Development of SPME with liquid chromatography-tandem mass spectrometry For high throughput analysis of pharmaceuticals in urban wastewater

Togunde, O P[1], Cudjoe, E[1], Servos, M R[2] , Pawliszyn, J[1]

University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

Abstract

- This research aims to develop a simple analytical method based on solid-phase microextraction (SPME) to simultaneously extract a variety of pharmaceutical compounds possessing differing physico-chemical properties from urban wastewater.
- This study presents a sensitive and specific method for the quantitative determination of pharmaceuticals in waste waters. As a sample preparation method, SPME is an environmentally-friendly analytical technique which isolations and pre-concentrates trace organic pollutants from water samples in a single step.
- The coupling of SPME to liquid chromatography with tandem mass spectrometry capability (LC-ESI-MS/MS – TSQ Vantage) allows for identification/confirmation and quantitation in a single analysis. The quantification limits range between 10 - 50 ng/L with relative standard deviations less than 15 % (RSD < 15 %).
- Preliminary results suggest this method is capable of detecting and quantifying a range of environmental pharmaceuticals in urban wastewater effluents collected from the Grand River near Kitchener, Ontario.

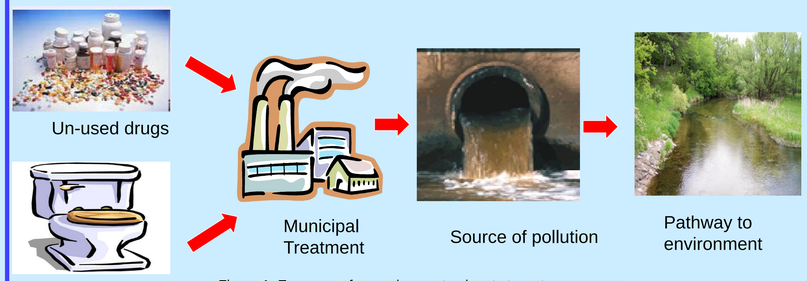


Figure 1: Exposure of emerging contaminants to water resources

Introduction

- Frequent detection and widespread distribution of emerging contaminants, such as pharmaceuticals and personal care product,, are cause for increasing concern from both environmental and human health perspective
- These emerging contaminants are continually infused to aquatic ecosystem through municipal wastewater discharge, while some pharmaceuticals are removed during wastewater treatment, others are.[1-3]
- There is need to develop analytical method that can measure trace concentrations of these compounds in water resources .
- Recently, solid phase microextraction was proposed as a simple sample preparation method suitable for extraction of pharmaceutical due to availability of sensitive tandem mass spectrometry which can be coupled with SPME.

Research Objectives

- To develop a simple analytical method based on SPME for high throughput analysis of target pharmaceuticals in waste water
- To understand the occurrence, fate and partitioning of emerging contaminant in environmental water samples

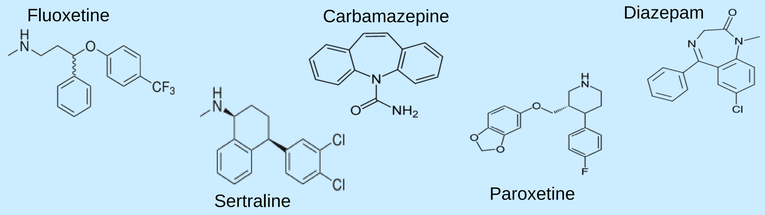


Figure 2: Structures of target pharmaceuticals under study

Experimental- SPME method (workflow)

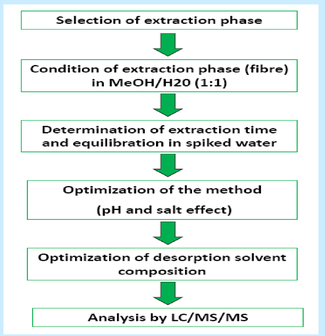
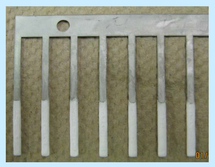


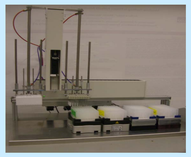
Figure 3: Overview of SPME method

SPME sampling procedure and LC/MS/MS method for analysis:

- PDMS and C18 fibers were compared during the selection of extraction phase.
- The extraction phase is conditioned in a mixture of water and methanol (1:1) for about 30 mins.
- Method optimization and validation in spiked water was carried out to evaluate method accuracy/precision.
- The extracted analytes were desorbed in a mixture of methanol/ water for 90 mins.
- The sample extract was analyzed using liquid chromatography coupled to tandem mass spectrometry



a) SPME thin film (C18 as extraction phase)



b) SPME thin film in a 96- well plate

c) LC/MS/MS TSQ (Thermo fisher)

Figure: 4 Description of method development format

$$n_e = \frac{K_{fs} V_f V_s}{K_{fs} V_f + V_s} C_0$$

SPME equation for equilibrium extraction

n_e: amount of analyte extracted by the fibre.
k_{fs} : the distribution coefficient between sample and coating
V_f: the volume of fiber coating; Vs : the volume of sample.
C_0: the initial concentration of the analyte in the sample.

Method validation in spiked wastewater

- Limit of detection (LOD) and quantification (LOQ) of the method was determined using signal-to-noise ratios of 3 and 10, respectively in spiked wastewater (3 ng/ml).
- Precision of the method (measured as relative standard deviation, RSD < 10%) is acceptable

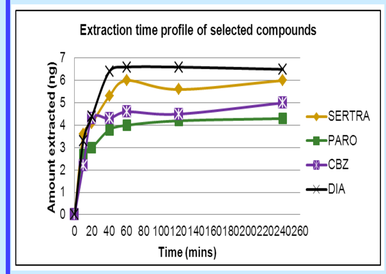
Spiked concentration - 3ng/ml

Compounds	LOD (ng/ml)	LOQ (ng/ml)	Precision (RSD %) (n =10)
Sertraline	0.006	0.02	5
Fluoxetine	0.009	0.03	2
Paroxetine	0.013	0.05	8
CBZ	0.002	0.01	3
Diazepam	0.005	0.02	4

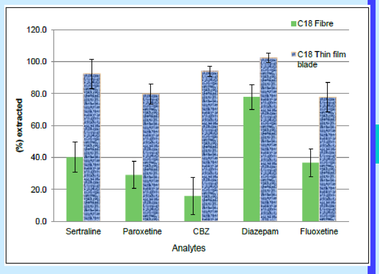
Extraction conditions	
Extraction time	90 mins
Extraction sample	Spiked water
Spiked conc.	3 ng/ml
Agitation condition	1000 rpm

Desorption conditions	
Desorption time	90 mins
Desorption solvent	MeOH/water (1:1)
Desorption volume	800uL
Agitation speed	1000 rpm

Optimization of extraction time



a) Extraction time profile of target compound



b) Comparison of C18 fiber and C18- thin film extraction phases

Figure 5: SPME method development and validation in spiked waste water samples

Optimization strategies

- Increased volume of the extraction phase to improve sensitivity of the method
- Increased surface area of the extraction phase for faster extraction
- Using a highly sensitive detector like mass spectrometry – TSQ MS

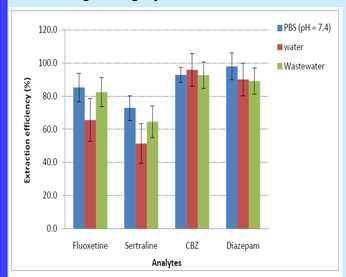


Figure 6 a) Comparison of extraction efficiency in PBS (pH = 7.4), Pure water and waste water

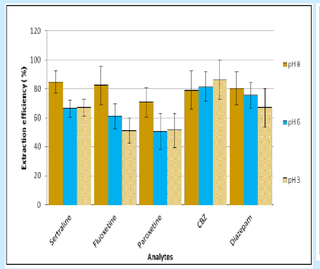


Figure 6b) Effect of pH on the extraction efficiency of SPME method

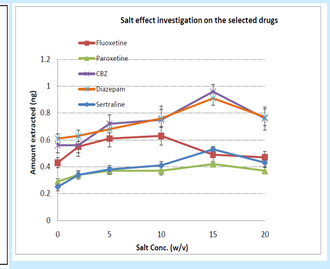


Figure 6c) Effect of salt on the extraction efficiency of the SPME method

Results and discussions

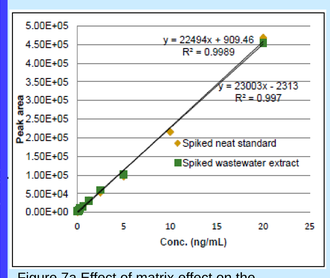


Figure 7a Effect of matrix effect on the method using fluoxetine

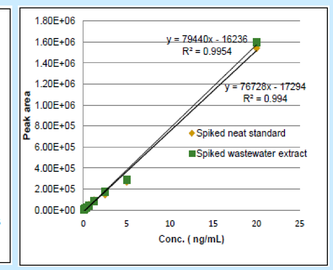


Figure 7b Effect of matrix effect on the method using carbamazepine

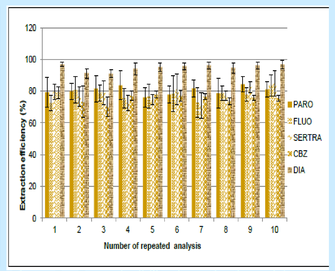


Figure 7c Reproducibility investigation of the method

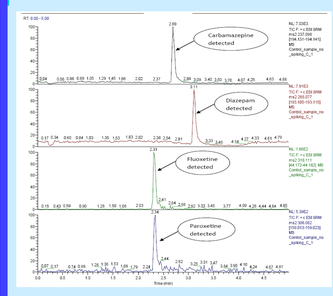


Figure 7d) Chromatogram of waste water of SPME extract collected from Kitchener Grand River

Compounds	BNR		CAS		CAS-N	
Paroxetine	0.24	0.06	0.35	0.09	0.32	0.06
Fluoxetine	1.05	0.11	1.45	0.15	1.23	0.32
Sertraline	1.41	0.27	1.93	0.18	1.91	0.34
Carbamazepine	0.64	0.12	0.75	0.08	0.69	0.11
Diazepam	ND		ND		ND	

16-Sep

Compounds	BNR		CAS		CAS-N	
Paroxetine	0.32	0.06	0.37	0.14	0.61	0.13
Fluoxetine	2.29	0.41	3.82	0.32	3.01	0.46
Sertraline	1.31	0.24	1.52	0.19	1.47	0.18
Carbamazepine	0.46	0.11	0.53	0.09	0.44	0.10
Diazepam	<LOQ		<LOQ		<LOQ	

Figure 7e) Concentration of target analytes detected in Burlington effluent

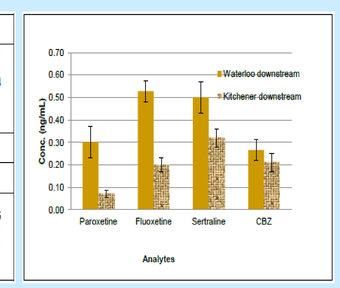


Figure 7f) Concentration of analytes detected in the downstream effluent along Grand River

Summary

- Solid phase microextraction may be a promising method of analysis of pharmaceuticals in waste water
- This study demonstrates that the developed method (SPME-thin film /LC/MS/MS) has acceptable detection limit (low ng/L range) capable of detecting the selected pharmaceuticals in environmental water samples
- The study demonstrates the utility of SPME as a simple analytical method for microextraction of selected pharmaceuticals in waste water

Acknowledgements

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3. Vuckovic D., Cudjoe E., Hein D., Pawliszyn J. *Anal. Chem.* 2008, *80,* 6870–6880