for reliable quantification in complex environmental samples. In the present study, matrix effects were assessed for seawater, sediment, and algae extracts using the post-extraction addition approach. Representative surface water (upstream and downstream) and wastewater samples (influent and effluent) were subjected to SPE, while sediment and algae were processed using the optimized UAE protocol. The resulting extracts were spiked

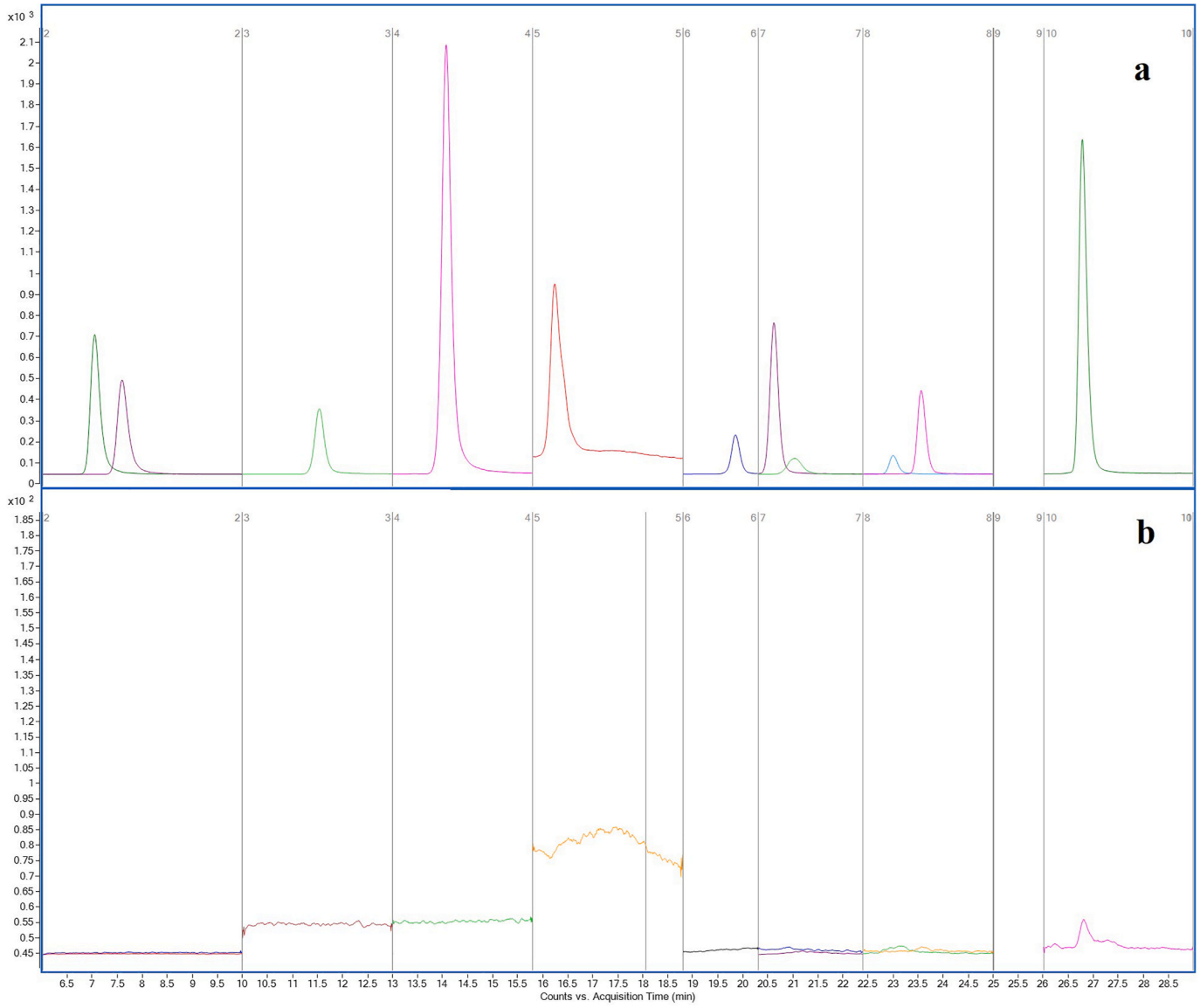

Fig. 6. Comparison of chromatograms obtained for (a) a blank water sample, in which no interferences were detected at the MRM transitions or retention times of the target OPFRs, and (b) a 100 ng/mL standard solution. The absence of background peaks in the blank confirms the selectivity of the developed method.

post-extraction with a known concentration of the analyte mixture (50 ng/mL) and analyzed under identical instrumental conditions as calibration standards. Matrix effects (ME, %) were calculated according to Eq. (1):

$$ME\ (\%) = \frac{A_{post-spike} - A_{blank}}{A_{standard}} \times 100 \quad (1)$$

where $A_{post-spike}$ is the peak area measured in the matrix extract spiked after SPE, A_{blank} is the peak area of the native analyte in the same extract, and $A_{standard}$ is the peak area of the calibration standard at the same concentration.

This approach enables quantitative differentiation between matrix-induced ion suppression (<100%) and enhancement (>100%), independent of extraction recovery. The obtained ME values (Table 8) indicate compound- and matrix-dependent variability. In seawater, ME ranged from 84% to 108%, suggesting limited ionization perturbation under saline conditions and moderate suppression for most analytes. Slight enhancement was observed for TDCPP (108%), likely attributable to co-eluting components promoting droplet desolvation or charge transfer efficiency. Sediment extracts exhibited more pronounced suppression (77–91%), consistent with the higher organic carbon content and co-extracted matrix constituents capable of competing during the ESI process.

The strongest suppression was observed for TPHP (77%) and BEHP (78%), compounds characterized by strong hydrophobic interactions with sediment-derived organic matter. Algal matrices showed comparable behavior (83–102%), reflecting their complex biogenic composition. TDCPP displayed slight enhancement (102%), while BEHP and TEHP showed moderate suppression (83–84%), indicating that matrix composition differentially affects analytes depending on polarity and ionization efficiency. The observed ME range (77–108%) falls within values commonly reported for LC–ESI–MS/MS determination of OPFRs in complex environmental matrices. Although suppression was dominant in sediment and algae, variability remained controlled and reproducible. The systematic evaluation of matrix effects, combined with matrix-matched calibration where necessary, ensured reliable quantification across all investigated matrices.

3.3.5. Accuracy and recovery

Method accuracy was evaluated through recovery experiments performed in representative marine matrices following the optimized extraction procedures (SPE for seawater; UAE for sediment and algae). Samples were spiked at 1 μg/L (aqueous matrices) or equivalent concentration in solids and processed through the complete analytical workflow. Recovery experiments were performed at a single spiking level selected to ensure reliable quantification above method quantification limits while avoiding detector saturation and maintaining comparable signal response across analytes with markedly different ionization efficiencies. Because the primary objective of the study was to evaluate the applicability and quantitative consistency of a harmonized LC–MS/MS workflow across chemically distinct marine matrices, the same spike level was systematically applied to seawater, sediment, and algae to enable direct comparison of extraction performance, precision, and matrix effects under identical validation conditions. This validation strategy is consistent with the fitness-for-purpose principles outlined in the Eurachem Guide (2025) for single-laboratory validation of environmental analytical methods. In addition, extraction efficiency was expected to remain stable across the investigated working range due to the demonstrated proportional detector response ($R^2 \geq 0.993$), controlled matrix effects (77–108%), and the absence of concentration-dependent deviations observed during calibration residual analysis. Since the selected fortification level was substantially above method quantification limits while remaining well within the validated linear range of all analytes, it was considered representative for evaluating method accuracy under routine analytical conditions. Within the Eurachem fitness-for-purpose framework, recovery assessment at a single representative fortification level is considered acceptable when linearity, precision, and matrix effects collectively demonstrate quantitative consistency across the intended working range.

Recoveries were calculated by comparing peak areas obtained after extraction (corrected for native background levels) with those of solvent-based calibration standards at identical concentrations. Absolute recoveries for the eleven OPFRs are summarized in Table 8. In seawater, recoveries ranged from 87% to 103%, demonstrating efficient analyte retention and elution under the optimized SPE conditions. For sediment and algae, recoveries were slightly lower (74–89%), reflecting stronger analyte–matrix interactions and increased physicochemical complexity in solid matrices. A recovery exceeding 100% was observed for TDCPP in algae (105%), which is attributed to matrix-induced signal enhancement rather than extraction over-efficiency, consistent with the matrix effect evaluation discussed in Section 3.3.4. Importantly, recovery variability remained within commonly accepted performance criteria (70–120%) for quantitative trace analysis in environmental matrices. Representative MRM chromatograms of spiked seawater, sediment, and algae extracts (1 μg/L) are presented in Fig. 7, confirming efficient analyte extraction, chromatographic resolution, and selective MS/MS detection across all matrices. The recovery data demonstrates that the optimized extraction protocols provide accurate and reproducible quantification of OPFRs in both aqueous and solid marine matrices, supporting the suitability of the method for routine environmental monitoring.

3.3.6. Sensitivity of the method. Limit of detection and limit of quantitation

Method sensitivity was evaluated at both instrumental and procedural levels to distinguish intrinsic MS detector performance from the overall analytical workflow efficiency. Instrumental limits of detection (LOD) and quantification (LOQ) were determined by sequential dilution of standard solutions until signal-to-noise (S/N) ratios of 3 and 10 were experimentally achieved, respectively. Instrumental quantification limits (IOQ) ranged from 0.06 to 0.28 μg/L across the eleven OPFRs (Table 9), reflecting compound-dependent ionization efficiencies and

Table 8
Method recovery and matrix effects for OPFRs at a spike level of 1 μg/L (n = 3).

Compound	Seawater R% ± SD	Seawater ME% ± SD	Sediment R% ± SD	Sediment ME% ± SD	Algae R% ± SD	Algae ME% ± SD
DPHP	87 ± 4.35	91 ± 3.82	77 ± 5.39	82 ± 4.02	83 ± 4.56	88 ± 3.96
DBP	94 ± 4.71	96 ± 4.03	80 ± 5.60	86 ± 4.21	89 ± 4.89	92 ± 4.14
TCEP	89 ± 4.45	91 ± 3.82	79 ± 5.53	85 ± 4.17	86 ± 4.73	89 ± 4.01
TPP	91 ± 4.55	89 ± 3.74	81 ± 5.67	87 ± 4.26	85 ± 4.67	86 ± 3.87
TCPP	89 ± 4.45	85 ± 3.57	78 ± 5.46	82 ± 4.02	83 ± 4.56	85 ± 3.83
TDCPP	103 ± 5.15	108 ± 4.54	75 ± 5.25	79 ± 3.87	105 ± 5.77	102 ± 4.59
TPHP	94 ± 4.72	93 ± 3.91	76 ± 5.32	77 ± 3.77	86 ± 4.73	84 ± 3.78
BEHP	89 ± 4.45	84 ± 3.53	74 ± 5.18	78 ± 3.82	78 ± 4.29	83 ± 3.74
TDBPP	87 ± 4.35	86 ± 3.61	89 ± 6.23	91 ± 4.46	82 ± 4.51	89 ± 4.01
TMPP	96 ± 4.82	91 ± 3.82	80 ± 5.60	86 ± 4.21	88 ± 4.84	93 ± 4.19
TEHP	89 ± 4.45	93 ± 3.91	84 ± 5.88	90 ± 4.41	81 ± 4.46	84 ± 3.78

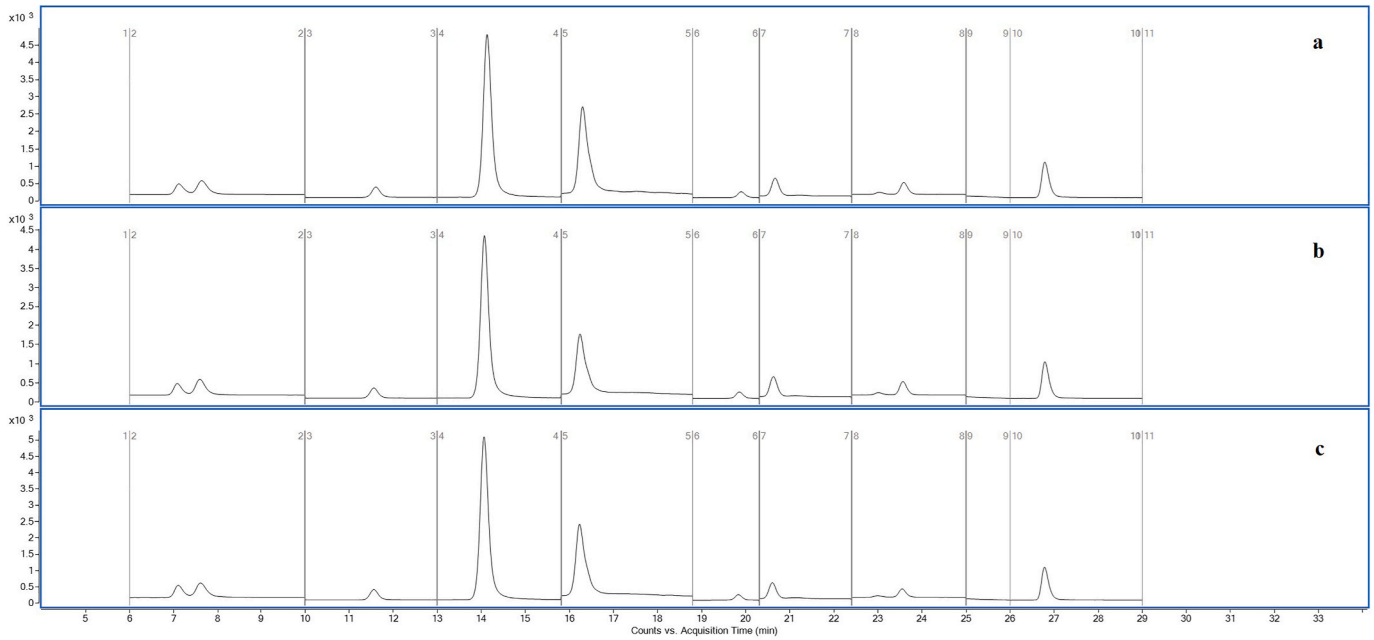

Fig. 7. MRM chromatograms of a mixed standard solution of OPFRs (1 μg/L) spiked into (A) seawater, (B) sediment, and (C) algae extracts.

Table 9
Method sensitivity for OPFR determination in different environmental matrices.

Compound	IOQ (μg/L)	Seawater (LOD–LOQ, ng/L)	Sediment (LOD–LOQ, ng/g)	Algae (LOD–LOQ, ng/g)
DPHP	0.11	0.22–0.61	0.08–0.21	0.06–0.18
DBP	0.11	0.20–0.57	0.09–0.24	0.08–0.21
TCEP	0.09	0.40–1.12	0.08–0.22	0.06–0.16
TPP	0.06	0.23–0.63	0.05–0.13	0.04–0.11
TCPP	0.09	0.24–0.66	0.04–0.11	0.03–0.09
TDCPP	0.15	0.19–0.52	0.13–0.36	0.10–0.28
TPHP	0.1	0.21–0.59	0.08–0.22	0.06–0.18
BEHP	0.28	0.26–0.73	0.23–0.63	0.12–0.34
TDBPP	0.22	0.22–0.61	0.17–0.48	0.12–0.34
TMPP	0.12	0.20–0.55	0.07–0.20	0.06–0.18
TEHP	0.07	0.21–0.59	0.06–0.16	0.04–0.12

MRM response characteristics. Aromatic triesters exhibited generally lower IOQs compared to bulkier brominated or highly hydrophobic analytes, consistent with their fragmentation behavior and signal intensity under optimized ESI conditions. Method detection limits (MDL) and method quantification limits (MQL) were calculated by incorporating sample preconcentration factors and experimentally determined recoveries, thereby accounting for the entire extraction and analytical procedure. In seawater, MDLs ranged from 0.19 to 0.40 ng/L, achieved with a moderate sample volume (200 mL). For sediment and algae, slightly higher MDLs were obtained due to increased matrix complexity and extraction variability; nevertheless, LOQs for most analytes remained below 0.5 ng/g. The sensitivity achieved for aqueous samples demonstrates an efficient sensitivity-to-volume ratio compared to previously reported approaches requiring larger sample volumes for comparable detection limits. In solid matrices, the combination of UAE preconcentration and optimized MRM acquisition ensured trace-level quantification despite pronounced matrix effects. The developed LC–MS/MS method provides ultra-trace detection capability across both aqueous and solid marine matrices, supporting reliable environmental monitoring of OPFRs at environmentally relevant concentration levels.

3.3.7. Robustness

Method robustness was assessed by deliberately introducing controlled variations in critical analytical parameters and evaluating their impact on chromatographic behavior, signal response, and quantitative performance. The tested parameters included small changes in mobile phase composition (±1% organic modifier), flow rate (±0.05 mL/min), and column temperature (±1 °C), as well as variation between SPE cartridge batches and minor deviations in sonication time during extraction of solid matrices. Under all tested conditions, retention times remained stable (no significant shift relative to optimized conditions), and peak symmetry and MRM signal intensity were not adversely affected. Variations in peak areas, recoveries, and precision remained within ±10% of nominal values, indicating limited sensitivity of the method to small operational fluctuations. Importantly, no systematic changes in matrix effects were observed under modified chromatographic or extraction conditions, confirming that ionization variability remained controlled. The absence of pronounced sensitivity to minor procedural deviations demonstrates that the method maintains quantitative reliability without requiring highly restrictive operating tolerances. Overall, the robustness evaluation confirms that the developed LC–MS/MS workflow tolerates routine analytical variability while preserving accuracy, precision, and sensitivity. This stability supports its applicability in long-term monitoring campaigns and multi-operator laboratory environments.

4. Application of the method to environmental marine samples

To demonstrate the practical applicability of the developed LC–MS/MS method, authentic marine samples collected from the Romanian Black Sea coastline, including seawater, sediment, and algae, were analyzed following the optimized extraction and analytical workflow. The method enabled the successful determination of eleven target OPFRs across all investigated matrices, confirming its suitability for environmental monitoring under real-world conditions. Mean concentrations and variability, expressed as relative standard deviation (RSD, %), are summarized in Table 10.

In seawater, ΣOPFR concentrations reached a mean value of 905 ng/L, indicating widespread contamination by OPFRs along the investigated coastline. Among the target compounds, TPP was the predominant contaminant (558 ± 3.0% ng/L), followed by TCPP (87.9 ± 3.0% ng/L) and TCEP (72.4 ± 3.2% ng/L). Other compounds such as DPHP, DBP, TPHP, TMPP, BEHP, and TEHP occurred at comparatively lower

Table 10
Mean concentrations (±RSD, %) of OPFRs in seawater (ng/L), sediment and algae (ng/g d.w.) from the Romanian Black Sea coastline.

Compound	Seawater (ng/L, mean ± RSD%)	Sediment (ng/g d.w., mean ± RSD%)	Algae (ng/g d.w., mean ± RSD%)
TCEP	72.4 ± 3.2	18.8 ± 3.2	122 ± 3.2
DPHP	39.9 ± 3.0	3.60 ± 3.1	23.8 ± 3.0
DBP	35.7 ± 4.3	1.68 ± 4.2	6.24 ± 4.3
TPP	558 ± 3.0	238 ± 3.0	5830 ± 3.0
TCPP	87.9 ± 3.0	108 ± 3.0	104 ± 3.0
TDCPP	3.72 ± 4.0	0.26 ± 3.8	1.21 ± 4.1
TPHP	35.9 ± 3.9	0.87 ± 3.4	8.99 ± 3.9
TDBPP	3.21 ± 6.2	<LOQ	0.24 ± 4.2
TMPP	34.4 ± 3.4	0.87 ± 3.4	2.90 ± 3.4
BEHP	34.2 ± 3.7	0.85 ± 3.5	2.09 ± 3.8
TEHP	35.5 ± 4.0	1.72 ± 4.1	5.44 ± 4.0

concentrations (34–40 ng/L), while TDCPP and TDBPP were detected at trace levels (<5 ng/L). Detection rates exceeded 92% for all analytes, demonstrating broad environmental occurrence. Sediment samples showed lower overall contamination, with a mean ΣOPFR concentration of 375 ng/g d.w. Similar to seawater, TPP (238 ± 3.0% ng/g d.w.) and TCPP (108 ± 3.0% ng/g d.w.) were the dominant compounds, whereas most other OPFRs occurred below 20 ng/g d.w. The relatively lower concentrations observed for several analytes may reflect limited sediment accumulation and the predominantly sandy nature of the investigated marine sediments, which are characterized by lower organic matter content compared with fine-grained riverine sediments. Algae exhibited the highest contamination burden among the investigated matrices, with mean ΣOPFR concentrations of 6106 ng/g d.w., suggesting strong accumulation potential within marine biota. TPP was by far the dominant analyte (5830 ± 3.0% ng/g d.w.), accounting for the majority of total OPFRs, while TCEP (122 ± 3.2% ng/g d.w.) and TCPP (104 ± 3.0% ng/g d.w.) were also consistently detected. Other analytes were present at substantially lower levels (<25 ng/g d.w.), although detection rates remained high across compounds.

The successful quantification of OPFRs in authentic Black Sea samples, combined with high detection frequencies and reproducible analytical performance, demonstrates the applicability of the proposed LC–MS/MS workflow for routine multi-matrix environmental monitoring of OPFRs in marine ecosystems.

5. Comparison with other methods

A wide range of analytical strategies has been reported for the determination of OPFRs in aqueous environmental matrices, with differences primarily arising from extraction approaches, instrumental configurations, and achievable sensitivity (Table 2). Solid-phase extraction (SPE) remains the predominant preconcentration technique, frequently employing Oasis HLB, ENVI-18, or Strata-X sorbents. However, reported sample volumes vary substantially, from 100 mL in wastewater applications [24] to more than 10 L for river water monitoring [25], reflecting the trade-off between detection limits and operational feasibility. While large-volume extraction improves sensitivity, it increases processing time, solvent consumption, and risk of analyte loss or contamination. Alternative extraction techniques, including liquid–liquid extraction (LLE), solid-phase microextraction (SPME), and stir-bar sorptive extraction (SBSE), have been explored to reduce solvent usage and simplify sample handling [19]. Although these approaches may offer practical advantages, they often present limitations in terms of reproducibility, analyte coverage across wide polarity ranges, or quantitative robustness in complex matrices. Consequently, method development for OPFRs continues to require careful optimization of sensitivity-to-volume ratio, matrix compatibility, and analytical throughput.

5.1. Aqueous samples

In aqueous matrices, GC–MS/MS and LC–MS/MS represent the predominant analytical platforms for OPFR determination (Table 11). GC-based approaches, typically employing electron ionization (EI) in SIM or MRM mode, have achieved very low detection limits (0.02–0.54 ng/L) [30]. However, these methods frequently require extensive sample preparation, large extraction volumes, and, in some cases, derivatization steps to improve volatility and thermal stability. For example, river water methods using 12 L sample volumes [30] demonstrate excellent sensitivity but involve significant processing effort and solvent consumption. LC–MS/MS, particularly in electrospray ionization (ESI) mode, offers broader applicability to polar and thermolabile OPFRs and their metabolites. Reported LOQs in the range of 0.03–0.1 ng/L have been achieved using UHPLC–MS/MS systems [37], while Hu et al. (2014) reported MQLs of 0.5–1 ng/L in seawater using SPE-LC–MS/MS [35]. Compared to GC-based methods, LC–MS/MS generally reduces the need for derivatization and expands analyte coverage; nevertheless, many studies rely on sample volumes between 500 mL and 1 L, reflecting the ongoing trade-off between sensitivity and operational practicality [29,33,34,36]. Alternative extraction strategies such as LLE [28,30], SPME [27], or combined SPE/LLE approaches [31] have been explored to optimize recovery and reduce solvent consumption [38]. However, these techniques often exhibit compound-dependent variability, particularly for highly hydrophobic analytes. Reported recoveries span a broad range (43–165%), with notable variability depending on matrix composition and extraction protocol [32–34]. Extremely low recoveries (24–40%) have been documented for TEHP in certain studies [31], highlighting the analytical challenges associated with strongly hydrophobic OPFRs. In contrast, chlorinated compounds such as TCPP and TDCPP typically exhibit more consistent recoveries (>80%) across aqueous matrices [33]. When benchmarked against the methods summarized in Table 10, the present approach offers several analytical advantages. First, method detection limits of 0.19–0.40 ng/L in seawater were achieved using a moderate sample volume (200 mL), resulting in a favorable sensitivity-to-volume ratio compared with approaches requiring ≥500 mL or multi-liter extractions. Second, the method ensures consistent recoveries (87–103%) within accepted validation criteria while maintaining controlled matrix effects.

Third, unlike most previously reported studies focused exclusively on freshwater or wastewater matrices, the present method was validated across multiple marine compartments. This multi-matrix validation enhances transferability and supports integrated monitoring of OPFR distribution in coastal and marine environments.

5.2. Sediment samples

Numerous extraction and clean-up strategies have been reported for OPFR determination in sediment matrices (Table 12), reflecting the analytical challenges posed by high organic carbon content and strong analyte–matrix interactions. Soxhlet extraction remains one of the most frequently applied approaches, typically employing dichloromethane/hexane or hexane/acetone mixtures, often followed by SPE clean-up using Florisil or Oasis HLB cartridges. Under these conditions, recoveries generally range between 67 and 125%, with reported detection or quantification limits as low as 0.0054–0.098 μg/kg dw [28,39]. Despite satisfactory analytical performance, Soxhlet extraction is inherently time- and solvent-intensive, limiting its suitability for high-throughput or large-scale monitoring applications. To address these limitations, pressurized liquid extraction (PLE) and microwave-assisted extraction (MAE) have been introduced as accelerated alternatives. Giulivo et al. (2017) reported recoveries of 67–103% using PLE with hexane:acetone (1:1) coupled to LC–MS/MS detection; however, lower efficiency was observed for specific compounds such as TCEP (~51%), indicating compound-dependent extraction challenges [40]. Similarly, García-López et al. (2009) demonstrated recoveries

Table 11
Reported extraction techniques, instrumental configurations, and performance characteristics for OPFR determination in aqueous environmental samples.

Matrix type	Extraction (sample vol., sorbent, solvent)	Instrument & mode	Column	LOD/LOQ/MDL (ng L^{-1})	Recovery (%)	Reference
River water	SPE, 500 mL, Oasis HLB, elution DCM/Hex/Ac	GC–MS/MS, EI, MRM	DB-5MS	8–44	–	[26]
River water	SPE, 12 L, Amberlite XAD-2, elution DCM	GC–MS/MS, EI, MRM	DB-5MS	0.082–8.6 (MDL)	–	[25]
River + drinking water	SPME, 10 mL, graphene-oxide fiber	GC–NPD	HP5-MS	1.4–135.6	–	[27]
River water	LLE, 600 mL, elution DCM	GC–MS/MS, EI, MRM	DB-5MS	0.13–9.6 (LOQ)	67–121	[28]
River water	SPE, 1 L, Oasis HLB, elution ethyl acetate	GC–MS, EI, SIM	RXI-17SIL MS	0.084–1.51	–	[29]
River + wastewater	SPE, 1 L, Strata-X, elution MeOH/DCM	UHPLC–MS/MS, ESI+, SRM	C18	0.03–0.1 (LOQ)	94–106	[25]
River water	LLE, 800 mL, elution DCM	GC–MS, EI, SIM	HP-5MS	0.02–0.54 (MDL)	67–95	[30]
River water	SPE/LLE, 500 mL, SERDOLITH PAD III, elution DCM	GC–MS/MS, EI, MRM	HP5-MS	2.7–26.6	43–78 (SPE); 45–77 (LLE)	[31]
Fish farming water	SPE, 700 mL, Oasis HLB, elution ethyl acetate + DCM	GC–MS, EI, SIM	DB5-MS	0.13–20.7	28–165	[32]
Surface, wastewater, runoff	SPE, 200 mL, ENVI-18, elution DCM/ACN	LC–MS/MS, ESI+, MRM	HILIC-1	0.3–16	65–104	[33]
Surface water	SPE, 500 mL, Oasis HLB, elution MeOH:MTBE 1:1	GC–MS/MS, EI, MRM	HP5-MS	0.5–14	85–132	[34]
Seawater	SPE, 500 mL, ENVI-18, elution ACN	LC–MS/MS, ESI+, MRM	BEH phenyl	0.5–1 (MQL)	67–104	[35]
Seawater	SPE, 1 L, Oasis HLB, elution ethyl acetate	GC–MS, EI, SIM	DB-5MS	30–529 (pg/L, MDL)	80–101	[36]
Seawater	SPE, Strata-X polymeric reverse-phase cartridges (500 mg/6 mL, 33 μm), ACN	LC-MS/MS, ESI+, ESI-	C18	0.52-1.12	87-103	This study

Table 12
Reported extraction, clean-up, and analytical conditions for OPFR determination in sediments.

Extraction & Solvent	Clean-up	Detection system	Column & Ionization	LOD/LOQ (μg kg^{-1} dw)	Recovery (%)	Reference
Soxhlet, DCM:Hex (1:4)	Florisil SPE, elution EtOAc	GC–MS/MS, MRM	DB-5MS, EI	0.02–1.44 (LOQ)	67–125	[28]
Soxhlet, Hex:Acetone (1:1)	SPE, Oasis HLB, elution EtOAc	GC–MS, SIM	DB-5MS, ECNI	0.0054–0.098 (MDL)	79.5–123.3	[39]
PLE, Hex:Acetone (1:1)	–	LC–MS/MS, SRM	Purosphere RP-18, ESI	0.05–1.25	67–103 (TCEP: 51)	[40]
SPME, PDMS fiber ($CaCl_2$ + NaN_3)	–	GC–MS, SIM	DB-5MS, EI	–	–	[41]
Tenax extraction, Acetone:Hex (1:1)	–	GC–MS, SIM	DB-5MS, EI	–	–	
Sonication, Hex:Acetone (1:1)	Florisil SPE, elution EtOAc	GC–MS, EI	DB-5MS	0.02–4.10	56.9–98.0	[42]
Sonication, ACN	-	LC-MS/MS	C18	0.13-0.63 (LOQ)	74-89	This study

between 78 and 105% using Soxhlet or MAE followed by GC–ICP-MS analysis, highlighting the versatility of these techniques for phosphorus-containing flame retardants [43]. Ultrasound-assisted extraction (UAE) has emerged as a simplified and less resource-intensive alternative. Reported recoveries range from 56.9 to 98%, with LODs between 0.02 and 4.1 μg/kg dw [42]. Nevertheless, increased variability and lower recoveries for certain hydrophobic analytes (e.g., TBEP, 48%) have been documented, underscoring the importance of solvent optimization and adequate clean-up [41]. More recently, passive sampling approaches such as SPME and Tenax extraction have been applied to estimate the bioavailable fraction of OPFRs in sediments [44]. While these techniques provide valuable ecotoxicological insight, they do not quantify total concentration and are therefore not directly comparable to exhaustive extraction methods.

When benchmarked against these approaches, the present method demonstrates competitive analytical performance while offering improved operational efficiency. The optimized UAE protocol combined with SPE clean-up and LC–MS/MS detection achieved recoveries of 74–105% in sediment and algae, with method detection limits below 0.5 ng/g. These limits are comparable to or lower than those reported for conventional Soxhlet, PLE, or MAE-based methods, but with significantly reduced solvent consumption and shorter processing times. Importantly, the achieved sensitivity-to-effort ratio supports routine application in monitoring programs without compromising quantitative reliability. The method therefore provides a balanced compromise between extraction efficiency, sensitivity, and sustainability, making it particularly suitable for large-scale assessment of OPFR contamination in marine sediments.

5.3. Marine biota and algae

Although numerous analytical methods have been reported for persistent organic pollutants in marine biota and algae, most studies have focused on legacy contaminants such as PAHs, PCBs, OCPs, PFASs, or hydrocarbons rather than organophosphate flame retardants (OPFRs). Previous algae-based monitoring studies commonly relied on labor-intensive extraction and clean-up procedures to address the complexity of biological matrices. For instance, El-Maradny et al. employed repeated ultrasonic extraction of freeze-dried algae using hexane/dichloromethane (4:1, v/v), followed by an extensive multi-layer clean-up step consisting of silica, alumina, florisil, copper powder, and sodium sulfate columns prior to GC–MS analysis of POPs in marine algae from the Mediterranean Sea [45]. This workflow required large sample amounts (≈5 g d.w.), multiple extraction cycles, and substantial solvent consumption, which reduced analytical throughput despite achieving satisfactory recoveries (78–108%) and low detection limits for hydrophobic contaminants.

By comparison, recent LC–MS/MS methods for OPFRs and PFASs in aquatic systems have increasingly favored simplified sample preparation strategies combined with isotope-labelled internal standards and matrix-matched calibration to compensate for matrix effects and improve quantification reliability. For example, studies investigating

OPFR occurrence in riverine environments, such as the Upper Yangtze River, relied on SPE-based enrichment followed by UHPLC–MS/MS, demonstrating excellent sensitivity but focusing primarily on aqueous matrices rather than marine biota [46]. However, analytical approaches specifically optimized for algae remain scarce, despite the recognized role of algae as efficient bioaccumulators and biomonitors for hydrophobic and semi-polar contaminants. Likewise, algae have been increasingly explored for their pollutant uptake potential and environmental remediation capacity, highlighting their strong interaction with hydrocarbons, metals, and organic contaminants, although analytical workflows for contaminant determination in algal tissues remain highly matrix-dependent [47].

In the present study, a simplified ultrasound-assisted extraction (UAE) method using acetonitrile as a universal extraction solvent was developed for algae and sediment samples, eliminating the need for dedicated sorbent clean-up while maintaining robust analytical performance. Compared with previously reported methods employing non-polar solvent mixtures and extensive fractionation, the proposed approach reduced sample preparation time, solvent consumption, and procedural complexity. Despite the absence of an intensive clean-up step, acceptable matrix effects (83–102%), reproducible recoveries (78–105% in algae), and low method detection limits (LOQs generally <0.5 ng/g d.w.) were achieved for all target OPFRs. Moreover, the streamlined UAE–LC–MS/MS protocol improved analytical throughput and enabled the simultaneous determination of triester OPFRs and more polar transformation products within a single workflow. The successful application of the method to authentic Black Sea algae samples further confirms its practical suitability for marine biomonitoring and environmental assessment of OPFR contamination.

6. Environmental relevance

The validated LC–MS/MS workflow enables ultra-trace quantification of organophosphate flame retardants (OPFRs) across multiple marine compartments, with method detection limits of 0.19–0.40 ng/L in seawater and <0.5 ng/g in solid matrices. These sensitivity levels fall below or within the lower range of concentrations commonly reported for urban and coastal environments, ensuring reliable detection not only in impacted areas but also under background conditions. The favorable sensitivity-to-volume ratio achieved with moderate sample volumes enhances practical applicability for routine monitoring.

A key strength of the method lies in its multi-compartment validation. By integrating seawater, sediment, and algal matrices within a harmonized analytical framework, the approach supports coherent assessment of contaminant distribution and partitioning behavior. Water samples reflect short-term inputs and transport dynamics, sediments represent long-term accumulation and historical deposition, while algae provide insight into early-stage biological uptake. The ability to quantify both relatively polar OPFRs (e.g., tris(2-chloroethyl) phosphate, TCEP, and tris(1-chloro-2-propyl) phosphate, TCPP) and more hydrophobic compounds (e.g., triphenyl phosphate, TPHP, and tris(2-ethylhexyl) phosphate, TEHP) within a single workflow facilitates a more comprehensive evaluation of environmental fate and exposure pathways.

The environmental monitoring of OPFRs is particularly relevant in the current regulatory context. These compounds have been increasingly used as replacements for legacy brominated flame retardants restricted under international agreements such as the Stockholm Convention on Persistent Organic Pollutants, which has phased out several polybrominated flame retardants due to their persistence and bioaccumulation potential [15]. Consequently, OPFRs have emerged as widely used alternatives in polymers, textiles, electronics, and polyurethane foams, leading to their widespread detection in environmental compartments [16]. Although most OPFRs are not currently listed as persistent organic pollutants under the Stockholm Convention, they are increasingly recognized as “regrettable substitutes” due to growing evidence of environmental occurrence and potential toxicity [48].

Regulatory attention to OPFRs has intensified in recent years. Within the European Union, the European Chemicals Agency (ECHA) regulates these substances under the REACH Regulation (EC No. 1907/2006) and the CLP Regulation (EC No. 1272/2008). For example, TCEP has been identified as a Substance of Very High Concern (SVHC) due to its carcinogenic and reproductive toxicity properties, while TPHP was added to the REACH Candidate List in 2024 because of its endocrine-disrupting potential [17]. In addition, regulatory initiatives have addressed chlorinated OPFRs such as TCEP, TCPP, and TDCPP, particularly regarding their presence in consumer products and materials intended for children [49]. Similar regulatory scrutiny exists in the United States, where the U.S. Environmental Protection Agency (EPA) has prioritized several chlorinated OPFRs, including TCEP, for risk evaluation under the Toxic Substances Control Act (TSCA) due to concerns related to human health and environmental exposure [50].

Within this evolving regulatory landscape, reliable analytical data are essential for exposure assessment, risk evaluation, and regulatory decision-making. The validated method addresses this need by providing reproducible and comparable measurements across matrices with distinct physicochemical properties. Such harmonized datasets are critical for identifying contamination trends, assessing environmental partitioning processes, and supporting science-based management strategies for emerging contaminants. Therefore, the developed analytical platform offers a transferable and robust tool for integrated environmental surveillance of OPFRs in marine and freshwater ecosystems, bridging the gap between high-sensitivity instrumental analysis and the growing demand for regulatory-relevant monitoring data.

7. Strengths and limitations of the study

A principal strength of this study is the development and comprehensive validation of a multi-matrix LC–MS/MS method enabling simultaneous determination of eleven OPFRs across seawater, sediment, and algal samples within a harmonized analytical framework (Fig. 8). In contrast to many previous investigations focused exclusively on aqueous matrices, the present approach demonstrates applicability to both abiotic and biotic compartments, supporting integrated assessment of contaminant distribution and partitioning behavior.

The method achieved low detection limits (0.19–0.40 ng/L for seawater; <0.5 ng/g for solids) while maintaining moderate sample volumes, resulting in a favorable sensitivity-to-effort ratio suitable for routine monitoring applications. Another key strength lies in the systematic validation of analytical performance parameters, including linearity over a broad dynamic range, controlled matrix effects, reproducible recoveries (74–105%), and precision values consistently below 10%. The robustness assessment further demonstrated that minor variations in chromatographic or extraction conditions did not significantly affect quantitative performance, confirming the method's stability under routine laboratory conditions. Together, these elements support the generation of reliable and comparable datasets across matrices with markedly different physicochemical properties. Nevertheless, several limitations should be acknowledged. First, analyte coverage, although representative of major OPFR classes, does not encompass the full spectrum of commercially used flame retardants and emerging transformation products, primarily due to the limited availability of certified reference standards. Second, while the selected SPE and UAE procedures provide reproducible and efficient extraction, they remain more time- and solvent-demanding than miniaturized microextraction techniques (e.g., SPME or SBSE), which may offer advantages in high-throughput applications. Additionally, moderate matrix effects were observed in sediment and algal extracts, indicating that further refinement of clean-up strategies could enhance quantitative robustness, particularly for highly hydrophobic compounds. Overall, the study delivers a validated and transferable analytical workflow for OPFR determination across multiple environmental compartments. Future research should focus on

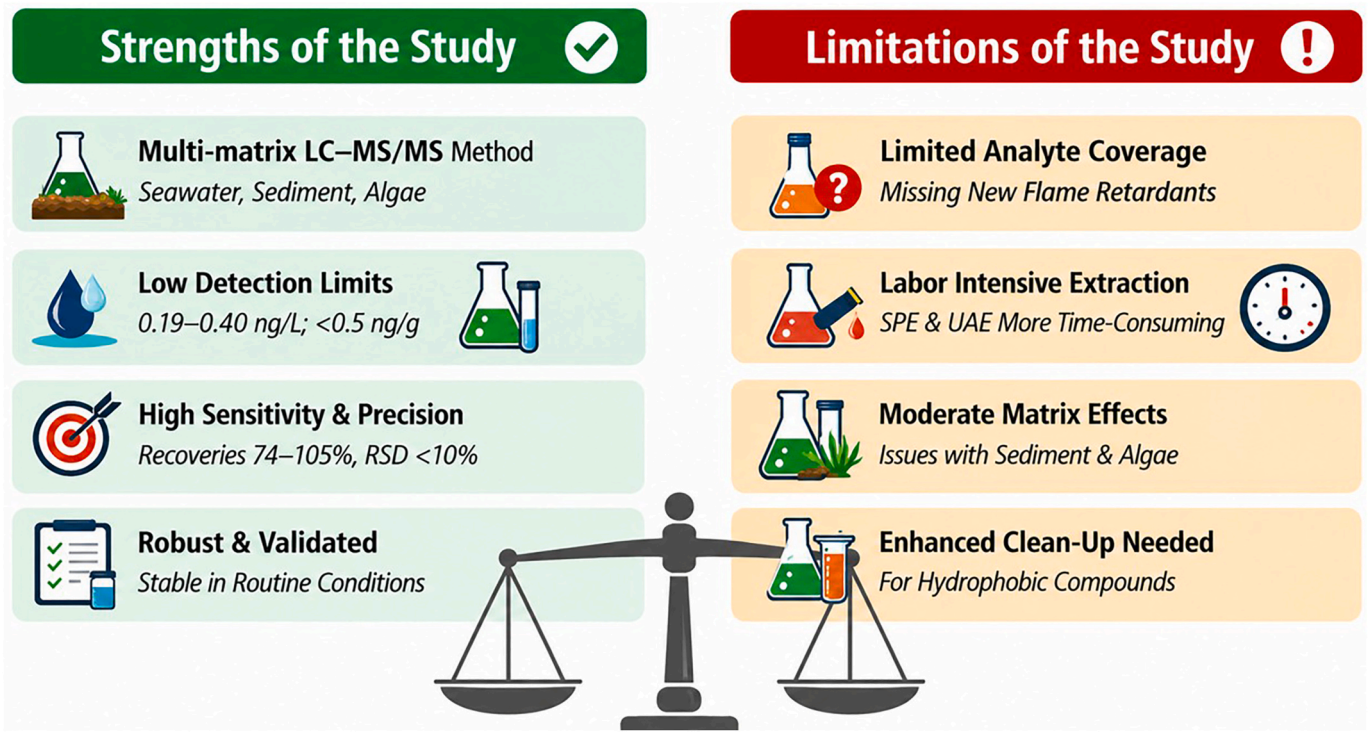

Fig. 8. Strengths and limitations of the developed multi-matrix LC–MS/MS method for OPFR determination.

expanding analyte coverage, integrating high-resolution mass spectrometry for transformation product identification, and further optimizing extraction strategies to improve analytical efficiency and sustainability.

8. Conclusions

A multi-matrix LC–MS/MS method was developed and comprehensively validated for the simultaneous determination of eleven organophosphate flame retardants in seawater, sediment, and algae. The method demonstrated excellent linearity over more than three orders of magnitude ($R^2 \geq 0.993$), reproducible recoveries (74–105%), and precision values consistently below 10%, confirming reliable quantitative performance across matrices with distinct physicochemical characteristics. Ultra-trace sensitivity was achieved, with method detection limits of 0.19–0.40 ng/L in seawater and <0.5 ng/g in solid matrices, obtained using moderate sample volumes and simplified extraction procedures. Matrix effects were systematically evaluated and remained within controlled limits (77–108%), ensuring robust quantification without significant ionization bias. Robustness testing further confirmed that minor variations in chromatographic and extraction conditions did not compromise analytical performance. By integrating validated protocols for aqueous, sediment, and biotic samples within a single analytical workflow, this study provides a transferable platform for coherent multi-compartment monitoring of OPFRs. This method bridges the gap between high-sensitivity instrumental analysis and practical environmental surveillance, supporting improved assessment of contaminant distribution, partitioning behavior, and exposure pathways in aquatic ecosystems.

CRediT authorship contribution statement

Florentina Laura Chiriac: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Iuliana Paun:** Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Florinela Pirvu:** Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Visualization, Writing – original draft, Writing – review & editing. **Vasile Ion Iancu:** Conceptualization, Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. **Victor Constantin Cojocaru:** Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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