

DOI: <http://doi.org/10.21698/simi.2022.ab24>

ANALYTICAL STRATEGY BASED ON NEW CHROMATOGRAPHIC METHOD FOR PHARMACEUTICAL RESIDUES IN SEAWATER

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Keywords: *analgesic contaminants, pharmaceuticals residues, seawater, SPE-LC-MS/MS*

Introduction

The pharmaceuticals occurrence has been widely described in surface freshwater and groundwater, but their presence in the marine environment has been less investigated, mainly due to the complexity of the matrices and the pharmaceutical dilution in marine waters. However, there has been a growing interest in recent years for this compartment as the last recipient of continental contamination. Pharmaceutical compounds can pose a risk to the health of non-target marine organisms and act as additional stressors on marine ecosystems already affected by climate change, overfishing and eutrophication.

Materials and methods

The analgesics analytical standards: piroxicam, ketoprofen, naproxen, indomethacin, diclofenac, ibuprofen, acetaminophen, and a caffeine were acquired from Sigma Aldrich (Germany). The analytical technique applied was based on liquid-chromatography coupled with mass detection (LC-MS/MS, Agilent Technologies, 1260-6410). The equipment used for the extraction of analgesics from the seawater samples was the automated SPE (solid phase extractor) 280 Autotrace (Dionex -Thermo- Scientific). The novelty of the method developed, optimized and validated on real seawater samples involved the variation of some extraction parameters (type of polymeric adsorbent phase Strata X, octa-dodecyl silica Strata C18, methanol and acetonitrile extraction solvent) but also some chromatographic parameters (mobile phase: formic acid, acetic acid, collision and fragmentation energies in the mass spectrometer). The parameters obtained during the analytical chromatographic separation process included: Zorbax Eclipse XDB C18 chromatographic column (100x2.1mm, 3.5 µm), column temperature 30°C, mobile flow rate of 0.3 mL/min, injected extract volume 10 µL, mobile phase composition 0.1% formic acid: acetonitrile with gradient elution (0-2 min: 10% ACN and 90% HCOOH, 2.01 - 6 min: 50% ACN, 6.01 - 9 min: 100% ACN, and the equilibration was carried out for 5 minutes with the starting mobile phase). For the detection of analytes with the quadruple triple mass spectrometer (QQQ MS) detector, the following optimal operating parameters were established: positive ionization mode (ESI +), nitrogen temperature at source of 300°C, type of ESI source (positive electrospray ionization source), 8 L/min nitrogen flow for drying ions, nebulizing

gas pressure of 40 psi, voltage applied to the capillary 4000 V, collision energies 5-35 V, fragmentation voltages 75-140 V. Also, a voltage of 5-8 V was applied on the collision cell and for each compound, 2 MRM transitions (multiple reaction monitoring) were recorded from the precursor ion to the most abundant product ions (Quantifier, qualifier).

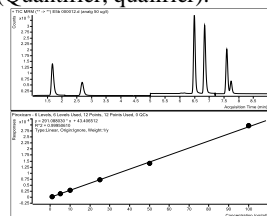


Fig. 1. ESI MRM chromatogram of a analgesic calibration standard (50 µg/L) and the linear regression for piroxicam

For the processing of seawater samples (salinity of ~17‰), the following optimal parameters of solid-phase selective extraction were established: sample pH = 2 with HCl, methanol cartridges (polymeric Strata X, 0.5g/6 mL, Phenomenex) conditioning (10 mL), seawater sample (0.5 L) percolation through cartridge, adsorbent washing with 20 mL ultra-pure water (pH 2), SPE adsorbent drying with air for 20 minutes, elution of analytes with 2x3 mL MeOH, evaporation of extracts to dryness and dissolution of residue in 1 mL of the mobile phase.

Results and conclusions

For the extraction and analysis of analgesic cotaminants from seawaters a new method was developed, optimized and validated. The novelty of the method consists in approaching of sea water as a complex matrix that was investigated from a chromatographic point of view. The linear regressions were obtained using the external standard method and the afferent coefficients of determination (R^2) were higher than 0.99 on the calibration range of 1-100 µg/L, as can be seen in Table 1. The limits of quantification, calculated based on signal to noise ratio, varied in the range of 0.1-3.3 ng/L. These limits allow the quantification of analgesic compounds in seawater taken from Black Sea, at trace levels (ng/L). RSD values obtained for repeatability ranged from 5.9% to 8.5% for all compounds. For intermediate precision experiments, the RSD values were below 16.5%. The results obtained from repeatability and intermediate precision experiments indicate that the method was repeatable and reproducible with standard deviations <20%. By testing the method on the real seawater samples with known addition of the standard, recovery yields were calculated in accordance with the chromatographic methods, with values in the range of 76.5 to 95.1%. The obtained method presents the accuracy corresponding to the LC methods.

Table 1. Validation data for detection of analgesic contaminants in seawater

Compound	R^2	Calibration range (µg/L)	Recovery (%)	Precision (%)		LOQ, ng/L
				RSD _r	RSD _R	
Acetaminophen	0,9964	1-100	80,4	7,1	13,7	3,3
Caffeine	0,9942	1-100	82,0	8,0	12,2	1,2
Piroxicam	0,9994	1-100	76,5	7,5	10,4	0,1
Ketoprofen	0,9994	1-100	86,5	8,5	16,5	1,1
Naproxen	0,9998	1-100	86,9	6,9	15,6	2,9
Indomethacin	0,9976	1-100	95,1	5,9	13,6	0,2
Diclofenac	0,9993	1-100	84,2	4,9	13,8	0,8
Ibuprofen	0,9995	1-100	88,3	5,2	14,6	1,5

Acknowledgement. The work was funded by Ministry of Research, Innovation and Digitalization of Romania through Programme Nucleu, contract no. 20N/2019, Project PN 19 04 01 01.