CEST 2015

A workflow for the orthogonal identification of biotransformation products by HILIC-QTOFMS

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TrAMS
Trace Analysis and Mass Spectrometry Group

Scope

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- Determine the degradation rate of emerging contaminants (pharmaceuticals, illicit drugs) in the aquatic environment under aerobic conditions
- Study the occurrence and formation of transformation products
- Develop an integrated workflow for suspect and non-target screening for the identification of biotransformation products
- Study the use of HILIC as a complementary technique for the identified TPs
- Further investigation for suspect and unknown TPs which may be eluted better in HILIC conditions

Experimental

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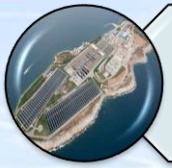
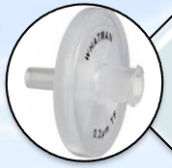


Samples of activated sludge from active bioreactors and effluent waste water from the WWTP of Athens (Psitalia)

Batch reactors seeded with activated sludge under aerobic conditions in room temperature

Spike in a concentration of 2 mg/L

Sampling immediately after spiking and after several time intervals (depending on the compound)



Filtration through glass fiber syringe and then through 0.2 μm RC filter

LC-HRMS (qTOF) both RP and HILIC

Experimental

Prior to the spiking pH and TSS, were determined.

Check for sorption to the suspended sludge particles

Check for abiotic degradation processes (hydrolysis, volatilization)

•Check for contamination between sampling
•Subtraction of the background

Aerobic biodegradation

All reactors were covered with perforated caps and placed on a magnetic stirrer to aerate the sludge.

Instrumentation

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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

Bruker Maxis Impact™
Quadrupole-Time-of-Flight

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

Levels of identification confidence

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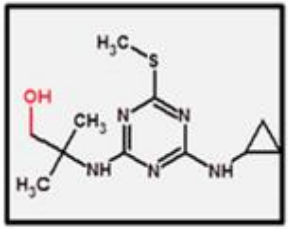
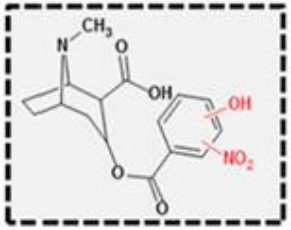
Example	Identification confidence	Minimum data requirements
	***Level 1: Confirmed structure*** by reference standard	MS, MS^2, RT, Reference Std.
	Level 2: Probable structure a) by library spectrum match	MS, MS^2, Library MS^2
	b) by diagnostic evidence	MS, MS^2, Exp. data
	***Level 3: Tentative candidate(s)*** structure, substituent, class	MS, MS^2, Exp. data
$C_6H_5N_3O_4$	***Level 4: Unequivocal molecular formula***	MS isotope/adduct
192.0757	***Level 5: Exact mass*** of interest	MS

Schymanski et al. Environmental Science and Technology (2014) 48(4):2097

Suspect screening-Workflow

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Compilation of suspect list: Metabolite prediction tools+ literature

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

Evaluation of candidates (Tentative): meet the set criteria, absence in the blank, chromatographic retention time plausibility (time trend)

MassBank

Acquire MS/MS spectra with inclusion list both in RP and HILIC: Interpretation of the fragmentation pathway

MetFrag

MetFusion

Confirmation, if it's possible with reference standard (RT and MS/MS spectra)

Suspect screening-Workflow

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Compilation of the suspect list

EAWAG-BBD Pathway Prediction System

http://eawag-bbd.ethz.ch/predict/

Rule based

Microbial catabolic reactions

(reactions found in the EAWAG-BBD database/

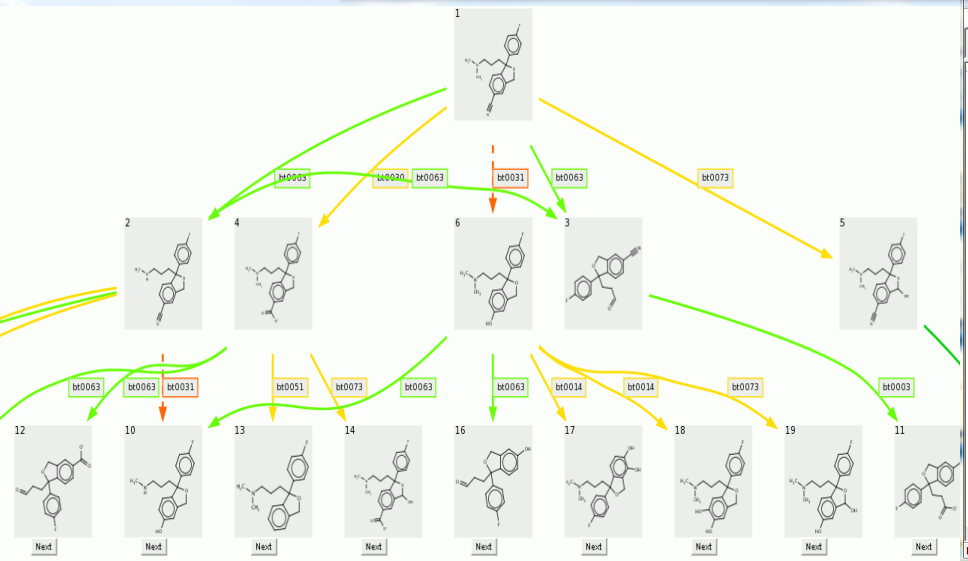
scientific literature)

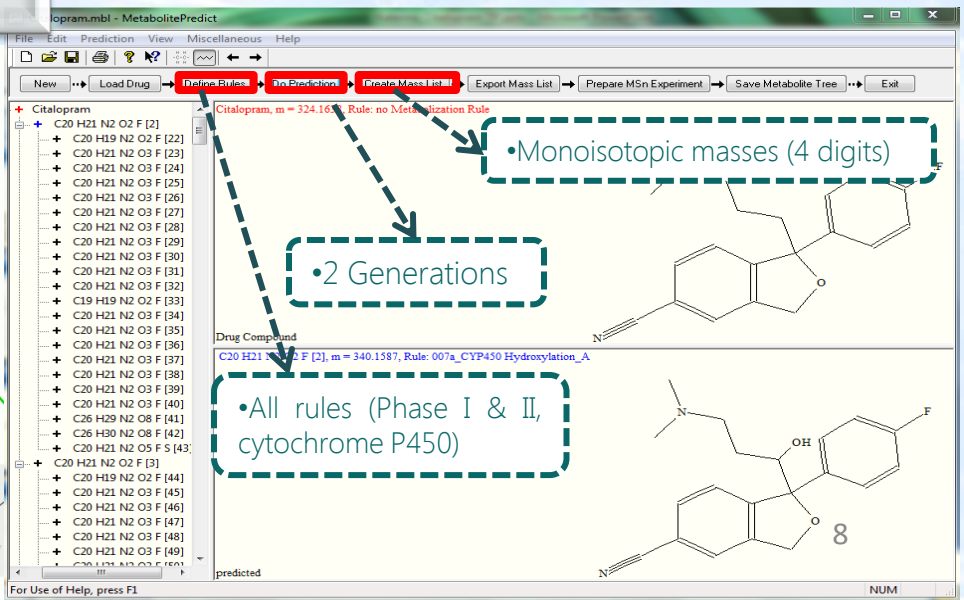
Metabolite Predict

Metabolomic software

Rule based

(Phase I & II metabolism, cytochrome P450)





Suspect screening-Workflow

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

Bruker Compass Target Analysis 1.3

Compile the suspect list
Save as .csv file

Identification Criteria

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

- Run Bruker Compass Target Analysis 1.3
- Set appropriate thresholds (m/z tolerance and mSigma)
- Consider mass accuracy and isotopic pattern to identify plausible TPs

A1: m/z [detected ion POS]

	A	B	C	D	E	F	G	H	I	J	K
1	m/z [dete	RT POS	sum formula	name	CAS	comment	comment	relativeRe	minimum	indivSigm	indivMass
2			C17H27NO2	Venlafaxine							
3			C16H25NO2	N-DESMETHYLVENLAFAXINE							
4			C15H20O3	2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl)acetaldehyde							
5			C16H25NO2	O-Desmethylvenlafaxine							
6			C17H27NO3	1-[2-(dimethylamino)-1-hydroxy-1-(4-methoxyphenyl)ethyl]cyclohexan-1-ol							
7			C15H23NO2	N,N-Didesmethylvenlafaxine							
8			C15H23NO2	N,O-Didesmethyl Venlafaxine							
9			C15H19O4	2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl)acetate							
10			C16H25NO3	4-[2-(dimethylamino)-1-(1-hydroxycyclohexyl)ethyl]benzene-1,2-diol							
11			C16H25NO3	1-[1-hydroxy-1-(4-methoxyphenyl)-2-(methylamino)ethyl]cyclohexan-1-ol							
12			C14H18O3	2-(1-hydroxycyclohexyl)-2-(4-hydroxyphenyl)acetaldehyde							
13			C16H25NO3	4-[2-(dimethylamino)-1-hydroxy-1-(1-hydroxycyclohexyl)ethyl]phenol							

Clear Results | Screen | General Unknown | Save | Print | DataAnalysis | Print with Excel

Comment:

Multi Target Screening with 'C:\Users\Katerina\Desktop\Databases\Venlafaxine_TPs.csv'

#	Id	Cmpd.Name	Formula	PMI	m/z calc.	m/z meas.	Err [ppm]	Err [mDa]	mSigma	RT exp. [min]	RT meas. [min]	deltaRT [min]
8	+	N-DESMETHYLVE...	C 16 H 25 N 1 O 2	[...	264.1958	264.1943	5.6	1.5	6.3	0.00	7.48	-7.48
9	+	O-Desmethylvenl...	C 16 H 25 N 1 O 2	[...	264.1958	264.1943	5.6	1.5	6.3	0.00	7.48	-7.48
11	+	VEN 294	C 17 H 28 N 1 O 3	[...	295.2142	295.2137	-1.6	-0.5	13.8	0.00	10.51	-10.51
5	+	N	C 15 H 23 N 1 O 2	[...	250.1802	250.1788	5.5	1.4	13.8	0.00	6.46	-6.46
6	+	N	C 15 H 23 N 1 O 2	[...	250.1802	250.1788	5.5	1.4	13.8	0.00	6.46	-6.46
46	+	1-[2-(dimethylami...	C 17 H 27 N 1 O 3	[...	294.2064	294.2055	-2.8	-0.8	13.8	0.00	13.63	-13.63
47	+	1-[2-(dimethylami...	C 17 H 27 N 1 O 3	[...	294.2064	294.2055	-2.8	-0.8	13.8	0.00	13.63	-13.63
1	+	N-DESMETHYLVE...	C 16 H 25 N 1 O 2	[...	264.1958	264.1950	3.0	0.8	14.6	0.00	6.16	-6.16
2	+	O-Desmethylvenl...	C 16 H 25 N 1 O 2	[...	264.1958	264.1950	3.0	0.8	14.6	0.00	6.16	-6.16

Suspect screening-Workflow

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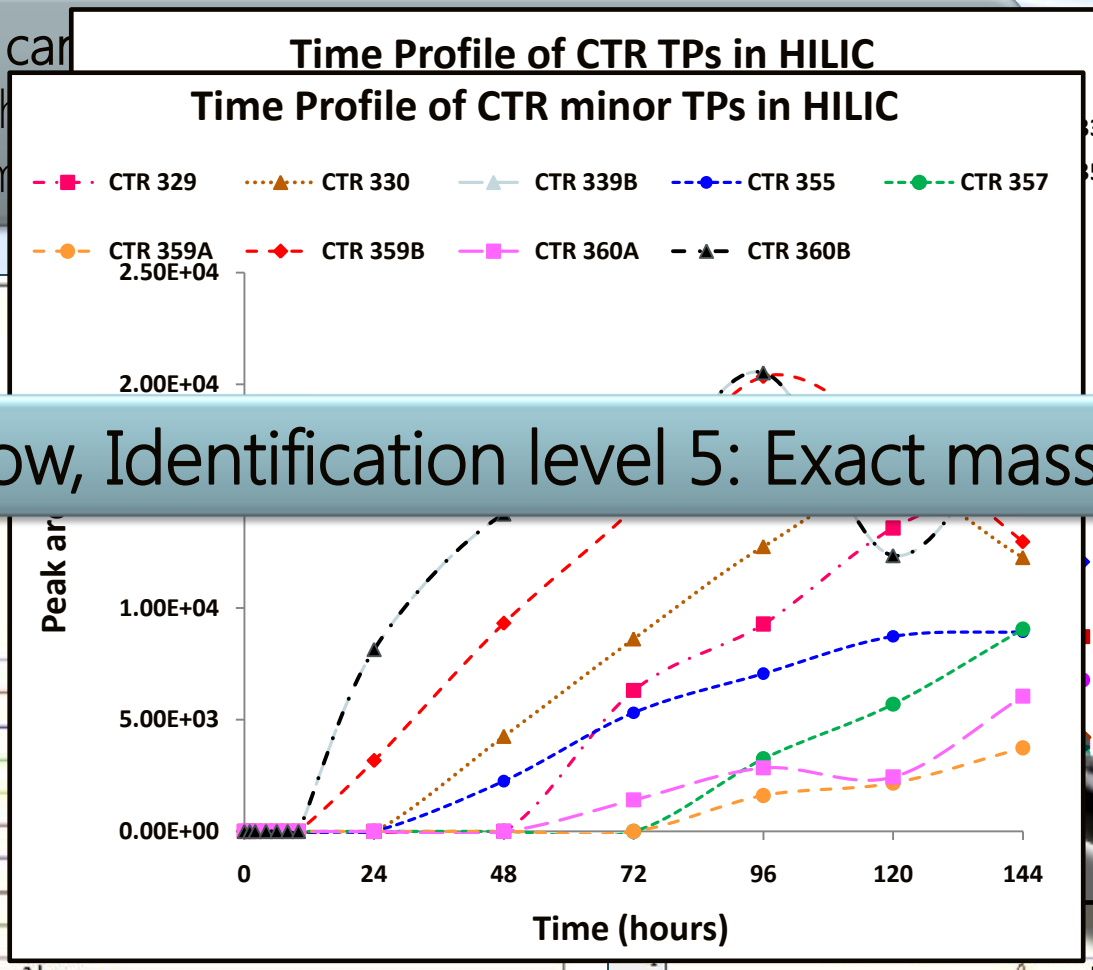
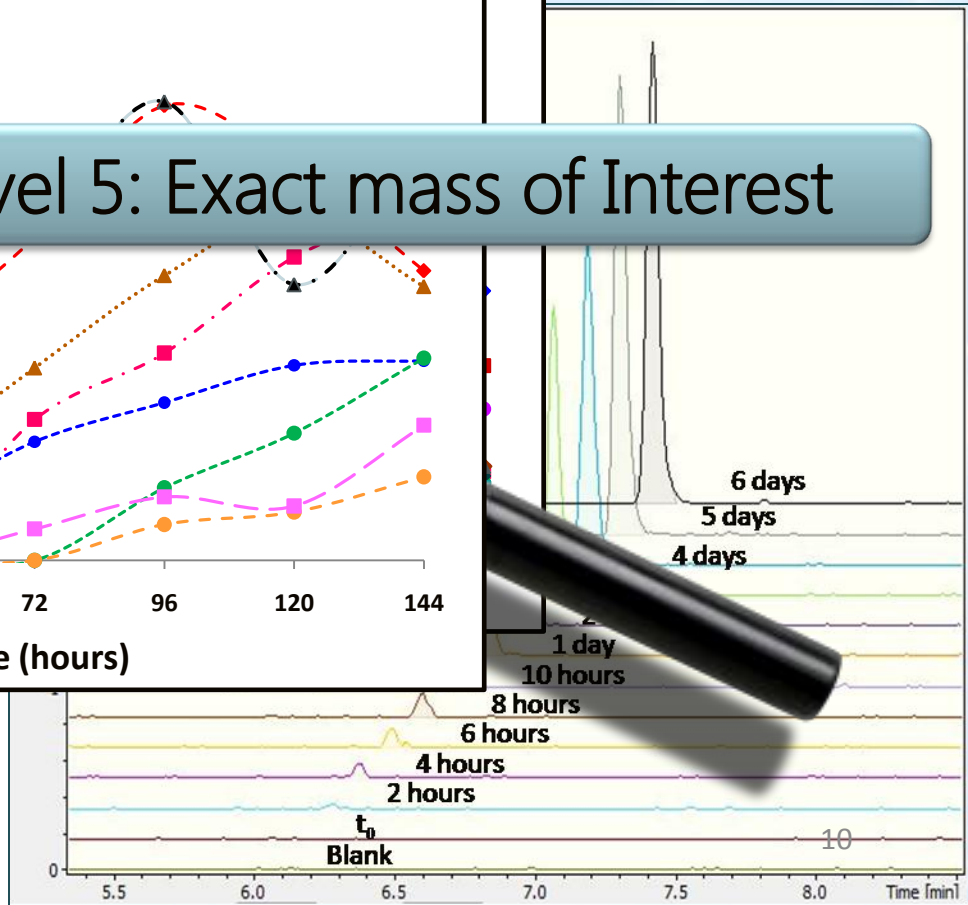
Evaluation of car
absence in th
plausibility (tim



Until now, Identification level 5: Exact mass of Interest



Suspect screening-Workflow

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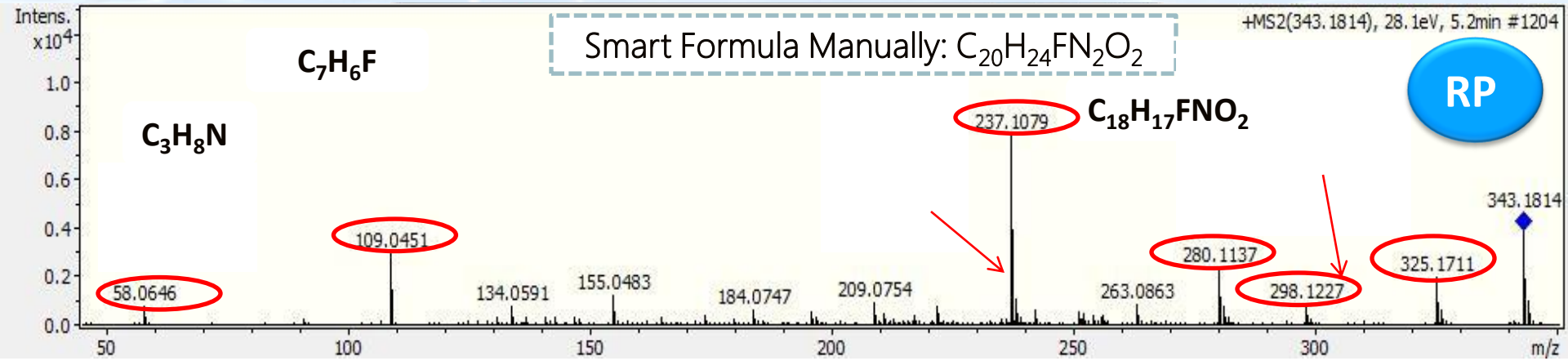
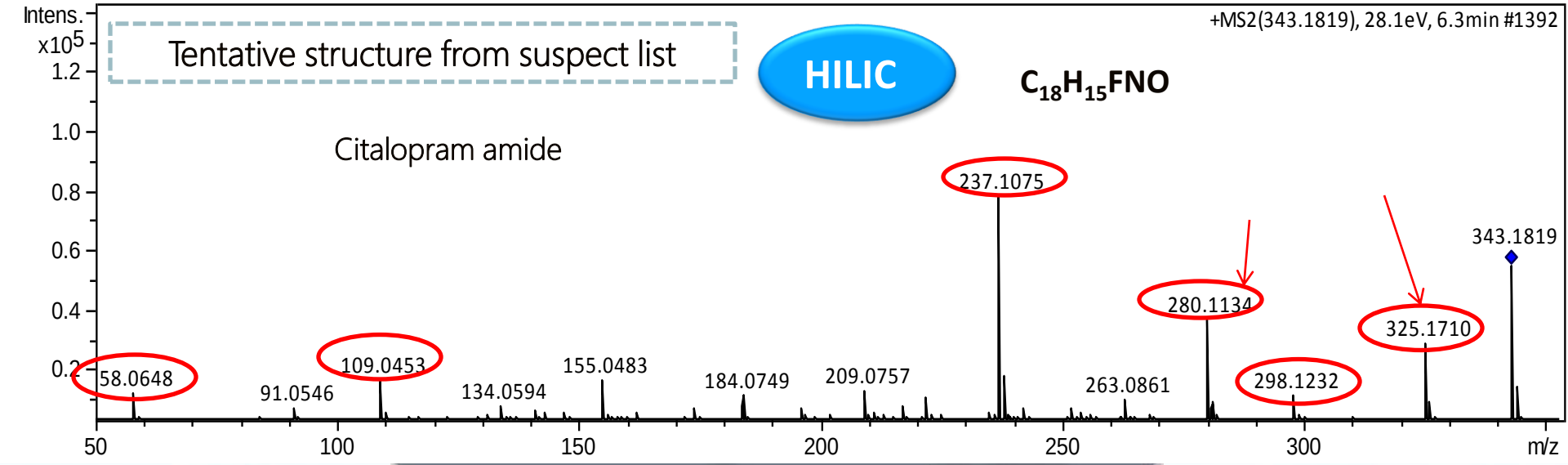
Acquire MS/MS spectra with inclusion list both in RP and HILIC: Interpretation of the fragmentation pathway

Data analysis

CTR 343





Suspect screening-Workflow

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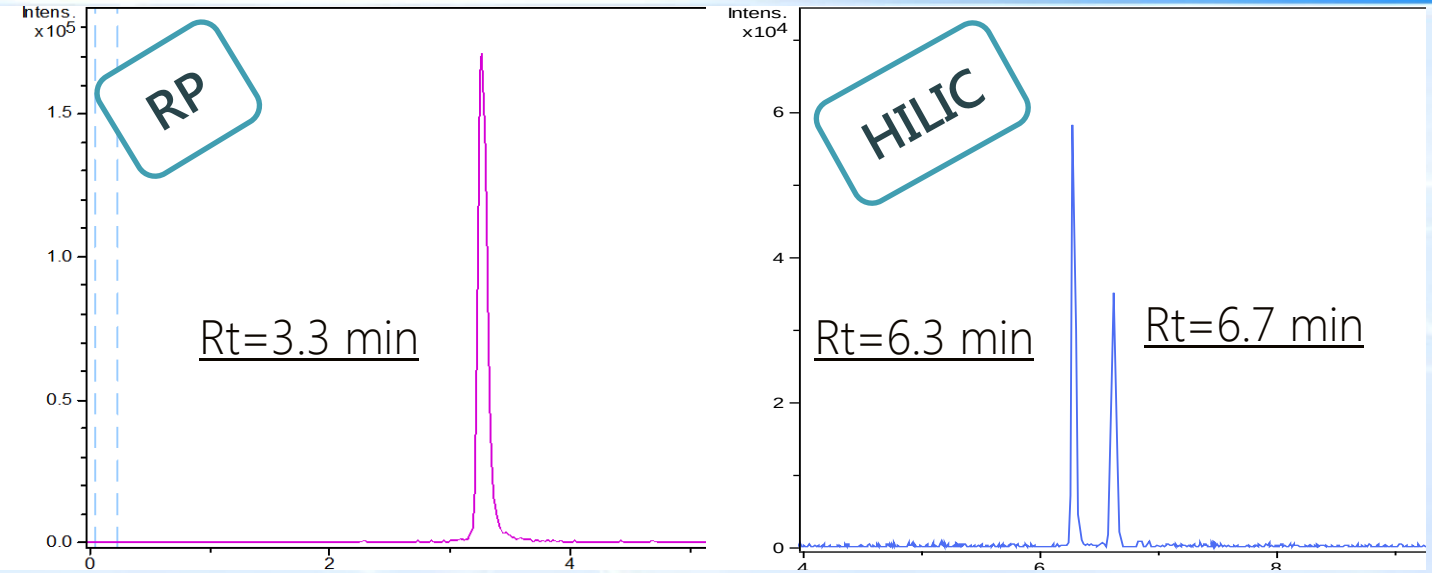
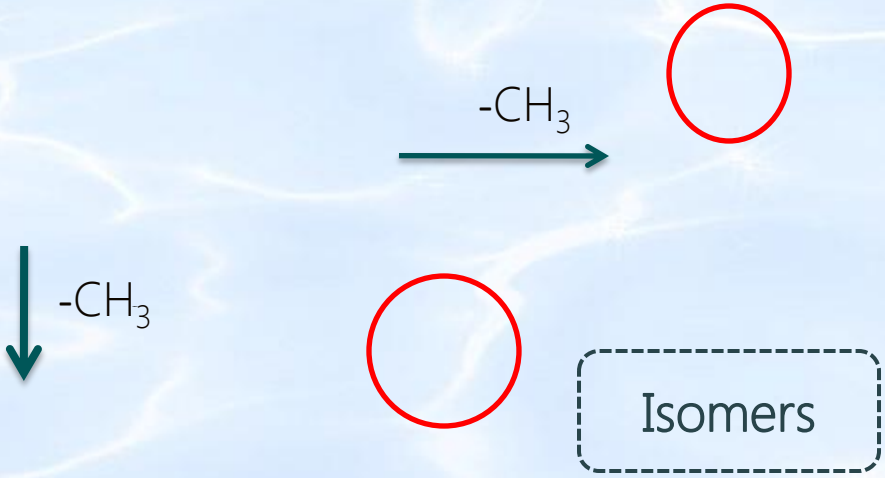
RAN301 (desmethyl ranitidine)
Probable structure by diagnostic evidence





Suspect screening-Workflow

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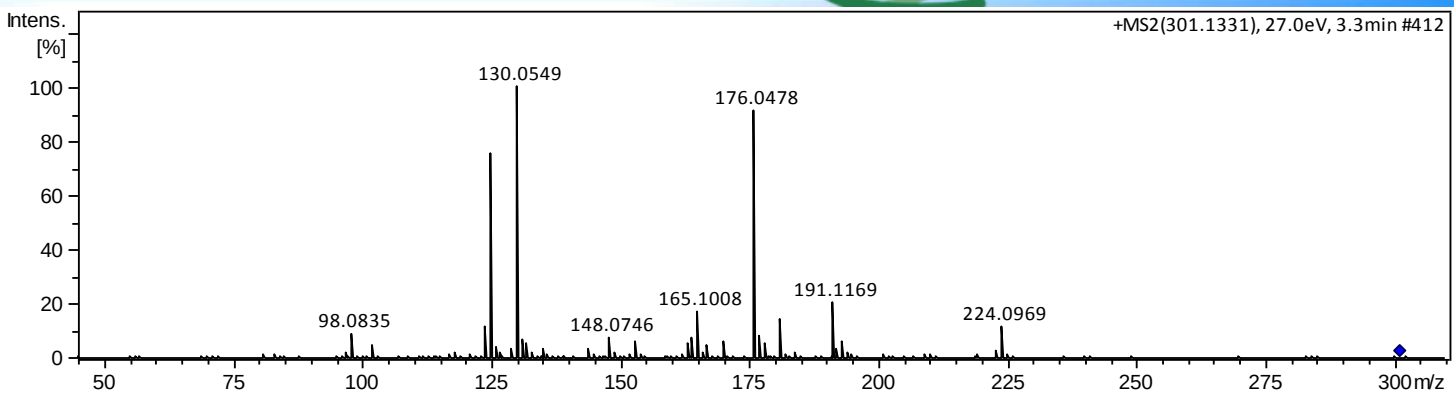
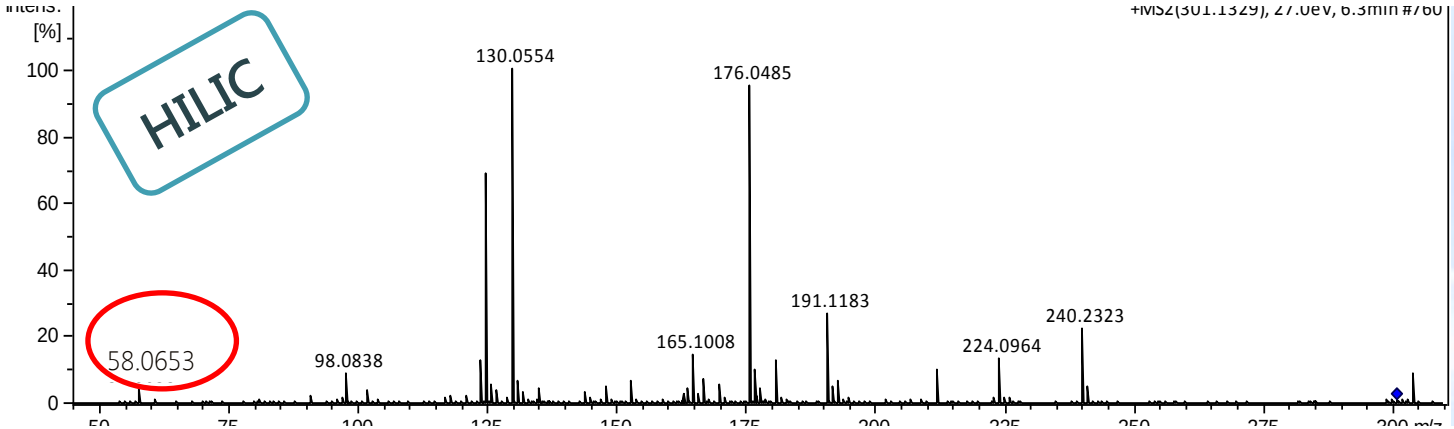
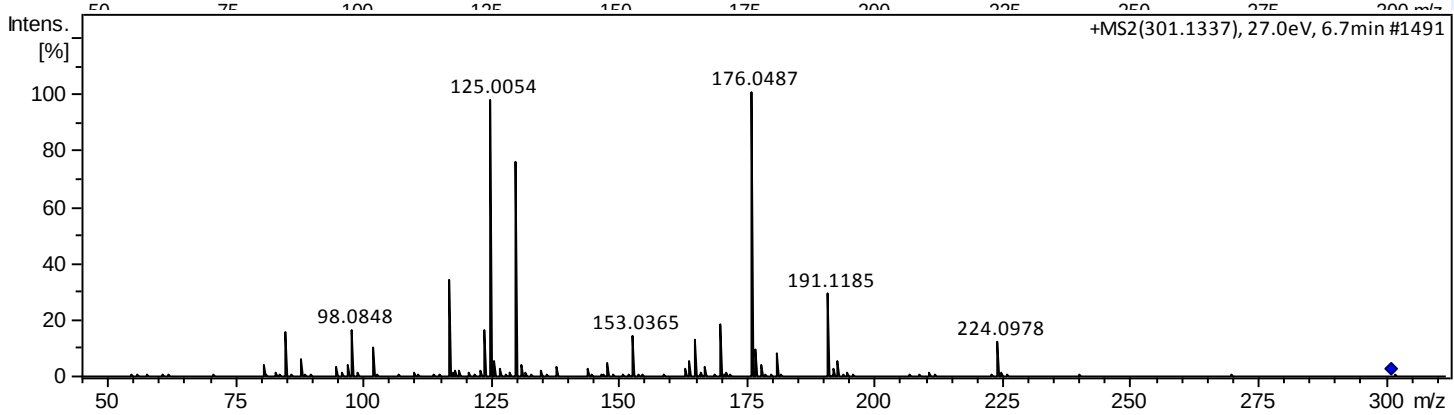
✓Separation of isomers

RAN301 (desmethyl ranitidine)
Probable structure by diagnostic evidence

m/z= 58.0651 characteristic fragment for N-demethylation







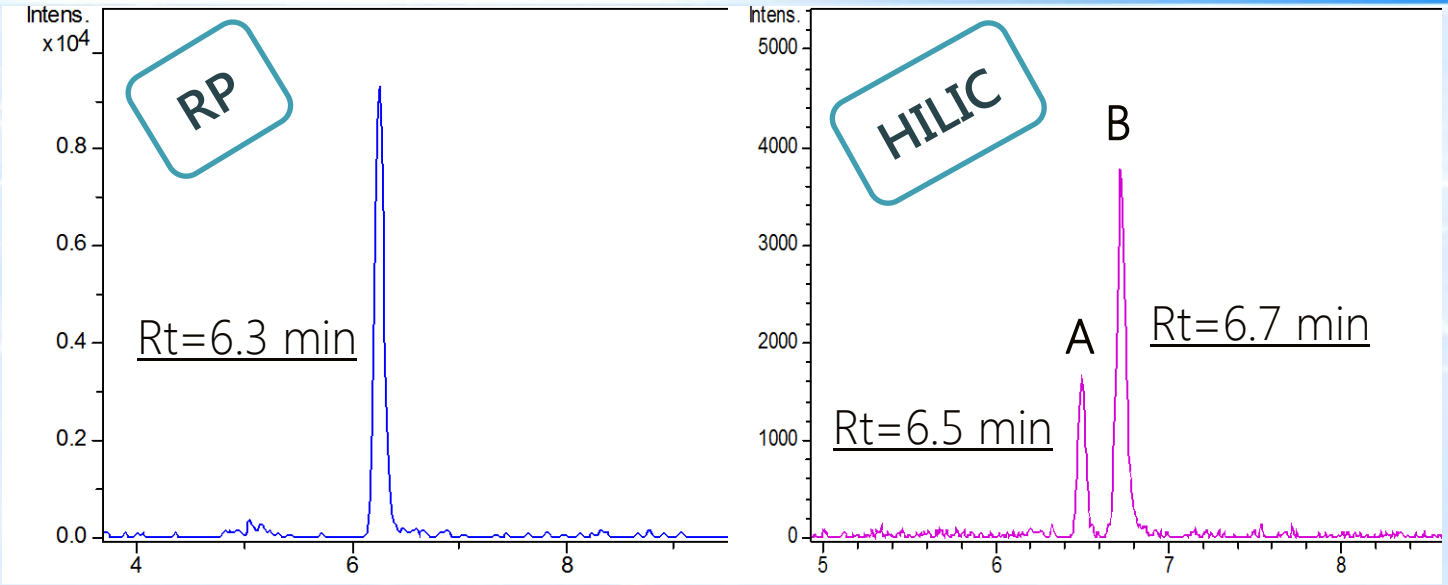
Suspect screening-Workflow

CEST 2015

✓Separation of isomers

CTR360B
Probable structure by diagnostic evidence

CTR360A
Tentative candidate



Isomers

Carboxylic acid of CTR N-oxide

Hydroxylated derivative of CTR carboxylic acid

Suspect screening-Workflow

CEST 2015

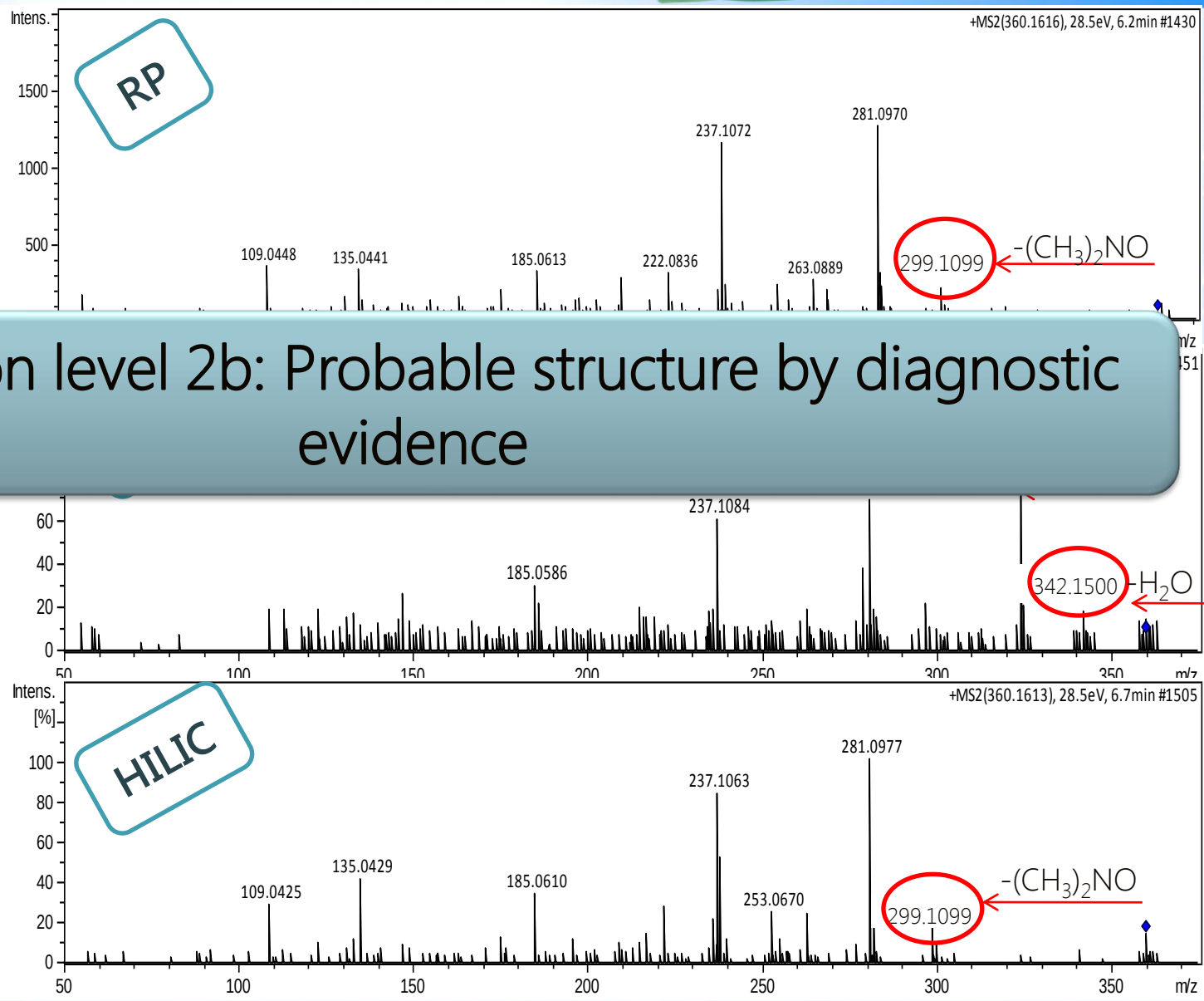
✓Separation of isomers

CTR360
Probable structure by diagnostic

Identification level 2b: Probable structure by diagnostic evidence

characteristic fragmentation pattern that <u>not occur in CTR N-oxide</u>

m/z= 299.1099
Elimination of $(CH_3)_2NO$: characteristic fragmentation pattern only <u>in CTR N-oxide</u>



Suspect screening-Workflow

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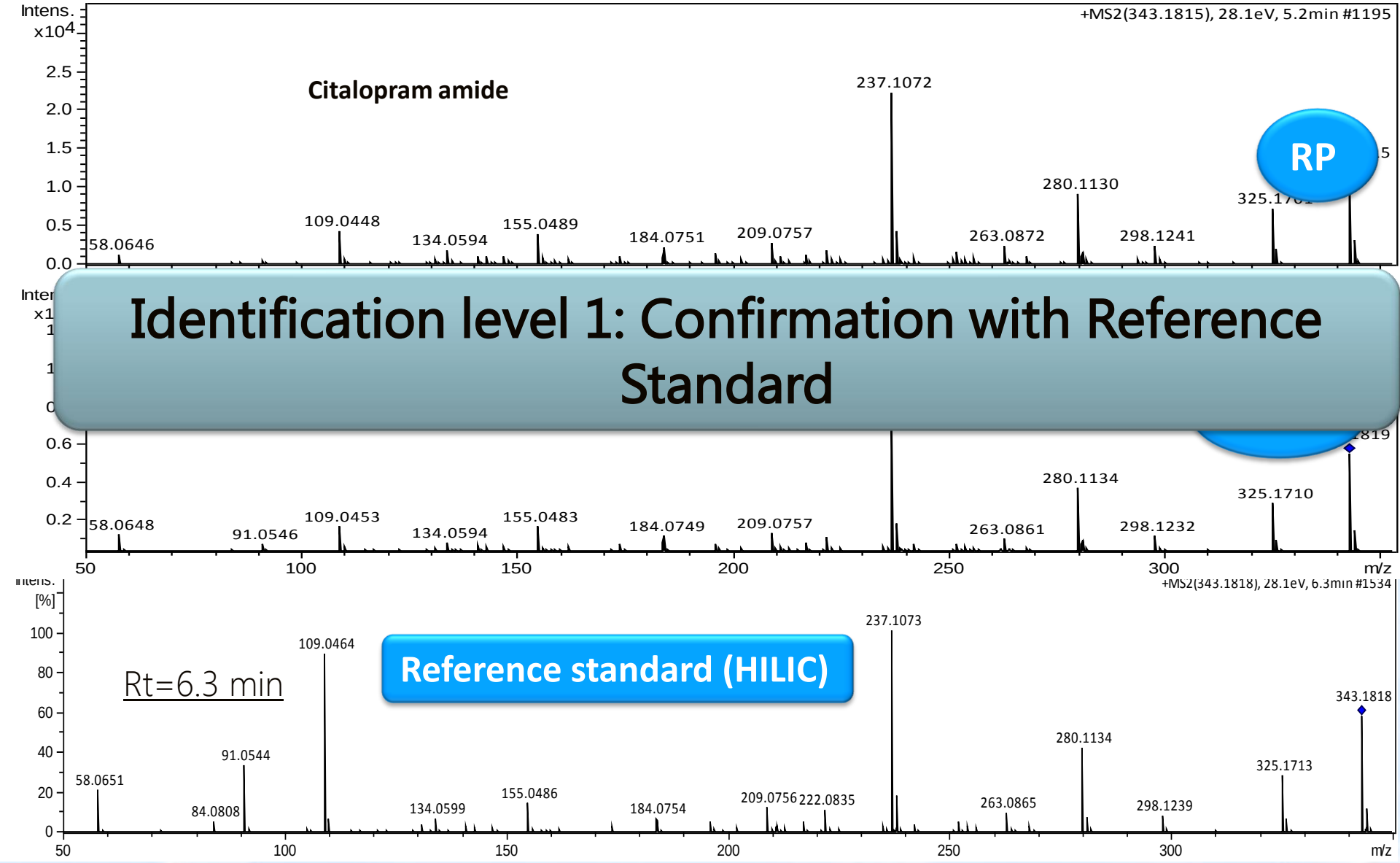

Non-target screening-Workflow

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Subtraction of the background (Bruker's MetaboliteDetect 2.0)

Generation of a **peak list** of unknown

Treat further as "suspect peaks"

Background Subtraction Parameters

Difference

eXpose mode, Ratio: 3

Detect -Mass spectrum Parameters

Int. threshold: 30%

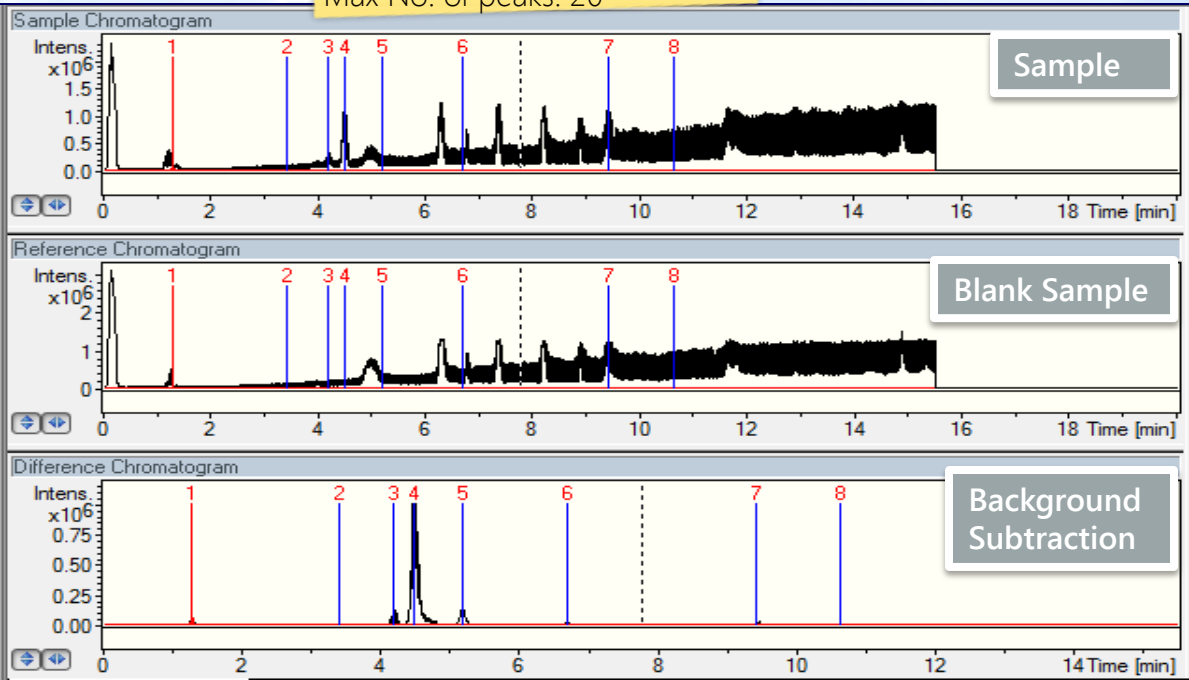
Max No. of peaks: 20

Metabolite Detect

detected | expected

Generation of peak list

No.	Chrom.	Gp.	RT-S[min]	m/z-det.	MS/MS	Comment
A		-	Base Peak	-	-	Base Peak Chr
B	+	-	BPC Int.	-	-	Base Peak Chr
C	+	-	TIC	-	-	Total Ion Curr
D	+	-	EIC	-	-	Extracted Ion
1		1*	1.3	107.9681	(yes)	Det. Trace 1
2	+	1	1.3	123.9411	(yes)	Det. Trace 2
3	+	1	1.3	139.9146	(yes)	Det. Trace 3
4	+	2*	3.4	216.1231	(yes)	Det. Trace 4
5	+	3a1*	4.2	207.1489	(yes)	Det. Trace 5
6	+	3b1	4.2	266.1975	(yes)	Det. Trace 6
7	+	4*	4.5	86.0973	(yes)	Det. Trace 7
8	+	5*	5.2	86.0963	(yes)	Det. Trace 8
9	+	6*	6.7	219.1491	(yes)	Det. Trace 9
10	+	7*	9.4	652.2653	(yes)	Det. Trace 10
11	+	7	?	694.2812	(yes)	Det. Trace 11
12	+	8*	10.6	910.5027	(yes)	Det. Trace 12



Non-target screening-Workflow

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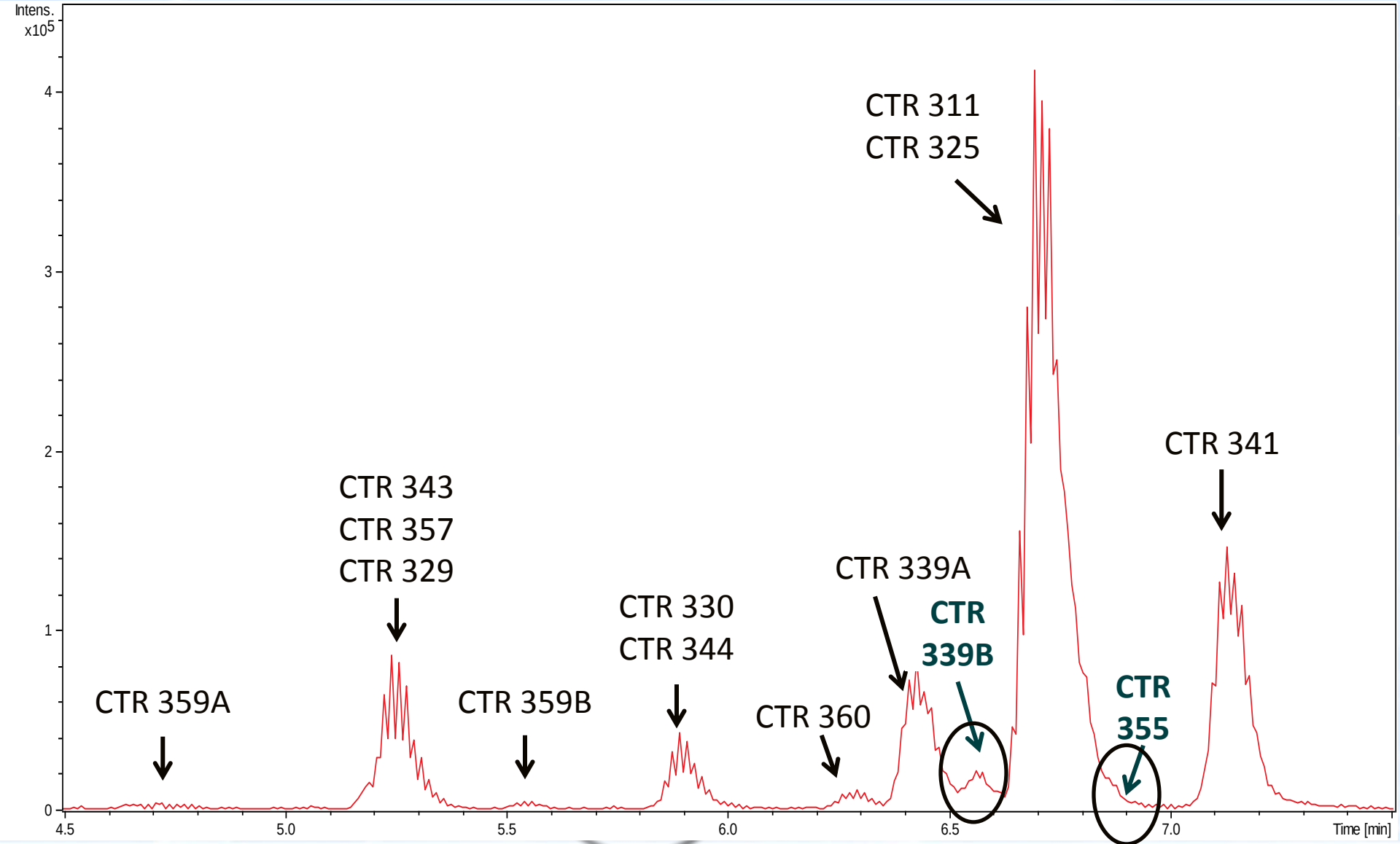

Results

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PC`	TP	Mass $[M+H]^+$	Molecular Formula	Appearance of max peak area	Time trend	Id. Level	Name
Lidocaine	LDC207	207.1504	C12H18N2O	7d	↗↘	1	MEGX
	LDC251	251.1756	C14H22N2O2	2d	↗↘	1	Lidocaine-N-oxide
	LDC219	219.1485	C13H18N2O	1d	↗↘	2b	
	LDC233	233.1282	C13H16N2O2	7d	↗→	4	
	LDC265	265.1538	C14H20N2O3	9d	↗→	4	
Citalopram	CTR311	311.1554	C19H19FN2O	6 d	↗	1	Desmethyl CTR
	CTR329	329.1660	C19H21FN2O2	6 d	↗	2b	Desmethyl CTR amide
	CTR330	330.1500	C19H20FNO3	6 d	↗	2b	Desmethyl CTR carboxylic acid
	CTR339A	339.1503	C20H19FN2O2	6 d	↗	1	3-Oxo-CTR
	CTR339B	339.1880	C21H23FN2O	6 d	↗	2b	Methylated derivative of CTR
	CTR341	341.1660	C20H21FN2O2	3 d	↗↘	2a	CTR-N-oxide
	CTR343	343.1816	C20H23FN2O2	6 d	↗	1	CTR amide
	CTR344	344.1656	C20H22FNO3	5 d	↗	1	CTR carboxylic acid
	CTR355	355.1457	C20H19FN2O3	6 d	↗	3	Hydroxylated derivative of 3-Oxo-CTR
	CTR357	357.1609	C20H21FN2O3	6 d	↗	2b	Amide of 3-Oxo-CTR
	CTR359A	359.1765	C20H23FN2O3	6 d	↗	3	Hydroxylated derivative of CTR amide
	CTR359B			4 d	↗↘	2b	Amide of CTR-N-oxide
	CTR360A	360.1606	C20H22FNO4	6 d	↗	3	Hydroxylated derivative of CTR carb.acid
	CTR360B				↗	2b	Carboxylic acid of CTR-N-oxide
Mtf	MTF103	103.0614	C2H6N4O	2d	↗	1	Guanylurea

Results

CEST 2015

PC	TP	Mass $[M+H]^+$	Molecular Formula	Appearance of max peak area	Time trend	Id. Level	Name
Ranitidine	RAN286A	286.0856	C11H15N3O4S	1d	↗↘	4	-
	RAN286B			2d	↗→	4	-
	RAN286C			10h	↗↘	4	-
	RAN301A	301.1329	C12H20N4O3S	10h	↗↘	2b	desmethyl RAN
	RAN301B					2b	
	RAN302A	302.0805	C11H15N3O5S	1d	↗	3	
	RAN302B			1d	↗↘	3	
	RAN316A (2peaks)	316.1331		3d	↗	3	
	RAN316B			3d	↗	3	
	RAN317 (2peaks)	317.1278	C12H20N4O4S	3d	↗	3	Desmethyl RAN-S-oxide
	RAN331A (2peaks)	331.1435	C13H22N4O4S	1d	↗↘	1	RAN-S-oxide
	RAN331B			1d	↗↘	1	RAN-N-oxide
Atorvastatin	ATR515	515.2341	C31H31FN2O4	2d	↗	2b	β-oxidation product of ATR
	ATR471	471.2078	C29H27FN2O3	2d	↗	2b	β-oxidation product of ATR515
	ATR487A	487.2028	C29H27FN2O4	2d	↗	3	Monohydroxylation of ATR471
	ATR487B			2d	↗	3	

Conclusions

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- 5 pharmaceuticals (metformin, ranitidine, lidocaine, citalopram and atorvastatine) 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.
- 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 characterisation of isomeric TPs, due to the better detection sensitivity that provided clearer spectra for interpretation.

European Union
European Social Fund

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

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programme for development
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Eleni Georgantzi

Our collaborators:

Kathrin Fenner Rebekka Gulde

eawag
aquatic research

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Thank you for your attention

TrAMS
Trace Analysis and Mass Spectrometry Group

http://trams.chem.uoa.gr/
http://tremepol.chem.uoa.gr/