أكاديمية كاوست
KAUST ACADEMY

جامعة الملك عبدالله للعلوم والتقنية
King Abdullah University of Science and Technology

وزارة التعليم
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BESE
BIOLOGICAL AND ENVIRONMENTAL SCIENCE AND ENGINEERING DIVISON

Emerging Contaminants in Marine Samples: Advancing Analytical Methods for Water Sustainability

Mashael Meshaal Alghamdi

Supervised by Mariana Rodrigues, Dr. Andreia Farinha, Micaela Justo, Fern Mary Temple, Prof. Susana Carvalho

mashsel2979@gmail.com, Mariana.rodrigues@kaust.edu.sa, susana.carvalho@kaust.edu.sa, andreia.farinha@kaust.edu.sa, fernmarygilbert.lynetemple@kaust.edu.sa, micaelasofia.dossantosjusto@kaust.edu.sa.

Abstract

Pharmaceuticals and personal products are among the contaminants of emerging concern (**CECs**) that are often found in aquatic settings and could endanger marine ecosystems. The complexity of marine matrix and their prevalence at trace concentrations make it difficult to accurately identify and quantify them, underscoring the necessity for trustworthy analytical techniques. Reliable assessment of these contaminants is essential for water sustainability. Understanding their occurrence, distribution, and potential accumulation in marine ecosystems supports water-quality monitoring, ecosystem protection, and sustainable management of coastal resources. The goal of this work was to use solid-phase extraction (**SPE**) and high-performance liquid chromatography-Orbitrap mass spectrometry (**HPLC-Orbitrap MS**) to determine **CECs** in fish and seawater samples.

Dual polarity electrospray ionization (**ESI+** and **ESI-**) with targeted selected ion monitoring (**tSIM**) was used to assess a multi-residue approach that targeted 33 **CECs** from various chemical classes. Validation parameters that include recovery, matrix effects, precision, linearity, and sensitivity were used to evaluate the performance of the method. Several analytes displayed values below the predetermined acceptable range in the recovery data, which indicated compound-dependent variation and the need for additional extraction condition modification. Although ion suppression was seen for some analytes, matrix effects were typically acceptable for the majority of substances, justifying the use of matrix-matched calibration. With matrix-matched calibration correlation coefficients close to 1.000, the technique showed excellent linearity for many substances.

With all factors considered, the validated **SPE-HPLC-Orbitrap MS** procedure offers an acceptable analytical method for identifying **CECs** in marine matrices. The method supports future monitoring studies investigating the occurrence and potential bioaccumulation of emerging contaminants in marine environments. By strengthening the capacity to characterize chemical pressures in seawater and marine organisms, this analytical approach contributes to water sustainability through improved water-quality assessment, early identification of emerging contamination, and science-based protection and management of marine and coastal ecosystems.

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1 Introduction

Contaminants of emerging concern (**CECs**) are a heterogeneous group of chemicals that are currently increasingly detected in aquatic environments due to their constant inflow and incomplete removal in conventional wastewater treatment processes **[1]**. Among all, pharmaceuticals and personal care products (**PPCPs**) are a significant group of contaminants, such as antibiotics, analgesics, hormones, cosmetic ingredients, and other biologically active compounds **[1]**. Their widespread use and constant discharge into aquatic environments have raised concerns about their potential impacts on aquatic ecosystems **[3]**. Although many **CECs** are not classified as persistent pollutants, their continuous input into the aquatic environment can lead to long-term exposure of marine organisms **[1]**. These compounds have been found in different environmental compartments such as water, sediments, and biological matrices **[2]**. In marine environments, **CECs** may be present at trace concentrations and could be bioaccumulated in organisms, leading to potential ecological effects **[3]**. However, their occurrence and fate in marine organisms have been less studied than in wastewater and surface waters **[1]**.

Monitoring emerging contaminants from a water sustainability perspective is critical for the protection of aquatic ecosystems and long-term water quality. Evaluation of **CECs** plays a vital role in sustainable water resource management and environmental protection in regions like the Red Sea, where marine environments are characterized by high biodiversity and are affected by increasing anthropogenic activities **[3]**. The accurate determination of **CECs** in marine organisms is challenging as these compounds are present at trace levels and biological matrices are complex **[2]**. These complex matrices may influence analyte extraction and instrumental response; therefore, sensitive and reliable analytical methods are needed for accurate detection and quantification **[2]**. Furthermore, variations in analyte properties and matrix-related interferences may affect extraction efficiency and analytical performance, representing key challenges in **CEC** determination.

A step of crucial importance for **CEC** analysis is the sample preparation, as efficient extraction and purification can enhance analytical performance. In this step, solid-phase extraction (**SPE**) has been extensively used for the pre-concentration and cleanup of **CECs** from environmental matrices **[1]**. The coupling of SPE with liquid chromatography-mass spectrometry (**LC-MS**) offers a sensitive and selective approach for the determination of trace-level contaminants in complex marine matrices **[2]**. Therefore, an analytical workflow combining effective sample preparation with advanced instrumental analysis can provide a reliable solution for overcoming challenges associated with **CEC** detection in marine samples.

For reliable quantitative analysis, analytical methods require proper validation to assess their performance and suitability. Validation parameters, including recovery, matrix effect, precision, linearity, and sensitivity, are essential to confirm the reliability of analytical measurements. Thus, this study aimed to apply and validate an analytical method for the determination of **CECs** in fish and seawater samples using solid-phase extraction (**SPE**) followed by high-performance liquid chromatography-Orbitrap mass spectrometry (**HPLC-Orbitrap MS**).

2 Methodology

2.1 Overview of the analytical workflow

The analytical workflow was designed for the determination of contaminants of emerging concern (**CECs**) in seawater and fish matrices using solid-phase extraction (**SPE**) followed by high-performance liquid chromatography coupled with Orbitrap mass spectrometry (**HPLC-Orbitrap MS**). The workflow consisted of sample preparation, internal standard addition, SPE extraction, extract concentration and reconstitution, chromatographic separation, mass spectrometric detection, and data evaluation. The method employed targeted selected ion monitoring (**tSIM**) with dual polarity electrospray ionization (ESI+ and ESI-) for the detection of target compounds.

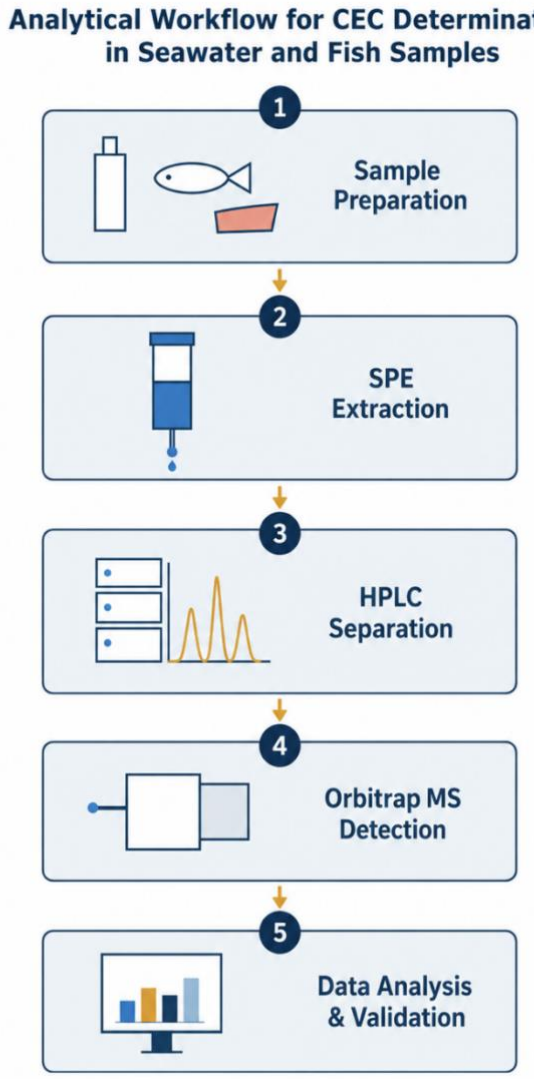


Figure 1: Analytical workflow for CECs determination in seawater and fish samples using SPE-HPLC-ORBITRAP MS.

2.2 Target compounds and standards preparation

A total of 33 target compounds were selected, representing different chemical classes including pharmaceuticals, hormones, industrial chemicals, personal care products, and pesticides. Two LC-MS injections were performed for each sample, including positive and negative electrospray ionization modes. Sulfamethoxazole was monitored in both ionization modes due to its suitable detection in ESI+ and ESI-.

Five multi-analyte stock solutions were prepared at appropriate concentrations using different solvent systems according to analyte properties. Working standards and calibration solutions were prepared by serial dilution before analysis.

2.3 Sample preparation and SPE extraction

Seawater samples

Synthetic seawater was prepared using sea salts (40 g L^{-1}) dissolved in Milli-Q water to mimic Red Sea salinity. Seawater samples (100 mL) were spiked at selected concentration levels and processed using the same SPE-based extraction procedure applied to fish samples. The grinding step is not necessary for seawater samples due to their liquid matrix.

Fish samples

Fish muscle samples were freeze-dried and homogenized into a uniform powder. Aliquots of 0.5 g dry weight were prepared for extraction and spiked at low and medium concentration levels to evaluate extraction performance.

SPE procedure

Fish and seawater extracts were pre-concentrated using Oasis HLB cartridges (6 mL, 500 mg, Waters). Cartridges were conditioned with 5mL methanol and water before sample loading. After loading, cartridges were rinsed with water, air-dried, and eluted using methanol/ethyl acetate (50:50, v/v). Extracts were evaporated at 35 °C and reconstituted in acetonitrile/water (30:70, v/v), followed by filtration through 0.22 µm PTFE filters prior to HPLC-Orbitrap MS analysis.

2.4 HPLC-Orbitrap MS analysis

Chromatographic separation was achieved using a C18 column (150 mm × 4.6 mm) maintained at 30 °C with a flow rate of 0.5 mL/min and an injection volume of 10 µL. The mobile phases consisted of water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B) with the following 20 min gradient: 95% eluent A and 5% eluent B for the first 0.5 min, followed by a linear change to 5% eluent A and 95% eluent B, which was maintained until 16.6 min. The initial composition was then restored to 95% eluent A and 5% eluent B for the remainder of the run. Mass spectrometric analysis was performed using an Orbitrap Exploris 120 mass spectrometer equipped with a heated electrospray ionization source operating in positive and negative modes. Data acquisition was performed using targeted Selected Ion Monitoring (**tSIM**) at high resolution.

2.5 Evaluation Procedure

Method validation was performed using spiked seawater and fish samples to evaluate analytical performance. Different sample groups were prepared, including unspiked samples, low and medium spike samples, and matrix-matched calibration samples.

2.6 Evaluation parameters

The analytical method was evaluated using several validation parameters, including recovery, matrix effect, precision, linearity, and sensitivity.

Recovery was evaluated by comparing the measured analyte concentrations, determined using the matrix matched calibration curve, with the expected concentrations based on the spiked amounts. Recovery was expressed as the percentage of the expected concentration recovered after extraction.

Matrix effects were determined by comparing the slopes of matrix-matched calibration curves with solvent calibration curves to assess ion suppression or enhancement.

Precision was assessed using relative standard deviation (RSD%) between replicate measurements.

Linearity was evaluated using matrix-matched calibration curves and correlation coefficients (R^2).

Sensitivity was assessed based on limits of detection and quantification using signal-to-noise criteria.

2.7 Data analysis and acceptance criteria

Analytical performance was evaluated according to predefined acceptance criteria, including

Recovery **(70-120%),**

RSD values **below 20%,**

Matrix effect evaluation **(85-115%),**

and acceptable Calibration Linearity **($R^2 > 0.995$).**

Matrix effects were calculated using the ratio between matrix-matched and solvent calibration slopes. as shown in the Equation (1) below. Values **below 100%** indicate **ion suppression**, whereas values **above 100%** indicate **ion enhancement**.

$$ME(\%) = \frac{Slope_{matrix}}{Slope_{solvent}} \times 100$$

Equation (1):

The List of Target CEC compounds

Injection	Compound	Chemical class
ESI+	Salbutamol	β_2-agonist
ESI+	Acetaminophen	Analgesic
ESI+	Caffeine	Stimulant
ESI+	Metronidazole	Nitroimidazole antibiotic
ESI+	Atenolol	β-blocker
ESI+	Ciprofloxacin	Fluoroquinolone antibiotic
ESI+	Primidone	Anticonvulsant
ESI+	Ranitidine	H_2-receptor antagonist
ESI+	Trimethoprim	Antibiotic
ESI+	Sulfamethoxazole	Sulfonamide antibiotic
ESI+	Tramadol	Opioid analgesic
ESI+	Carbamazepine	Anticonvulsant
ESI+	Atrazine	Herbicide
ESI+	Benzophenone	UV filter
ESI+	Erythromycin	Macrolide antibiotic
ESI+	Fluoxetine	Antidepressant (SSRI)
ESI+	Progesterone	Steroid hormone
ESI+	17-β-Estradiol	Estrogen
ESI+	Estrone	Estrogen
ESI+	17-α-Ethynylestradiol	Synthetic estrogen
ESI+	Testosterone	Androgen
ESI+	Bisphenol A	Industrial EDC
ESI+	4-para-Nonylphenol	Alkylphenol EDC
ESI+	4-tert-Octylphenol	Alkylphenol EDC
ESI-	Sulfamethoxazole	Sulfonamide antibiotic
ESI-	Propylparaben	Preservative (paraben)
ESI-	Phenytoin	Anticonvulsant
ESI-	Gemfibrozil	Lipid regulator (fibrate)
ESI-	Naproxen	NSAID
ESI-	Bezafibrate	Lipid regulator (fibrate)
ESI-	Ibuprofen	NSAID
ESI-	Diclofenac	NSAID
ESI-	Triclosan	Antimicrobial agent
ESI-	Triclocarban	Antimicrobial agent

3 Results

3.1 Recovery Performance

Recovery was evaluated to assess the extraction efficiency of the SPE-HPLC-Orbitrap MS method. The acceptable recovery range was defined as **70-120%** based on commonly used acceptance criteria for analytical method validation in environmental analysis. Recovery values varied considerably among the analyzed **CECs** and ionization modes, indicating differences in extraction efficiency depending on compound properties and matrix interactions.

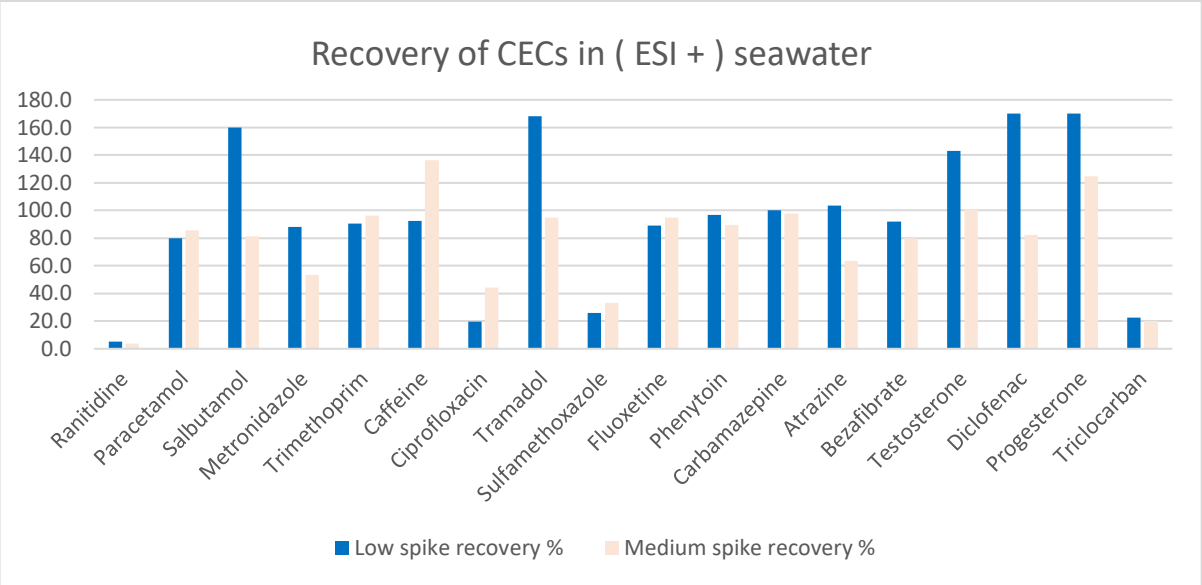


Figure 2: Recovery of CECs in seawater samples under ESI+ mode at low and medium spike levels. Most compounds showed variable recovery, with higher responses observed for tramadol, diclofenac, and progesterone.

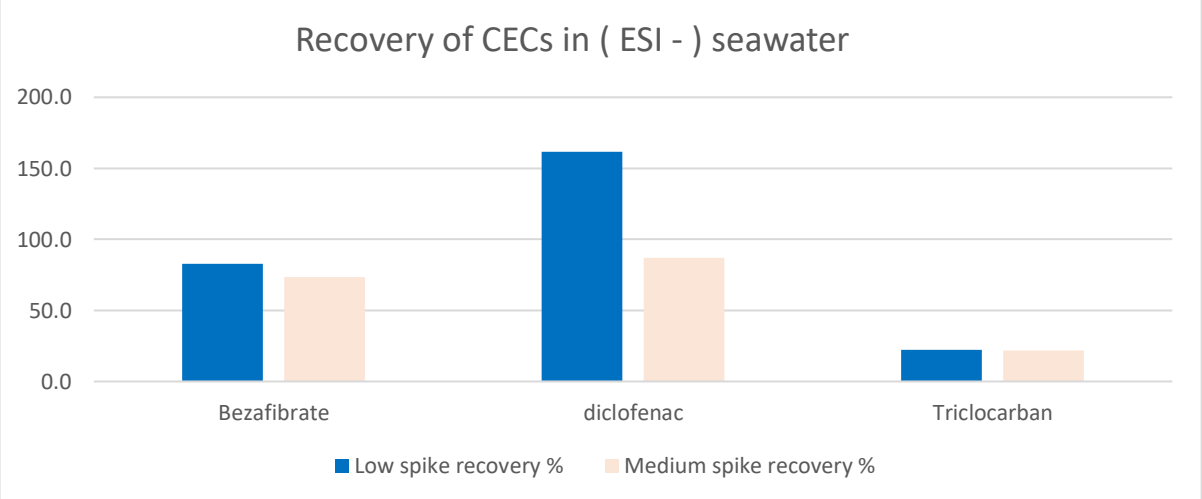


Figure 3: Recovery of CECs in seawater samples under ESI- mode at low and medium spike levels. Recovery values showed compound-specific variations, with diclofenac exhibiting higher recovery compared with other analytes.

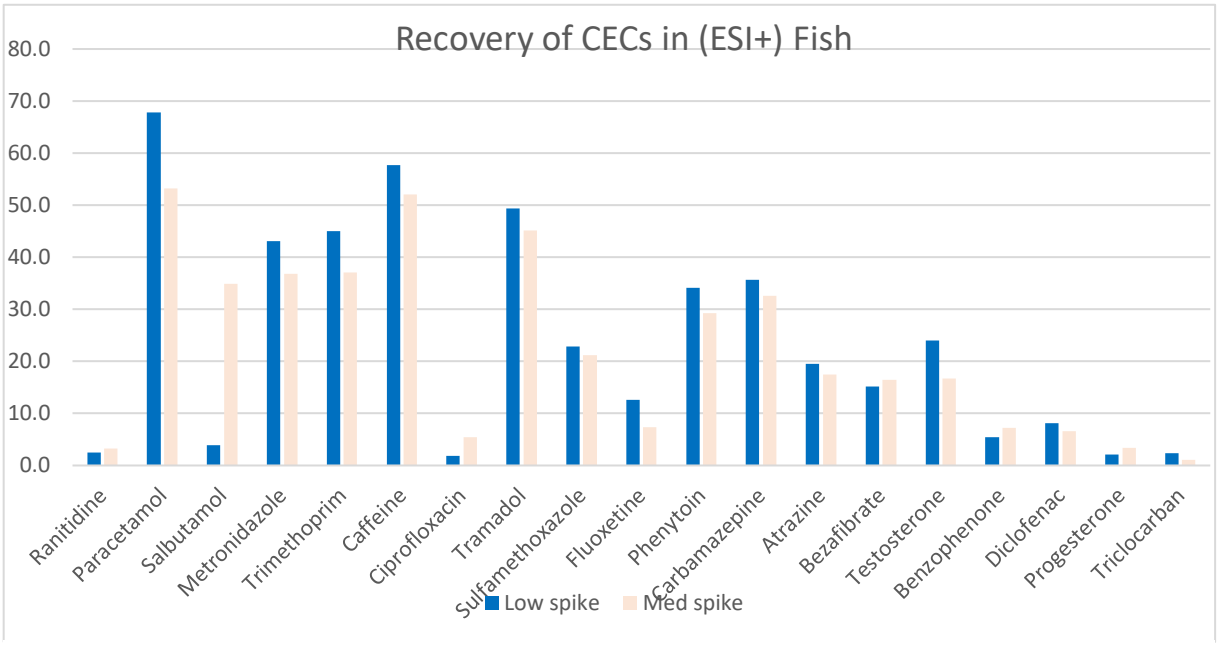


Figure 4: Recovery of CECs in fish samples under ESI+ mode at low and medium spike levels, showing compound-dependent variations in extraction performance

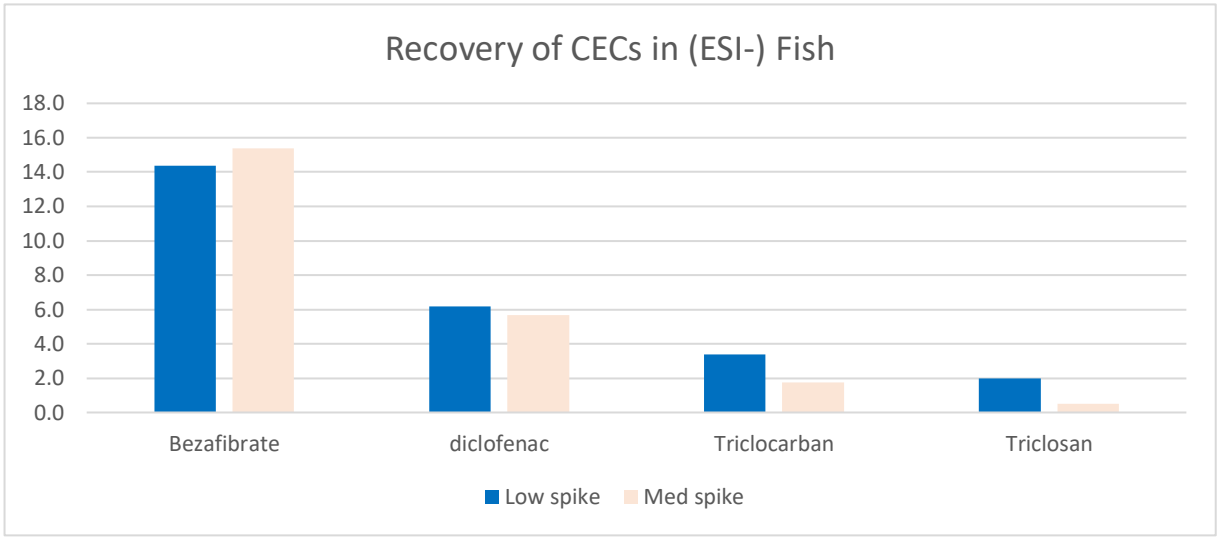


Figure 5: Recovery of CECs in fish samples under ESI- mode at low and medium spike levels, showing variations in recovery among the analyzed compounds.

3.2 Matrix Effect Evaluation

Matrix effects were evaluated to assess the influence of sample matrix components on analyte ionization during Orbitrap MS analysis. The acceptable matrix effect range was defined as **85-120%**, where values **below 85%** indicate **ion suppression** and values **above 120%** indicate **ion enhancement**.

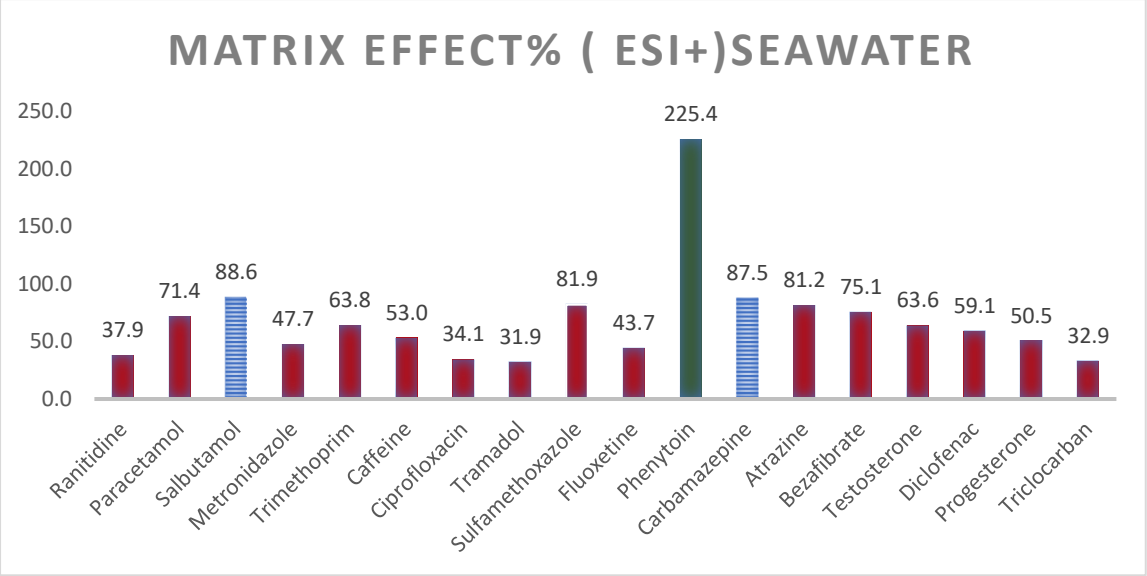


Figure 6: Matrix effect % of CECs in seawater samples under ESI+ mode, showing analyte-dependent variations in ion suppression and enhancement effects.

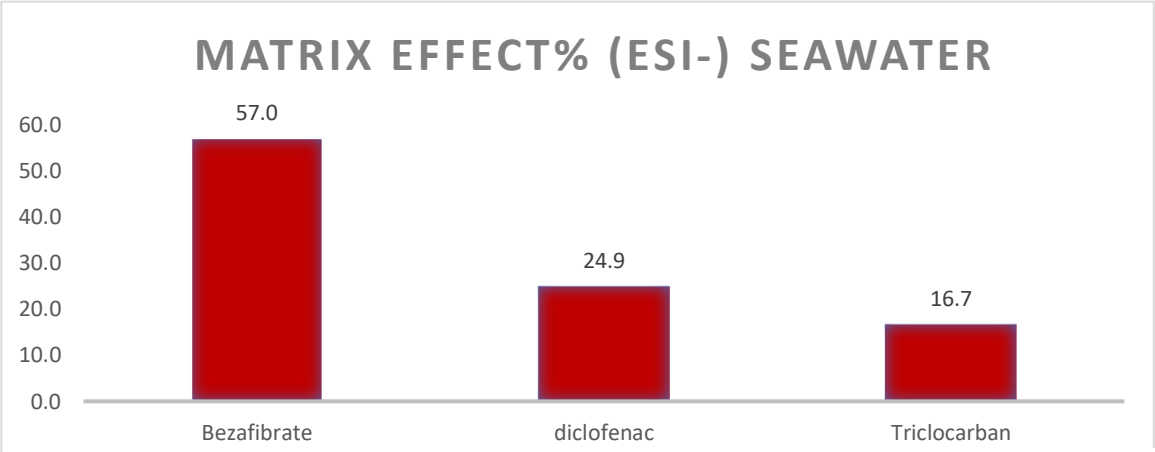


Figure 7: Matrix effect % of selected CECs in seawater samples under ESI- mode, showing variable matrix-related effects on analyte response.

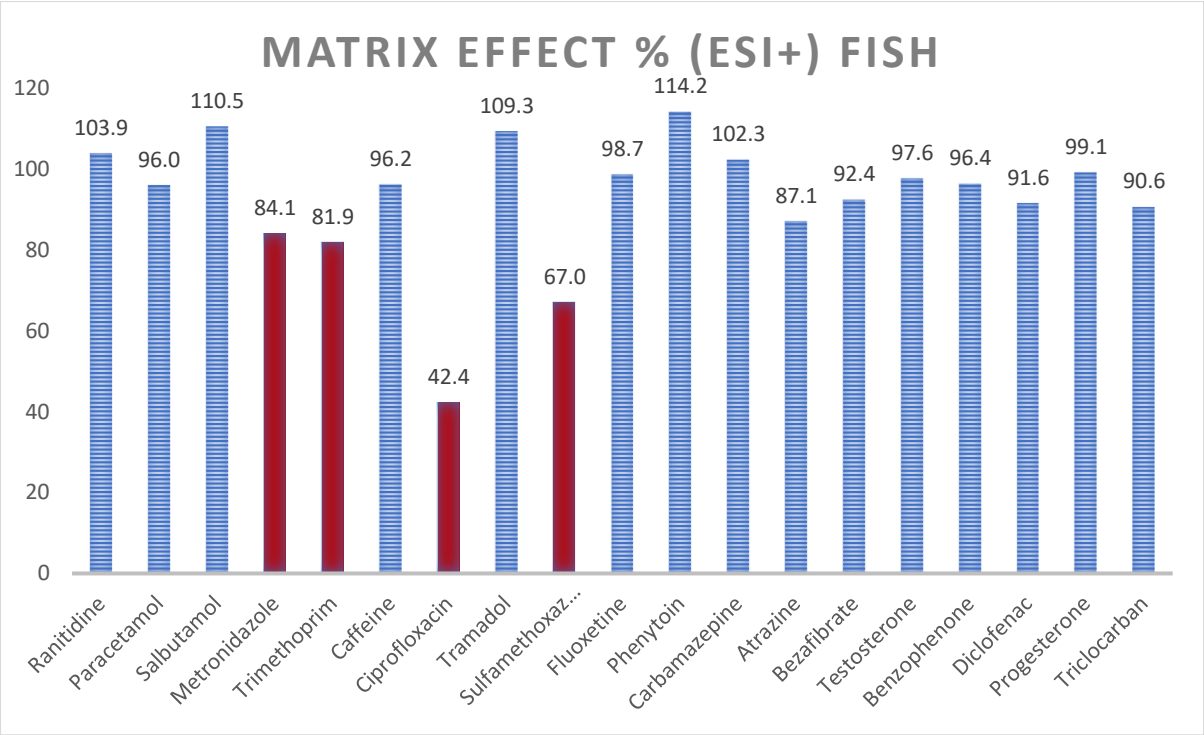


Figure 8: Matrix effect % of CECs in fish samples under ESI+ mode, with most compounds within the acceptable range and selected analytes showing ion suppression.

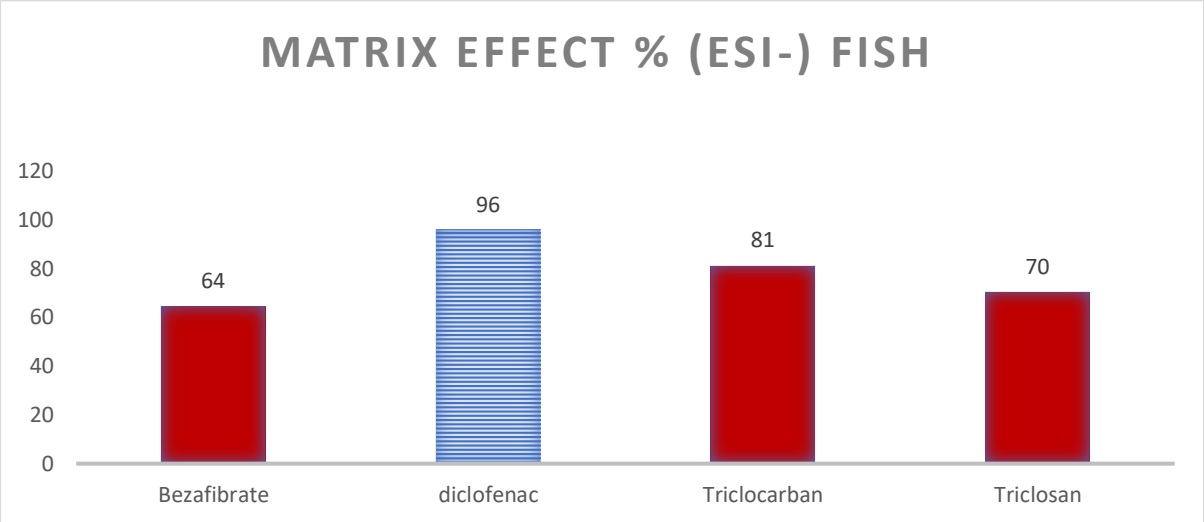


Figure 9: Matrix effect % of selected CECs in fish samples under ESI- mode, with diclofenac showing minimal matrix effect while other analytes exhibited ion suppression.

3.3 Precision Assessment (%RSD)

Precision was evaluated by calculating the relative standard deviation (RSD%) across replicate analyses to assess the repeatability of the analytical method. The acceptance criterion was defined as **RSD ≤20%,** indicating acceptable measurement variability.

The percentage of compounds meeting the precision criterion varied depending on ionization mode and spike level. In **ESI+** mode, acceptable precision was observed for **58%** of compounds at the **low spike level** and increased to **63%** at the **medium spike level**. In **ESI-** mode, the **low spike level** showed the highest proportion of compounds within the acceptance criterion **75%,** while the **medium spike level** showed greater variability, with **50%** of compounds exceeding the acceptable RSD limit.

These results indicate that precision performance was influenced by both ionization polarity and spike concentration. The observed variability highlights the importance of optimizing extraction conditions and controlling matrix-related effects to improve method reproducibility

Table 1: Percentages represent the proportion of compounds meeting each RSD criterion (e.g., 58% of compounds with RSD ≤20%).

Matrix	Ion Mode / Spike	RSD ≤20%	RSD >20%
Fish	ESI+ Low Spike	58%	42%
Fish	ESI+ Medium Spike	63%	37%
Fish	ESI− Low Spike	75%	25%
Fish	ESI− Medium Spike	50%	50%
Seawater	ESI+ Low Spike	58%	42%
Seawater	ESI+ Medium Spike	63%	37%
Seawater	ESI− Low Spike	75%	25%
Seawater	ESI− Medium Spike	50%	50%

3.4 **Linearity Assessment (**Matrix-Matched Calibration Curve **R^2)**

Linearity was evaluated using matrix-matched calibration (MMCAL) curves to assess the relationship between analyte concentration and analytical response. The acceptance criterion for linearity was defined as **R^2 >0.995**.

Table 2: Range of matrix-matched calibration curve (MMCAL) R^2 values obtained for CECs in fish and seawater samples compared with the acceptance criterion.

Matrix	MMCAL R^2 Range	Acceptance Criterion
Fish	0.984 – 1.000	$R^2 > 0.995$
Seawater	0.967 – 1.000	$R^2 > 0.995$

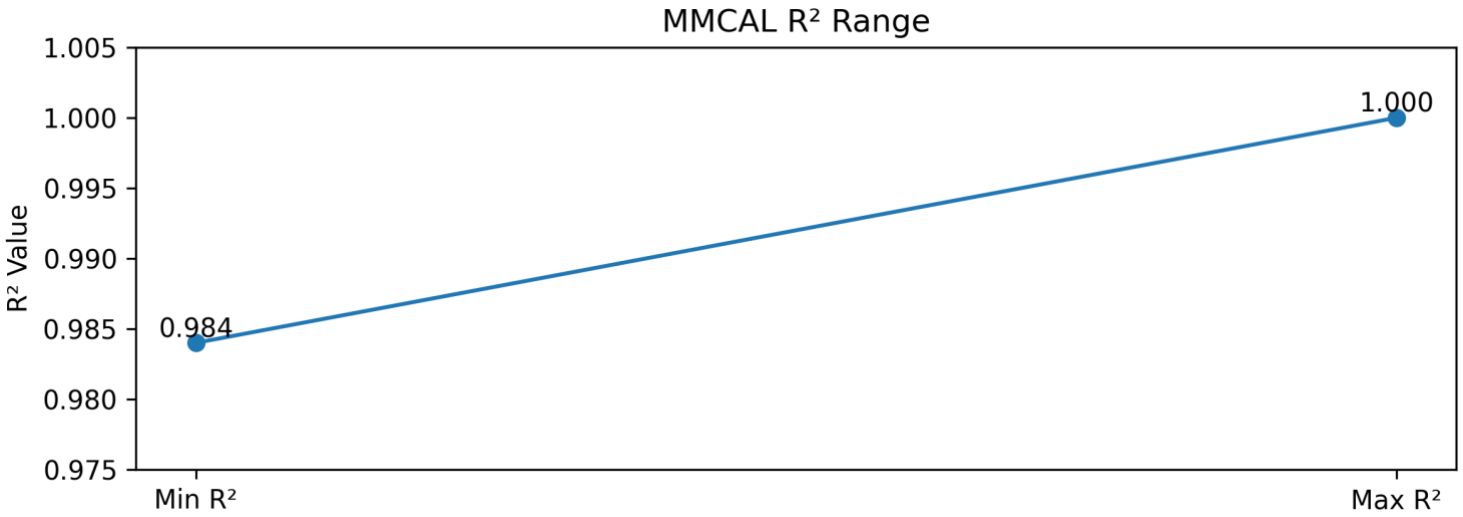


Figure 10: Range of matrix-matched calibration curve (MMCAL) R^2 values obtained for CECs in fish and seawater samples.

3.5 Sensitivity Evaluation (LOD/LOQ)

Sensitivity was evaluated to determine the ability of the analytical method to detect and quantify **CECs** at low concentration levels. **The limits of detection (LOD)** and **quantification (LOQ)** were defined based on signal-to-noise **(S/N)** criteria, where LOD was established at S/N >3 and LOQ at S/N >10.

These criteria ensure that detected compounds can be reliably distinguished from background noise and accurately quantified at low concentration levels.

Sensitivity Parameter	Acceptance Criterion
LOD	S/N > 3
LOQ	S/N > 10

Table 3: Sensitivity criteria used for LOD and LOQ determination.

Overall, method performance varied among validation parameters, with linearity and matrix effects showing more consistent results compared with recovery and precision.

4 Discussion

4.1 Overall Method Performance

The validation results demonstrated that the performance of the SPE-HPLC-Orbitrap MS method varied among the evaluated parameters and target compounds. Differences in analytical performance were observed between recovery, matrix effects, precision, and linearity, reflecting the challenges associated with determining **CECs** in different marine matrices. The comparison between fish and seawater samples showed that matrix composition influenced method performance, as biological samples generally presented more complex interactions compared with seawater. Previous studies have highlighted that the analysis of **CECs** in environmental and biological matrices is challenging due to their trace-level concentrations and matrix complexity **[1,3]**.

4.2 Recovery Performance

Recovery performance varied among compounds and between sample matrices. Fish samples generally showed lower recovery values, particularly under ESI- mode, suggesting that the biological matrix may have influenced extraction efficiency. In contrast, seawater samples showed a wider recovery range, with some compounds approaching or exceeding the acceptable range (70-120%), while others showed reduced recovery. These differences indicate that extraction efficiency depends on analyte properties, including chemical characteristics and interactions with the sample matrix. Previous studies have reported that CEC determination in complex matrices requires optimized extraction procedures due to differences in analyte behavior and matrix composition **[2,3]**. Therefore, further optimization of extraction conditions may improve recovery for compounds showing poor performance.

4.3 Matrix Effects

Matrix effects were observed in both fish and seawater samples, demonstrating the influence of sample composition on analyte response during HPLC-Orbitrap MS analysis. In fish samples, most compounds under ESI+ mode showed acceptable matrix effects, while selected analytes **exhibited ion suppression**, particularly **ciprofloxacin** and **sulfamethoxazole**. Under ESI- mode, several compounds showed suppression effects, indicating stronger matrix influence for some analytes. Seawater samples also showed compound-dependent matrix effects. Several analytes exhibited ion suppression, while **phenytoin** showed **ion enhancement**, indicating that seawater components could either decrease or increase ionization efficiency depending on the analyte. These differences highlight the importance of using matrix-matched calibration to compensate for matrix-induced variability and improve quantitative accuracy.

4.4 Influence of Ionization Mode and Spike Level on Method Performance

The comparison between ESI+ and ESI- modes showed that analytical performance was compound-dependent. As expected, some analytes showed better response and recovery under ESI+ mode, whereas others were more suitable for ESI- detection, reflecting differences in ionization behavior between chemical classes. For example, **diclofenac** in seawater showed slightly higher recovery under ESI- mode compared with ESI+ mode (**86.8% vs. 82.2% at medium spike level**), while **bezafibrate** showed higher recovery under ESI+ mode compared with ESI- mode (**92.2% vs. 82.8% at low spike level**). Variations between low and medium spike levels were also seen in both fish and seawater samples, indicating that analyte concentration can influence method performance, particularly at low concentration levels. Therefore, evaluating multiple spike levels is important to ensure reliable quantification across a wide concentration range.

4.5 Linearity and Sensitivity

Matrix-matched calibration demonstrated strong linearity for **CEC** quantification in both fish and seawater samples, with most compounds showing R^2 values above the acceptance criterion. The obtained R^2 ranges (**0.984-1.000 for fish and 0.967-1.000 for seawater**) indicate a strong concentration-response relationship for the majority of analytes. The sensitivity evaluation based on LOD and LOQ criteria supports the suitability of the method for detecting **CECs** at trace concentrations. The use of high-resolution LC-MS techniques has been widely applied for the determination of emerging contaminants in complex environmental matrices due to their high sensitivity and selectivity **[2,3]**.

5 Limitations and Future Work

5.1 Compound-dependent recovery

Limitation:
The method showed variable recovery among different **CECs**, with some compounds showing recoveries below the acceptable range. This indicates that the current extraction procedure does not provide equal efficiency for all target analytes.

Future improvement:
Further optimization should focus on the **SPE** extraction conditions, including solvent composition and extraction parameters, or even the change of the **SPE** cartridge, could improve recovery for compounds with lower extraction efficiency.

5.2 Matrix-related effects

Limitation:
Some analytes showed ion suppression due to matrix-related interference, showing that complex marine matrices can affect analyte response during HPLC-Orbitrap MS analysis.

Future improvement:
Future work could focus on improving sample cleanup procedures and expanding matrix evaluation to reduce ion suppression and enhance quantitative accuracy.

5.3 Limited validation scope

Limitation:
The current validation was performed using selected fish and seawater matrices, which may not fully represent the variability of different marine organisms and environmental conditions.

Future improvement:
Further validation using additional marine organisms and field-collected samples from different locations would improve the robustness and applicability of the method for environmental monitoring.

6 Conclusion

This study successfully applied and validated an analytical method for the determination of contaminants of emerging concern (**CECs**) in marine matrices using solid-phase extraction (**SPE**) followed by high-performance liquid chromatography-Orbitrap mass spectrometry (HPLC-Orbitrap MS). The method showed a reliable analytical performance, particularly in terms of linearity and matrix effect evaluation, while recovery and precision showed compound-dependent variations requiring further optimization for specific analytes. The use of matrix-matched calibration improved the reliability of quantitative analysis by compensating for matrix-related variations. All in all, this validated workflow provides a valuable analytical approach for monitoring **CECs** in seawater and fish samples and supports future investigations of their occurrence and potential bioaccumulation in marine environments.

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