Waters[™]

Note d'application

Determination of Pharmaceuticals in Environmental Samples

Waters Corporation



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 C_{18} 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) | lonization (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 | 10.3 Negative | | 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 |
| Ibuprofen | Anti- inflammatory | 10.8 | Positive | 205.1 | 161.0 |
| Ketoprofen | Anti- inflammatory | 10.0 | Positive | 253.0 | 209.0 |
| Naproxen | Anti- inflammatory | 9.5 | 9.5 Positive | | 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 Condition | IS | | | | | |
|----------------------------------|--|--|--|--|--|--|
| 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:

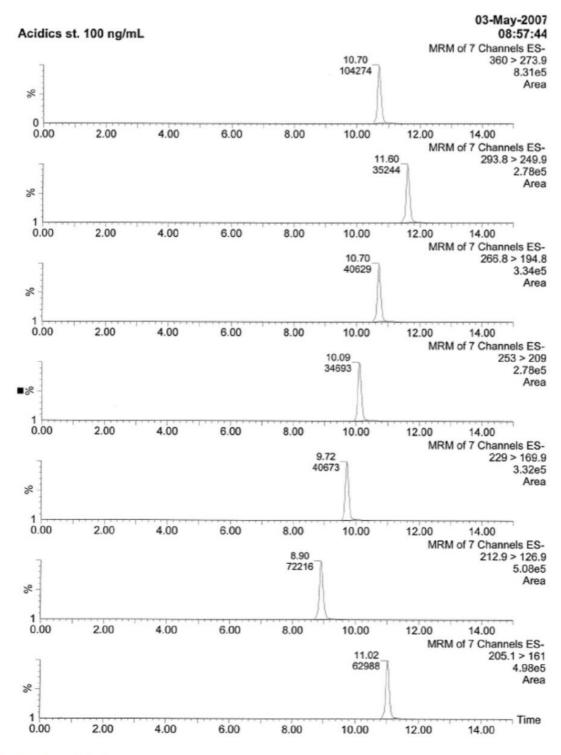
Also 10 mM NH₄OH was tested but the peak shape was more symmetrical with the 5 mM solution. Good peak shapes was obtained also by using 10 mM ammonium acetate as the aqueous eluent. However, since the negative ionization of compounds is hampered at lower pH, sensitivity was reduced with this eluent.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

| 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 | | |

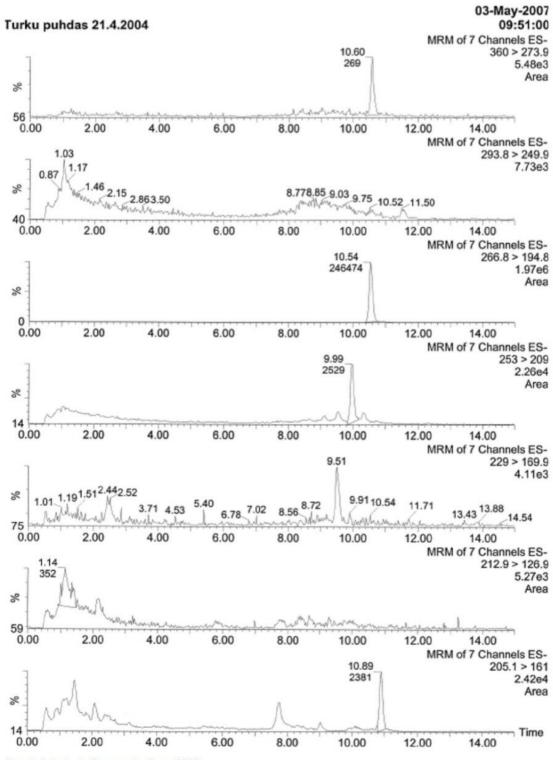
For the positively ionized compounds, the following LC method was used:

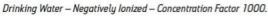
Also 10 mM ammonium acetate was tested but the antibiotics could not be well resolved with that eluent. Peak shapes were acceptable for all the other compounds except for atenolol and sotalol that eluted early in the run. Peaks representing these compounds were wide and tailing.

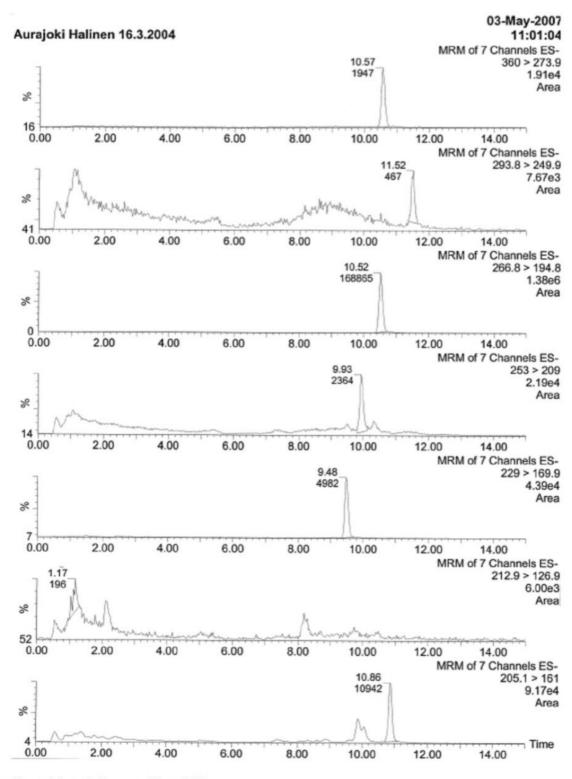
Results and Discussion



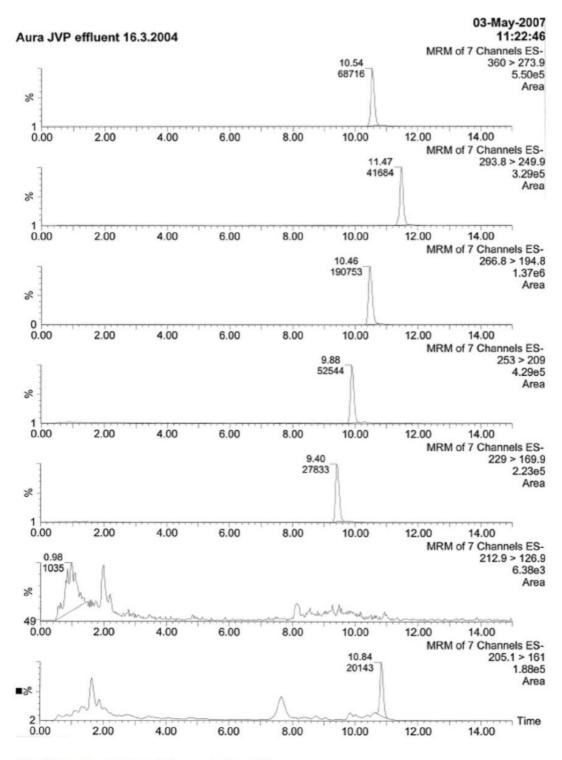
Standard - Negatively Ionized - 100 ng/mL.



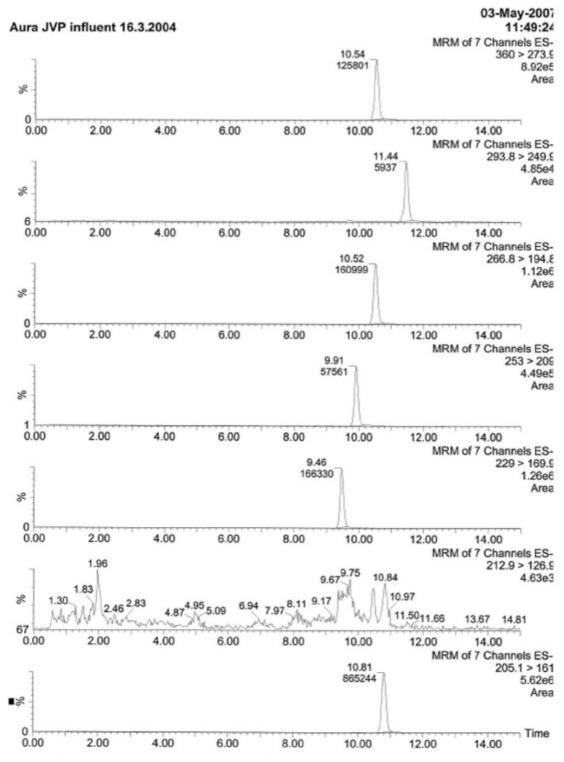




Surface Water - Negatively Ionized - Concentrated Factor 1000.



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



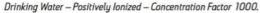
Sewage Treatment Plant Influent - Negatively Ionized - Concentration Factor 200.

28-May-2007

| 07052803 | | | | | 9.72 | | MRM of 11 | Channels E 361.8 > |
|------------------|-------------|-------------|--|------|-------|-------------|--------------------|---------------------------------------|
| ■% | | | | | 5945 | | | 6.0 A |
| 5 0.00 | 2.00 | 4.00 | 6.00 | 8.00 | 10.00 | 12.00 | 14.00 | 16.00 |
| 07052803 | | | | | 10.29 | | MRM of 11 | Channels E 359.9 > 3 |
| % | | | | | 1860 | | | 1.8 |
| 17 | | | | | | | | ······· / |
| 0.00 | 2.00 | 4.00 | 6.00 | 8.00 | 10.00 | 12.00 | 14.00 | 16.00 |
| 07052803 | | | | | 10.50 | | MRM of 11 | Channels 336.8 > |
| % | | | | | 66731 | | | 5.4 |
| 1 | | | | | | | | |
| 0.00 | 2.00 | 4.00 | 6.00 | 8.00 | 10.00 | 12.00 | 14.00 | 16.00 Channele |
| 07052803 | | | | | 9.84 | | MRM OF 11 | Channels 331.9 > 2 |
| % | | | | | 652 | | | 9.0 |
| 34 | | | | | ····· | | | · · · · · · · · · · · · · · · · · · · |
| 0.00 | 2.00 | 4.00 | 6.00 | 8.00 | 10.00 | 12.00 | 14.00 | 16.00 |
| 07052803 | | | | | 9.72 | | MKM OF 11 | Channels 319.8 > 2 |
| % | | | | | 243 | | | 5.3 |
| 58 | | | | | | | ***** | |
| 0.00 | 2.00 | 4.00 | 6.00 | 8.00 | 10.00 | 12.00 | 14.00 | 16.00 |
| 07052803 | | | | | 10.71 | | MRM of 11 | 267.9 > 1 |
| % | | | | | 4784 | | | 4. |
| 7 | | Arrent | ,,,,, ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, | | | ···· | | |
| 0.00 | 2.00 | 4.00 | 6.00 | 8.00 | 10.00 | 12.00 | 14.00 | 16.00 Channels |
| 07052803 | 3.43 | l Menna | | | | | MRM OF T | 267 > 1 |
| % | 6021 | | | | | | | 3.1 |
| 1 7 | | Anton | | | | | | |
| 0.00 07052803 | 2.00 | 4.00 | 6.00 | 8.00 | 10.00 | 12.00 | 14.00 | 16.00 Channels |
| 01052005 | 3.14 | | | | | | MICHI OF T | 254.8 > 1 |
| % | 41829 | λ | | | | | | 2.4 |
| 1 | | | | | | 40.00 | | |
| 0.00 07052803 | 2.00 | 4.00 | 6.00 | 8.00 | 10.00 | 12.00 | 14.00 | 16.00 Channels |
| | | | | | | 12.82 | | 249.9 > 1 |
| % | | | | | | 10904 | 7 | 9. |
| 3 | 2.00 | 4.00 | 6.00 | 8.00 | 10.00 | 12.00 | 14.00 | 10.00 |
| 0.00 07052803 | 2.00 | 4.00 | 6.00 | 8.00 | 10.00 | 12.00 | 14.00 MRM of 11 | 16.00 Channels |
| - | | | | | | | 3.52 | 239 > 1 |
| % | | | | | | 61 | 606 | 5.3 |
| 1 | 2.00 | 4 00 | 6.00 | 8.00 | 10.00 | 12.00 | 14.00 | 16.00 |
| 0.00 07052803 | 2.00 | 4.00 | 6.00 | 8.00 | 10.00 | 12.00 | | Channels |
| | | | | | | 13.4 | 40 | 237 > 1 |
| 8 | | | | | | 1509 | 985 | 1.3 |
| 0.00 | 2.00 | 4.00 | 6.00 | 8.00 | 10.00 | 12.00 | 14.00 | T 16.00 |

28-May-2007

Vaasa Puhdas 3.5.04 17:17:33 07052815 MRM of 11 Channels ES+ 10.21 361.8 > 318 3.25e4 3031 * Area 10 16.00 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 MRM of 11 Channels ES+ 07052815 10.21 359.9 > 315.9 % 7.77e4 8001 Area 4 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 MRM of 11 Channels ES+ 07052815 10.46 336.8 > 116 % 224 5.07e3 Area 62 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 07052815 MRM of 11 Channels ES+ 331.9 > 287.9 Area 3.77e3 % 83 ----mon in 1.20 16.00 12.00 14.00 0.00 2.00 4.00 6.00 8.00 10.00 07052815 MRM of 11 Channels ES+ 319.8 > 275.9 My May My May March wild 3.72e3 8 And Area 85 really 16.00 0.00 10.00 12.00 2.00 4.00 6.00 8.00 14.00 07052815 MRM of 11 Channels ES+ 10.01 10.63 11.12 267.9 > 190.9 12.90 13.1914.06 8 0.991.07 1.282.19 March 4.38e3 8.56 9.26 5.83 6.78 4.47.4.71 72 minut WWW. 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 07052815 MRM of 11 Channels ES+ 6.41.6.57 8.14 8.93 9.63 10.75 11.20 12.57 12.82 13.89 267 > 144.9 6.55e3 3.27 1.32 3.47 16.00 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 07052815 MRM of 11 Channels ES+ 13.27 13.68 254.8 > 132.9 3.02 11.08 6.41 7.19 8.14 9.63 9.84 % 0.87 1.32 2.27 3.31 4.47 5.29 month man An 74 her 120 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 07052815 MRM of 11 Channels ES+ 12.73 249.9 > 172.9 1.64e5 19191 8 Area 2 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 48 239 > 193.9 % 1.79e6 191699 Area 0 0.00 16.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 07052815 MRM of 11 Channels ES+ 13.35 237 > 193.9∎% 1805 1.69e4 TARE 19 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00



28-May-2007 VAJ V84 16.6.05 17:46:13 07052816 MRM of 11 Channels ES+ 10.21 361.8 > 318 3.38e4 3371 % Area 9 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 07052816 MRM of 11 Channels ES+ 10.21 359.9 > 315.9 9882 9.33e4 8 Area 3 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 MRM of 11 Channels ES+ 07052816 10.42 336.8 > 116 1975 2.13e4 8 Area 15 0.00 4.00 12.00 16.00 2.00 6.00 8.00 10.00 14.00 MRM of 11 Channels ES+ 07052816 331.9 > 287.9 10.01;107 % Area 84 0.00 2.00 4.00 8.00 12.00 16.00 6.00 10.00 14.00 07052816 MRM of 11 Channels ES+ 14.39;147 319.8 > 275.9 M. M. M. M. M. 3.8883 % moundar how 80 0.00 2.00 4.00 8.00 12.00 14.00 16.00 6.00 10.00 07052816 MRM of 11 Channels ES+ 10.63 267.9 > 190.9 % 1593 1.62e4 Area 19 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 07052816 MRM of 11 Channels ES+ 3.31 267 > 144.9 3595 2.55e4 8 Area 12 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 07052816 MRM of 11 Channels ES+ 3.06 254.8 > 132.9 4308 2.97e4 % 11 Area 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 07052816 MRM of 11 Channels ES+ 12.69 249.9 > 172.9 32669 2.79e5 8 Area 1 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 07052816 MRM of 11 Channels ES+ 13.48 239 > 193.9 % 256907 2.42e6 Area 0 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 07052816 MRM of 11 Channels ES+ 13.35 237 > 193.9 % 23805 2.36e5 1 TARP 0.00 2.00 4.00 6.00 8.00 14.00 10.00 12.00 16.00

Surface Water - Positively Ionized - Concentration Factor.

28-May-2007 Riihimaki lahteva 17.6.05 18:14:50 07052817 MRM of 11 Channels ES+ 9.63 361.8 > 318 178 4.89e3 % Area 64 0.00 8.00 10.00 12.00 14.00 16.00 2.00 4.00 6.00 07052817 MRM of 11 Channels ES+ 10.17 359.9 > 315.9 15567 1.33e5 % Area 2 0.00 6.00 14.00 16.00 2.00 4.00 8.00 10.00 12.00 07052817 MRM of 11 Channels ES+ 10.42 336.8 > 116 7.17e4 7417 8 Area 4 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 MRM of 11 Channels ES+ 07052817 9.80 331.9 > 287.9 4.10e3 77 A % N 76 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 MRM of 11 Channels ES+ 07052817 12.28 319.8 > 275.9 165 5.06e3 ∎% Area 62 AN 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 07052817 MRM of 11 Channels ES+ 10.63 267.9 > 190.9 5759 5.06e4 8 Area 6 0.00 16.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 07052817 MRM of 11 Channels ES+ 3.27 267 > 144.9 15963 1.03e5 %3 Area 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 07052817 MRM of 11 Channels ES+ 3.02 254.8 > 132.9 22489 1.46e5 % Area 2 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 07052817 MRM of 11 Channels ES+ 249.9 > 172.9 7.37e3 % Area 43 2.00 4.00 6.00 0.00 8.00 10.00 12.00 14.00 16.00 07052817 MRM of 11 Channels ES+ 13.48 239 > 193.9 236729 2.21e6 8 Area 0.00 2.00 4.00 6.00 10.00 14.00 16.00 8.00 12.00 07052817 MRM of 11 Channels ES+ 13.35 237 > 193.9 64685 6.24e5 % TARE 1 8.00 0.00 2.00 4.00 6.00 16.00 10.00 12.00 14.00

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

| Riihimak | i tuleva 17.6 | .05 | | | | | | 28-May-200 18:43:2 |
|-----------------------------|-----------------------|-----------|-------------|-----------|---------------|------------------------|---------------------------------|--|
| 07052818 & | | | | | .63 J | | MRM of 11 | Channels ES 361.8 > 31 6.46e |
| 48 | 2.00 | 4.00 | 6.00 | 8.00 | 10.00 | 12.00 | 14.00 | Are 16.00 |
| 07052818 * | | | | | 10.21 5529 | | MRM of 11 | Channels ES 359.9 > 315 4.98e Are |
| 6 0.00 07052818 | 2.00 | 4.00 | 6.00 | 8.00 | 10.00 | 12.00 | 14.00 MRM of 11 | 16.00 Channels ES |
| % | | | | | 10.42 6749 | | | 336.8 > 11 6.48e |
| 5 0.00 07052818 | 2.00 | 4.00 | 6.00 | 8.00 | 10.00 | 12.00 | 14.00 MRM of 11 | 16.00 Channels ES |
| » 75 | 1.41 2.19 2.94 | 3.84 4.05 | 5.54 7.07 | 7.36 8.48 | 9.639.72 | 12.36 | 2.86 13.23 | 331.9 > 287. 15.30 4.20e |
| 0.00 | 2.00 | 4.00 | 6.00 | 8.00 | 10.00 | 12.00 | 14.00 MRM of 11 | 16.00 Channels ES |
| % 41 | | | | | | 12.24 560 | | 319.8 > 275 7.54e Are |
| 0.00 | 2.00 | 4.00 | 6.00 | 8.00 | 10.00 | 12.00 | 14.00 MRM of 11 | 16.00 Channels ES |
| % 12 | | | | ~ | 10.67 2716 | | | 267.9 > 190. 2.70e Are |
| 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 2.68e |
| 1 0.00 07052818 | 2.00 3.02 14798 | 4.00 | 6.00 | 8.00 | 10.00 | 12.00 | 14.00 MRM of 11 | Are 16.00 Channels ES 254.8 > 132 |
| 3 0.00 | 2.00 | 4.00 | 6.00 | 8.00 | 10.00 | 40.00 | | 1.20e |
| 07052818 | 1. mars | 4.00 | 0.00 | 0.00 | 10.00 | 12.00 | 14.00 MRM of 11 14.59;103 | 16.00 Channels ES 249.9 > 172 4.00e |
| 78 0.00 0.00 07052818 | 2.00 | 4.00 | 6.00 | 8.00 | 10.00 | | .44 | 16.00 Channels ES 239 > 193. |
| % | **** | | | | | 224 | 267 | 1.94e Are |
| 0.00 07052818 | 2.00 | 4.00 | 6.00 | 8.00 | 10.00 | 12.00 13. [] 191 | 31 | 16.00 Channels ES 237 > 193. 1.06e |
| 3 | 2.00 | 4.00 | 6.00 | 8.00 | 10.00 | 12.00 | | TAR |

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

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