Analysis of Pharmaceutical Residues in Wastewater Samples using High Performance Liquid Chromatography coupled to Quadrupole-Orbitrap Mass Spectrometry

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Pharmaceuticals in water

Pharmaceuticals in water

- Can represent a threat to aquatic species

- The fostering of antimicrobial resistance genes

Analytical procedures

GC

- Preferable for the analysis of non-polar and volatile compounds

- Addition of a derivatization step for the analysis of low concentrations of pharmaceuticals

- Derivatization step can affect the accuracy of the method, because of the losses of analytes that can occur.

LC

- Polar organic pollutants

- Shorter analysis time, necessary for monitoring studies

- The main drawback of HPLC analysis of pharmaceuticals in environmental samples is matrix effects (the ion-suppression phenomenon)

- For the detection of the analytes, tandem MS–MS is increasingly being used, replacing other detectors


Analytical procedures

- Recent trend is focused toward the use of powerful high resolution MS detectors like TOF and Orbitrap

- Hybrid high resolution mass spectrometers Q-TOF-HRMS and Q-Orbitrap-HRMS allows the combination of non-targeted screening in full scan mode and targeted MS/MS analysis

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Selected compounds in the study

- *Analgesic* (acetaminophen)
- *Antidepressant* (fluoxetine)
- *Anti-epileptic* (carbamazepine)
- *Veterinary sedative* (xylazine)
- *Anti-hypertensive* (losartan, valsartan)
- *β-blocker* (atenolol, metoprolol, propranolol)

- *Antibiotics* (ciprofloxacin, azithromycin, erythromycin, clarithromycin, sulfamethoxazole, trimethoprim)
- *Lipid regulator* (atorvastatin, simvastatin, pravastatin)
- *NSAIDs* (ketoprofen, naproxen, ibuprofen, diclofenac)
- *CNS* stimulant (caffeine)

Sample preparation – SPE optimization
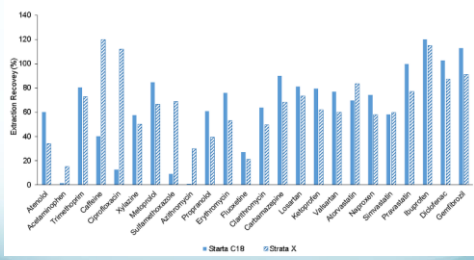
Sample preparation

Add 20 µL of 0.5 M Na$_2$EDTA solution and 100 µL of acetic acid to 200 mL of sample and agitate.

- Activate with 3 mL of methanol and 3 mL of deionized water.
- Load the sample on column
- Dry the cartridge under vacuum for 10 min.
- Elute sample with 6 mL of methanol.

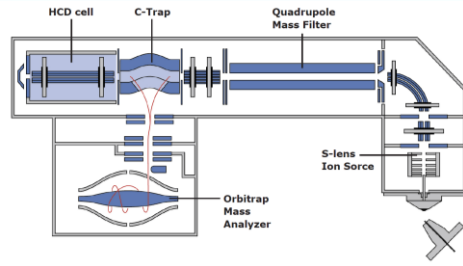Evaporate the elute to dryness under nitrogen stream at 40ºC.

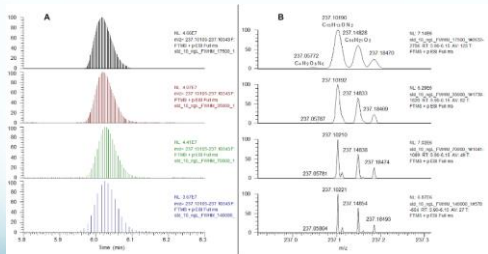Reconstitute the sample in 100 µL of water/methanol (80/20, v/v).

Orbitrap analyzer
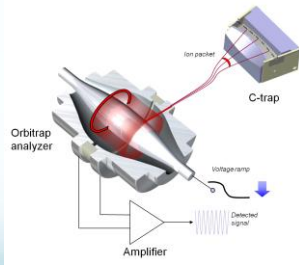
Q-Exactive Orbitrap HRMS

Resolution

UHPLC-Q-Orbitrap-MS parameters

- Column: Phenomenex®, Kinetex C18, 100 x 2,1 mm, 2.6 µm
- Mobile phases: «A» 0.1 % formic acid in water, «B» methanol
- Analysis time: 10 min

- Polarity: +/–
- Full MS/dd-MS^2
- Full scan (125 to 800 m/z; 70,000 FWHM)

For confirmatory purpose:

- Parallel reaction monitoring, 17,500 FWHM
- Inclusion list with exact product ion mass, collision energies and expected RT

Performance of the method

- Linearity $R^2 > 0.992$
- Recovery ranged from 79% to 133%
- Repeatability ranged from 7.0% to 42%
- The LOQ values varied from 10 to 1000 pg/L
- Matrix effect: the majority of the analytes were subject to ion suppression, with the exception of azithromycin, caffeine, ciprofloxacin, metoprolol, and trimethoprim, which showed signal enhancement.

| Compound	RSD,% (n = 15)	Recovery, % (n=15)	LOQ, pg/L	ME, %
Acetaminophen	9.4	98	100	64
Atenolol	12	92	500	43
Atorvastatin	18	102	500	62
Azithromycin	27	126	1000	104
Caffeine	42	132	100	125
Carbamazepine	6.4	102	10	82
Ciprofloxacin	35	115	1000	130
Clarithromycin	12	87	10	77
Diclofenac	7.0	92	100	51
Erythromycin	33	90	100	71
Fluoxetine	31	86	500	44
Gemfibrozil	13	79	100	57
Ibuprofen	27	95	500	56
Ketoprofen	8.1	94	100	48
Losartan	16	91	50	46
Metoprolol	9.7	113	50	112
Naproxen	13	86	10	48
Pravastatin	6.7	78	10	39
Propranolol	22	109	10	82
Simvastatin	16	103	500	68
Sulfamethoxazole	10	94	50	35
Trimethoprim	8.4	133	10	104
Valsartan	15	97	100	74
Xylazine	11	109	10	63

Chromatograms
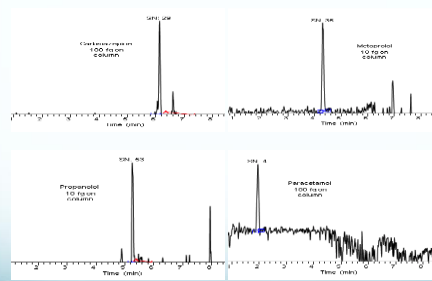
Collection of samples

- 21 samples of untreated wastewaters
- 3 samples per day during 7 days (April 2016)
- Central wastewater treatment plant "Daugavgriva" of the Riga city.
- Samples were collected in glass amber bottles and kept at +4ºC during transportation.
- Once delivered to the laboratory, the samples were filtered and extracted within 24 hrs.

Analysis of wastewater samples

- The concentration range for most of the pharmaceuticals varied between 10-200 ng/L
- The concentrations for caffeine and acetaminophen were in low µg/L range, at 7-12 µg/L and 1.8-4.2 µg/L
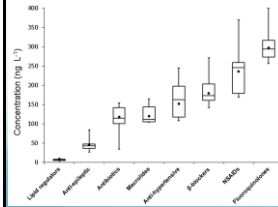
Analyte	Drug type	Conc. range, ng/L
Acetaminophen	Analgesic	1800-4200
Fluoxetine	Antidepressant	ND
Carbamazepine	Anti-epileptic	18-50
Xylazine	Veterinary sedative	2-180
Losartan	Anti-hypertensive	2-5
Valsartan	Anti-hypertensive	30-80
Caffeine	CNS stimulant	7000-12000
Ciprofloxacin	Fluoroquinolone antibiotic	250-400
Gemfibrozil	Lipid regulator	ND
Atorvastatin	Lipid regulator	3-10
Simvastatin	Lipid regulator	ND
Pravastatin	Lipid regulator	0.2-0.8
Azithromycin	Macrolide antibiotic	70-150
Erythromycin	Macrolide antibiotic	1-5
Clarithromycin	Macrolide antibiotic	1-21
Ketoprofen	NSAID	8-16
Naproxen	NSAID	9-20
Ibuprofen	NSAID	100-325
Diclofenac	NSAID	4-12
Sulfamethoxazole	Sulfanilamide antibiotic	50-120
Trimethoprim	Sulfanilamide antibiotic	15-43
Atenolol	β-blocker	50-150
Metoprolol	β-blocker	50-125
Propranolol	β-blocker	ND

Thank you for your attention!