Supplementary Material

Exploring the efficiency of UHPLC-Orbitrap MS for the determination of 20 pharmaceuticals and acesulfame K in hospital and urban wastewaters with the aid of FPSE

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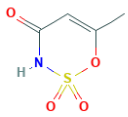
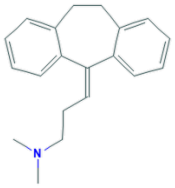
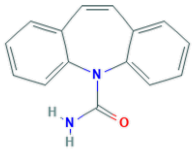
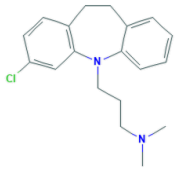
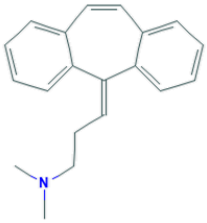
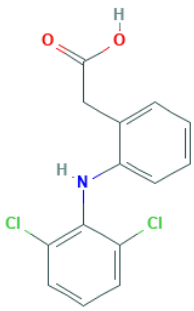
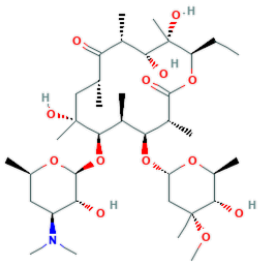
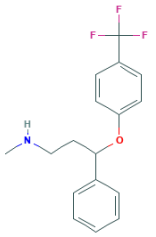
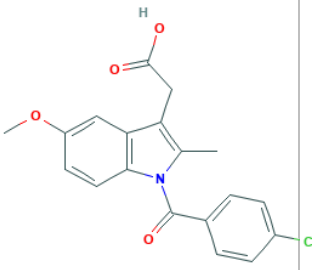
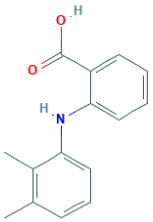
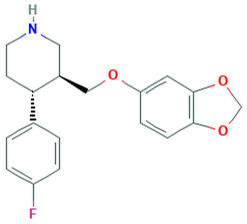
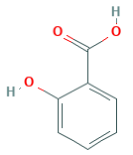
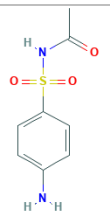
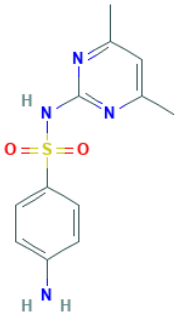
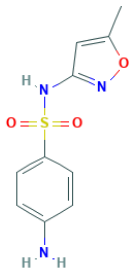
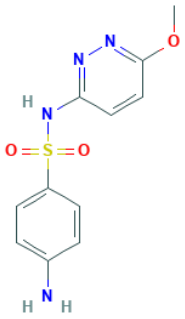
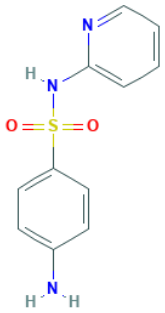
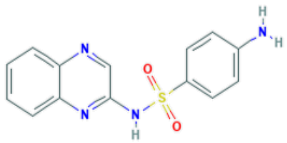
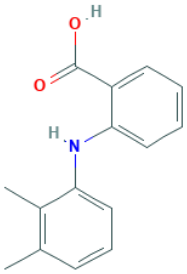
Table S1: Physicochemical properties and chemical structures of target analytes

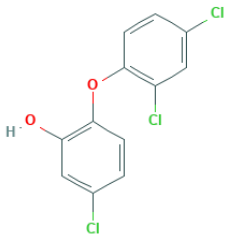
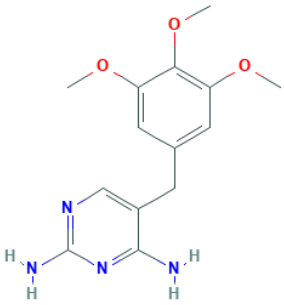
Compound	Chemical Structure	Molecular Formula	pKa	[Ref.]	logP	Therapeutic class
Acesulfame		$C_4H_5NO_4S$	2.0	[1]	-0.552	Artificial Sweetener
Amitriptiline		$C_{20}H_{23}N$	9.4	[2]	4.81	tricyclic antidepressants
Carbamazepine		$C_{15}H_{12}N_2O$	7.0/13.9	[3]	2.766	Psychiatric Antiepileptic Drugs
Clomipramine		$C_{19}H_{23}ClN_2$	8.98	[4]	4.883	Psychiatric Antidepressant Drugs
Cyclobenzaprine		$C_{20}H_{21}N$	8.47	[5]	4.613	Psychiatric Antidepressant Drugs
Diclofenac		$C_{14}H_{11}Cl_2NO_2$	4.2	[3]	4.259	Non-steroidal anti-inflammatory drug (NSAID)

Erythromycin		$C_{37}H_{66}NO_{12}$	8.9	[3]	2.596	Macrolide Antibiotic
Fluoxetine		$C_{17}H_{18}F_3NO$	10.1	[6]	4.173	Antidepressant Drugs
Indomethacin		$C_{19}H_{16}ClNO_4$	4.27	[3]	3.53	Analgesic-anti-inflammatory drug
Mefenamic Acid		$C_{15}H_{15}NO_2$	4.2	[7]	5.398	Nonsteroidal anti-inflammatory drugs (NSAID)
Paroxetine		$C_{19}H_{20}O_3NF$	9.6	[6]	3.148	Psychiatric Antidepressant Drugs

Salicylic acid		$C_7H_6O_3$	2.3/3.5	[6]	1.977	Non-steroidal anti-inflammatory drug (NSAID)
Sulfacetamide		$C_8H_{10}N_2O_3S$	5.4	[8]	-0.3	Sulfonamide Antibiotic
Sulfamethazine		$C_{12}H_{14}N_4O_2S$	7.6/2.65	[9]	0.65	Sulfonamide Antibiotic

Sulfamethoxazole		$C_{10}H_{11}N_3O_3S$	5.7/1.6	[9]	0.791	Sulfonamide Antibiotic

Sulfamethoxy-pyridazine		$C_{11}H_{12}N_4O_3S$	6.7	[9]	0.466	Sulfonamide Antibiotic
Sulfapyridine		$C_{11}H_{11}N_3O_2S$	6.24/2.63	[9]	1.009	Sulfonamide Antibiotic
Sulfaquinoxaline		$C_{14}H_{12}N_4O_2S$	6.79/2.16	[9]	1.552	Sulfonamide Antibiotic
Tolfenamic Acid		$C_{14}H_{12}ClNO_2$	4.3	[3]	5.488	Lipid regulator

Triclosan		$C_{12}H_7Cl_3O_2$	4.5/8.1	[3]	4.982	Disinfectant
Trimethoprim		$C_{14}H_{18}N_4O_3$	6.6/7.2	[3]	1.284	Sulfanilamide Antibiotic

Data concerning chemical structures, obtained from international database https://pubchem.ncbi.nlm.nih.gov/

S2. Materials and Methods

S2.1. Standard Solutions and reagents

Sulfacetamide, sulfapyridine, sulfamethazine, trimethoprim, sulfamethoxy-pyridazine, sulfamethoxazole, sulfaquinoxaline, Acesulfame-K, erythromycin, diclofenac, indomethacin, triclosan, mefenamic acid, tolfenamic acid were purchased from Sigma-Aldrich (Athens, Greece), salicylic acid was obtained from Merck KGaA (Darmstadt, Germany), paroxetine, cyclobenzaprine, carbamazepine, amitriptyline, fluoxetine, clomipramine was obtained from TCI Tokyo Chemical Industry (Zwijndrecht, Belgium)

Individual stock solutions of each compound, were prepared on a weight basis solution in methanol, (at a concentration of 1000 mg L^{-1}) with exception of sulfaquinoxaline that was prepared in acetonitrile since this compound is slightly soluble in pure methanol and freely soluble in acetonitrile. In addition, since erythromycin is easily degraded in the aquatic environment and converted into anhydroerythromycin (ERY-H2O) is always detected as this metabolite [10][11], therefore, preparation of ERY-H2O standard solution was performed according to procedure described elsewhere [10].

All stock standard solutions were stored at −20 ◦ C and refreshed every three months apart from antibiotics that were prepared monthly because of their limited stability. The mix of working solution containing all target compounds was prepared in methanol: ultrapure water (10:90, v/v) with 0.1% f. a v/v by diluting appropriate volume of stock solution. Working solutions were prepared before each analytical run.

Methanol, acetonitrile, formic acid (all MS grade), were purchased from Fisher Scientific (Leicester, UK). Ultrapure water (resistivity of 18.2 MΩ-cm) was obtained by using an Evoqua purification system (Evoqua, Pittsburg, USA). Ethylenediaminetetraacetic acid disodium salt 2-hydrate (Na_2EDTA) (assay 99.9–101.0%) was obtained from Panreac (Barcelona, Spain), sodium chloride (NaCl), and ammonium hydroxide (NH_4OH) from Riedel de Haën (Hannover, Germany). The starting material used to be coated as FPSE media were Whatman microfiber glass filters 110 mm (Boston, Massachusetts, USA). Organic polymer polyethylene glycol (PEG 300) was purchased from Sigma-Aldrich (Athens, Greece). Trimethoxymethylsilane (MTMS), trifluoroacetic acid (TFA) and sodium hydroxide and hydrochloric acid were supplied from Merck (Darmstadt, Germany).

S2.2. UHPLC–LTQ Orbitrap MS analysis

For the instrument method concerning positive ionization mode the gradient program started at 95% mobile phase A and was maintained for 1 min; the next minute the amount of mobile phase B increased to 70% followed by an increase to 100 % within 3 min, where it stayed stable for additional 2 min. Afterwards, the mobile phase was restored to the initial conditions of 95% A and maintained over 3 min for re-equilibration of the column. The total running time was 10 min with a flow rate of 250μL min-1 and injection volume set at 10μL. A gradient program with slight modifications was used for the separation of compounds ionized in negative mode: 90% of mobile phase (A) was used from 0- 0.5min, followed by consecutive linear declines to 30%A from 0.5 to 2.0 min, to 10% A from 2.0 to3.0 and 5%A from 3.0-3.9. In the 4.5 min of total run the percentage of methanol (B) increased to 100% and this composition was maintained for half a minute. Finally, the column was re-equilibrated with 90%A from 5.1 to 8.0minutes. The mobile phase was delivered at the flow rate of 200μL min−1 in a 35oC of thermostatted column. 20μL aliquot of sample was injected. Water-Methanol (30:70, v/v) mixture was employed as the solvent system for washing the sample loop and injector's needle.

Table S2: Parameters for full MS/dd-MS2 analysis in positive ionization mode.
Rt [a]: Retention time, NCE [b] Normalized Collision Energy

COMPOUND	Rt[a] (min)	Elemental formula	Theoretical mass (m/z)	Empirical mass (m/z)	Mass Error (ppm)	Fragm. Ion	Elemental Formula	NCE [b] %
Sulfacetamide	3.25	$C_8H_{11}N_2O_3S+$	215.0485	215.0486	0.514	108.0448 92.0500 156.0112	C_6H_6NO+ C_6H_6N+ $C_6H_6NO_2S+$	30
Sulfapyridine	3.71	$C_{11}H_{12}N_3O_2S+$	250.0645	250.0645	0.105	156.0112 108.0440 184.0867	C_6HNO_2S+ C_6H_6NO+ $C_{11}H_{10}N_3+$	30
Trimethoprim	3.75	$C_{14}H_{19}N_4O_{3+}$	291.1452	291.1453	0.457	230.1161 261.0980	$C_{12}H_{14}N_4O+$ $C_{12}H_{13}N_4O_3+$	30
Sulfamethazine	3.90	$C_{12}H_{15}N_4O_2S+$	279.0910	279.0909	-0.441	123.0662	C_6H_9N3+	30
Sulfamethoxy-pyridazine	3.92	$C_{11}H_{13}N_4O_3S+$	281.0703	281.0703	0.045	156.0113 108.0430 92.0486	$C_6H_6NO_2S+$ C_6H_6NO+ C_6H_6N+	20
Sulfamethoxazole	4.01	$C_{10}H_{12}N_3O_3S+$	254.0594	254.0592	-0.742	188.0818 156.0113 147.0789	$C_{10}H_{10}N_3O+$ $C_6H_6NO_2S+$ $C_8H_9N_3+$	20
Sulfaquinoxaline	4.28	$C_{14}H_{13}N_4O_2S+$	301.0754	301.0754	0.090	156.0113 108.0440	C_6HNO_2S+ C_6H_6NO+	30
Paroxetine	4.49	$C_{19}H_{21}O_3NF+$	330.1500	330.1502	0.611	192.1180 151.0387	$C_{12}H_{15}NF+$ $C_8H_7O_3+$	35
Cyclobenzaprine	4.52	$C_{20}H_{22}N+$	276.1747	276.1749	0.810	58.0659 84.0814	C_3H_8N+ $C_5H_{10}N+$	30
Erythromycin-H_2O	4.57	$C_{37}H_{66}NO_{12}$	716.4580	716.4591	1.601	158.1175 558.3635	$C_8H_{16}O2N+$ $C_{26}H_{54}O_{12}$	40
Amitriptiline	4.62	$C_{20}H_{24}N+$	278.1903	278.1905	0.624	233.1322 191.0854 155.0854 117.0695	$C_8H_{17}+$ $C_{15}H_{11}+$ $C_{12}H_{11}+$ C_9H_9+	30
Fluoxetine	4.67	$C_{17}H_{19}F_3NO+$	310.1413	310.1415	0.563	148.1119 247.0918	$C_{10}H_{14}N+$ $C_4H_{18}FN_3+$	15
Carbamazepine	4.73	$C_{15}H_{13}N_2O$	237.1022	237.1024	0.677	194.0964	$C_{14}H_{12}N+$	35

Clomipramine	4.79	$C_{19}H_{24}ClN_{2+}$	315.1623	315.1627	1.418	220.0756$C_{15}H_{10}NO+$ 192.0808$C_{14}H_{10}N+$ 86.0940 $C_5H_{12}N+$ 58.059 C_3H_8N+	30

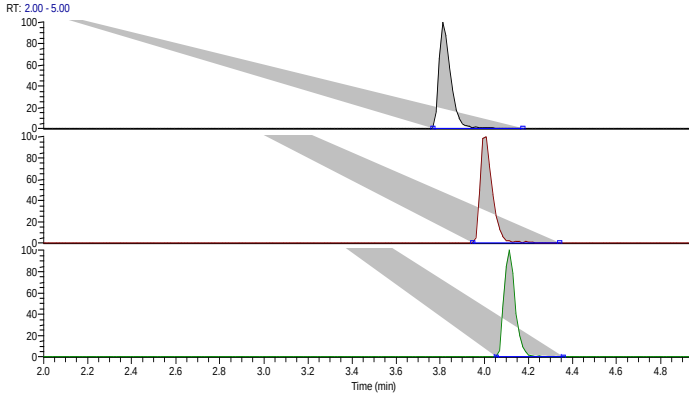
Table S3: Parameters for full MS/dd-MS2 analysis in negative ionization mode.

COMPOUND	Rt (min)	Elemental formula	Theoretical mass (m/z)	Empirical mass (m/z)	Mass Error (ppm)	Fragm. Ion	Elemental Formula	NCE % [b]
Acesulfame	2.44	$C_4H_4NO_4S^-$	161.9867	161.9868	0.916	77.9642 82.0285	NO_2S- C_4H_4NO-	30
Salicylic acid	5.04	$C_7H_5O_3^-$	137.0244	137.0250	4.252	93.00334 65.0384	C_6H_5O- C_5H_5-	30
Indomethacin	5.97	$C_{19}H_{15}ClNO_4^-$	356.0676	356.0675	-2.281	312.0796	$C_{18}H_{15}NO_2Cl$-	30
Diclofenac	6.04	$C_{14}H_{10}Cl_2NO_2^-$	294.0094	294.0089	-1.725	250.0193	$C_{13}H_{10}CL_2N$-	35
Mefenamic Acid	6.51	$C_{15}H_{14}NO_2^-$	240.103	240.1026	-1.674	196.1133 240.1029	$C_{14}H_{14}N$- $C_{15}H_{14}NO_2$-	20
Triclosan	6.54	$C_{12}H_6Cl_3O_2^-$	286.9439	286.9433	-2.042	161.2632	$C_6H_3CL_2O$-	25
Tolfenamic Acid	6.76	$C_{14}H_{11}ClNO_2^-$	260.0484	260.0480	-1.460	216.0580	$C_{14}H_{11}ClNO_2$-	20

Rt [a]: Retention time, NCE [b] Normalized Collision Energy

Figure.S1 Chromatograms of selected pharmaceuticals of standard solution at concentration of 5 µg/L. A) Hypersil Gold C18 (100mmx2.1, 1.9µm), B) Speedcore- Diphenyl (50mmx2.1, 2.6µm).

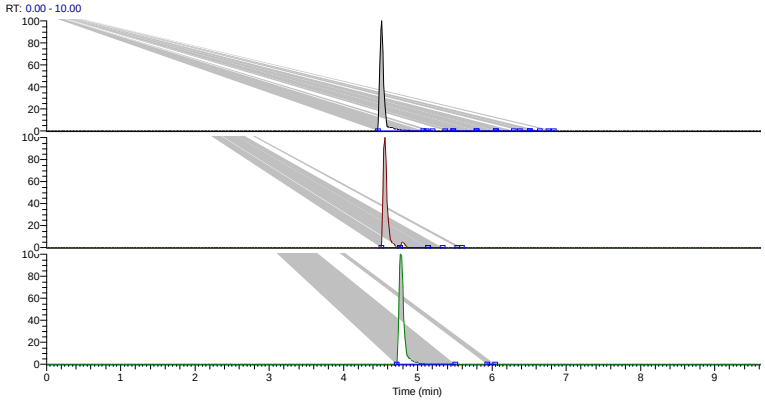
A) Hypersil Gold C18 (100mmx2.1, 1.9µm)



SULFAPYRIDINE

SULFAMETHOXAZOLE

SULFAQUINOXALINE

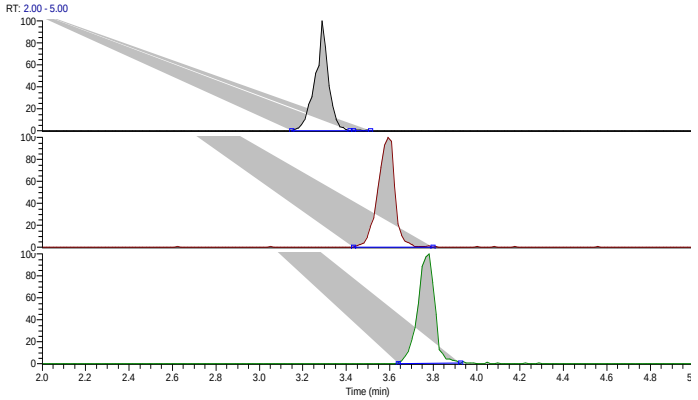


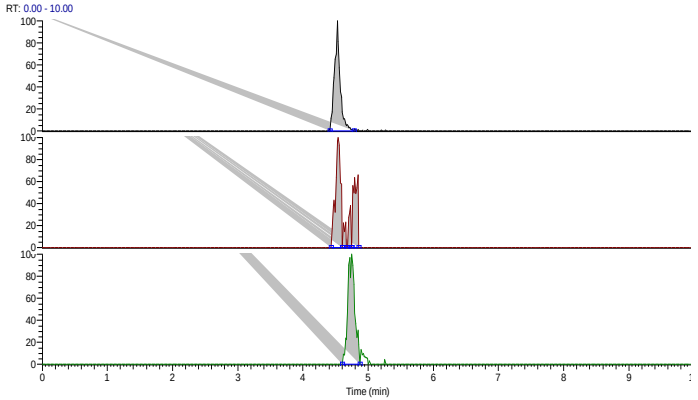
CYCLOBENZAPRINE

ERYTHROMYCIN-H₂O

CLOMIPRAMINE

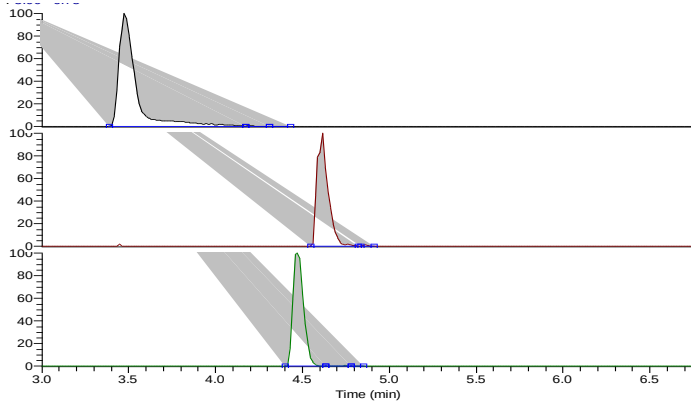
B) Speedcore- Diphenyl (50mmx2.1, 2.6µm)





A) Hypersil Gold C18 (100mmx2.1, 1.9µm)

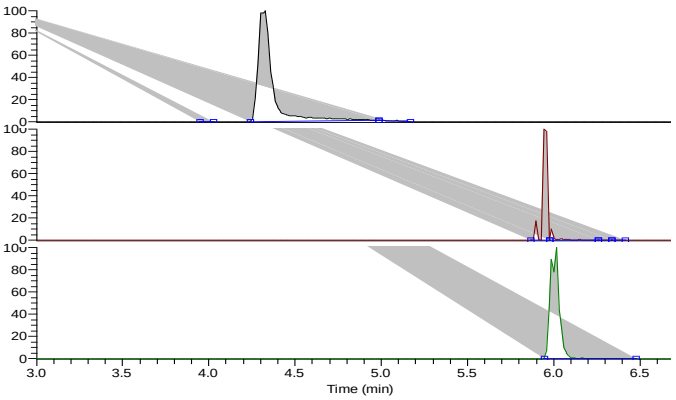
B) Speedcore- Diphenyl (50mmx2.1, 2.6µm)

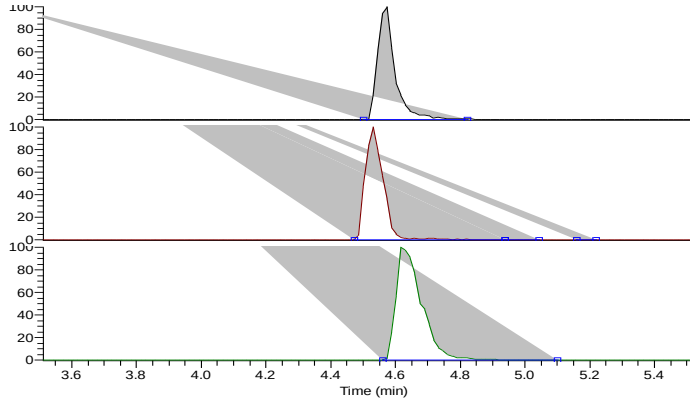


SALICYLIC ACID

INDOMETHACIN

DICLOFENAC





MEFENAMIC ACID

TRICLOSAN

TOLFENAMIC ACID

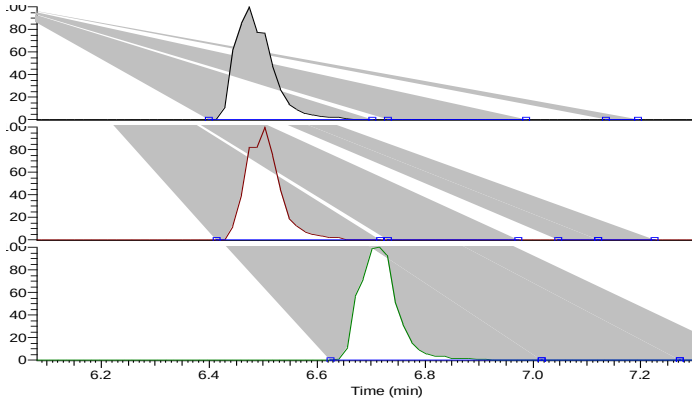
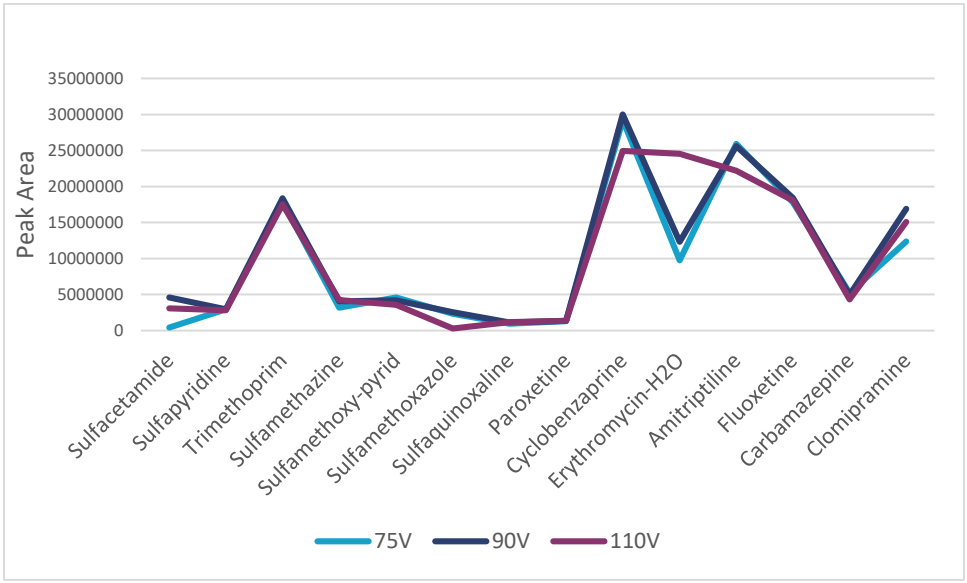


Figure S2: Response variance with different voltage of Tube Lens a) Positive Ionization, b) Negative Ionization.

(a)



(b)

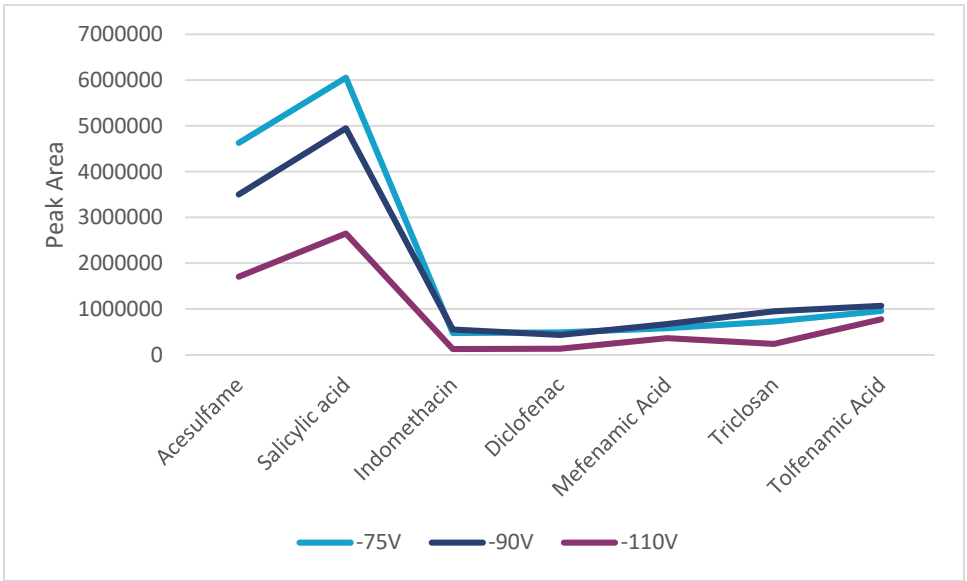
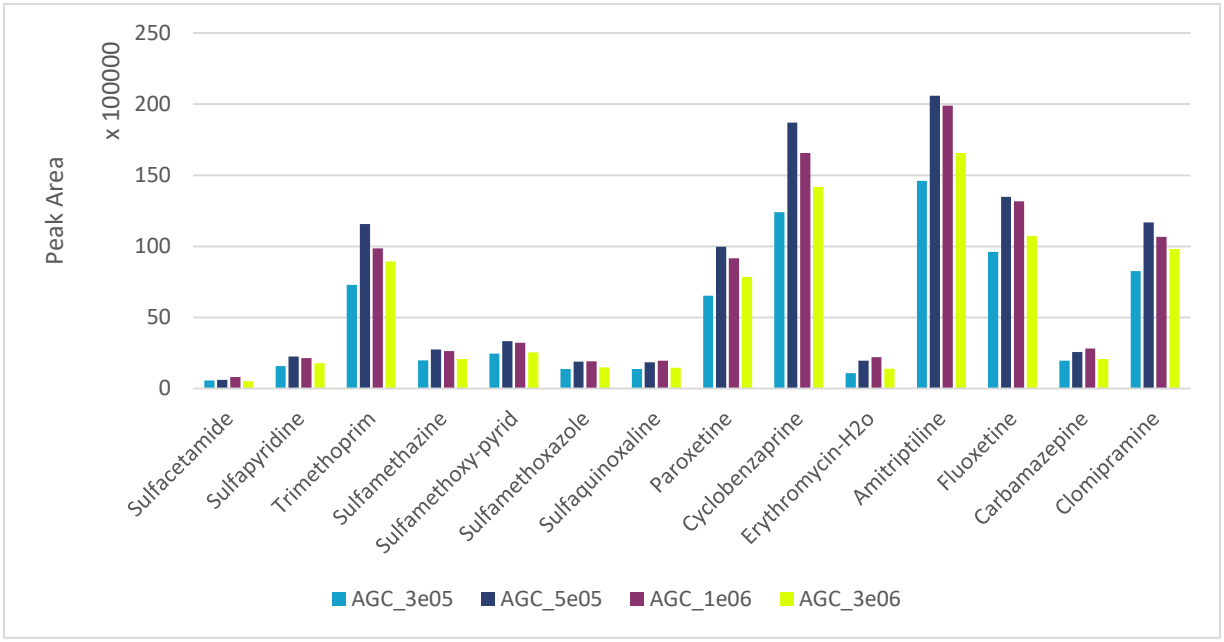


Figure S3: Effect of AGC target values on the response of studied analytes a) positive and b) negative ionization mode.

(a)



(b)

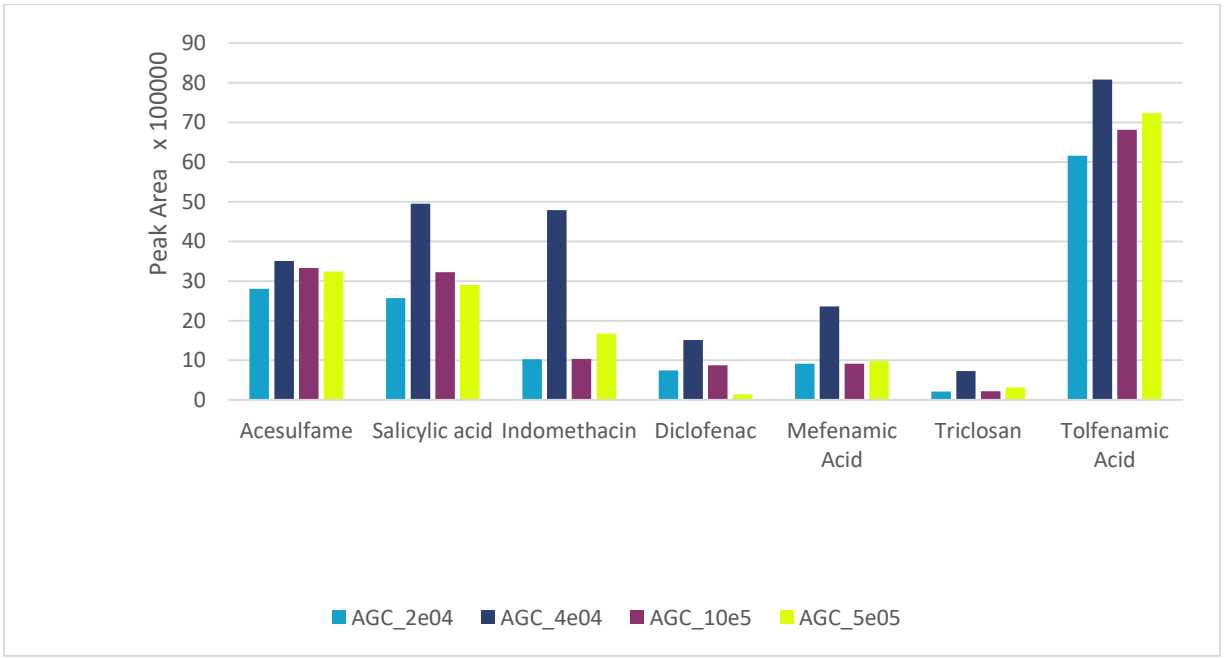
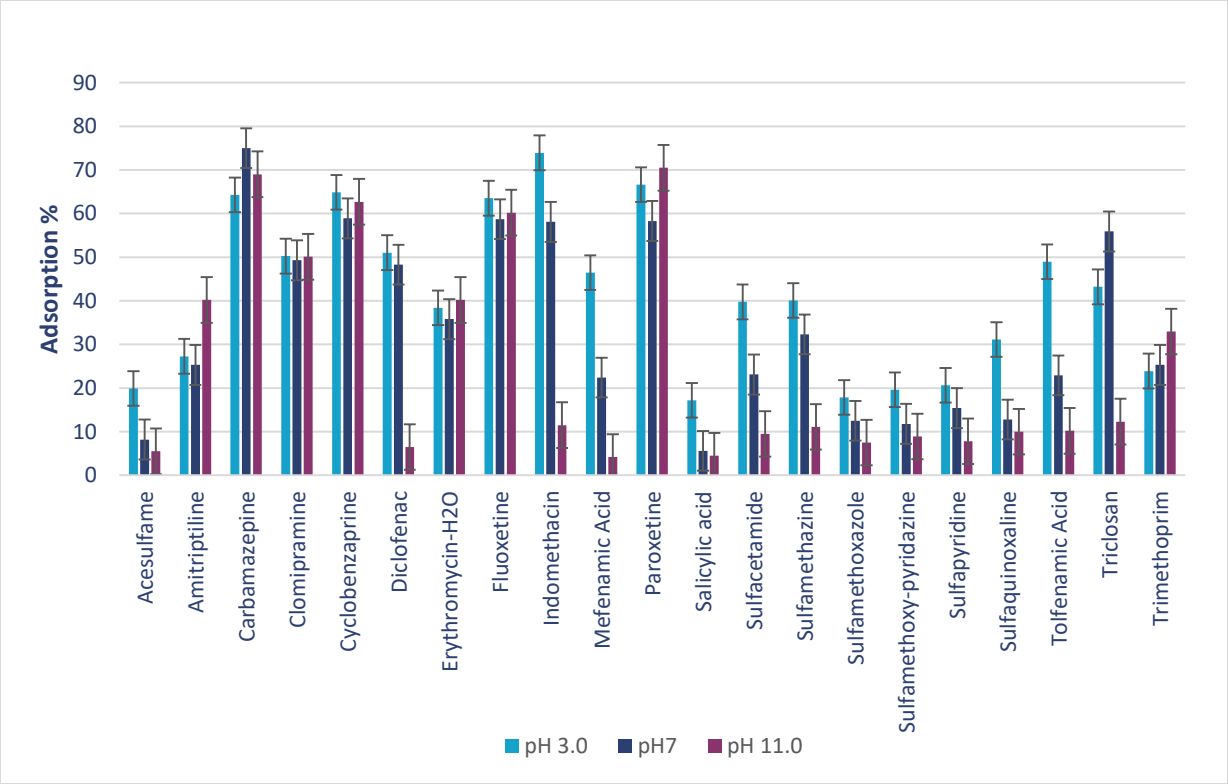
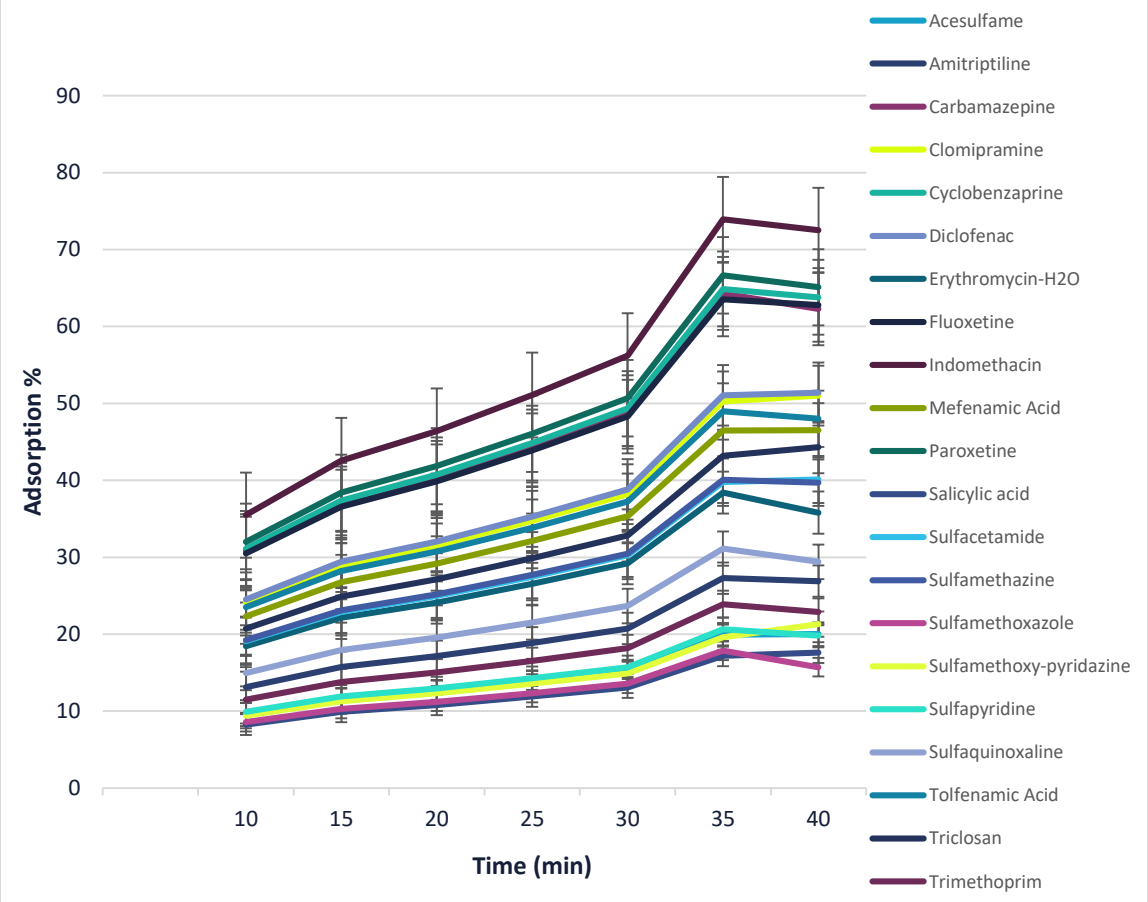


Figure S4: Optimization of FPSE extraction: (a) sample pH, (b) extraction time, (c) ionic strength

(a)



(b)


Adsorption %
Time (min)
0
10
20
30
40
50
60
70
80
90
10
15
20
25
30
35
40
Acesulfame
Amitriptiline
Carbamazepine
Clomipramine
Cyclobenzaprine
Diclofenac
Erythromycin-H2O
Fluoxetine
Indomethacin
Mefenamic Acid
Paroxetine
Salicylic acid
Sulfacetamide
Sulfamethazine
Sulfamethoxazole
Sulfamethoxy-pyridazine
Sulfapyridine
Sulfaquinoxaline
Tolfenamic Acid
Triclosan
Trimethoprim

(c)

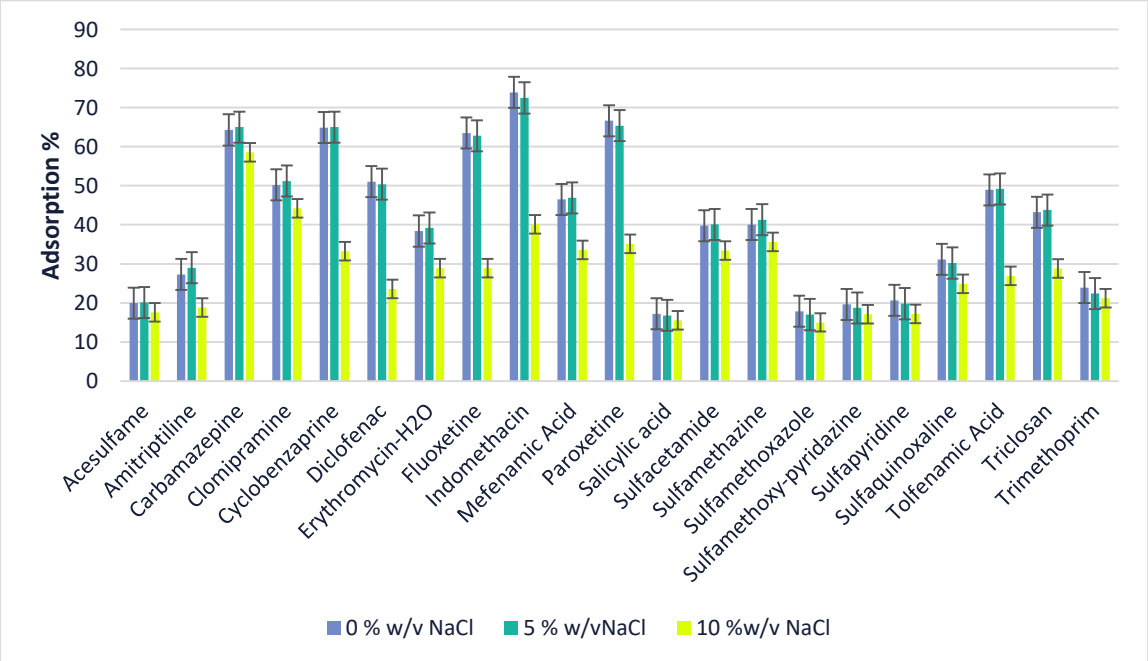
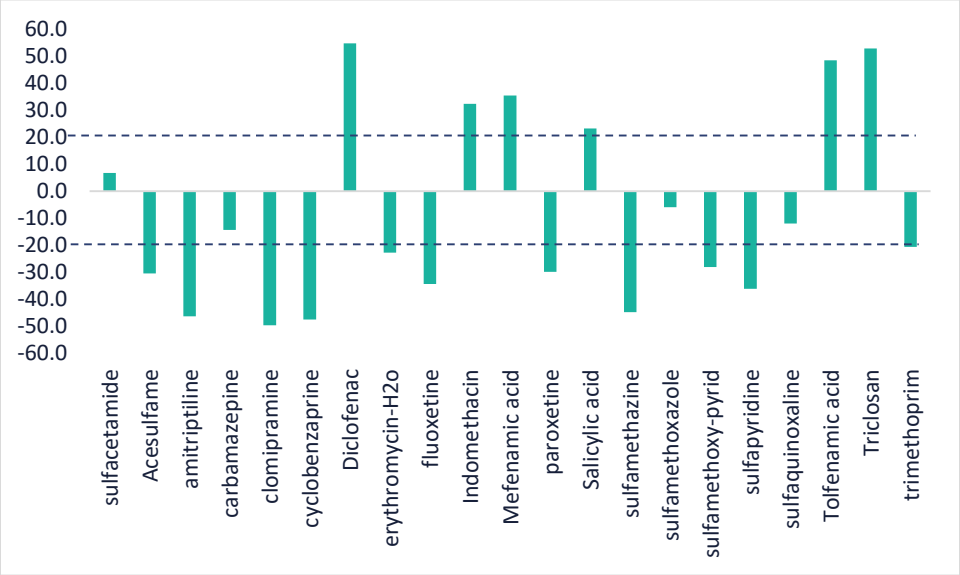

Adsorption %
0
10
20
30
40
50
60
70
80
90
Acesulfame
Amitriptiline
Carbamazepine
Clomipramine
Cyclobenzaprine
Diclofenac
Erythromycin-H2O
Fluoxetine
Indomethacin
Mefenamic Acid
Paroxetine
Salicylic acid
Sulfacetamide
Sulfamethazine
Sulfamethoxazole
Sulfamethoxy-pyridazine
Sulfapyridine
Sulfaquinoxaline
Tolfenamic Acid
Triclosan
Trimethoprim
0 % w/v NaCl
5 % w/vNaCl
10 %w/v NaCl

Table S4: Recoveries and precision results expressed as RSD_r and RSD_R: within each spiking level

Compound	RR% n=3	RSD_r% n=5	RSD_R% n=15	RR% n=3	RSD_r% n=5	RSD_R% n=15	R% n=3	RSD_r% n=5	RSD_R% n=15
		LOQ			10 xLOQ			100 xLOQ	
Acesulfame	83.2	1.7	5.8	92.0	1.9	2.7	93.0	3.1	4.0
Amitriptyline	81.1	6.1	7.9	86.8	4.7	5.2	82.5	3.9	4.2
Carbamazepine	96.3	5.4	8.2	98.6	2.3	3.3	97.1	1.9	3.3
Clomipramine	90.7	2.4	5.0	94.3	2.0	2.5	91.9	1.8	2.5
Cyclobenzaprine	99.2	4.9	6.9	100.1	3.7	4.0	98.7	2.8	3.2
Diclofenac	90.5	7.9	10.2	99.4	7.8	8.3	97.7	6.9	9.5
Erythromycin-H2o	90.1	2.1	4.0	95.6	2.5	2.8	94.1	1.7	2.5
Fluoxetine	96.8	2.8	4.2	99.1	1.8	2.8	97.8	2.2	2.9
Indomethacin	120.1	6.5	8.5	114.0	5.8	6.9	101.3	5.4	6.2
Mefenamic acid	102.3	7.2	118.0	98.5	7.2	10.7	97.9	7.7	11.0
Paroxetine	105.9	3.8	6.2	85.3	2.1	3.1	84.6	3.1	3.9
Salicylic acid	84.9	7.7	13.3	95.3	7.4	11.0	92.1	7.9	10.9
Sulfacetamide	101.3	1.2	5.0	111.1	1.3	1.8	105.3	1.8	2.5
Sulfamethazine	79.5	1.3	7.1	83.7	1.1	2.1	81.2	1.2	2.0
Sulfamethoxazole	80.1	0.9	3.6	88.6	0.7	1.8	87.5	1.0	1.4
Sulfamethoxy-pyrid	79.3	1.0	5.2	90.2	0.9	2.4	90.3	0.8	1.3
Sulfapyridine	95.6	0.8	4.6	99.8	0.1	1.9	95.6	0.7	1.9
Sulfaquinoxaline	101.2	0.7	3.9	100.2	1.2	2.0	112.3	2.0	2.8
Tolfenamic acid	79.1	8.0	13.5	85.3	7.2	11.0	88.1	7.2	10.8
Triclosan	97.7	8.0	12.0	104.0	7.1	10.9	99.7	6.1	10.4.
Trimethoprim	82.1	1.8	7.3	85.6	1.7	3.1	84.7	1.4	2.0

Figure S5. Matrix effects for the target analytes in the effluent water



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