Accurate Mass Screening Of Pharmaceuticals In Water And Sediment By Uhplc-Orbitrap Mass Spectrometry

Christina Nannou, Vasiliki Boti*, Triantafyllos Albanis

Lab of Analytical Chemistry, Dept. of Chemistry, University of Ioannina, GR 45110 Ioannina, Greece

Corresponding author: chnannou@cc.uoi.gr

Abstract

The extended use of pharmaceuticals is a potential contamination source of surface water, groundwater, sea water and soils. Pharmaceuticals are among the most prescribed pharmaceutically active substances throughout the world. Their presence and persistence in environmental matrices and the toxicity posed by them as well, according to the first studies carried out, indicate that environmental monitoring programs are crucial to assess the fate of such substances ending up to the environment. The present study focuses on the determination at trace levels of psychiatric drugs in environmental matrices (water, sediment) taking at the same time advantage of the innovative hybrid technology and versatility of the high resolution-accurate mass LTQ Orbitrap MS platform.

Keywords: *pharmaceuticals, waters, sediments, LC-LTQ-Orbitrap MS*

Introduction

Pharmaceuticals are chemical substances used in the treatment, cure, prevention, or diagnosis of disease or used to otherwise enhance physical or mental well-being. The growing use of pharmaceutical products is becoming a new environmental problem. Besides other micropollutants, drug residues have become a notable contaminant of surface water during recent years. Human and veterinary applications are the main sources of pharmaceuticals in the environment and the major pathways are excretion and discharge to the environment through sewage treatment plants (STP's). STP's input constituents have to deal with complex mixture of various organic and inorganic substances and detailed information on potential wastewater composition is often scarce. Even the processing of communal wastewater in sewage treatment plants cannot avoid the entry of drugs into surface water because of the high stability of some drugs or their metabolites against biological degradation. Finally, these compounds may even enter groundwater as well as drinking water produced from groundwater as recent studies have shown. Pharmaceutical residues are usually present in environmental water samples in trace levels (1-6). The most common sample isolation and pre-concentration technique is solid phase extraction (SPE) where as well as isolation and pre-concentration, the matrix-solvent (water) is exchanged with a more volatile organic solvent suitable for gas chromatography (GC) (7-8).

In this study, the determination of twelve pharmaceutical compounds belonging to various therapeutic categories (Salycilic Acid, Ibuprofen, Paracetamol, Naproxen, Diclofenac, Gemfibrozil, Caffeine, Carbamazepine, Fenofibrate, Bezafibrate, Phenazone and Triclosan) were investigated in water samples, based on solid phase extraction (SPE) and GC-MS analysis. The analytical performance of the SPE procedure using the SDB-RPS sulfonated sorbents for water samples proved to be effective for the above compounds.

Materials And Methods

Reagents and standards

All Pharmaceuticals were supplied from Promochem (Wesel, Germany). Methanol (MeOH), ethyl acetate and acetone were supplied from Pestiscan (Labscan, Ltd, Dublin, Ireland) and anhydrous sodium sulfate from Merck (Darmstadt, Germany). Empore SDB-RPS disks (47 mm diameter, 0.5 mm thickness) were purchased from 3M (Saint Paul, MN, USA). Physicochemical characteristics of the target analytes are shown in Table I.

Area description

Water samples used in this study were collected from Kalamas River. Kalamas is one of the major rivers in North West Greece. Its sources are to the northern side of Ioannina prefecture. The river flows through the mountains Tymfi and Kasidiaris and, 20km to the W of Ioannina, it turns towards the W and, flowing through Thesprotia prefecture, empties into the Ionian Sea. Its length is 113 km. The Ioannina STP is located by the Kalams River, just south of the city (population 120,000) and discharges the treated sewage into the Kalamas River. The total area of the Kalamas River basin is about 1800 km^2 and the soil types are mainly sandy clay loam with inclusions of fine and coarse sediments.

Sampling process

A monitoring program was carried out for the four seasons of the year 2006. The sampling months were May, July, October and December of 2006. Sixteen water samples from four sampling stations of the River were collected. The sampling stations of the Kalamas River were vrosina, vrontismeni, paliouri, fragma ragio (Figure 1). At each sampling station of the river, between 1 and 2 L of sample were collected and delivered to the laboratory within 36h. Temperature, pH, and conductivity were measured at each

Table I. Overview of physicochemical properties of studied pharmaceuticals

Pharmaceutical class	Compound	Molecular formula	MW	pKa	Log*Kow*	Pv (mmHg)
Non steroidal/	Salicylic acid	$C_7H_6O_3$	138.123	2.97	1.13	8.20E-05
Antinflammatory	Ibuprofen	$C_{13}H_{18}O_2$	206.28	4.91	3.97	1.86E-04
	Paracetamol	$C_8H_9NO_2$	151.17	9.38	0.46	7.00E-06
	Naproxen	$C_{14}H_{14}O_3$	230.26	4.15	3.5	1.89E-06
	Diclofenac	$C_{14}H_{10}C_{l2}NO_2\times K$	334.23	4.15	4.51	6.14E-08
Lipid lowering	Gemfibrozil	$C_{15}H_{22}O_{13}$	250.34	4.7	4.77	n.d
agents	Fenofibrate	$C_{20}H_{21}ClO_4$	360.831	4.46	5.19	n.d
	Bezafibrate	$C_{19}H_{20}ClNO_4$	361.82	3.6	4.25	n.d
Antiepileptic	Carbamazepine	$C_{15}H_{12}N_2O$	236.27	7	2.47	1.84E-07
Sychomotor stimulant	Caffeine	$C_8H_{10}N_4O_2$	194.2	10.4	n.d	15
Analgesic/ Antipyretic	Phenazone	$C_{11}H_{12}N_2O$	188.226	1.5	0.38	3.06E-05
Disinfectant	Triclosan	$C_{12}H_7Cl_3O_2$	289.5	4.5	4.8	6.45E-07

sample. The samples were acidified to pH 3-3.5 to enhance trapping of the acidic compounds on the solid-phase extraction (SPE) sorbent and stored at 4°C prior to solid-phase extraction SPE.

Solid Phase Extraction (SPE)

Isolation of the pharmaceuticals from the water samples were performed off-line using a standard SPE-system from Supelco (Bellefonte, PA, USA) connected to a vacuum pump. In the water samples, extraction disks were pre-conditioned with 10 ml of acetone and 10 ml of ethyl acetate. Then they were washed with 10 ml of methanol and 10 ml of ultra-pure water and without letting the disk become dry, a 500 ml water sample was applied to a speed of 10 ml/min. Next the disks were dried under vacuum for 10 min. The analytes were eluted with 3x5 ml ethyl acetate. The extract was dried over anhydrous sodium sulfate. Extracts were dried under a gentle stream of nitrogen. The final volume extract was 100 µl. After that, they were stored at -20 ^{0}C until being analysed by GC-MS.

GC-MS Analysis

A GC-MS, QP 5000 Shimadzu equipped with capillary column DB-5-MS, 30 x 0.25 mm x 0.25 µm, contained 5% phenyl-methylpolysiloxane (J& W Scientific) was used at the following chromatographic conditions: Injector temperature 240^{O}C, oven temperature program: 70^{O}C (2 min) to 250^{O}C (5 min) at 10^{O}C/min and finally from 250^{O}C to 280^{O}C (10 min) at 6^{O}C/min. Helium was used as the carrier gas at 1.0 ml/min. The interface was kept at 290^{O}C and the spectra were obtained at 70 eV. To achieve better detection limits and enhanced selectivity subsequent SPE analyses were performed in the selected ion monitoring mode (SIM) (Table II).

Table II: Retention times and diagnostic (m/z) ions of the twelve pharmaceuticals in the GC-MS system

Pharmaceuticals	t_R	Diagnostic ions SIM		
	(min)	*(m/z)*		
Salicylic Acid	10.06	92	120	138
Ibuprofen	14.66	161	163	107

Paracetamol	15.70	109	151	80
Caffeine	16.43	194	109	55
Phenazone	17.80	188	281	96
Gemfibrozil	18.25	122	107	129
Naproxen	20.06	185	230	
Triclosan	20.20	288	289	218
Fenofibrate	21.43	121	232	139
Diclofenac	22.54	214	242	295
Carbamazepine	23.36	193	236	165
Bezafibrate	29.24	120	220	139

Results And Discussion

A SPE/GC-MS analytical method was developed, which allowed the simultaneous determination of twelve pharmaceutical compounds in surface waters. Pharmaceutical concentrations ranged between 60-2712 ng/L in all sampling stations. The sampling station Vrontismeni presented the highest mean concentrations due to the fact that there were detected high concentrations of Salicylic acid and Caffeine. This may attribute to the fact that this sampling station is near small villages. Salicylic acid and Caffeine were detected in 100% of the analysed samples with mean concentrations of 1125 ng/L and 614 ng/L, respectively. Diclofenac and Carbamazepine were detected in relatively high concentrations with mean concentrations of 479 ng/L and 585 ng/L, respectively. Gemfibrozil, Ibuprofen and Naproxen were detected in lower concentrations while Phenazone and Triclosan were detected in only one sample, in concentration of 108 ng/L and 150 ng/L, respectively. Paracetamol was detected in the 50% of the analysed samples in mean concentration of 90 ng/L while fenofibrate was not detected at any sample. Bezafibrate was detected in concentrations below limit of detection. The occurrence of pharmaceuticals in Kalamas River is attributed to the fact that it is connected to the STP of Ioannina city, which discharges the treated sewage into the river.

Conclusions

A SPE followed by GC-MS determination has been proposed for simultaneous analysis of twelve pharmaceutical compounds. The detection of these compounds in surface waters, weight to the argument that the occurrence of drugs in the environment is a global issue. It is likely that other high volume pharmaceuticals with appropriate physicochemical properties that were not analysed in this study were also present and may also contaminate the aquatic environment exposing aquatic organisms to complex mixtures of compounds.

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