Development and validation of an analytical method for quantification of selected pharmaceuticals in surface water samples by liquid chromatography coupled with mass spectrometry

University of Ljubljana
Faculty of Pharmacy

J. Umnik[1], A. Klančar[1], R. Roškar[1]

[1]Faculty of Pharmacy, University of Ljubljana, Slovenia

(E-mail: janja.umnik@gmail.com, anita.klancar@ffa.uni-lj.si, robert.roskar@ffa.uni-lj.si)

INTRODUCTION

Increased use of pharmaceuticals brings along increased risk of potential negative effects that these compounds could have on various organisms. Their occurrence in the environment can be measured not only in water samples but also in soil and living organisms (e.g. fish). Wastewater treatment plant effluent concentration of pharmaceuticals as the most frequently reported data have been the basis for our pre-selection of 44 pharmaceuticals that could occur in surface waters in a detectable concentration. The aim of our study was to optimise a solid phase extraction (SPE) method for selected pharmaceuticals, to validate the method and to apply it to samples from Slovene surface waters.

METHODS

Sample preparation method:

Step	Optimised method
Phase activation	10 mL methanol
Equilibration	10 mL 25 mL buffer pH 4
Sample loading	200 mL surface water adjusted to pH 4
10 min drying	
Elution	2 mL
Dried volume	2 mL
Reconstitution volume	150 µL

Table 1: Extraction steps for manual SPE procedure.

Liquid chromatography-tandem mass spectrometry conditions: The Agilent 1290 Infinity LC coupled to 6460 triple quadrupole mass spectrometer (Agilent Technologies, USA); 2 µL of sample was injected on Poroshell EC-C18 100 × 3.0 mm, 2.7 µm column (Agilent Technologies, USA) by gradient elution using 0.05 % formic acid and acetonitrile with flow rate of 0.5 mL/min.

Samples: 4 river and 1 lake sample, collected using a grab sampling technique from a depth of 1-2 m from water surface. They were collected into plastic bottles and kept cool in the fridge at 4°C until extraction was carried out within 24 hours.

RESULTS

- Determination coefficients mostly over 0.9990.
- Vast majority of analytes have limit of quantification (LOQ) less than 5 ng/L.
- All analytes were detected in at least one sample.
- 20% of target pharmaceuticals were above LOQ in all samples (bisoprolol, clarithromycin, caffeine, irbesartan, theophylline, tramadol, trimethoprim, valsartan and venlafaxine).
- Highest measured concentrations were for valsartan, tramadol and ranitidine.

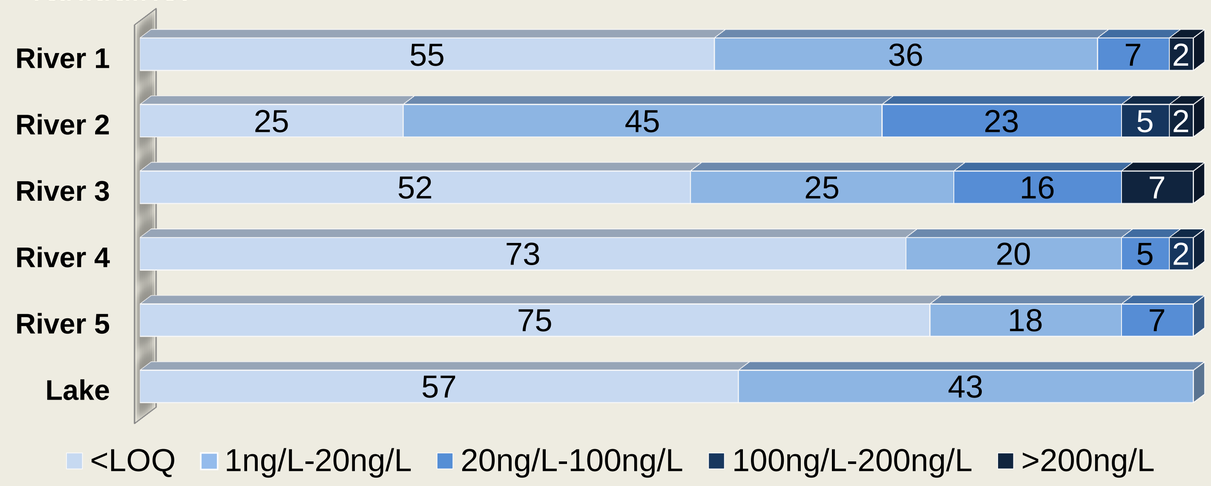

Figure 1: Percentages (%) of detected pharmaceuticals in a particular concentration range for 5 samples.

Table 2: Validation parameters for selected pharmaceuticals

Analyte	Linear range (ng/L)	R^2	LOQ (ng/L)	RSD (%) n=3
amantadine	0.59-78.64	0.9999	0.59	7
antipyrin	0.32-64.00	0.9998	0.32	2
atenolol	80.00-1600	0.9995	80.00	7
atorvastatin	10.00-400	0.9936	10.00	4
azithromycin	50.00-200	0.9560	50.00	3
bisoprolol	0.16-32.00	0.9999	0.16	2
ciprofloxacin	0.80-160	0.9998	0.80	1
diazepam	0.40-80.00	0.9997	0.40	2
diclofenac	1.60-320	0.9966	1.60	1
escitalopram	0.08-16.00	0.9997	0.08	2
fexofenadine	0.16-32.00	0.9996	0.16	3
furosemide	8.00-1600	0.9999	8.00	2
gabapentin	40.00-8000	0.9998	40.00	2
gemfibrozil	4.00-800	0.9986	4.00	3
irbesartan	0.032-6.40	0.9994	0.03	3
carbamazepine	0.16-32.00	0.9997	0.16	0
clarithromycin	0.16-3.20	0.9985	0.16	3
caffeine	0.48-64.00	0.9996	0.48	5
lacosamide	40.00-800	0.9961	40.00	4
lamotrigine	1.60-320	0.9980	1.60	2
levetiracetam	32.00-6400	0.9999	32.00	6
licarbazepine	4.00-80.00	0.9977	4.00	7
metformin	160-3200	0.9996	160.00	5
metoprolol	0.40-80.00	0.9999	0.40	0
naproxen	16.00-3200	0.9970	16.00	3
nifedipine	100-400	0.9858	100.00	16
norfloxacin	0.80-160	0.9992	0.80	3
oxazepam	0.40-80.00	0.9995	0.40	1
oxcarbazepine	1.60-320	0.9998	1.60	9
pantoprazole	160-3200	0.9998	160.00	12
paracetamol	6.40-128	0.9997	6.40	5
paroxetine	0.64-12.80	0.9988	0.64	2
primidone	3.20-640	0.9997	3.20	1
propyphenazone	4.00-16.00	0.0144	4.00	3
propranolol	0.22-44.64	0.9976	0.22	3
ranitidine	21.44-1715	0.9994	21.44	8
rosuvastatin	1.65-330.9	0.9995	1.65	3
sertraline	0.08-16.00	0.9994	0.08	2
sulfamethoxazole	4.00-320	0.9998	4.00	5
theophylline	3.20-640	0.9999	3.20	4
tramadol	0.18-36.48	0.9997	0.18	1
trimethoprim	0.40-80.00	0.9998	0.40	2
valsartan	0.80-160	0.9999	0.80	3
venlafaxine	0.08-16.00	0.9999	0.08	2

CONCLUSION

The optimised method was successfully validated and applied to surface water samples. The results revealed a great amount of pharmaceuticals releasing into surface water from wastewater treatment plant effluents. Consequently, the presented method may provide an important insight into the occurrence of pharmaceuticals in surface water which can be used in future ecotoxicological studies in order to protect aquatic biota.