



The Development of Methodology Addressing the European Water Framework Directive2013/39/EU

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EU Directive 2013/39/EU

- Introduced in August 2013 amending EU directive 2000/60/EC and 2008/105/EC;
- It lays down **a strategy against the pollution of water** to be applied to all European Union member states;
- It involves the **identification of priority substances** and the **monitoring of different classes of contaminants**;
- Includes the **first watch list** (used for future prioritization exercises);
- Member States have the flexibility to apply an **EQS** (environmental quality standard) for an alternative matrix or, where relevant, an alternative biota taxon, for example sub-phylum *Crustacea*, paraphylum “fish”, class *Cephalopoda* or class *Bivalvia* (mussels and clams);
- Encourages the development of novel monitoring methods such as passive sampling and other tools.

Priority Hazardous Substances/Technique To Be Used

Priority Hazardous Substances	GC-MS	LC-MS	ICP-MS
<u>Anthracene</u>	✓		
<u>Brominated Diphenylethers</u>	✓		
<u>Cadmium and its compounds</u>			✓
<u>C₁₀₋₁₃ Chloroalkanes</u>	✓		
<u>Di-(2-ethylhexyl)phthalate (DEHP)</u>	✓		
<u>Endosulfan</u>	✓		
<u>Hexachlorobenzene (HCB)</u>	✓		
<u>Hexachlorobutadiene (HCBD)</u>	✓		
<u>Hexachlorocyclohexane</u>	✓		
<u>Mercury and its compounds</u>			✓
<u>Nonylphenols</u>	✓		
<u>Pentachlorobenzene</u>	✓		
<u>Polyaromatic Hydrocarbons (PAHs)</u>	✓		
<u>Tributyltin compounds</u>	✓		
<u>Trifluralin</u>	✓		
<u>Dicofol</u>	✓		
<u>Perfluorooctane sulfonic acid and its derivatives (PFOS)</u>		✓	
<u>Quinoxyfen</u>	✓	✓	
<u>Dioxins and Dioxin-like compounds</u>	✓		
<u>Hexabromocyclododecane (HBCDD)</u>		✓	
<u>Heptachlor and heptachlor epoxide</u>	✓		

Priority Substances/Technique To Be Used

Priority Substances	GC-MS	LC-MS	ICP-MS
<u>Alachlor</u>	√		
<u>Atrazine</u>	√	√	
<u>Benzene</u>	√		
<u>Chlorfenvinphos</u>	√	√	
<u>Chlorpyrifos (ethyl)</u>	√		
<u>1,2-dichloroethane</u>	√		
<u>Dichloromethane</u>	√		
<u>Diuron</u>		√	
<u>Fluoranthene</u>	√		
<u>Isoproturon</u>		√	
<u>Lead and its compounds</u>			√
<u>Naphthalene</u>	√		
<u>Nickel and its compounds</u>			√
<u>Octylphenols</u>	√		
<u>Pentachlorophenol</u>	√		
<u>Simazine</u>	√	√	
<u>Trichlorobenzenes</u>	√		
<u>Trichloromethane (chloroform)</u>	√		
<u>Aclonifen</u>	√		
<u>Bifenoxy</u>	√		
<u>Cybutryne</u>	√		
<u>Cypermethrin</u>	√		
<u>Dichlorvos</u>	√		
<u>Terbutryn</u>	√	√	

Watch List /Technique To Be Used

Watch List ³	GC-MS	LC-MS	ICP-MS
<u>Diclofenac</u>		√	
<u>17-beta-estradiol</u>	√	√	
<u>17-alpha-ethinylestradiol</u>	√	√	

³ Substances to be monitored in order to gather information on the risk posed by those substances into the environment

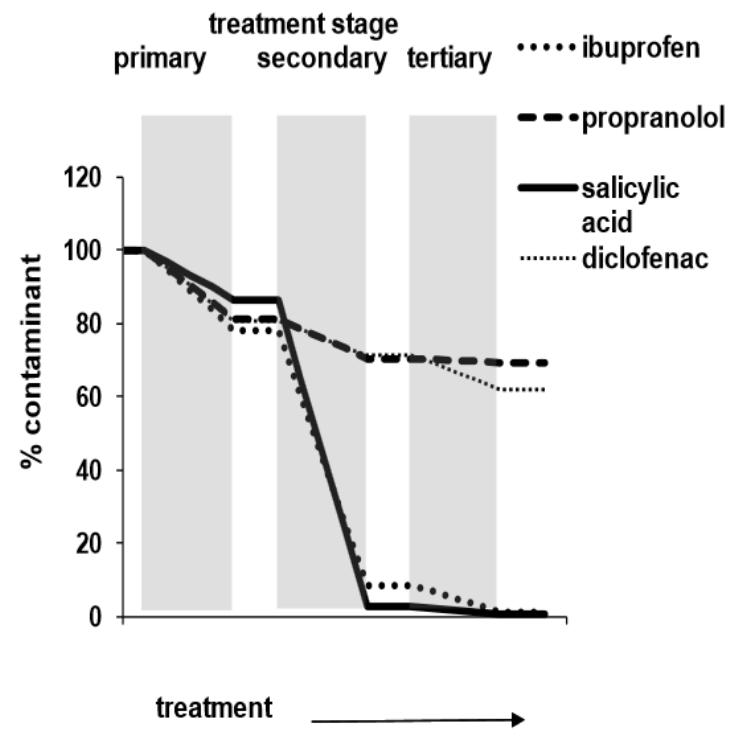
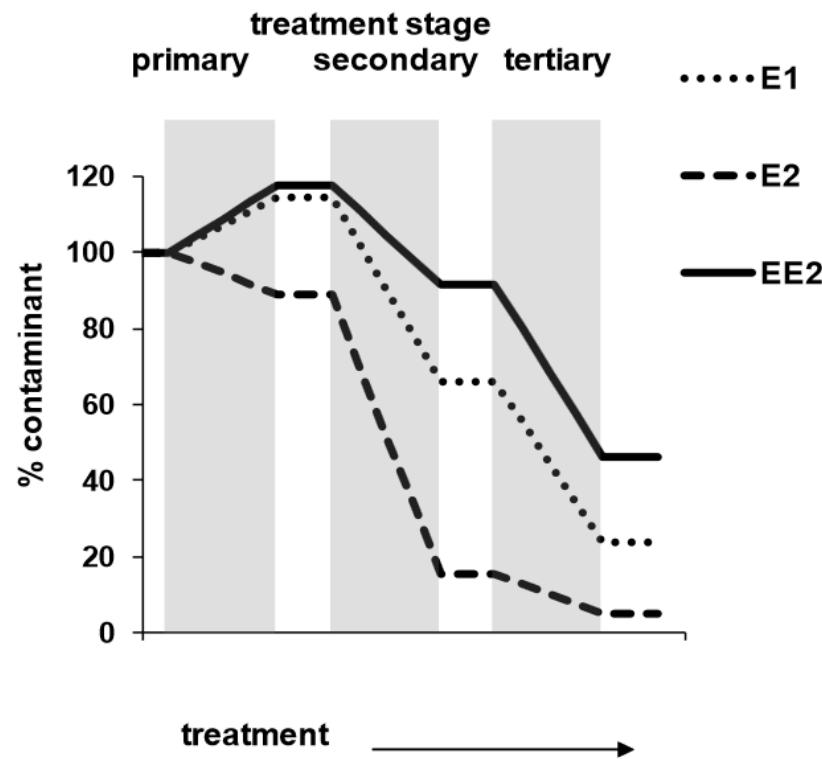
Chemicals Investigation Programme (CIP)

- Investigations into the **occurrence, sources and removal of trace substances in wastewater** treatment works effluents.
- Helps to establish priorities with respect to action to be taken to ensure surface waters meet new Environmental Quality Standards
- **70 priority chemicals** were determined in 162 STW effluents
- **11 pharmaceuticals** (EQS proposed for 3 – diclofenac, E2 and EE2)
- All substances selected for analysis were detected in effluent
- Levels of many priority chemicals in effluent exceeded EQS



Current - CIP2

- CIP 2 is a £100M programme – reporting March 2017
- Focus on pharmaceuticals (LC-MS)



CIP 2 Pharmaceuticals

Compound	Required Influent & Effluent LOD (µg/L)
<u>Diclofenac</u> (WFD)	0.01
<u>Ibuprofen</u>	0.01
<u>Atorvastatin</u>	0.01
<u>ortho-hydroxyatorvastatin</u>	0.01
<u>para-hydroxyatorvastatin</u>	0.01
<u>Propanolol</u>	0.01
<u>Atenolol</u>	0.01
<u>Erythromycin</u>	0.1
<u>Norerythromycin</u>	0.1
<u>Azithromycin</u>	0.005
<u>Clarithromycin</u>	0.01
<u>Ciprofloxacin</u>	0.01
<u>Metformin</u>	0.1
<u>Ranitidine</u>	0.1
<u>Carbamazepine</u>	0.1
<u>10,11-epoxycarbamazepine</u>	0.1



Information collected from DRAFT CIP2 Technical Specification and Guidance

CIP 2 - Cont. and Others

Compound	Required Influent & Efluent LOD (µg/L)	Required River LOD (µg/L)
<u>Estrone</u>	0.001	-
<u>17-β-estradiol (WFD)</u>	0.0003	-
<u>17-α-ethinylestradiol (WFD)</u>	0.00003	-
<u>PFOS (WFD)</u>	0.00065	0.00009
<u>PFOA (WFD)</u>	0.00065	0.00009
<u>HBCDD (WFD)</u>	0.0016	0.00023
<u>Nonylphenol, 4 –nonylphenol and ethoxylates (WFD)</u>	0.1	0.04
<u>Octylphenols (WFD)</u>	0.1	0.014
<u>Trixylenyl phosphate</u>	0.01	-
<u>Benzotriazole</u>	0.002	-
<u>Tolytriazole</u>	0.002	-
<u>Sertraline</u>	0.01	-
<u>Norsertraline</u>	0.01	-
<u>Fluoxetine</u>	0.01	-
<u>Tamoxifen</u>	0.005	-

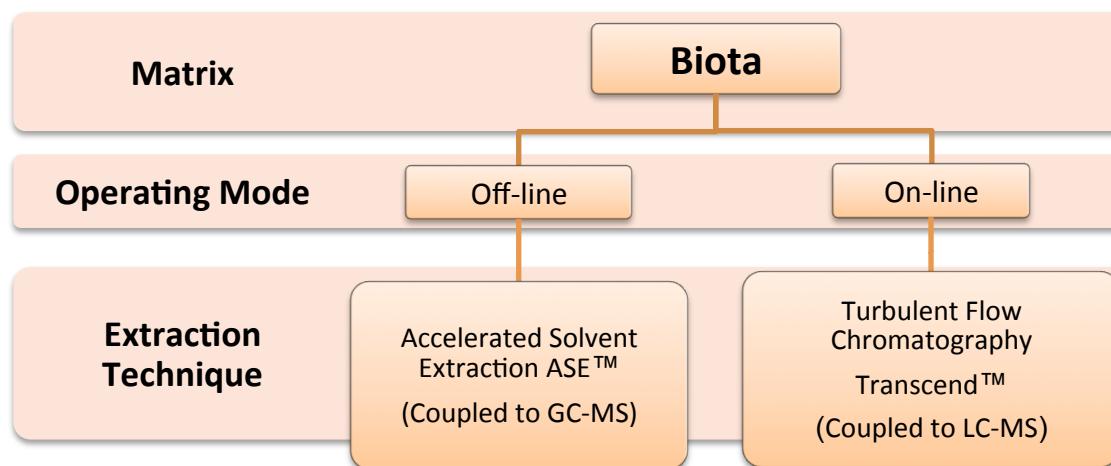
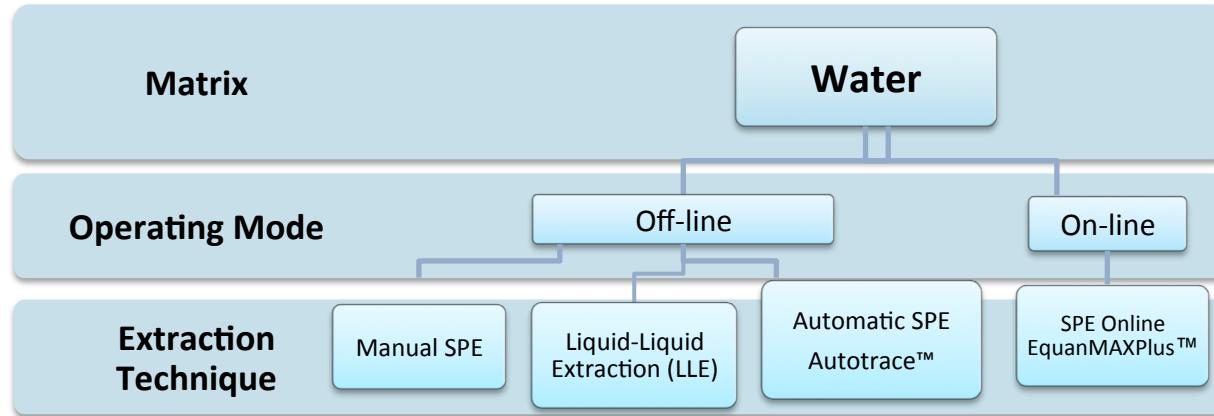
Information collected from DRAFT CIP2 Technical Specification and Guidance

● **Sample Preparation**



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Recommended Automated (& Semi) Workflows



Guidelines for General Offline SPE Procedure (LC-MS)

Thermo Scientific™ HyperSep™
Retain PEP Cartridges

Loading

- pH 2

Wash

- 10% MeOH/H₂O

Elution

- ACN (0.1%FA) – ACN – ACN (0.1% NH₄OH)
- EtOAc (PFCs, HBCDD, Phenols, Triclosan, Triazoles)

Evaporation

- Final Volume (0.5 mL)



Reconstitution

- Addition of 4.5 mL of 0.1% FA

Analysis

- 10 uL direct injection
- 1-5 mL Equan Injection

Polymeric Mixed Mode Ion Exchange Material are also available
(improved recoveries – class dependent)

● **Contaminants amenable to analysis by
LC-MS**



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Recommended (General) Instrumental Conditions

LC parameters

System: UltiMate 3000 LC

Column: Thermo Scientific™ Accucore™ C18 LC columns; 100 x 2.1 mm, 2.6 µm at 30 °C

Mobile Phase: (A) 2mM ammonium acetate (B) MeOH

Gradient: Start at 10% B (Hold 1 min), Ramp to 90% B in 5min. Ramp to 100% B in 1 min (Hold 1 min). Ramp to 10% B in 0.10 min (Hold 4 min)

Flow Rate: 0.4 mL/min

Injection Volume: 10 µL

MS Parameters (TSQ Quantiva)

Ionization and Spray Voltage: ESI at 3500V (+) 2500 V (-)

Scan Mode: SRM Cycle Time: 0.3 s Chrom Filter : 4 s

Resolution: 0.7 Th Q1/Q3

Collision Gas: Ar (2 mTorr)

Capillary Temperature (°C): 270

Vaporizer Temperature (°C): 400

Sheath Gas Pressure (Arb): 50

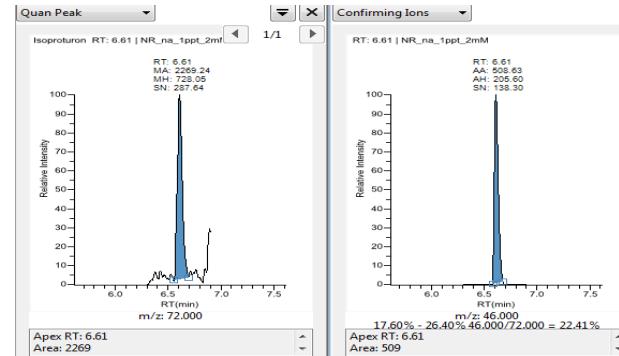
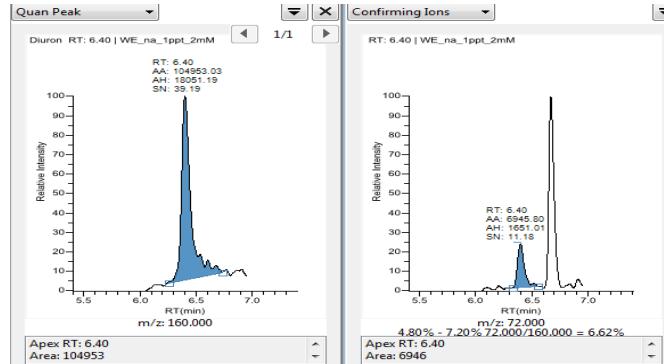
Aux Gas Pressure (Arb): 20

Sweep Gas Pressure (Arb): 1

Pesticides - Results

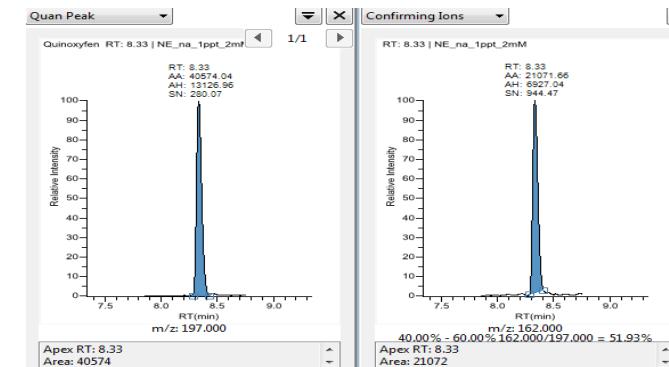
Compounds	2008/105/EC		2013/39/EU		Required LOD (pg on column)	River LOD (pg on column)	Effluent LOD (pg on column)	Influent LOD (pg on column)
	AA-EQS inland surface water (µg/ L)	EQS/3 (µg/L)	AA-EQS inland surface water (µg/L)	EQS/3 (µg/L)				
Atrazine	0.6	0.2	0.6	0.2	2	0.01	0.01	0.01
Chlорfenvinphos	0.1	0.034	0.1	0.034	0.34	0.01	0.01	0.01
Diuron	0.2	0.067	0.2	0.067	0.67	0.1	0.1	0.1
Isoproturon	0.3	0.1	0.3	0.1	1	0.01	0.01	0.01
Simazine	1	0.34	1	0.34	3.4	0.03	0.03	0.01
Quinoxifen	-	-	0.15	0.05	0.5	0.01	0.01	0.01
Terbutryn	-	-	0.065	0.022	0.22	0.05	0.01	0.1

Pesticides – Chromatogram at LOD



Diuron

Isoproturon



Quinoxifen

Pharmaceuticals I

Compounds	CIP 2		River LOD (pg on column)	Effluent A (pg on column)	Influent (pg on column)
	Required LOD (µg/L)	LOD (pg on column)			
Diclofenac (WFD)	0.01	0.1	0.03	0.1	0.03
Ibuprofen	0.01	0.1	1	0.8	0.8
Atorvastatin	0.01	0.1	0.05	0.05	0.05
ortho-hydroxyatorvastatin	0.01	0.1	0.05	0.1	0.1
para-hydroxyatorvastatin	0.01	0.1	0.05	0.1	0.1
Propanolol	0.01	0.1	0.4	0.2	0.2
Atenolol	0.01	0.1	0.2	0.15	0.1
Amoxicillin	Not on draft - March 2014		1	1	1
Erythromycin	0.1	1	0.6	0.15	0.15
Norerythromycin	0.1	1	Standard not available		
Azithromycin	0.005	0.05	2	tbd	0.4

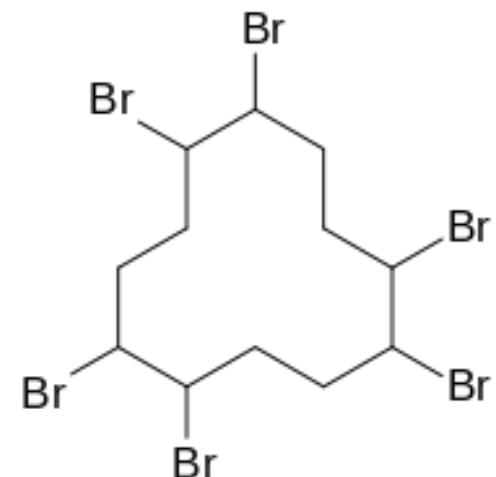
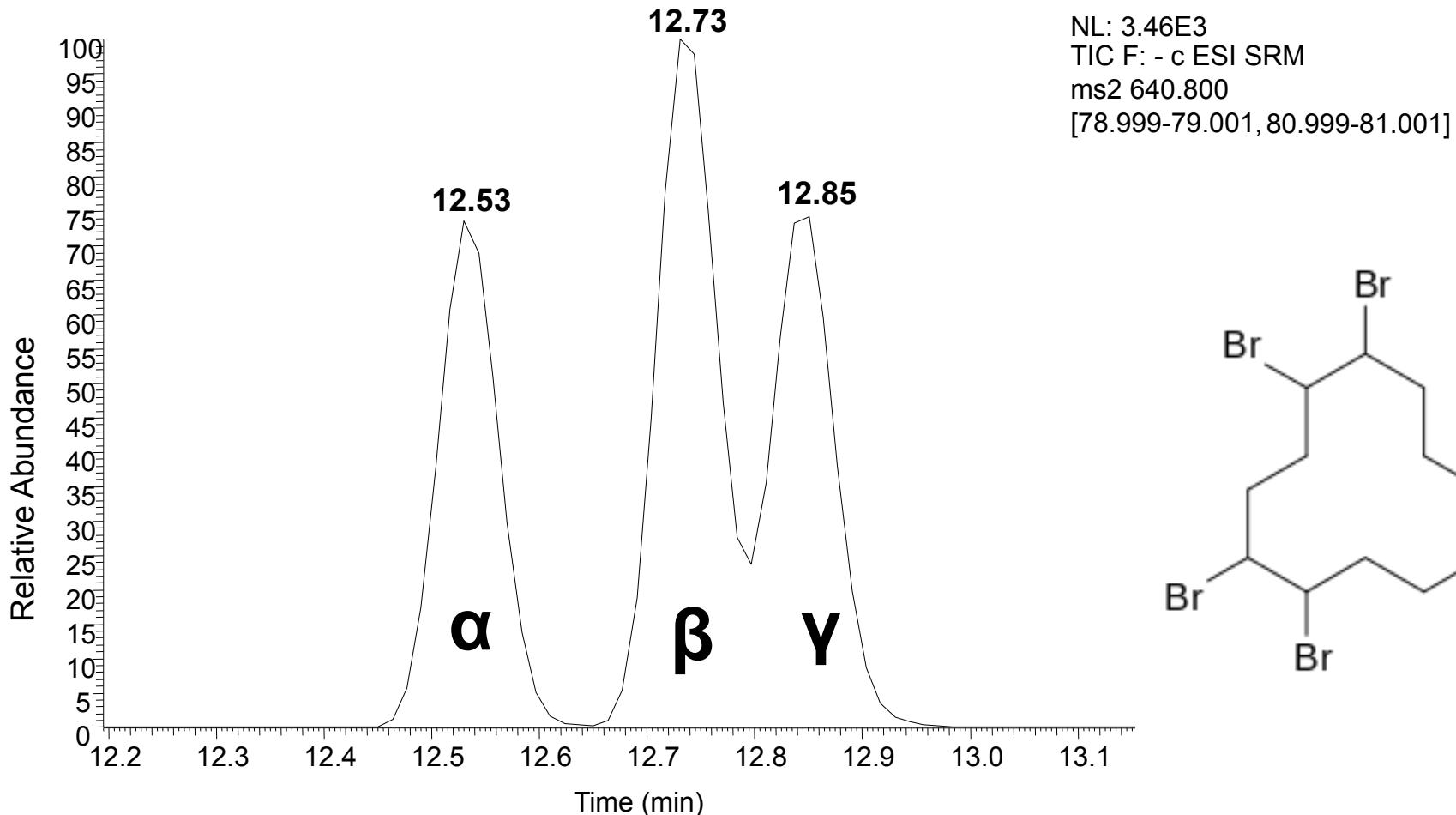
Pharmaceuticals II

Compound	CIP 2		River LOD (pg on column)	Effluent LOD (pg on column)	Influent LOD (pg on column)
	Required LOD (µg/L)	LOD (pg on column)			
Clarithromycin	0.01	0.1	0.1	0.1	0.03
Ciprofloxacin	0.01	0.1	1	tbd	tbd
Ranitidine	0.1	1	0.3	0.1	0.1
Carbamazepine	0.1	1	0.01	0.1	0.1
10,11-epoxycarbamazepine	0.1	1	0.01	0.1	0.04
Sertraline	0.01	0.1	0.5	1	0.1
Norsertraline	0.01	0.1	0.5	1	0.1
Fluoxetine	0.01	0.1	0.15	0.1	0.1
Tamoxifen	0.005	0.05	To be determined		
Metformin	0.1	1	To be determined (on different LC method)		

HBCDD

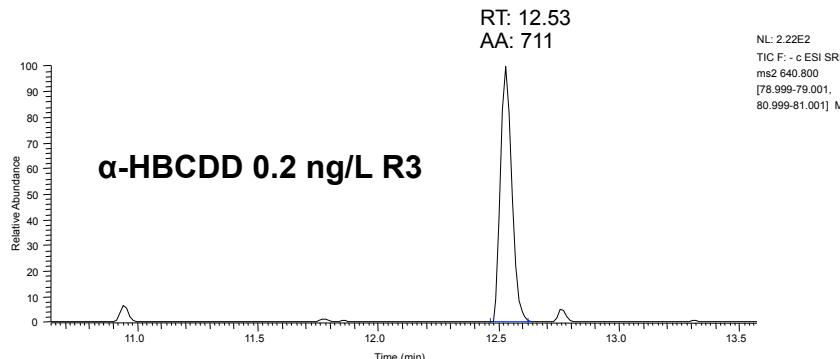
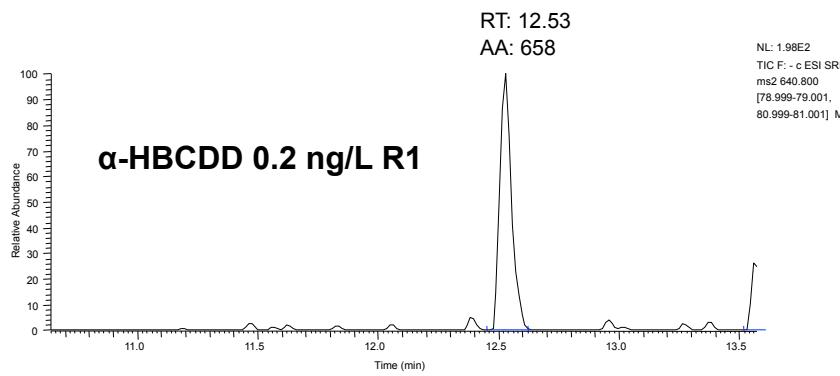
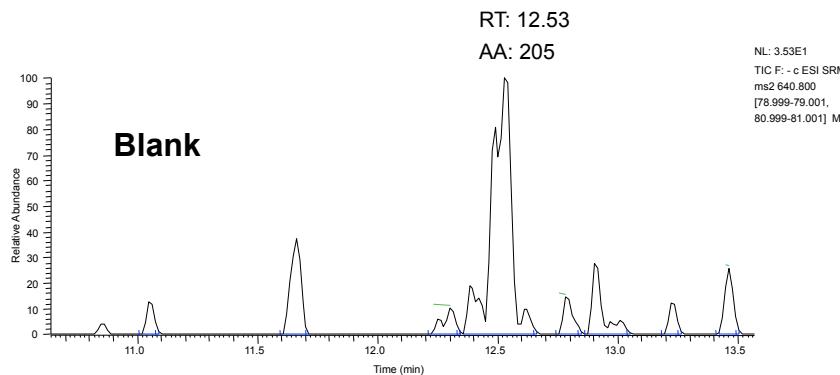
Compound	2013/39/EU			Required LOD (pg on column)	LOD in Solvent (pg on column)
	AA-EQS inland surface water (µg/L)	AA-EQS other surface waters (µg/ L)	EQS Biota (µg/Kg)		
HBCDD	0.0016	0.0008	167	2000	2000 (measured for α- HBCDD)

Chromatographic Separation of HBCDD Isomers

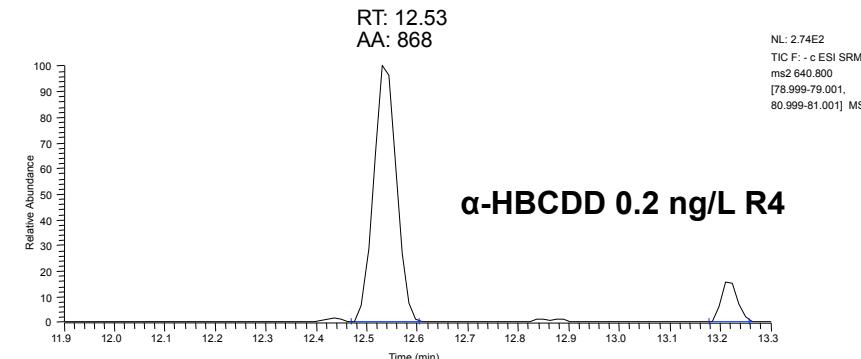
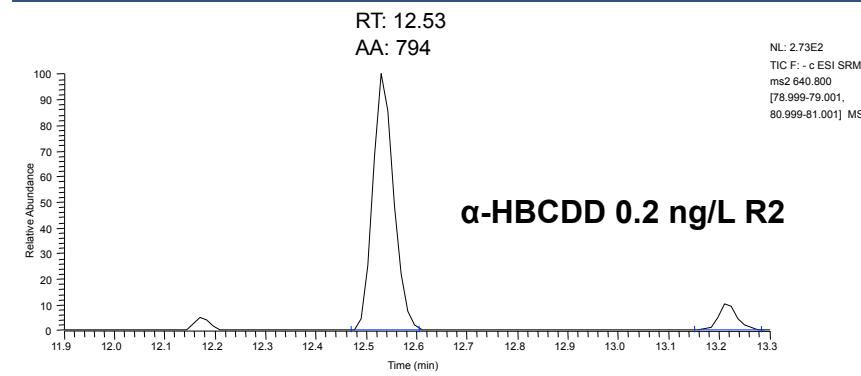


**Gradient elution using 2mM ammonium acetate and MeOH
Accucore C18 LC column - 100 x 2.1mm, 2.6 µm**

α -HBCDD – Blank vs 0.2 ng/L (Std in ACN)



12% RSD - based on area
10 μ L injection



Steroids - Conditions

LC Parameters

System: Ultimate 3000 RSLC system

Column: Thermo Scientific™ Hypersil GOLD™ columns - 50 x 2.1 mm, 1.9 µm at 30°C

Mobile Phase: (A) 0.02 % Ammonium Hydroxyde in H₂O (B) 0.02 % Ammonium Hydroxyde in MeOH

Flow Rate: 0.4 mL/min

Injection Volume: 50 µL

MS Parameters (TSQ Quantiva)

Ionization: ESI

Scan Mode: Timed SRM

Cycle Time: 100 ms

Resolution: 0.7 Th Q1/Q3

Collision Gas: Ar (1.5 mTorr)

Capillary Temperature (°C): 320

Vaporizer Temperature (°C): 350

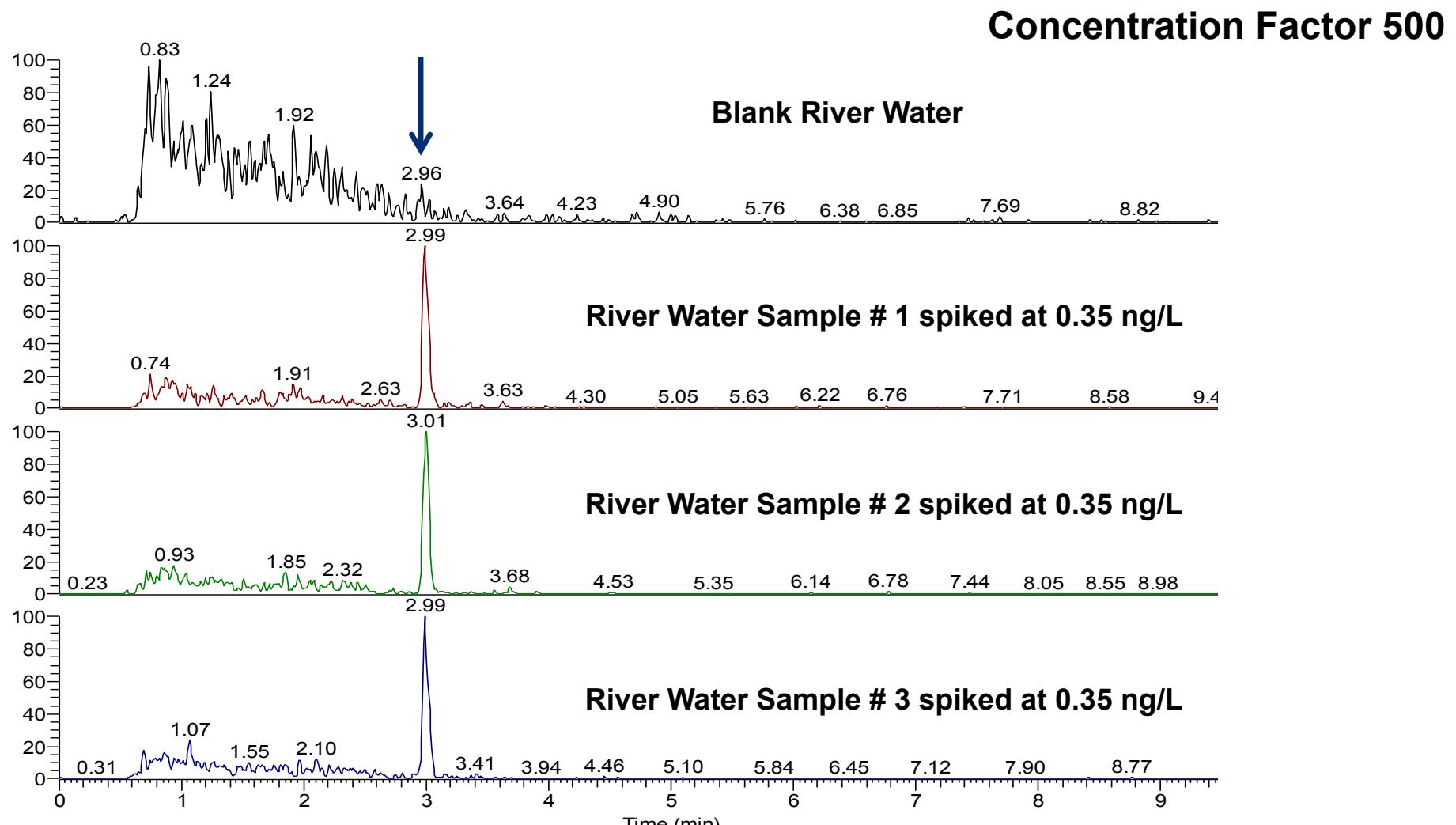
Sheath Gas Pressure (Arb): 50

Aux Gas Pressure (Arb): 40

Steroids

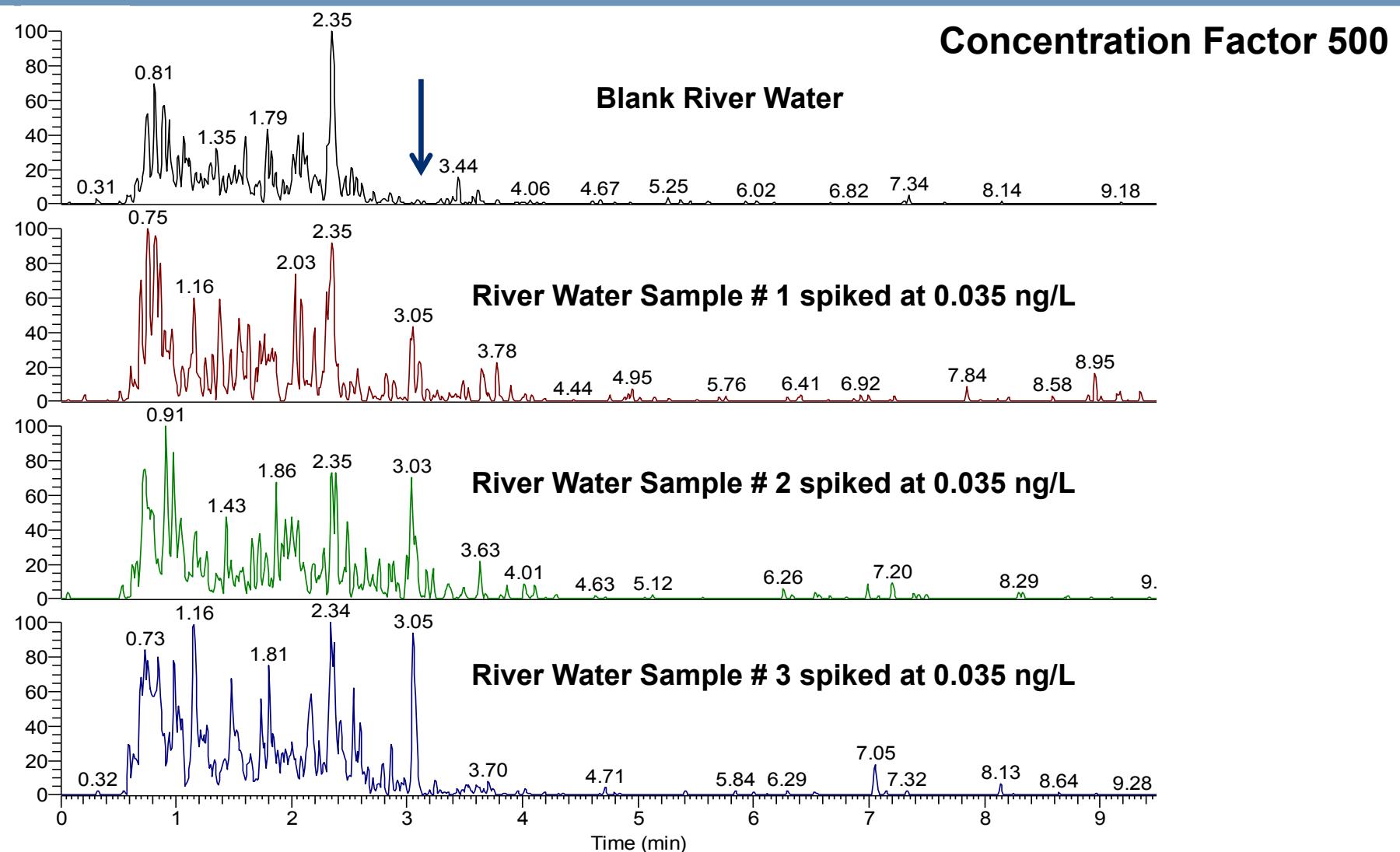
Compounds	CIP 2	COM(2011)876	2013/39/EU	LOD Influent/ Effluent (ng/L)
	Required LODs Influent/Effluent (ng/L)	AA-EQS inland surface water (ng/L)	AA-EQS inland surface water	
17-alpha-ethynodiol (EE2)	0.03	0.35	Watch List	100
17-beta-estradiol (E2)	0.3	4	Watch List	10
Estrone	1	-	-	15

E2 in River Water Sample - Chromatogram



Chromatogram representing Quan Transition (m/z 271- m/z 145) at TSQ Quantiva MS

EE2 in River Water Sample



Chromatogram representing Quan Transition (m/z 271- m/z 145) at TSQ Quantiva MS

Conclusions

- An efficient sample preparation in combination with a sensitive LC-MS/MS methodology is crucial for achieving the established EQSs.



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