DETERMINATION OF EMERGING ORGANIC POLLUTANTS IN WASTEWATER BY "ON-LINE SPE" LC/QqLIT AND IDENTIFICATION BY MS/MS SPECTRUM

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INTRODUCTION

Last years have been growing the interest in the quantitation of pharmaceuticals and drugs, called Emerging Organic Contaminants (EOPs), in drinking water or wastewater, in order to control the presence of these compounds and to avoid future problems on the people. LC/MS is the appropriate technique because of the selectivity, good sensitivity, linearity and robustness. Problems had begun when confirmation of target compounds must to be done and identification of unknown compounds is also needed. Mainly at very low concentrations, MRM ratios are not so consistent for confirmation. Hybrid QTrap MS/MS (QqLIT) systems allow to do that confirmation issues.

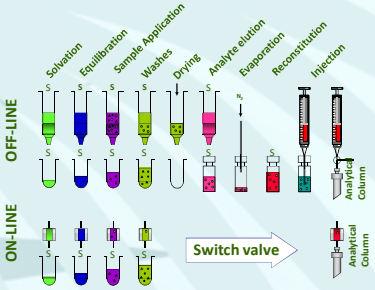
"On-Line SPE" offer several features to improve the performance of the method: less sample volume, less timework required, better recoveries. Only 1 ml sample is needed to achieve the detection limit required.

INSTRUMENTATION

Symbiosis Pico XLC System

3200 QTrap™ LC/MS/MS System

Off-Line vs On-Line SPE



EXPERIMENTAL

Symbiosys Pico "On-Line SPE" automated system from Spark Holland coupled with 3200 QTrap™ LC/MS/MS system from ABSCIEX, have been the instrumentation used. "On-Line SPE" basics are depicted in the figure above.
1 ml of sample was injected to the system. Previously cartridge (Hysphere 8µm GP Resin, Spark Holland, Emmen, Holland).was conditioned and prepared for the extraction. Once the sample was applied, cartridge was washed and, then, mobile phase from the LC pump pass thru the cartridge to elute the analytes to the column. Steps, solvents and conditions are summarized in Table I. Mobile phases were: Water, 2mM ammonia formiate, 0.1% formic acid (A) and acetonitrile (B). LC Gradient is shown in Table II. LC Column used was Luna C18(2) 50mm, 2.1mm, 3µm (Phenomenex, Torrance, CA, USA).

Table I. "On-line" SPE Conditions

Step	Solvent	Flowrate (µl/min)	Volume (µl)
Conditioning	MeOH	3000	2000
Equilibration	Water	3000	1000
Sample Extraction	Water	1000	1500
Sample Wash	Water	2000	1000
System wash	Water	2000	1000
System wash 2	Water	2000	1000
Elution Time (min)	10:00		

Table II. Chromatographic Conditions

Time (min)	Flowrate (ml/min)	A (%)	B (%)
00:01	0.30	80	20
01:00	0.30	80	20
07:00	0.30	0	100
10:00	0.30	0	100
10:01	0.30	80	20
15:00	0.30	80	20

Table I. LC/MS Source Conditions

Parameter	Value	Units
Curtain Gas	20	psi
Nebulizer Voltage	5500	V
Turbo Temperature	500	ºC
Nebulizer Gas	40	psi
Turbo Gas	70	psi
Collision Gas	High	

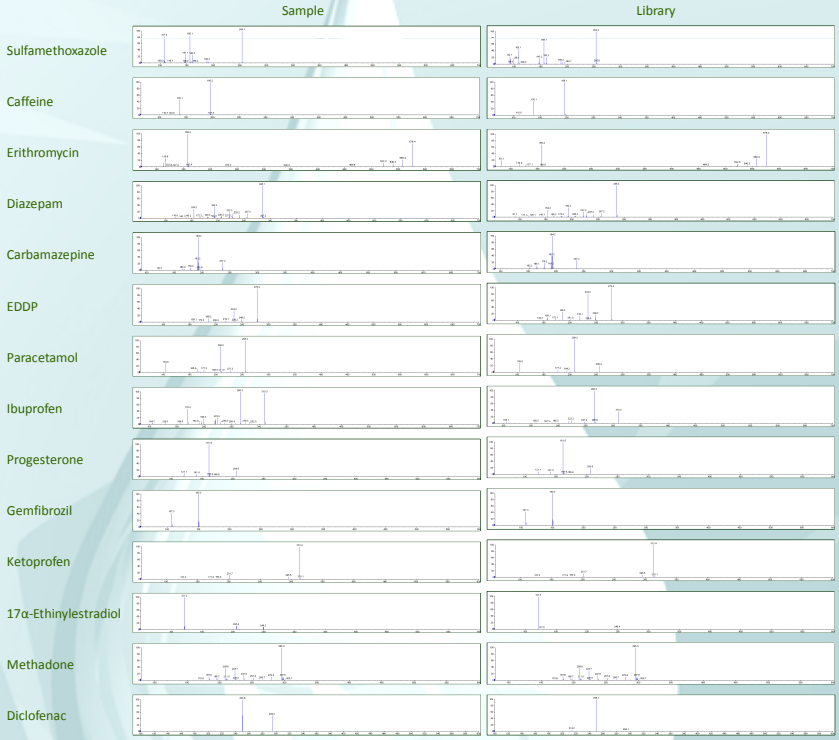
Mass Spectrometer Turbo V™ source conditions are summarized in Table III. Acquisition has been made in MRM Mode and Identification of analytes has been made by QTrap via EPI and thru IDA criteria. Recognition of the acquired MS/MS spectra was done by comparison with commercial available "Forensic" Library from ABSCIEX.

RESULTS AND DISCUSSION

The method developed in this work include 114 compounds from different families shown In Table IV, together with the MRM conditions. This MRMs (one per compound) allow us to quantitate. Method has been validated according EC 657/2002. CCα was found to be lower than 1 µg/l for all compounds arriving, individually, to 0.05 µg/. Linearity, precision and Accuracy wee inside accetable limits.

Table IV. Analytes characteristics and MS/MS Conditions

Analyte	Family	Pol.	[M+H]+ (m/z)	Product (m/z)	DP (V)	CE (eV)
Enalapril	ACE inhibitor	+	377.0	234.0	61	29
Acetaminophen	Analgesic	-	150.0	107.0	-50	-25
Acetylsalicylic acid	Analgesic	-	179.0	137.0	-15	-6
Diazepam	Ansiolytic	+	285.0	193.0	51	43
Meprobamate	Ansiolytic	+	219.0	158.0	36	13
Amoxicillin	Antibiotic	-	364.0	286.0	-25	-14
Carbadox	Antibiotic	-	261.0	122.0	-50	-25
Chloramphenicol	Antibiotic	-	321.0	152.0	-50	-25
Chlorotetracycline	Antibiotic	+	479.0	444.0	40	25
Ciprofloxacin	Antibiotic	+	332.0	314.0	40	30
Doxycycline	Antibiotic	+	445.0	428.0	40	25
Enrofloxacin	Antibiotic	+	360.0	342.0	40	30
Erythromycin	Antibiotic	+	734.0	158.0	40	32
Lasalocid A	Antibiotic	-	589.0	235.0	-50	-45
Lincomycin	Antibiotic	+	407.0	126.0	40	32
Meclocycline	Antibiotic	+	477.0	460.0	40	35
Monensin sodium	Antibiotic	+	694.0	676.0	40	40
Norfloxacin	Antibiotic	+	320.0	302.0	40	25
Oxytetracline	Antibiotic	+	461.0	426.0	40	22
Penicillin G	Antibiotic	+	335.0	176.0	40	25
Roxithromycin	Antibiotic	+	838.0	158.0	40	40
Sulfachloropyridazine	Antibiotic	+	285.0	156.0	40	20
Sulfadiazine sodium	Antibiotic	-	249.0	185.0	-50	-25
Sulfadimethoxine	Antibiotic	+	311.0	156.0	40	30
Sulfamerazine	Antibiotic	+	265.0	156.0	40	23
Sulfamethazine	Antibiotic	+	279.0	186.0	40	23
Sulfamethizole	Antibiotic	+	271.0	156.0	40	20
Sulfamethoxazole	Antibiotic	+	254.0	156.0	51	23
Sulfathiazole	Antibiotic	+	256.0	156.0	40	20
Tetracycline	Antibiotic	+	445.0	410.0	40	22
Triclosan	Antibiotic	-	287.0	35.0	-45	-38
Trimethoprim	Antibiotic	+	291.0	261.0	81	37
Tylosin	Antibiotic	+	916.0	174.0	40	50
Virginiamycin M1	Antibiotic	-	524.0	245.0	-50	-25
Warfarin	Anticoagulant	-	307.0	161.0	-50	-25
Fluoxetine	Antidepressive	+	310.0	44.0	51	37
Norfluoxetine	Antidepressive	+	296.0	134.0	31	11
Carbamazepine	Antiepileptic	+	237.0	194.0	31	25
Dilantin	Antiepileptic	+	253.0	182.0	66	29
Diclofenac	Anti-inflammatory	-	294.0	250.0	-25	-18
Flunisolide	Anti-inflammatory	+	435.0	321.1	6	19
Fluocinolone acetonide	Anti-inflammatory	+	453.1	413.2	11	17
Ibuprofen	Anti-inflammatory	-	205.0	161.0	-40	-6
Indomethacin	Anti-inflammatory	-	356.0	312.0	-50	-15
Ketoprofen	Anti-inflammatory	+	255.0	105.0	40	20
Naproxen	Anti-inflammatory	+	231.0	185.0	40	20
Triamcinolone	Anti-inflammatory	+	391.2	375.0	46	15
Triamcinolone acetonide	Anti-inflammatory	+	435.0	415.1	6	15
Risperidone	Antipsychotic	+	411.0	110.0	76	69
Acebutolol	Beta blocker	+	337.0	116.2	21	29
Alprenolol	Beta blocker	+	249.9	116.2	16	23
Atenolol	Beta blocker	+	267.0	145.0	61	37
Bisoprolol	Beta blocker	+	326.1	116.2	21	25
Metoprolol	Beta blocker	+	268.0	116.3	21	23
Nadolol	Beta blocker	+	310.1	254.1	21	23
Pindolol	Beta blocker	+	248.9	116.2	16	23
Propranolol	Beta blocker	+	259.9	116.3	16	23
Sotalol	Beta blocker	+	272.9	255.1	6	17
Timolol	Beta blocker	+	317.0	261.1	16	23
Atorvastatin	Cholesterol reducer	+	559.0	440.0	66	31
o-Hydroxy atorvastatin	Cholesterol reducer	+	575.0	440.0	91	31
p-Hydroxy atorvastatin	Cholesterol reducer	+	575.0	440.0	91	31
Simvastatin	Cholesterol reducer	-	399.0	115.0	-90	-30
Simvastatin hydroxy acid	Cholesterol reducer	-	435.0	319.0	-60	-24
Betamethasone	Corticoid	+	393.0	373.1	41	13
Budesonide	Corticoid	+	431.0	413.3	6	15
Cortisone	Corticoid	+	361.0	163.1	26	31
Dexamethasone	Corticoid	+	393.1	373.1	41	13
Diethylsilbestrol	Corticoid	-	267.0	251.0	-50	-35
Hydrocortisone	Corticoid	+	362.9	327.2	31	29
Prednisolone	Corticoid	+	360.9	343.1	11	15
Prednisone	Corticoid	+	358.9	313.0	21	17
PFBS	Desinfectant	-	299.0	80.0	-50	-45
PFOA	Desinfectant	-	413.0	169.0	-50	-25
PFOS	Desinfectant	-	499.0	80.0	-50	-75
Bisphenol A	Endocrine disruptor	-	227.0	212.0	-80	-28
Bezafibrate	Fibrate drug	-	360.0	274.0	-50	-25
Cemfibrozil	Fibrate drug	-	249.0	121.0	-45	-28
Clofibric acid	Fibrate drug	-	213.0	127.0	-50	-20
Gemfibrozil	Fibrate drug	-	249.0	121.0	-50	-15
Atrazine	Herbicide	+	216.0	174.0	71	27
Linuron	Herbicide	+	249.0	160.0	61	27
17-α-Ethynyl Estradiol	hormone	-	295.0	145.0	-50	-50
17-β-Estradiol	hormone	-	271.0	145.0	-65	-52
19-Norethersterone	hormone	-	297.0	269.0	-50	-35
Equilin	hormone	-	267.0	143.0	-50	-45
Esterone	hormone	-	269.0	145.0	-50	-50
Estriol	hormone	-	287.0	171.0	-50	-50
Progesterone	hormone	+	315.0	109.0	40	30
6-acetylmorphine	Illicit drug	+	328.4	165.0	90	80
Acetylcodeine	Illicit drug	+	342.2	225.2	131	37
Amphetamine	Illicit drug	+	136.1	90.9	46	15
Cocaethylene	Illicit drug	+	318.4	196.0	70	30
Cocaine	Illicit drug	+	304.4	182.0	70	30
Codeine	Illicit drug	+	300.1	215.1	151	37
EDDP	Illicit drug	+	278.2	234.1	26	43
Ephedrine	Illicit drug	+	166.2	148.0	40	20
Heroin	Illicit drug	+	370.4	268.0	70	50
LSD	Illicit drug	+	324.4	208.0	70	40
MDMA	Illicit drug	+	194.3	163.0	50	20
Methadone	Illicit drug	+	310.2	265.2	41	17
Methamphetamine	Illicit drug	+	150.2	91.0	50	30
Morphine	Illicit drug	+	286.3	152.0	90	75
Nor-LSD	Illicit drug	+	310.4	193.0	60	40
Nor-THC	Illicit drug	-	343.5	299.5	-100	-35
OH-LSD	Illicit drug	+	356.4	237.0	50	35
OH-THC	Illicit drug	-	329.5	311.2	-70	-25
THC	Illicit drug	+	315.0	193.0	50	35
Δ9-THC	Illicit drug	-	313.5	245.1	-70	-40
Benzoylecgonine	Illicit drug metabolite	+	290.3	168.0	80	35
Morphine 3-ß-D-glucuron.	Illicit drug metabolite	+	462.5	286.0	80	45
Morphine 6-ß-D-glucuron.	Illicit drug metabolite	+	462.6	286.0	80	45
THC-COOH	Illicit drug metabolite	+	345.3	327.3	60	19
Caffeine	Licit drug	+	195.1	138.2	41	27



Antibiotics, anti-inflammatory, analgesics, licit and illicit drugs, among other are included in the list (Table IV). In the figure above, spectra of several compounds of the method (the most common appeared in the samples are shown (left side), compared with the spectra coming from the commercial library (on the right), showing all very good fit, over 80 %.

CONCLUSIONS

This job shows a very rapid and effective method using all the most of the features of a QqLIT system to get very good sensitivity and identification capabilities. Using MRM transition to quantitate and MS/MS spectrum obtained with the Linear Ion Trap for identification purposes is possible to work with a low resolution system and found unknown compounds from a pre-target list.