應用手冊

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

Waters Corporation

Abstract

In this Application, we monitor the presence of pharmaceuticals in drinking water supplies and examining their long term effects on human health.

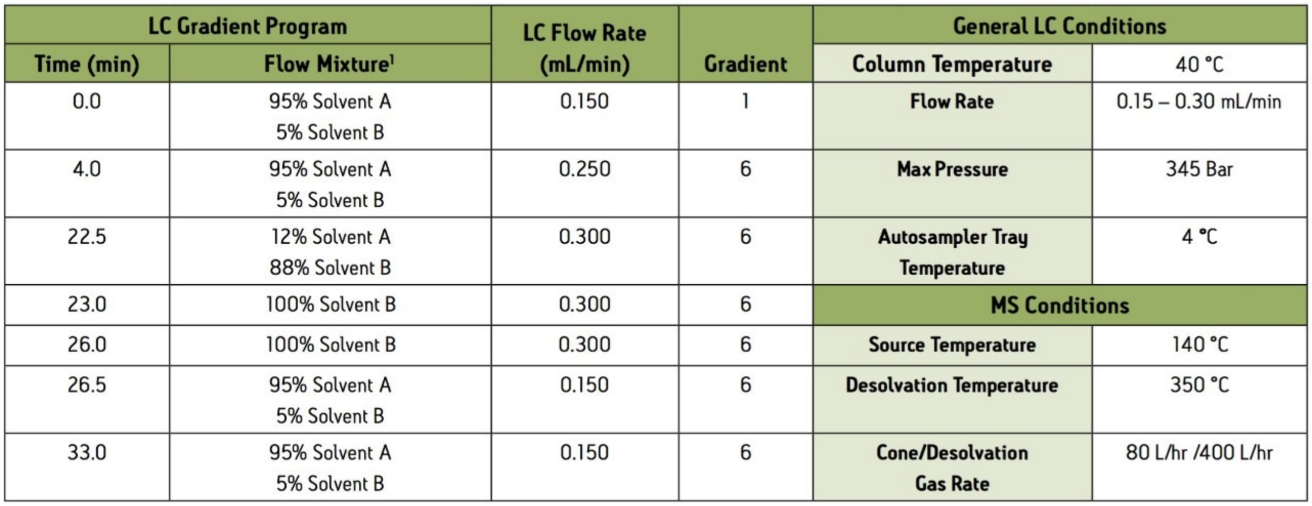
Introduction

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.

Experimental

HPLC conditions

Instrument:	Waters 2690 HPLC or Waters 2795 HPLC, Quattro Ultima MS/MS
LC Column:	Waters XTerra C_{18}, 3.5 µm, 10.0 cm, 2.1 mm
Ionizatoin:	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[1]			**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	**MS Conditions**	
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

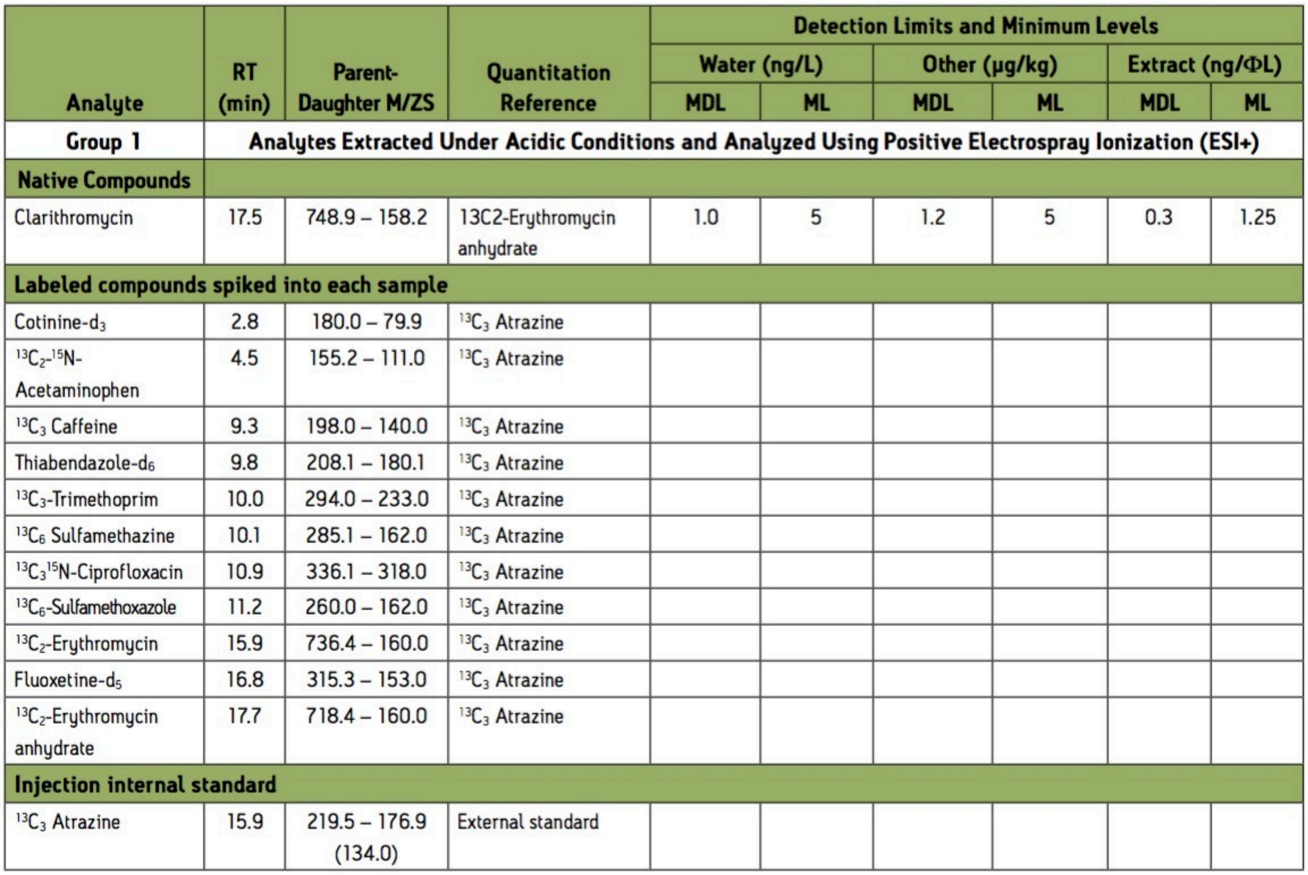
[1] Solvent A = 0.3% Formic Acid and 0.1% Ammonium Formate in HPLC water

Solvent B = 1:1 Acetonitrile:Methanol

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

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
Group 1	**Analytes Extracted Under Acidic Conditions and Analyzed Using Positive Electrospray Ionization (ESI+)**								
Native Compounds									
Sulfanilamide	2.5	190.0 - 155.8	$^{13}C_6$-Sulfamethazine	8.9	50	48	200	2.2	12.5
Cotinine	2.8	177.0 – 98.0	Cotinine-d_3	3.4	5	1.1	5	0.9	1.25
Acetaminophen	4.6	152.2 – 110.0	$^{13}C_2$-^{15}N-Acetaminophen	27	200	35	200	6.7	50
Sulfadiazine	6.0	251.2 – 156.1	$^{13}C_6$-Sulfamethazine	0.4	5	2.7	10	0.1	1.25
1,7-Dimethylxanthine	6.9	181.2 – 124.0	$^{13}C_3$-Caffeine	120	500	270	1000	30	125
Sulfathiazole	7.7	256.3 – 156.0	$^{13}C_6$-Sulfamethoxazole	0.5	5	1.9	50	0.1	1.25
Codeine	8.3	300.0 – 152.0	$^{13}C_3$-Trimethoprim	1.5	10	3.4	10	0.4	2.5
Sulfamerazine	8.7	265.0 – 156.0	$^{13}C_6$-Sulfamethazine	0.3	2	1.4	5	0.1	0.5
Lincomycin	9.3	407.5 – 126.0	$^{13}C_3$-Trimethoprim	0.8	10	4.7	10	0.2	2.5
Caffeine	9.3	195.0 – 138.0	$^{13}C_3$-Caffeine	15	50	5.4	50	3.6	12.5
Sulfamethizole	10.0	271.0 – 156.0	$^{13}C_6$-Sulfamethoxazole	0.4	2	0.88	5	0.1	0.5
Trimethoprim	10.0	291.0 – 230.0	$^{13}C_3$-Trimethoprim	1.1	5	3.3	10	0.3	1.25
Thiabendazole	10.0	202.1 – 175.1	Thiabendazole-d_6	0.7	5	2.1	10	0.2	1.25
Sulfamethazine	10.1	279.0 – 156.0	$^{13}C_6$-Sulfamethazine	0.6	2	0.83	5	0.2	0.5
Cefotaxime	10.2	456.4 – 396.1	$^{13}C_3$-Trimethoprim	10	20	18	50	2.5	5
Carbadox	10.5	263.2 – 231.2	$^{13}C_3$-Trimethoprim	2.3	5	2.1	10	0.6	1.25
Ormetoprim	10.5	275.3 – 259.1	$^{13}C_3$-Trimethoprim	0.3	2	0.50	2	0.1	0.5
Norfloxacin	10.7	320.0 – 302.0	$^{13}C_3$ ^{15}N-Ciprofloxacin	28	50	15	50	7.0	12.5
Sulfachloropyridazine	10.8	285.0 – 156.0	$^{13}C_6$-Sulfamethazine	1.2	5	1.9	5	0.3	1.25
Ofloxacin	10.8	362.2 – 318.0	$^{13}C_3$ ^{15}N-Ciprofloxacin	1.8	5	3.4	10	0.4	1.25
Ciprofloxacin	10.9	332.2 – 314.2	$^{13}C_3$ ^{15}N-Ciprofloxacin	5.1	20	8.1	20	1.3	5
Clinafloxacin	12.2	366.3 – 348.0	$^{13}C_3$ ^{15}N-Ciprofloxacin	6.9	20	14	50	1.7	5
Digoxigenin	12.6	391.2 – 355.2	$^{13}C_3$-Trimethoprim	5.7	20	9.4	20	1.4	5
Oxolinic acid	13.1	261.8 – 243.8	$^{13}C_3$-Trimethoprim	0.6	2	0.62	2	0.2	0.5
Sulfadimethoxine	13.2	311.0 – 156.0	$^{13}C_6$-Sulfamethoxazole	0.1	1	0.55	2	0.03	0.25
Diphenhydramine	14.5	256.8 – 168.1	$^{13}C_3$-Trimethoprim	0.4	2	0.66	2	0.1	0.5
Penicillin G	14.6	367.5 – 160.2	$^{13}C_3$-Trimethoprim	2.4	10	13	50	0.6	2.5
Azithromycin	14.8	749.9 – 591.6	$^{13}C_3$-Trimethoprim	1.3	5	1.6	5	0.3	1.25
Flumeqine	15.2	262.0 – 173.7	$^{13}C_3$-Trimethoprim	2.7	5	1.4	5	0.7	1.25
Ampicillin	15.3	350.3 – 160.2	$^{13}C_3$-Trimethoprim	-	5	-	5	-	1.25
Diltiazem	15.3	415.5 – 178.0	$^{13}C_3$-Trimethoprim	0.6	2	0.30	2	0.2	0.25
Carbamazepine	15.3	237.4 – 194.2	$^{13}C_3$-Trimethoprim	1.4	5	1.6	5	0.4	1.25
Penicillin V	15.4	383.4 – 160.2	$^{13}C_3$-Trimethoprim	4.4	20	19	50	1.1	5
Erythromycin	15.9	734.4 – 158.0	$^{13}C_2$-Erythromycin	-	1	-	2	-	0.25
Tylosin	16.3	916.0 – 772.0	$^{13}C_2$-Erythromycin anhydrate	13	50	8.1	50	3.2	5
Oxacillin	16.4	434.3 – 160.1	$^{13}C_3$-Trimethoprim	3.3	10	9.4	20	0.8	2.5
Dehydronifedipine	16.5	345.5 – 284.1	$^{13}C_3$-Trimethoprim	0.6	2	0.41	2	0.2	0.5

Continuation of above table

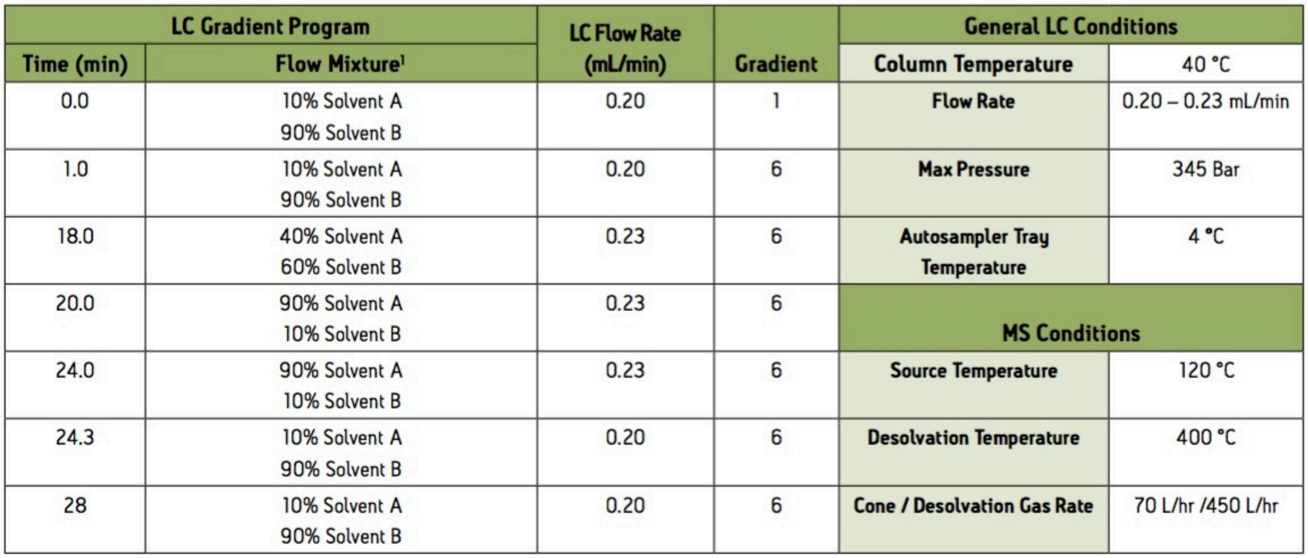


Analyte	RT (min)	Parent-Daughter M/ZS	Quantitation Reference	Detection Limits and Minimum Levels					
				Water (ng/L)		Other (µg/kg)		Extract (ng/ΦL)	
				MDL	ML	MDL	ML	MDL	ML
Group 1	**Analytes Extracted Under Acidic Conditions and Analyzed Using Positive Electrospray Ionization (ESI+)**								
Native Compounds									
Clarithromycin	17.5	748.9 – 158.2	13C2-Erythromycin anhydrate	1.0	5	1.2	5	0.3	1.25
Labeled compounds spiked into each sample									
Cotinine-d$_3$	2.8	180.0 – 79.9	$^{13}C_3$ Atrazine						
$^{13}C_2$-^{15}N-Acetaminophen	4.5	155.2 – 111.0	$^{13}C_3$ Atrazine						
$^{13}C_3$ Caffeine	9.3	198.0 – 140.0	$^{13}C_3$ Atrazine						
Thiabendazole-d$_6$	9.8	208.1 – 180.1	$^{13}C_3$ Atrazine						
$^{13}C_3$-Trimethoprim	10.0	294.0 – 233.0	$^{13}C_3$ Atrazine						
$^{13}C_6$ Sulfamethazine	10.1	285.1 – 162.0	$^{13}C_3$ Atrazine						
$^{13}C_3{}^{15}$N-Ciprofloxacin	10.9	336.1 – 318.0	$^{13}C_3$ Atrazine						
$^{13}C_6$-Sulfamethoxazole	11.2	260.0 – 162.0	$^{13}C_3$ Atrazine						
$^{13}C_2$-Erythromycin	15.9	736.4 – 160.0	$^{13}C_3$ Atrazine						
Fluoxetine-d$_5$	16.8	315.3 – 153.0	$^{13}C_3$ Atrazine						
$^{13}C_2$-Erythromycin anhydrate	17.7	718.4 – 160.0	$^{13}C_3$ Atrazine						
Injection internal standard									
$^{13}C_3$ 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.

HPLC conditions

Instrument:	Waters 2690 HPLC or Waters 2795 HPLC, Quattro Ultima MS/MS
LC Column:	Waters XTerra C_{18}, 3.5 µm, 10.0 cm, 2.1 mm
Ionization:	Negative Ion Electrospray
Acquisition:	MRM mode, unit resolution
Injection Volume:	5 µL

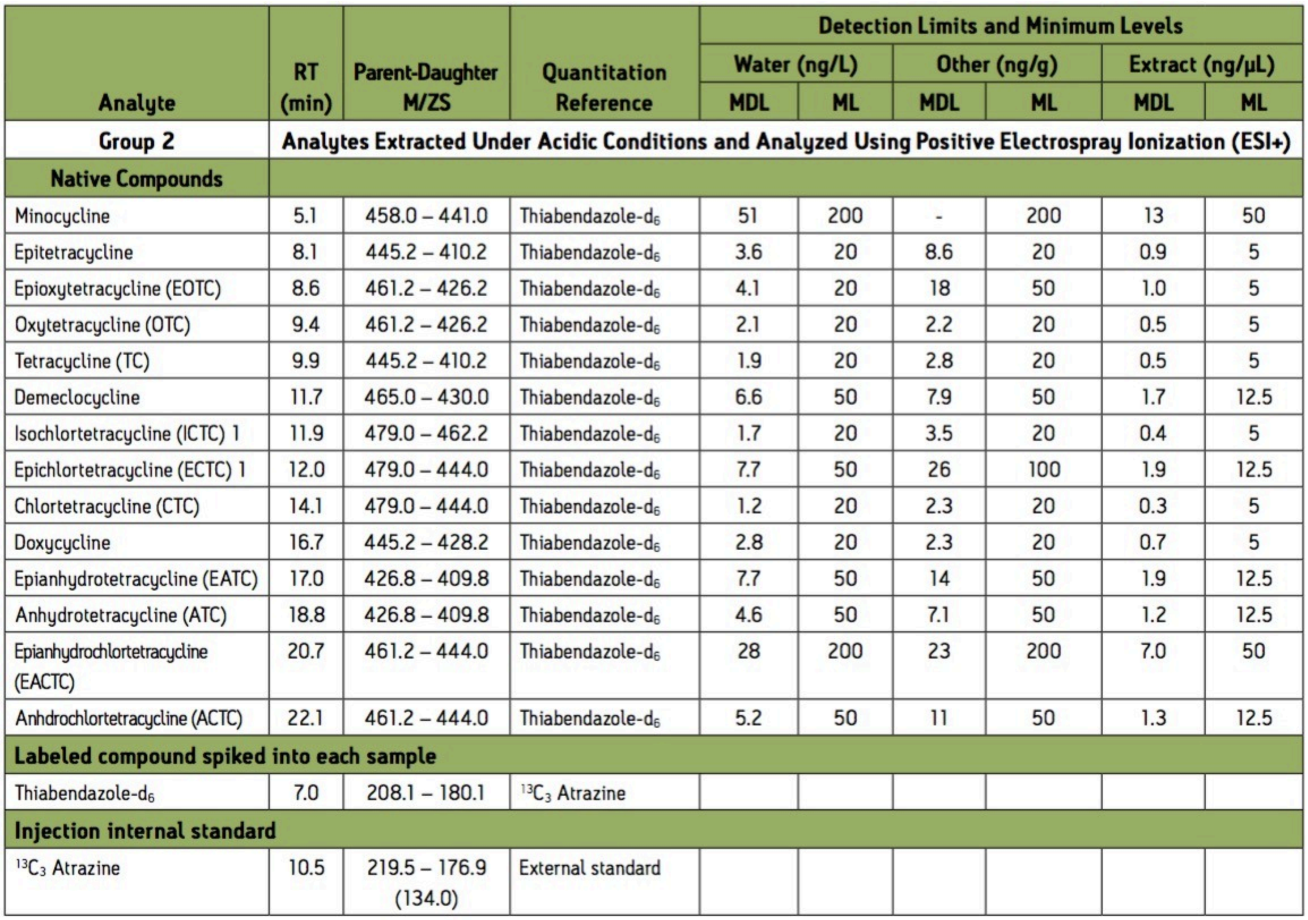


LC Gradient Program		LC Flow Rate (mL/min)	Gradient	General LC Conditions	
Time (min)	Flow Mixture[1]			**Column Temperature**	40 °C
0.0	10% Solvent A 90% Solvent B	0.20	1	**Flow Rate**	0.20 – 0.23 mL/min
1.0	10% Solvent A 90% Solvent B	0.20	6	**Max Pressure**	345 Bar
18.0	40% Solvent A 60% Solvent B	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

[1] Solvent A = 1:1 acetonitrile:methanol, with 5 mM Oxalic Acid

Solvent B = HPLC H_2O, 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
Group 2	**Analytes Extracted Under Acidic Conditions and Analyzed Using Positive Electrospray Ionization (ESI+)**								
Native Compounds									
Minocycline	5.1	458.0 – 441.0	Thiabendazole-d_6	51	200	-	200	13	50
Epitetracycline	8.1	445.2 – 410.2	Thiabendazole-d_6	3.6	20	8.6	20	0.9	5
Epioxytetracycline (EOTC)	8.6	461.2 – 426.2	Thiabendazole-d_6	4.1	20	18	50	1.0	5
Oxytetracycline (OTC)	9.4	461.2 – 426.2	Thiabendazole-d_6	2.1	20	2.2	20	0.5	5
Tetracycline (TC)	9.9	445.2 – 410.2	Thiabendazole-d_6	1.9	20	2.8	20	0.5	5
Demeclocycline	11.7	465.0 – 430.0	Thiabendazole-d_6	6.6	50	7.9	50	1.7	12.5
Isochlortetracycline (ICTC) [1]	11.9	479.0 – 462.2	Thiabendazole-d_6	1.7	20	3.5	20	0.4	5
Epichlortetracycline (ECTC) [1]	12.0	479.0 – 444.0	Thiabendazole-d_6	7.7	50	26	100	1.9	12.5
Chlortetracycline (CTC)	14.1	479.0 – 444.0	Thiabendazole-d_6	1.2	20	2.3	20	0.3	5
Doxycycline	16.7	445.2 – 428.2	Thiabendazole-d_6	2.8	20	2.3	20	0.7	5
Epianhydrotetracycline (EATC)	17.0	426.8 – 409.8	Thiabendazole-d_6	7.7	50	14	50	1.9	12.5
Anhydrotetracycline (ATC)	18.8	426.8 – 409.8	Thiabendazole-d_6	4.6	50	7.1	50	1.2	12.5
Epianhydrochlortetracycline (EACTC)	20.7	461.2 – 444.0	Thiabendazole-d_6	28	200	23	200	7.0	50
Anhdrochlortetracycline (ACTC)	22.1	461.2 – 444.0	Thiabendazole-d_6	5.2	50	11	50	1.3	12.5
Labeled compound spiked into each sample									
Thiabendazole-d_6	7.0	208.1 – 180.1	$^{13}C_3$ Atrazine						
Injection internal standard									
$^{13}C_3$ Atrazine	10.5	219.5 – 176.9 (134.0)	External standard						

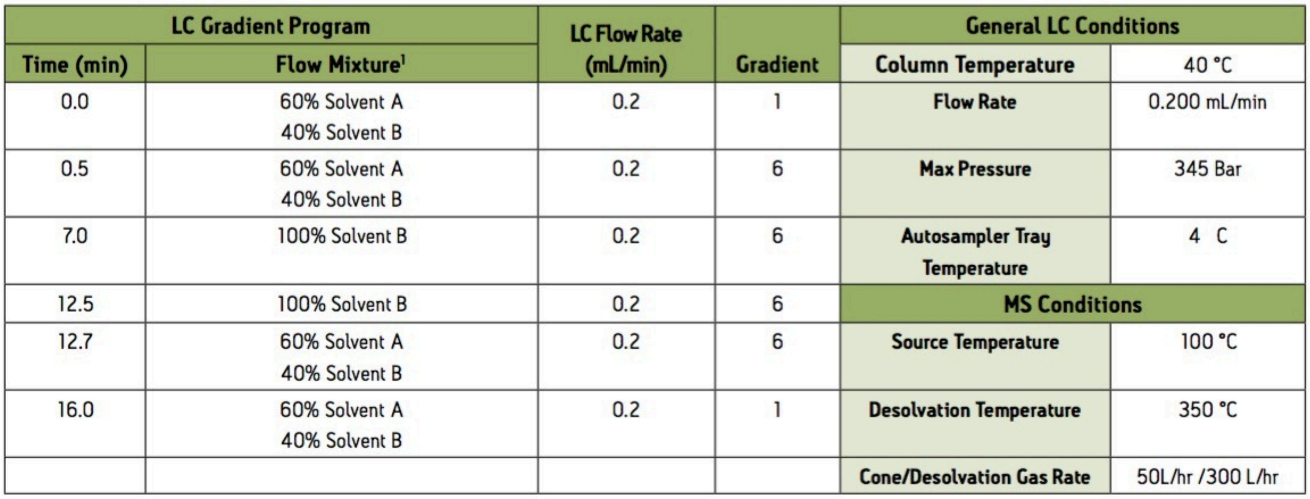
[1] Isochlortetracycline (ICTC) is reported as the sum ICTC + ECTC due to a common transition ion.

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.

HPLC conditions

Instrument:	Waters 2690 HPLC or Waters 2795 HPLC, Quattro Ultima MS/MS
LC Column:	Waters XTerra C_{18}, 3.5 µm, 10.0 cm, 2.1 mm
Ionization:	Negative Ion Electrospray
Acquisition:	MRM mode, unit resolution

Injection Volume: 5 µL

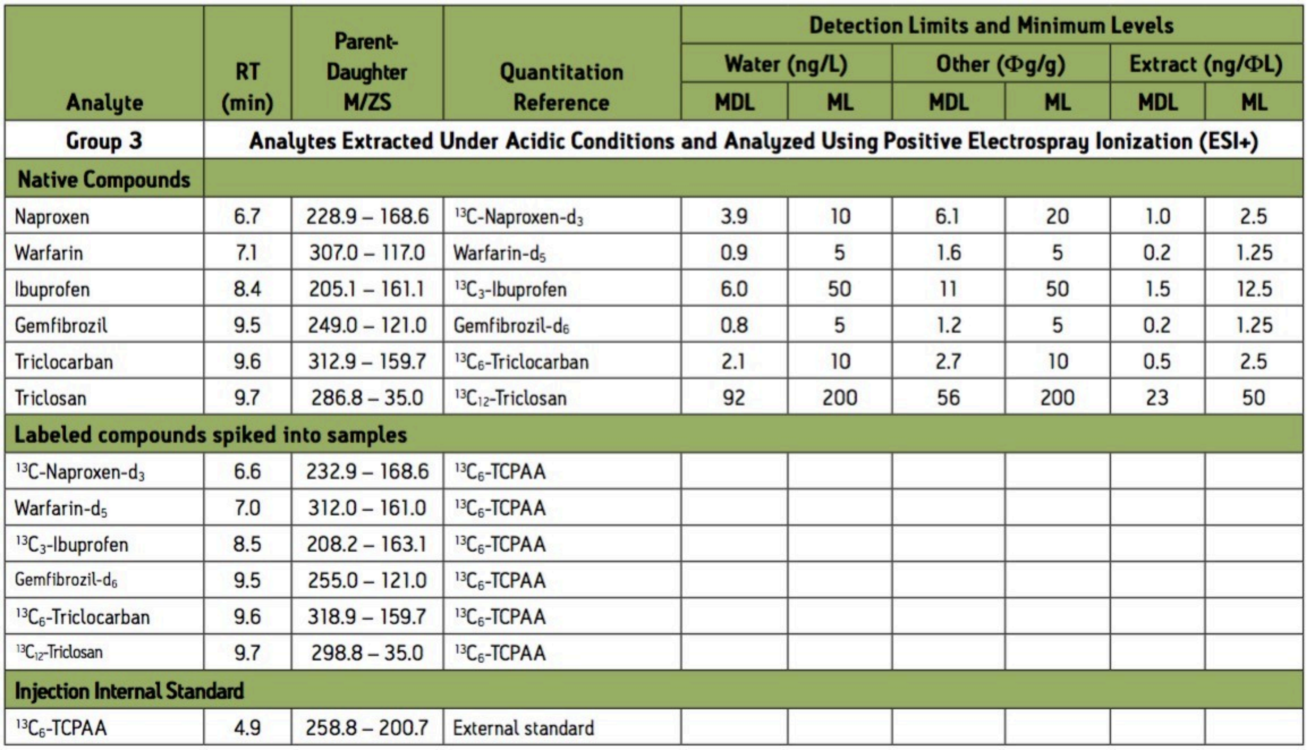


LC Gradient Program		LC Flow Rate (mL/min)	Gradient	General LC Conditions	
Time (min)	Flow Mixture[1]			Column Temperature	40 °C
0.0	60% Solvent A 40% Solvent B	0.2	1	**Flow Rate**	0.200 mL/min
0.5	60% Solvent A 40% Solvent B	0.2	6	**Max Pressure**	345 Bar
7.0	100% Solvent B	0.2	6	**Autosampler Tray 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

[1] Solvent A = 0.1% Ammonium Acetate and 0.1% Acetic Acid in HPLC water

Solvent B = 1:1 MethanolAcetonitrile

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

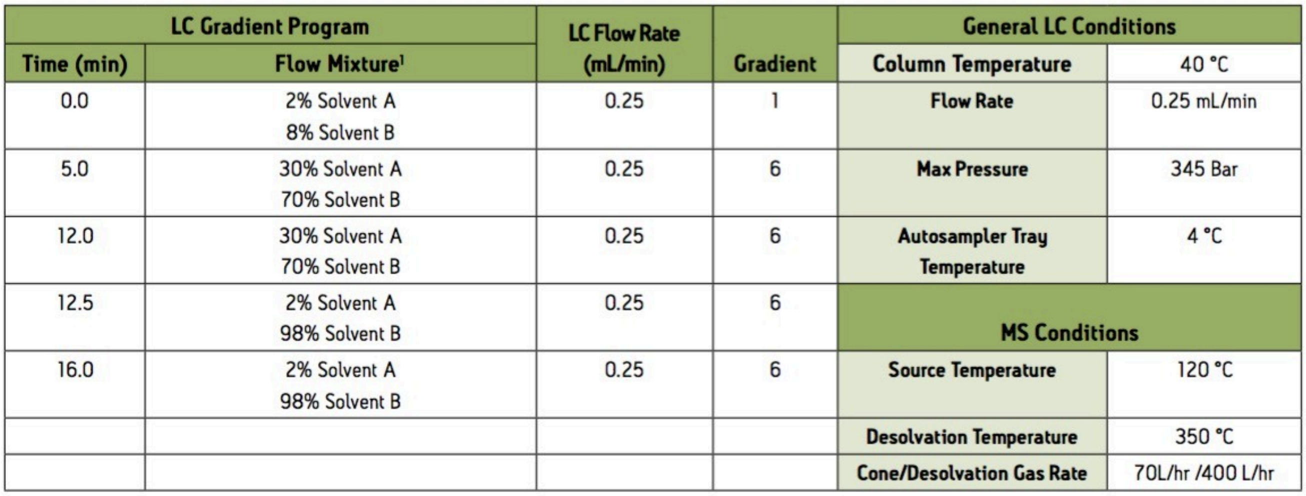


Analyte	RT (min)	Parent-Daughter M/ZS	Quantitation Reference	Detection Limits and Minimum Levels					
				Water (ng/L)		Other (Φg/g)		Extract (ng/ΦL)	
				MDL	ML	MDL	ML	MDL	ML
Group 3	**Analytes Extracted Under Acidic Conditions and Analyzed Using Positive Electrospray Ionization (ESI+)**								
Native Compounds									
Naproxen	6.7	228.9 – 168.6	^{13}C-Naproxen-d$_3$	3.9	10	6.1	20	1.0	2.5
Warfarin	7.1	307.0 – 117.0	Warfarin-d$_5$	0.9	5	1.6	5	0.2	1.25
Ibuprofen	8.4	205.1 – 161.1	^{13}C$_3$-Ibuprofen	6.0	50	11	50	1.5	12.5
Gemfibrozil	9.5	249.0 – 121.0	Gemfibrozil-d$_6$	0.8	5	1.2	5	0.2	1.25
Triclocarban	9.6	312.9 – 159.7	^{13}C$_6$-Triclocarban	2.1	10	2.7	10	0.5	2.5
Triclosan	9.7	286.8 – 35.0	^{13}C$_{12}$-Triclosan	92	200	56	200	23	50
Labeled compounds spiked into samples									
^{13}C-Naproxen-d$_3$	6.6	232.9 – 168.6	^{13}C$_6$-TCPAA						
Warfarin-d$_5$	7.0	312.0 – 161.0	^{13}C$_6$-TCPAA						
^{13}C$_3$-Ibuprofen	8.5	208.2 – 163.1	^{13}C$_6$-TCPAA						
Gemfibrozil-d$_6$	9.5	255.0 – 121.0	^{13}C$_6$-TCPAA						
^{13}C$_6$-Triclocarban	9.6	318.9 – 159.7	^{13}C$_6$-TCPAA						
^{13}C$_{12}$-Triclosan	9.7	298.8 – 35.0	^{13}C$_6$-TCPAA						
Injection Internal Standard									
^{13}C$_6$-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.

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_3CN (changed from H2O)
Injection Volume:	2.0 µL

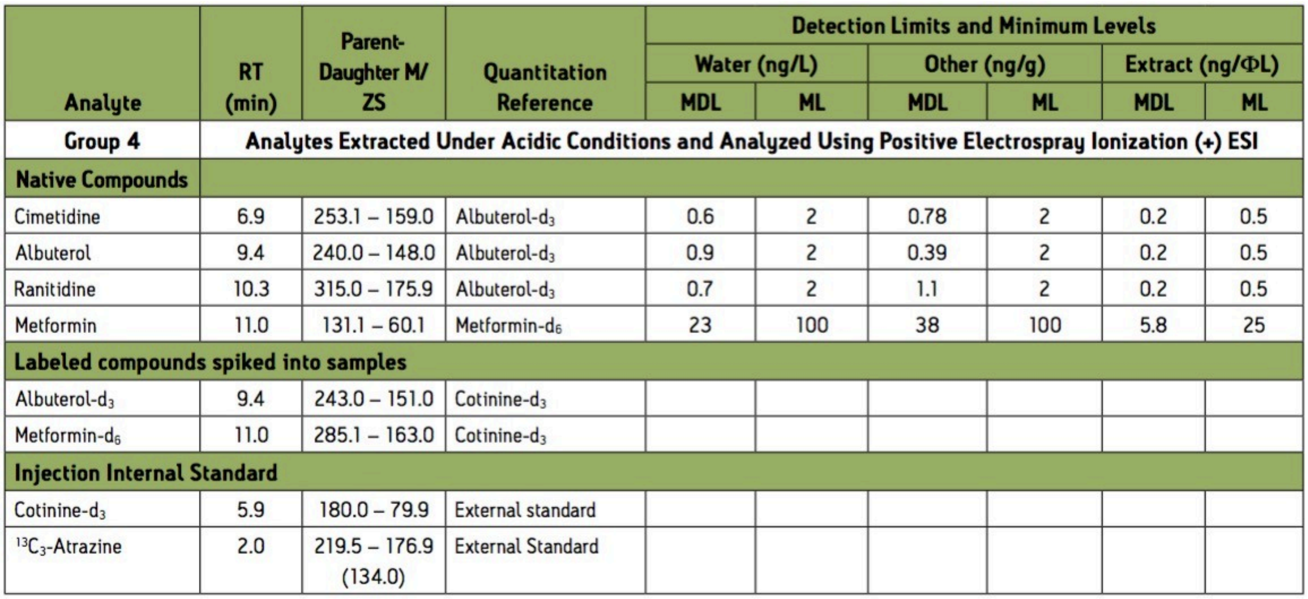


LC Gradient Program		LC Flow Rate (mL/min)	Gradient	General LC Conditions	
Time (min)	Flow Mixture[1]			Column Temperature	40 °C
0.0	2% Solvent A 8% Solvent B	0.25	1	Flow Rate	0.25 mL/min
5.0	30% Solvent A 70% Solvent B	0.25	6	Max Pressure	345 Bar
12.0	30% Solvent A 70% Solvent B	0.25	6	Autosampler Tray 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

[1] 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
Group 4	**Analytes Extracted Under Acidic Conditions and Analyzed Using Positive Electrospray Ionization (+) ESI**								
Native Compounds									
Cimetidine	6.9	253.1 – 159.0	Albuterol-d$_3$	0.6	2	0.78	2	0.2	0.5
Albuterol	9.4	240.0 – 148.0	Albuterol-d$_3$	0.9	2	0.39	2	0.2	0.5
Ranitidine	10.3	315.0 – 175.9	Albuterol-d$_3$	0.7	2	1.1	2	0.2	0.5
Metformin	11.0	131.1 – 60.1	Metformin-d$_6$	23	100	38	100	5.8	25
Labeled compounds spiked into samples									
Albuterol-d$_3$	9.4	243.0 – 151.0	Cotinine-d$_3$						
Metformin-d$_6$	11.0	285.1 – 163.0	Cotinine-d$_3$						
Injection Internal Standard									
Cotinine-d$_3$	5.9	180.0 – 79.9	External standard						
$^{13}C_3$-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.

References

1. Total Solutions for Environmental Applications: 720002163EN
2. LC-MS Determination of Pharmaceutical Residues in Environmental Samples: 720000421EN

Featured Products

- [Alliance HPLC System <https://www.waters.com/534293>](https://www.waters.com/534293)

720002734, August 2008