Development and Validation of an Extraction Method for commonly used Antidepressants from Wastewater Samples

J. Trontelj*, A. Klančar*, R. Roškar*, A.Kristl*

*Faculty of Pharmacy, University of Ljubljana
(E-mail: *jurij.trontelj@ffa.uni-lj.si*; *anita.klancar@ffa.uni-lj.si*, *robert.roskar@ffa.uni-lj.si*, *albin.kristl@ffa.uni-lj.si*)

Abstract
This paper presents a new method for extraction and quantification in wastewater of some of the most commonly prescribed antidepressants. Three extraction methods were tested: liquid-liquid extraction, classic manual solid phase extraction (SPE) and an automatic SPE using the disk sorbents. The extracts were analyzed by a highly sensitive LC-MS/MS method. The SPE methods were optimized and validated and then applied to wastewater samples from Slovenia.

Keywords: antidepressants, automatic solid-phase extraction, wastewater, LC-MS/MS

INTRODUCTION

The use of antidepressants has more than doubled within the last 10 years. Antidepressants are highly active compounds and they can have a significant effect on aquatic organisms at ecologically relevant concentrations (Ford, 2015). The aim of our study was to select the most appropriate extraction method for antidepressants from wastewater samples, to validate the method and to apply it to effluent samples from a wastewater treatment plant. The following antidepressants were selected: venlafaxine, escitalopram, amitriptyline, fluoxetine, and sertraline.

METHODS

The following extraction methods were evaluated: a liquid-liquid extraction (LLE), a manual SPE on cartridges and an automatic SPE on extraction discs and 4790 SPE-DEX system (Horizon Technology, USA). For all three extraction procedures, the main conditions affecting the recovery were optimized (pH, type of organic solvents, and sorbent material.) Final optimized LLE involved 100 mL buffered (pH 7; 50 mM phosphate) aqueous sample with 10 mL of dichloromethane as the organic phase. The extraction mixture was shaken on an orbital shaker with 150 RPM for 1 hour. The optimized extraction procedures for solid phase extractions are summarized on table 1.

Table 1. Extraction steps for manual and automatic SPE procedures

Step	Manual SPE	Automatic SPE
phase activation	10 mL methanol	methanol, 15 s soak
equilibration	3 mL 50 mM buffer pH 3	2 x MilliQ water (15+10) s soak
sample treatment	0.45 µm filtration	not required
sample loading	250 mL + 50 mL buffer pH 3	250 mL + 250 mL buffer pH 3
elution	4 mL ACN:MeOH:IPA=2:1:1	same solvent as manual SPE; 3 min soak time, volume: 25 mL

Among several tested sorbents, the best choice proved to be 60 mg Strata X (Phenomenex, Torrance, USA), and the Atlantic HLB extraction disks (Horizon Technology, USA), for manual and automatic SPE, respectively. The matrix effect (ME) was determined as a ratio (B-C)/A, where B is the response of the extracted matrix spiked after extraction, C is the response from extracted unspiked matrix, and A is the response from the neat solvent spiked at the same concentration level. Both SPE methods were validated in terms of accuracy, precision, working linear range, recovery,

matrix effects, and limit of quantification. The extracts were analyzed by an ultra high performance liquid chromatograph Agilent Infinity 1290 coupled to an Agilent 6460 triple quadrupole mass spectrometer (Agilent, Santa Clara, USA). The effectiveness and usability of both SPE methods was demonstrated on real wastewater samples (250 mL aliquots) obtained from an effluent of a wastewater treatment plant in the suburbs of Ljubljana, Slovenia. The sampling with subsequent analysis was performed at two separate time points.

RESULTS AND DISCUSSION

The recoveries of LLE were too low (20-65%) with high RSDs too high (33-48%), therefore this type of extraction was not developed any further. For both SPE method, the linear ranges were demonstrated from 0.01 to 1 µg/L with R^2 values greater than 0.9994. The manual SPE recoveries were higher compared to automatic SPE (table 2). The repeatabilities of both methods were very good, with RSD values below 4% at concentration 1 µg/L. The matrix effects were not significant since the obtained values were close to 100% at both tested concentrations (0.2 and 2 µg/L). The observed difficulty with manual SPE compared to automatic SPE was lower sample loading throughput and possible cartridge blocking.

Table 2. The validation parameters for all three extraction methods.

	Parameter	Escitalopram	Venlafaxine	Amitriptyline	Fluoxetine	Sertraline
manual SPE	recovery (RSD) [%]	106.5 (0.1)	108.6 (0.7)	105.2 (0.5)	107.0 (3.4)	107.0 (0.5)
	LOQ [ng/L]	50	50	50	10	50
	ME [%]	99.9	92.0	102.4	90.5	101.5
automatic SPE	recovery (RSD) [%]	74.8 (2.0)	64.4 (4.0)	69.1 (1.4)	69.1 (1.6)	68.3 (2.1)
	LOQ [ng/L]	10	10	50	50	10
	ME [%]	91.4	95.4	96.2	95.2	93.7
LLE	recovery (RSD) [%]	11.8 (13.2)	0.2 (8.2)	22.1 (28.3)	1.5 (15.3)	5.6 (31.2)
	LOQ [ng/L]	-	-	-	-	-
	ME [%]	-	-	-	-	-

In the collected wastewater samples, escitalopram and venlafaxine were determined in measurable concentrations: 57 and 290 ng/L, respectively. Detected (but below LOQ) were also desipramine and sertraline. The results from both SPE methods were highly similar.

CONCLUSION

The method of choice for extraction of antidepressants from wastewater is automatic SPE-DEX, mainly due to its better tolerance towards particulate matter within the sample and ease of use.

ACKNOWLEDGMENTS

REFERENCES

Ford A.T., Fong P.P. (2015) The effects of antidepressants appear to be rapid and at environmentally relevant concentrations. Environmental Toxicology and Chemistry, **9999**,1-5.