

Fast and sensitive analysis of drug residues in water using on line SPE-UHPLC-MS/MS with ultra-fast polarity switching

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Introduction

As monitoring of drug residue in drinking and surface water is becoming of major concern in western countries, the environmental laboratories must face the increase in analysis request as well as the increase in the number of compounds to be screened. In parallel, as long as no consensus as been found on regulatory reporting levels, the assay sensitivity must be very high to not miss any compound. Thus, laboratories need a simple, rapid, reliable, sensitive and cost effective method to screen and to accurately quantify drug residues in a variety of water samples.

A method was then set up to fulfill these requirements. On line SPE is a useful tool to enable large sample volume injections. But due to the chemical diversity of target compounds, sample clean up cannot be pushed too far to not lose polar compounds. Then matrix effect or ion suppression can be problematic for accurate quantification. Then, a very sensitive Mass Spectrometer as well as very efficient chromatography were necessary to compensate the relatively low sample volume injection. An innovative SPE set up was developed to combine low pressure on line SPE and high pressure UHPLC analysis.

Materials and Methods

Online SPE-UHPLC-MS/MS

System	: Shimadzu Nexera X2 with online SPE features (fig. 1)
Columns	: SPE : Spark Holland cartridge OASIS HLB 15µm 10x2mm (@RT) UHPLC : Restek Raptor C18 2.7µm 100x2mm (@30°C)
Mobile Phases	: SPE : A : Water B : Methanol C : Methanol/Isopropanol 1/1 UHPLC : A : Water + NH ₄ OH 0.05% B : Methanol + NH ₄ OH 0.05%
Flow rate:	: SPE : Loading : 2 mL/min Elution : 0.4 mL/min Cleaning : 3 mL/min UHPLC : 0.5 mL/min
Injection volume	: 1000 µL
Sample Temperature	: +5°C

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Time Program

Online SPE				UHPLC		
Time (min)	Step	Action	Value	Time (min)	Step	Value
	Loading			0.01	Initial conditions	0%B
0.90		Loading	2 mL/min			
0.91		Changing flow rate	0.4 mL/min			
1.00	Elution	Switching Elution loop	Elution position			
1.00		Switching Storage loop	Storage position			
1.50		Switching Storage loop	Elution position			
1.60	Cleaning	Changing Solvent	C			
1.70		Changing flow rate	3 mL/min			
				2.50	Starting Gradient	0%B
6.00	Filling elution loop	Changing Solvent	B			
8.90		Switching Elution loop	Loading position			
9.00		Changing Solvent	A			
				10.50	Holding gradient	90%B
				12.50	Ending gradient	90%B
				12.60	Reconditioning	0%B
14.00		Stop		14.00	Stop	

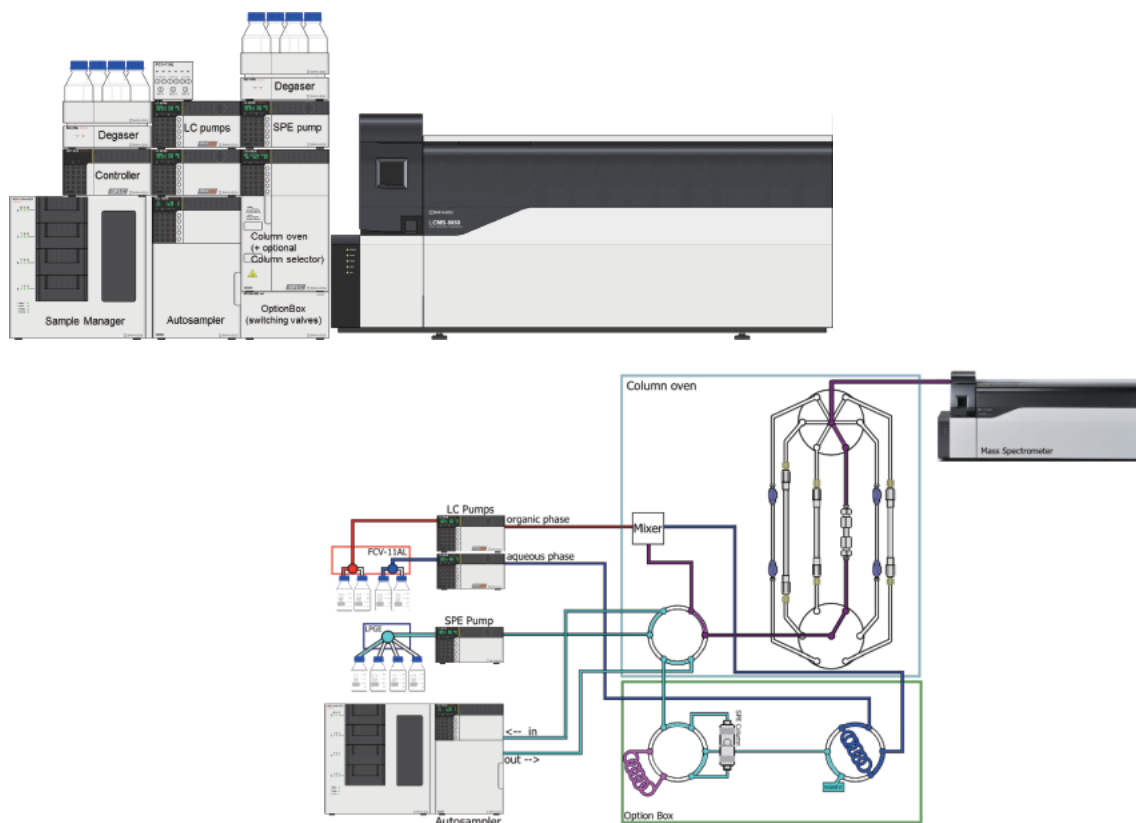


Figure 1 – scheme of the analytical system (patent pending)

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Mass Spectrometry

System	: Shimadzu LCMS-8050
Ionization mode	: Heated ESI
Temperatures	: Interface : 400°C DL : 250°C Heater Block : 400°C
Gas flow	: Nebulizer : 3L/min HESI : 10 L/min Drying : 10 L/min
Polarity switching time	: 5 ms
Pause time	: 1 ms
Dwell time	: 5 to 30 ms to obtain at least 25 points per peak,
Compound table	: see table 1

Results

Out of the 130 compounds assayed with this method, 40 compounds were selected for evaluation of sensitivity and matrix effect.

A calibration curve ranging from 0.1 to 100 ng/L was prepared by spiking Evian water (spring water). Independent QCs in Evian, tap water and superficial water

were prepared for accuracy and matrix effect evaluation. ISTD compounds added in the calibration standards and QCs were only use for process and injection monitoring. Therefore external calibration was performed and ion suppression/enhancement was not compensated by the internal standards.

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Table 1: List of the compounds

Name	Polarity	MRM Quantifier	MRM Qualifier
14 Hydroxy-clarithromycin	Pos	764.3 > 158.25	764.3 > 606.1
19-Norethisterone	Pos	299.2 > 109.2	299.2 > 91.1
1-OH-ibuprofen	Pos	221 > 159.1	221 > 177.2
2-OH-ibuprofen	Pos	221.4 > 177.35	N/A
4-epitetracycline	Pos	444.9 > 410	444.9 > 347
4-Methylbenzylidene camphor	Pos	255.2 > 237	255.2 > 171.15
Acebutolol	Pos	337.2 > 116.2	337.2 > 319.1
Alprazolam	Pos	309.1 > 281.1	309.1 > 205.1
Aminopyrine (aminophenazone)	Pos	232.1 > 113.25	232.1 > 111.2
Amitriptyline	Pos	278.2 > 91.05	278.2 > 105.05
Amlodipine	Pos	410.1 > 239	410.1 > 238.1
Androstenedione	Pos	286.7 > 97.2	286.7 > 109.15
Antipyrindine	Pos	189.2 > 57	189.2 > 77.1
Atenolol	Pos	266.8 > 145.05	266.9 > 190.1
Betaxolol	Pos	308.2 > 116.2	308.2 > 72.2
Bezafibrate	Pos	361.9 > 316	361.9 > 138.95
Bezafibrate	Pos	360.1 > 274.1	360.1 > 154.05
Bezafibrate d6 (ISTD)	Pos	367.9 > 138.95	N/A
Bisoprolol	Pos	326.2 > 116.2	326.2 > 74.15
Bromazepam	Pos	316 > 182	317.7 > 182.2
Bupivacaine	Pos	289.2 > 140.25	289.2 > 84.05
Cafeine	Pos	194.9 > 138.2	194.9 > 42.2
Carbamazepine	Pos	237.1 > 194.2	237.1 > 192.15
Carbamazepine 10,11 epoxide	Pos	253.1 > 180.2	253.1 > 236
Carbamazepine c13 (ISTD)	Pos	239.1 > 194.2	239.1 > 192.15
Cetiofur	Pos	524 > 240.85	524 > 165.95
Chloramphenicol	Pos	320.8 > 152.1	323 > 152.1
Chlormadinone	Pos	405.1 > 309	405.1 > 345
Chlorophene	Neg	217 > 181.2	217 > 153.2
Chlortetracycline	Neg	479.1 > 444.05	479.1 > 260
Ciprofloxacin	Neg	332 > 231.15	332.1 > 288.15
Clarithromycin	Neg	748.5 > 158.2	748.5 > 83
Clenbuterol	Neg	279.2 > 204.9	279.2 > 181.2
Clindamycin	Neg	425.2 > 126.2	425.2 > 377.15
Clofibrac acid	Neg	213 > 127	213 > 85.1
Cortisol	Neg	362.7 > 121.2	362.7 > 327.1
Cotinine	Neg	177.2 > 80.1	177.2 > 98.15
Cyclophosphamide	Neg	260.7 > 140	263 > 142.05
Danofloxacin	Neg	358.1 > 314	358.1 > 283.05
Dexamethasone	Neg	393.2 > 373.05	393.2 > 355.05
Diazepam	Pos	285.1 > 154.1	285.1 > 193.05
Diclofenac	Neg	296 > 214	298 > 217.1
Dihydrocodeine	Neg	302.3 > 199.1	302.3 > 128.1
Dilantin	Neg	251 > 101.9	251 > 208.25
Diltiazem	Neg	415.1 > 178.05	415.1 > 150.05
Doxepine	Neg	280.2 > 107.1	280.2 > 235
Doxycycline	Neg	444.9 > 428.15	444.9 > 147.3
Enoxacin	Neg	321.1 > 303	321.1 > 232.05
Enrofloxacin	Neg	360 > 316.05	360 > 245.15
Erythromycin A	Neg	734.3 > 158.2	734.3 > 576.25
Fenobric acid	Neg	317 > 231	317 > 249.1
Fenofibrate	Neg	362.1 > 234.15	362.1 > 139.1
Fenopropfen	Neg	241.1 > 197.2	241.1 > 93
Florfenicol	Neg	356 > 335.85	356 > 185.1
Flumequine	Neg	261.9 > 202.15	261.9 > 126.2
Fluoxetine	Neg	310.1 > 251	310.1 > 44.1
FOSA	Neg	497.9 > 77.95	497.9 > 477.9
Furosemide	Neg	329 > 285.05	329 > 205
Gemfibrozil	Neg	249.2 > 121	249.2 > 106.1
Ibuprofen	Neg	204.9 > 161.1	N/A
Imipramine	Neg	281.2 > 86.1	281.2 > 58.15
Indomethacin	Neg	355.8 > 297.1	355.8 > 312.1
Irbesartan	Neg	429.2 > 206.95	429.2 > 195.25
Isfosamide	Neg	261 > 154	261 > 92.1
Isoquinoline	Pos	130.1 > 77.15	130.1 > 103.15
Ketoprofen	Pos	254.8 > 105.1	254.8 > 209
Ketorolac	Pos	256.1 > 105.1	256.1 > 77.15
Levonorgestrel	Pos	313.2 > 245	313.2 > 91.15
Lincomycin	Pos	407.2 > 126.3	407.2 > 359
Lorazepam	Pos	320.8 > 275.05	322.6 > 276.9
Marbofloxacin	Pos	363.1 > 72.25	363.1 > 320
Meclocycline	Pos	476.9 > 460	476.9 > 235
Mefenamic acid	Pos	242.1 > 223.95	242.1 > 209
Mepivacaine	Pos	247.2 > 98.15	247.2 > 70.15
Methotrexate	Pos	454.9 > 308	454.9 > 175.2
Metoprolol	Pos	268 > 116.35	268 > 55.95
Monensin sodium	Pos	693.2 > 675.2	693.2 > 461.05
Morphine	Pos	286.2 > 165.2	286.2 > 152.1
Nadolol	Pos	310.2 > 254	310.2 > 201.15
Naproxen	Pos	229 > 169.3	229 > 170.2
n-ETFOA	Pos	526 > 169.15	526 > 219
Nicotine	Pos	163.2 > 130.1	163.2 > 117.2
Norfloxacin	Pos	320.1 > 276.05	320.1 > 233.2
Norflouxetine	Pos	296 > 213.95	296 > 214.95
Odesmethyl-tramadol	Pos	250.2 > 58.15	250.2 > 171.05
Ofloxacin	Pos	361.9 > 318.05	361.8 > 260.95
Ofloxacin d3 (ISTD)	Pos	364.9 > 321.05	364.8 > 260.95
Oxazepam	Pos	286.7 > 241.1	286.7 > 268.95
Oxycodone	Pos	316.1 > 298	316.1 > 241
Oxytetracycline	Pos	460.9 > 426.1	460.9 > 201
Pentoxifylline	Pos	279.2 > 205.1	279.2 > 181.15
PFBA	Pos	213 > 169.1	213 > 93
PFBS (L-PFBS)	Pos	298.9 > 79.9	298.9 > 98.95
PFDA	Pos	512.9 > 468.9	512.9 > 269
PFDoA	Pos	612.9 > 568.75	612.9 > 169.05
PFDS (L-PFDS)	Pos	598.9 > 79.9	598.9 > 98.95
PFHDA (PFHxDA)	Pos	768.9 > 269	768.9 > 168.9
PFHpA	Pos	363 > 318.95	363 > 169.15
PFHpS (L-PFHpS)	Pos	448.9 > 79.9	448.9 > 98.9
PFHxA (PFHxA)	Pos	313 > 269.1	313 > 91.05
PFHxS (PFHxS)	Pos	398.9 > 79.9	398.9 > 99
PFNA	Pos	463.1 > 418.9	463.1 > 219
PFOA	Pos	412.9 > 368.9	412.9 > 169.15
PFODA	Pos	912.9 > 868.8	912.9 > 168.95
PFOS	Pos	498.9 > 98.95	498.9 > 79.95
PFPeA	Pos	263 > 219	263 > 18.8
PFTeA	Pos	712.9 > 219	712.9 > 219
PFTrA (PFTrDA)	Pos	663 > 618.85	663 > 169
PFUnDA	Pos	562.9 > 518.8	562.9 > 219.05
Piroxicam	Pos	330.1 > 146.15	330.1 > 266.1
Prednisolone	Pos	361.2 > 343.05	361.2 > 307
Prilocaine	Pos	221.2 > 86	221.2 > 44.2
Pristinamycin IIA	Pos	526.1 > 355.15	526.1 > 508.05
Progesterone	Pos	314.8 > 109.2	314.8 > 97.15
Propranolol	Pos	260.15 > 183.15	260.15 > 116.15
Propyphenazone	Pos	231.1 > 189.2	231.1 > 56.1
Ramiprilat	Pos	389.2 > 206	389.2 > 156.2
Ranitidine	Pos	314.8 > 176.1	315.1 > 130.1
Roxithromycin	Pos	837.3 > 158.05	837.3 > 679.3
Salbutamol	Pos	240.2 > 148.15	240.2 > 222
Sertraline	Pos	306.1 > 159.05	306.1 > 274.95
Simvastatine	Pos	419.1 > 199.2	419.1 > 225.05
Sotalol	Pos	272.9 > 213.1	273.1 > 133.1
Spiramycin	Pos	422.3 > 174.05	422.3 > 101.15
Sulfamethazine	Pos	278.9 > 185.95	278.9 > 124.2
Sulfamethoxazole	Pos	253.9 > 156.1	253.9 > 92.1
Sulfaquinoxaline	Pos	302 > 156.1	302 > 157.15
sulfaquinoxaline	Pos	300.8 > 156.05	300.8 > 92.1
Terbutaline	Pos	226.2 > 152	226.2 > 107.15
Testosterone	Pos	289.1 > 96.9	289 > 109
Tetracycline	Pos	444.9 > 410	444.9 > 241
Ticlopidine	Pos	264.1 > 125.15	264.1 > 154.15
Timolol	Pos	317.1 > 260.95	317.1 > 74.15
Tramadol	Pos	264.2 > 58.15	N/A
Trimethoprim	Pos	290.8 > 230	290.8 > 123
Trimethoprim d3 (ISTD)	Pos	293.8 > 230	293.8 > 123
Zolpidem	Pos	308.1 > 237.05	308.1 > 235.05

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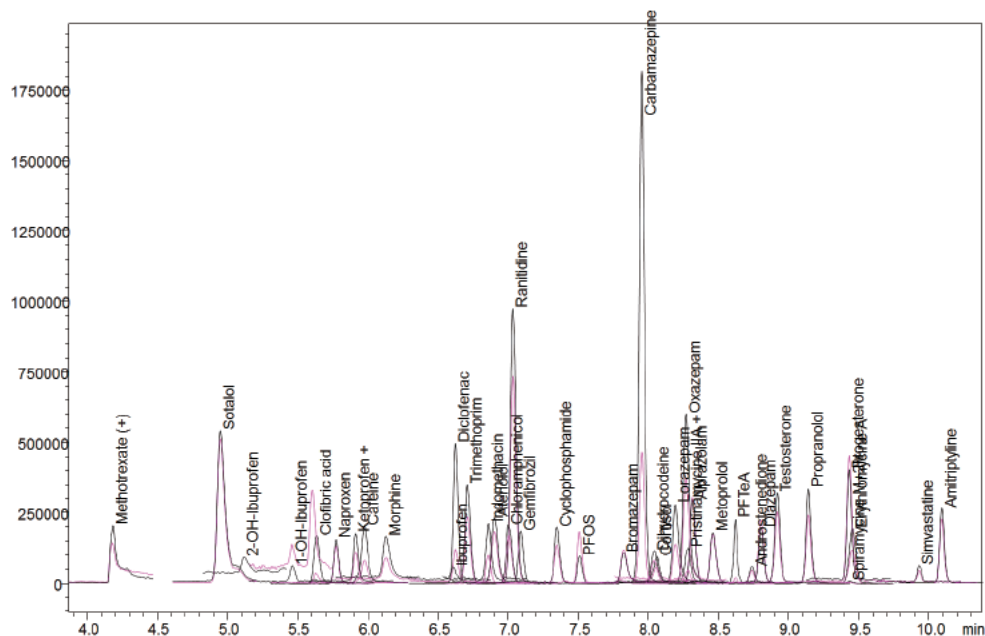


Figure 2 – Superficial water spiked at 10 ng/L

Table 2: Quantitative results

Compound	LOQ (ng/L)	Accuracy in QC (50 ng/L)		
		Evian	Tap water	Superficial water
1-OH-ibuprofen	5	75%	90%	17%
2-OH-ibuprofen	10	80%	95%	43%
Alprazolam	0.5	95%	100%	99%
Amitriptyline	1	84%	84%	51%
Androstenedione	0.5	98%	113%	20%
Atenolol	0.5	99%	116%	89%
Bromazepam	0.5	100%	104%	97%
Cafeine	5	104%	162%	98%
Carbamazepine	0.1	95%	103%	110%
Chloramphenicol	1	106%	183%	123%
Clofibric acid	0.5	90%	102%	72%
Cortisol	2.5	110%	161%	70%
Cyclophosphamide	0.5	104%	126%	104%
Diazepam	1	97%	99%	104%
Diclofenac	0.5	107%	160%	104%
Dihydrocodeine	0.5	96%	106%	116%
Erythromycine A	0.1	92%	96%	74%
Gemfibrozil	0.5	89%	93%	74%
Ibuprofen	10	67%	61%	67%
Indomethacin	2.5	90%	124%	69%

Compound	LOQ (ng/L)	Accuracy in QC (50 ng/L)		
		Evian	Tap water	Superficial water
Ketoprofen	0.5	110%	272%	107%
Lorazepam	0.5	103%	128%	103%
Methotrexate	0.5	64%	57%	5%
Metoprolol	0.5	96%	96%	93%
Morphine	2.5	88%	122%	69%
Naproxen	1	94%	96%	49%
n-ETFOSA	5	18%	17%	5%
Oxazepam	0.5	104%	111%	100%
PFOS	0.5	52%	73%	58%
PFTeA	0.1	72%	117%	52%
Pristinamycine IIA	1	93%	97%	80%
Progesterone	2.5	91%	98%	14%
Propranolol	0.5	93%	92%	72%
Ranitidine	0.5	97%	105%	92%
Simvastatine	2.5	41%	48%	7%
Sotalol	0.5	93%	131%	122%
Spiramycin	0.5	85%	128%	37%
Testosterone	0.5	97%	109%	18%
Trimethoprim	0.5	99%	110%	86%

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Conclusion

A fast and sensitive method was set up to assay a large number of drug residue in water. Limits of quantification are ranging from 0.1 to 5 ng/L with only 1 mL of sample and an automated sample preparation. Most of the compounds do not suffer from matrix effect without using internal standards. Some investigations are running to improve accuracy using selected internal standards.