

# Determination of pharmaceutical residues in natural waters using high performance liquid chromatography coupled to quadrupole-Orbitrap mass spectrometry

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## Abstract

The presence of residual pharmaceuticals in the aquatic environment is an important environmental issue. Since the drugs are designed to cause biological responses, they could pose a risk to organisms in their natural environment. In this study, the presence of selected pharmaceutical compounds (paracetamol, phenazone, ketoprofen, budenonide and atenolol) in surface waters was studied. Six sampling points along the aquatic system of the River Louros, close to the city of Ioannina (Epirus, NW Greece), were selected for the assessment of their pollutant load. Analytical method was based on solid-phase extraction of water samples, using Oasis HLB cartridges, followed by high performance liquid chromatography coupled to Orbitrap high resolution mass spectrometry (UHPLC-LTQ/Orbitrap-MS). The methodology exhibited good analytical characteristics ( $R > 65\%$ ,  $R^2 > 0.9990$ , LOQs between 1.5 and 11.0 ng/L). Results revealed the presence of only paracetamol (although in levels below LOQ) in two sampling stations. The proposed analytical methodology proved to be fast, easy and reliable for the systematic monitoring of pharmaceuticals residues in natural waters.

**Keywords:** Pharmaceuticals, SPE, LC-ORBITRAP/MS, river water, monitoring.

## 1. Introduction

Water pollution research has recently been shifted from the conventional organic priority pollutants to the so-called emerging contaminants, where many of them are not regulated yet. This category of pollutants includes different substances with both industrial and domestic applications and various potential harmful effects (e.g. endocrine disruption, carcinogenicity, etc.). Among others, they include pharmaceutically active compounds (PhACs), personal care products and disinfectants (Archer et al., 2017). PhACs are one of the most relevant groups of substances in the aquatic ecosystems due to their universal use, physicochemical properties and known mode of action in aquatic organisms at low concentrations. Despite these relatively low concentrations, PhACs may pose a risk to aquatic organisms, because they are designed to modify

biochemical pathways in the human body at low doses. In Europe and the USA, around 4000 different PhACs are commercialised to be used as human and veterinary drugs. Residues from industrial production, improper disposal of expired and unused medication via the toilet, landfills, leachates and accidental spills during manufacturing and distribution are possible sources of water pollution. However, the main source of such pollution is that after administration many drugs and their transformation products are sometimes insufficiently retained in wastewater treatment plants (WWTPs), entering therefore the aquatic environment in considerable very high amounts. Many studies worldwide investigated and reported the occurrence of pharmaceuticals and personal care products (PPCPs) in surface waters (Kosma et al., 2014; 2019; Nannou et al, 2015).

**Table 1.** Pharmaceutical substances studied

Therapeutic category	Pharmaceutical substances
Analgesic – Antipyretic	Paracetamol
Analgesic - Antipyretic	Phenazone
Non-steroidal anti-inflammatory	Ketoprophen
Glucocorticoid steroids	Budenonide
Exclusives $\beta$ -adrenergic receptors	Atenolol

In the present study, the occurrence of pharmaceutical compounds in the aquatic region of Epirus and especially in the River Louros, close to the city of Ioannina (Epirus, NW Greece), was investigated. This region is intensively subjected to anthropogenic activity and the aim of the study is to provide a better understanding of the eventual sinks and fates of these pharmaceuticals.

## 2. Methodology

### 2.1. Standards and reagents

All pharmaceutical analytical standards were purchased from Sigma-Aldrich (Steinheim, Germany) and were of high purity grade ( $>95\%$ ). Methanol and water (LC-MS

grade) were received from Fisher Scientific (Leicestershire, UK). Formic acid (purity, 98–100%) was obtained from Merck KGaA (Darmstadt, Germany). Oasis HLB (200 mg, 6 cm<sup>3</sup>) cartridges were purchased from Waters Corporation (Milford, MA, U.S.A.). A UHPLC/ LTQ-Orbitrap-MS system was used (Thermo Fischer Scientific, Bremen, Germany) for the determination of the analytes in the six sampling points along the aquatic system of the River Louros. The samples were analyzed using positive ionization (PI) mode.

## 2.2 Sampling collection and sample preparation

Six sampling points along the aquatic system of the River Louros, close to the city of Ioannina (Epirus, NW Greece), were selected for the assessment of their pollutant load. The sampling points were (Σ1) Louros Sources, (Σ2) Lake Zirou, (Σ3) Kaloghrou Bridge A, (Σ4) Kaloghrou Bridge B, (Σ5) Lagoon of Tsopeli and (Σ6) Amvrakikos gulf.



To isolate the selected substances, the solid phase extraction method, SPE followed (UHPLC-LTQ/Orbitrap-MS) was used. Oasis HLB extraction cartridges were used as sorbents, which were activated with 5 mL methanol and 5 mL water. The volume of the aqueous sample passing through each time was 250 mL, while methanol (2 x 6 mL) was selected as the eluent solvent. The extract was concentrated under a gentle stream of nitrogen and reconstituted to 250 µL of Methanol (MEOH) : Water (H<sub>2</sub>O) (10:90) and then injected into the liquid chromatography system.

## 3. Results

Quantification limits for all substances ranged from 1.5 ng/L to 11.0 ng/L. Recoveries were in all cases greater than 64%. Concerning the concentration levels found for drug residues, paracetamol was detected only at two sampling stations (Bridge of Kaloghrou A and Amvrakikos gulf) below the quantification limit, while none of the other compounds were detected.

## 4. Conclusions

In conclusion, the optimized SPE-LC / LTQ-Orbitrap-MS method used in the present study appears to be simple, fast and reliable for the determination of pharmaceutical residues and can be applied for the systematic monitoring of the levels of concentrations in natural waters.

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