Applikationsbericht

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 C_{18} 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.

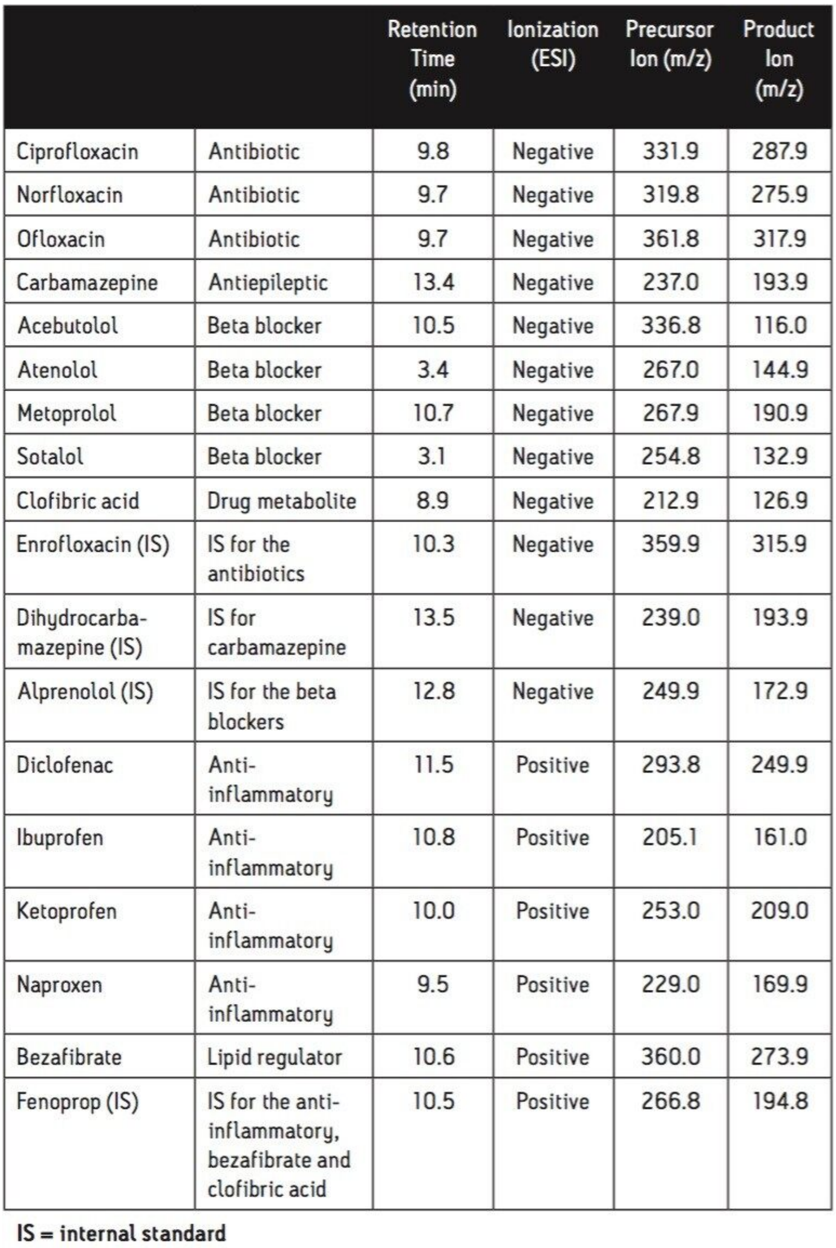
Additional sources of drugs in the environment include agricultural runoff (verterinary drugs excreated by domesticated animals) and landfill leachate (disgarded drugs from households). The effects of the drugs and their metabolics on aquatic environments and organisms are largely unknown, as are the 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)	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
Dihydrocarbamazepine (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	Positive	229.0	169.9
Bezafibrate	Lipid regulator	10.6	Positive	360.0	273.9
Fenoprop (IS)	IS for the anti-inflammatory, bezafibrate and clofibric acid	10.5	Positive	266.8	194.8

IS = internal standard

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

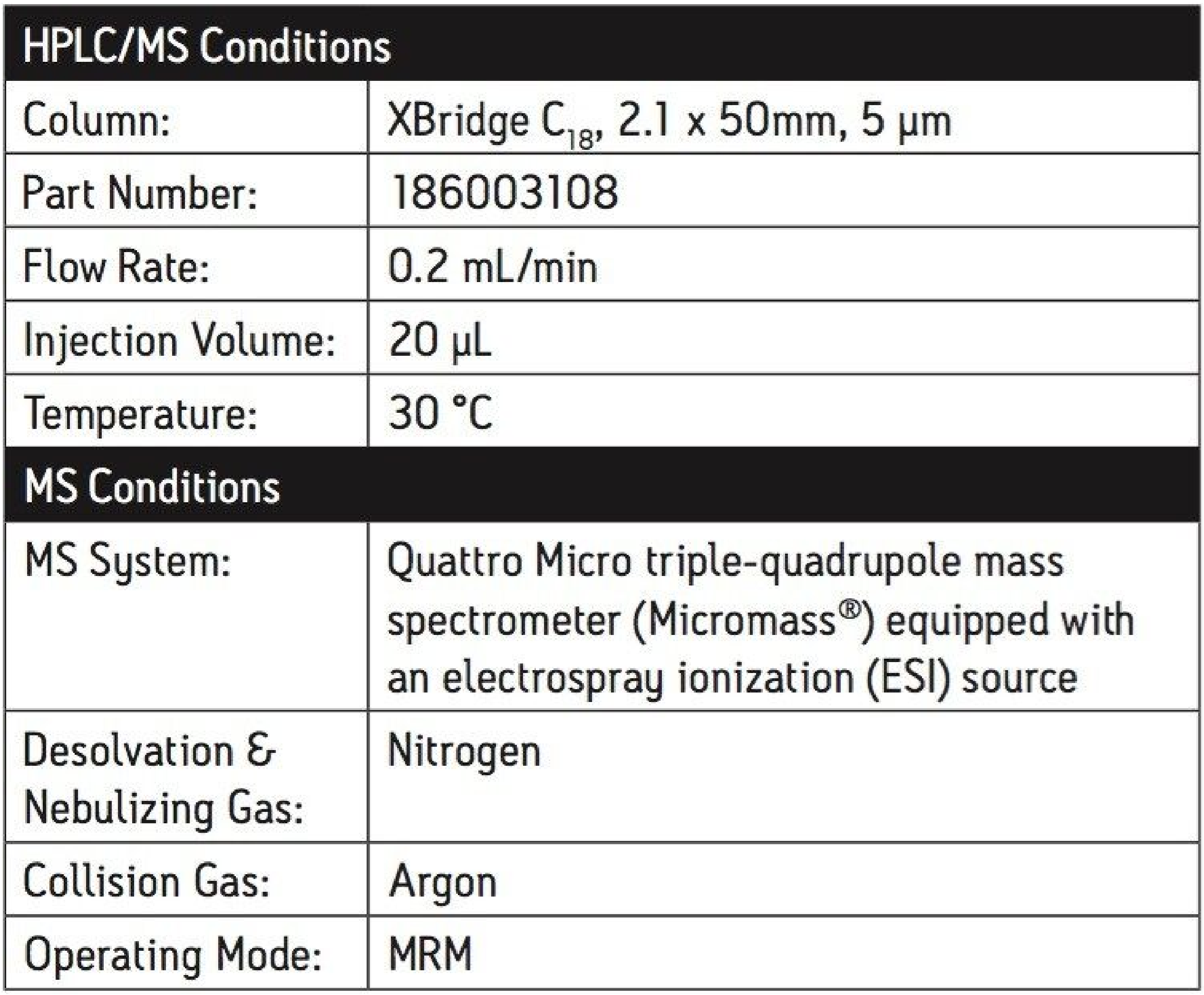
Sewage samples (influents and effluents) were obtained as composite samples over 24 hours and stored at -18 °C. River water samples were collected as grad samples, obtained using glass bottles and stored at 4 °C.

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 µL with nitrogen and 100 µL of methanol was added. Evaporation continued until the volume was 50 µL. 450 µL of ammonium hydroxide was added and the extracts stored at -18 °C.

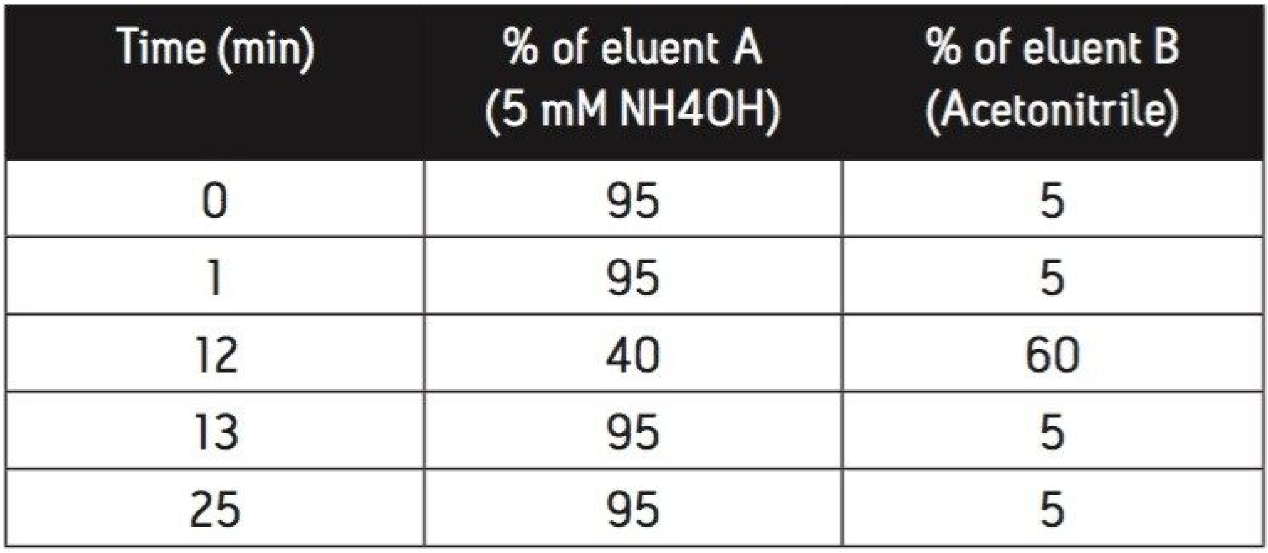


HPLC/MS Conditions	
Column:	XBridge C_{18}, 2.1 x 50mm, 5 µm
Part Number:	186003108
Flow Rate:	0.2 mL/min
Injection Volume:	20 µL
Temperature:	30 °C
MS Conditions	
MS System:	Quattro Micro triple-quadrupole mass spectrometer (Micromass®) equipped with an electrospray ionization (ESI) source
Desolvation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

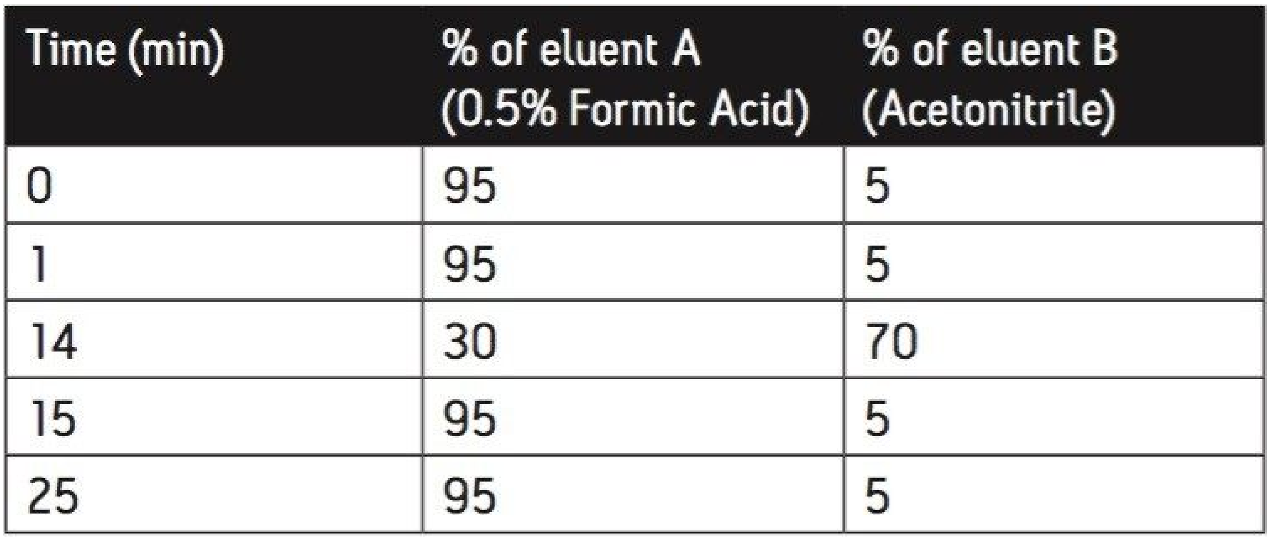
The LC method for the negatively ionized compounds was:



Time (min)	% of eluent A (5 mM NH4OH)	% of eluent B (Acetonitrile)
0	95	5
1	95	5
12	40	60
13	95	5
25	95	5

Also 10 mM NH_4OH 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.

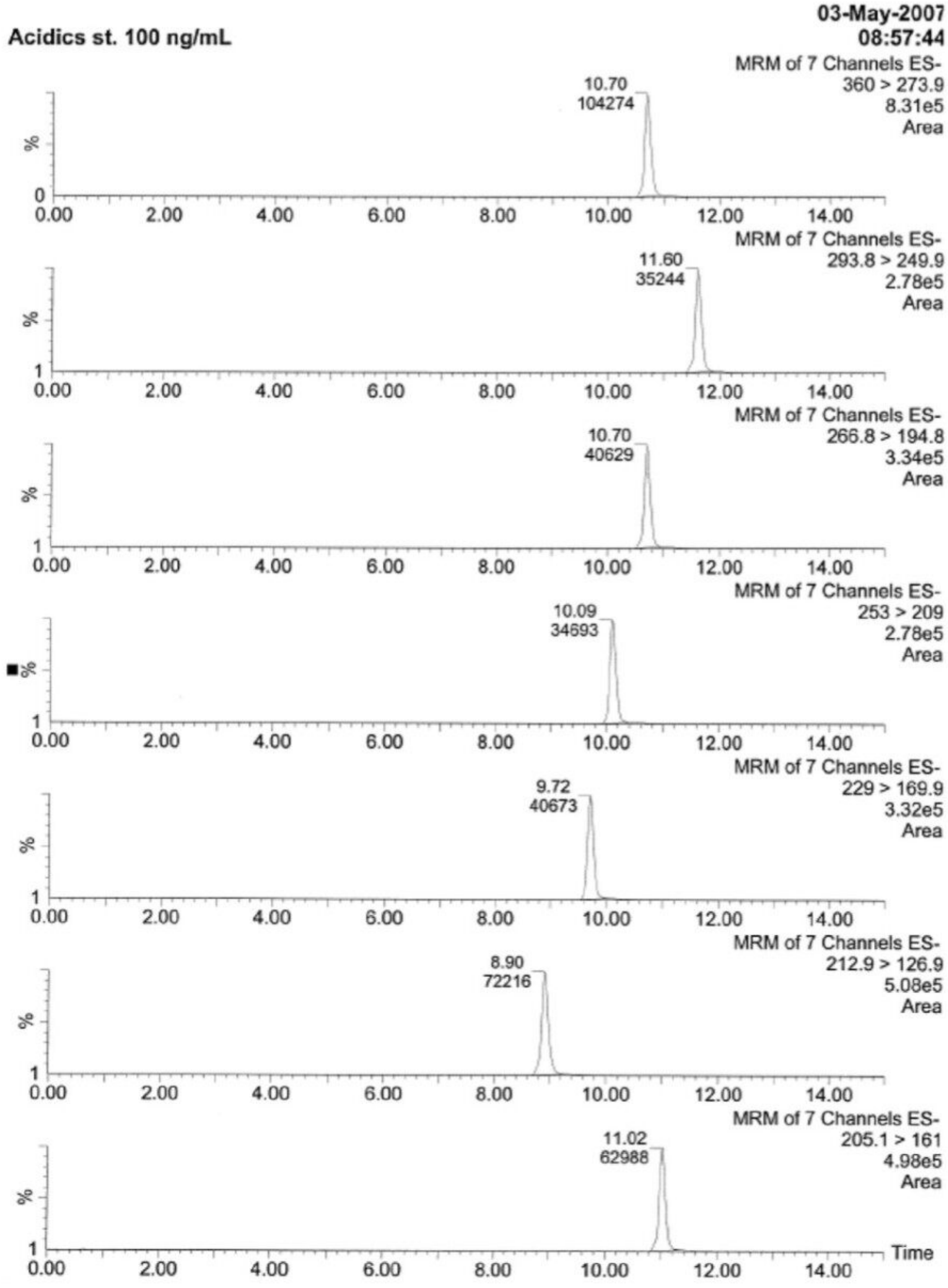
For the positively ionized compounds, the following LC method was used:



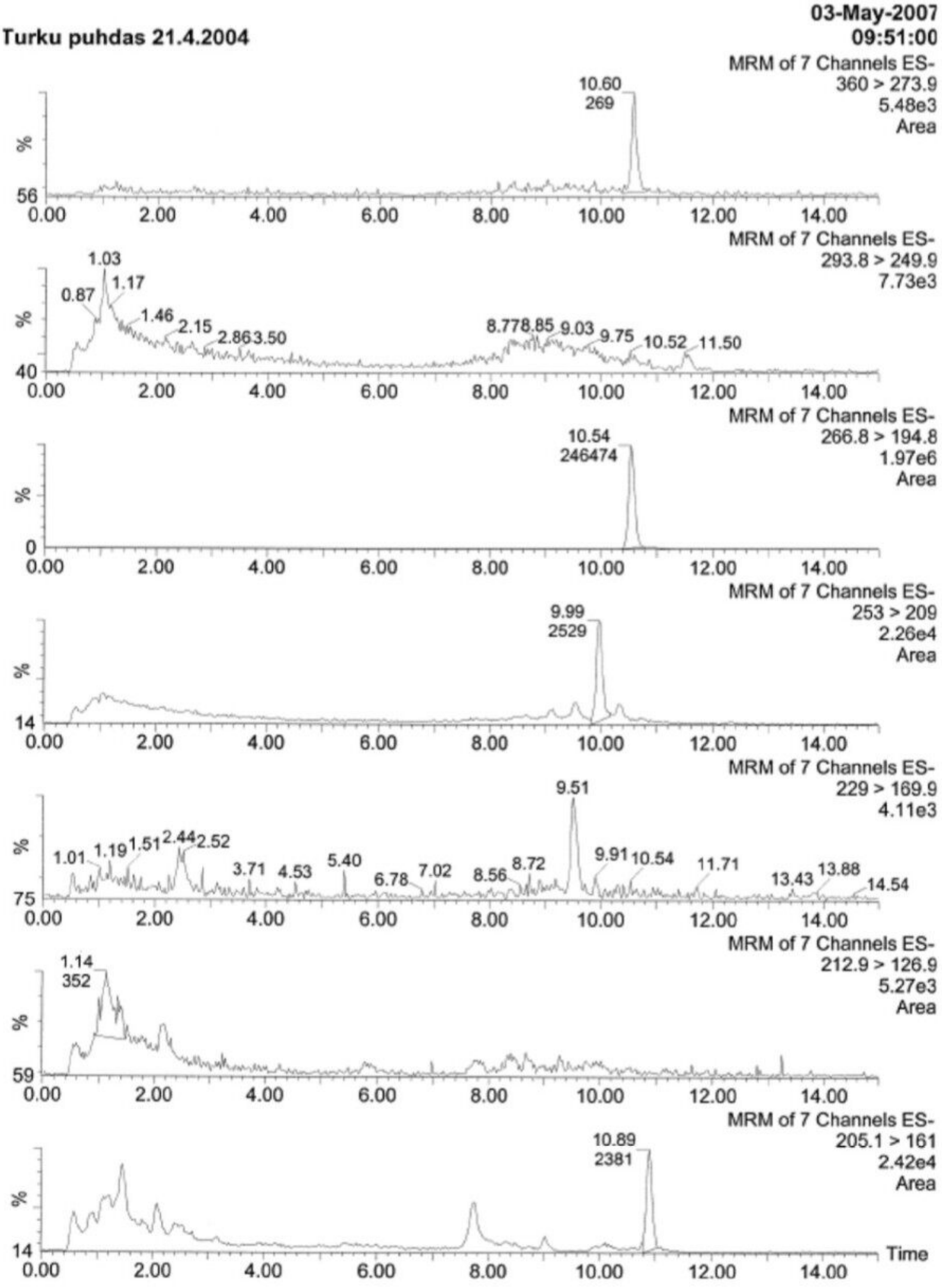
Time (min)	% of eluent A (0.5% Formic Acid)	% of eluent B (Acetonitrile)
0	95	5
1	95	5
14	30	70
15	95	5
25	95	5

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.

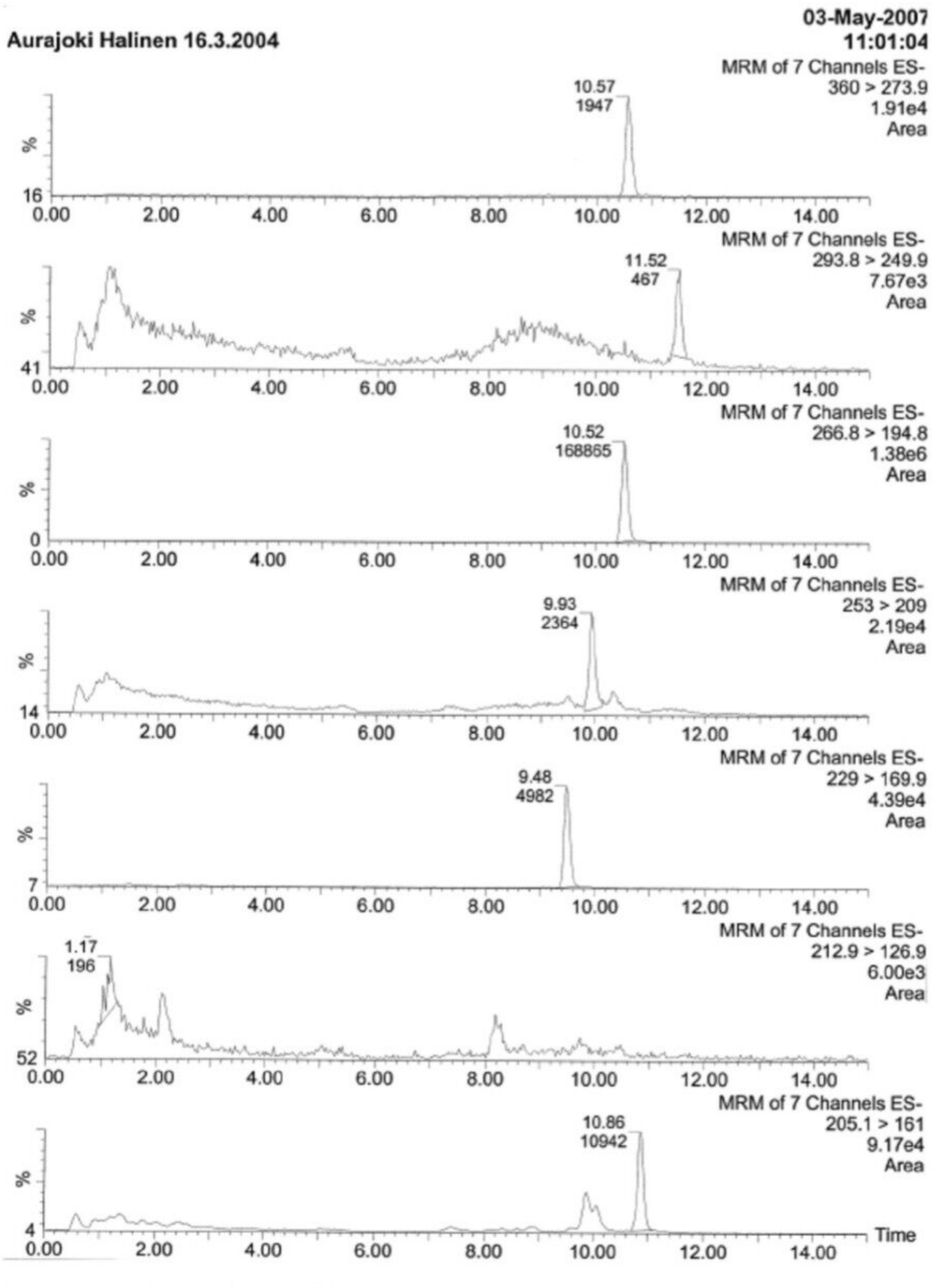
Results and Discussion



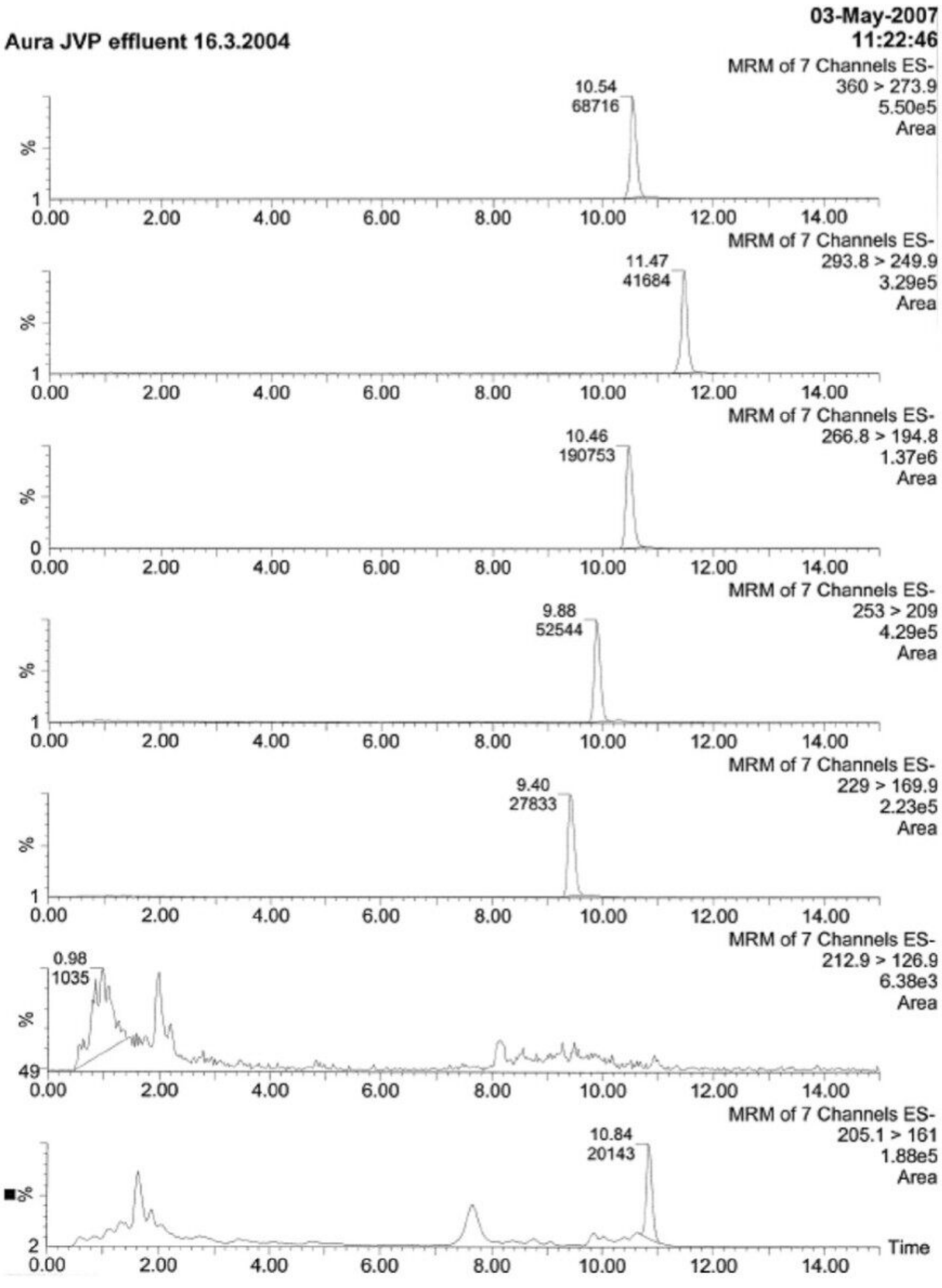
Standard – Negatively Ionized – 100 ng/mL.



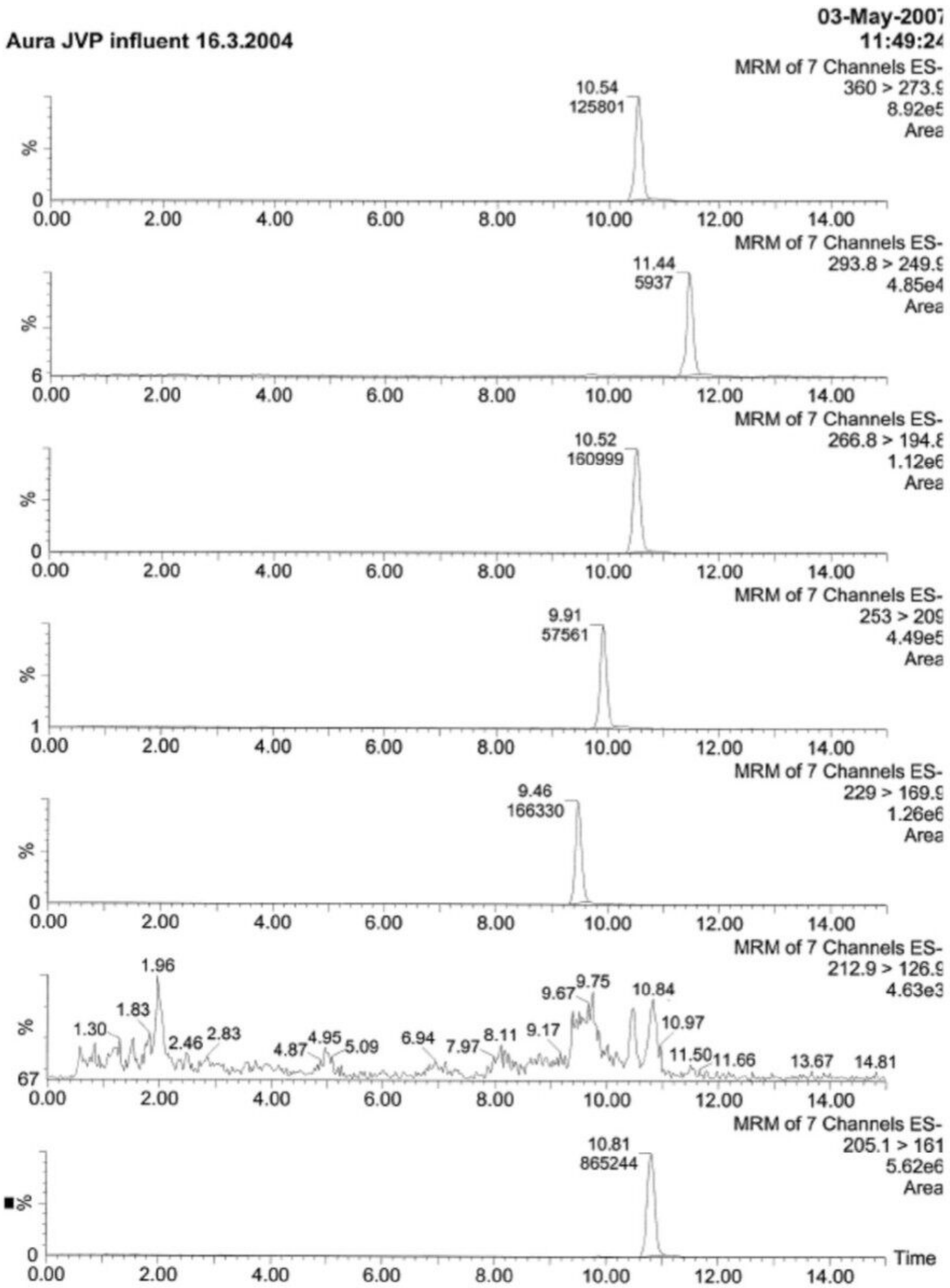
Drinking Water – Negatively Ionized – Concentration Factor 1000.



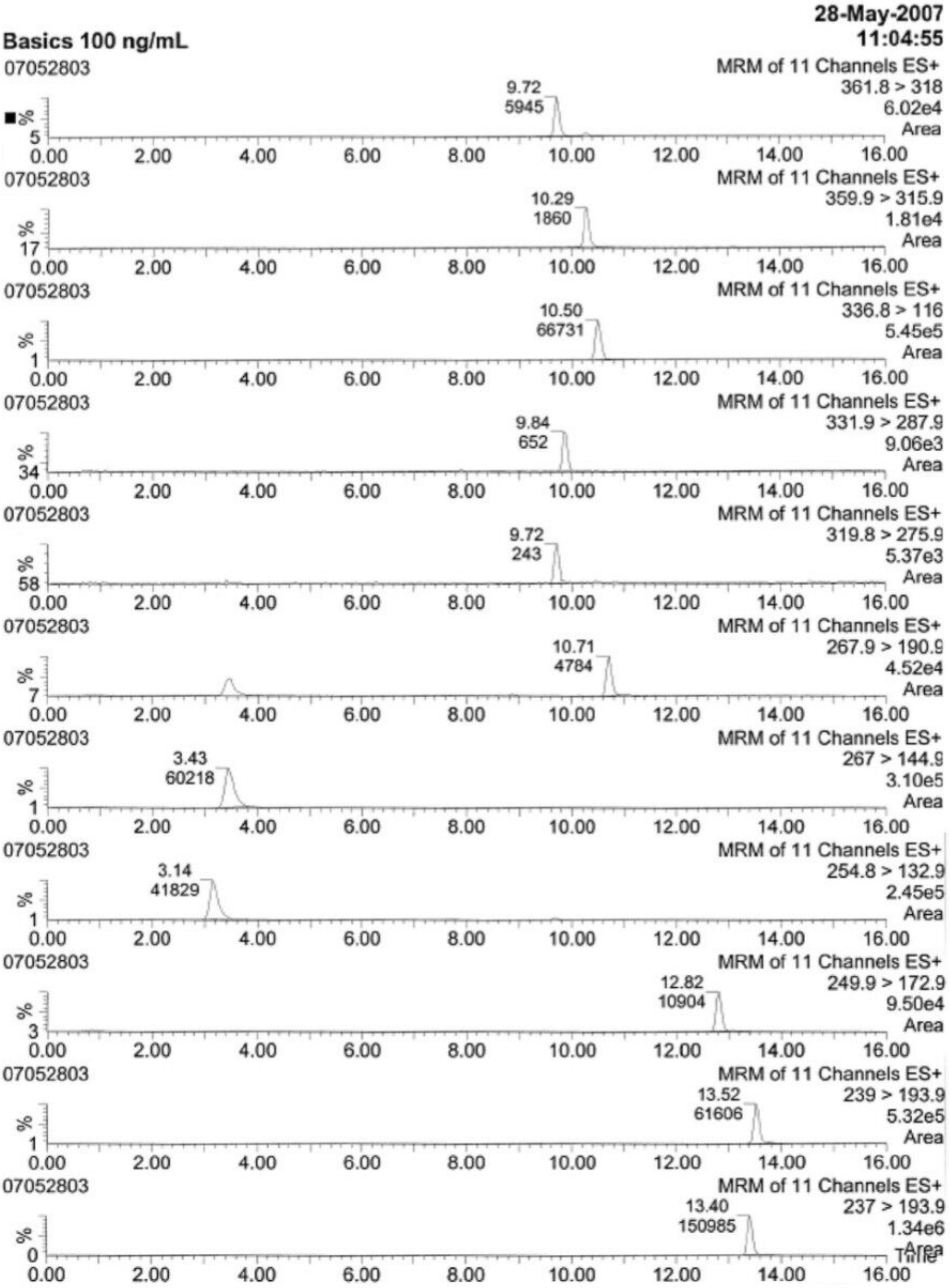
Surface Water – Negatively Ionized – Concentrated Factor 1000.



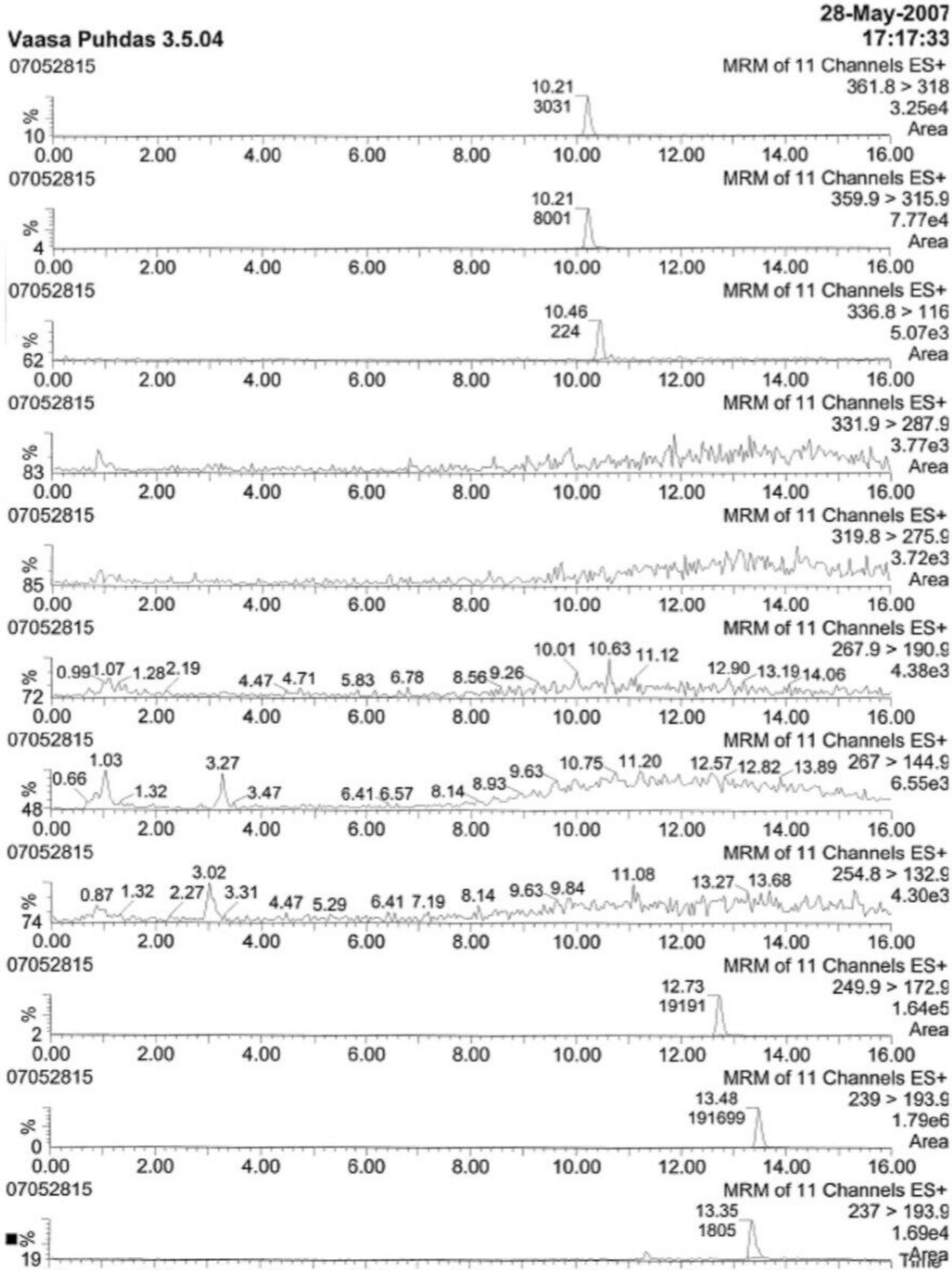
Sewage Treatment Plant Effluent – Negatively Ionized – Concentration Factor 500.



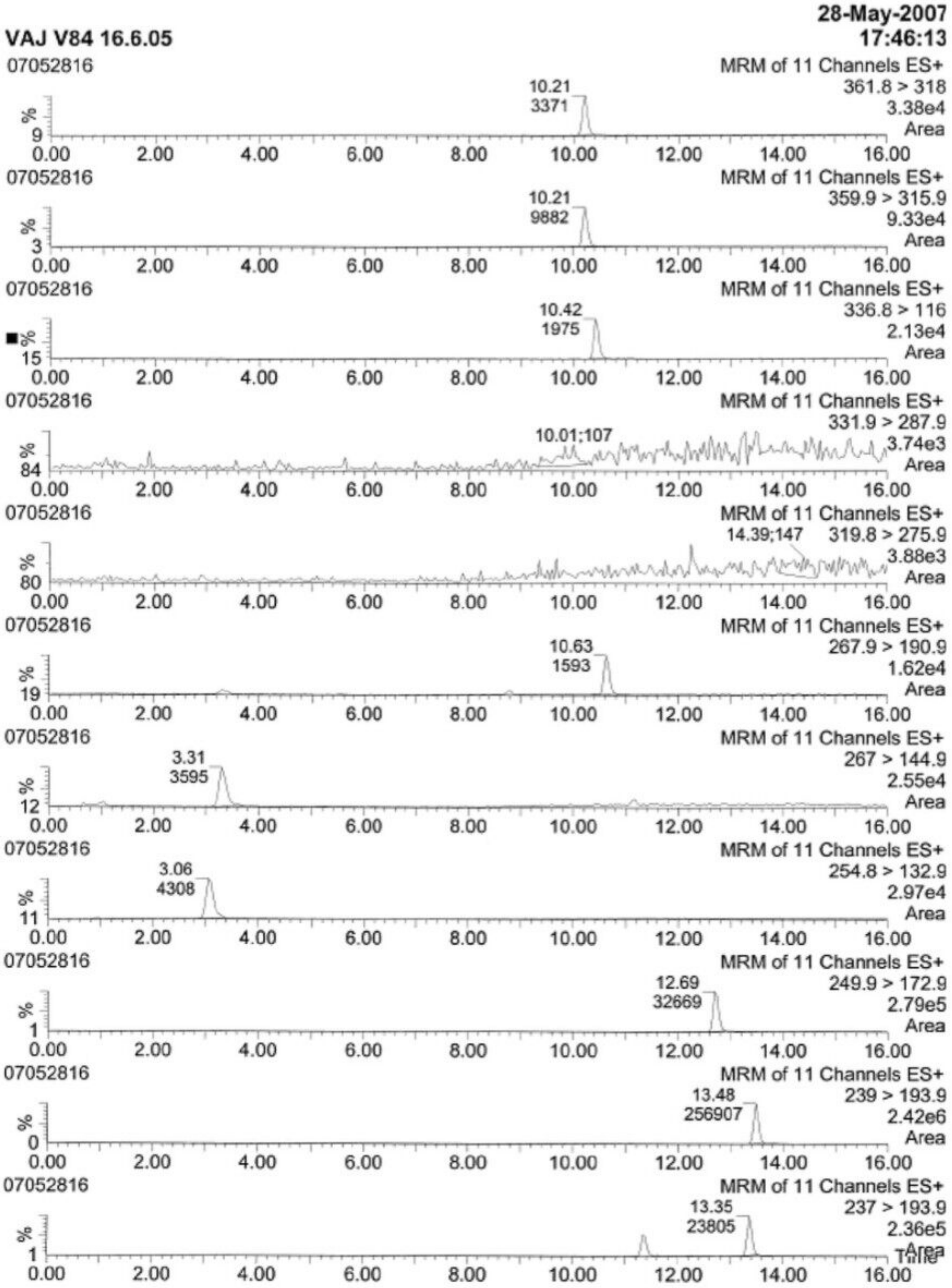
Sewage Treatment Plant Influent – Negatively Ionized – Concentration Factor 200.



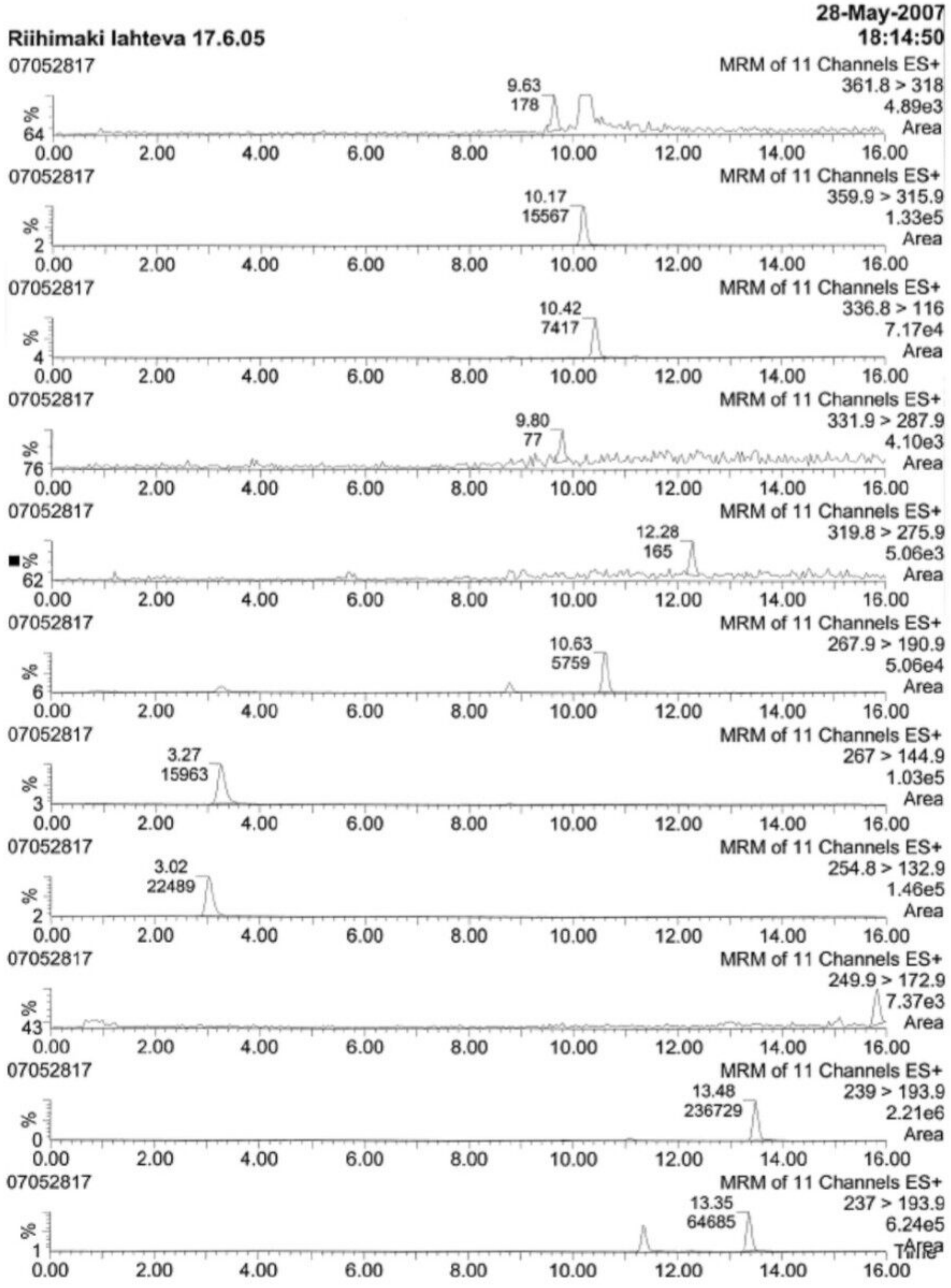
Standard – Positively Ionized – 100ng/mL



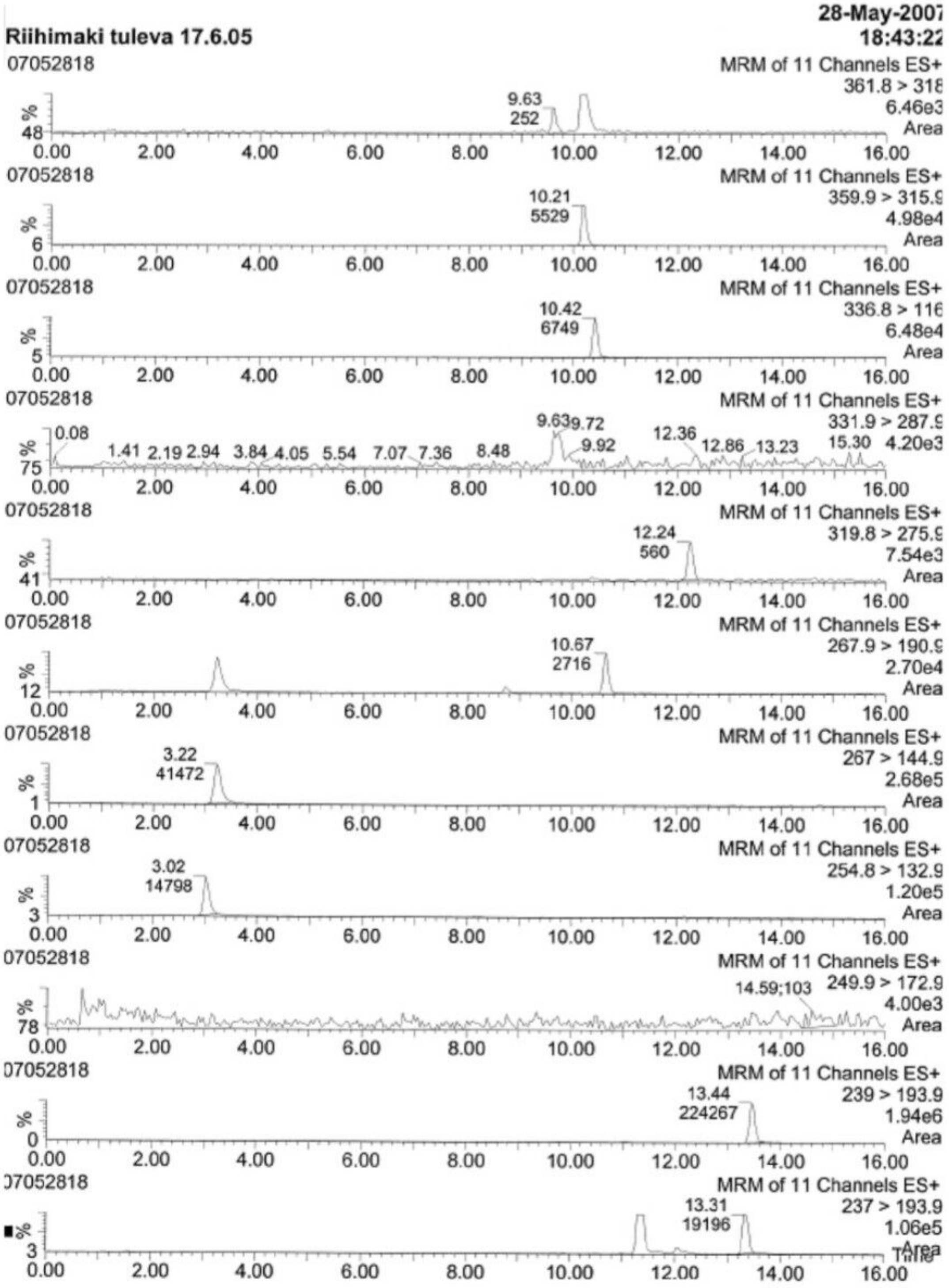
Drinking Water – Positively Ionized – Concentration Factor 1000.



Surface Water – Positively Ionized – Concentration Factor.



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



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

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