

Many hundreds of active compounds are used in both human and veterinary drug formulations. Due to the many different applications related to pharmaceuticals, their residues can reach the environment in multiple ways including excretion and manufacturing discharge. These compounds are not completely eliminated via sewage treatment plants, thus they can reach surface and groundwater supplies. Recently, there has been increased interest in monitoring for the presence of pharmaceuticals in drinking water supplies and examining their long term effects on human health.

## HPLC CONDITIONS

Instrument: Waters 2690 HPLC or Waters 2795 HPLC, Quattro Ultima<sup>®</sup> MS/MS

LC Column: Waters XTerra<sup>®</sup> C<sub>18</sub>, 3.5 µm, 10.0 cm, 2.1 mm

Ionization: Electrospray Positive (ES+)

Acquisition: MRM mode, unit resolution

Injection Volume: 15 µL

LC Gradient Program		LC Flow Rate (mL/min)	Gradient	General LC Conditions	
Time (min)	Flow Mixture <sup>1</sup>			Column Temperature	40 °C
0.0	95% Solvent A 5% Solvent B	0.150	1	Flow Rate	0.15 – 0.30 mL/min
4.0	95% Solvent A 5% Solvent B	0.250	6	Max Pressure	345 Bar
22.5	12% Solvent A 88% Solvent B	0.300	6	Autosampler Tray Temperature	4 °C
23.0	100% Solvent B	0.300	6	<b>MS Conditions</b>	
26.0	100% Solvent B	0.300	6	Source Temperature	140 °C
26.5	95% Solvent A 5% Solvent B	0.150	6	Desolvation Temperature	350 °C
33.0	95% Solvent A 5% Solvent B	0.150	6	Cone/Desolvation Gas Rate	80 L/hr /400 L/hr

<sup>1</sup> Solvent A = 0.3% Formic Acid and 0.1% Ammonium Formate in HPLC water

Solvent B = 1:1 Acetonitrile:Methanol

Group 1 – Acidic extraction, positive electrospray ionization (ESI+) instrument conditions.

Continue...

Analyte	RT (min)	Parent-Daughter M/ZS	Quantitation Reference	Detection Limits and Minimum Levels					
				Water (ng/L)		Other (µg/kg)		Extract (ng/ΦL)	
				MDL	ML	MDL	ML	MDL	ML
<b>Group 1</b>	<b>Analytes Extracted Under Acidic Conditions and Analyzed Using Positive Electrospray Ionization (ESI+)</b>								
<b>Native Compounds</b>									
Sulfanilamide	2.5	190.0 - 155.8	<sup>13</sup> C <sub>6</sub> -Sulfamethazine	8.9	50	48	200	2.2	12.5
Cotinine	2.8	177.0 – 98.0	Cotinine-d <sub>3</sub>	3.4	5	1.1	5	0.9	1.25
Acetaminophen	4.6	152.2 – 110.0	<sup>13</sup> C <sub>2</sub> - <sup>15</sup> N-Acetaminophen	27	200	35	200	6.7	50
Sulfadiazine	6.0	251.2 – 156.1	<sup>13</sup> C <sub>6</sub> -Sulfamethazine	0.4	5	2.7	10	0.1	1.25
1,7-Dimethylxanthine	6.9	181.2 – 124.0	<sup>13</sup> C <sub>3</sub> -Caffeine	120	500	270	1000	30	125
Sulfathiazole	7.7	256.3 – 156.0	<sup>13</sup> C <sub>6</sub> -Sulfamethoxazole	0.5	5	1.9	50	0.1	1.25
Codeine	8.3	300.0 – 152.0	<sup>13</sup> C <sub>3</sub> -Trimethoprim	1.5	10	3.4	10	0.4	2.5
Sulfamerazine	8.7	265.0 – 156.0	<sup>13</sup> C <sub>6</sub> -Sulfamethazine	0.3	2	1.4	5	0.1	0.5
Lincomycin	9.3	407.5 – 126.0	<sup>13</sup> C <sub>3</sub> -Trimethoprim	0.8	10	4.7	10	0.2	2.5
Caffeine	9.3	195.0 – 138.0	<sup>13</sup> C <sub>3</sub> -Caffeine	15	50	5.4	50	3.6	12.5
Sulfamethizole	10.0	271.0 – 156.0	<sup>13</sup> C <sub>6</sub> -Sulfamethoxazole	0.4	2	0.88	5	0.1	0.5
Trimethoprim	10.0	291.0 – 230.0	<sup>13</sup> C <sub>3</sub> -Trimethoprim	1.1	5	3.3	10	0.3	1.25
Thiabendazole	10.0	202.1 – 175.1	Thiabendazole-d <sub>6</sub>	0.7	5	2.1	10	0.2	1.25
Sulfamethazine	10.1	279.0 – 156.0	<sup>13</sup> C <sub>6</sub> -Sulfamethazine	0.6	2	0.83	5	0.2	0.5
Cefotaxime	10.2	456.4 – 396.1	<sup>13</sup> C <sub>3</sub> -Trimethoprim	10	20	18	50	2.5	5
Carbadox	10.5	263.2 – 231.2	<sup>13</sup> C <sub>3</sub> -Trimethoprim	2.3	5	2.1	10	0.6	1.25
Ormetoprim	10.5	275.3 – 259.1	<sup>13</sup> C <sub>3</sub> -Trimethoprim	0.3	2	0.50	2	0.1	0.5
Norfloracin	10.7	320.0 – 302.0	<sup>13</sup> C <sub>3</sub> <sup>15</sup> N-Ciprofloxacin	28	50	15	50	7.0	12.5
Sulfachloropyridazine	10.8	285.0 – 156.0	<sup>13</sup> C <sub>6</sub> -Sulfamethazine	1.2	5	1.9	5	0.3	1.25
Ofloxacin	10.8	362.2 – 318.0	<sup>13</sup> C <sub>3</sub> <sup>15</sup> N-Ciprofloxacin	1.8	5	3.4	10	0.4	1.25
Ciprofloxacin	10.9	332.2 – 314.2	<sup>13</sup> C <sub>3</sub> <sup>15</sup> N-Ciprofloxacin	5.1	20	8.1	20	1.3	5
Clinafloxacin	12.2	366.3 – 348.0	<sup>13</sup> C <sub>3</sub> <sup>15</sup> N-Ciprofloxacin	6.9	20	14	50	1.7	5
Digoxigenin	12.6	391.2 – 355.2	<sup>13</sup> C <sub>3</sub> -Trimethoprim	5.7	20	9.4	20	1.4	5
Oxolinic acid	13.1	261.8 – 243.8	<sup>13</sup> C <sub>3</sub> -Trimethoprim	0.6	2	0.62	2	0.2	0.5
Sulfadimethoxine	13.2	311.0 – 156.0	<sup>13</sup> C <sub>6</sub> -Sulfamethoxazole	0.1	1	0.55	2	0.03	0.25
Diphenhydramine	14.5	256.8 – 168.1	<sup>13</sup> C <sub>3</sub> -Trimethoprim	0.4	2	0.66	2	0.1	0.5
Penicillin G	14.6	367.5 – 160.2	<sup>13</sup> C <sub>3</sub> -Trimethoprim	2.4	10	13	50	0.6	2.5
Azithromycin	14.8	749.9 – 591.6	<sup>13</sup> C <sub>3</sub> -Trimethoprim	1.3	5	1.6	5	0.3	1.25
Flumequine	15.2	262.0 – 173.7	<sup>13</sup> C <sub>3</sub> -Trimethoprim	2.7	5	1.4	5	0.7	1.25
Ampicillin	15.3	350.3 – 160.2	<sup>13</sup> C <sub>3</sub> -Trimethoprim	-	5	-	5	-	1.25
Diltiazem	15.3	415.5 – 178.0	<sup>13</sup> C <sub>3</sub> -Trimethoprim	0.6	2	0.30	2	0.2	0.25
Carbamazepine	15.3	237.4 – 194.2	<sup>13</sup> C <sub>3</sub> -Trimethoprim	1.4	5	1.6	5	0.4	1.25
Penicillin V	15.4	383.4 – 160.2	<sup>13</sup> C <sub>3</sub> -Trimethoprim	4.4	20	19	50	1.1	5
Erythromycin	15.9	734.4 – 158.0	<sup>13</sup> C <sub>2</sub> -Erythromycin	-	1	-	2	-	0.25
Tylosin	16.3	916.0 – 772.0	<sup>13</sup> C <sub>2</sub> -Erythromycin anhydrate	13	50	8.1	50	3.2	5
Oxacillin	16.4	434.3 – 160.1	<sup>13</sup> C <sub>3</sub> -Trimethoprim	3.3	10	9.4	20	0.8	2.5
Dehydronifedipine	16.5	345.5 – 284.1	<sup>13</sup> C <sub>3</sub> -Trimethoprim	0.6	2	0.41	2	0.2	0.5

Analyte	RT (min)	Parent-Daughter M/ZS	Quantitation Reference	Detection Limits and Minimum Levels					
				Water (ng/L)		Other (µg/kg)		Extract (ng/ΦL)	
				MDL	ML	MDL	ML	MDL	ML
<b>Group 1</b>	<b>Analytes Extracted Under Acidic Conditions and Analyzed Using Positive Electrospray Ionization (ESI+)</b>								
<b>Native Compounds</b>									
Clarithromycin	17.5	748.9 – 158.2	<sup>13</sup> C <sub>2</sub> -Erythromycin anhydrate	1.0	5	1.2	5	0.3	1.25
<b>Labeled compounds spiked into each sample</b>									
Cotinine-d <sub>3</sub>	2.8	180.0 – 79.9	<sup>13</sup> C <sub>3</sub> Atrazine						
<sup>13</sup> C <sub>2</sub> - <sup>15</sup> N-Acetaminophen	4.5	155.2 – 111.0	<sup>13</sup> C <sub>3</sub> Atrazine						
<sup>13</sup> C <sub>3</sub> Caffeine	9.3	198.0 – 140.0	<sup>13</sup> C <sub>3</sub> Atrazine						
Thiabendazole-d <sub>6</sub>	9.8	208.1 – 180.1	<sup>13</sup> C <sub>3</sub> Atrazine						
<sup>13</sup> C <sub>3</sub> -Trimethoprim	10.0	294.0 – 233.0	<sup>13</sup> C <sub>3</sub> Atrazine						
<sup>13</sup> C <sub>6</sub> Sulfamethazine	10.1	285.1 – 162.0	<sup>13</sup> C <sub>3</sub> Atrazine						
<sup>13</sup> C <sub>3</sub> <sup>15</sup> N-Ciprofloxacin	10.9	336.1 – 318.0	<sup>13</sup> C <sub>3</sub> Atrazine						
<sup>13</sup> C <sub>6</sub> -Sulfamethoxazole	11.2	260.0 – 162.0	<sup>13</sup> C <sub>3</sub> Atrazine						
<sup>13</sup> C <sub>2</sub> -Erythromycin	15.9	736.4 – 160.0	<sup>13</sup> C <sub>3</sub> Atrazine						
Fluoxetine-d <sub>5</sub>	16.8	315.3 – 153.0	<sup>13</sup> C <sub>3</sub> Atrazine						
<sup>13</sup> C <sub>2</sub> -Erythromycin anhydrate	17.7	718.4 – 160.0	<sup>13</sup> C <sub>3</sub> Atrazine						
<b>Injection internal standard</b>									
<sup>13</sup> C <sub>3</sub> Atrazine	15.9	219.5 – 176.9 (134.0)	External standard						

Group 1 acidic extraction, positive electrospray ionization (ESI+) compound retention times (RTs), parent-daughter transitions, quantitation references, method detection limits, and minimum levels of quantitation.

Continue...

## HPLC CONDITIONS

Instrument: Waters 2690 HPLC or Waters 2795 HPLC, Quattro Ultima MS/MS

LC Column: Waters XTerra C<sub>18</sub>, 3.5 µm 10.0 cm, 2.1 mm

Ionization: Electrospray Positive (ES+)

Acquisition: MRM mode, unit resolution

Injection Volume: 5 µL

LC Gradient Program		LC Flow Rate (mL/min)	Gradient	General LC Conditions	
Time (min)	Flow Mixture <sup>1</sup>			Column Temperature	40 °C
0.0	10% Solvent A 90% Solvent B	0.20	1	Flow Rate	0.20 – 0.23 mL/min
1.0	10% Solvent A 90% Solvent B	0.20	6	Max Pressure	345 Bar
18.0	40% Solvent A 60% Solvent B	0.23	6	Autosampler Tray Temperature	4 °C
20.0	90% Solvent A 10% Solvent B	0.23	6	MS Conditions	
24.0	90% Solvent A 10% Solvent B	0.23	6	Source Temperature	120 °C
24.3	10% Solvent A 90% Solvent B	0.20	6	Desolvation Temperature	400 °C
28	10% Solvent A 90% Solvent B	0.20	6	Cone / Desolvation Gas Rate	70 L/hr /450 L/hr

<sup>1</sup> Solvent A = 1:1 acetonitrile:methanol, with 5 mM Oxalic AcidSolvent B = HPLC H<sub>2</sub>O, with 5 mM Oxalic Acid.

Group 2 – Acidic extraction positive electrospray ionization (ESI+) instrument conditions.

Analyte	RT (min)	Parent-Daughter M/ZS	Quantitation Reference	Detection Limits and Minimum Levels					
				Water (ng/L)		Other (ng/g)		Extract (ng/μL)	
				MDL	ML	MDL	ML	MDL	ML
<b>Group 2</b>	<b>Analytes Extracted Under Acidic Conditions and Analyzed Using Positive Electrospray Ionization (ESI+)</b>								
<b>Native Compounds</b>									
Minocycline	5.1	458.0 – 441.0	Thiabendazole-d <sub>6</sub>	51	200	-	200	13	50
Epitetracycline	8.1	445.2 – 410.2	Thiabendazole-d <sub>6</sub>	3.6	20	8.6	20	0.9	5
Epioxytetracycline (EOTC)	8.6	461.2 – 426.2	Thiabendazole-d <sub>6</sub>	4.1	20	18	50	1.0	5
Oxytetracycline (OTC)	9.4	461.2 – 426.2	Thiabendazole-d <sub>6</sub>	2.1	20	2.2	20	0.5	5
Tetracycline (TC)	9.9	445.2 – 410.2	Thiabendazole-d <sub>6</sub>	1.9	20	2.8	20	0.5	5
Demeclocycline	11.7	465.0 – 430.0	Thiabendazole-d <sub>6</sub>	6.6	50	7.9	50	1.7	12.5
Isochlortetracycline (ICTC) 1	11.9	479.0 – 462.2	Thiabendazole-d <sub>6</sub>	1.7	20	3.5	20	0.4	5
Epichlortetracycline (ECTC) 1	12.0	479.0 – 444.0	Thiabendazole-d <sub>6</sub>	7.7	50	26	100	1.9	12.5
Chlortetracycline (CTC)	14.1	479.0 – 444.0	Thiabendazole-d <sub>6</sub>	1.2	20	2.3	20	0.3	5
Doxycycline	16.7	445.2 – 428.2	Thiabendazole-d <sub>6</sub>	2.8	20	2.3	20	0.7	5
Epianhydrotetracycline (EATC)	17.0	426.8 – 409.8	Thiabendazole-d <sub>6</sub>	7.7	50	14	50	1.9	12.5
Anhydrotetracycline (ATC)	18.8	426.8 – 409.8	Thiabendazole-d <sub>6</sub>	4.6	50	7.1	50	1.2	12.5
Epianhydrochlortetracycline (EACTC)	20.7	461.2 – 444.0	Thiabendazole-d <sub>6</sub>	28	200	23	200	7.0	50
Anhydrochlortetracycline (ACTC)	22.1	461.2 – 444.0	Thiabendazole-d <sub>6</sub>	5.2	50	11	50	1.3	12.5
<b>Labeled compound spiked into each sample</b>									
Thiabendazole-d <sub>6</sub>	7.0	208.1 – 180.1	<sup>13</sup> C <sub>3</sub> Atrazine						
<b>Injection internal standard</b>									
<sup>13</sup> C <sub>3</sub> Atrazine	10.5	219.5 – 176.9 (134.0)	External standard						

<sup>1</sup> Isochlortetracycline (ICTC) is reported as the sum ICTC + ECTC due to a common transition ion.

Group 2 acidic extraction positive electrospray ionization (ESI+) compound retention times (RTs), parent-daughter transitions, quantitation references, method detection limits, and minimum levels of quantitation.

Continue...

## HPLC CONDITIONS

Instrument: Waters 2690 HPLC or Waters 2795 HPLC, Quattro Ultima MS/MS

LC Column: Waters XTerra C<sub>18</sub>, 3.5 µm, 10.0 cm, 2.1 mm

Ionization: Negative Ion Electrospray

Acquisition: MRM mode, unit resolution

Injection Volume: 5 µL

LC Gradient Program		LC Flow Rate (mL/min)	Gradient	General LC Conditions	
Time (min)	Flow Mixture <sup>1</sup>			Column Temperature	40 °C
0.0	60% Solvent A 40% Solvent B	0.2	1	Flow Rate	0.200 mL/min
0.5	60% Solvent A 40% Solvent B	0.2	6	Max Pressure	345 Bar
7.0	100% Solvent B	0.2	6	Autosampler Tray Temperature	4 °C
12.5	100% Solvent B	0.2	6	MS Conditions	
12.7	60% Solvent A 40% Solvent B	0.2	6	Source Temperature	100 °C
16.0	60% Solvent A 40% Solvent B	0.2	1	Desolvation Temperature	350 °C
				Cone/Desolvation Gas Rate	50L/hr /300 L/hr

<sup>1</sup> Solvent A = 0.1% Ammonium Acetate and 0.1% Acetic Acid in HPLC water

Solvent B = 1:1 Methanol:Acetonitrile

Group 3 Acidic extraction negative electrospray ionization (ESI-) instrument conditions.

# PHARMACEUTICALS AND PERSONAL CARE PRODUCTS

EPA METHOD

Pharmaceuticals and Personal Care Products in Water, Soil, Sediment and Biosolids by HPLC/MS/MS

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Analyte	RT (min)	Parent-Daughter M/ZS	Quantitation Reference	Detection Limits and Minimum Levels					
				Water (ng/L)		Other (Φg/g)		Extract (ng/ΦL)	
				MDL	ML	MDL	ML	MDL	ML
<b>Group 3</b>	<b>Analytes Extracted Under Acidic Conditions and Analyzed Using Positive Electrospray Ionization (ESI+)</b>								
<b>Native Compounds</b>									
Naproxen	6.7	228.9 – 168.6	<sup>13</sup> C-Naproxen-d <sub>3</sub>	3.9	10	6.1	20	1.0	2.5
Warfarin	7.1	307.0 – 117.0	Warfarin-d <sub>5</sub>	0.9	5	1.6	5	0.2	1.25
Ibuprofen	8.4	205.1 – 161.1	<sup>13</sup> C <sub>3</sub> -Ibuprofen	6.0	50	11	50	1.5	12.5
Gemfibrozil	9.5	249.0 – 121.0	Gemfibrozil-d <sub>6</sub>	0.8	5	1.2	5	0.2	1.25
Triclocarban	9.6	312.9 – 159.7	<sup>13</sup> C <sub>6</sub> -Triclocarban	2.1	10	2.7	10	0.5	2.5
Triclosan	9.7	286.8 – 35.0	<sup>13</sup> C <sub>12</sub> -Triclosan	92	200	56	200	23	50
<b>Labeled compounds spiked into samples</b>									
<sup>13</sup> C-Naproxen-d <sub>3</sub>	6.6	232.9 – 168.6	<sup>13</sup> C <sub>6</sub> -TCPAA						
Warfarin-d <sub>5</sub>	7.0	312.0 – 161.0	<sup>13</sup> C <sub>6</sub> -TCPAA						
<sup>13</sup> C <sub>3</sub> -Ibuprofen	8.5	208.2 – 163.1	<sup>13</sup> C <sub>6</sub> -TCPAA						
Gemfibrozil-d <sub>6</sub>	9.5	255.0 – 121.0	<sup>13</sup> C <sub>6</sub> -TCPAA						
<sup>13</sup> C <sub>6</sub> -Triclocarban	9.6	318.9 – 159.7	<sup>13</sup> C <sub>6</sub> -TCPAA						
<sup>13</sup> C <sub>12</sub> -Triclosan	9.7	298.8 – 35.0	<sup>13</sup> C <sub>6</sub> -TCPAA						
<b>Injection Internal Standard</b>									
<sup>13</sup> C <sub>6</sub> -TCPAA	4.9	258.8 – 200.7	External standard						

Group 3 acidic extraction negative electrospray ionization (ESI-) compound retention times (RTs), parent-daughter transitions, quantitation references, method detection limits, and minimum levels of quantitation.

Continue...

## HPLC CONDITIONS

Instrument: Waters 2690 HPLC or Waters 2795 HPLC, Quattro Ultima MS/MS

LC Column: Waters Atlantis® HILIC, 3.0 µm, 2.1x 100 mm

Ionization: Electrospray Positive (ES+)

Acquisition: MRM mode, unit resolution

Purge Solvent: 100% CH<sub>3</sub>CN (changed from H<sub>2</sub>O)

Injection Volume: 2.0 µL

LC Gradient Program		LC Flow Rate (mL/min)	Gradient	General LC Conditions	
Time (min)	Flow Mixture <sup>1</sup>			Column Temperature	40 °C
0.0	2% Solvent A 8% Solvent B	0.25	1	Flow Rate	0.25 mL/min
5.0	30% Solvent A 70% Solvent B	0.25	6	Max Pressure	345 Bar
12.0	30% Solvent A 70% Solvent B	0.25	6	Autosampler Tray Temperature	4 °C
12.5	2% Solvent A 98% Solvent B	0.25	6	MS Conditions	
16.0	2% Solvent A 98% Solvent B	0.25	6	Source Temperature	120 °C
				Desolvation Temperature	350 °C
				Cone/Desolvation Gas Rate	70L/hr /400 L/hr

<sup>1</sup> Solvent A = 0.1% Acetic Acid/Ammonium Acetate Buffer

Solvent B = Acetonitrile

Group 4 – Basic extraction positive electrospray ionization (ESI+) instrument conditions.

Analyte	RT (min)	Parent-Daughter M/ZS	Quantitation Reference	Detection Limits and Minimum Levels					
				Water (ng/L)		Other (ng/g)		Extract (ng/ΦL)	
				MDL	ML	MDL	ML	MDL	ML
<b>Group 4</b>	<b>Analytes Extracted Under Acidic Conditions and Analyzed Using Positive Electrospray Ionization (+) ESI</b>								
<b>Native Compounds</b>									
Cimetidine	6.9	253.1 – 159.0	Albuterol-d <sub>3</sub>	0.6	2	0.78	2	0.2	0.5
Albuterol	9.4	240.0 – 148.0	Albuterol-d <sub>3</sub>	0.9	2	0.39	2	0.2	0.5
Ranitidine	10.3	315.0 – 175.9	Albuterol-d <sub>3</sub>	0.7	2	1.1	2	0.2	0.5
Metformin	11.0	131.1 – 60.1	Metformin-d <sub>6</sub>	23	100	38	100	5.8	25
<b>Labeled compounds spiked into samples</b>									
Albuterol-d <sub>3</sub>	9.4	243.0 – 151.0	Cotinine-d <sub>3</sub>						
Metformin-d <sub>6</sub>	11.0	285.1 – 163.0	Cotinine-d <sub>3</sub>						
<b>Injection Internal Standard</b>									
Cotinine-d <sub>3</sub>	5.9	180.0 – 79.9	External standard						
<sup>13</sup> C <sub>3</sub> -Atrazine	2.0	219.5 – 176.9 (134.0)	External Standard						

Group 4 basic extraction positive electrospray ionization (ESI+) compound retention times (RTs), parent-daughter transitions, quantitation references, method detection limits, and minimum levels of quantitation.

## ORDERING INFORMATION

Related Parts	Part Number
XTerra MSC18, 3.5 μm, 2.1 x 100 mm	186000404
Atlantis HILIC, 3.0 μm, 2.1 x 100 mm	186002013
Oasis® HLB Extraction Cartridge, 20 cc, 1 g, LP	186000117
EPA Method 1694 Analysis Kit	176001634
LC/MS Certified Vials	see catalog

Related Documents	Literature Code
Total Solutions for Environmental Applications	720002163EN
LC/MS Determination of Pharmaceutical Residues in Environmental Samples	720000421EN

[www.waters.com/library](http://www.waters.com/library)