Determination of Pharmaceuticals in Water by SPE and LC/MS/MS in Both Positive and Negative Ion Modes

Application Note

Environmental

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Abstract

Using solid phase extraction (SPE) and liquid chromatography/tandem mass spectrometry (LC/MS/MS), 19 pharmaceuticals in positive ion mode and 11 pharmaceuticals in negative ion mode were analyzed at low picogram level on column without any derivatization. Good linearity was observed for analytes from 1 pg to 1 ng on column.

Repeatability from six injections of analytes at 5 pg on column showed RSDs below 15%, for all target compounds except for fluoxetine at 23%.

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Introduction

Many articles in leading medical journals and newspapers reported sexual development and reproductive problems in animals and humans, for example, low sperm counts, genital deformities, male fish making eggs, and others. Scientists suggested that man-made chemicals (for example, pesticides and pharmaceuticals) are disrupting the endocrine system.

Compounds like antibiotics, over-the-counter medicines, and caffeine drain through the sewage system largely unaltered into rivers and streams, and even get into the drinking water supply in very small amounts. In order to monitor the trace pharmaceuticals in surface and ground water, an effective sample preparation and analysis method is required.

In 1999, the U.S. Geological Survey National Water Quality Laboratory (NWQL) developed and implemented an Oasis HLB, solid-phase extraction (SPE), and a high-performance liquid chromatography (HPLC)-mass spectrometry (MS) method to analyze pharmaceuticals.

Using the Multiple Reaction Monitoring (MRM) technique, any interference and matrix signal from organic matters in the water can be minimized from the target compound signals for better confirmation and quantitation. In this application note, SPE and LC/MS/MS methods are described to analyze 19 pharmaceuticals in positive ion mode and 11 pharmaceuticals in negative ion mode.

Experimental

Sample Preparation Procedure

See Reference 1 for more information.

1. Filter water samples in the field or laboratory using 0.7-µm glass fiber filters.
2. Pump 1 L of the filtered water sample, at a flow rate of 10 mL/min, through an Oasis HLB (SPE) cartridge containing 0.5 g of sorbent.
3. Elute the HLB column with 6 mL of methanol followed by 4 mL of 0.1% trifluoroacetic acid (TFA) in methanol.

Instrumentation

Positive Ion Mode

LC:	1200 LC
Column:	ZORBAX Extend-C-18, RRHT, 2.1 mm × 100 mm, 1.8 µm
Column temperature:	40 °C
Mobile phases:	A: 0.1% formic acid in water, add NH_4OH buffer to pH 5.5 B: Acetonitrile (ACN)
Flow rate:	0.3 mL/min

Gradient:

Time	%B
0	0
15	100
20	100
21.5	0

Injection volume:	1.0 µL
MS:	G6410A QQQ
Ionization:	ESI–(+)
Mass range:	125 to 800 amu
Scan time:	300 ms
Capillary:	3500 V
Nebulizer P:	40 psi
Drying gas:	9 L/min
Gas temperature:	350 °C
Skimmer:	35 V

Negative Ion Mode

LC:	1200 LC
Column:	ZORBAX Extend-C-18, RRHT, 2.1 mm × 100 mm, 1.8 µm
Column temperature:	60 °C
Mobile phases:	A: 0.04% Glacial acetic acid in water B: Acetonitrile (ACN)
Flow rate:	0.3 mL/min

Gradient:

Time	%B
0	0
1	40
2	52
3	70
6	100
13	100
14	0

Injection volume:	1.0 µL
MS:	G6410A QQQ
Ionization:	ESI (–)
Mass range:	120–800 amu
Scan time:	300 ms
Capillary:	3500 V
Nebulizer P:	40 psi
Drying gas:	9 L/min
Gas temperature:	200 °C
Skimmer:	35 V

The MRM parameters for positive ion mode and negative ion mode are listed in Tables 1 and 2, respectively.

Table 1. Positive Ion Mode MRM Method Parameters

Name	RT	MW	Precursor	Quant ion	Collision V	Dwell	Segment
Metformin HCl	0.856	129	130.4	71.5	15	300	1
Acetaminophen	4.591	151	152.3	110.3	18	30	2
Salbutamol	4.717	239	240.4	148.4	15	30	2
Cimetidine	4.815	252	253.4	94.9	17	30	2
1,7,-Dimethylxanthine	4.89	180	181.3	123.9	20	30	2
Cotinine	5.24	176	177.3	118.3	29	30	2
Codeine	5.321	299	300.4	164.9	30	30	2
Caffeine	5.493	194	195.3	137.9	22	30	2
Trimethoprim	5.935	290	291.4	122.8	25	30	2
Thiabendazole	7.194	201	202.3	131.3	35	100	3
Sulfamethoxazole	7.309	253	254.3	156.0	15	100	3
Azithromycin	7.326	749	375.5	157.9	16	100	3
Diphenhydramine	8.446	255	256.5	167.1	5	100	4
Diltiazem HCl	8.693	414	415.4	177.6	18	100	4
Carbamazepine	8.912	236	237.4	194.0	20	100	4
Fluoxetine HCl	9.71	309	310.4	148.5	0	100	5
Dehydronifedipine	10.635	344	345.4	283.9	27	100	5
Warfarin	11.152	308	309.4	163.3	15	100	5
Miconazole nitrate salt	12.865	416	417.2	159.3	30	300	6

Table 2. Negative Ion Mode MRM Method Parameters

Name	RT	MW	Precursor	Quant ion	Frag. V	Collision V	Dwell	Segment
Hydrochlorothiazide	3.42	297	296	269	140	20	70	1
Aspirin	3.49	180	179	122	120	15	70	1
Enalaprilat	3.71	348	347	114	120	10	70	1
Furosemide	4.51	330	329	285	140	15	70	1
Ketoprofen	5.17	254	253	209	80	5	70	2
Clofibric acid	5.20	214	213	127	80	10	70	2
Napoxen	5.20	230	229	170	80	10	70	2
Diclofenac sodium salt	5.84	294	294	250	100	10	100	3
Ibuprofen	6.03	206	205	161	80	0	100	3
Ibuprofen-d3	6.03	209	208	164	80	0	100	3
Gemfibrozil	6.49	250	249	121	120	25	150	4
Triclocarban	6.66	314	313	160	140	15	150	4

Results and Discussion

The total ion chromatogram (TIC) in negative ion mode is shown in Figure 1. The analysis time in negative ion mode is less than 7 minutes for the 11 analytes. Their peak widths are about 0.1 minute, using a 1.8-µm particle size column. The narrower peak width gives a higher signal-to-noise (s/n) ratio compared to a 3.5-µm or larger particle size column.

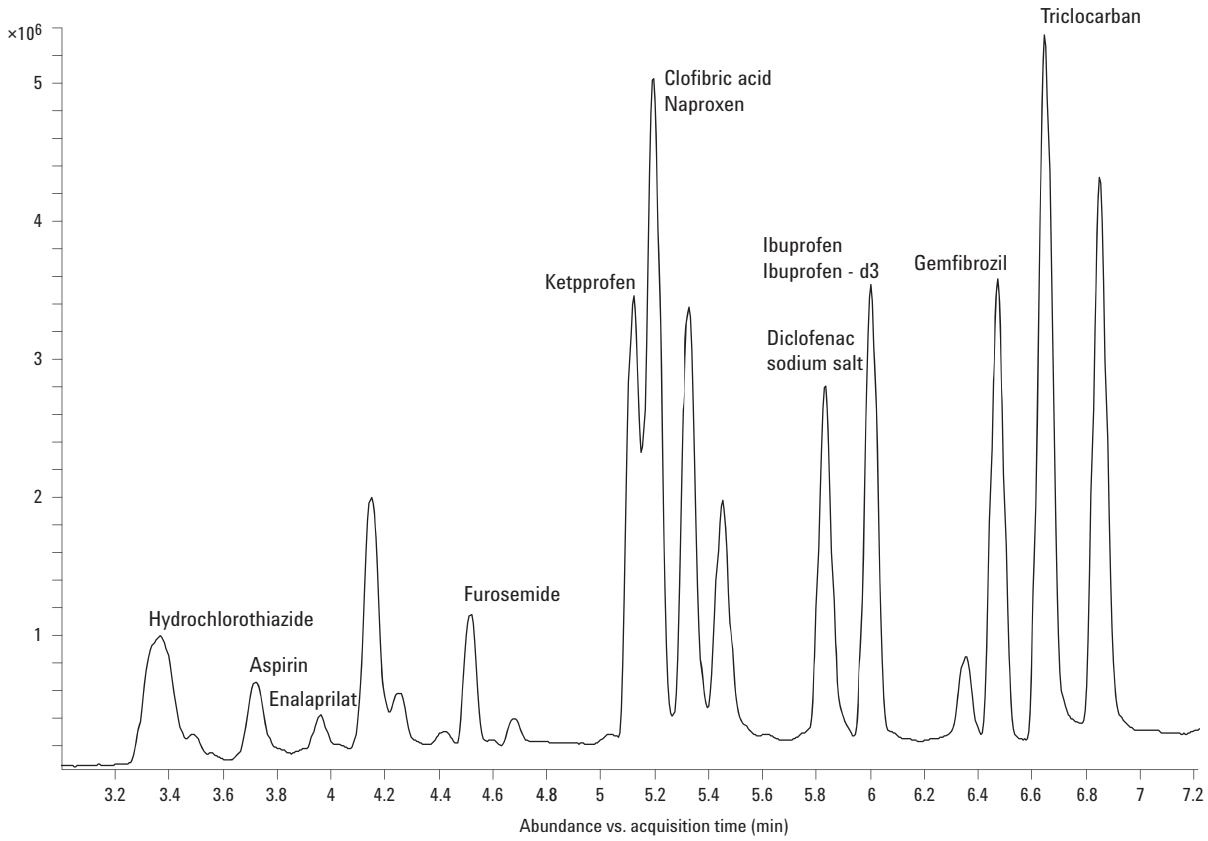

Figure 1. Negative ion mode TIC of 11 pharmaceuticals.

A few compounds, for example, ketoprofen (Figure 2), are sensitive to heat from the drying gas. Higher drying gas temperature (350 °C) lowers the intensity of the precursor ions. Therefore, in the negative ion mode, the drying gas temperature was set to 200 °C.

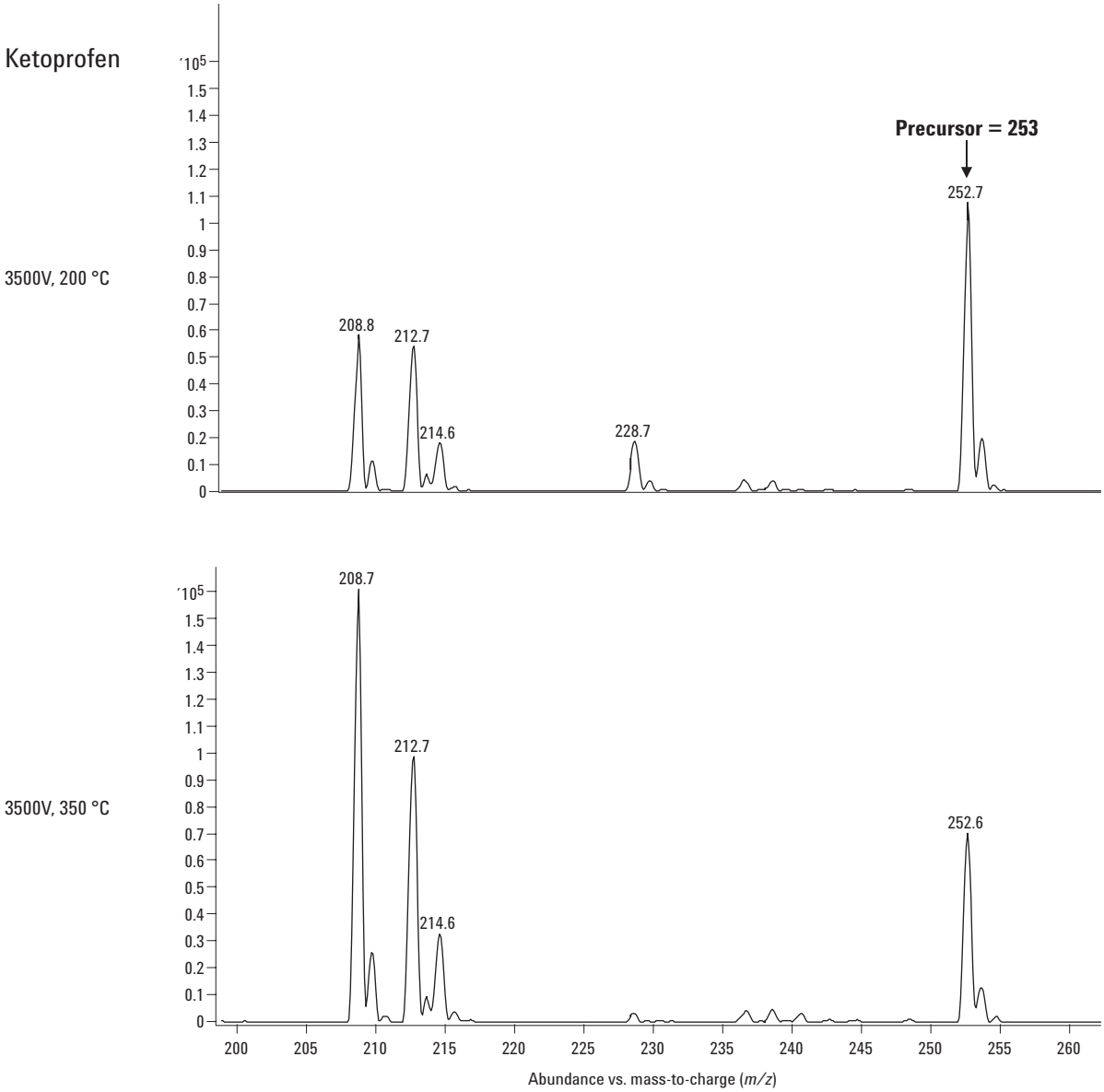

Figure 2. Higher drying gas temperature lowers precursor intensity for certain compounds.

In Figure 3, it was interesting to see that the fragment ion actually had a higher *m/z* value than the precursor ion. For azithromycin, the doubly charged ion showed higher intensity than the singly charged ion and was chosen as the precursor. Therefore, depending on the precursor chosen, it is sometimes necessary to set the upper mass of the product ion scan to be higher than the precursor ion.

Figure 4 shows the overlaid chromatograms of 19 pharmaceuticals, each at 5 pg on column, from the positive ion mode MRM analysis.

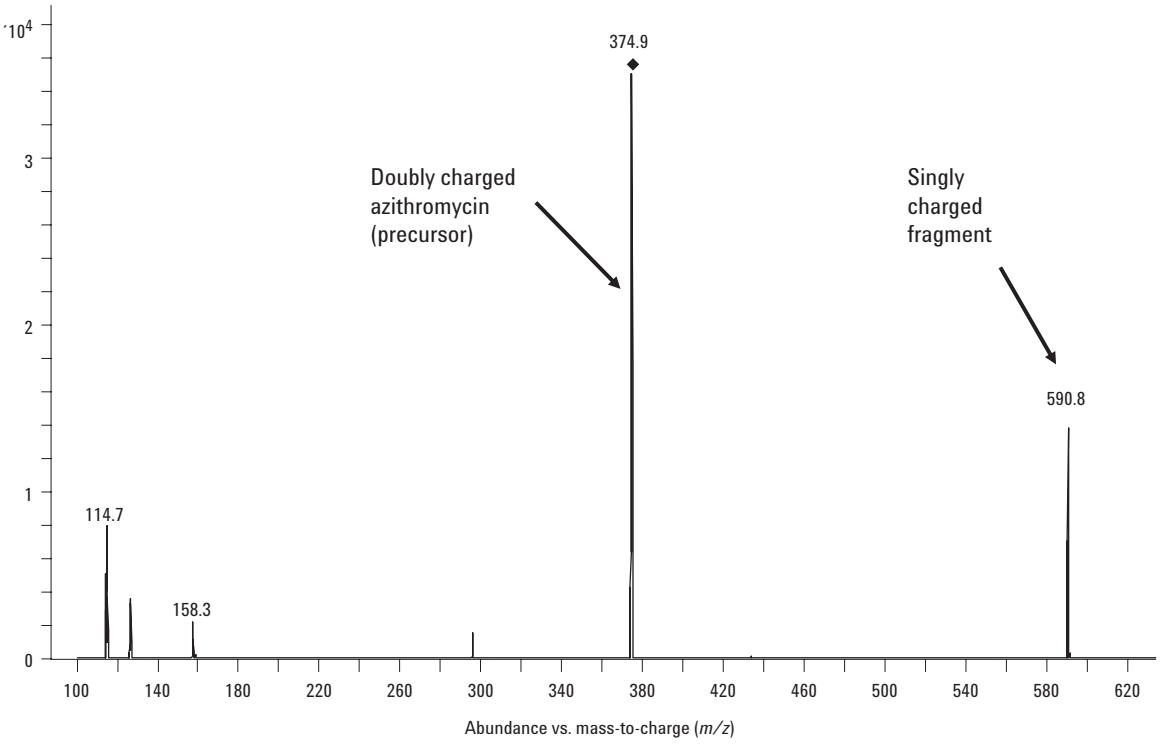

Figure 3. Doubly charged precursor results in a fragment at higher m/z.

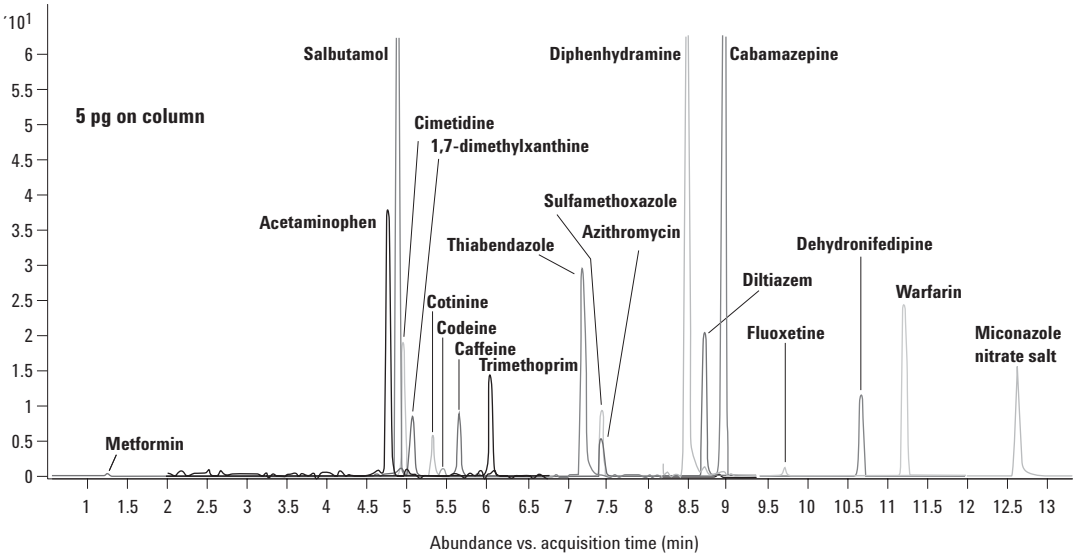

Figure 4. Overlaid MRM chromatograms of the 19 pharmaceuticals in positive ion mode.

Figure 5 shows the overlaid chromatograms of 10 pharmaceuticals, each at 10 pg on column, from the negative ion mode MRM analysis. In both Figures 4 and 5, the analysis times were relatively short and s/n ratios were high.

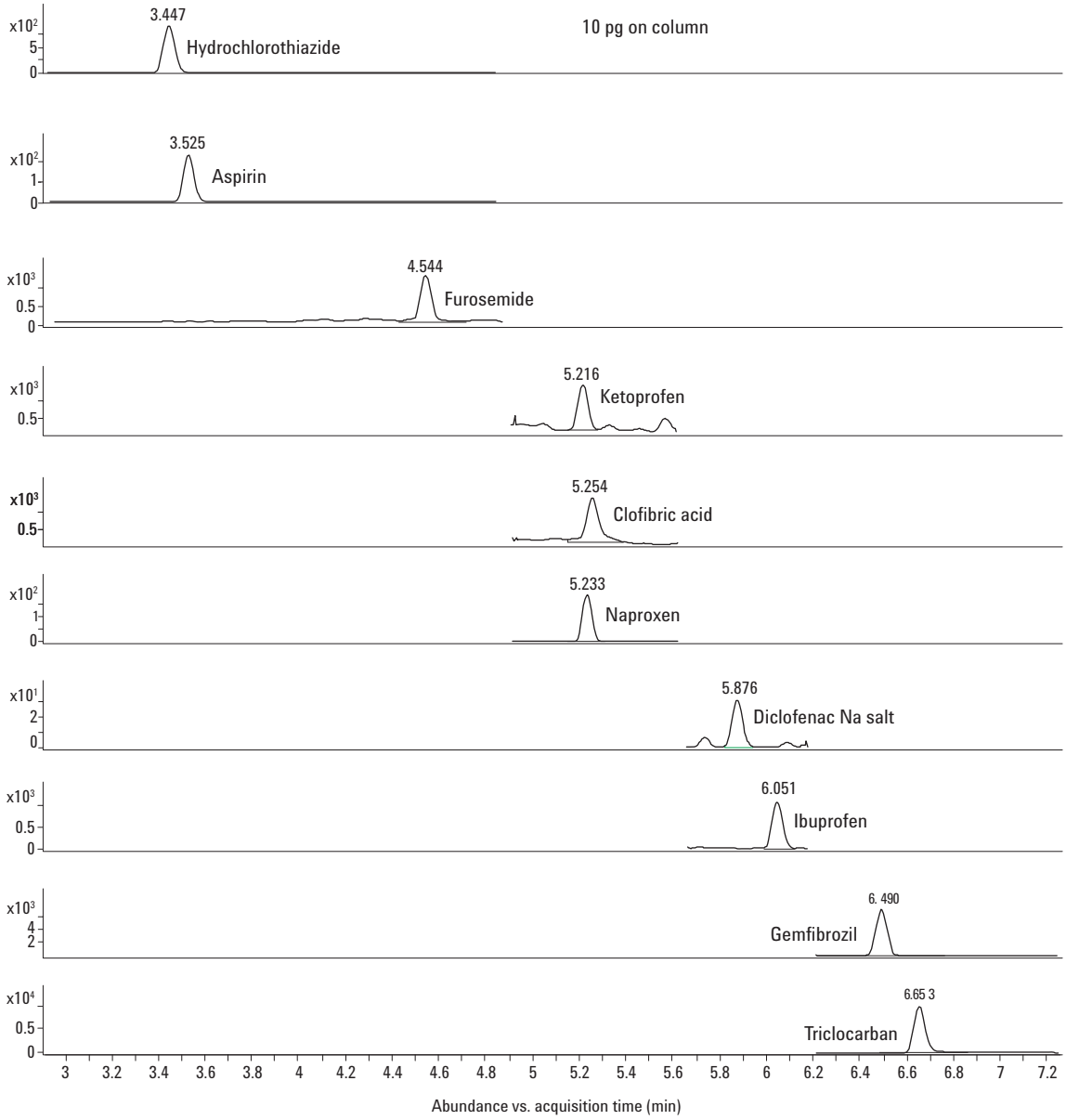

Figure 5. Overlay of MRM results from the 10 pharmaceuticals in negative ion mode.

Table 3 shows the linearity results of all 19 pharmaceuticals (ESI+) over the range of 1, 5, 10, 20, 40, 100, 200, 400, and 1,000 pg on column. Two calibration models were used: a linear model and a quadratic model that both included origin with no weighting. Some of the compounds showed significant fitting improvement from the linear model to the quadratic model. This is the nature of these compounds.

Table 3. Linearity: 1, 5, 10, 20, 40, 100, 200, 400, and 1,000 pg on Column (ESI+), Origin Included, No Weighting

Compound	R^2 (linear fit)	R^2 (quadratic fit)
Metformin HCl	0.9975	0.9999
1,7,-Dimethylxanthine	0.9998	0.9998
Acetaminophen	0.9852	0.9999
Caffeine	0.9992	0.9997
Cimetidine	0.9968	0.9998
Codeine	0.9989	0.9997
Cotinine	0.9971	0.9998
Salbutamol	0.9850	0.9994
Trimethoprim	0.9980	0.9999
Azithromycin	0.9633	0.9998
Sulfamethoxazole	0.9998	0.9999
Thiabendazole	0.9997	0.9998
Carbamazepine	0.9926	0.9999
Diltiazem HCl	0.9997	0.9997
Diphenhydramine	0.9975	0.9998
Dehydronifedipine	0.9985	0.9993
Fluoxetine HCl	0.9984	0.9997
Warfarin	0.9989	0.9997
Miconazole nitrate salt	0.9989	0.9995

Table 4 shows the repeatability results from six injections of 5 pg of each analyte on column. In general, the RSDs are below 15%, except for fluoxetine, which was at 23%.

Table 4. Repeatability from Six Injections at 5 pg/µL (5 pg on column), ESI(+)

Compound	%RSD
Metformin HCl	12.4
1,7,-Dimethylxanthine	8.6
Acetaminophen	6.1
Caffeine	5.7
Cimetidine	4.1
Codeine	16.2
Cotinine	10.5
Salbutamol	3.7
Trimethoprim	3.6
Azithromycin	9.4
Sulfamethoxazole	10.7
Thiabendazole	5.3
Carbamazepine	2.8
Diltiazem HCl	4.7
Diphenhydramine	3.7
Dehydronifedipine	5.4
Fluoxetine HCl	23.4
Warfarin	4.4
Miconazole nitrate salt	2.9

Table 5 shows the linearity results of all 11 pharmaceuticals (ESI–) over the range of 10, 20, 40, 80, 400, and 800 pg on column. All the R^2 values were above 0.99, except triclocarban, which was about 0.97.

Table 5. Linearity: 10, 20, 40, 80, 400, and 800 pg on Column (ESI–), Origin Included, No Weighting

Compound	R^2 (linear fit)
Hydrochlorothiazide	0.9999
Aspirin	0.9977
Enalaprilat	0.9981
Furosemide	0.9997
Ketoprofen	0.9988
Clofibric acid	0.9997
Naproxen	0.9994
Diclofenac Na salt	0.9993
Ibuprofen	0.9997
Ibuprofen-d3	0.9998
Gemfibrozil	0.9993
Triclocarban	0.9655

Once the method is established, one can screen and quantitate target analytes in water. Figures 6, 7, and 8 are MRM analyses of actual water sample extracts in positive and negative ion modes.

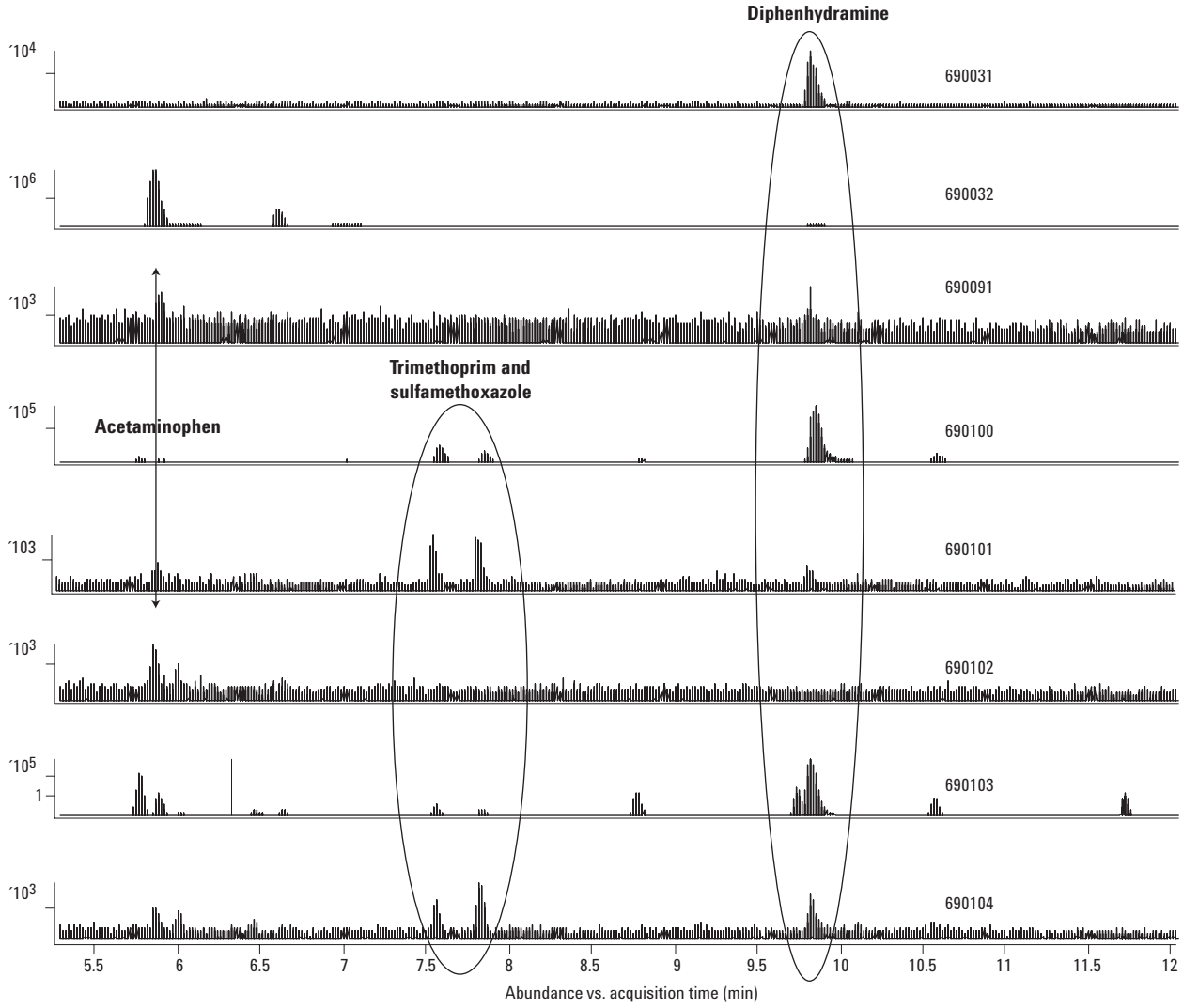

Figure 6. Pharmaceuticals screening in positive ion mode.

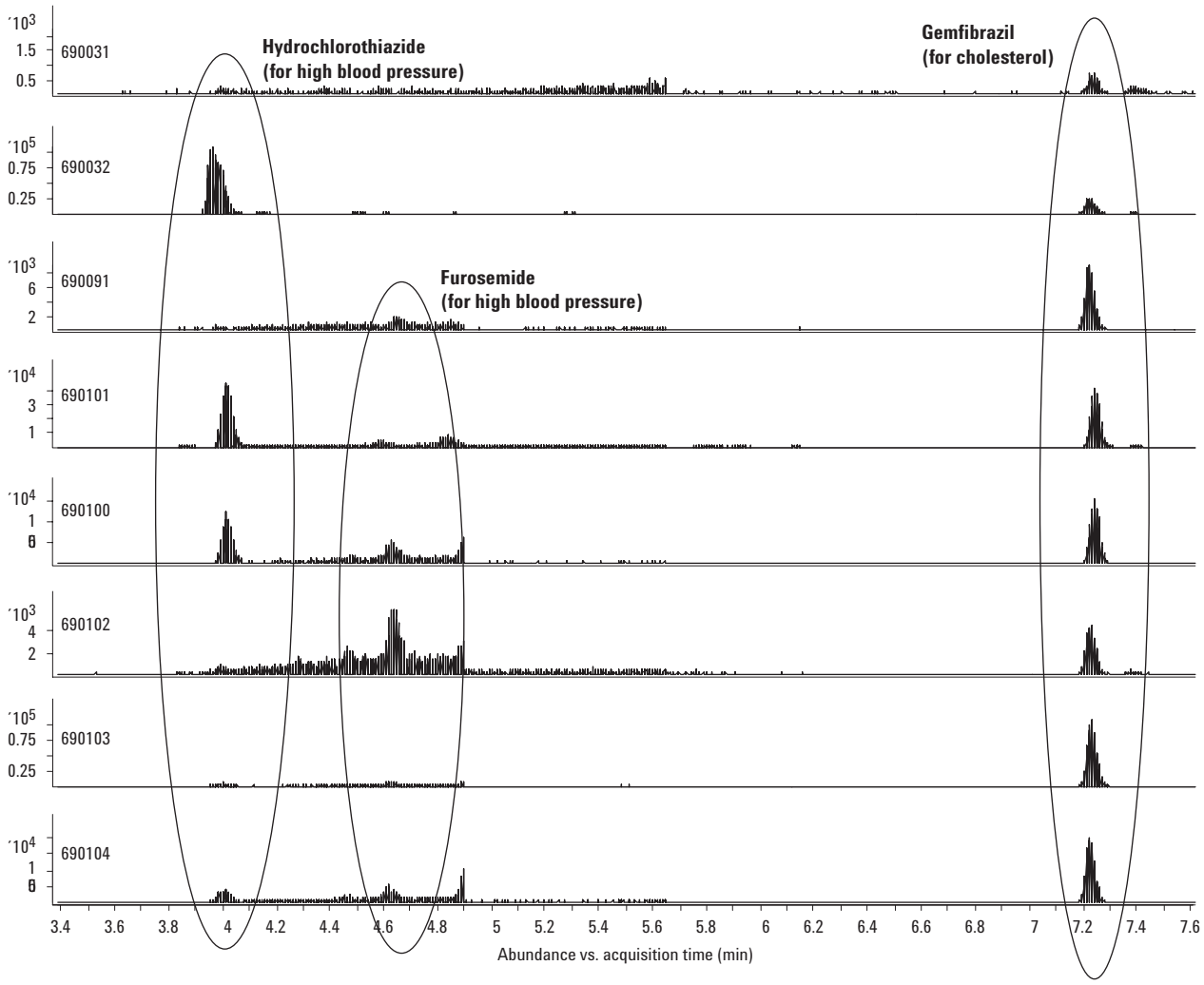

Figure 7. Pharmaceuticals screening in negative ion mode.

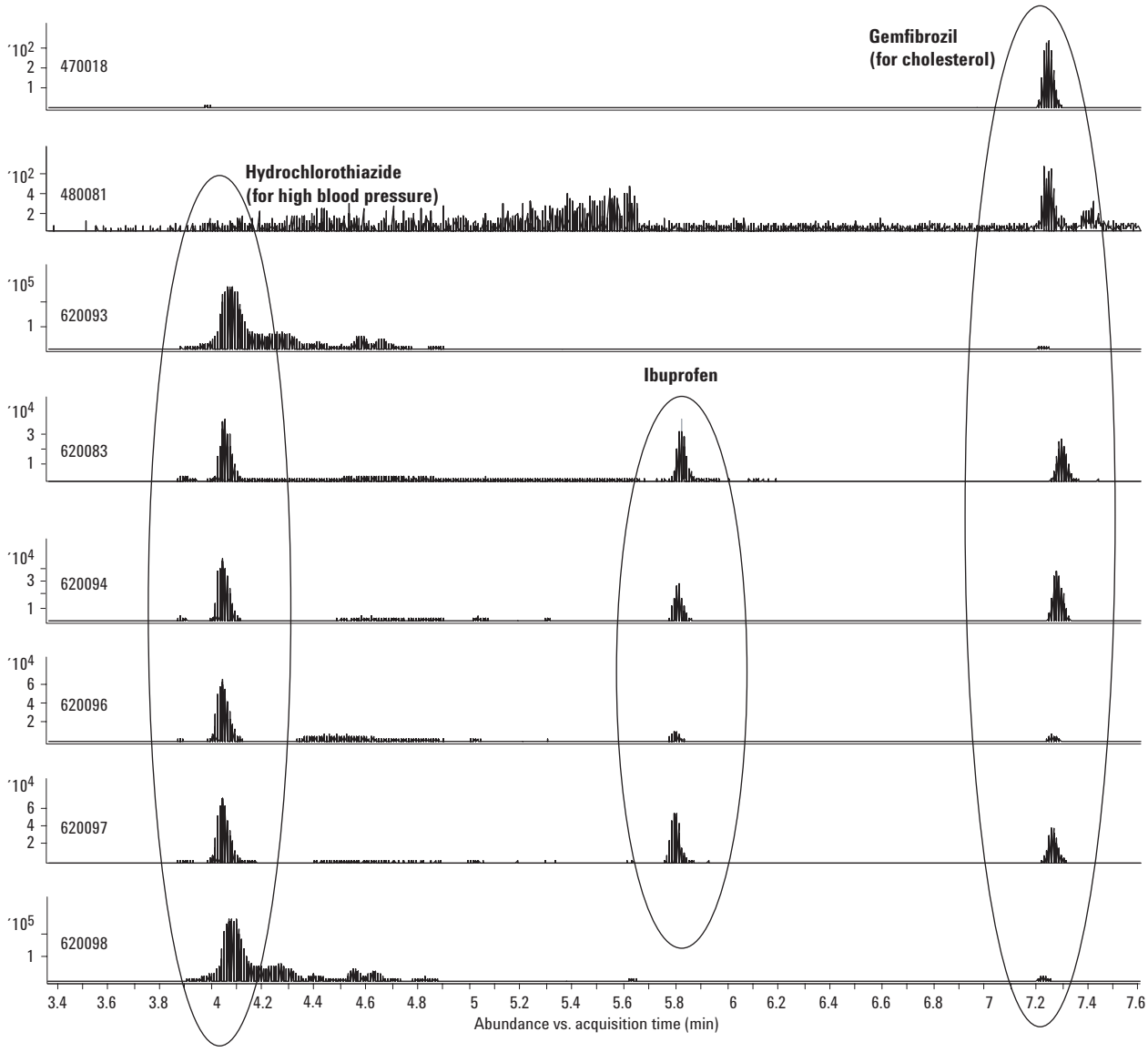

Figure 8. Pharmaceuticals screening in negative ion mode.

Figure 6 shows several of the pharmaceuticals, for example, diphenhydramine and acetaminophen, that were common to several of the water samples. Some of the antibiotics were also found in the samples. Interestingly enough, in Figures 7 and 8, the most common pharmaceuticals in the water samples were related to high blood pressure and cholesterol medications.

Conclusions

Using SPE and LC/MS/MS, 19 pharmaceuticals in positive ion mode and 11 pharmaceuticals in negative ion mode were analyzed at low picogram level on column without any derivatization. Good linearity was observed for analytes from 1 pg to 1 ng on column.

Repeatability study from six injections of target analytes at 5 pg on column showed RSDs below 15%, except for fluoxetine at 23%.

This method was applied to water sample extracts, finding that several target pharmaceutical drugs were commonly found among the analyzed samples.

Reference

1. USGS SOP: Instrumental Analysis for Determination of Human Health Pharmaceuticals in Water by Chemically Modified Styrene-Divinylbenzene Resin-Based Solid-Phase Extraction and High-Performance Liguid Chromatography/Mass Spectrometry, by Steve Werner, 2006.

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