

Determination of Pharmaceuticals in Environmental Samples

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Abstract

This application note describes the use of two LC-MS methods for the examination of pharmaceuticals in environmental samples (concentrated sewage, surface water and drinking water). These analyses were performed utilizing an XBridge C₁₈ HPLC column for the chromatographic separations

Introduction

The full effects of pharmaceutical substances in the environment are largely unknown however the risk is significant enough that many new studies are being initiated by such organizations as the U.S. Environmental Protection Agency and the World Health Organization in order to better understand the fate of these chemicals.

Worldwide, thousands of tons of active pharmaceutical substances are utilized in preserving human and animal health but very little is know about the ultimate fate of these drugs. A large portion of each dose can be excreted and unused medicine is often disposed of via sewer systems. Recent studies have indicated that a significant portion of urban sewage contains drug compounds that many pharmaceuticals are not completely eliminated in sewage treatment plants.

Additional sources of drugs in the environment include agricultural runoff (verterinary drugs excreated by domesticated animals) and landfill leachate (disgarded drugs from households). The effects of the drugs and their metabolics on aquatic environments and organisms are largely unknown, as are the potential inpacts on human health. They are, however, likely to have some impact and their presence to grow more common, thus additional research in this area is warranted.

This report will describe the use of two LC-MS methods for the examination of pharmaceuticals in environmental samples (concentrated sewage, surface water and drinking water). These analyses were performed utilizing an XBridge C₁₈ HPLC column for the chromatographic separations

Experimental

Samples

The analyses included standard solutions of pharmaceuticals as well as environmental samples (concentrated sewage, surface water and drinking water samples). Fourteen pharmaceuticals (and four internal standards) were analyzed (Table 1) using two LC.

		Retention Time (min)	Ionization (ESI)	Precursor Ion (m/z)	Product Ion (m/z)
Ciprofloxacin	Antibiotic	9.8	Negative	331.9	287.9
Norfloxacin	Antibiotic	9.7	Negative	319.8	275.9
Ofloxacin	Antibiotic	9.7	Negative	361.8	317.9
Carbamazepine	Antiepileptic	13.4	Negative	237.0	193.9
Acebutolol	Beta blocker	10.5	Negative	336.8	116.0
Atenolol	Beta blocker	3.4	Negative	267.0	144.9
Metoprolol	Beta blocker	10.7	Negative	267.9	190.9
Sotalol	Beta blocker	3.1	Negative	254.8	132.9
Clofibric acid	Drug metabolite	8.9	Negative	212.9	126.9
Enrofloxacin (IS)	IS for the antibiotics	10.3	Negative	359.9	315.9
Dihydrocarba- mazepine (IS)	IS for carbamazepine	13.5	Negative	239.0	193.9
Alprenolol (IS)	IS for the beta blockers	12.8	Negative	249.9	172.9
Diclofenac	Anti- inflammatory	11.5	Positive	293.8	249.9
lbuprofen	Anti- inflammatory	10.8	Positive	205.1	161.0
Ketoprofen	Anti- inflammatory	10.0	Positive	253.0	209.0
Naproxen	Anti- inflammatory	9.5	Positive	229.0	169.9
Bezafibrate	Lipid regulator	10.6	Positive	360.0	273.9
Fenoprop (IS)	enoprop (IS) IS for the anti- inflammatory, bezafibrate and clofibric acid		Positive	266.8	194.8

IS = internal standard

Table 1. The analyzed compounds, their retention times and MS

parameters.

Sewage samples (influents and effluents) were obtained as composite samples over 24 hours and stored at -18

°C. River water samples were collected as grad samples, obtained using glass bottles and stored at 4 °C.

Sample Preparation

Solid-phase extraction was used to separate the pharmaceuticals from the water component of the sample. The

samples were filtered through 0.45 μm filters which were pre-washed with hexane, acetone, methanol and water. The pH of the samples was adjusted to 2.0 using concentrated HCL. Oasis MCX 3 cc (60 mg – Part Number 186000253) was used as the solid-phase adsorbent.

The adsorbent was pre-conditioned with 2 mL of hexane, 2 mL of acetone and 10 mL of methanol and 10 mL of non-contaminated groundwater (pH adjusted to 2.0). The samples were added to the cartridges at a flow rate of 8 mL/min. The cartridges were dried with nitrogen for 1 hour and the pharmaceuticals eluted using 4 x 1 mL of acetone. The extracts were then evaporated to 100 μ L with nitrogen and 100 μ L of methanol was added. Evaporation continued until the volume was 50 μ L. 450 μ L of ammonium hydroxide was added and the extracts stored at -18 °C.

HPLC/MS Conditions							
Column:	XBridge C ₁₈ , 2.1 x 50mm, 5 µm						
Part Number:	186003108						
Flow Rate:	0.2 mL/min						
Injection Volume:	20 µL						
Temperature:	30 °C						
MS Conditions							
MS System:	Quattro Micro triple-quadrupole mass spectrometer (Micromass®) equipped with an electrospray ionization (ESI) source						
Desolvation & Nebulizing Gas:	Nitrogen						
Collision Gas:	Argon						
Operating Mode:	MRM						

The cone voltage and collision energy were optimized for each analyte by direct infusion of pure compound to the MS/MS compartment (Table 1).

The compounds were analyzed using two LC-MS/MS methods, one to analyze the negatively ionized compounds and another to analyze the positively ionized compounds (Table 1). It should be noted that the LC methods were not optimized to a great detail. Therefore, even better chromatograms can be expected in the future.

Time (min)	% of eluent A (5 mM NH40H)	% of eluent B (Acetonitrile)		
0	95	5		
1	95	5		
12	40	60		
13	95	5		
25	95	5		

The LC method for the negatively ionized compounds was:

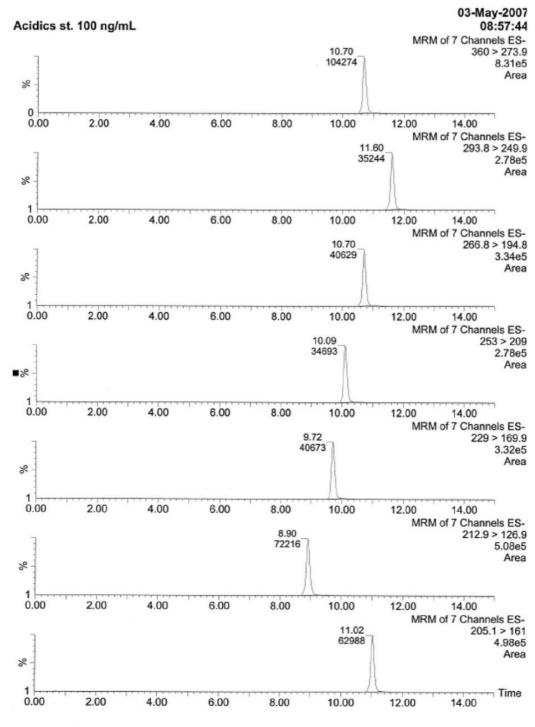
Also 10 mM NH₄OH was tested but the peak shape was more symmetrical with the 5 mM solution. Good peak shapes was obtained also by using 10 mM ammonium acetate as the aqueous eluent. However, since the negative ionization of compounds is hampered at lower pH, sensitivity was reduced with this eluent. Peak shapes were acceptable for all the analysed compounds.

For the positively ionized compounds, the following LC method was used:

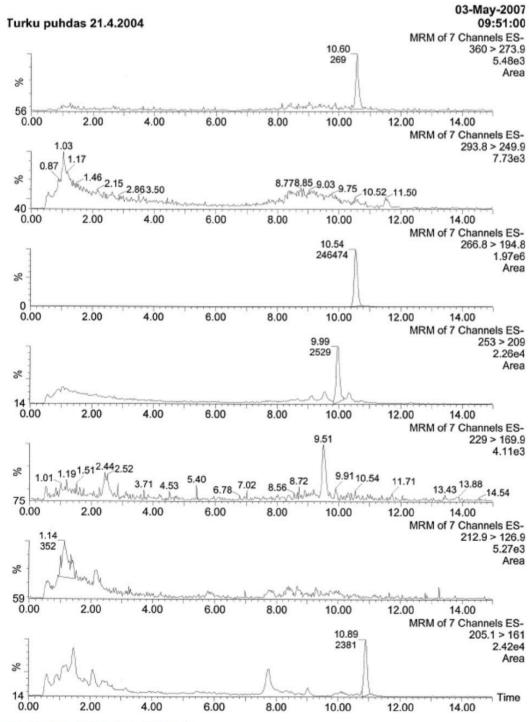
Time (min)	% of eluent A (0.5% Formic Acid)	% of eluent B (Acetonitrile)
0	95	5
1	95	5
14	30	70
15	95	5
25	95	5

Also 10 mM ammonium acetate was tested but the antibiotics could not be well resolved with that eluent. Peak shapes were acceptable for all the other compounds except for atenolol and sotalol that eluted early in the run. Peaks representing these compounds were wide and tailing.

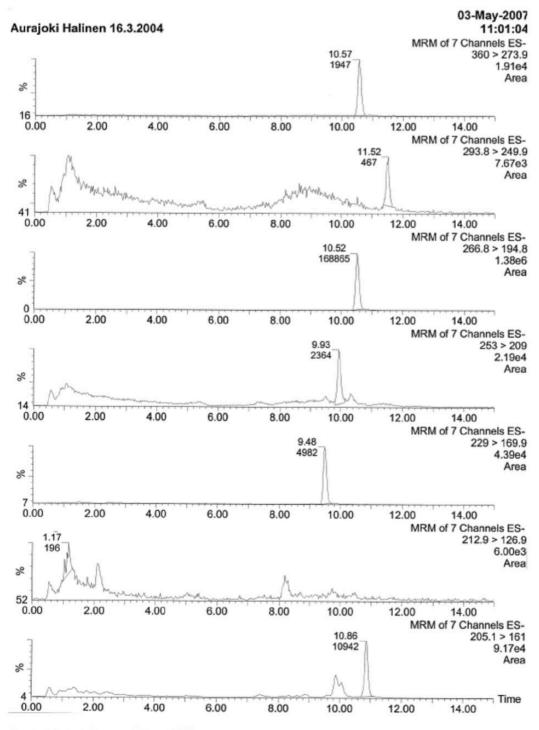
Results and Discussion



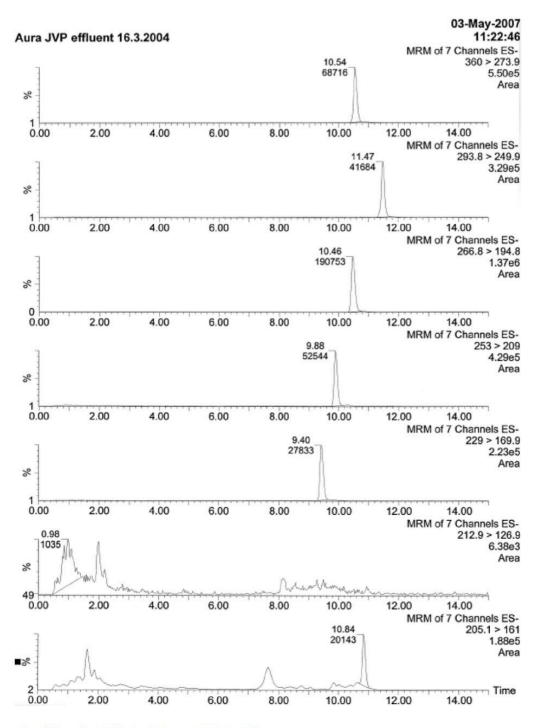
Standard - Negatively Ionized - 100 ng/mL.



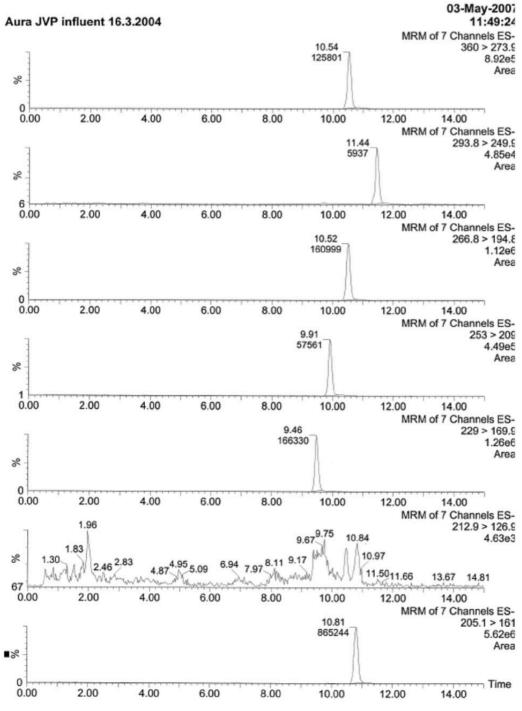
Drinking Water - Negatively Ionized - Concentration Factor 1000.







Sewage Treatment Plant Effluent - Negatively Ionized - Concentration Factor 500.



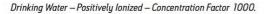


28-May-2007

Basics 100	na/mL						11:04:5
07052803 ∎%					9.72 5945	MRM of	11 Channels ES 361.8 > 31 6.02e
5 0.00 07052803	2.00	4.00	6.00	8.00	10.00	12.00 14. MRM of	11 Channels ES
» 17					10.29 1860		359.9 > 315 1.81e Are
0.00	2.00	4.00	6.00	8.00	10.00	12.00 14.	
07052803 ∦					10.50 66731	MRM of	11 Channels ES 336.8 > 11 5.456 Are
0.00	2.00	4.00	6.00	8.00	10.00	12.00 14.	
07052803					9.84 652	MRM of	11 Channels ES 331.9 > 287 9.066
0.00 07052803	2.00	4.00	6.00	8.00	10.00 9.72 243	12.00 14. MRM of	
58					A		Are
0.00 07052803	2.00	4.00	6.00	8.00	10.00 10.71 4784	12.00 14. MRM of	00 16.00 11 Channels ES 267.9 > 190 4.520
7					A		An
0.00 07052803	2.00 3.43 60218		6.00	8.00	10.00	12.00 14. MRM of	11 Channels ES 267 > 144 3.100
1 0.00 07052803	2.00	4.00	6.00	8.00	10.00	12.00 14. MRM of	11 Channels ES
% 	3.14 41829	Λ					254.8 > 132 2.45 Are
0.00	2.00	4.00	6.00	8.00	10.00	12.00 14.	00 16.00
07052803						12.82_ 10904	11 Channels ES 249.9 > 172 9.500 Are
3 0.00 7052803	2.00	4.00	6.00	8.00	10.00	12.00 14. MRM of 13.52 61606	
1 0.00 07052803	2.00	4.00	6.00	8.00	10.00	12.00 14. MRM of 13.40 150985	00 16.00 11 Channels ES 237 > 193 1.34
0.00	2.00	4.00	6.00	8.00	10.00	12.00 14.	TAM 00 16.00

Standard - Positively Ionized - 100ng/mL

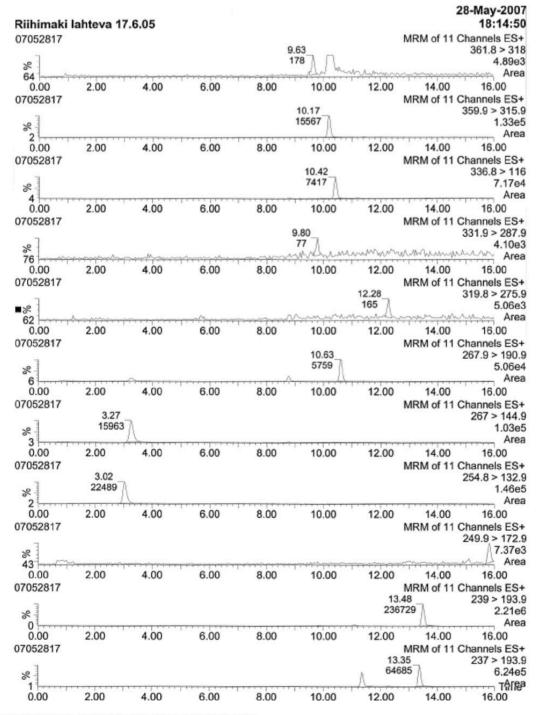
28-May-2007 Vaasa Puhdas 3.5.04 17:17:33 07052815 MRM of 11 Channels ES+ 10.21 361.8 > 318 3031 3.25e4 % Area 10 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 MRM of 11 Channels ES+ 07052815 10.21 359.9 > 315.9 8001 7.77e4 % Area 4 10.00 14.00 16.00 2.00 4.00 6.00 8.00 12.00 07052815 MRM of 11 Channels ES+ 10.46 336.8 > 116 87 224 5.07e3 Area 62 -16.00 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 07052815 MRM of 11 Channels ES+ 331.9 > 287.9 while the stress while the stress of the str % white 83 -----24 ma 16.00 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 07052815 MRM of 11 Channels ES+ 319.8 > 275.9 myy My March much 3.72e3 % Mum Area 85 16.00 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 07052815 MRM of 11 Channels ES+ 10.01 10.63 11.12 267.9 > 190.9 12.90 13.19 14.06 8 0.991.07 1.282.19 4.38e3 8.56 9.26 5.83 6.78 . Am 4.47 4.71 Mynn mann when m sou 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 07052815 MRM of 11 Channels ES+ 1.03 10.75 11.20 12.57 12.82 13.89 267 > 144.9 3.27 » 0.66 ∫ 9.63 8.14 8.93 mannam 6.55e3 1.32 3.47 6.41.6.57 1 48 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 07052815 MRM of 11 Channels ES+ 3.02 11.08 13.27 13.68 254.8 > 132.9 0.87 1.32 2.27 3.31 4.47 5.29 8.14 9.63 9.84 May Wall 4.30e3 * 6.41 7.19 wV 74 0.00 2.00 4.00 6.00 12.00 14.00 16.00 8.00 10.00 07052815 MRM of 11 Channels ES+ 12.73 249.9 > 172.9 19191 1.64e5 % 2 Area 0.00 2.00 4.00 6.00 8.00 10.00 14.00 16.00 12.00 07052815 MRM of 11 Channels ES+ 13.48 239 > 193.9 191699 1.79e6 8 Area 0 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 07052815 MRM of 11 Channels ES+ 13.35 237 > 193.9 1805 1.69e4 ∎% тАгеа 19 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00



28-May-2007

VAJ V84 16 07052816	5.6.05						MPM of 11	28-May-200 17:46:1 Channels ES
%]					10.21 3371		MIRENI OF TT	361.8 > 31 3.38e Are
0.00 07052816	2.00	4.00	6.00	8.00	10.00 10.21 9882	12.00	14.00 MRM of 11	16.00 Channels ES 359.9 > 315. 9.33e Are
0.00 07052816	2.00	4.00	6.00	8.00	10.00 10.42 1975	12.00	14.00 MRM of 11	16.00 Channels ES 336.8 > 11 2.13e Are
0.00 07052816	2.00	4.00	6.00	8.00	10.00 10.01;107	12.00 Marthallard	14.00 MRM of 11	16.00 Channels ES 331.9 > 287. MM 3.74e Are
0.00 07052816	2.00	4.00	6.00	8.00	10.00	12.00	14.00 MRM of 11 14.39;147	16.00 Channels ES 319.8 > 275. 3.88e Are
0.00 07052816	2.00	4.00	6.00	8.00	10.00 10.63_ 1593	12.00	14.00 MRM of 11	16.00 Channels ES 267.9 > 190 1.62e Are
0.00 07052816	2.00 3.31 3595	4.00	6.00	8.00	10.00	12.00	14.00 MRM of 11	16.00 Channels ES 267 > 144 2.55e Are
0.00 07052816	2.00 3.06 4308	4.00	6.00	8.00	10.00	12.00	14.00 MRM of 11	16.00 Channels ES 254.8 > 132 2.97e Are
0.00	2.00	4.00	6.00	8.00	10.00	12.00 12.69 32669	14.00 MRM of 11	16.00 Channels ES 249.9 > 172 2.79e Are
0.00	2.00	4.00	6.00	8.00	10.00		14.00 MRM of 11 .48 .907	16.00 Channels ES 239 > 193 2.42e Are
0.00 07052816	2.00	4.00	6.00	8.00	10.00	12.00 13 238	.35	16.00 Channels ES 237 > 193. 2.36e
1 0.00	2.00	4.00	6.00	8.00	10.00	12.00	14.00	16.00

Surface Water - Positively Ionized - Concentration Factor.



Sewage Treatment Plant Effluent – Positively Ionized – Concentration Factor 500.

Riihimaki	i tuleva 17.6	.05						28-May-200 18:43:2
07052818							MRM of 11	Channels ES
%					63 52			361.8 > 3 6.466 Are
48 0.00 07052818	2.00	4.00	6.00	8.00	10.00 10.21 5529	12.00	14.00 MRM of 11	16.00 Channels ES 359.9 > 315 4.986
6 0.00 07052818	2.00	4.00	6.00	8.00	10.00	12.00	14.00 MRM of 11	Are 16.00 Channels ES 336.8 > 1
%5	2.00	1.00			6749	40.00		6.48e
0.00 07052818	2.00	4.00	6.00	8.00	10.00 9.639.72	12.00	14.00 MRM of 11	16.00 Channels ES 331.9 > 287
% 0.08 75	1.41 2.19 2.94	3.84 4.05	5.54 7.07	7.36 8.48	9.92	12.36 1	2.86 13.23	15.30 4.200
0.00 07052818	2.00	4.00	6.00	8.00	10.00	12.00	14.00 MRM of 11	16.00 Channels ES 319.8 > 275 7.54e Are
41 0.00 07052818	2.00	4.00	6.00	8.00	10.00 10.67 2716	12.00	14.00 MRM of 11	16.00 Channels ES 267.9 > 190 2.706 Are
12 0.00 07052818	2.00 3.22 41472	4.00	6.00	8.00	10.00	12.00	14.00 MRM of 11	16.00 Channels ES 267 > 144 2.68 Are
1 0.00 7052818	2.00 3.02 14798	4.00	6.00	8.00	10.00	12.00	14.00 MRM of 11	16.00 Channels ES 254.8 > 132 1.20 Are
3 0.00 7052818	2.00	4.00	6.00	8.00	10.00	12.00	14.00 MRM of 11 14.59;103	16.00 Channels ES 249.9 > 172 4.00
78 0.00 7052818	2.00	4.00	6.00	8.00	10.00	12.00 13. 224	.44	16.00 Channels ES 239 > 193 1.94
0 0.00 7052818	2.00	4.00	6.00	8.00	10.00	12.00 13. 191	31	Are 16.00 Channels ES 237 > 193 1.066
3	2.00	4.00	6.00	8.00	10.00	12.00	14.00	TAIS 16.00

Sewage Treatment Plant Influent - Positively Ionized - Concentration Factor 200.

Conclusion

It was determined that the XBridge C_{18} could detect the compounds in sewage, surface and drinking water samples. The peak shapes and sensitivity were good and the column was proven to be highly usable in the environmental analysis of pharmaceuticals.

Acknowledgement

Data for this report was provided courtesy of Abo Akademi University, Abo, Finland

Featured Products

WA60205, April 2008

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