HILIC-QTOF-HR-MS/MS for the orthogonal (complementary) screening and identification of polar micropollutants in environmental samples

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Target Screening

Wide-scope method for 2327 EPs

Suspect Screening

Human metabolites – Environmental TPs

Non-Target Screening

Unknown compounds in wastewaters

Technique of choice:

Reverse-Phase Liquid Chromatography (RP) - MS

Smart alternative for polar compounds

Hydrophilic Interaction LIquid Chromatography (HILIC) - MS

Scope of the study

HILIC wide-scope target method

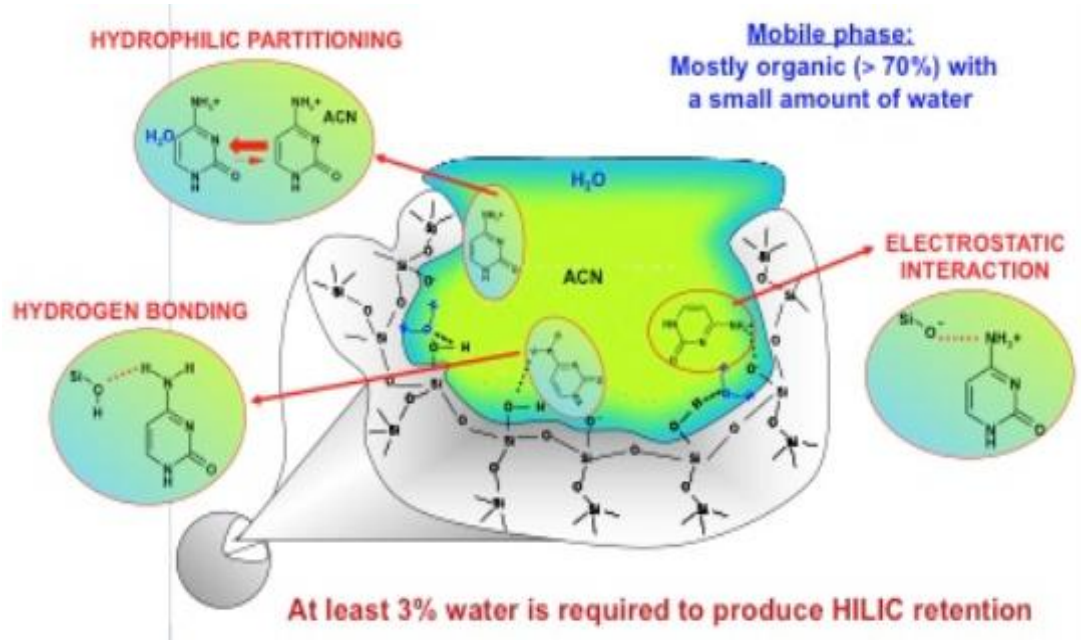
Optimization-validation-application to real samples

Application of HILIC method for additional confirmation in Suspect & Non-Target Screening

Advantages

- ✓ Retention of polar components → higher intensity
- ✓ Several different stationary phases available
- ✓ MS compatible
- ✓ Use of ACN (low viscosity solvent) → higher flow rates & better ionization
- ✓ Combination of 3 major LC techniques (NR, RP, IEX)

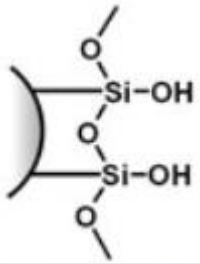
- Complex mechanistic separation (Adsorption, Partitioning, H bonding, Ion exchange)
- Great effort for the method optimization and development
- More equilibration time



HILIC stationary phases

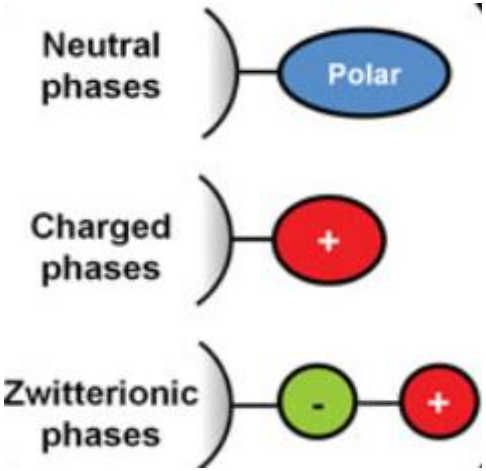
Unmodified bare silica gels

Polar chemically boned phases



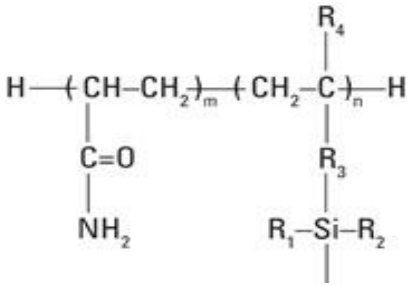
(a) 

BEH HILIC Acquity
(2.1 × 100 mm, 1.7 µm)

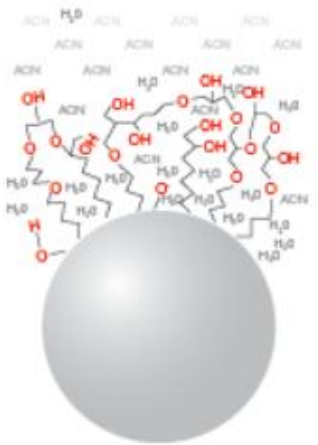


(b) 

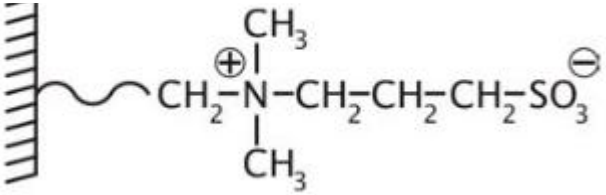
BEH Amide Acquity
(2.1 × 100 mm, 1.7 µm)

(c) 

TSKgel Amide Tosoh Bioscience
(2.0 × 150 mm, 3 µm)

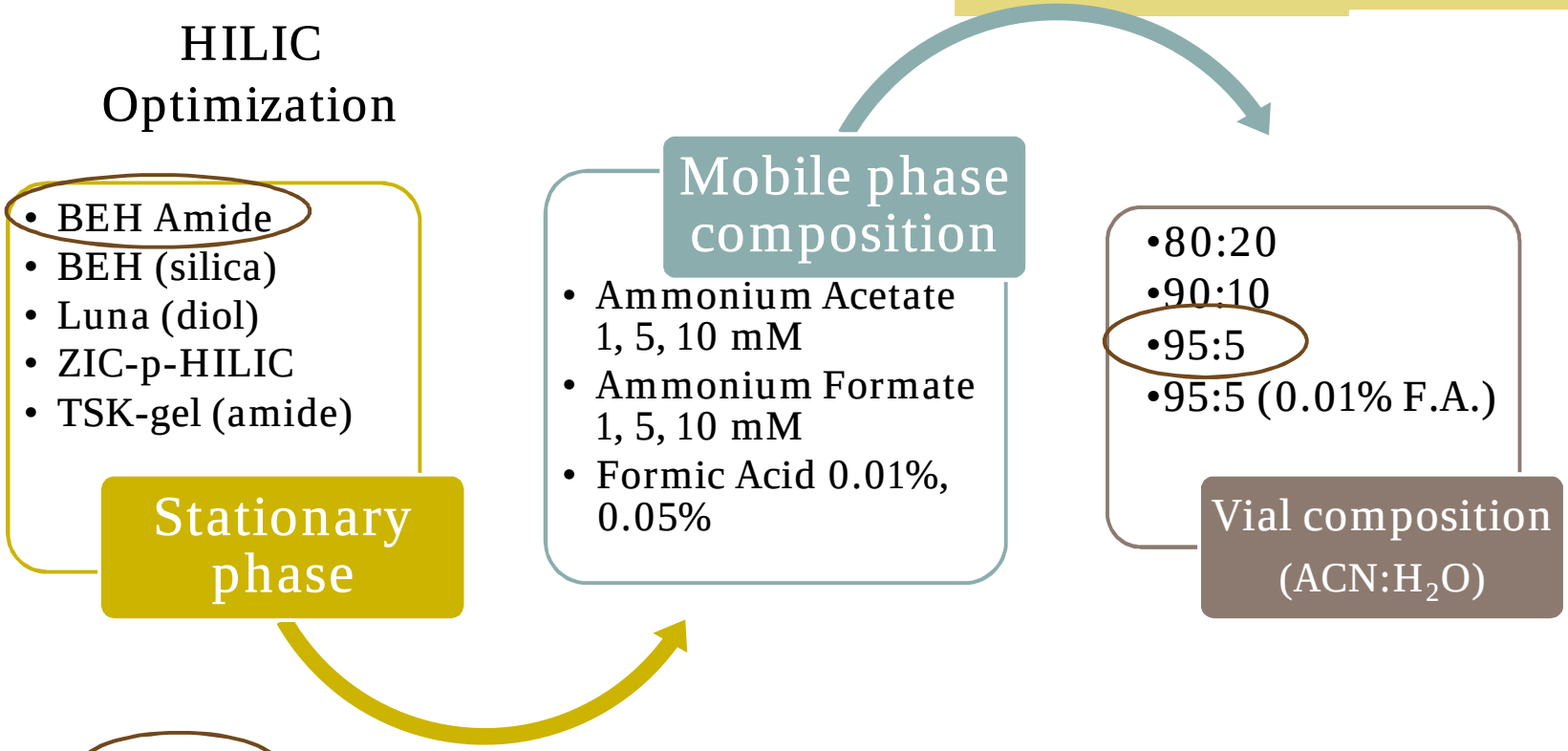
(d) 

Luna HILIC
PHENOMENEX
(2.0 × 100 mm, 3 µm)

(e) 

ZIC- pHILIC SeQuant
(2.1 × 150 mm, 5 µm)

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

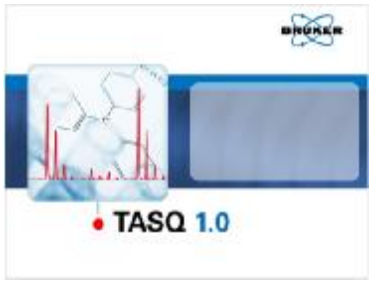
MaXis Impact
Ultra High Resolution
Time-of-Flight Mass
Spectrometer

- 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

bbCID mode
† Target Analysis
AutoMS mode
† Suspect Screening
† Non-target screening

Database

EPs, belonging to a diverse group of compounds

902 compounds

601 well-retained compounds (k'>1)

Chosen according to environmental relevance & HILIC chromatographic behavior

Sampling & Sample preparation

Location: **WWTP of Athens, Greece**

Period: 8th March 2015 (Sunday)

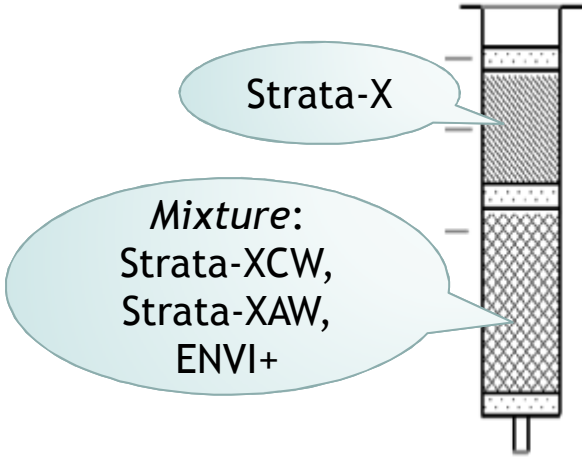
Samples: 24-h composite flow-proportional influent & effluent wastewater

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*

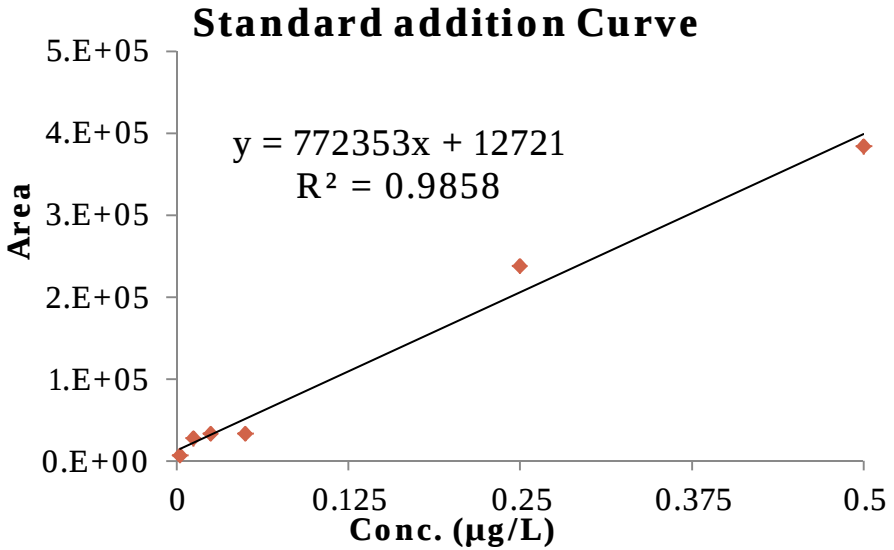
- **validation dataset**
  - 85 compounds
  - 10% of the compounds of the total database
  - Representative physicochemical properties
  - Compounds from every class of EPs
- **Calibration curves** (**solvent**, **matrix** & **spiked** samples) (6 levels of concentration)
- **Repeatability**, **recoveries** 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;

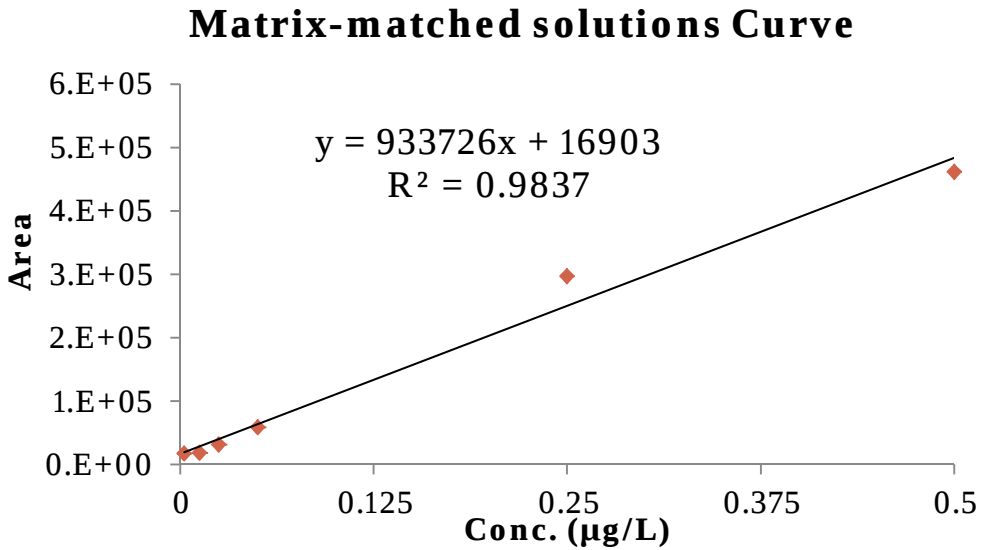
    t_R + Precursor ion = 2 Identification Points (2 IPs)
  - **LOI**: the lowest concentration tested for which a compound was satisfactorily identified in all spiked samples;

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

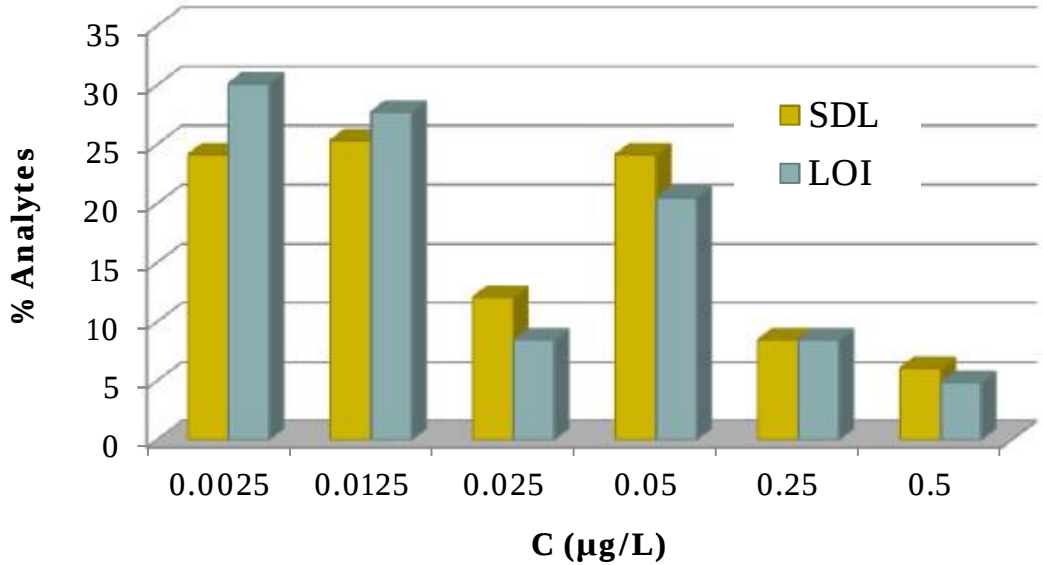
Linearity

Amisulpride-N-oxide

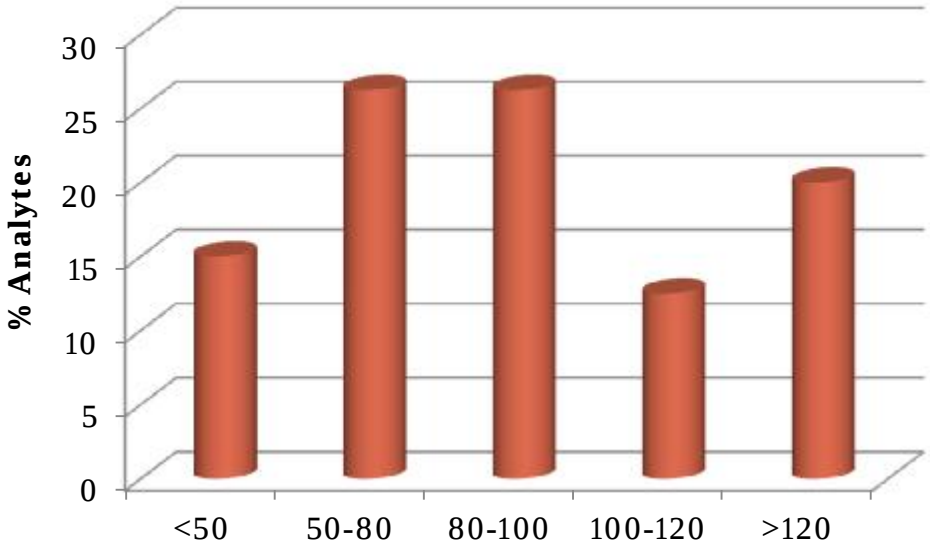




Screening Detection Limits (SDL) – Limits of Identification (LOI)



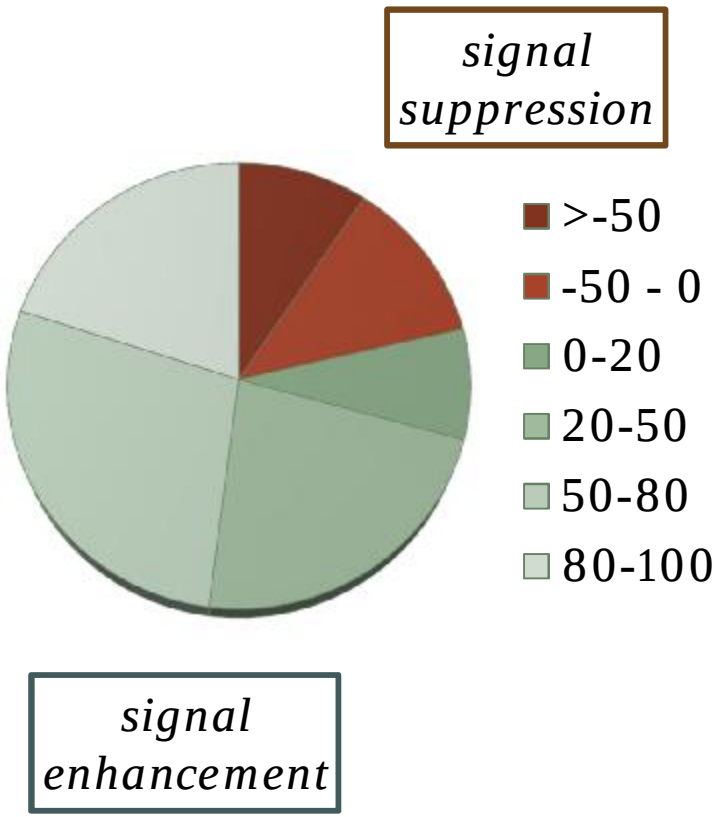
% Recoveries



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

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

% Matrix Effect

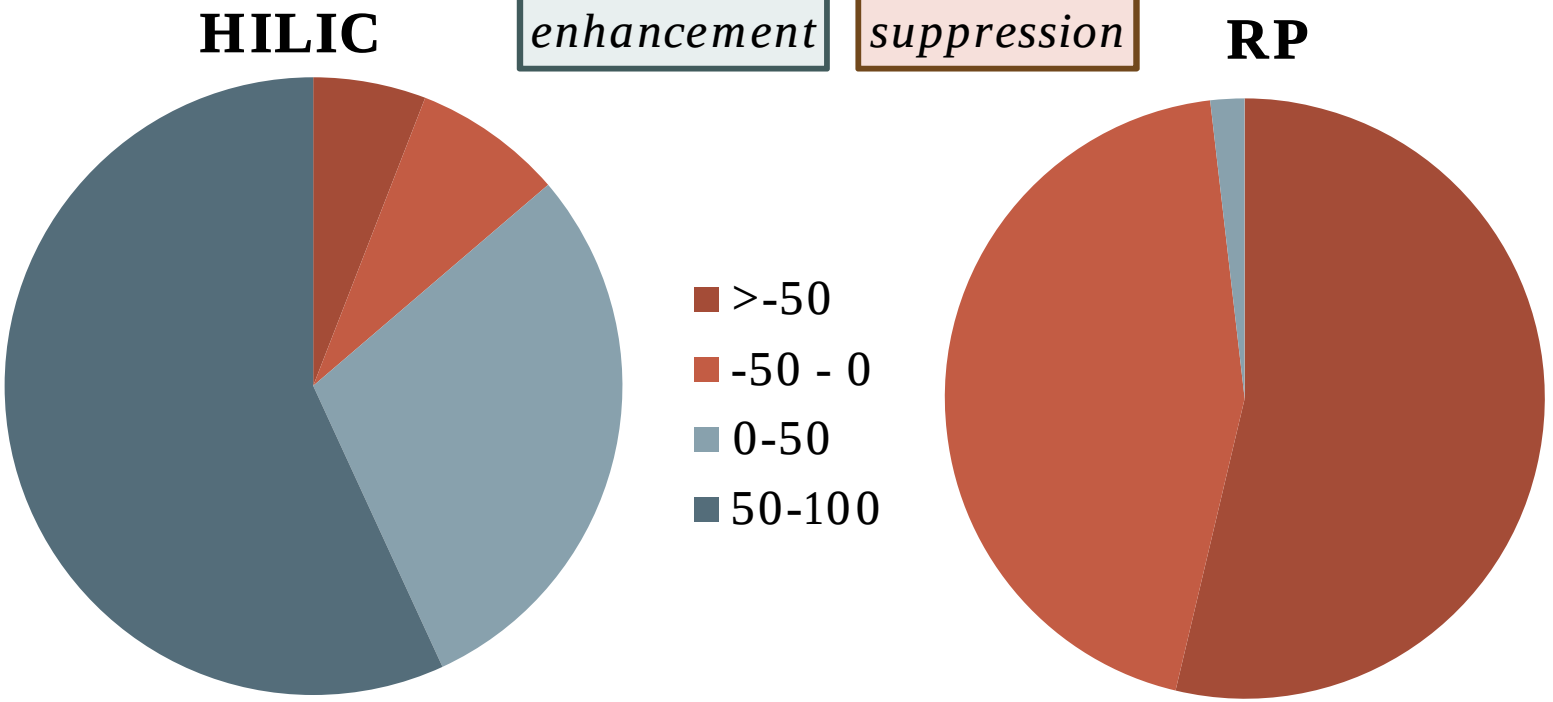


º Comparison RP – HILIC »

58 compounds
Common in RP & HILIC validation
(representative t_R)

Matrix Effect

signal enhancement | *signal suppression*



° Comparison RP – HILIC »

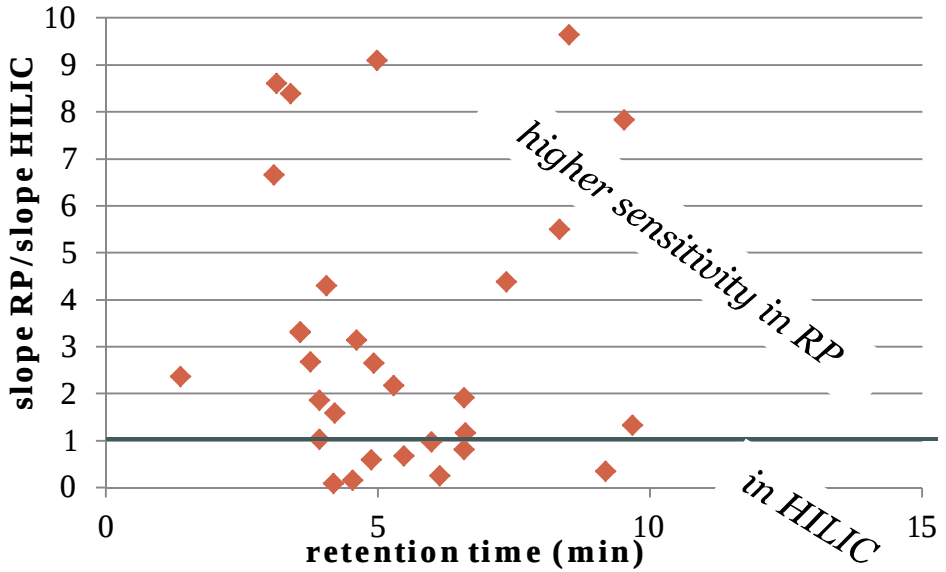
Sensitivity

Slope (b), standard addition curve

- 19% compounds → higher sensitivity in HILIC

~~Due to t_R ?~~

Due to physicochemical properties ?



Screening Detection Limit –Limit of Identification

SDL-LOI

- 38% compounds → lower SDL-LOI in HILIC
- 51% compounds → lower SDL-LOI in RP
- 11% compounds → equal SDL-LOI

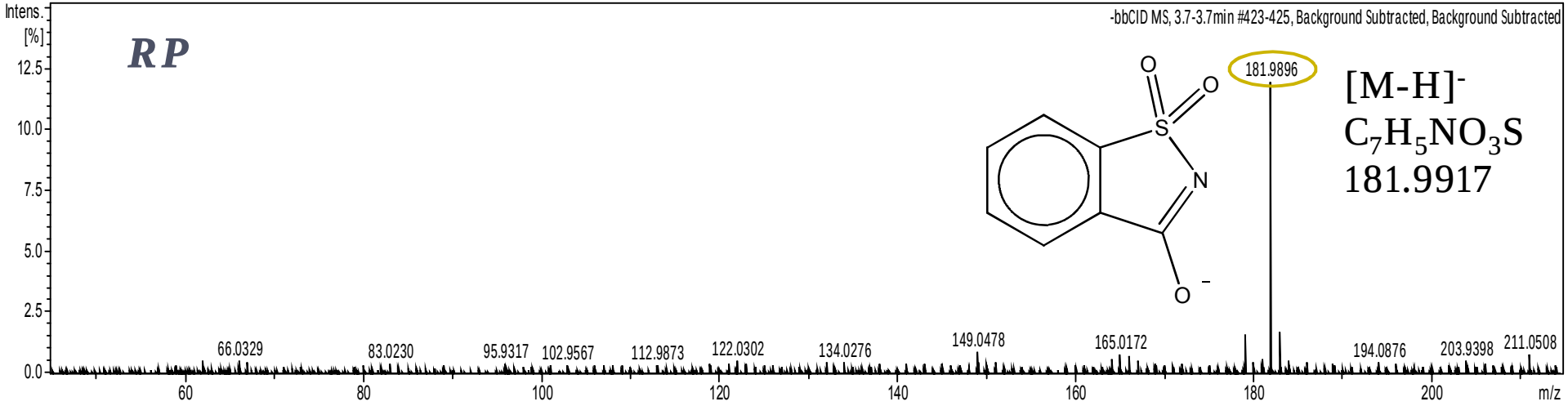
...compounds different fragmentation pattern RP-HILIC

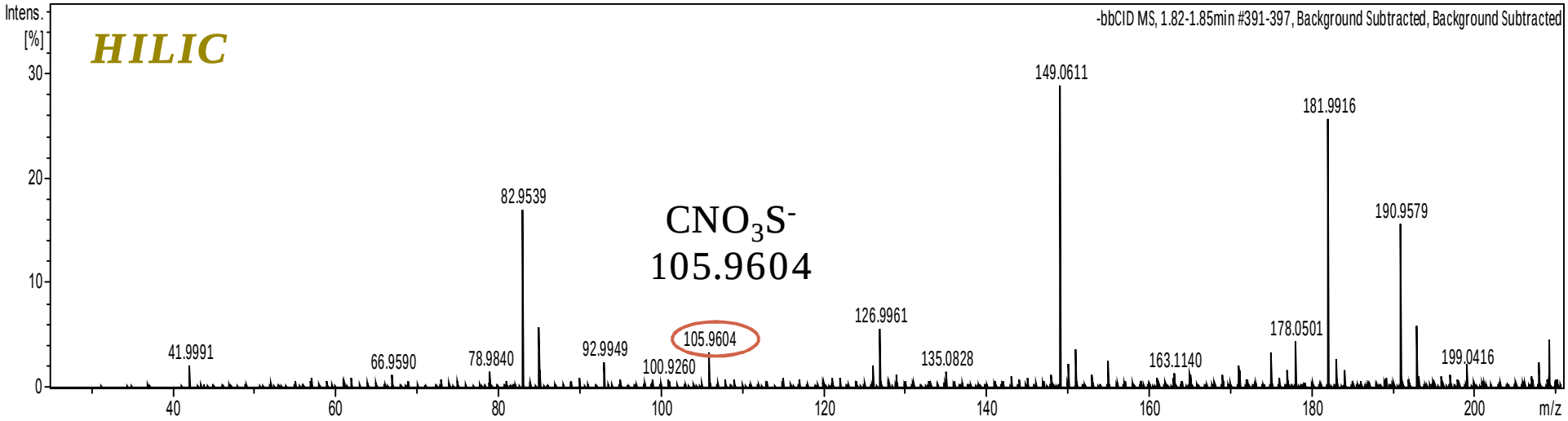
° Comparison RP – HILIC »

Different fragmentation pattern

Saccharin

MS/MS spectra
Spiked sample 0.5 µg/L





Wastewater Results

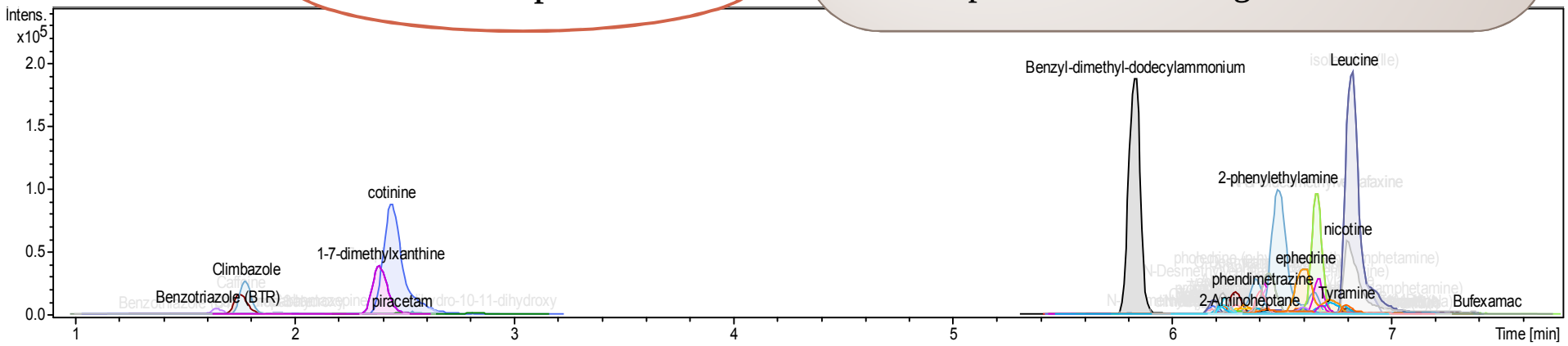
336 compounds
detected in total

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

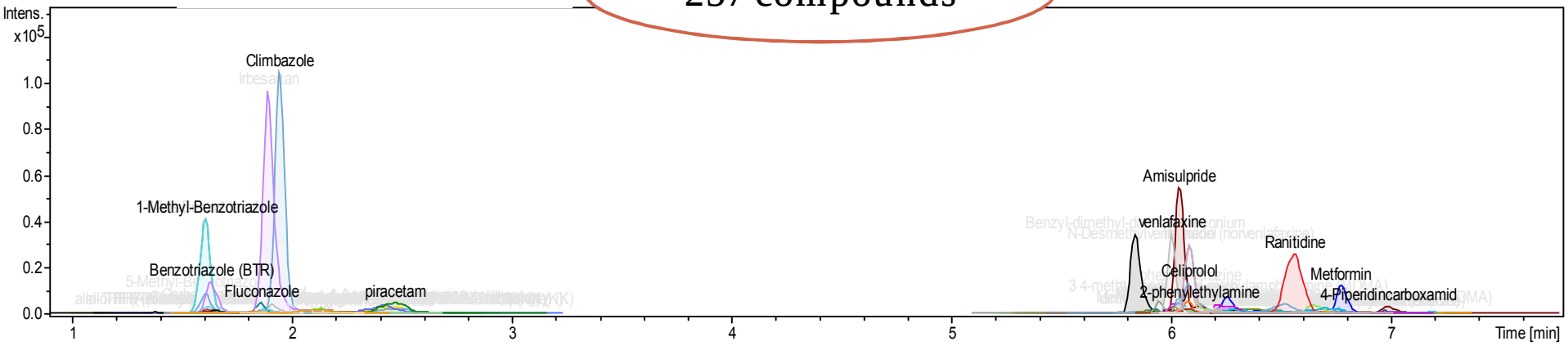
Influent wastewater

256 compounds



Effluent wastewater

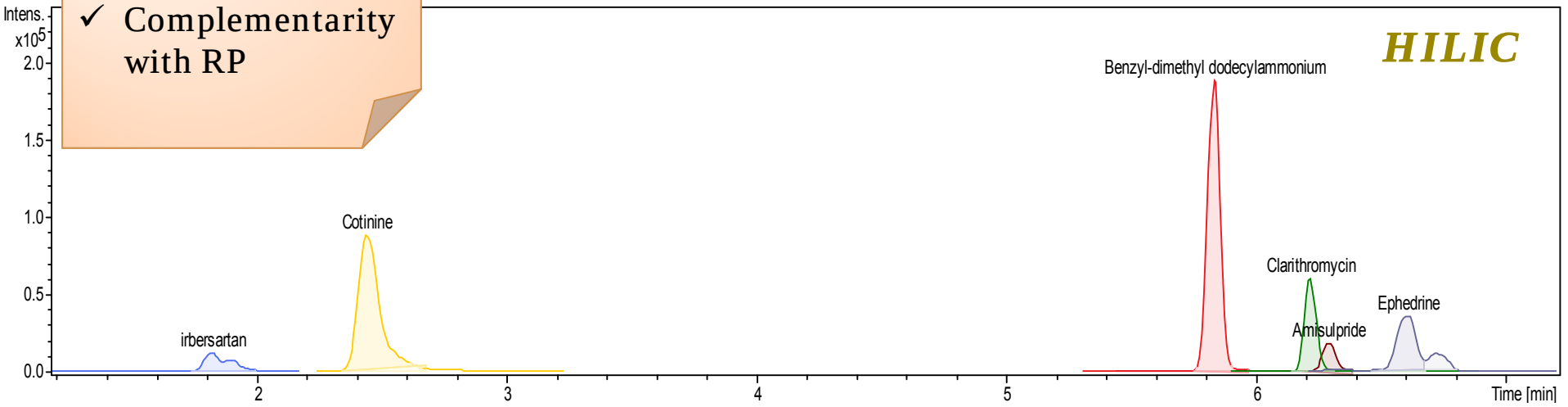
257 compounds

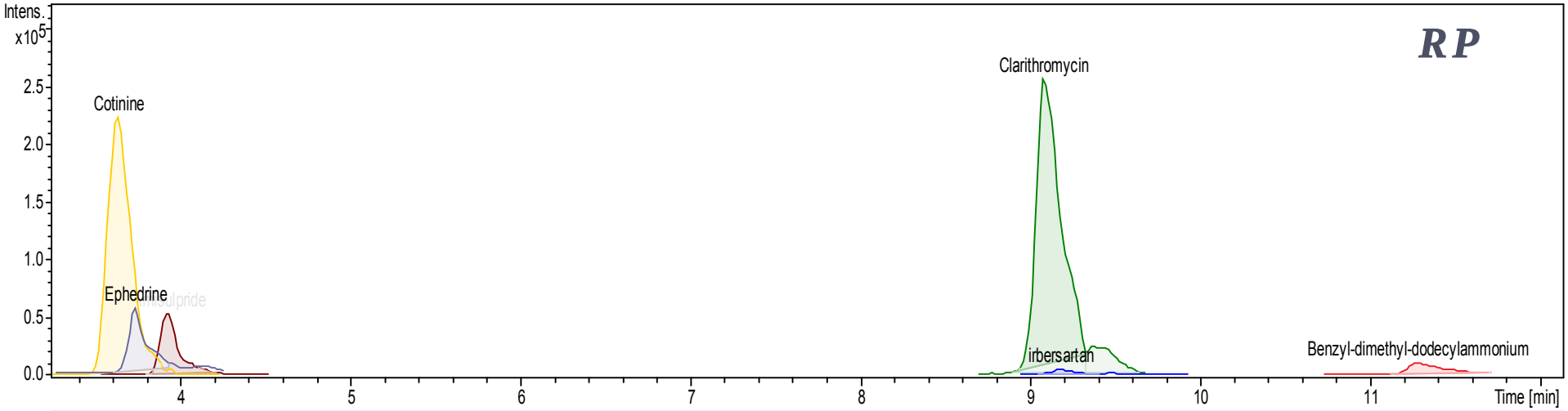


HILIC

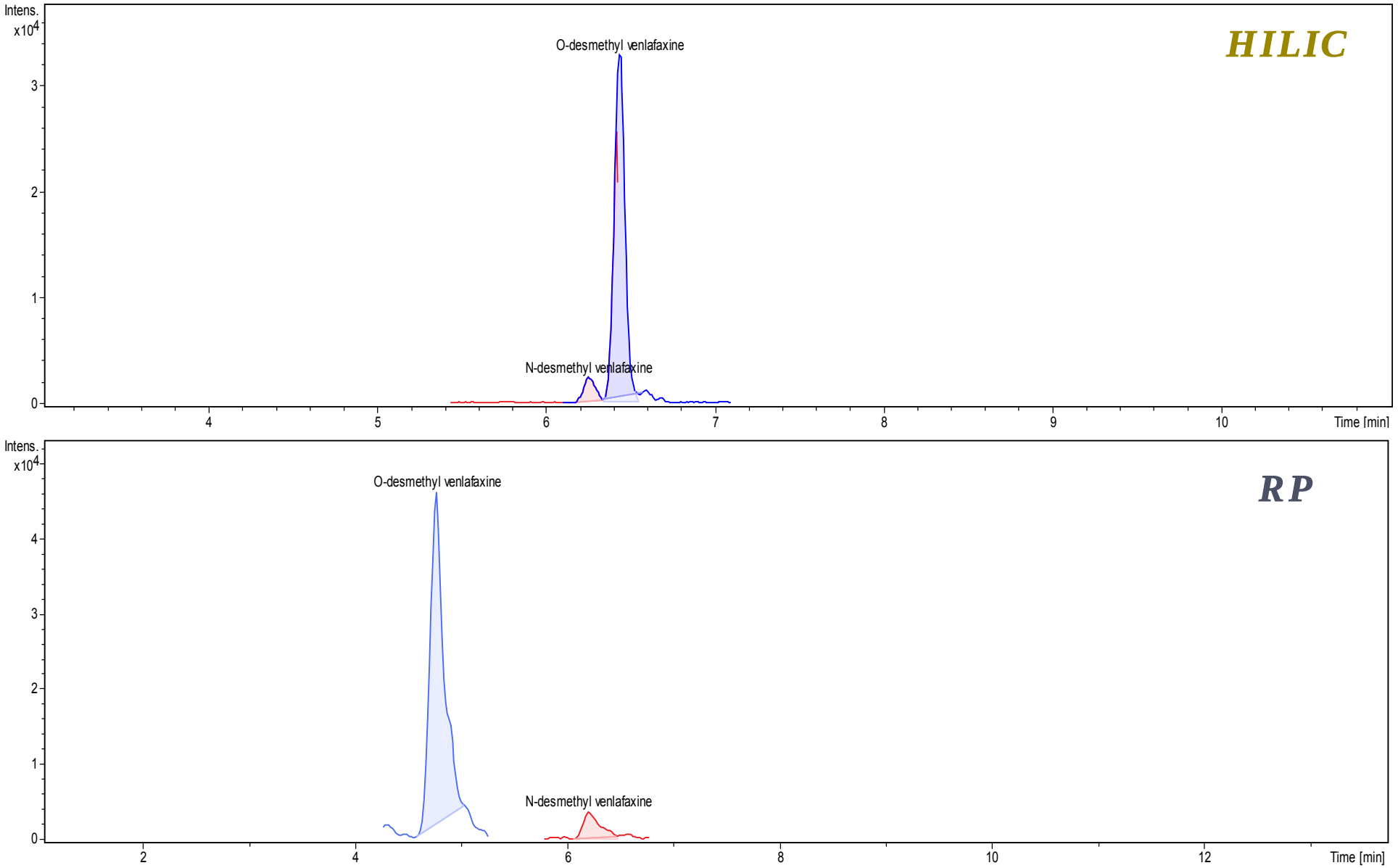
- ✓ Complex mechanism of separation
- ✓ Complementarity with RP

Comparison of retention of selected analytes

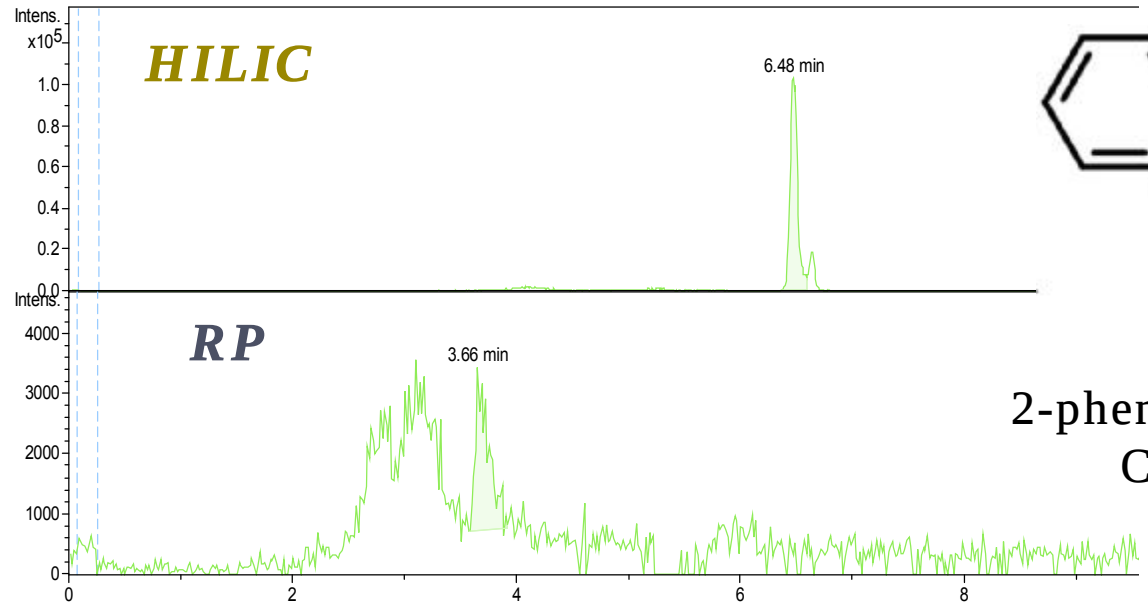


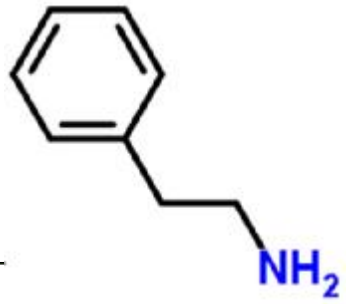


Comparison of retention of isomers



Wastewater Results



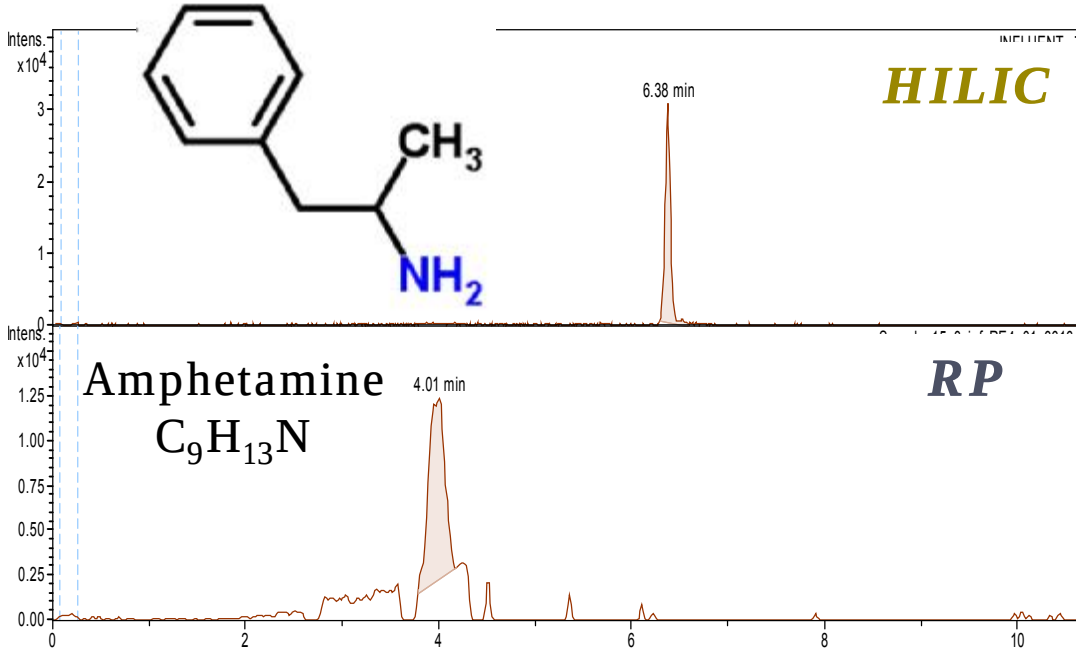


2-phenethyamine
$C_8H_{11}N$

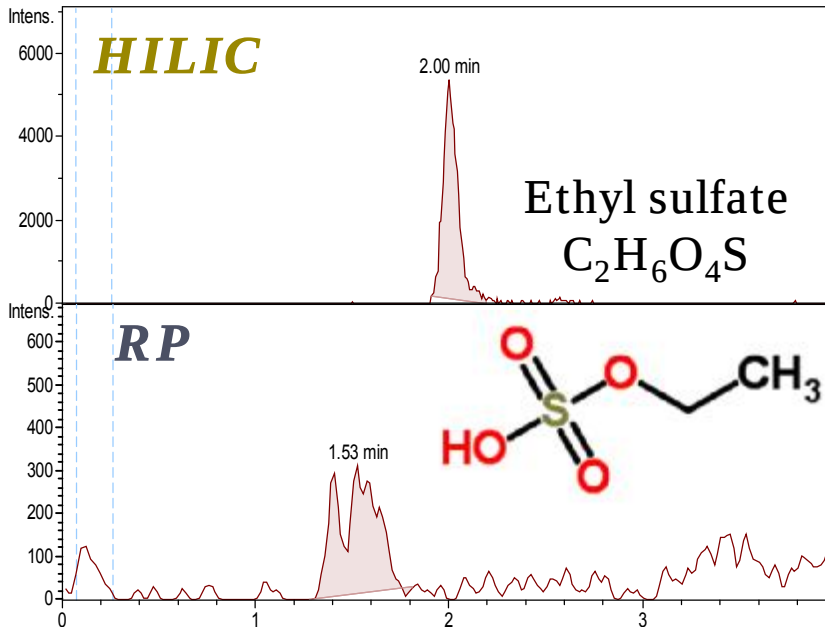
HILIC

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

(-) ESI



Amphetamine
$C_9H_{13}N$



Ethyl sulfate
$C_2H_6O_4S$

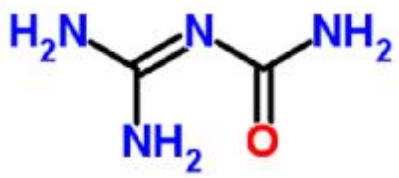
Wastewater Results

HILIC

- ✓ Better retention
- ✓ Better peak shape

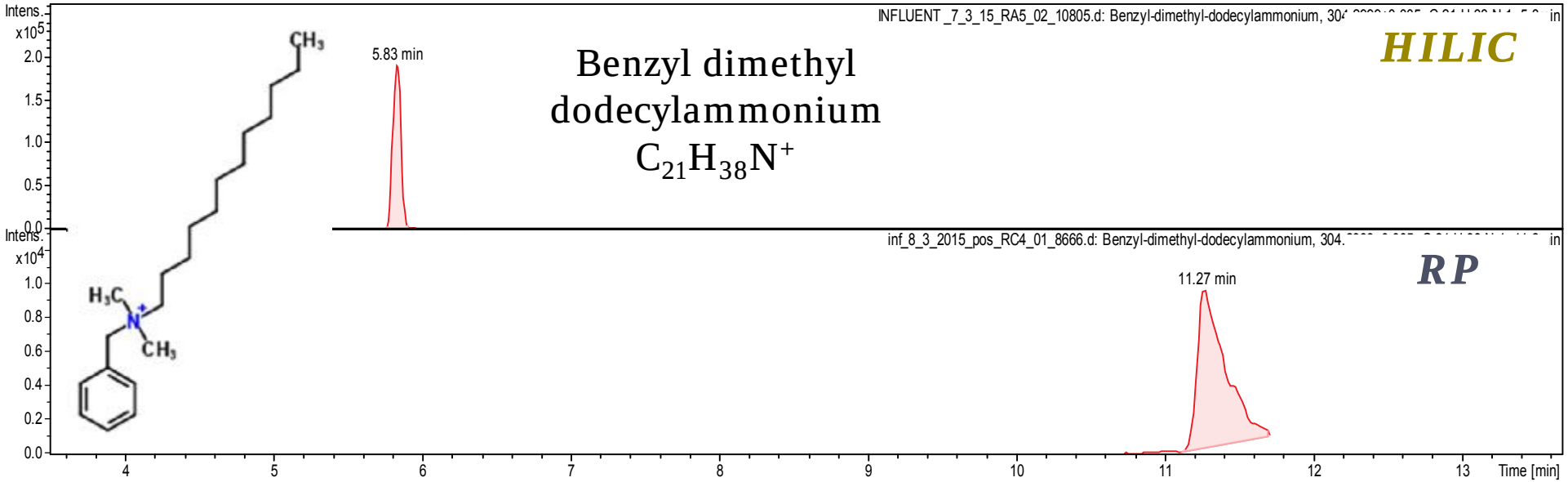
Guanylurea

$C_2H_6N_4O$



Benzyl dimethyl dodecylammonium

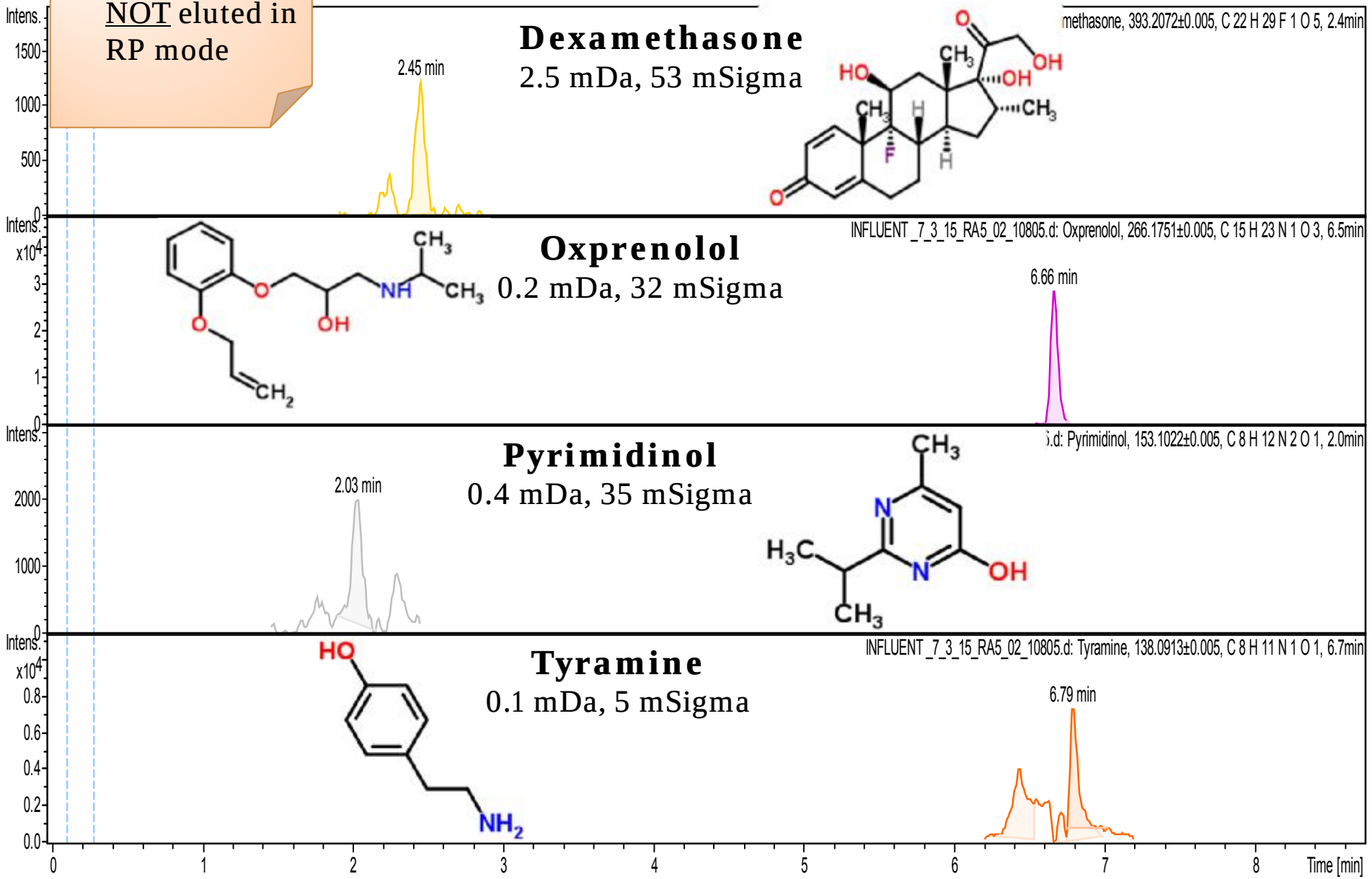
$C_{21}H_{38}N^+$



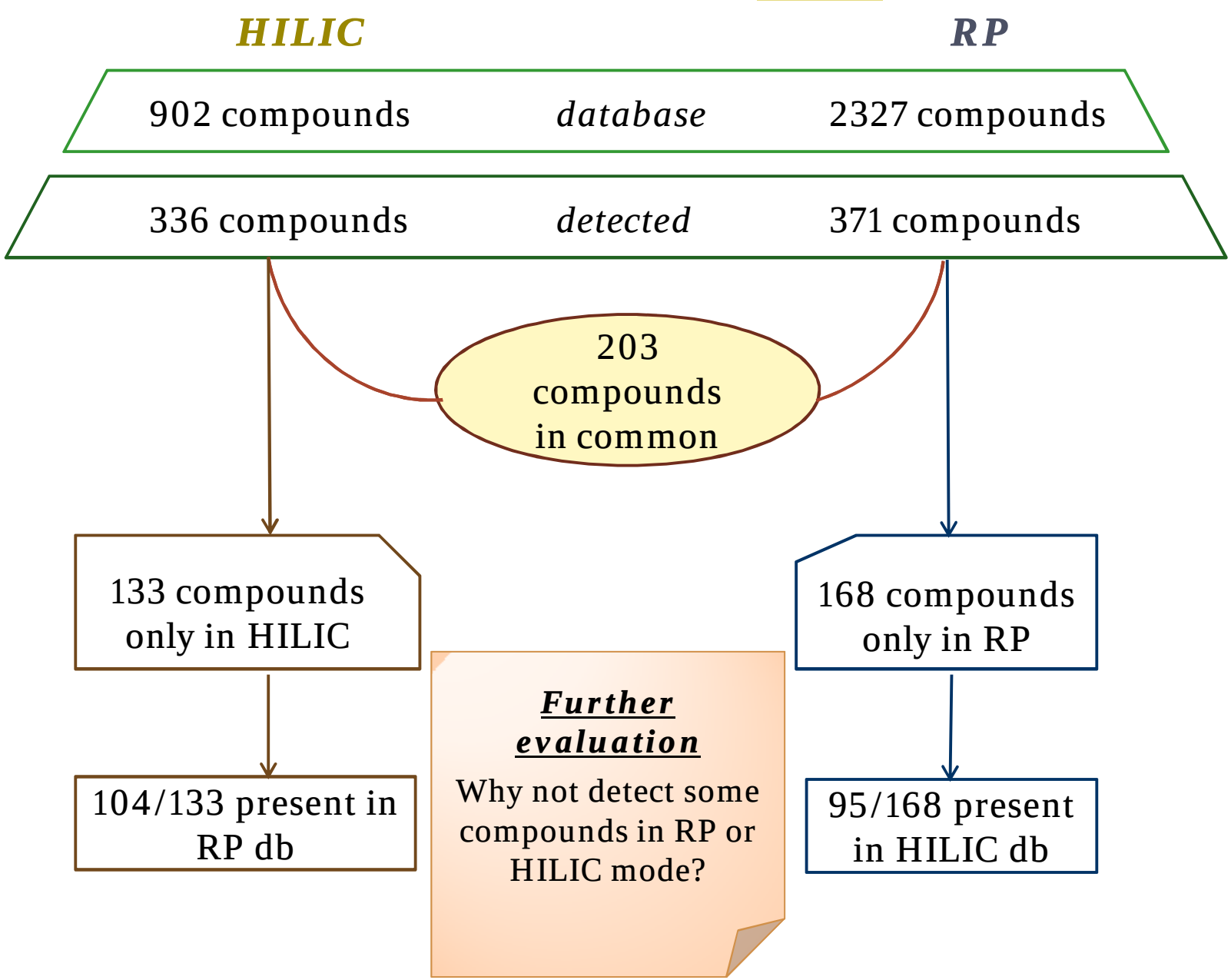
HILIC

HILIC

✓ Compounds NOT eluted in RP mode



Comparison HILIC- RP



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..

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& our collaborators from eawag aquatic research ooc

Thank you for your attention!

Laboratory of Analytical Chemistry
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TrAMS Trace Analysis and Mass Spectrometry Group