Waters[™]

Applikationsbericht

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		General LC Conditions		
Time (min)	Flow Mixture ¹	(mL/min)	Gradient	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 Condit	ions	
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.

				Detection Limits and Minimum Levels					
	RT	Parent-	Quantitation	Water	(ng/L)	Other ((µg/kg)	Extract (ng/ΦL)
Analyte	(min)	Daughter M/ZS	Reference	MDL	ML	MDL	ML	MDL	ML
Group 1	Ana	lytes Extracted I	Under Acidic Condition	ns and An	alyzed Using	g Positive El	ectrospray l	onization (ESI+)
Native Compounds									
Sulfanilamide	2.5	190.0 - 155.8	¹³ C ₅ -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 - I	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	5.5	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

				Detection Limits and Minimum Levels					
	RT	Parent-	Quantitation	Water (ng/L)		Other (µg/kg)		g) Extract (ng/ΦL)	
Analyte	(min)	Daughter M/ZS	Reference	MDL	ML	MDL	ML	MDL	ML
Group 1	Ana	lytes Extracted l	Jnder Acidic Conditio	ons and Ana	alyzed Usin	g Positive El	ectrospray l	onization (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	s spiked	into each sample				19			
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-d6	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-d5	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 s	tandard							-82 	
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), parentdaughter 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
	MDM model with recelution
Acquisition:	MRM mode, unit resolution
Injection Volume	5.11
	~ M=

	LC Gradient Program	LC Flow Rate		General LC Con	ditions
Time (min)	Flow Mixture ¹	(mL/min)	Gradient	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 Conditi	ons
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.

	Detection Limits and Minimum Lev				m Levels				
	RT	Parent-Daughter	Quantitation	Water	Water (ng/L)		Other (ng/g)		(ng/µL)
Analyte	(min)	M/ZS	Reference	MDL	ML	MDL	ML	MDL	ML
Group 2	Analy	tes Extracted Und	ler Acidic Condition	s and Ana	lyzed Usi	ng Positiv	e Electrospr	ay lonizati	on (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	d			82 X					
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	MDM mode unit resolution
Acquisition:	WHW MODE, UNIT RESOlUTION

	LC Gradient Program	LC Flow Rate		General LC Con	ditions
Time (min)	Flow Mixture ¹	(mL/min)	Gradient	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 Conditi	ons
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

 $^{\rm 1}$ 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.

		Parent			Detection Limits and Minimum Levels						
	RT	Daughter	Quantitation	Water	(ng/L)	Other ((Φg/g)	Extract	(ng/@L)		
Analyte	(min)	M/ZS	Reference	MDL	ML	MDL	ML	MDL	ML		
Group 3	Ana	lytes Extracted	Under Acidic Condition	s and Analy	zed Using I	Positive Ele	ctrospray I	onization	(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-d6	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 i	nto samples									
¹³ C-Naproxen-d ₃	6.6	232.9 - 168.6	¹³ C ₆ -TCPAA								
Warfarin-d5	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 Stan	dard										
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
Purae Solvent	100% CH ₂ CN (changed from H2O)
Injection Volume:	2.0 µL

LC	Gradient Program	LC Flow Rate		General LC Conditions		
Time (min)	Flow Mixture ¹	(mL/min)	Gradient	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.

		Parent- RT Daughter M/	Quantitation	Detection Limits and Minimum Levels					
	RT			Water (ng/L)		Other (ng/g)		Extract (ng/ΦL)	
Analyte	(min)	ZS	Reference	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), parentdaughter 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>

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