



1st NORMAN workshop on analysis of problematic compounds

Orthogonal identification of biotransformation products by HILIC-QTOFMS

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<http://trams.chem.uoa.gr/>
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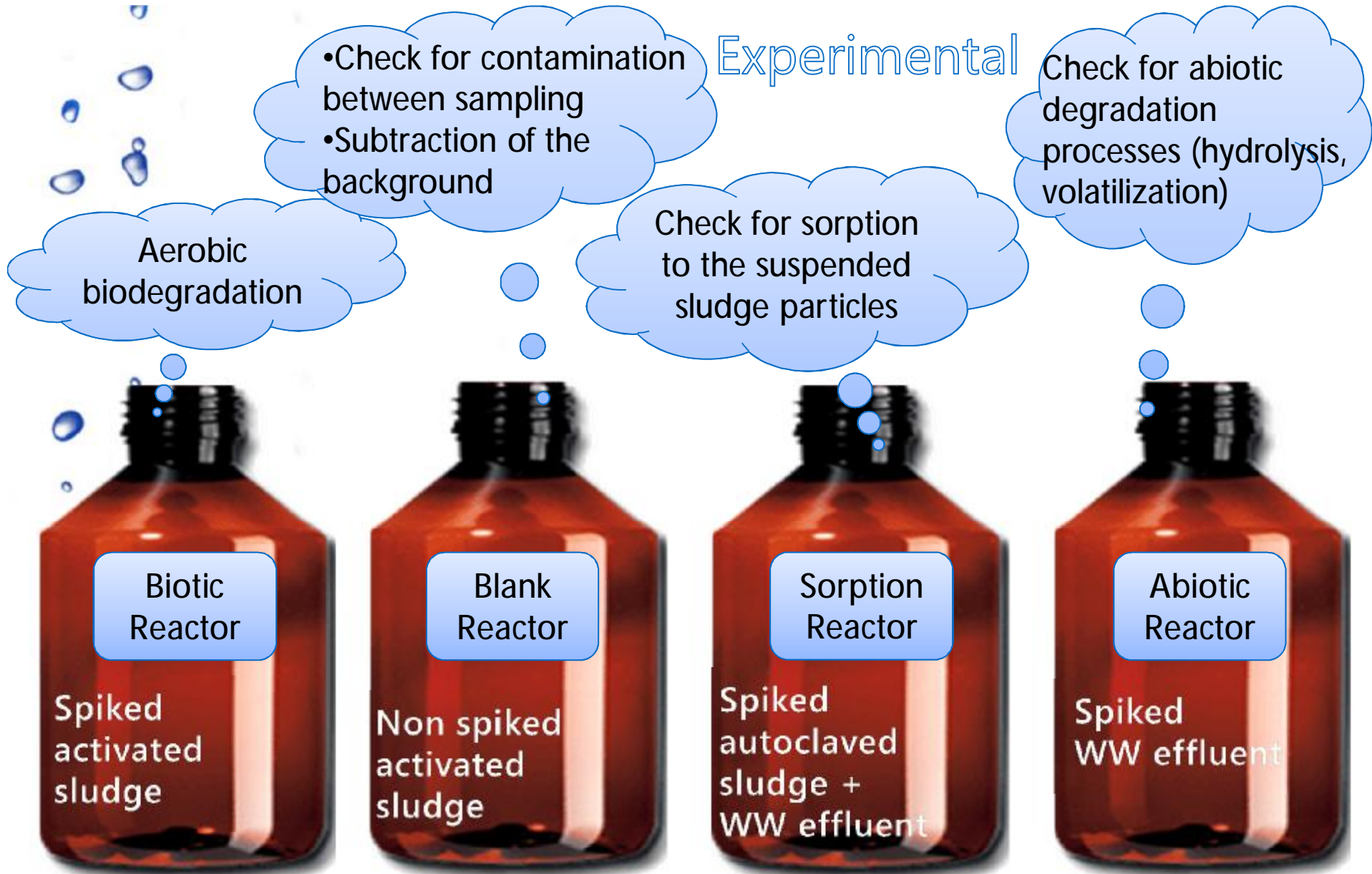
HILIC:

Orthogonal
identification of
biotransformation
products

Aim of the research

- To use HILIC as a complementary approach to RP for the identification of biotransformation products
- To further investigate for suspect and unknown TPs which may be eluted better in HILIC conditions
- To point out the advantages of HILIC as a complementary technique to RP.

Experimental



□ Sampling immediately after spiking and after several time intervals.

Instrumentation

Scan: 50-1000 m/z
Spectra rate: 2Hz
MS mode: bbCID &
Auto MS with
inclusion list



UltiMate 3000™
RSLC (Dionex) UHPLC System

Column: Thermo Acclaim RSLC
C18, 2.2µm 120 Å, 2.1x100 mm
Mobile Phase & gradient
program: Bruker Pesticide
screener

Inj. Volume: 5 µL

RP



Column: Waters BEH Amide
Acquity 1.7 µm, 2.1 × 100 mm
Inj. Volume: 5 µL

HILIC

Acquity
UPLC®



Bruker Maxis Impact™
Quadrupole-Time-of-Flight MS

Workflow

Suspect screening

Compilation of suspect list

Screening all the time interval chromatograms both in RP and HILIC (+ESI/-ESI)

Annotate as possible TP: meet the set criteria, absence in the blank, occurrence of a time trend

Acquire MS/MS spectra with inclusion list both in RP and HILIC → Spectra interpretation → Structure elucidation

Confirm, if it's possible with reference standard

Non-Target screening

Subtraction of the background

Generation of a peak list of unknown

Background Subtraction Parameters

Difference

eXpose mode

Ratio: 3

Detect -Mass spectrum Parameters

Int. threshold: 30%

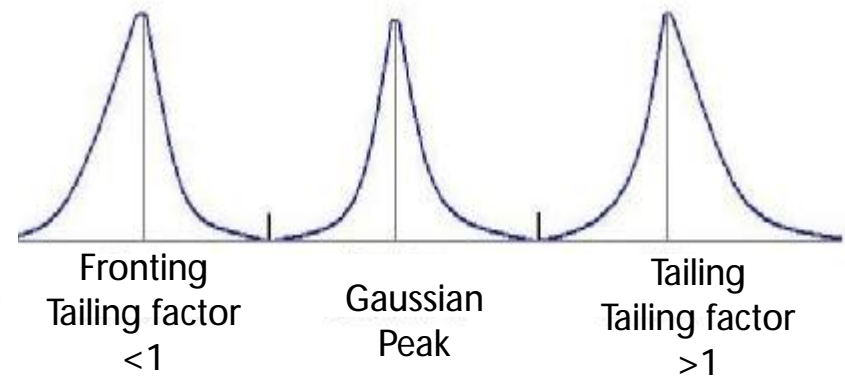
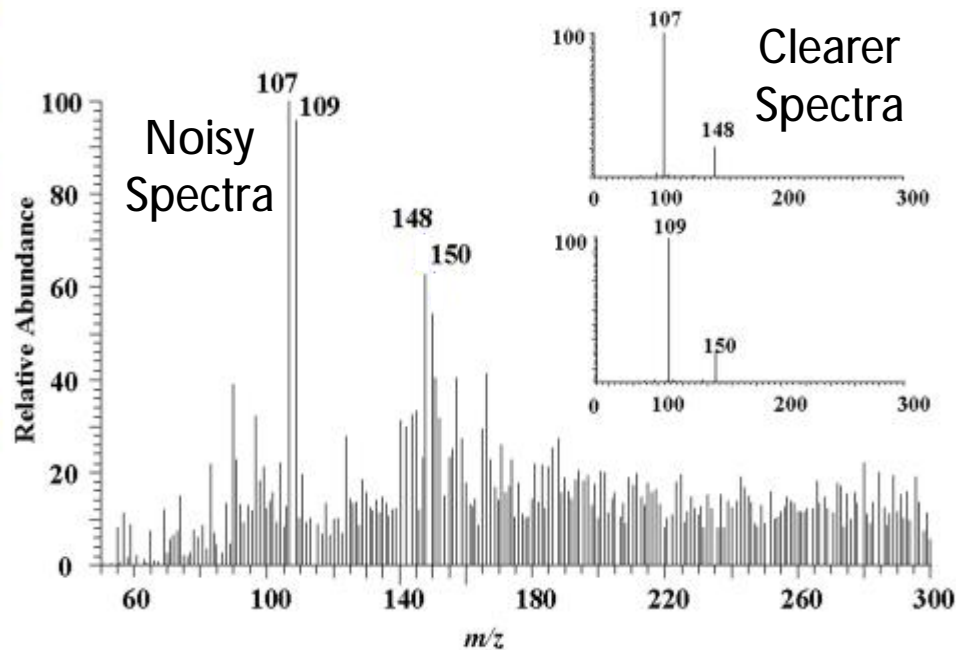
Max No. of peaks: 20

Identification Criteria

- Mass Accuracy: Error 5 ppm
- Distinctive Isotopic fit: 100 mSigma
- Intensity > 1000 (+ESI) / 200 (-ESI)

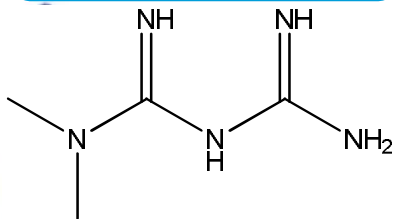
Advantages of HILIC

- ✓ Elution in the reverse order compared to RP (Orthogonality)
- ✓ Improved detection sensitivity (mobile phase composition fully compatible with ESI)
- ✓ Retention of the more polar TPs of pharmaceuticals that are not retained in RP columns
- ✓ Better peak shape (Gaussian peaks)

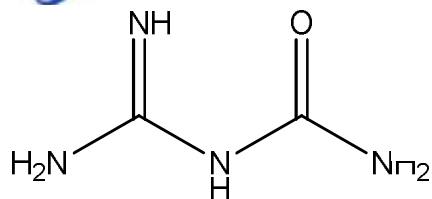


✓Elution of more polar TPs
 ✓Improvement of peak shape

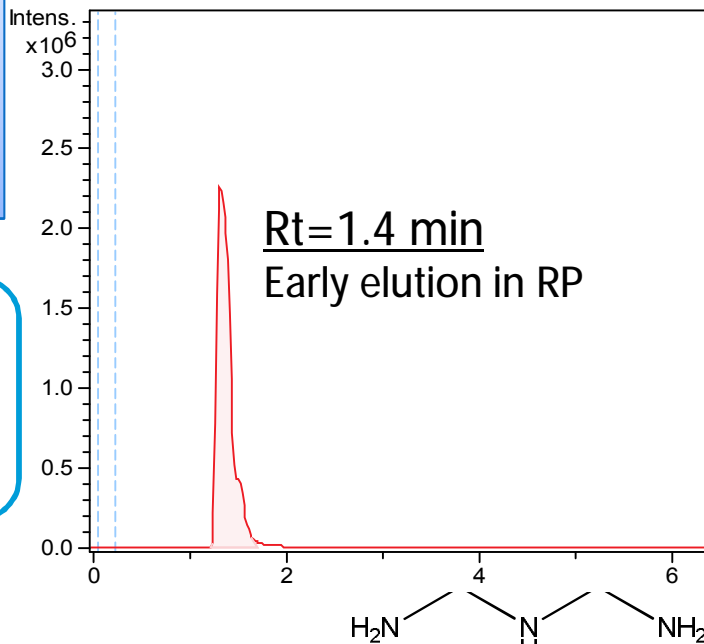
Metformin
 Very polar pharmaceutical



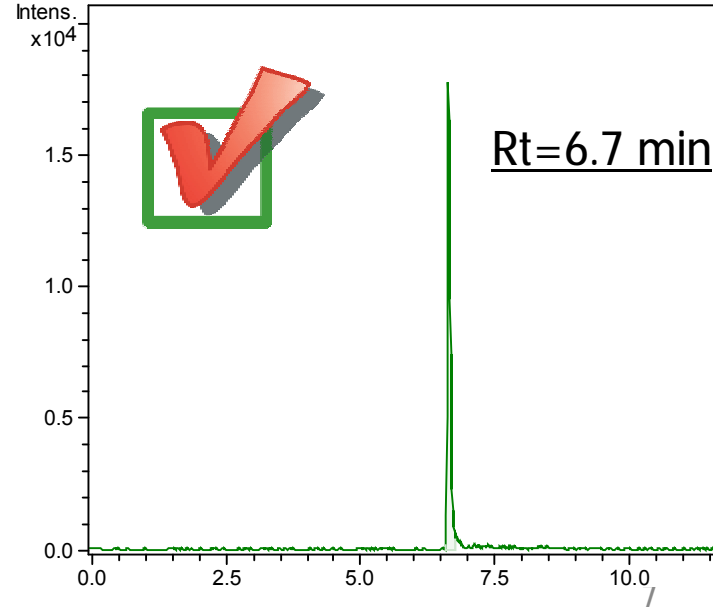
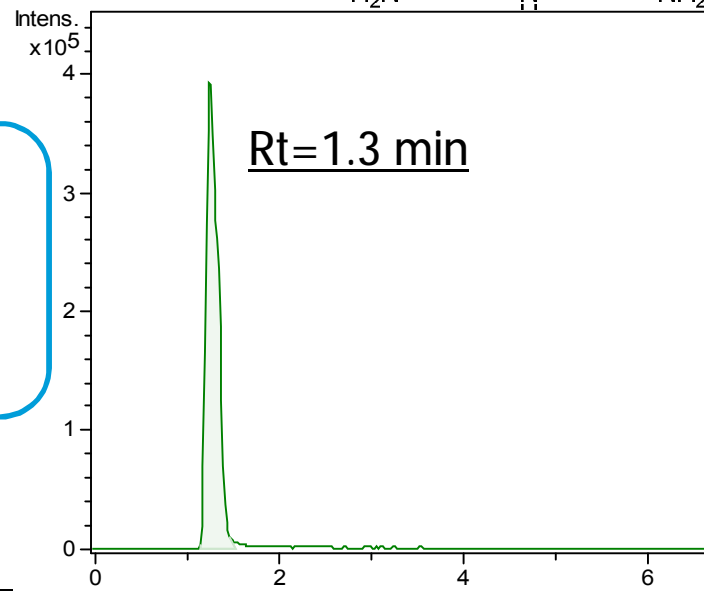
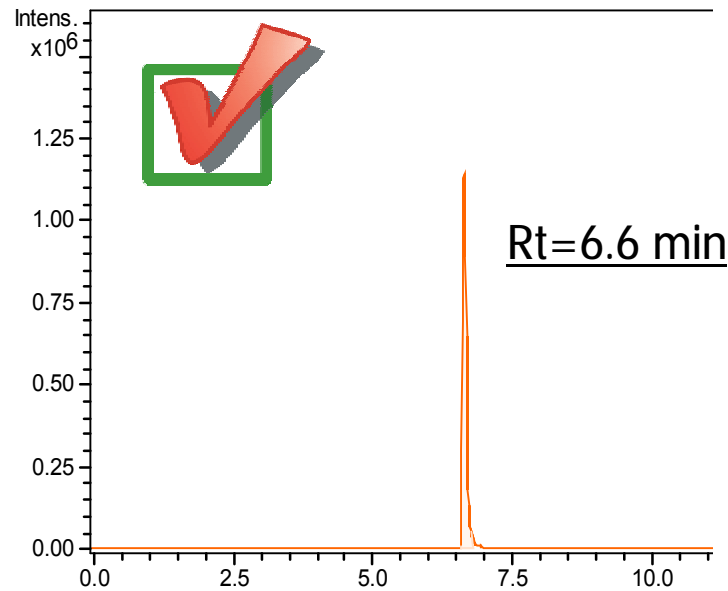
MTF103
 (guanylurea)
 More polar TP
 Confirmed with reference standard



RP

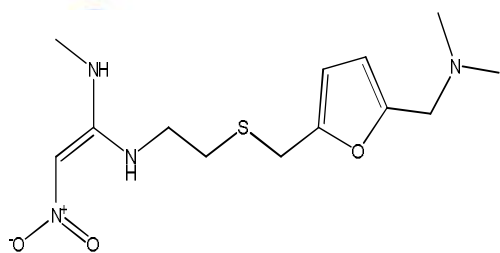


HILIC

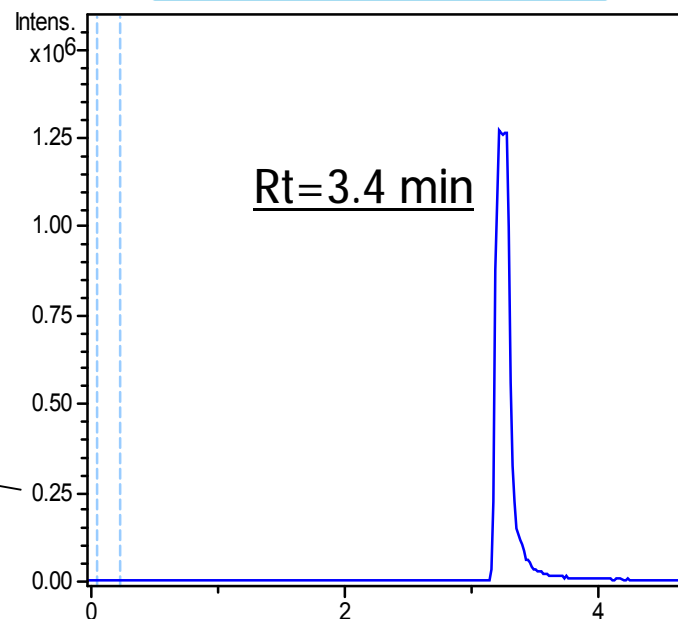


✓Elution of more polar TPs
✓Improvement of peak shape

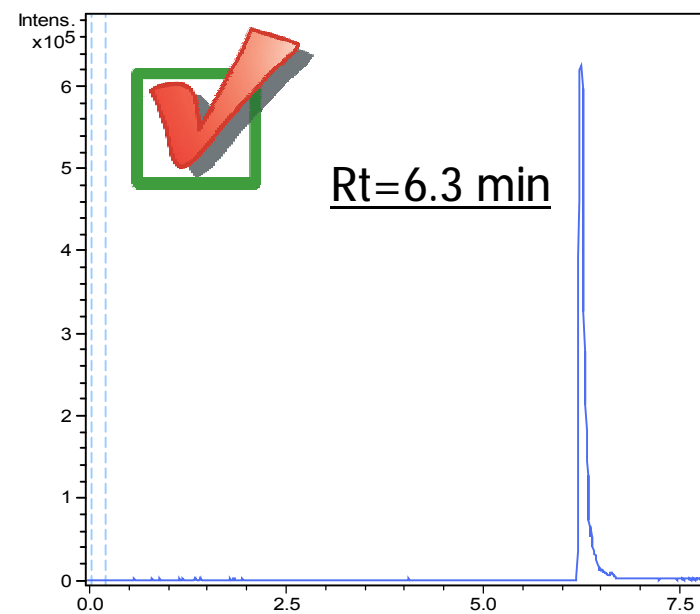
Ranitidine



RP

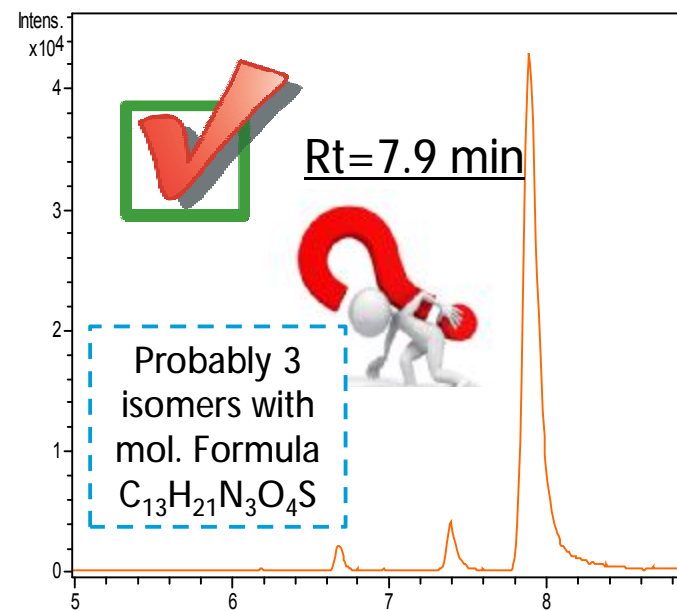
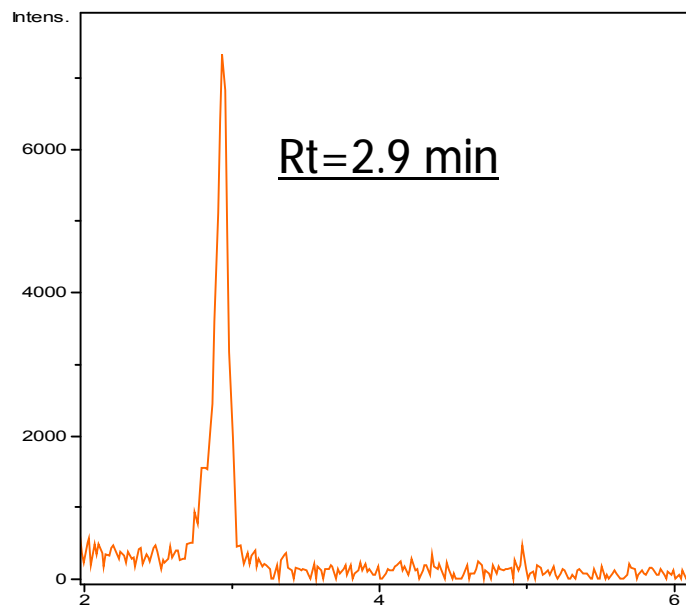


HILIC



RAN316

Unequivocal mol.
Formula
 $C_{13}H_{21}N_3O_4S$

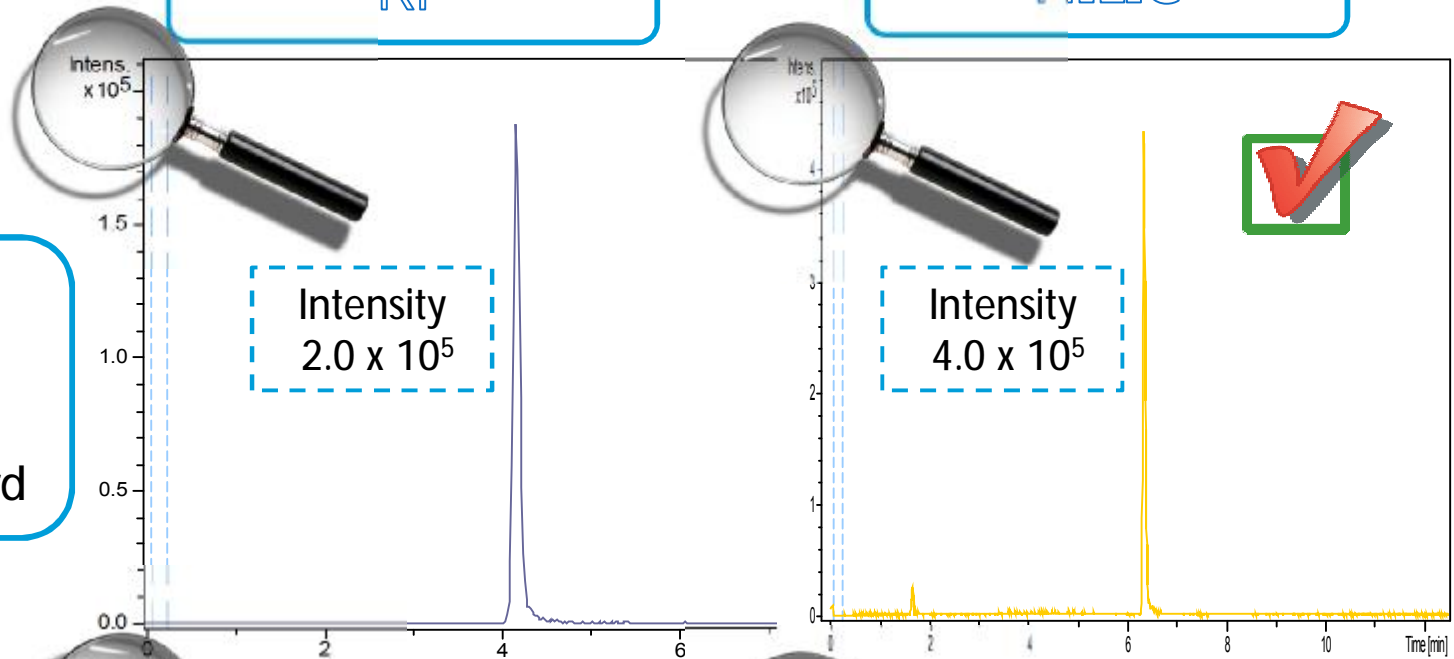
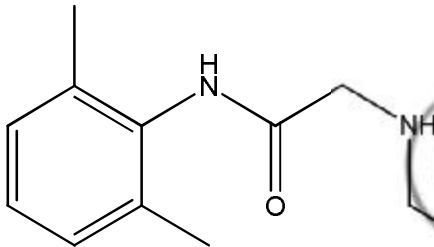


✓ Better detection sensitivity

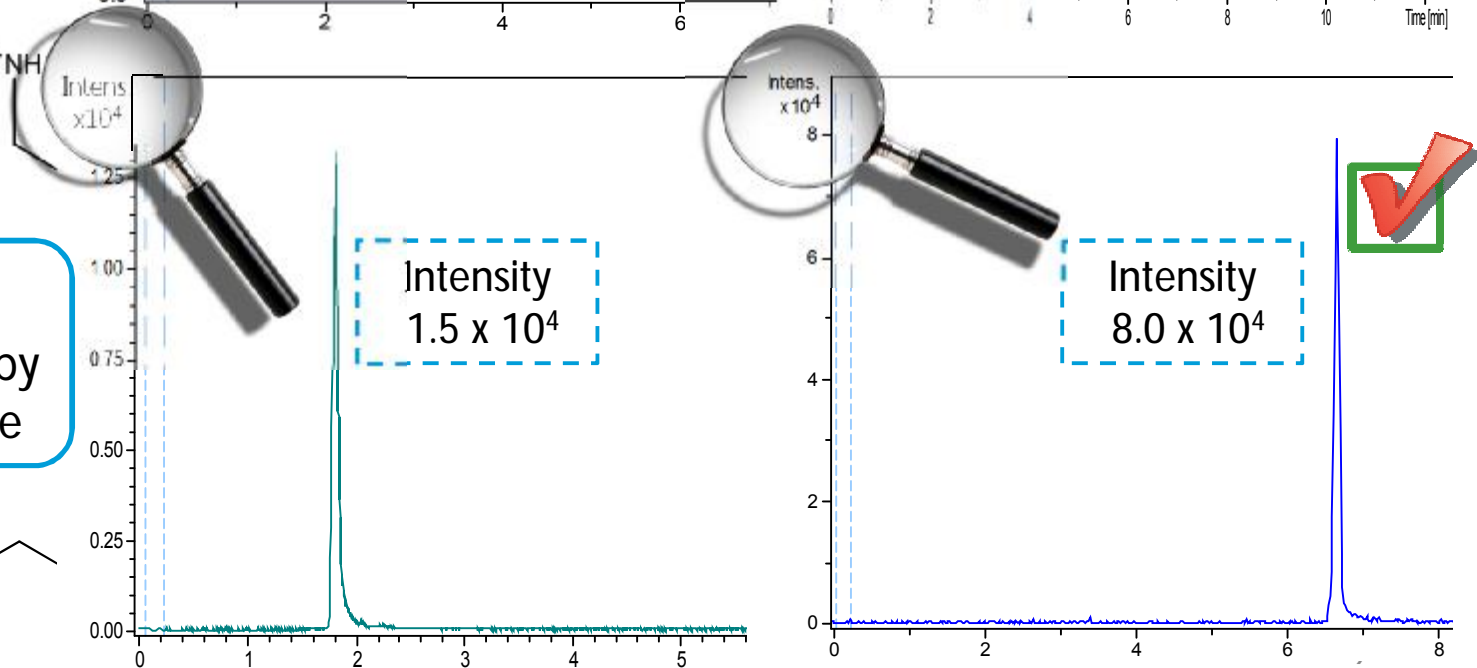
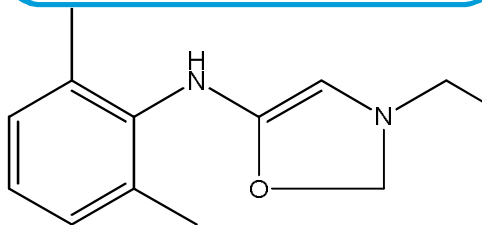
RP

HILIC

LDC207
(MEGX)
Confirmed with reference standard



LDC219
Probable structure by diagnostic evidence



✓TPs identified only in HILIC-Separation of isomers

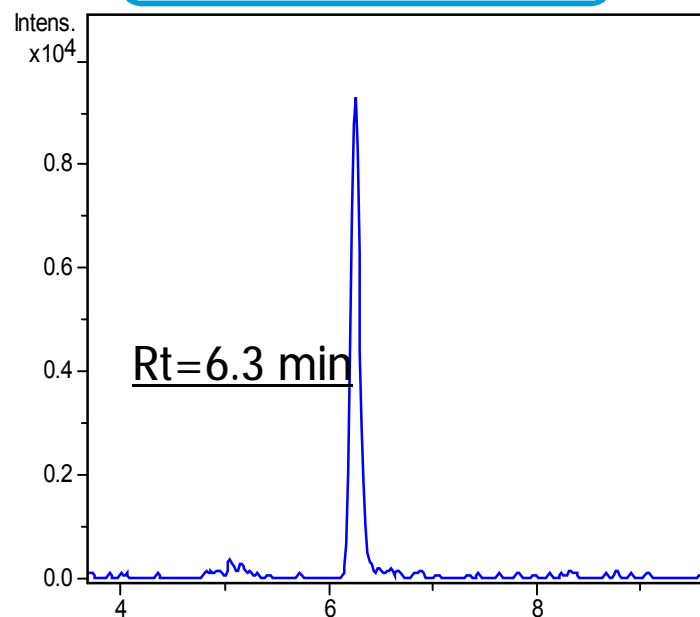
CTR360B

Probable structure by diagnostic evidence

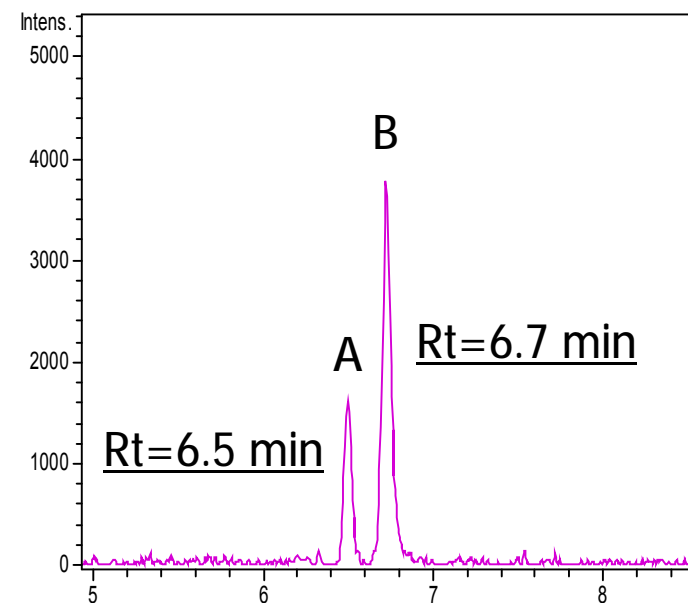
CTR360A

Tentative candidate

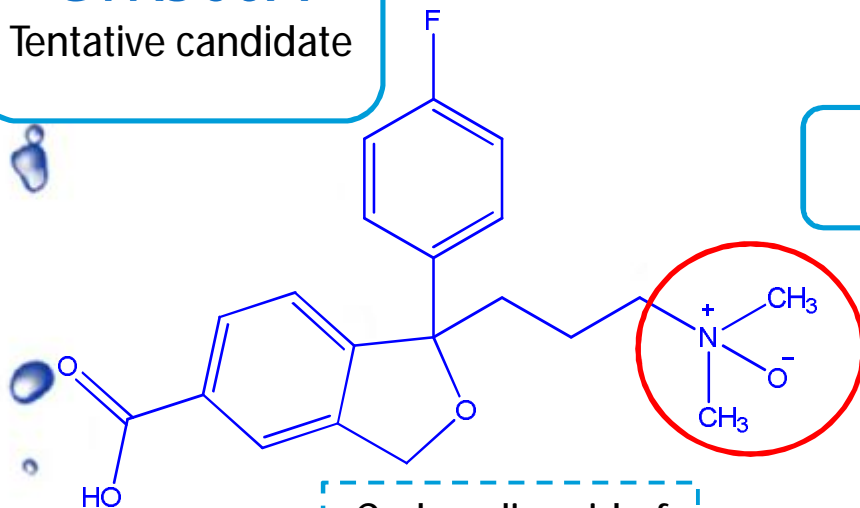
RP



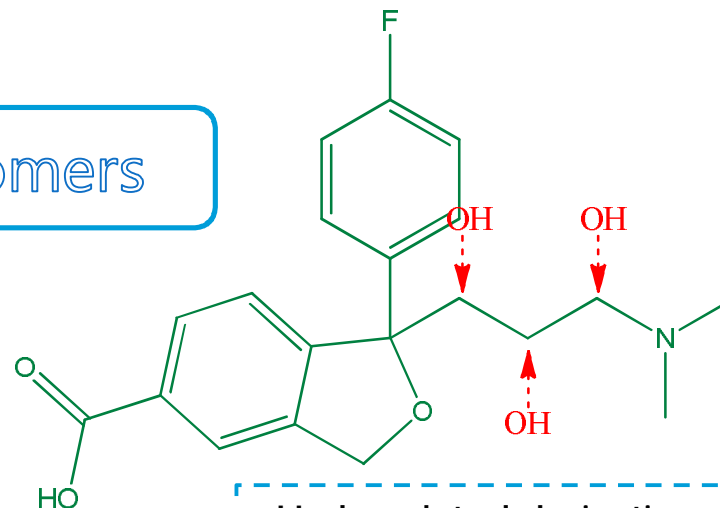
HILIC



Isomers



Carboxylic acid of CTR N-oxide



Hydroxylated derivative of CTR carboxylic acid

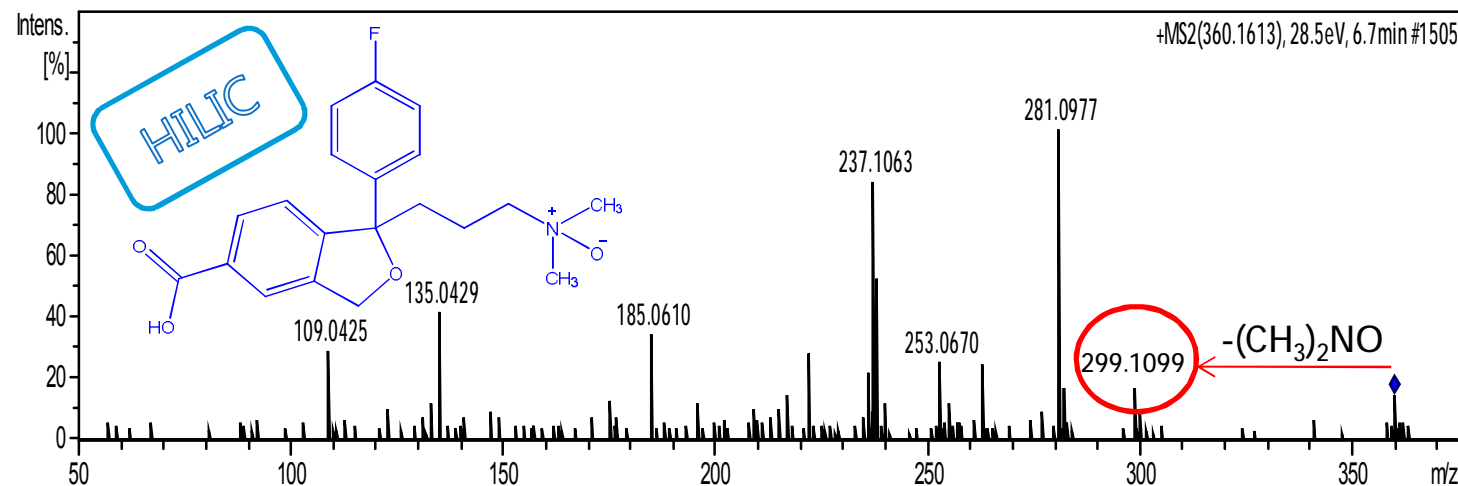
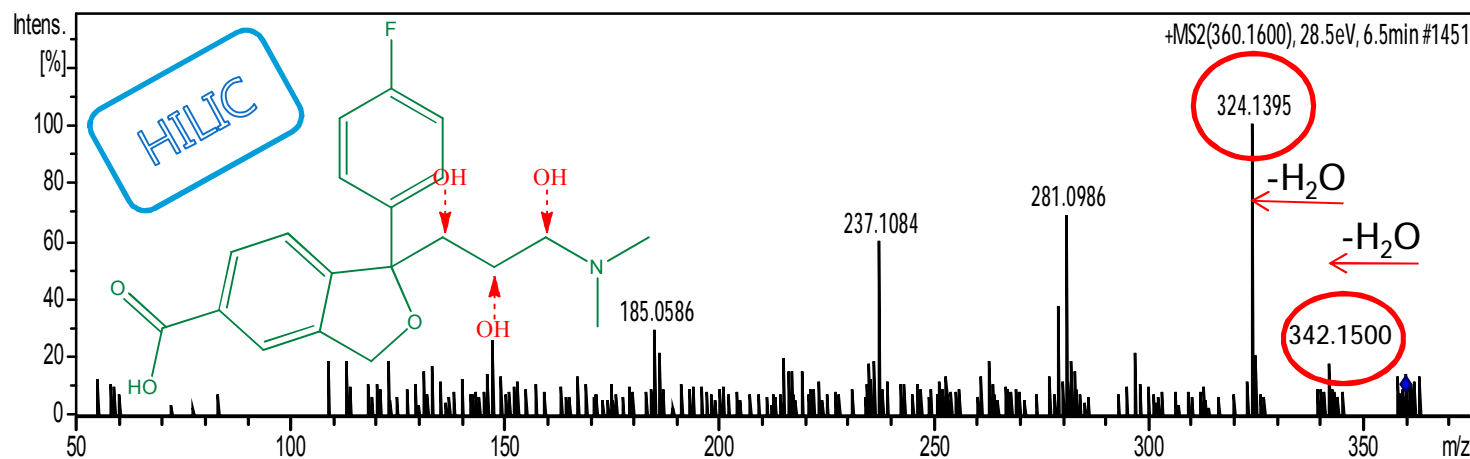
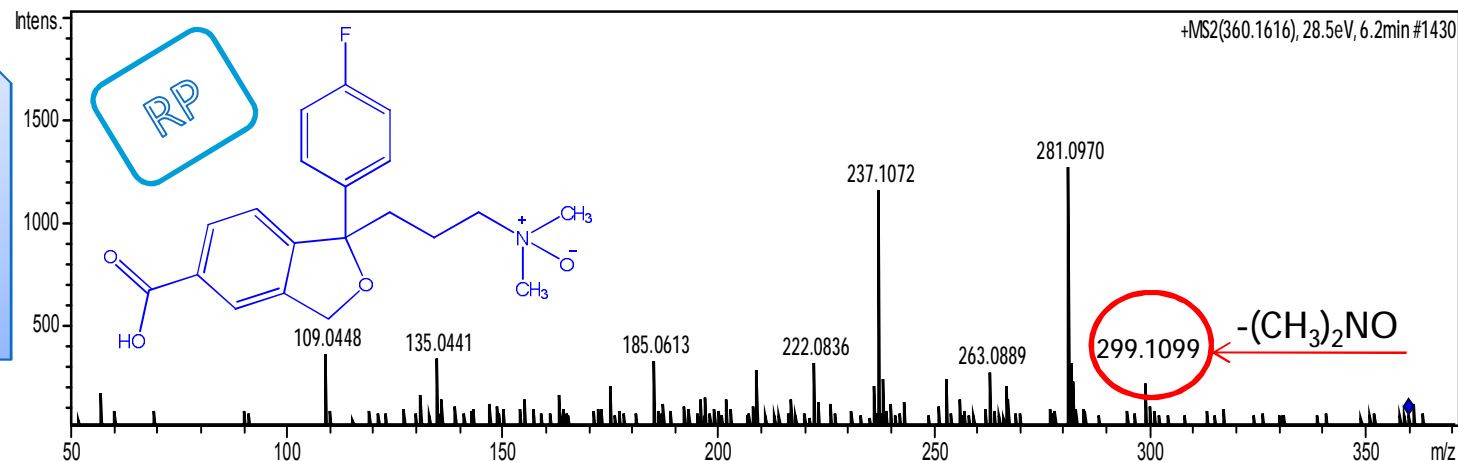
✓TPs identified only in HILIC-Separation of isomers

CTR360

Probable structure by diagnostic evidence

m/z = 342.1395
elimination of H₂O:
characteristic fragmentation pattern that not occur in CTR N-oxide

m/z = 299.1099
Elimination of (CH₃)₂NO:
characteristic fragmentation pattern only in CTR N-oxide



Conclusions



HILIC was presented to be fit for the orthogonal identification of TPs and suitable for screening of more polar metabolites



HILIC provided better peak shapes and greater intensities in some of the identified TPs.



HILIC also, permitted the identification and characterization of isomeric TPs, due to the better detection sensitivity that provided clearer spectra for interpretation.



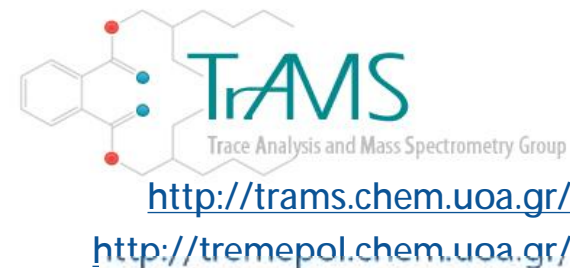
Concluding, 5 pharmaceuticals (metformin, ranitidine, lidocaine, citalopram and atorvastatin) were investigated for their TPs under aerobic biodegradation experiments and 34 TPs were identified, in total.



Nine of them were confirmed by a reference standard and the majority of the rest reached Id. Level 2: probable structure by diagnostic evidence.



National and Kapodistrian
UNIVERSITY OF ATHENS
Faculty of Chemistry



Acknowledgments

Vassiliki Beretsou Pablo Gago Ferrero
Eleni Georgantzi



Thank you for your attention

This research has been co-financed by the European Union and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) – ARISTEIA 624 (TREMEPOL project).



Co-financed by Greece and the European Union

