Development of multi-residue analysis of pharmaceuticals in water samples by stir-bar sorptive extraction using liquid chromatography-mass spectrometry

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Abstract
The present study covered the development of stir-bar sorptive extraction process and the new rapid analytical LC-MS/MS method for target pharmaceutical compounds determination. A list of 15 representative pharmaceuticals from a wide range of therapeutic classes was selected according to their chemical properties, in particular the octanol-water partition coefficient ($2.7 \leq \log K_{O/W} \leq 5.2$). First, extraction conditions, such as time, temperature and solvent of extraction and desorption, addition of NaCl and MeOH were evaluated. The optimized extraction method provided remarkable recoveries (55-101 %) and a wide linear range (mainly 1.25-1250 ng/L, R^2 >0.99) with very low limit of detection for all analytes (1.25 ng/L). The proposed method enables a rapid and simple extraction process, what result in high throughput and less workload per sample. Consequently, it is highly suitable to be involved in routine monitoring of particular pharmaceuticals in various environmental water samples.

Keywords
stir-bar sorptive extraction, pharmaceuticals, water samples, LC-MS/MS

INTRODUCTION

In recent years, several studies have been published about the occurrence of pharmaceuticals in wastewater, surface and even in tap and drinking water. Nowadays, the availability of advanced and highly sensitive analytical equipment enables their confident determination in wide array of environmental samples. Pharmaceuticals residues (except for diclofenac) are not currently included in any list of priority substances in routine water quality monitoring practice. However, they have become an environmental concern since they may cause a risk to the aquatic organisms as well as human beings and it seems to be only a matter of time when their monitoring will be obligatory. Consequently, the development of fast, simple and effective extraction processes coupled to sensitive analytical methodology is a subject of increasing interest. Among the several sorptive sample preparation techniques developed in the last decades, stir-bar sorptive extraction (SBSE) takes an important place in the field of green chemistry, since it enables a very low organic solvent consumption and simplification of the extraction. It is highly appropriate for analytes with a high octanol-water partition coefficient (log Ko/w $\geq$ 2.7) and is less effective for more polar compounds (Prieto, et al., 2010). Thus, the main aim of our work was to develop and optimise the SBSE in water samples using LC-MS/MS method for selected non-polar pharmaceuticals from different therapeutic groups.

MATERIAL AND METHODS

Selected standards were purchased from various suppliers, mainly from Sigma-Aldrich. The reagents for standard and sample preparation were from Merck and ultra-pure Milli-Q water was used. Firstly, all stir-bars (1×10 mm) were preconditioned in 2 mL of MeOH for 30 minutes. Thereafter, they were dried and placed into 20 mL of heated water samples with

adjusted pH value. The extraction lasted for 150 minutes at 990 rpm. Subsequently, the stir-bars were dried and placed into a 2 mL mixture of organic solvents acetonitrile and MeOH (50/50, v/v). Liquid desorption was accomplished in 15 minutes at stirring speed 990 rpm. An aliquot of 500 µL was subjected to a LC-MS/MS analysis performed by the 1290 Infinity liquid chromatograph coupled to 6460 Triple Quad Mass Spectrometer (Agilent Technologies) using the dynamic MRM mode.

RESULTS

Regarding the optimization of the multi-residue SBSE, various parameters that may affect the extraction were evaluated. Firstly, the effect of modifying the pH value (between 3 and 9) of water samples was evaluated, where water samples adjusted to pH 9 provided the best recoveries for almost all tested analytes. Thereafter, the addition of NaCl, (modifies the ionic strength) and MeOH (organic modifiers – could reduce the wall adsorption effects) were tested, however, both proved to be ineffective. Finally, the optimal results were achieved after 150 minutes extraction at 50 °C. Similarly, the liquid desorption efficiency was optimized at 15 minutes and 50 °C. After optimisation, the complete method performance was evaluated. Wide linear range with high determination coefficients, very low limits of quantification (1.25 ng/L) with a high and consistent overall recovery (Table 1) are showing the suitability of this simple, fast and inexpensive extraction method for its intended use.

Table1: Method validation parameters

Analyte	log $K_{O/W}$	Linear range (ng/L)	R^2	RSD (%) n=6	Recovery (%)
amitriptyline	5.1	1.25-1250	0.9996	3.3	90.6
clomipramine	5.0	1.25-1250	0.9992	2.9	91.1
desipramine	4.0	1.25-500	0.9994	1.5	101.0
donepezil	4.1	1.25-1250	0.9939	0.9	92.0
escitalopram	3.6	1.25-1250	0.9950	1.7	80.6
fluoxetine	4.1	1.25-1250	0.9995	1.3	84.3
haloperidol	3.7	1.25-1250	0.9953	2.3	83.1
loperamide	4.4	1.25-500	0.9960	4.3	64.7
loratadine	4.8	1.25-1250	0.9993	3.2	99.8
promethazine	4.5	1.25-1250	0.9990	2.4	89.7
propranolol	3.0	1.25-500	0.9958	2.1	55.3
selegiline	3.1	1.25-1250	0.9996	2.1	90.1
sertraline	5.1	1.25-1250	0.9996	2.4	90.5
venlafaxine	2.7	1.25-500	0.9939	3.6	59.8
verapamil	5.2	1.25-1250	0.9996	1.8	65.7

CONCLUSION

The presented method was successfully validated and its application is currently in progress in monitoring of wastewater samples in Slovenia. Such a method is of a high importance in order to monitor the aquatic environmental contamination and support future protection measures.

REFERENCES

Prieto, A. et al. (2010) Stir-Bar Sorptive Extraction: A View on Method Optimisation, Novel Applications, Limitations and Potential Solutions., *J Chromatogr A*, **16**, 2642-66.