Laboratory of Analytical Chemistry
Department of Chemistry
University of Athens

HILIC-QTOF-HR-MS/MS FOR WIDE-SCOPE SCREENING OF POLAR MICROPOLLUTANTS IN ENVIRONMENTAL SAMPLES

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CEST
International Conference on Environmental Science and Technology

- Target screening
- Suspect screening
- Non-target screening

Technique of choice:

Reverse-Phase Liquid Chromatography

Smart alternative for polar compounds

Hydrophilic Interaction LIquid Chromatography (HILIC)

Sample & Sample preparation

LC-QTOF

Data Processing

Identification Confirmation

Why HILIC ?

- ✓ Retention of polar components → higher intensity
- ✓ Several different stationary phases available
- ✓ MS compatible
- ✓ Use of ACN (low viscosity solvent) → higher flow rates & better ionization

↳ Complex mechanistic separation

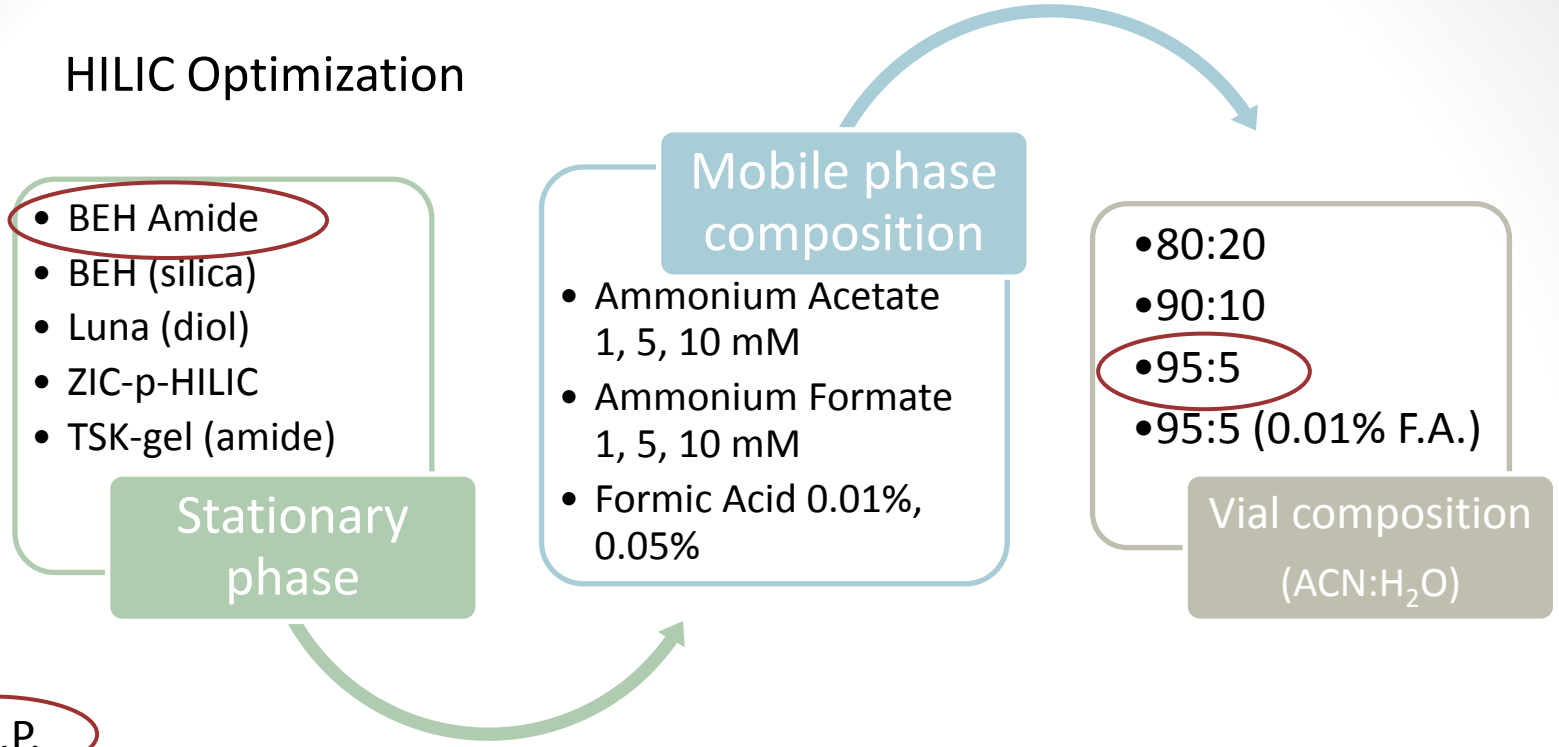
↳ Great effort for the method optimization and development

- Stationary phase
- Mobile phase (solvents, buffer, pH)
- Gradient elution program & flow rate
- Column temperature
- Vial composition

compromise

Wide-scope screening of emerging pollutants

HILIC Optimization



M.P.

(+) ESI: (A) H_2O, 1mM Amm. Form. 0.01% F.A.
(B) ACN:H_2O (95:5), 1mM Amm. Form. 0.01% F.A.
(-) ESI: (A) H_2O, 10mM Amm. Form.
(B) ACN:H_2O (95:5), 10mM Amm. Form.

- Flow rate: 200 µL/min
- Column T: 40 °C
- Chromatogram: 20 min (+5 min re-equilibration)

Method development

MaXis Impact

Ultra High Resolution

Time-of-Flight Mass Spectrometer

- Positive & Negative ESI
- bbCID mode

bbCID mode

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

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

TargetAnalysis

DataAnalysis

EPs, belonging to a diverse group of compounds

Database

902 compounds

- 601 well-retained compounds (k'>1) -

Chosen according to environmental relevance & HILIC chromatographic behavior

validation dataset

- 85 compounds
- 10% of the compounds of the total database
- Representative physicochemical properties
- Compounds from every class of EPs

- **Calibration curves** (**solvent**, **matrix** & **spiked** samples) (6 levels of concentration)
- **Repeatability**, **recoveries** and **matrix effect**
- The **screening detection limit (SDL)** and the **limit of identification (LOI)**:
  - **SDL:** the lowest concentration level tested for which a compound was detected in all samples;

    t_R + Precursor ion= 2 Identification Points (2 IPs)
  - **LOI:** the lowest concentration tested for which a compound was satisfactorily identified in all spiked samples;

    t_R + Precursor ion + fragment = 4 Identification Points (4 IPs)

Sample & Sample Preparation

Location: **WWTP of Athens, Greece**

Period: 8th March 2015 (Sunday)

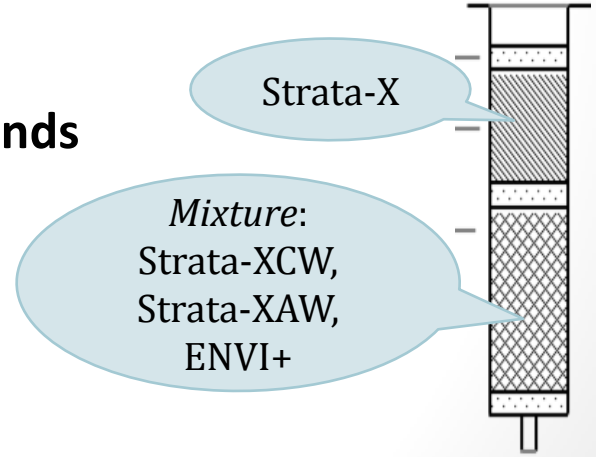
Samples: 24-h composite flow-proportional influent & effluent wastewater

Sample Preparation:

- ✓ **100 mL** wastewater (GFF filtration)
- ✓ **IS** spiking (100 ng/L)
- ✓ SPE ***Mixed-bed cartridges***
- ✓ Extraction: **Neutral, Basic & Acidic Compounds**

→ 100 times pre-concentration

as performed in RP target screening method.

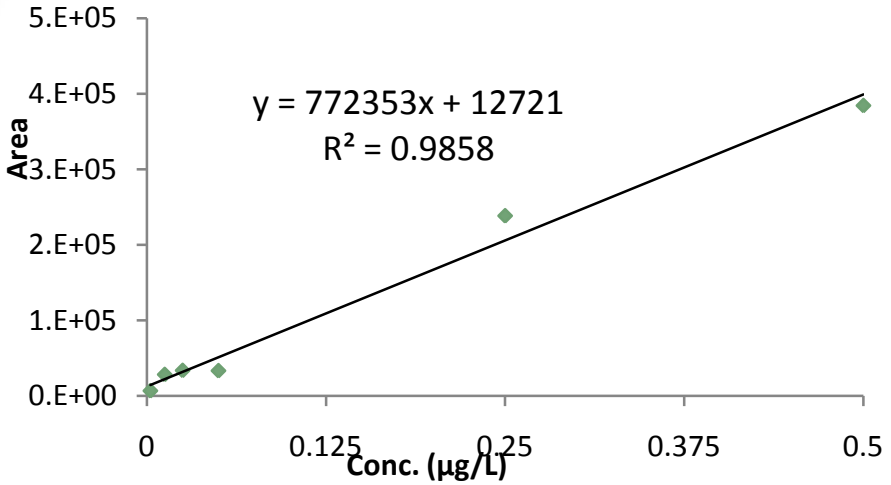


**Kern et al. EST (2009) 43(18):7039*

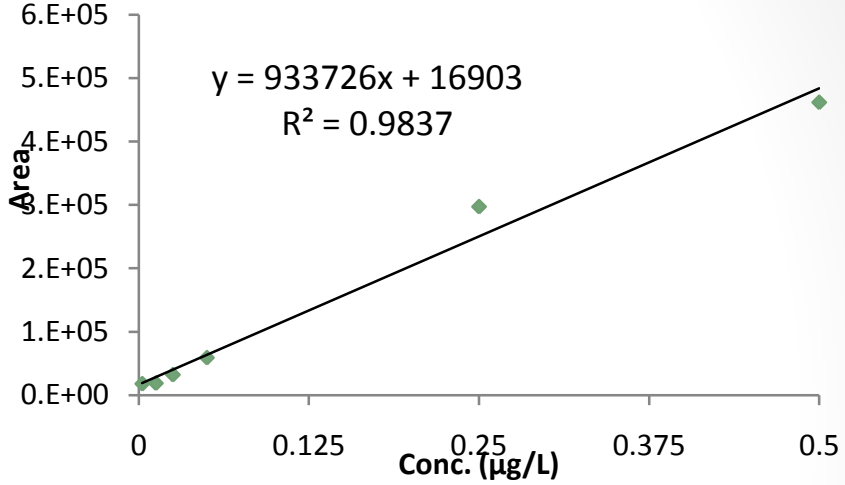
Linearity

Amisulpride-N-oxide

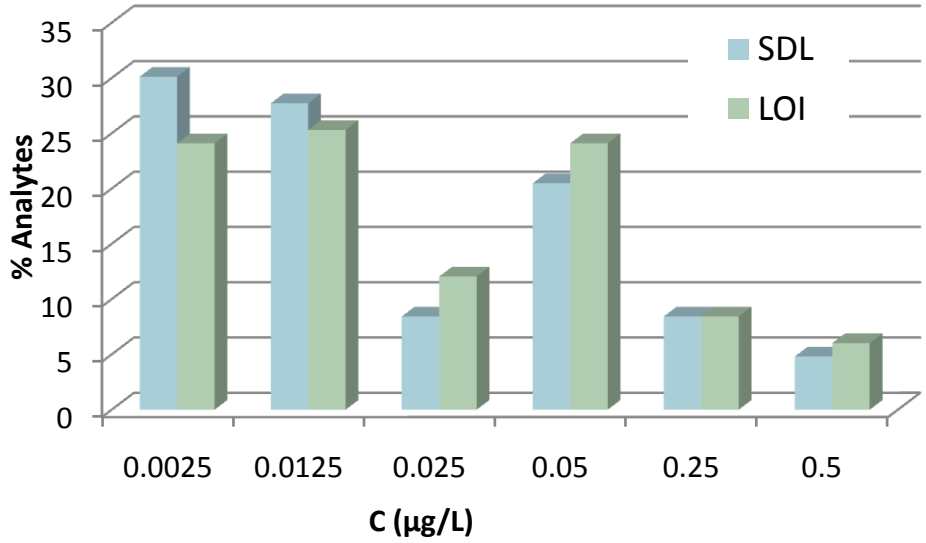
Standard addition Curve



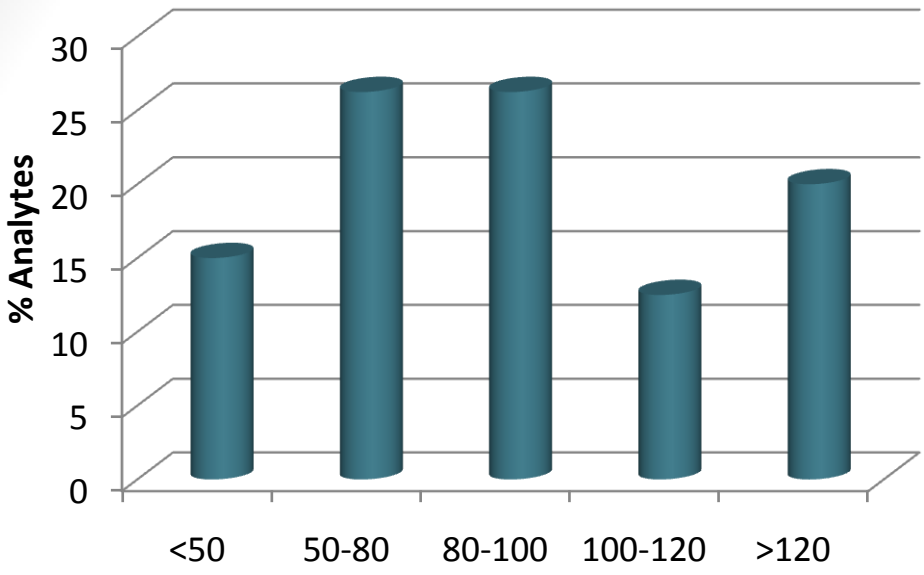
Matrix-matched solutions Curve



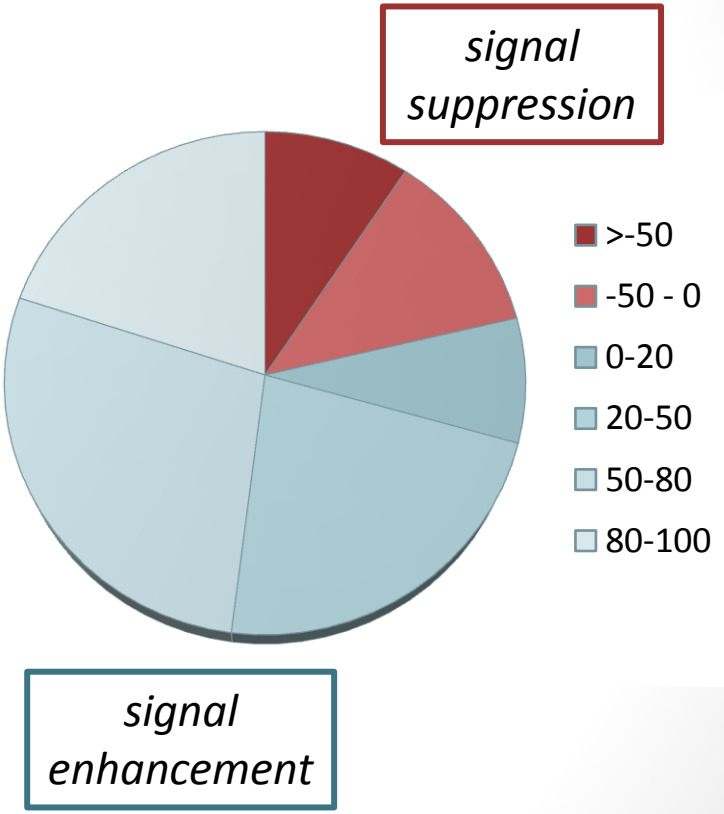
Screening Detection Limits (SDL) – Limits of Identification (LOI)



% Recoveries



% Matrix Effect



% Repeatability (*n=6*) (RSD%)

- 0.25 µg/L: **3.4-16 %**
- 0.025 µg/L: **6.0-17 %**
- 0.0025 µg/L: **11- 21 %**

Wastewater Results

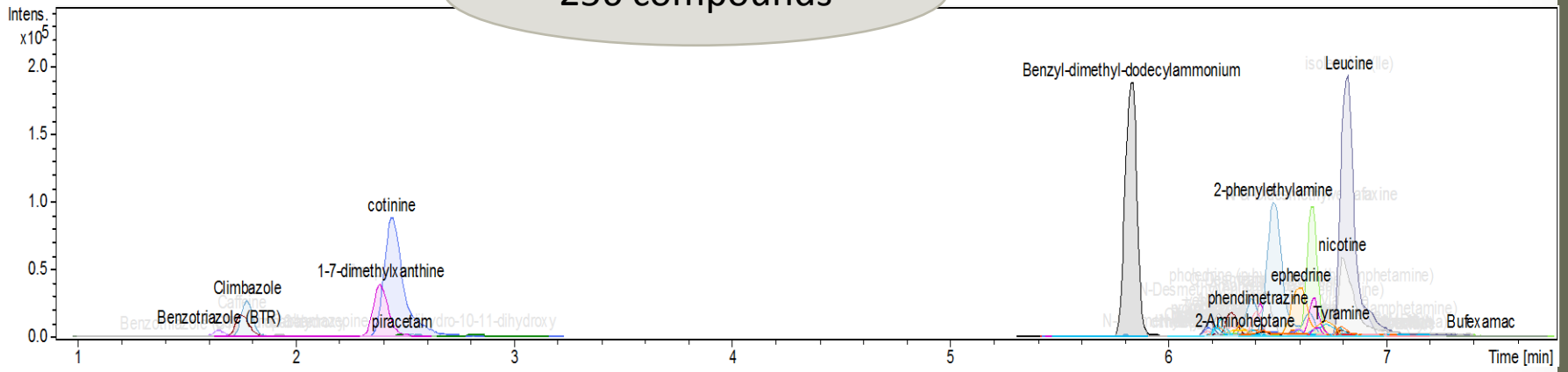
Criteria

- Ion Intensity > 250 (+ESI) / 150 (-ESI)
- Peak Area > 1000 (+ESI) / 600 (-ESI)
- deltaRT ≤ 0.4 min
- Accuracy: Error ≤ 2.5 mDa
- Isotopic fit: ≤ 100 mSigma

336 compounds detected in total

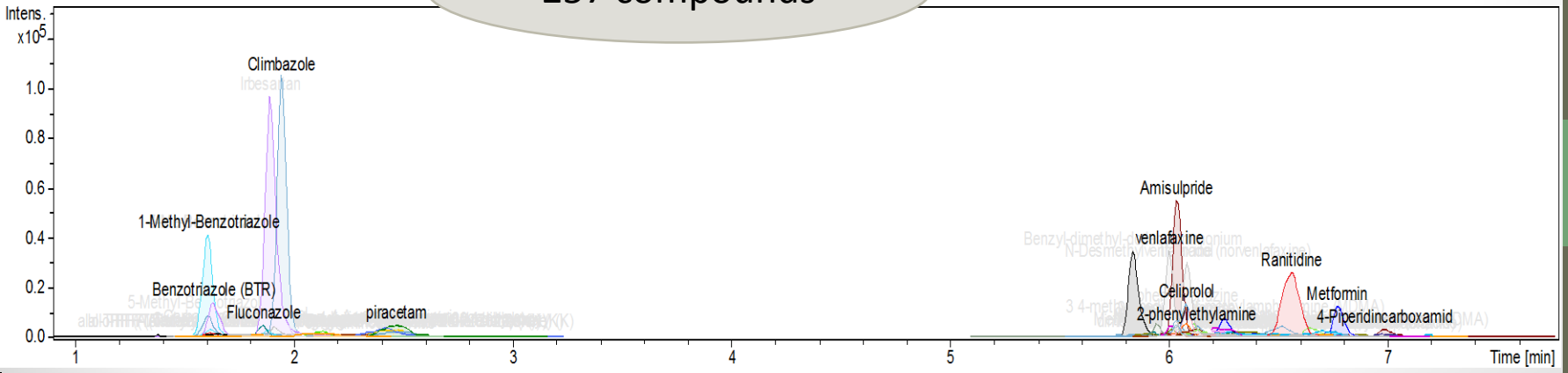
Influent wastewater

256 compounds



Effluent wastewater

257 compounds



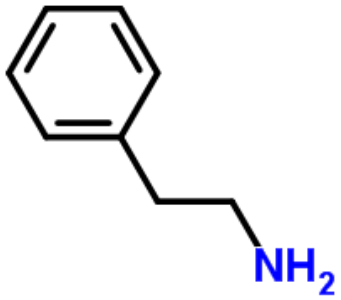
Wastewater Results

HILIC

- ✓ Better retention
- ✓ Better peak shape
- ✓ Higher intensity

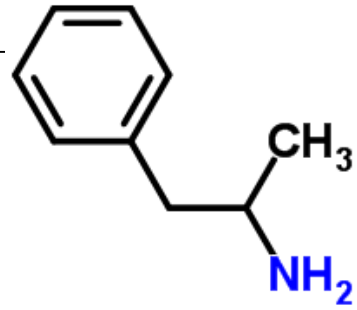
2-phenethyamine

$C_8H_{11}N$



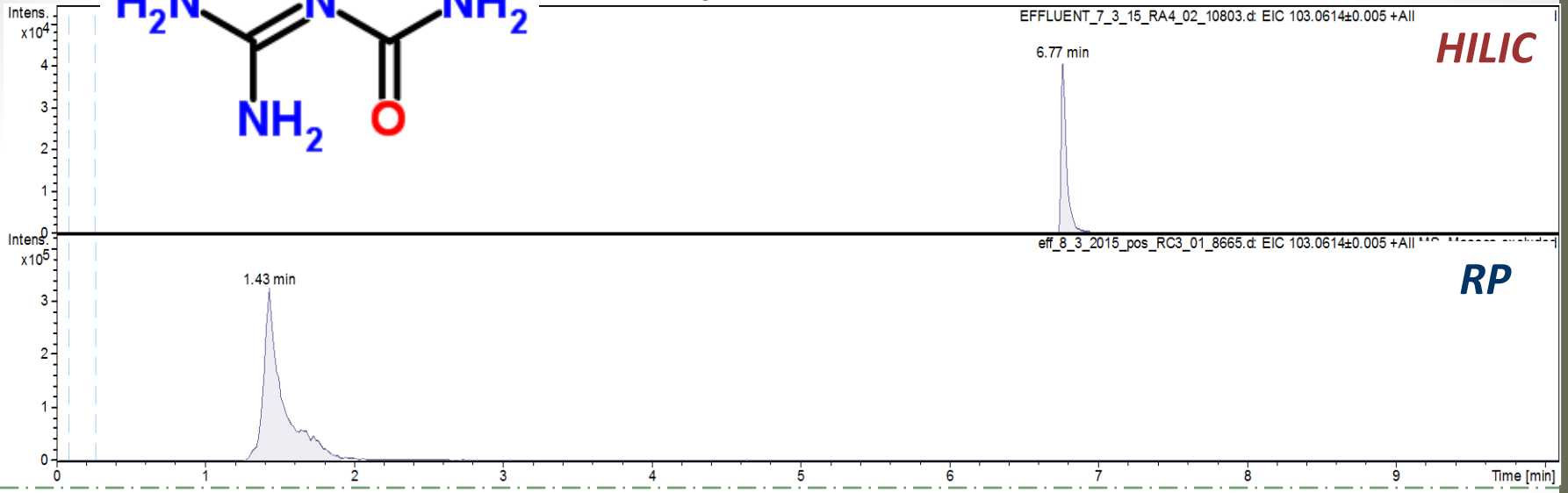
Amphetamine

$C_9H_{13}N$



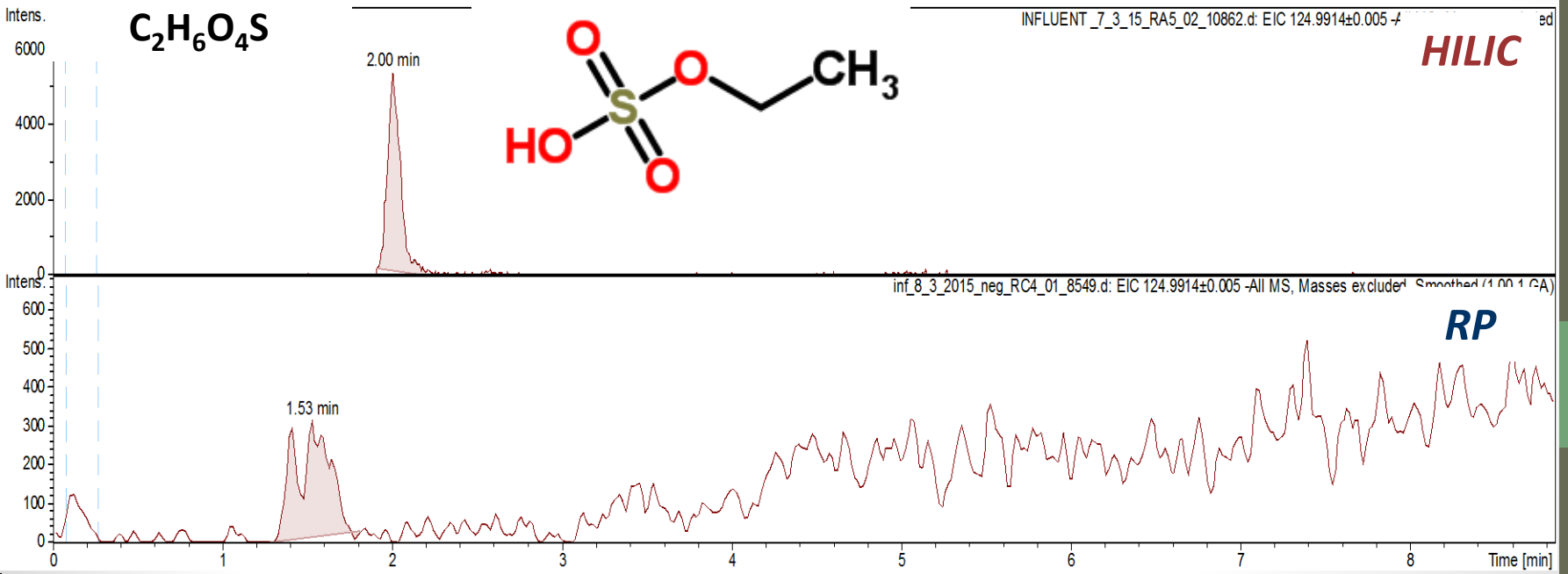
Wastewater Results

Guanylurea
$C_2H_6N_4O$



Ethyl sulfate
$C_2H_6O_4S$

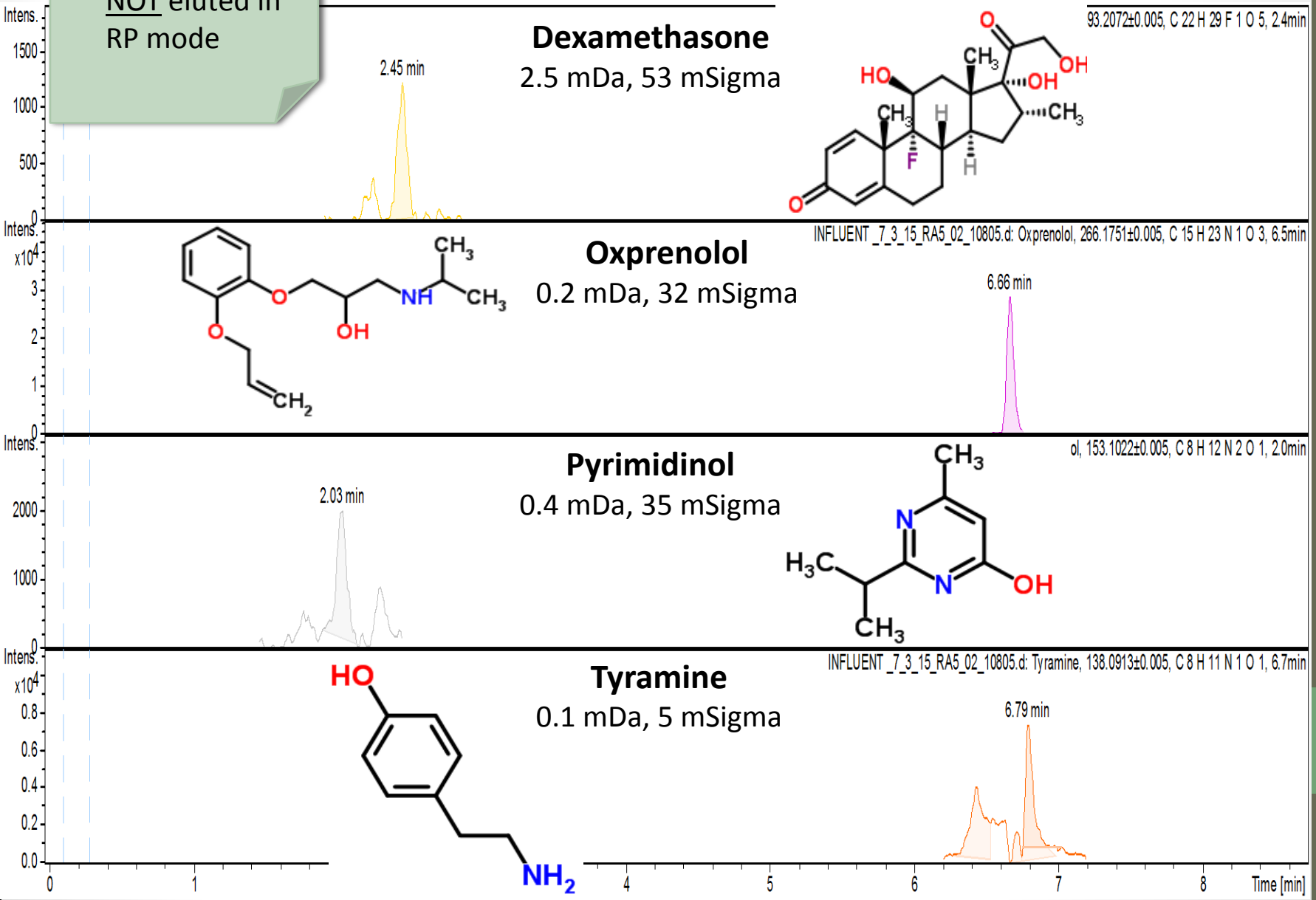
(-) ESI



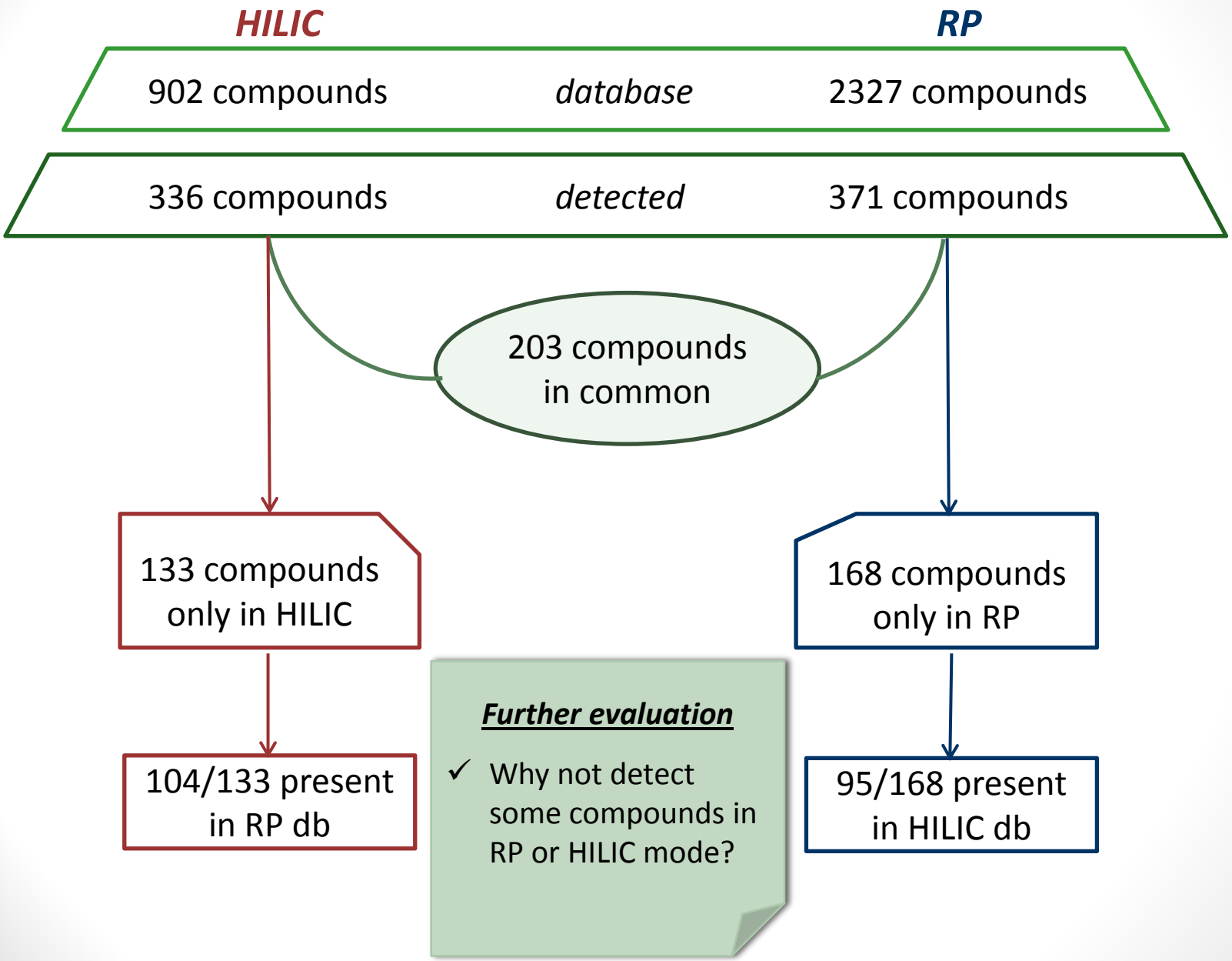
Wastewater Results

HILIC

- ✓ Compounds NOT eluted in RP mode



HILIC *Vs* RP


HILIC
RP
902 compounds
database
2327 compounds
336 compounds
detected
371 compounds
203 compounds in common
133 compounds only in HILIC
168 compounds only in RP
104/133 present in RP db
95/168 present in HILIC db
Further evaluation
✓ Why not detect some compounds in RP or HILIC mode?

Conclusions

- Development of HILIC wide-scope target method
- Optimization & validation of the HILIC method
- In-house database with information for 902 compounds
- Application in influent & effluent wastewater samples
- Comparison with RP target screening method

✓ Complementary technique for target screening

✓ Use in suspect & non-target screening for additional information

Acknowledgments to..

Alexandros Markatis

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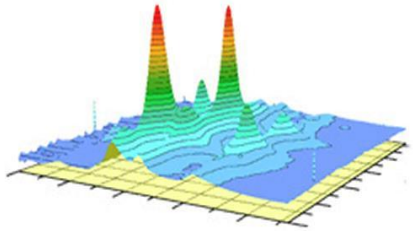
Thank you for your attention!

TrAMS Trace Analysis and Mass Spectrometry Group



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European Social Fund

OPERATIONAL PROGRAMME
EDUCATION AND LIFELONG LEARNING
investing in knowledge society
MINISTRY OF EDUCATION & RELIGIOUS AFFAIRS
MANAGING AUTHORITY

NSRF 2007-2013
programme for development
EUROPEAN SOCIAL FUND

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