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# QUALITATIVE SCREENING FOR EMERGING CONTAMINANTS AND THEIR METABOLITES/TRANSFORMATION PRODUCTS IN SEWAGE SLUDGE OF ATHENS BY UHPLC-QTOF MS



Viola Borova

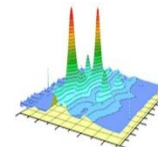
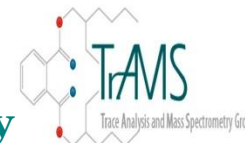
Ph.D Researcher



*Acknowledgements: Anna Bletsou and Nikolaos Thomaidis*



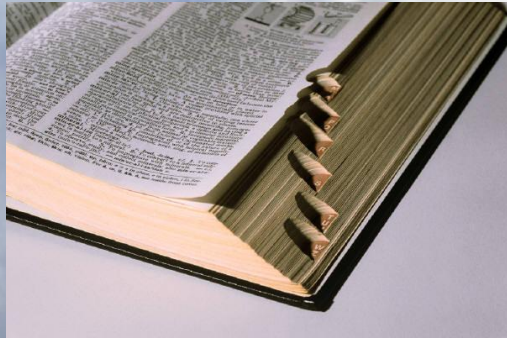
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Department of Chemistry



# Overview



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## Introduction

- Emerging contaminants, Issue of concern
- Their occurrence in the environment

## Analytical methodology Method Validation

## Application in real sewage sludge samples

## Conclusions

## Sources of PPCPs



ECs : **Pharmaceuticals, Illicit drugs, Personal care products, Endocrine disruptive compounds (EDCs), Flame retardants, Food additives, Disinfection by-products, Pesticides, PLUS metabolites & TPs**

They Come from "You"



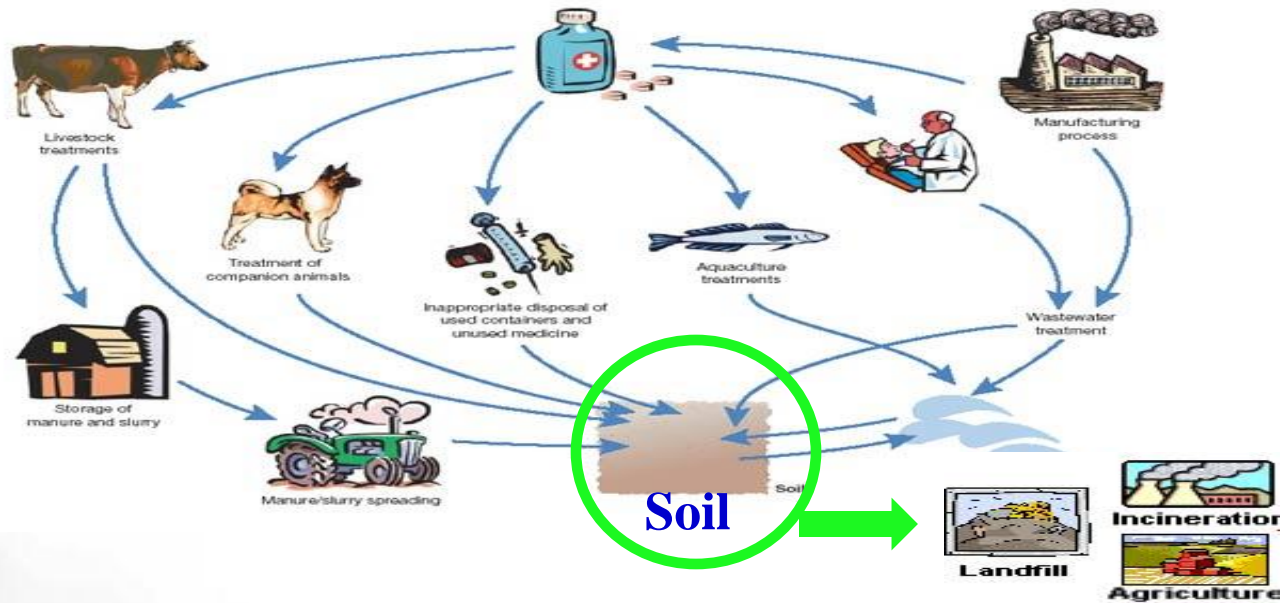
## What's in wastewater?

- ▷ human feces and urine
- ▷ food from sinks
- ▷ soaps and other cleaning agents
- ▷ runoff from streets and lawns
- ▷ industrial discharges





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So what's the problem with sewage sludge?

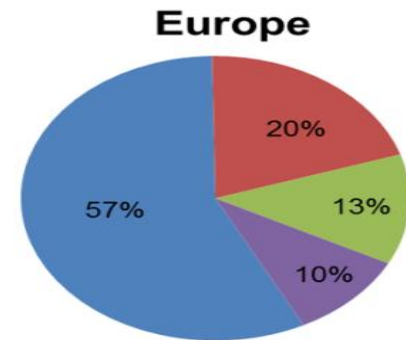


- ECs remain in the sewage sludge (SS) generated
- Efforts on improving water quality led to an increased sewage loads
- Sorption processes are complex and difficult to predict
- Additional route of entry of organic pollutants to the environment, Toxicity, Plant growth (amendment)

**Sewage Sludge** - any solid, semisolid, or liquid residue removed during the treatment of municipal waste water or domestic sewage

In Europe is estimated 90 g d.w. per person per day

- Land application
- Incineration
- Landfilling
- Sea disposal





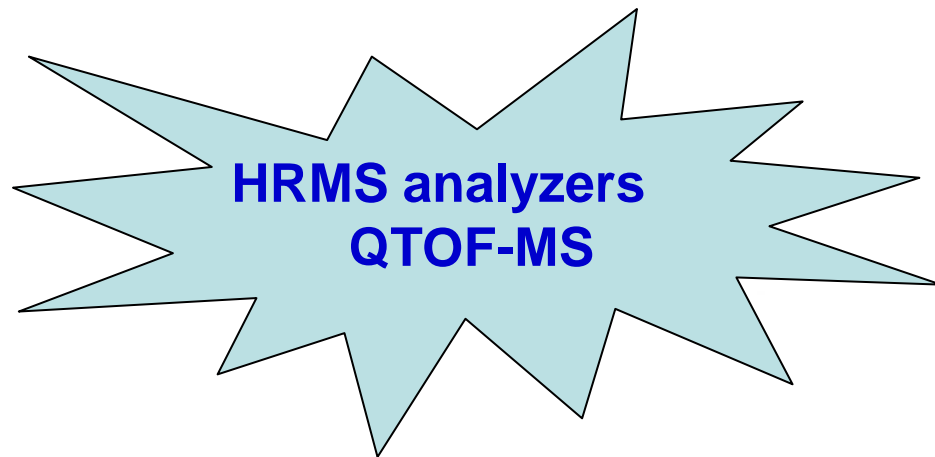
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## Issue of concern

➤ Detailed studies on the presence of ECs and their metabolites and TPs in sewage sludge **are necessary** in order **to have the whole picture** of the **distribution** of these emerging pollutants in the environment and to perform a reliable **risk assessment**.

**Imperative need for...**

Capable of monitoring **a large variety of compounds**, belonging to **different group of compounds**, with **different characteristics** with **one analytical procedure in one single run**.



# Analytical Methodology

## ~ Workflow for Screening of ECs in sewage sludge

**Sample Preparation**  
Extraction from sewage sludge

**UHPLC-QTOF-MS**  
(+), (-) ESI / bbCID mode  
High sensitivity & resolution  
Accurate mass data

**Processing: Target Analysis**  
In house database (2327 compounds)

**maXis Impact**  
Ultra High Resolution  
Time-Of-Flight Mass Spectrometer  
UHR-TOF-MS

- ✓ Unlimited number of analytes monitored
- ✓ No compound-specific method development
- ✓ Target and non-target approach



**Report**  
Identification, Confirmation



DataAnalysis



TargetAnalysis

# Analytical Methodology

## ~ Sample preparation\*

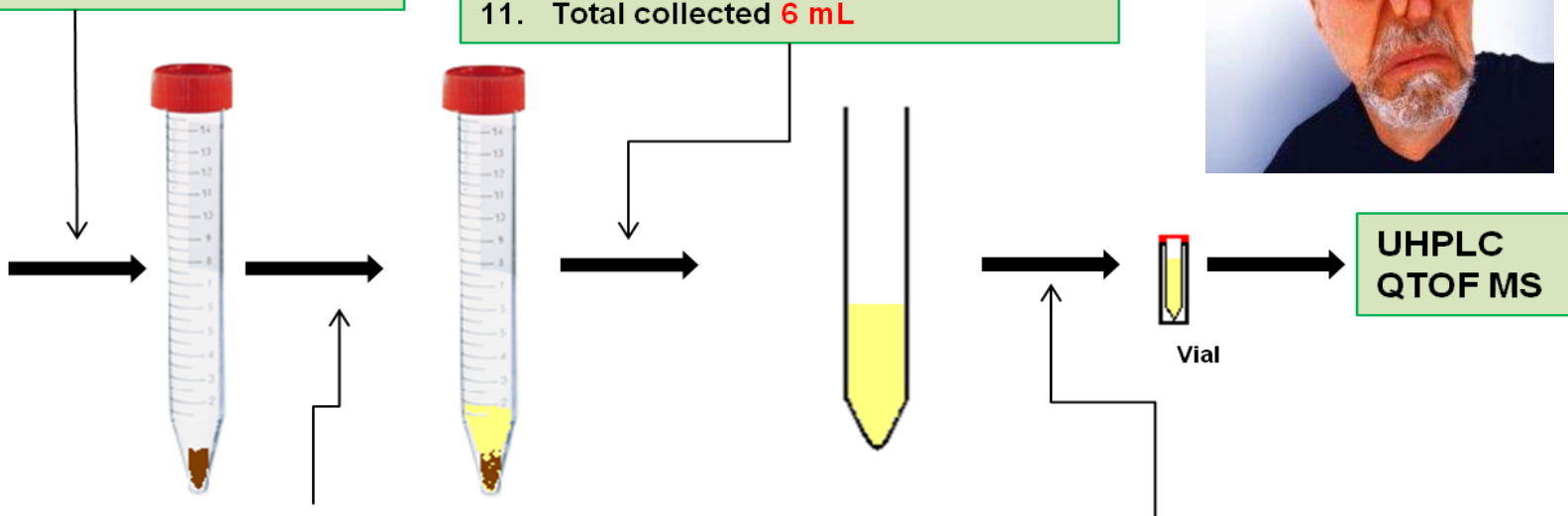


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\*Gago Ferrero et al. *Analytical and Bioanalytical Chemistry*, 2015, 407 (15): 4287-4297

1. Samples were collected after sewage sludge dewatering. Then, they were **freeze-dried** and stored in the dark at -20 °C until analysis
2. Finely homogenization in a mortar
3. Weigh **0.1 gr** of dried sludge
4. Internal deuterated standards of the compounds were added to all samples

7. 15 min in ultrasonic at 50°C
8. Centrifugation 4000 rounds for 10 min
9. The supernatant collected in glass tube
10. Steps 5,7,8,9 repeated two more times
11. Total collected **6 mL**



5. Addition of 2 mL mixture solution
6. Solution: **MeOH : Milli Q water (pH 2.5, FA 0.5% and 0.1% EDTA), 50:50 v/v)**

12. The extracts were evaporated to dryness under constant steam of nitrogen, N<sub>2</sub> (g) at 40°C.
13. Reconstitution **in 500 µL** of 25% MeOH and 75% ultra purified water with 0.05% v/v formic acid
14. 1-2 min vortex stirring
15. Final filtering step of the extract on a 0.2 mm syringe filter



# Analytical Methodology

## ~ UHPLC-QTOF-MS



### UHPLC

Dionex UltiMate

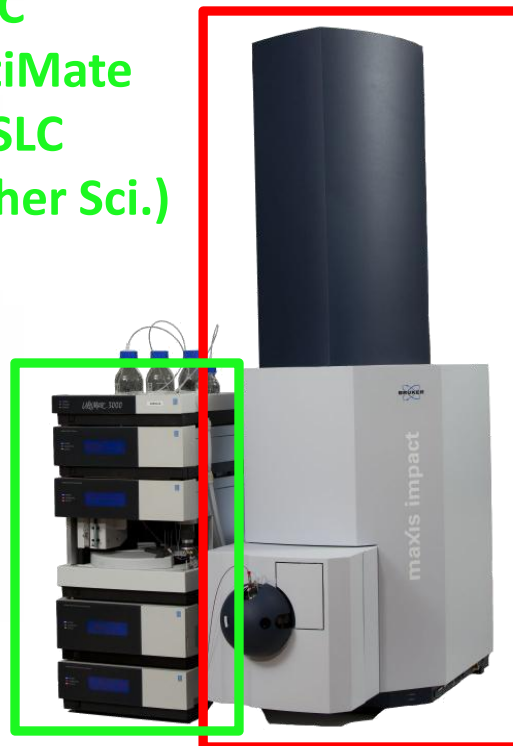
3000 RSLC

(Thermo Fisher Sci.)

**QTOF MAXIS IMPACT**  
(Bruker Daltonics)

Range:  $m/z$  50-1000

Scan: 2 Hz



### Mobile phase:

H<sub>2</sub>O:MeOH (gradient)

- both 0.01% HCOOH & 5  
mM NH<sub>4</sub>HCO<sub>2</sub> (ESI+)

-5 mM CH<sub>3</sub>COONH<sub>4</sub> (ESI-)

Flow rate: gradient

### Column

Acclaim<sup>TM</sup> RSLC 120 C18

(2.1 × 100 mm, 2.2 μm)

Injection volume: 5 μL

### Pre-column

VanGuard (Waters):

Acquity UPLC BEH C18

1.7 μm, 2.1 × 5 mm

Pesticide  
Screening  
Method

(+), (-) ESI  
bbCID mode

Low CE (4 eV) (*pass all*) → MS spectra

High CE (25 eV) (*fragment all*) → MS/MS spectra



# Analytical Methodology

## ~ Method development



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**In-house database: 2327 compounds**

**2224  
compounds  
for (+) ESI**

**580  
compounds  
for (-) ESI**

**> 700 pesticides**

**> 200 pharmaceuticals, illicit, DoA**

**~ 300 steroids & doping compounds**

**~ 100 compounds like industrial chemicals,  
food additives, dyes and natural occurring  
compounds (aminoacids)**

**~ 300 metabolites & TPs**

# Analytical Methodology

## ~ Method development



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	Q
1	m/z	RT	sum formula	name	CAS							Q1 1	Q1 2	Q1 3	Q
158	1.370.835.154	5.15	C8H11NO <sup>+1</sup>	Aminocarb (Metacil) Fragm 137	(2032-59-9)							1.521.072	1.370.836		
159	1.521.069.905	5.15	C9H14NO <sup>+1</sup>	Aminocarb (Metacil) Fragm 152	(2032-59-9)							1.521.072	1.370.836		
160	233.128.454	4.99	C13H16N2O2	Aminoglutethimide	(125-84-8)							146.096.426	94.065.126	18.810.699	
161	232.144.439	5.21	C13H17N3O1	Aminophenazone, Amidopyrin	(58-15-1)							56.049.476	97.076.025	111.091.675	
162	328.184.195	8.54	C19H25N3S	Aminpromazine	(58-37-7)							212.052.847	238.068.497	58.065.126	
163	1.630.865.894	3.67	C9H10N2O	Aminorex Isomer 1	(2207-50-3)							1.200.808	1.030.542		
164	120.080.776	3.67	C8H10N <sup>+1</sup>	Aminorex Isomer 1 Fragm 120	(2207-50-3)							1.200.808	1.030.542		
165	1.630.865.894	5.57	C9H10N2O	Aminorex Isomer 2	(2207-50-3)							1.200.808	1.030.542		
166	120.080.776	5.57	C8H10N <sup>+1</sup>	Aminorex Isomer 2 Fragm 120	(2207-50-3)							1.200.808	1.030.542		
167	64.603.097	12.1	C25H29N1O3I2	Amiodarone	(1951-25-3)							73.088.601	86.096.426	100.112.076	
168	2.941.964.742	13.3	C19H23N3	Amitraz	(33089-61-1)							163.122.975	122.096.426		
169	163.122.975	13.3	C10H15N2 <sup>+1</sup>	Amitraz Fragm 163	(33089-61-1)							163.122.975	122.096.426		
170	278.190.326	8.23	C20H23N1	Amitriptyline	(50-48-6)							117.069.877	218.109.002	233.132.477	
171	850.508.726	1.44	C2H4N4	Amitrole	(61-82-5)							57.044.725	58.039.974	68.024.323	
172	409.152.476	8.36	C20H25N2O5Cl1	Amlodipine	(88150-42-9)							238.062.933	294.089.148	334.084.062	
173	447.108.358	8.36	C20H25N2O5Cl1K <sup>+1</sup>	Amlodipine (K)	(88150-42-9)							238.062.933	294.089.148	334.084.062	
174	43.113.442	8.36	C20H25N2O5Cl1Na <sup>+1</sup>	Amlodipine (Na)	(88150-42-9)							238.062.933	294.089.148	334.084.062	
175	238.062.933	8.36	C12H13ClNO2 <sup>+1</sup>	Amlodipine Fragm 238	(88150-42-9)							238.062.933	294.089.148	334.084.062	
176	294.089.148	8.36	C15H17ClNO3 <sup>+1</sup>	Amlodipine Fragm 294	(88150-42-9)							238.062.933	294.089.148	334.084.062	
177	318.279.141	12.8	C21H35NO	Amorolfine	(78613-35-1)							130.122.641	161.132.477	11.610.699	
178	3.141.054.663	7.64	C17H16ClN3O	Amoxapine	(14028-44-5)							271.063.267	70.065.126	245.047.617	
179			C16H19N3O5S	Amoxicillin											
180	136.112.076	4.16	C9H13N	Amphetamine	(300-62-9)							91.054.227	65.038.577		
181	119.085.527	4.16	C9H11 <sup>+1</sup>	Amphetamine Fragm 119	(300-62-9)							91.054.227	65.038.577		
182	91.054.227	4.16	C7H7 <sup>+1</sup>	Amphetamine Fragm 91	(300-62-9)							91.054.227	65.038.577		

The in house database is a list of compounds for *identification*

Retention times for the matched UHPLC method

Adduct information

Isomer information

Fragment ions on MS data level

Isotopic confirmation

Qualifier ions for confirmation in broad band MS/MS mode

2327 compounds  
→ 4174 hits

# Analytical Methodology

## ~ Validation data set



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### Selection of target analytes

- ✓ Different classes of compounds
- ✓ Different properties
- ✓ Representative number
- ✓ Wide range retention time



### I. Dataset

**114 compounds: 106 in (+) ESI, 8 in (-) ESI, 5% of the compounds in the database**

### II. Optimization of the evaluation method (TargetScreening)

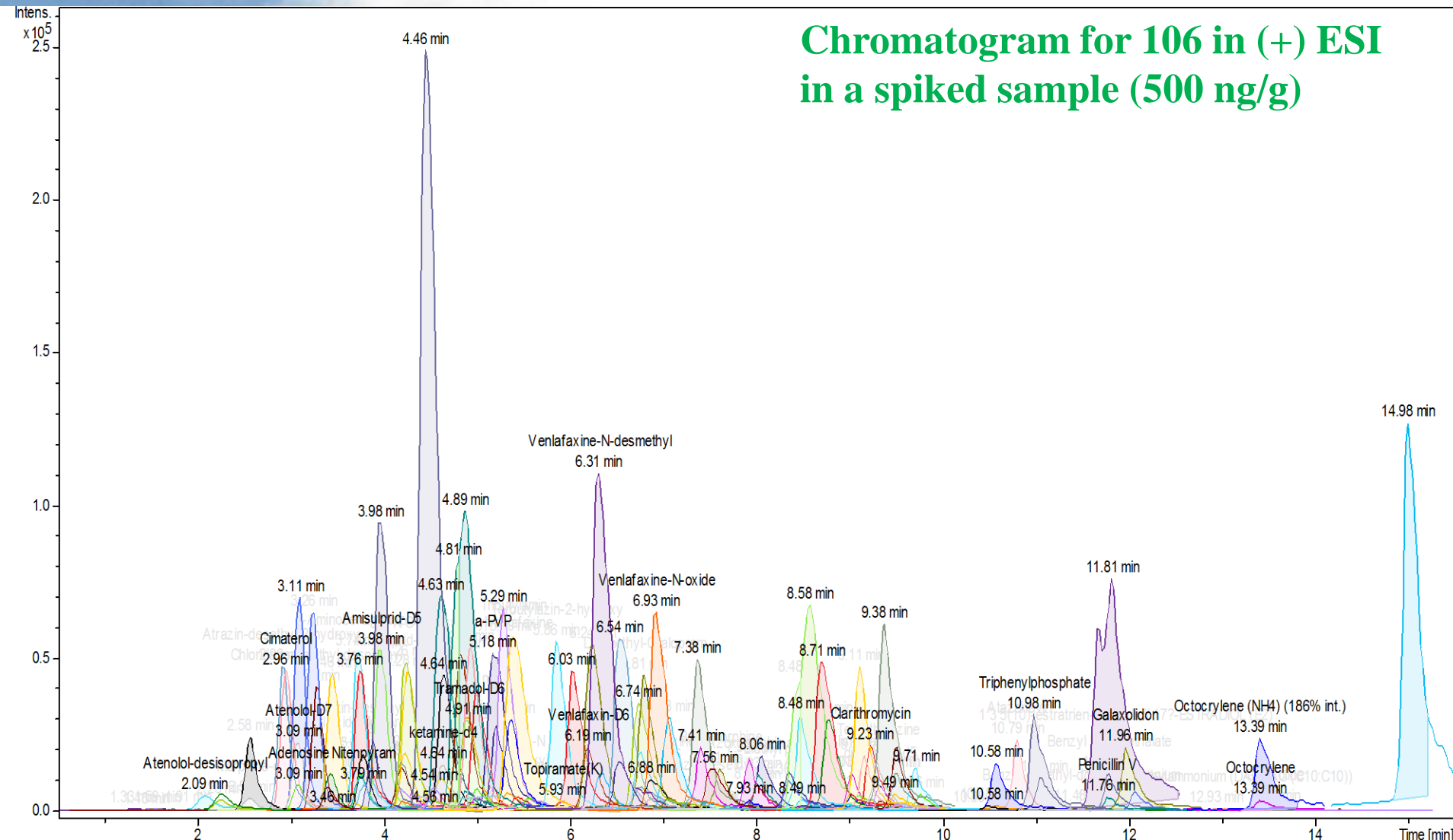
Find	Area	1000 (+)/ 600 (-)	
	Intensity	250(+)/ 150 (-)	
Scoring		min	max
	ret. Time (min)	0.1	0.4
	accuracy (ppm)	2.5	5
	mSigma threshold	100	200

# Analytical Methodology

## ~ Validation data set



### Chromatogram for 106 in (+) ESI in a spiked sample (500 ng/g)







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# Analytical Methodology

## ~ Validation data set

### Validation Parameters

- ❖ *Calibration curves* of standard solution in solvent and in spiked samples were built (6 levels of concentration)
- ❖ **Repeatability, Recoveries (in two levels of concentrations) and Matrix Effect**
- ❖ The screening detection limit (SDL) and the limit of identification (LOI): estimate the threshold concentration at which detection and identification become reliable, respectively.
  - **SDL:** the lowest concentration level tested for which a compound was **detected** in all samples; ( $t_R$  + precursor ion)
  - **LOI:** the lowest concentration tested for which a compound was satisfactorily **identified** in all spiked samples; ( $t_R$  + precursor ion + fragment ion)

A CRM 145R, sewage sludge from European Commission, was used for validation

# Analytical Methodology

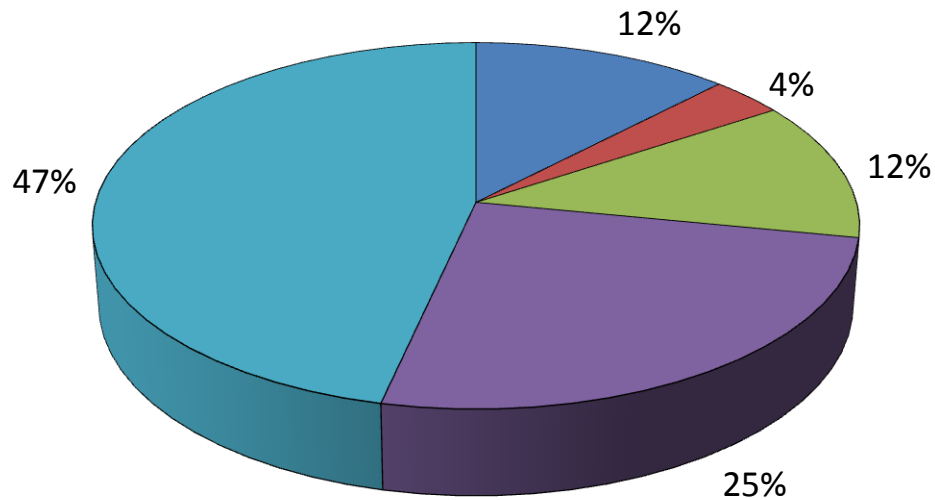
## ~ Validation data set



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### Recoveries , high level (500ng/g)

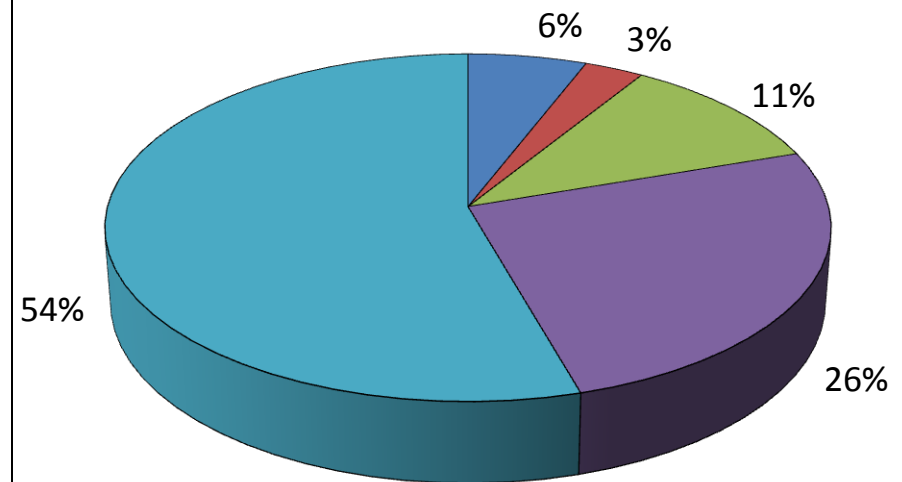
RSD% (n=6) : 0.4 – 23.0 %



> 30%      30% - 50%      50% - 70%  
70% - 90%      90% - 120%

### Recoveries , low level (50ng/g)

RSD% (n=6) : 0.7– 26.8%



> 30%      30% - 50%      50% - 70%  
70% - 90%      90% - 120%

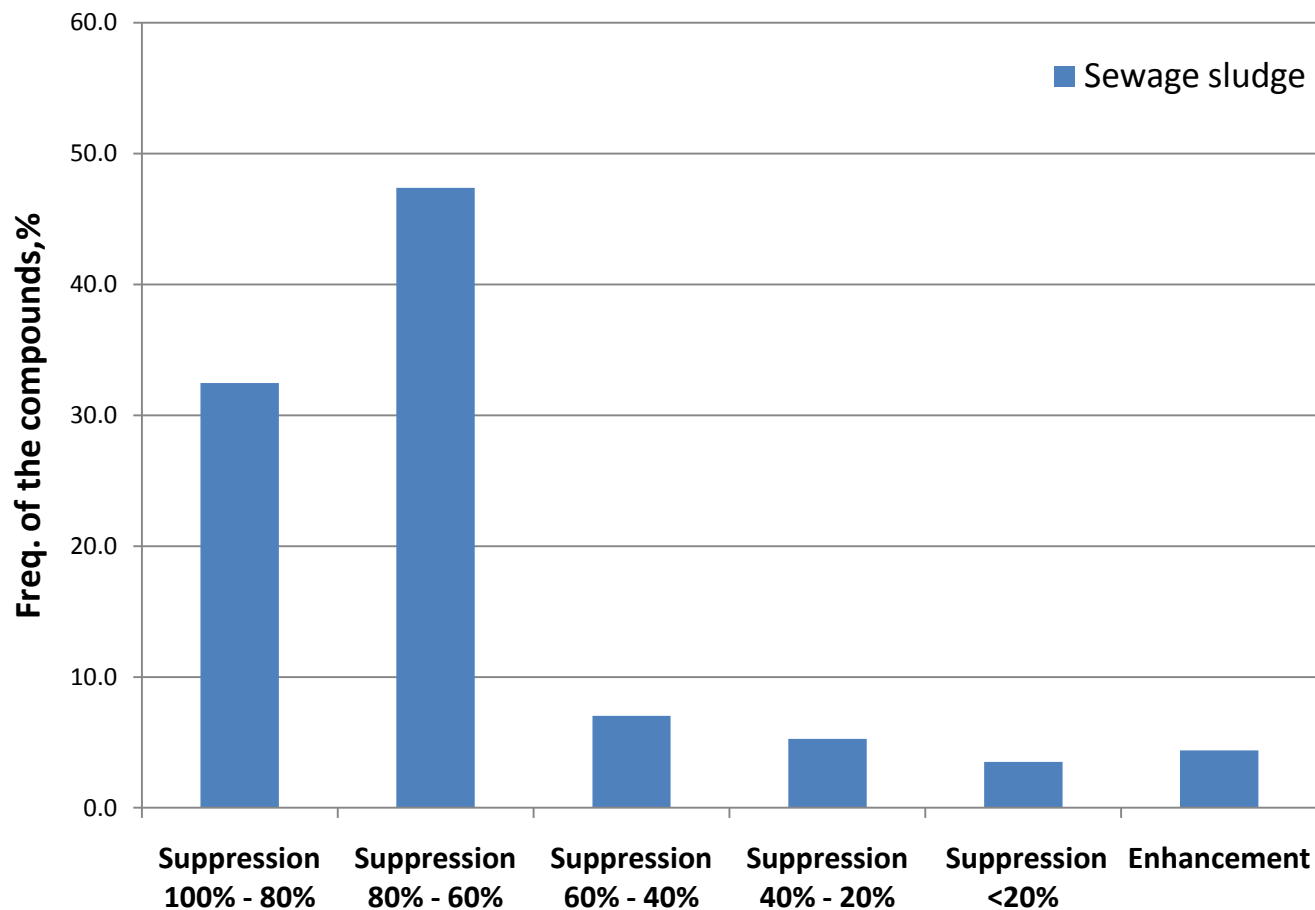
# Analytical Methodology

## ~ Validation data set



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### Matrix Effect

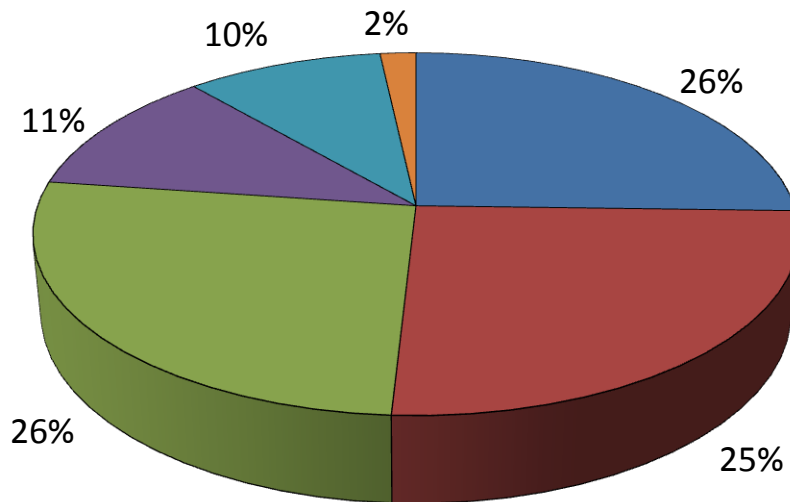


# Analytical Methodology

## ~ Validation data set

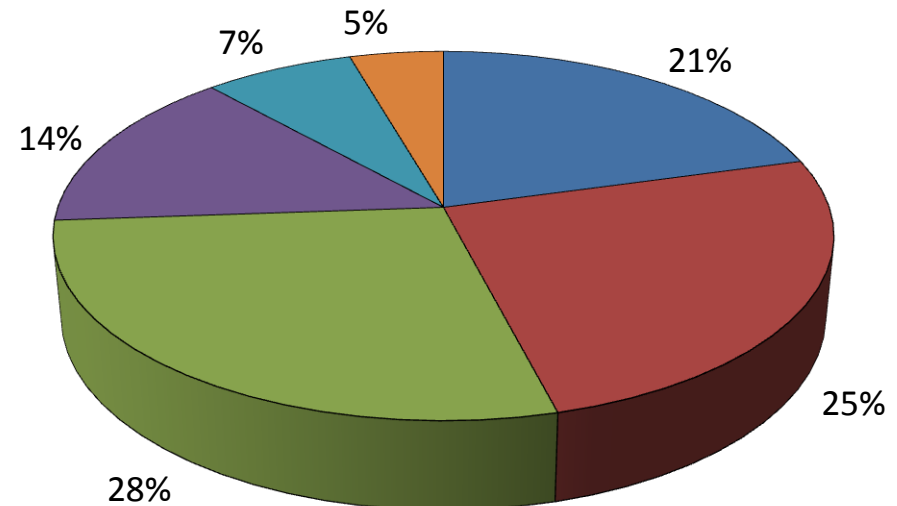


### Screening Detection Limits (SDLs)



- 2.5 ng/g d.w
- 5.0 ng/g d.w
- 10 ng/g - 25 ng/g d.w
- 50 ng/g d.w
- 100 ng/g - 250 ng/g d.w
- 500 ng/g d.w

### Limit of identification (LOI)



- 2.5 ng/g d.w
- 5.0 ng/g d.w
- 10 ng/g - 25 ng/g d.w
- 50 ng/g d.w
- 100 ng/g - 250 ng/g d.w
- 500 ng/g d.w





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# Application in real sewage sludge samples from WWTP of Athens

Location: **WWTP of Athens, Greece**

Period: **1 day in March 2014 & 1 day in March 2015**

Samples: **After sewage sludge dewatering**



## Results

March 2014

109 in  
(+) ESI

29 in  
(-) ESI

March 2015

112 in  
(+) ESI

25 in  
(-) ESI

Common in both years

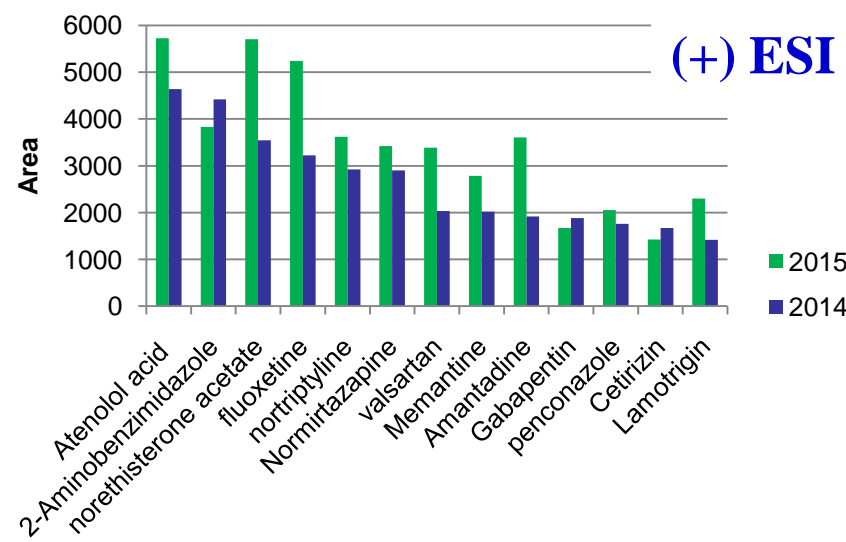
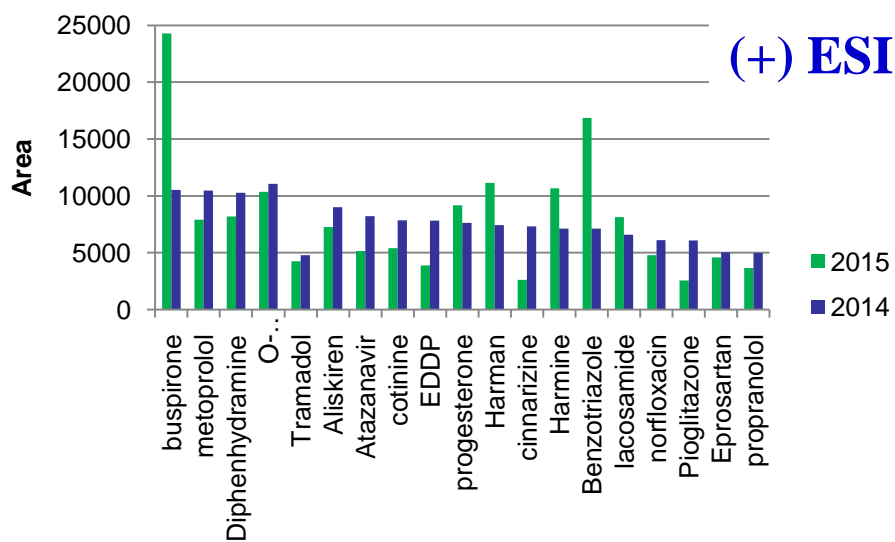
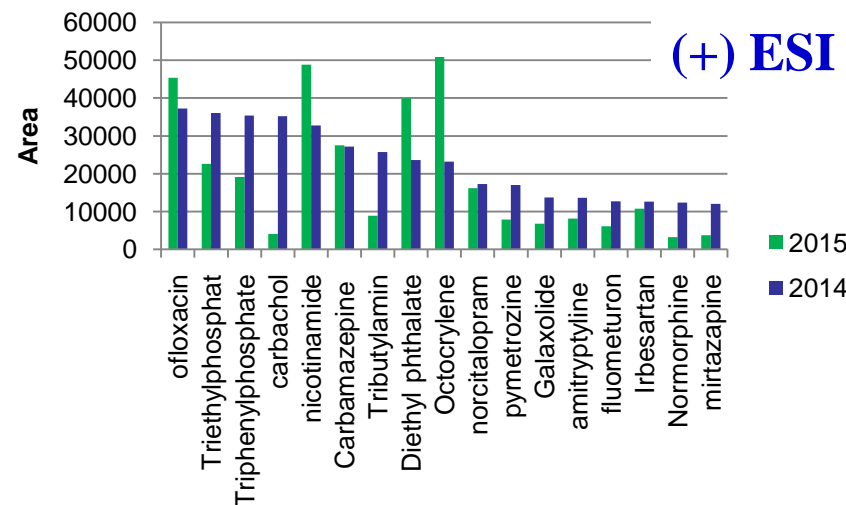
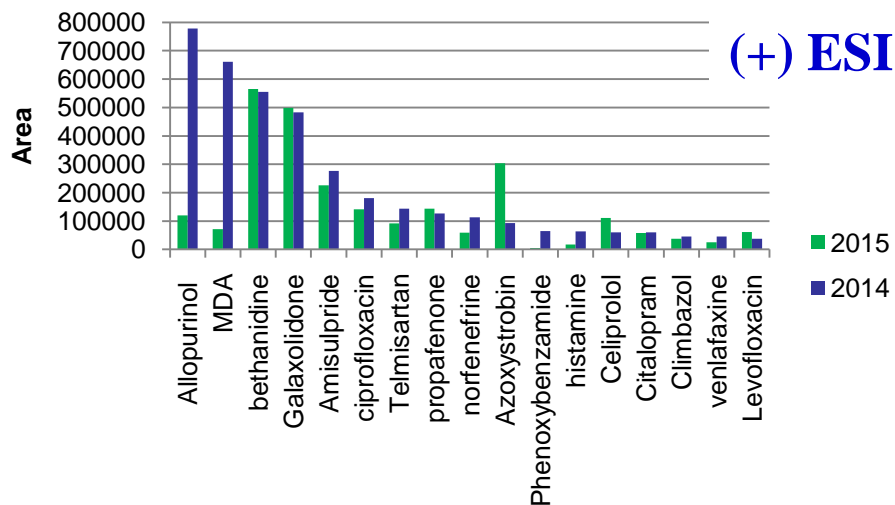
66 in (+)  
ESI

16 in (-)  
ESI



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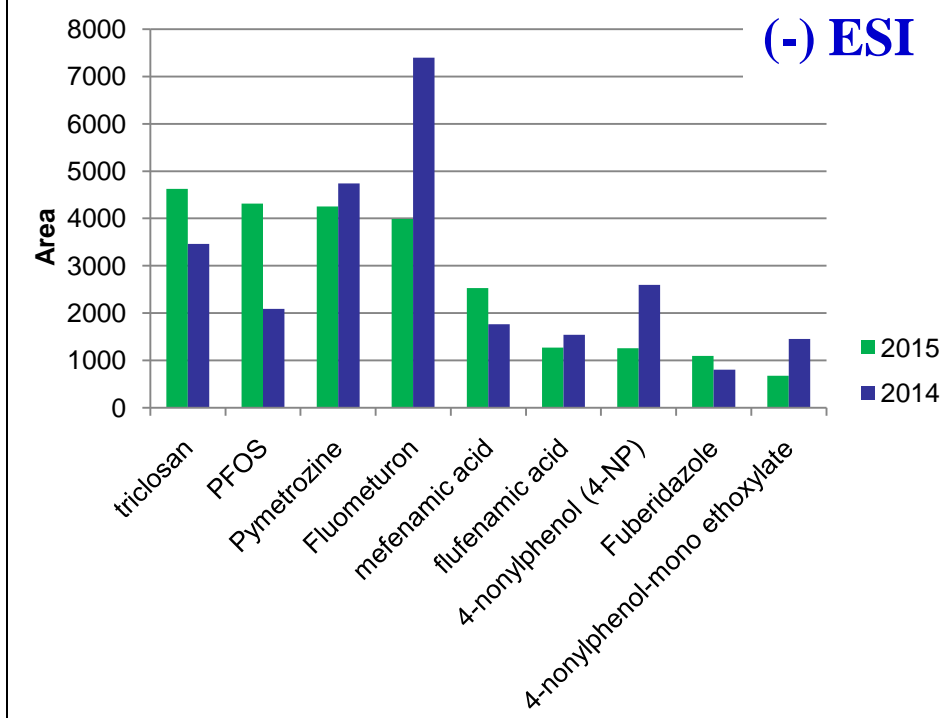
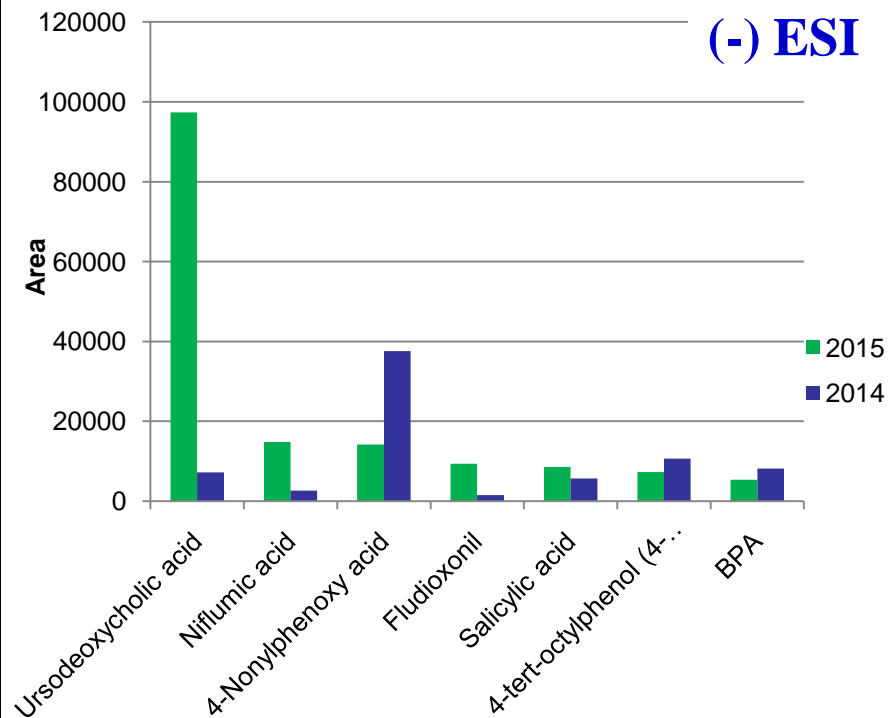
# Application in real sewage sludge samples from WWTP of Athens



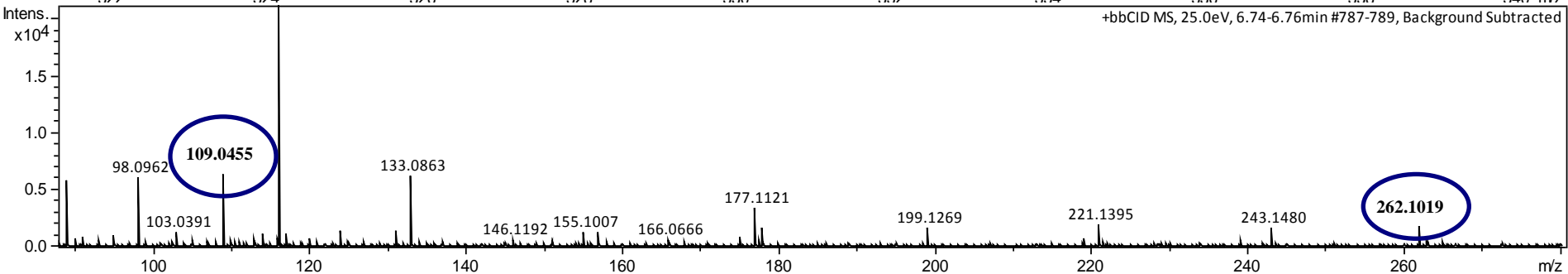
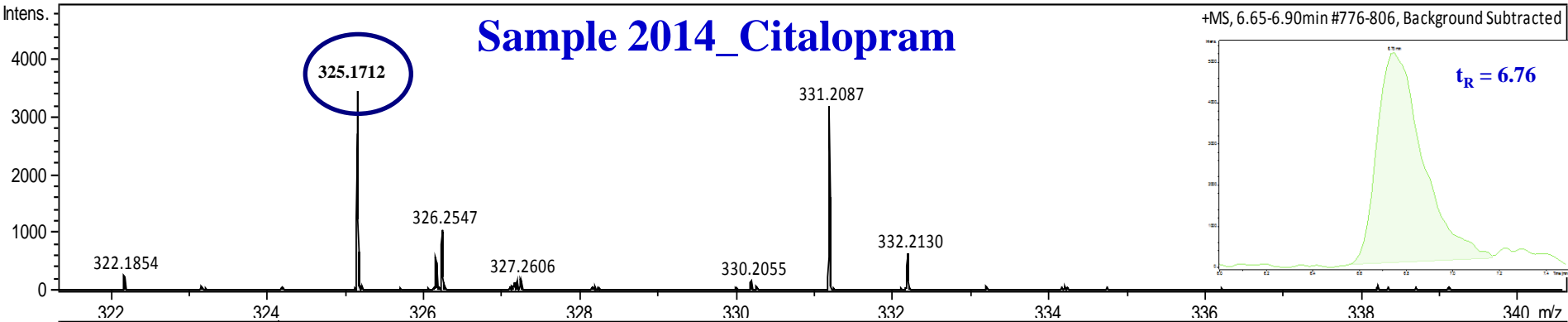


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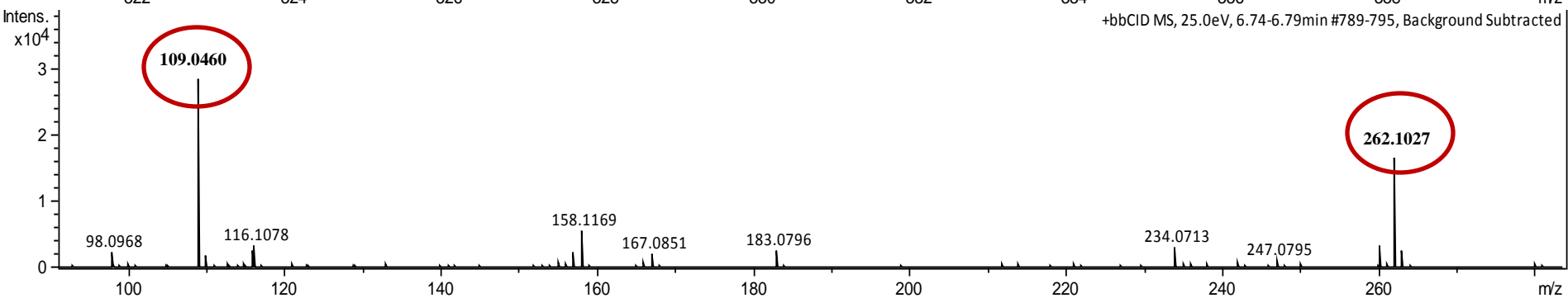
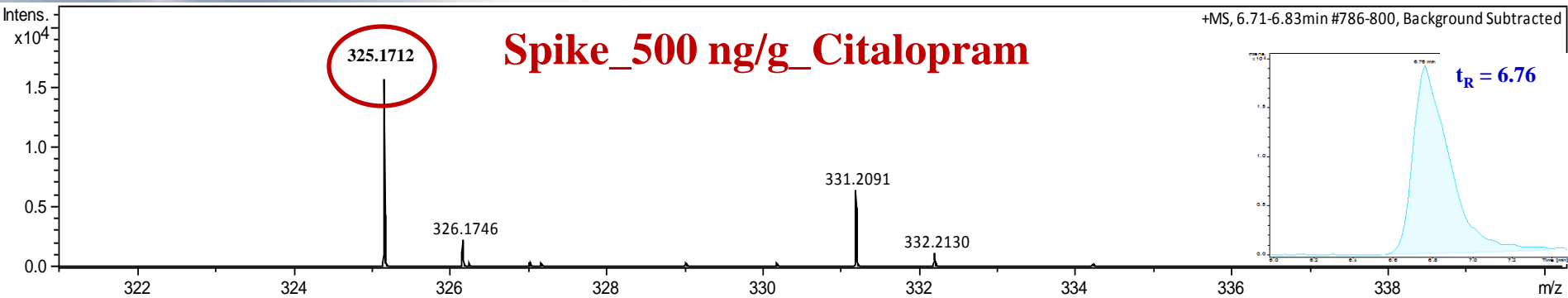
# Application in real sewage sludge samples from WWTP of Athens



# Sample 2014\_Citalopram



# Spike\_500 ng/g\_Citalopram





# Conclusions

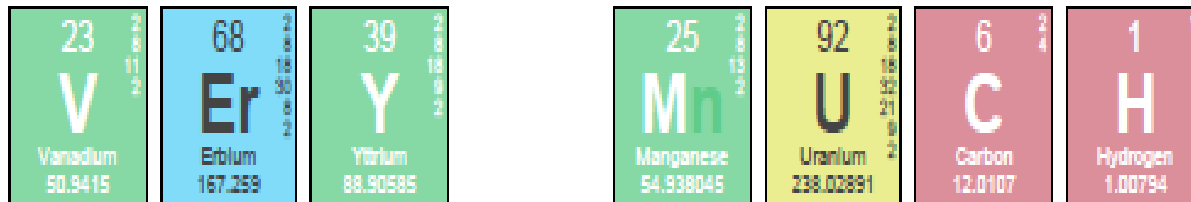


- ✓ **In-house database with information for 2327 compounds was applied in sewage sludge samples**
- ✓ **Generic solid liquid extraction of a wide range of compounds**
- ✓ **Validation of the target screening method**
- ✓ **Comparison of the results for 2 consecutive years**
- ✓ **Screening and Identification of the analytes (antihypertensives, antidepressants, pesticides etc.)**



### Acknowledgements:

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## Any Questions???

**E-mail Address:** [ntho@chem.uoa.gr](mailto:ntho@chem.uoa.gr)

[vborova@chem.uoa.gr](mailto:vborova@chem.uoa.gr)



 <b>European Union</b> European Social Fund	 OPERATIONAL PROGRAMME <b>EDUCATION AND LIFELONG LEARNING</b> <i>investing in knowledge society</i> MINISTRY OF EDUCATION & RELIGIOUS AFFAIRS, CULTURE & SPORTS MANAGING AUTHORITY Co-financed by Greece and the European Union	 <b>NSRF</b> 2007-2013 programme for development EUROPEAN SOCIAL FUND
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