Development of a Multi-class Analytical Method by SPE and LC-MS/MS for the Determination of Pharmaceuticals in Wastewater Samples

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Abstract Over the last decades the consumption of pharmaceuticals by humans and animals has resulted in the appearance of their residues in the environment that may cause unknown risks. Present study aims to investigate the existence and the residue concentration levels of pharmaceuticals in wastewaters before and after processing by a sewage treatment plant (STP) with a membrane bio-reactor. Additionally, samples were further purified by the reverse osmosis process. In this work, a multi-class method for the simultaneous determination of pharmaceuticals in wastewater samples, has been developed, using SPE and LC-MS/MS analysis. The sample pre-treatment consisted of a solid-phase extraction using Oasis MCX and HLB cartridges. Analytes were pharmaceuticals mainly used as antibiotics, non-steroidal anti-inflammatory drugs, antihypertensives, antidepressants, anticonvulsants and drugs for stomach diseases (proton pump inhibitors) and for the prevention of cardiovascular diseases (statins). It was found that in the first waste water treatment stage of the bioreactor membranes is achieved a substantial reduction of pharmaceuticals concentrations while in the second stage of reverse osmosis is achieved almost complete elimination of pollutants.

Keywords: pharmaceuticals, environment, wastewater, SPE, LC-MS/MS

Introduction

The increasing number of studies detailing occurrence, fate, effects and behavior of pharmaceuticals in the environment indicates their significance for human health and environment, especially in view of the increasing importance of freshwater resources. Pharmaceuticals of different classes can display a variety of removal efficiencies during wastewater treatment. Since receiving waters from wastewater treatment plants are used for potable supplies or agricultural applications, there is a need for detecting the occurrence of these compounds in water samples.

In this work, a multi-class method for the simultaneous determination of pharmaceuticals in wastewater samples, has been developed, using SPE and LC-MS/MS analysis. The sample pre-treatment consisted of a solid-phase extraction using Oasis MCX and HLB cartridges. Analytes were pharmaceuticals mainly used as antibiotics, anti-inflammatory, statins, antihypertensive, antidepressants et al. Recovery values (R%) were estimated in different pH values and different concentration levels to check the efficiency of each sorbent towards each compound. Analytical performance was estimated by determining Recovery (R%) and Repeatability Relative Standard deviations (RSD%) under optimized conditions for each compound; R% were in the range 70-120% with RSDr% below 20% in most of the cases.

Methods

Influent and effluent after processing by MBR (Membrane Bio-Reactor) wastewater samples from a STP of Athens, Greece after filtration, methanol addition and pH adjustment were subjected to solid-phase extraction (SPE) using the Water Oasis HLB and MCX cartridges. The final extract was analyzed by reversed-phase liquid chromatography (RP-HPLC) on a Phenomenex C18 Fusion-RP column (4um, 80A, 2.1x50mm), employing a gradient program consisting by an aqueous solution of 0.1% formic acid, ammonium formate 10mM and methanol solution of 0.1% formic acid and ammonium formate 10mM . Mass spectral acquisition was carried out on a triple quadrupole mass spectrometer (Thermo-Electron, TSQ Quantum Ultra) using electrospray (ESI) ionization in the positive ionization mode.

Results

Influent and effluent wastewater samples collected from a STP of Athens, Greece were analyzed for the presence of amlodipine besylate, atorvastatin, azithromycin, citalopram hydrobromide, diclofenac, gabapentin, ketoprofen, mefenamic acid, naproxen, omeprazole, roxythromycin, and venlafaxine hydrochloride. The analysis was performed by a SPE method using Oasis MCX and HLB cartridges and LC-ESI-MS/MS in positive ionization mode. Tandem mass spectrometric conditions were optimized for each analyte by using the automated tuning procedure. Detection was performed in SRM mode using two characteristic precursor/product ion transitions obtained from MS/MS optimization procedure. The matrix

effect arising by the complex matrix of wastewater effluent samples, during LC-ESI-MS-MS analysis of pharmaceuticals, was evaluated. Recovery values from fortified samples-shown in Table 1- were in most cases better than 70% with RSDs below 20%. Method detection limits in wastewater matrices were ranged between 5.0 and 10 ng/l. The efficiency of a successive STP step based on reverse osmosis was also tested by analyzing samples after treatment.

The results of mean concentration values (ng/L) of the identified pharmaceuticals in samples of influent, after processing by MBR (Membrane Bio-Reactor) and after treatment by reverse osmosis are shown in **Figure 1.**

Table 1. Recovery values (R%) and Relative Standard Deviation values (RSD%) for the two SPE methods and at different concentration levels

SPE Method	Pharmaceuticals	5 ng/L		10 ng/L		50 ng/L		100 ng/L	
		%R	%RSD	%R	%RSD	%R	%RSD	%R	%RSD
HLB pH=11	Atorvastatin	58,44	23,08	57,04	13,89	52,22	6,47	45,4	9,21
	Citalopram hydrobromide	65,04	2,75	89,56	7,07	89,95	13,36	88,79	5,27
	Diclofenac sodium	95,36	11,62	101,87	5,58	81,94	3,31	82,11	5,31
	Ketoprofen	92,92	16,39	106,56	12,34	79,03	2,84	75,9	5,18
	Mefenamic acid	73,46	10,22	72,93	3,39	77,41	3,9	81,38	6,3
	Naproxen	92,02	31,61	110,13	10,65	84,96	5,22	82,7	6,38
	Omeprazole	96,06	11,82	90,37	6,23	85,43	4,41	82,85	5,15
	Roxithromycin	82,6	21,14	72,31	9,74	61,01	2,4	57,99	9,09
	Venlafaxine hydrochloride	82,78	10,58	109,37	3.86	95.09	7,49	93,45	6,82
MCX pH=7	Amlodipine besylate	77,88	24,19	66,28	13,64	51,69	14,16	41,65	18,52
	Azithromycin	106,84	16,15	101,33	19,22	93,98	20,27	98,09	19,73
	Diclofenac sodium	84,74	15,4	86,96	14,09	87,62	10,5	72,31	7,95
	Gabapentin	88,3	6,2	103,25	2,18	92,79	8,39	101,02	1,73
	Ketoprofen	85,34	13,78	80,87	9,33	62,99	6,49	66,3	2,79
	Mefenamic acid	65,18	5,43	69,27	4,92	64,87	8,67	65,61	3,49
	Naproxen	70,2	8,4	90,05	13,54	76,1	8,68	85,95	5,39

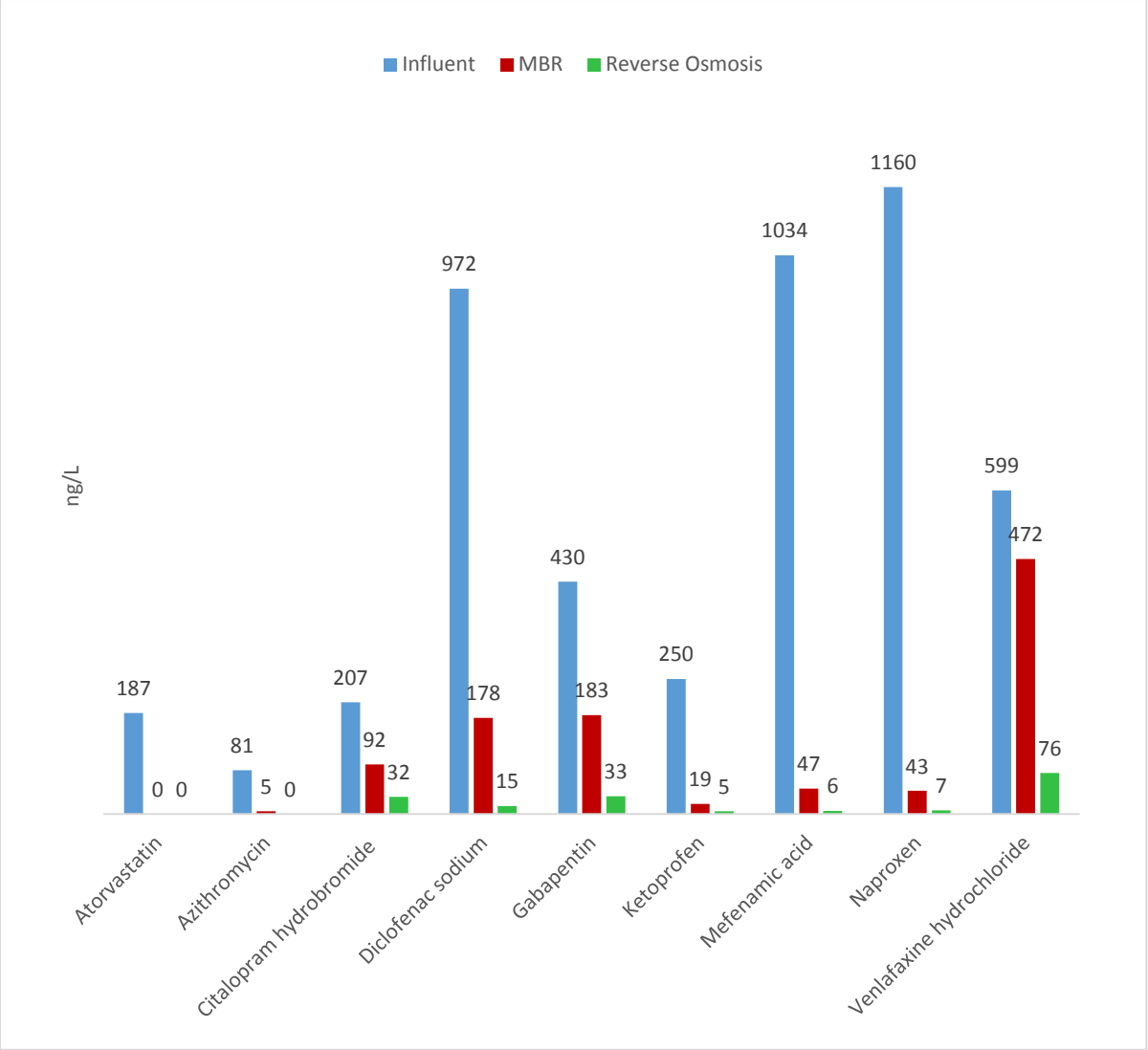

Figure 1. Concentration values (ng/L) of the identified pharmaceuticals in influent, after processing by MBR (Membrane Bio-Reactor) and after treatment by reverse osmosis

Conclusions

The proposed methodology, based on a SPE approach coupled to LC-MS/MS (QqQ) allowed the determination of atorvastatin, azithromycin, citalopram hydrobromide, diclofenac, gabapentin, ketoprofen, mefenamic acid, naproxen, omeprazole, roxythromycin, and venlafaxine hydrochloride, in influent and effluent samples collected from a wastewater treatment plant, located in the region of Athens, Greece. The levels of targeted pharmaceuticals ranged from 50-1500 ng/L in the influents and from 20 to 520 ng/L in the effluents showing a significant reduction-but not complete elimination-during the conventional treatment process. The efficiency of a successive treatment step based on reverse osmosis was tested; preliminary experiments showed an effective elimination of contaminants.

Further studies on occurrence, exposure and toxicological are required to provide a more comprehensive picture of the impact of pharmaceuticals in the environment as well as to perform reliable environmental risk assessment not only for the parent compounds but also for their metabolites/TPs.

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