Liquid chromatography-tandem mass spectrometry as a powerful tool for the determination of pharmaceuticals in environmental samples

Meritxell Gros, Mira Petrovic and Damià Barceló

IIQAB-CSIC, Environmental Chemistry Department, Barcelona, Spain

1st Thematic Workshop of the EU project NORMAN

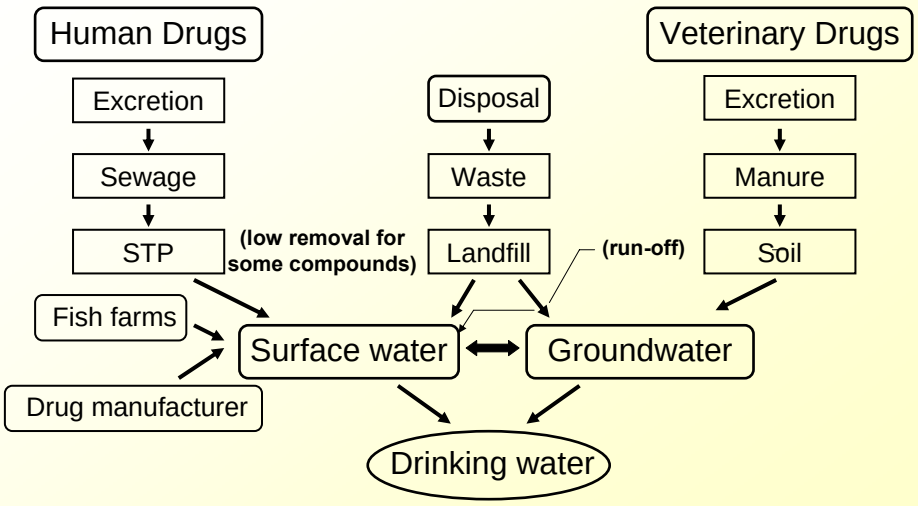
CHEMICAL ANALYSIS OF EMERGING POLLUTANTS

OUTLINE

- Introduction
  - Sources of pharmaceuticals
  - Suitability of LC-tandem MS for drug analysis in environmental samples according to EC directives
- Analytical protocols based on off line SPE -LC-tandem MS for the identification of pharmaceuticals in natural waters:
  - HPLC – QqQ
  - HPLC – Q-TRAP
  - UPLC-Q-TOF
- Performance comparison between the LC-tandem MS techniques
- Conclusions

Sources and fate

INTRODUCTION



Suitability of LC-tandem MS

INTRODUCTION

Identification and confirmation criteria for the analysis of drugs and other contaminants are defined in Directive 96/23/EC and Commision Decision 2002/657/EC, requiring a minimum of 3 identification points

Technique	Number of IP earned per ion	Example per ions	IP earned
LC-MS (Q)	1	SIM	1
LC-MS-MS (QqQ)	1 for precursor ion 1.5 for transition product	1 precursor 1 product (SRM)	2.5
		1 precursor 2 products (2 SRM)	4
		2 precursors, each with 1 product (2 SRM)	5
LC-Q-TOF-MS	2	One ion from a full scan	2
	2 for precursor ion 2.5 for transition product	1 precursor 1 product (MS/MS)	4.5
		1 precursor 2 products (MS/MS)	7.5

LC-TANDEM MS

Quantitative target analysis
Identification/Confirmation
Structural Information

(Semi)quantitative target analysis
Identification/Confirmation
Non Target analysis/Screening

HPLC – Quattro LC (QqQ)™

✓ MRM
✓MS/MS scans
✓Neutral loss scans

Waters Corporation (Manchester, UK)

HPLC – MDS Sciex (QTRAP)™

✓ MRM
✓MS^3
✓High sensitive MS/MS and MS scans

Applied Biosystems

ACQUITY UPLC™ – Q – TOF Micro™

ACQUITY UPLC™ BEH C18 1.7 μm (2.1 x 50 mm)

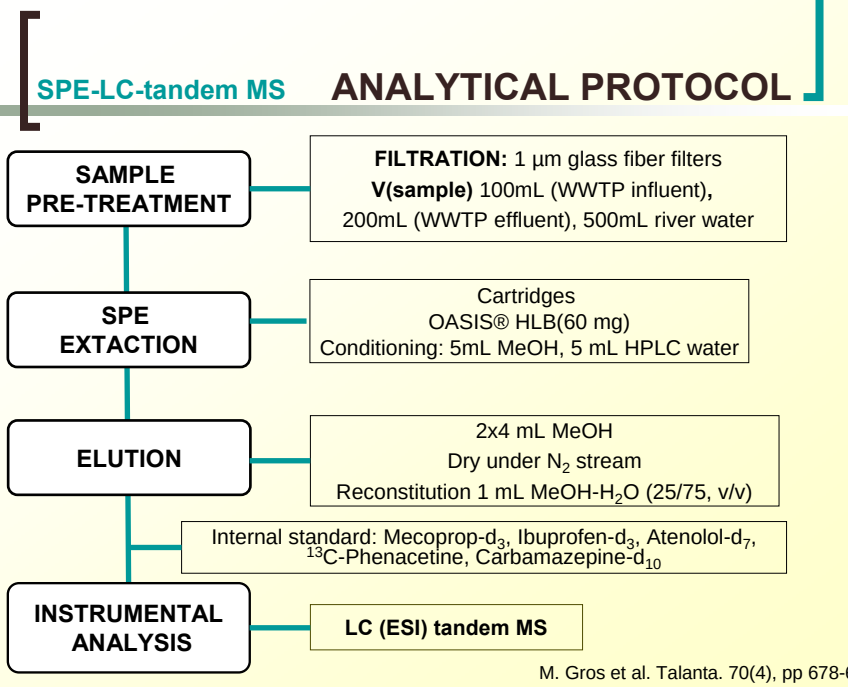
✓ TOF MS
✓ TOF MS/MS

Waters Corporation (Manchester, UK)

TARGET COMPOUNDS

Analgesics and antiinflammatories	Ibuprofen Ketoprofen Naproxen Diclofenac	Indomethacine Acetaminophen Mefenamic acid Phenylphenazone	To relief pain, inflammation and fever
Lipid regulator and cholesterol lowering statin drugs	Clofibric acid Gemfibrozil Bezafibrate	Pravastatin Mevastatin	To lower fat (lipids) level
Psychiatric drugs	Carbamazepine Fluoxetine	Paroxetine	Antidepressants Antiepileptics
Antiulcer agent	Lansoprazole		To prevent and treat ulcers
Anti-histaminics	Famotidine Ranitidine Loratadine		To relieve allergy symptoms
Antibiotics	Erythromycin Azithromycin Sulfamethoxazole	Trimethoprim Ofloxacin	Antibacterial agents
β-blockers	Atenolol Sotalol	Metoprolol Propanolol	Antianginal antihypertensive

SPE-LC-tandem MS — ANALYTICAL PROTOCOL



M. Gros et al. Talanta. 70(4), pp 678-690

HPLC conditions — HPLC-QqQ

Equipment: Waters 2690 HPLC (Milford, MA, USA)

Column: Purospher ® RP-18 (125x2mm) (5 µm)

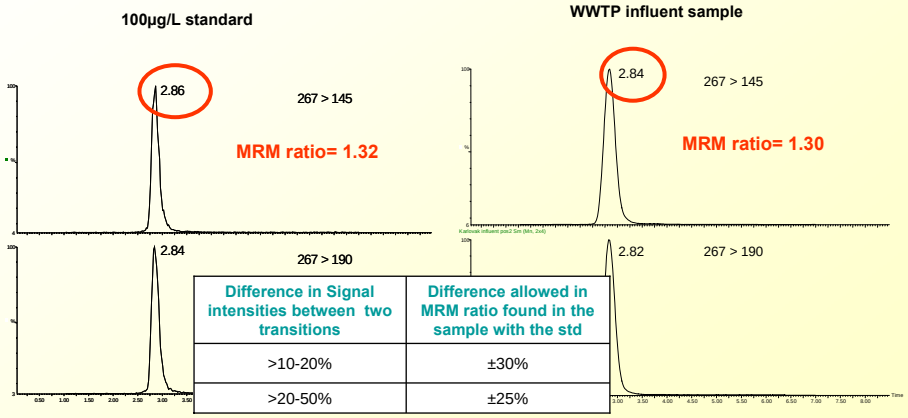
Flow: 0.2 mL/min

NI	
Mobile Phase	Solvent A: H_2O/ Solvent B: MeOH
Gradient	0 min (80%A-20%B), 0-20 min (20%A-80%B), 20-24 min (10%A-90%B), 24-27 min (10%A-90%B), 27-45 min (80%A-20%B) **TOTAL RUN: 45min**
PI	
Mobile Phase	Solvent A: NH_4A_C 5mM / HA_c (pH=4.8) / Solvent B: AcN / MeOH (2:1)
Gradient	0 min (85%A-15%B), 0-3 min (85%A-15%B), 3-25 min (5%A-95%B), 25-32 min (95%A-5%B), 32-37 min (85%A-15%B), 37-50 min (85%A-15%B) **TOTAL RUN: 50min**

Identification of target compounds — HPLC-QqQ

✓**Criteria for target analysis**: LC RT, 2 MRM transitions, ratio MRM transitions

Determination of the β-blocker Atenolol



Difference in Signal intensities between two transitions	Difference allowed in MRM ratio found in the sample with the std
>10-20%	±30%
>20-50%	±25%

Identification of target compounds — HPLC-QqQ

Compounds analyzed by NI mode

Target compounds	Rt window	Rt (min)	Precursor ion	MRM1	MRM2	MRM ratio
Clofibric acid	0-15.5min	11.93	213 $[M-H]^-$	213>127	213>85	4.95
Naproxen		13.89	229 $[M-H]^-$	229>169	229>185	1.40
Mecoprop-d_3		13.91	217 $[M-H]^-$	217>145	-	-
Ketoprofen		14.71	253 $[M-H]^-$	253>209	253>197	-
Bezafibrate	15.5-30min	16.02	360 $[M-H]^-$	360>274	360>154	5.79
Ibuprofen-d_3		17.99	208 $[M-H]^-$	208>164	-	-
Ibuprofen		18.04	205 $[M-H]^-$	205>160	-	-
Diclofenac		18.11	294 $[M-H]^-$	294>250	294>214	5.75
Mefenamic acid		19.00	240 $[M-H]^-$	240>196	240>180	16.17
Indomethacine		20.03	356 $[M-H]^-$	356>297	356>312	3.47
Gemfibrozil		21.19	249 $[M-H]^-$	249>121	-	-

Cone voltages: 20-30V; Collision energies: 10-15 eV

Identification of target compounds

HPLC-QqQ

Compounds analyzed by PI mode

Target compounds	Rt window	Rt (min)	Precursor ion	MRM1	MRM2	MRM ratio
Atenolol-d_7	0-8 min	3.06	274 $[M+H]^+$	274>190	-	-
Atenolol		3.09	260 $[M+H]^+$	267>190	267>145	1.05
Ranitidine		3.49	315 $[M+H]^+$	315>176	315>130	1.66
Acetaminophen		4.34	152 $[M+H]^+$	152>110	152>93	6.44
Trimethoprim	8-16 min	9.14	291 $[M+H]^+$	291>230	291>261	1.95
^{13}C-Phenacetin		15.55	181 $[M+H]^+$	181>139	-	-
Azythromycin		16.14	749 $[M+H]^+$	749>591	749>158	2.12
Propranolol	16-25 min	17.34	260 $[M+H]^+$	260>183	260>116	1.78
Carbamazepine-d_{10}		18.59	247 $[M+H]^+$	247>204	-	-
Erythromycin		18.62	734 $[M+H]^+$	734>576	734>558	4.96
Carbamazepine		18.71	237 $[M+H]^+$	237>194	237>192	4.09
Propyphenazone		19.34	231 $[M+H]^+$	231>189	231>201	3.04
Fluoxetine		21.26	310 $[M+H]^+$	310>148	310>44	2.88

Analytical Performances

HPLC-QqQ

Recovery ranges **51-106% surface water and WWTP effluent**

Compounds	Method detection limits (ng/L)		Instrumental detection limits (pg injected)
	Surface water	WWTP effluent	
Naproxen	7	9	30
Diclofenac	2	10	42
Ibuprofen	8	12	60
Propyphenazone	3	10	11
Clofibric acid	1	2	6
Gemfibrozil	1	1	21
Bezafibrate	1	2	5
Carbamazepine	2	10	6
Paroxetine	7	8	18
Ranitidine	2	20	23
Erythromycin	4	6	50
Sulfamethoxazole	5	20	27
Trimethoprim	1	10	13
Atenolol	9	10	10
Propranolol	2	7	16

LC-TANDEM MS

Quantitative target analysis
Identification/Confirmation
Structural Information

(Semi)quantitative target analysis
Identification/Confirmation
Non Target analysis/Screening

HPLC – Quattro LC (QqQ)™

✓ MRM
✓MS/MS scans
✓Neutral loss scans

Waters Corporation (Manchester, UK)

HPLC – MDS Sciex (QTRAP)™

✓ MRM
✓MS^3
✓High sensitive MS/MS and MS scans

Applied Biosystems

ACQUITY UPLC™ – Q – TOF Micro™

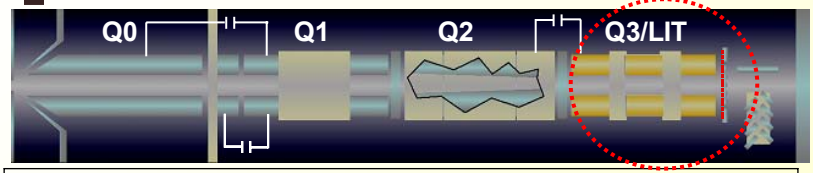
ACQUITY UPLC™ BEH C18 1.7 μm (2.1 x 50 mm)

✓ TOF MS
✓ TOF MS/MS

Waters Corporation (Manchester, UK)

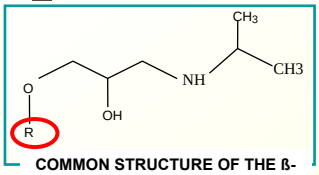
HPLC-QTRAP

QTRAP operation modes



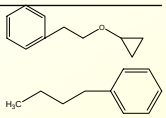
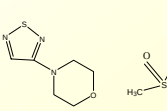
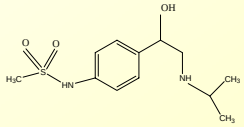
Q3 as aquadrupole analyzer			
Scan Type	**Q1**	**q2**	**Q3**
Q1 Scan	Resolving Scan	RF-only	RF-only
Q3 Scan	RF-only	RF-only	Resolving (Scan)
Product Ion Scan (PIS)	Resolving (Fixed)	Fragment	Resolving (Scan)
Precursor Ion Scan (PI)	Resolving (Scan)	Fragment	Resolving (Fixed)
Neutral Loss Scan (NL)	Resolving (Scan)	Fragment	Resolving (Scan Offset)
Selected Reaction Monitoring (SRM)	Resolving (Fixed)	Fragment	Resolving (Fixed)
Q3 as an ion trap analyzer			
Enhanced Product Ion Scan (EPI)	Resolving (Fixed)	Fragment	Trap/Scan
MS^3	Resolving (Fixed)	Fragment	Isolation/frag trap/scan
Time delayed frag capture Product Ion (TDF)	Resolving (Fixed)	Trap/No frag	Frag/trap/scan
Enhanced Q3 single MS (EMS)	RF-only	No frag	Trap/Scan
Enhanced Resolution Q3 Single MS (ERMS)	RF-only	No frag	Trap/Scan
Enhanced Multiply Charged	RF-only	No frag	Trap/empty/scan

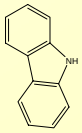
TARGET COMPOUNDS



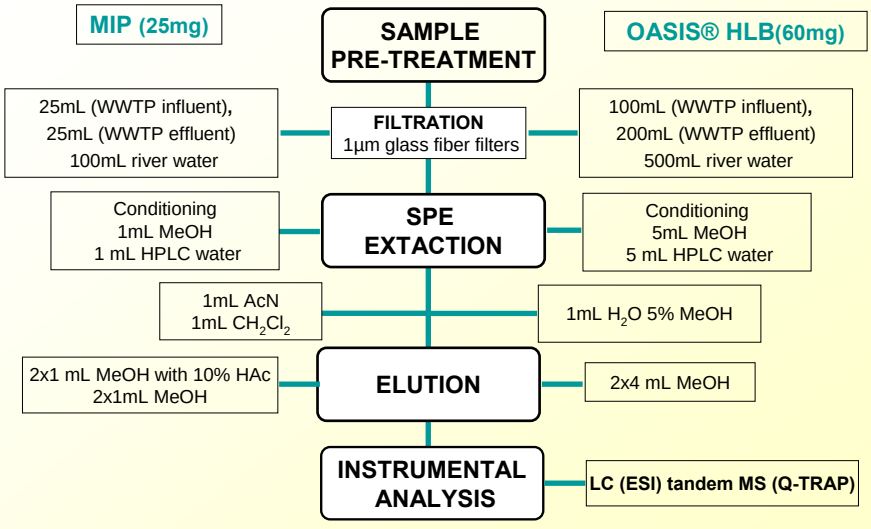
COMMON STRUCTURE OF THE ß-BLOCKERS STUDIED

- Used to treat cardiovascular disorders. Included in the list of prohibited substances of the IOC due to their sympathomimetic properties, similar to other central nervous system stimulants
  - ❖ hypertension
  - ❖ heart failure
  - ❖ arrhythmia
  - ❖ relief intraocular pressure

Compounds	R
Betaxolol	
Metoprolol	
Timolol	
Atenolol	
Sotalol	

Compounds	R
Pindolol	
Propranolol	
Carazolol	

ANALYTICAL PROTOCOL



HPLC Conditions

HPLC-QTRAP

Equipment: Agilent 1100 Series

Column: Purospher ® RP-18 (125x2mm) (5 µm)

Flow: 0.2 mL/min

POSITIVE IONIZATION MODE (PI)	
Mobile Phase	Solvent A: NH_4A_C 5mM / HA_c (pH=4.8) / Solvent B: AcN / MeOH (2:1)
Gradient	0 min (15%B), 3 min (15%B), 20 min (75%B), 25 min (15%B), 40 min (15%B) **TOTAL RUN: 40min**

QTRAP Operational parameters

Equipment: 4000 QTRAP (Applied Biosystems)

Curtain Gas (CUR): 30V	Interface Heater: On
Collision Gas (CAD): High	Resolution Q1: Unit
Ion Spray Voltage (IS): 5500V	Resolution Q3: Unit
Temperature (TEM): 700ºC	Pause between mass ranges: 5ms
Ion Source Gas (GS1): 50	Total scan time (includes Pause): 1.7850 sec.
Ion Source Gas (GS2): 50	

Identification of target compounds

HPLC-QTRAP

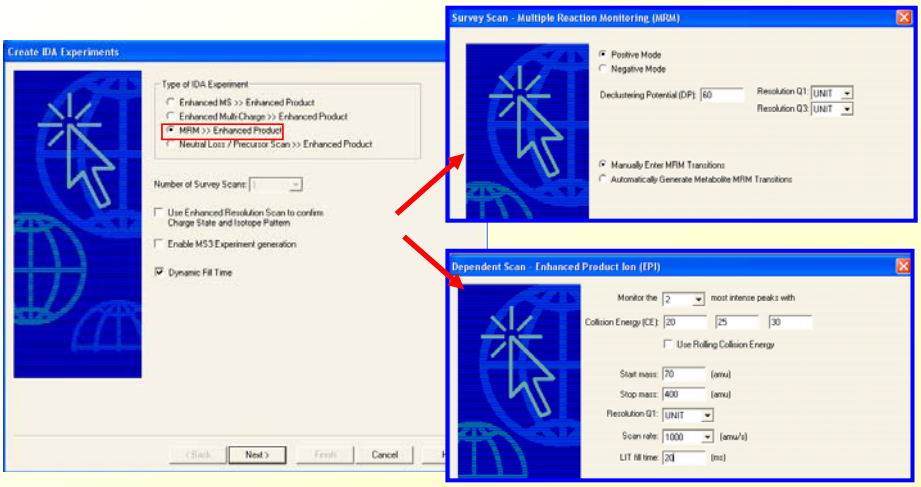
Target compounds	RT (min)	Precursor ion	MRM 1	DP-CE-CXP	MRM 2	DP-CE-CXP
Atenolol-d_7	3.01	274 $[M+H]^+$	274>190	60-20-10	-	-
Atenolol	3.08	267 $[M+H]^+$	267>145	60-35-8	267>190	60-35-14
Sotalol	3.40	273 $[M+H]^+$	273>213	60-25-6	273>255	60-25-6
Pindolol	10.29	249 $[M+H]^+$	249>116	60-30-8	249>98	60-30-14
Timolol	13.97	317 $[M+H]^+$	317>262	60-30-20	317>244	60-30-6
Metoprolol	14.42	268 $[M+H]^+$	268>121	60-35-10	268>133	60-35-8
Carazolol	17.06	299 $[M+H]^+$	299>116	60-35-8	299>222	60-35-2
Propranolol	18.25	260 $[M+H]^+$	260>116	60-30-8	260>183	60-30-10
Betaxolol	18.63	308 $[M+H]^+$	308>116	60-40-8	308>121	60-40-14

- **DP (Declustering Potential):** It is used to minimize the solvent clusters that may remain on the sample ions after they enter the vacuum chamber.
- **CXP (Collision Cell Exit Potential)**: It is used to focus and accelerate the ions out of the collision cell (Q2).
- **CE (Collision Energy)**: It is the amount of energy that the precursor ions receive as they are accelerated into the Q2 collision cell.

In addition, to obtain extra confirmation, an Information Dependent Acquisition experiment was performed

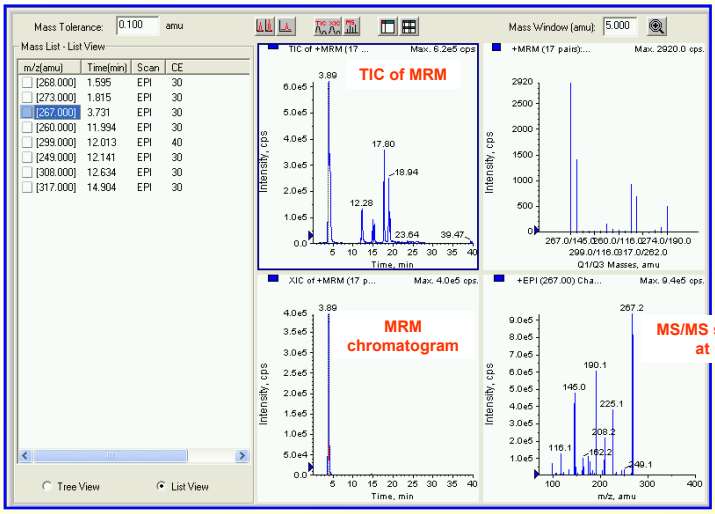
Information Dependent Acquisition HPLC-QTRAP

- It is a combination of a survey scan with an Enhanced Product Ion (MS/MS) scan in the same experiment and chromatographic run



Chromatogram of an IDA experiment HPLC-QTRAP

Identification of Atenolol in a WWTP influent sample



Analytical performances

HPLC-QTRAP

Recovery MIP **20-74% surface water; 50-110% WWTP effluent**

Recovery OASIS HLB **50-70% surface water; 50-81% WWTP effluent**

Compounds	Method detection limits (ng/L) MIP	
	Surface water	WWTP effluent
Atenolol	1	1.5
Pindolol	0.5	0.2
Timolol	0.3	0.4
Metoprolol	1.5	0.5
Carazolol	0.2	0.4
Propranolol	2.0	0.4
Betaxolol	0.3	3.0
	Method detection limits (ng/L) OASIS HLB	
Atenolol	2	4
Pindolol	1	1
Timolol	1	2
Metoprolol	3	3
Propranolol	1	1
Betaxolol	2	4

Compounds	Instrumental LOD (pg injected)
Atenolol	2.3
Sotalol	6.2
Pindolol	0.2
Timolol	0.4
Metoprolol	2.2
Carazolol	0.6
Propranolol	0.7
Betaxolol	2.0

IDL one and two orders of magnitude lower than QqQ and QTOF, respectively

LC-TANDEM MS

Quantitative target analysis
Identification/Confirmation
Structural Information

(Semi)quantitative target analysis
Identification/Confirmation
Non Target analysis/Screening

HPLC – Quattro LC (QqQ)™

✓ MRM
✓MS/MS scans
✓Neutral loss scans

Waters Corporation (Manchester, UK)

HPLC – MDS Sciex (QTRAP)™

✓ MRM
✓MS^3
✓High sensitive MS/MS and MS scans

Applied Biosystems

ACQUITY UPLC™ – Q – TOF Micro™

ACQUITY UPLC™ BEH C18 1.7 µm (2.1 x 50 mm)

✓ TOF MS
✓ TOF MS/MS

Waters Corporation (Manchester, UK)

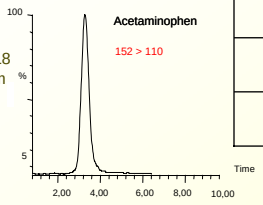
TARGET COMPOUNDS

Analgesics and antiinflammatories	Ibuprofen Ketoprofen Naproxen Diclofenac	Indomethacine Acetaminophen Mefenamic acid Phenylphenazone	To relief pain, inflammation and fever
Lipid regulator and cholesterol lowering statin drugs	Clofibric acid Gemfibrozil Bezafibrate	Pravastatin Mevastatin	To lower fat (lipids) level
Psychiatric drugs	Carbamazepine Fluoxetine	Paroxetine	Antidepressants Antiepileptics
Antiulcer agent	Lansoprazole		To prevent and treat ulcers
Anti-histaminics	Famotidine Ranitidine Loratadine		To relieve allergy symptoms
Antibiotics	Erythromycin Azithromycin Sulfamethoxazole	Trimethoprim Ofloxacin	Antibacterial agents
β-blockers	Atenolol Sotalol	Metoprolol Propanolol	Antianginal antihypertensive

UPLC vs HPLC

HPLC

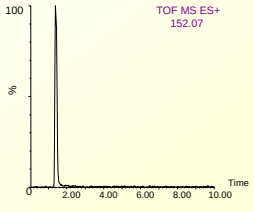
Purospher ® RP-18
(125 x 2mm) 5 µm
Flow 0.2 mL/min



Separation technique	**Peak width**	**Chromatographic run**
HPLC	0.5 – 1 min	30 min separation Total run **45 min**
UPLC	5 - 10 s	10 min separation Total run **14 min**

UPLC

ACQUITY UPLC™
BEH C18
(50 x 2.1 mm) 1.7 µm
Flow 0.4 mL/min



UPLC

Very low system volumes, and fast detection for increased throughput, sensitivity and peak capacity

Reduced peak width
Increased peak height
Improved sensitivity
Reduced spectral overlap in complex mixtures
Improved MS spectral data

UPLC Conditions

UPLC-Q-TOF

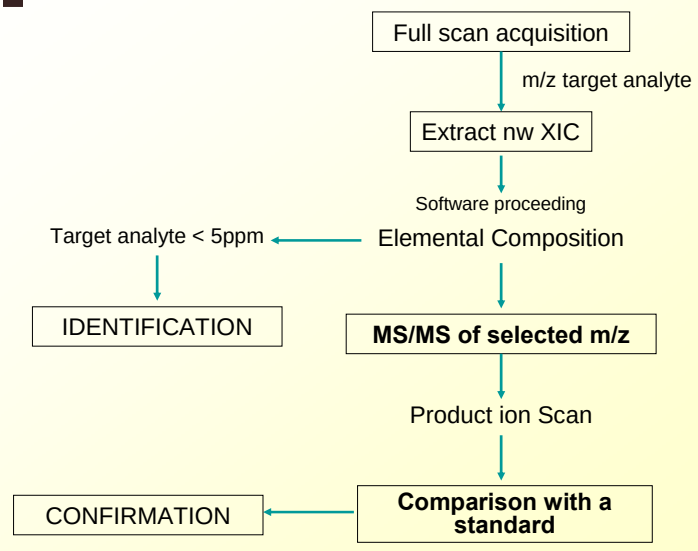
Equipment: Acquity UPLC - Q – Tof Micro (Waters, Milford, MA, USA)
Column: ACQUITY UPLC™ BEH C18 1.7 µm (2.1 x 50 mm)
Flow: 0.4 mL/min

NI	
Mobile Phase	Solvent A: H_2O Solvent B: MeOH
Gradient	0 min (5% B), 6 min (90% B), 8 min (95% B), total run **12 min**

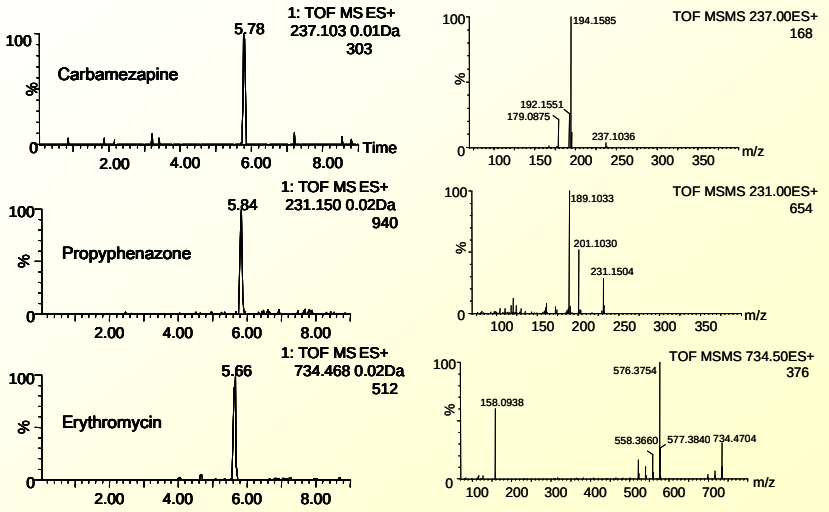
PI	
Mobile Phase	Solvent A: NH_4A_C 5mM / HA_c (pH=4.7) Solvent B: ACN / MeOH (2:1)
Gradient	0 min (5% B), 1 min (5% B), 8 min (60% B), 10 min (90% B), 11 min (90% B), total run **14 min**

Target analysis procedure

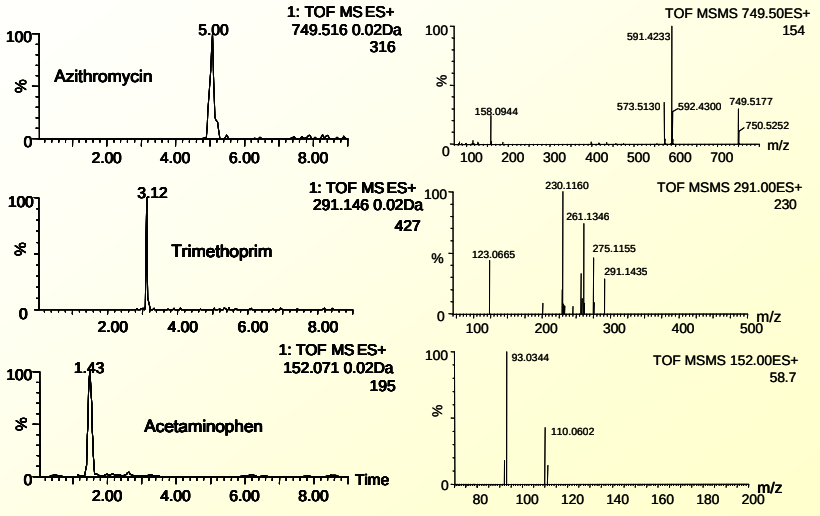
UPLC-Q-TOF



Identification of target compounds in wastewater samples



Identification of target compounds in wastewater samples



Identification of target compounds

UPLC-Q-TOF

The errors obtained for molecular ions (in the TOF mode) were between 0.7-4.4 ppm (root mean square (RMS = 2.02) and 0.2-1.2 mDa (RMS = 0.72)

Peak no	Compound	Retention time (min)	Elemental composition	Experimental mass (m/z)	Theoretical mass (m/z)	Error	
						mDa	ppm
1	Acetaminophen	1.43	$[M+H]^+$ C8H10NO2	152.0717	152.0712	0.5	3.6
2	Sotalol	1.83	$[M+H]^+$ C12H21N2O3S	273.1285	273.1273	1.2	4.4
3	Famotidine	1.91	$[M+H]^+$ C8H16N7O2S3	338.0531	338.0527	0.3	1.0
4	Atenolol	1.93	$[M+H]^+$ C14H23N2O3	267.1711	267.1708	0.2	0.9
5	Ranitidine	2.09	$[M+H]^+$ C13H23N4O3S	315.1500	315.1491	0.9	2.9
6	Trimethoprim	3.12	$[M+H]^+$ C14H19N4O3	291.1466	291.1457	0.9	3.0
7	Ofloxacine	3.36	$[M+H]^+$ C18H21FN3O4	362.1509	362.1516	-0.7	-2.0
8	Sulphametaxozole	3.91	$[M+H]^+$ C10H12N3O3S	254.0605	254.0599	0.6	2.2
9	Metoprolol	3.95	$[M+H]^+$ C15H26NO3	268.1921	268.1912	0.8	3.1

Identification of target compounds

UPLC-Q-TOF

Peak no	Compound	Retention time (min)	Elemental composition	Experimental mass (m/z)	Theoretical mass (m/z)	Error	
						mDa	ppm
10	Azithromycin	4.83	$[M+H]^+$ C38H73N2O12	749.5155	749.5163	-0.9	-1.1
11	Propranolol	5.35	$[M+H]^+$ C16H22NO2	260.1659	260.1650	0.8	3.3
12	Pravastatin	5.70	$[M+Na]^+$ C23H35NaO7	447.2349	447.2358	-1.0	-2.2
13	Carbamazepine	5.80	$[M+H]^+$ C15H13N2O	237.1033	237.1028	0.5	2.2
14	Propyphenazone	5.84	$[M+H]^+$ C14H19N2O	231.1502	231.1497	0.5	2.0
15	Erythromycin	5.88-6.00	$[M+H]^+$ C37H68NO13	734.4701	734.4690	1.0	1.4
16	Bezafibrate	5.93	$[M+H]^+$ C19H21ClNO4	362.1165	362.1159	0.6	1.6
17	Ketoprofen	6.32	$[M+H]^+$ C16H15O3	255.1017	255.1021	-0.4	-1.6
18	Paroxetine	6.40	$[M+H]^+$ C19H21FNO3	330.1499	330.1505	-0.6	-2.0
19	Lansoprazole	6.40	$[M+H]^+$ C16H15F3N3O2S	370.0841	370.0837	0.4	1.1
20	Fluoxetine	7.00	$[M+H]^+$ C17H19F3NO	310.1421	310.1418	0.2	0.7
21	Mefenamic acid	8.47	$[M+H]^+$ C15H16NO2	242.1191	242.1180	1.0	4.1
22	Loratadine	9.19	$[M+H]^+$ C22H24ClN2O2	383.1519	383.1526	-0.7	-1.9
23	Mevastatin	9.33	$[M+H]^+$ C23H35O5	391.2496	391.2484	1.2	2.9

Analytical Performances

UPLC-Q-TOF

Compound	Method detection limits (ng/L) WWTP influent*
Naproxen	10
Diclofenac	10
Ibuprofen	50
Propyphenazone	10
Clofibric acid	5
Gemfibrozil	10
Bezafibrate	10
Carbamazepine	50
Paroxetine	15
Ranitidine	20
Erythromycin	25
Sulfamethoxazole	50
Trimethoprim	1
Atenolol	10
Propranolol	20
Metoprolol	2

Compound	Instrumental LOD (pg injected)
Indomethacine	80
Mevastastine	150
Ketoprofen	150
Carbamazepine	100
Lansoprazole	120
Famotidine	200
Sulfametoxazole	150
Loratadine	50
Ibuprofen	150
Fluoxetine, Oflaxacine, Pravastatine	>200

10 -20x higher than with QqQ (MRM)

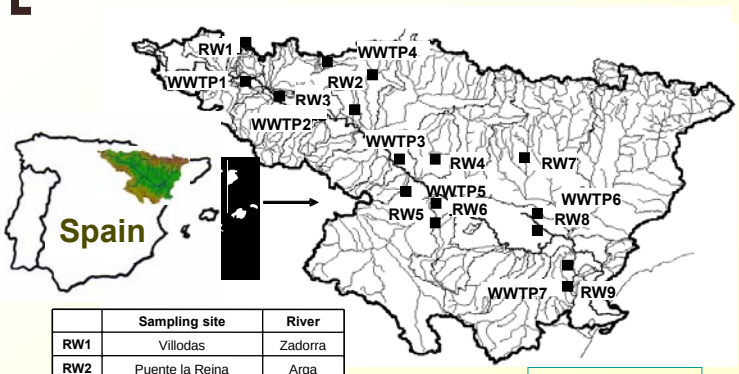
*TOF MS range 70-700 (PI), 70-500 (NI), 20 mDa window

Performance comparison

Technique	Sensitivity	Selectivity	Mass accuracy	Dynamic range	Number of IP
QqQ	Medium (full scan) High (SRM)	High	Low	High	1 for precursor ion 1.5 for transition product
QTOF	Medium	High	High	Medium	2 for precursor ion 2.5 for transition product
QqLIT	High (SRM)	High	Low	Medium-High	1 for precursor 1,5 for MS^2 product 1,5 for MS^3 products

Ebro river basin (NE Spain)

OCCURRENCE



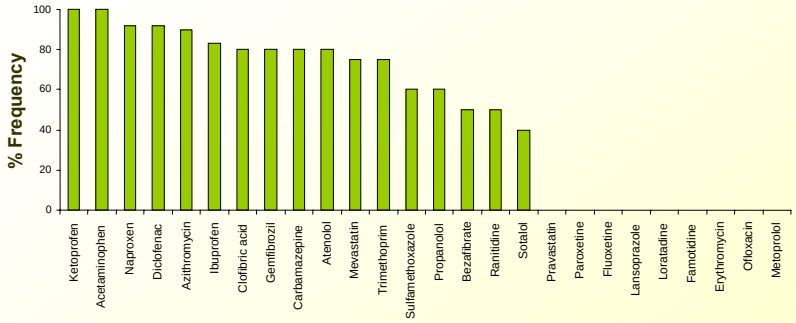
	Sampling site	River
RW1	Villodas	Zadorra
RW2	Puente la Reina	Arga
RW3	Logroño	Ebro
RW4	San Mateo Gállego	Gállego
RW5	Zaragoza	Huerva
RW6	Presa de Pina	Ebro
RW7	Alcolea de Cinca	Cinca
RW8	Torres de Segre	Segre
RW9	Tortosa	Ebro

OBJECTIVES

- To calculate removal rates of pharmaceuticals in WWTP and the loads that are entering in the environment
- To evaluate the contribution of WWTP effluents concerning to the presence of pharmaceuticals in the aquatic environment

Ebro river basin (NE Spain)

OCCURRENCE

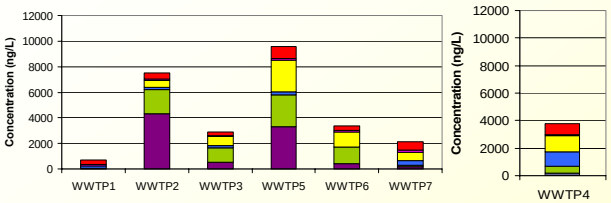


Groups of pharmaceuticals of major consumption in Spain in 2004				
Cholesterol lowering statin drugs	Analgesics	Antithrombotics	AINEs and antirheumatics	Glucocorticoids
Ulcer healings and anti-histaminics	Antagonsits of angiotensin II	Calcium antagonists	Macrolides Penicillins, Fluoroquinolones	Sexual hormones
Antidepressants, anseolitics	Inhalators	Diuretics	Cephalosporines	Vasodilators

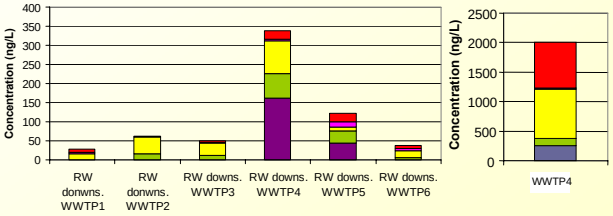
Ebro river basin (NE Spain)

OCCURRENCE

WWTP effluent



River water downstream WWTP



There is an important **dilution factor**: Levels found in surface waters downstream WWTP are in the low ng/range, whereas in WWTP effluent concentrations of target compounds are between low µg/L-high ng/L range.

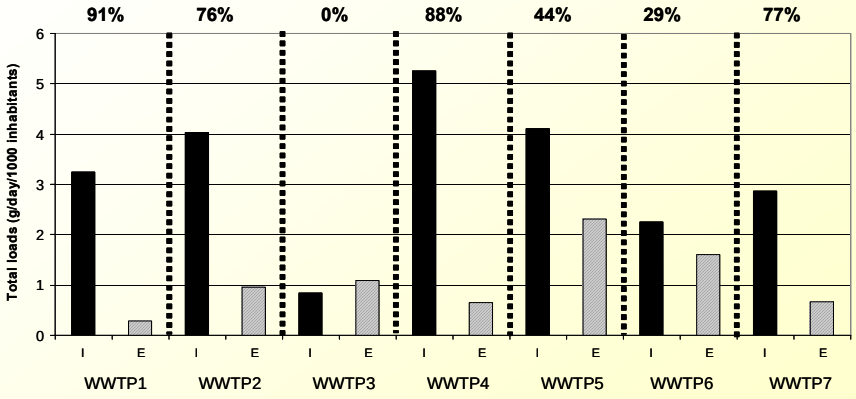
Environmental risks are reduced in river water due to the important dilution

Atenolol **Gemfibrozil** **Diclofenac** **Naproxen** **Ibuprofen** **Carbamazepine**

Ebro river basin (NE Spain)

OCCURRENCE

Overall removal efficiency



Total loads of pharmaceuticals were normalized for population equivalents and expressed as g/day/1000 inhabitants

CONCLUSIONS

❖ **LC-MS-MS** permits unequivocal identification of studied pharmaceutical classes. Pharmaceutical residues can be traced from wastewaters ("hot spots" are WWTP) and receiving waters (surface waters) down to coastal and drinking waters

❖ **UPLC** showed very good performances - reduced analysis time, improved separation and sensitivity than **HPLC**

❖ **Q-TOF** – high quality structural information for unequivocal identification of target compounds; rather good sensitivity and selectivity in full scan mode; lower capacity for trace quantitation

❖ **QqQ**– powerful tools for quantitative target analysis (LOD in low ng/L level. **QTRAP** is suitable for both quantitation and identification of pharmaceuticals (MRM combined with several scans in the same experiment), providing higher sensitivity than QTOF and QqQ.

❖ **UPLC-Q-TOF** is a complimentary tool for QqQ and QTRAP providing very good performances for pharmaceutical analysis in environmental matrices

ACKNOWLEDGEMENTS

This work has been supported by:

EU project **EMCO** (INCO CT 2004-509188)
Reduction of environmental risks, posed by Emerging Contaminants, through advanced treatment of municipal and industrial wastes

Spanish Ministerio de Ciencia y Tecnologia
Project CTM2004-06255-CO3-01

Thanks to MIP Technologies for providing the MIP cartridges, to Applied Biosystems for the 4000 QTRAP, to Waters Corporation for the SPE cartridges and to Merck for the gift of HPLC columns

Concern of drugs as pollutants

INTRODUCTION

- Due to their continuous introduction via WWTP effluents, they are referred to as "pseudo" persistent contaminants (i.e. high transformation/removal rates are compensated by their continuous introduction into environment)
- They often have the same type of physico-chemical behavior as other harmful xenobiotics (persistence in order to avoid the substance to be inactive before having a curing effect, and lipophilicity in order to be able to pass membranes).
- They are used by man in rather large quantities (i.e. similar to those of many pesticides).
- Some pharmaceuticals (antidepressants, anti-inflamamtories, ß-blockers) showed chronic toxicity to several aquatic organisms at the levels found in WWTP effluents.