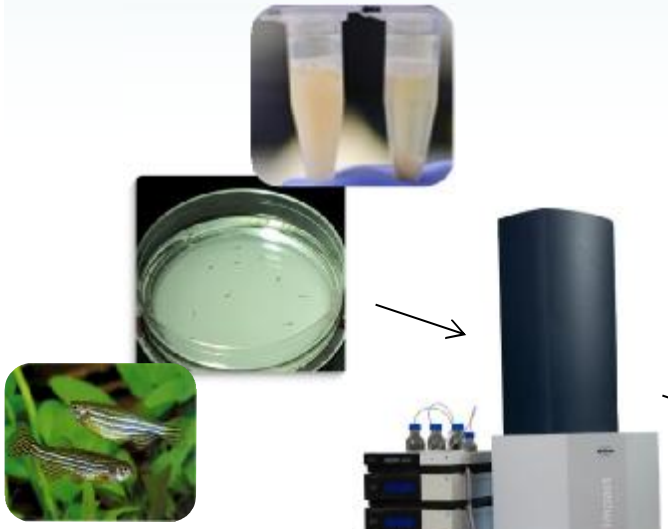
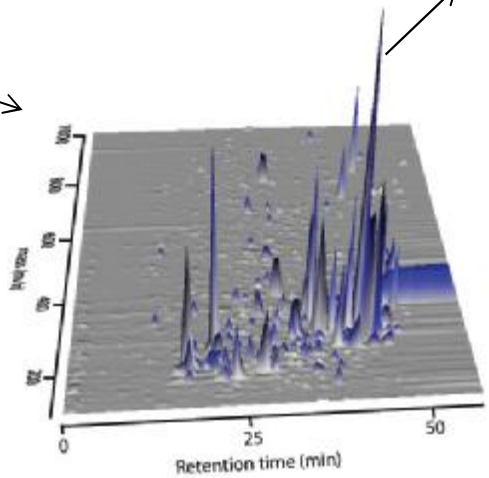
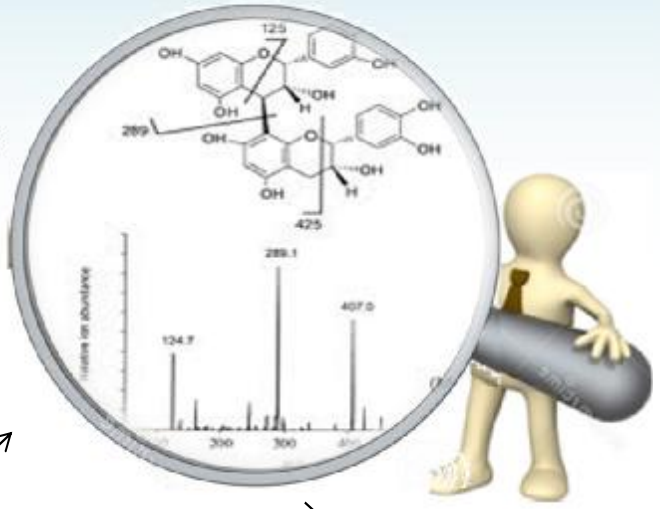
Study of the metabolism of zebrafish (danio rerio) embryo exposed to triclosan and benzotriazoles by HILIC-TOF-MS

Damalas Dimitris



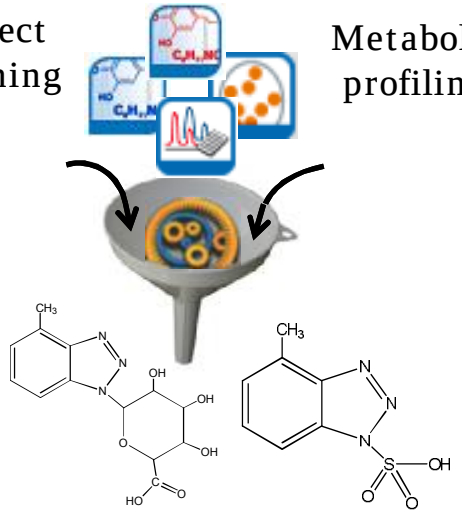




Non target screening

Suspect screening

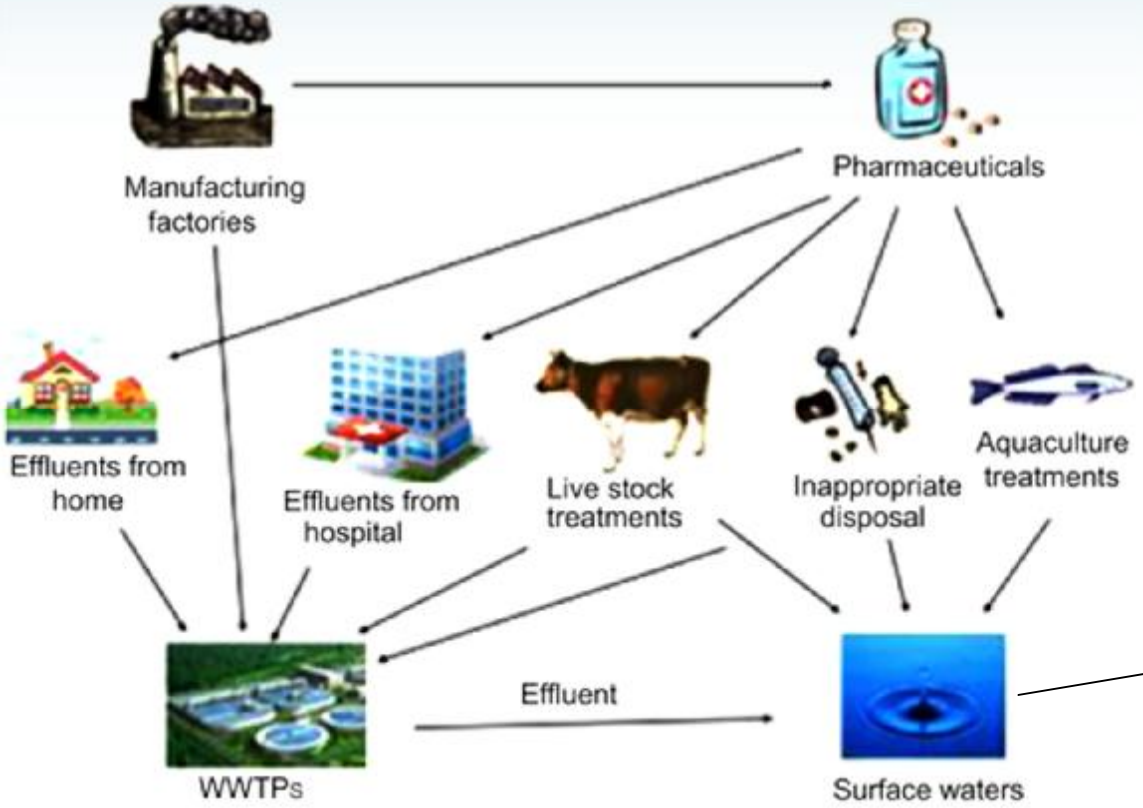
Metabolic profiling

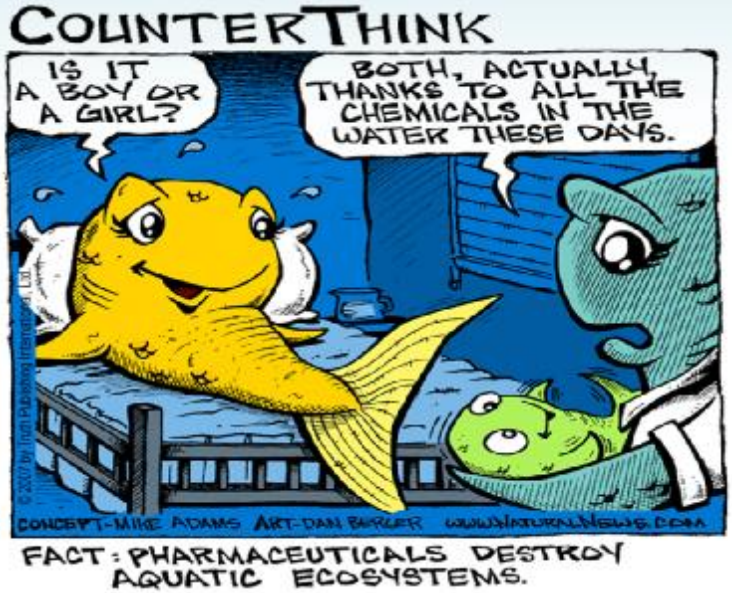


1st NORMAN workshop on analysis of problematic compounds

How can we analyse very polar and hardly-ionisable compounds

Aquatic environment contamination…





Bioaccumulation of xenobiotics and their bio-TPs in aquatic organisms

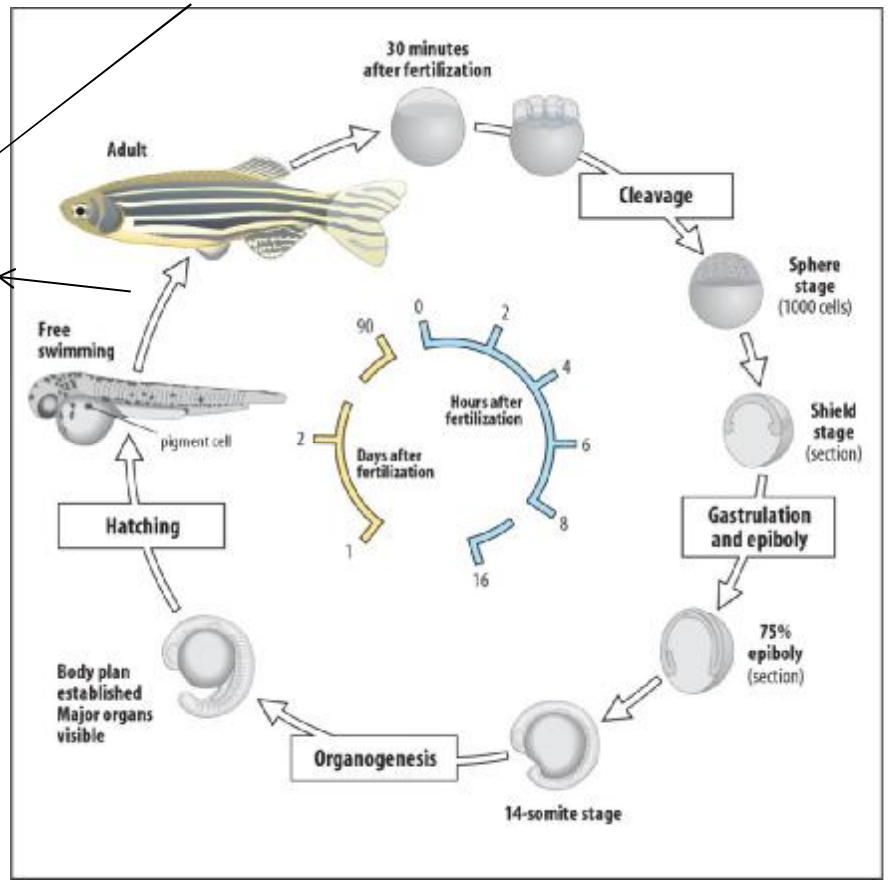
Xenometabolome = xenobiotics (unmodified) + bio-TPs

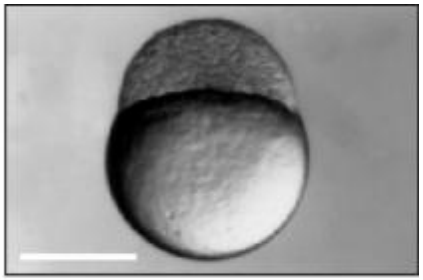
Endometabolome = endogenous organism metabolites

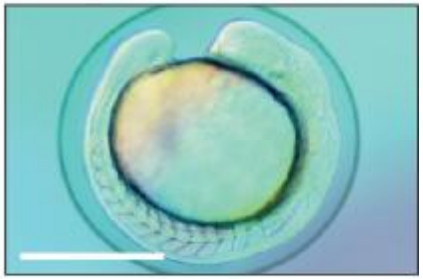
need for a model organism

- ❑ assess the toxicity of xenobiotics and their bio-TPs in aquatic organisms
- ❑ relate the toxic effect to metabolic phenotype variations
- ❑ evaluate the detoxification system of aquatic organisms

this is Zebrafish *(Danio rerio)* (96 hpf)







why *Zebrafish*...?

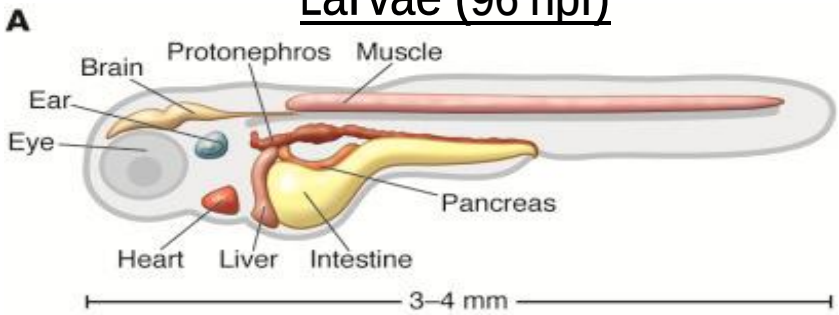
- ✓ Embryo and larval small size
- ✓ Rapid generation of large number of embryos
- ✓ Husbandry costs
- ✓ Recommended by the OECD for fish toxicity testing
- ✓ Similar xenobiotic metabolism pathways with those observed in mammals

Ideal for keeping in the lab

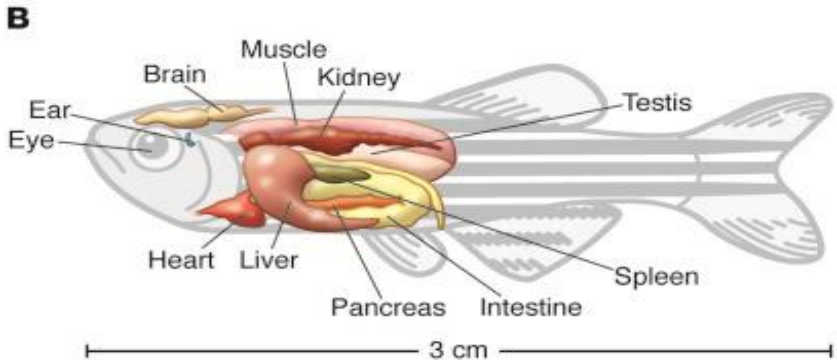
why 96 hpf...?

- ✓ The majority of major organs partially or fully formed at early life-stages
- ✓ Absorption of compounds from water across the skin at early life-stages

Larvae (96 hpf)



adult



experimental part

Incubation:

BTR | 4-Me-BTR | 5-Me-BTR | Triclosan | Control

~ 60 *Zebrafish* embryos (96 hpf) / petri

5 time intervals for each “exposure”: 30s | 2h | 4h | 8h | 24h

each time interval sample in different petri: e.g. for BTR

Time profiles observation

Estimation of skin adsorption

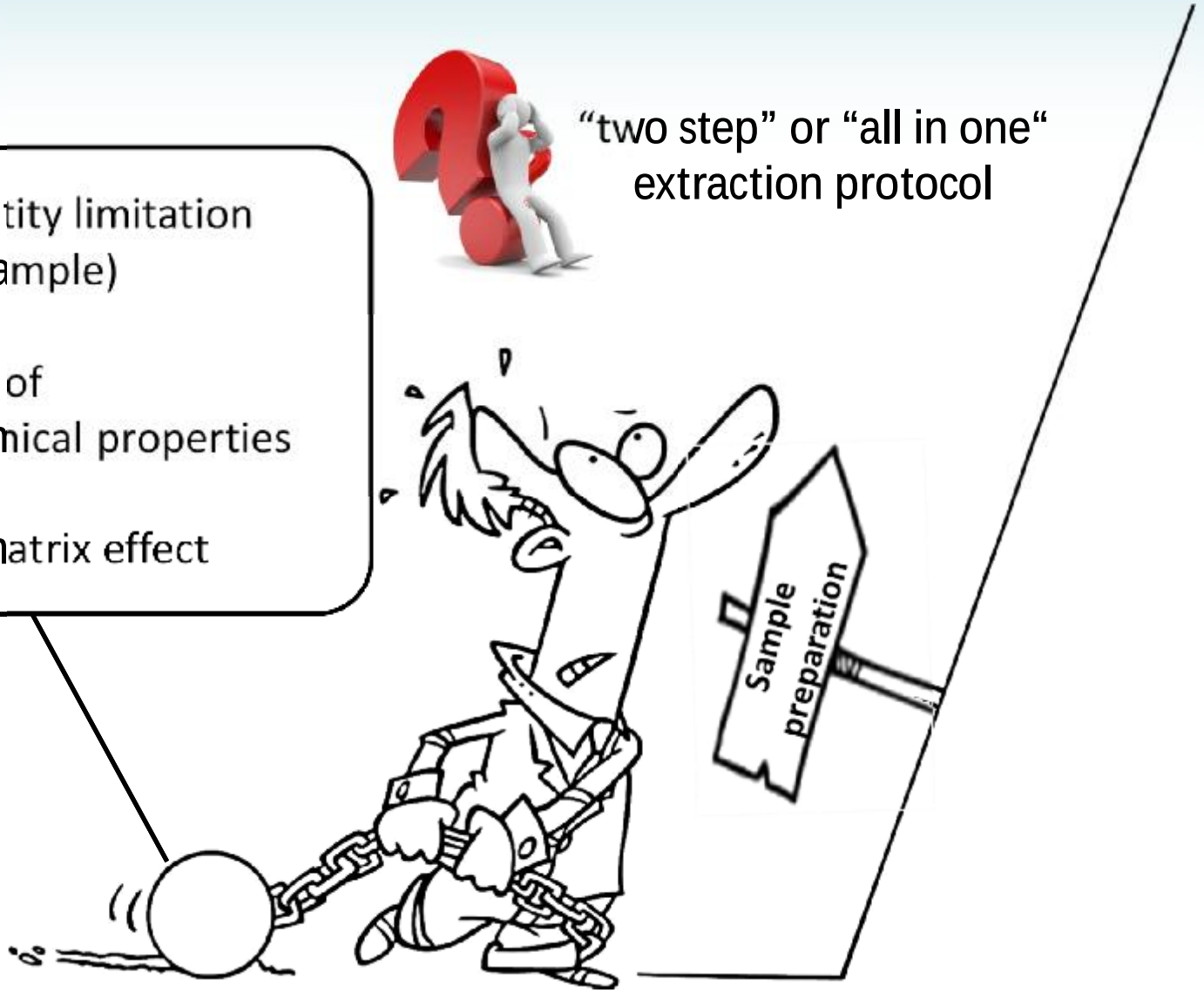
Short term effects

Long term effects

Sample preparation

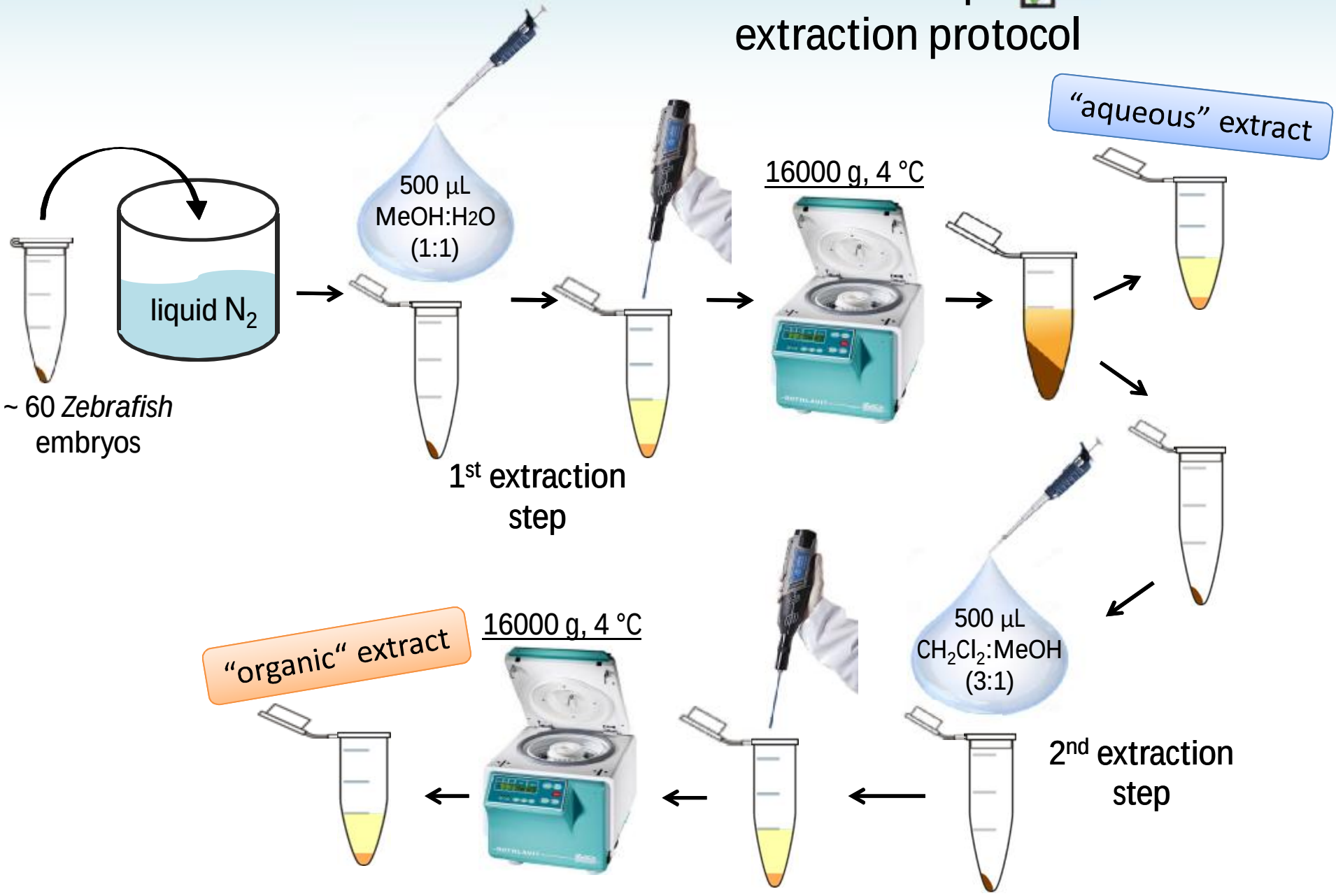
"two step" or "all in one" extraction protocol

- × Tissue quantity limitation (~ 15 mg/sample)
- × Wide range of physicochemical properties
- × Increased matrix effect



Sample preparation

"two step" extraction protocol



Evaluation part

Analysis

UHPLC-Q-TOF MS/MS

- RPLC (+/- ESI) (Acclaim C18)
- HILIC (+/- ESI) (Acquity BEH amide)

Data Acquisition modes

- Data-Independent (bb-CID)
- Data-Dependent (Auto-MS)

- Xenometabolome (low-medium polarity)
- Endometabolome (medium-high polarity)

Bio-TPs Identification

- Suspect Screening (knowledge based approach)

DataAnalysis TargetAnalysis Metabolite Predict

- Non-target Screening (treated/control comparison based)

Metabolite Detect DataAnalysis

Metabolic profiling

- Targeted approach (primary metabolites of interest)

DataAnalysis TargetAnalysis

- Untargeted approach (significant endogenous metabolic responses) ProfileAnalysis

Suspect screening

Suspect list compilation

Metabolite Predict

(*in-silico* drug metabolism prediction tool)

.csv

A	B	C	D
m/z	RT	sum formula	name
		C7 H7 N3	4 Me-BTR
		C7 H5 N3 O	4 Me-BTR-147
		C7 H7 N3 O	4 Me-BTR-149
		C8 H9 N3 O	4 Me-BTR-163
		C7 H7 N3 O2	4 Me-BTR-165

TargetAnalysis DataAnalysis

m/z ⟶ Tentative bio-TP

Criteria

✓ Accuracy ≤ 2 mDa

✓ Isotopic fit ≤ 100 mSigma

✓ Absence from control

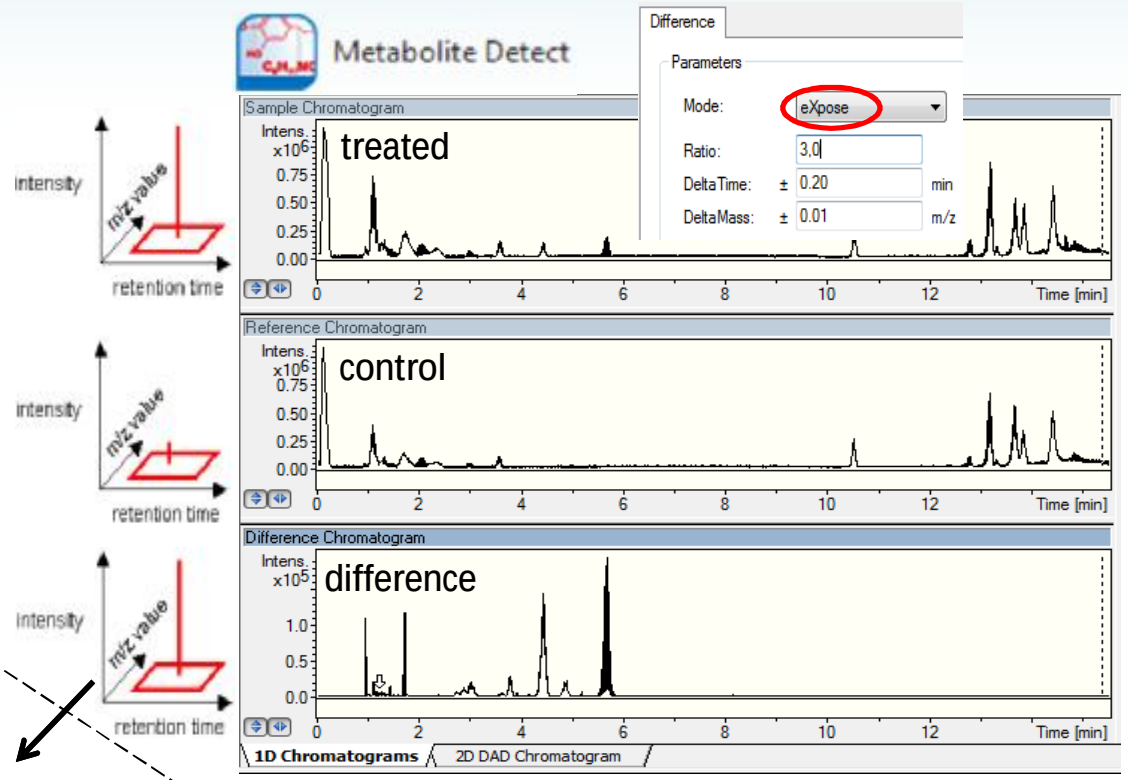
✓ Time profile existence

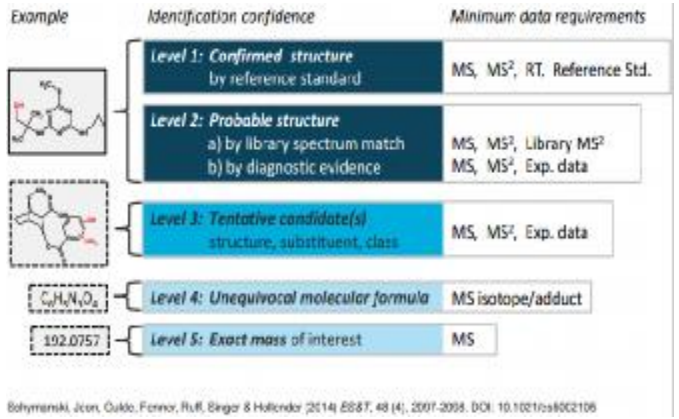
✦ Xenobiotic metabolism relevant

Non-target screening

Treated-control sample “comparison”:

Metabolite Detect





Targeted metabolic profiling

4 major classes of polar metabolites

- amines
- aminoacids
- organic acids
- sugars

standards available

B	C	D
RT POS	sum formula	name
2	C6H6N2O	Nicotinamide
2.5	C7H8N4O2	Theobromine
2.5	C5H6N2O2	Thymine
2.5	C5H10O5	Xylose
2.55	C5H10O5	Arabinose
2.7	C6H10O4	Adipic acid
2.76	C5H6O4	Itaconic acid
3	C10H11NO3	2-methyl-hippuric acid
3.45	C10H16N2O3S	Biotin
3.8	C9H9NO3	Hippuric acid
4	C4H4N2O2	Uracil

TargetAnalysis

DataAnalysis

Id	Cmpd.Name	Formula	Err [mDa]	mSigma	RT...	Area
+++	N-acetyl-histidine	C 8 H 11 N 3 O 3	-0.1	10.0	7.22	743052
+++	Alanine	C 3 H 7 N 1 O 2	0.3	3.8	7.50	11969
+++	Xanthine	C 5 H 4 N 4 O 2	0.1	2.7	6.00	12956
+++	Acetylcarnitine	C 9 H 17 N 1 O 4	0.2	6.1	7.05	1008280
+++	Inosine	C 10 H 12 N 4 O 5	-0.1	25.0	6.30	598996
+++	Uridine	C 9 H 12 N 2 O 6	0.2	16.2	5.30	55444
+++	Sarcosine	C 3 H 7 N 1 O 2	0.3	3.8	7.50	11969
++	Isoleucine	C 6 H 13 N 1 O 2	-0.5	5.7	6.40	7272
++	Monoisoamyla...	C 5 H 13 N 1	0.3	53.1	6.20	1894
++	Phenylalanine	C 9 H 11 N 1 O 2	0.4	21.1	6.90	1273
++	Proline	C 5 H 9 N 1 O 2	-0.5	n.a.	8.50	2335
++	Threonine	C 4 H 9 N 1 O 3	0.2	97.2	8.10	6839
++	Adipic acid	C 6 H 10 O 4	-0.5	38.5	2.70	2537

Untargeted metabolic profiling

Metabolic feature extraction DataAnalysis

- Peak detection, peak alignment
- Find Molecular Features (FMF) Algorithm

Data pretreatment ProfileAnalysis

- Normalization, scaling, missing values handling

Multivariate data analysis

- PCA ProfileAnalysis

Why an additional separation mode?

× Double chromatographic run

× Laborious

× Time consuming

× Double data treatment

Bio-TPs Identification

Metabolic profiling

Complementary metabolite separation technique to RPLC

Improved separation efficiency
- Better peak shape
- Smaller peak width

More comprehensive endometabolome coverage
- Adequate retention of highly polar primary metabolites (unretained in RPLC)

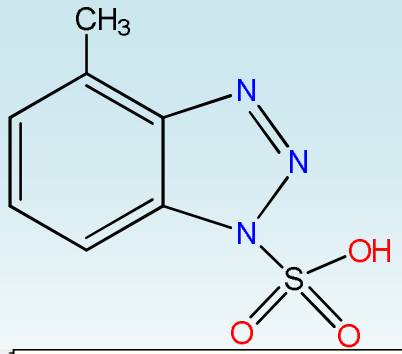
Orthogonality
- Elution in reversed order
- Enhance identification confidence

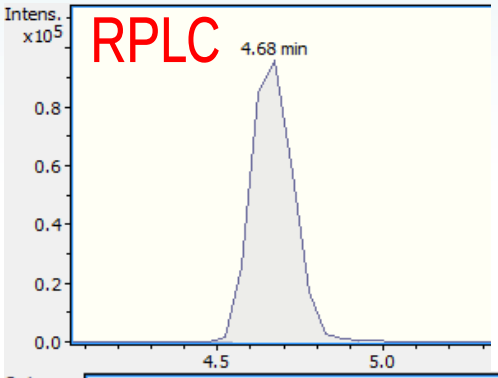
Improved detection sensitivity
- High organic content of mobile phase

Examples (bio-TPs identification)

4-Me-BTR-N-sulfate

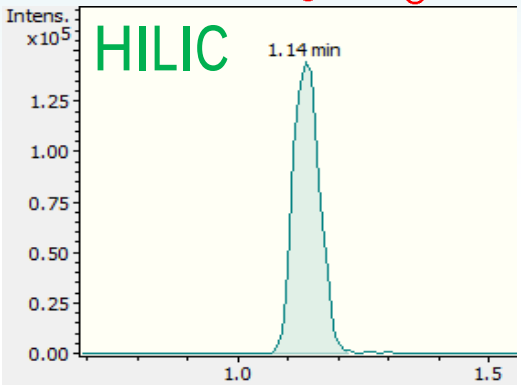
Negative

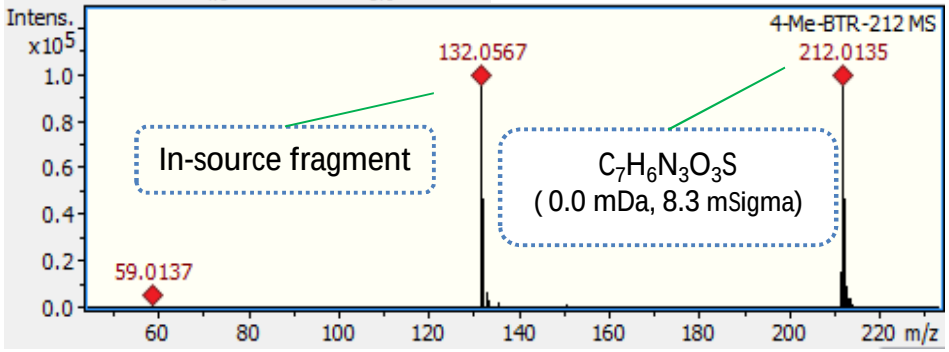


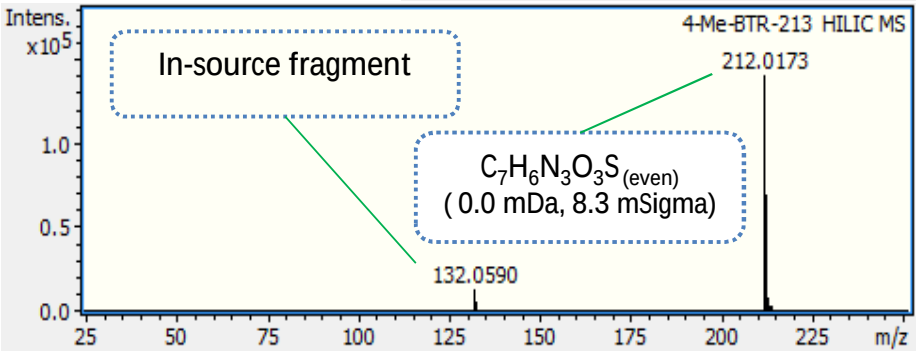


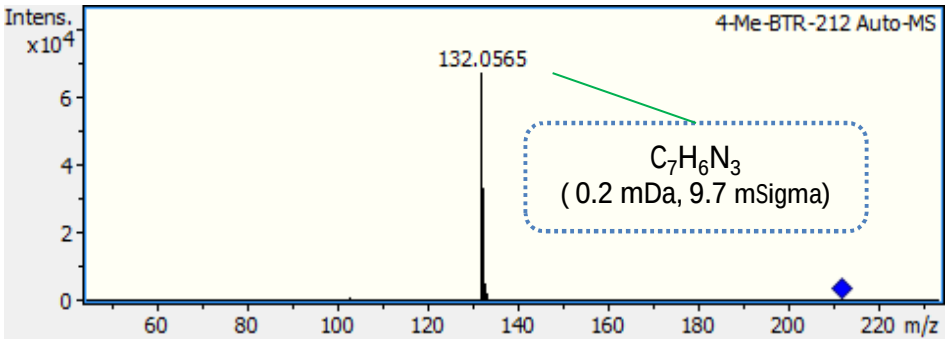
complementarity of HILIC mode

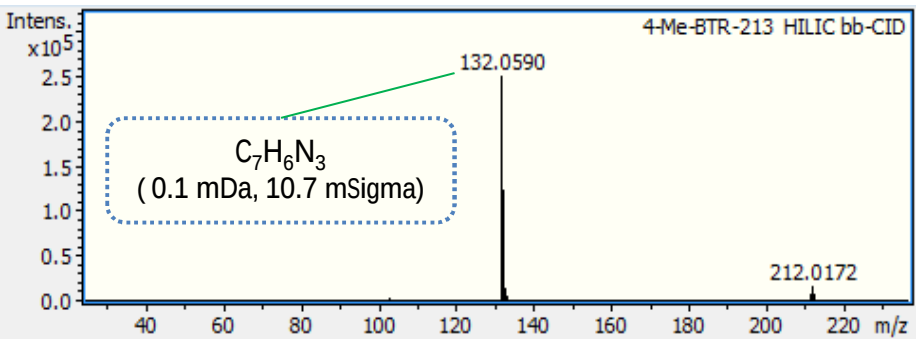
- ✓ Identical MS and MS/MS spectra





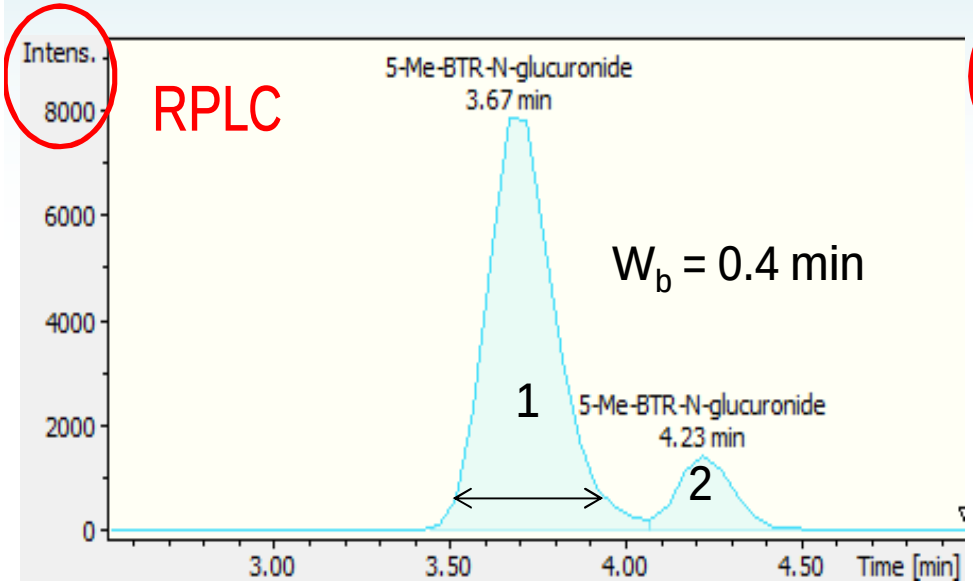


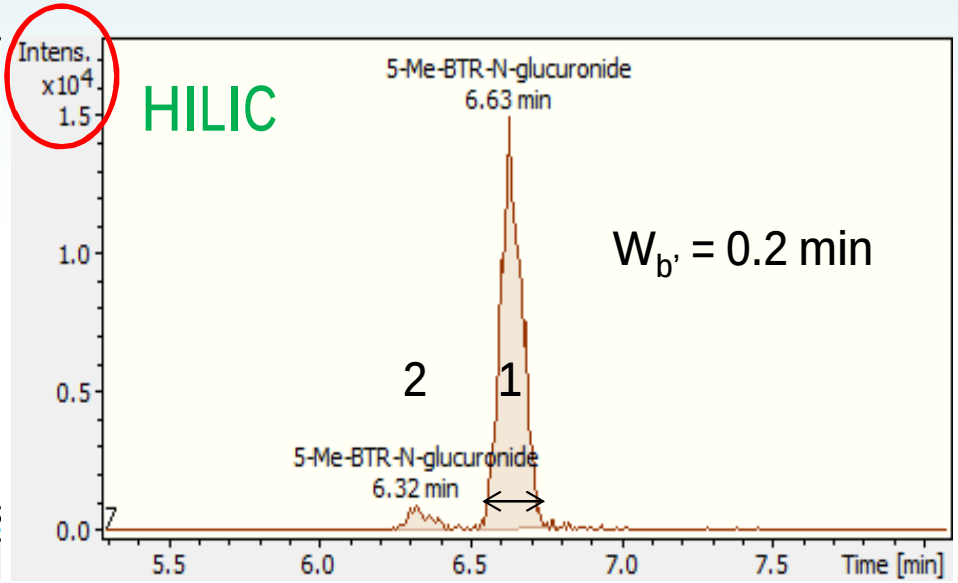




Examples (bio-TPs identification)

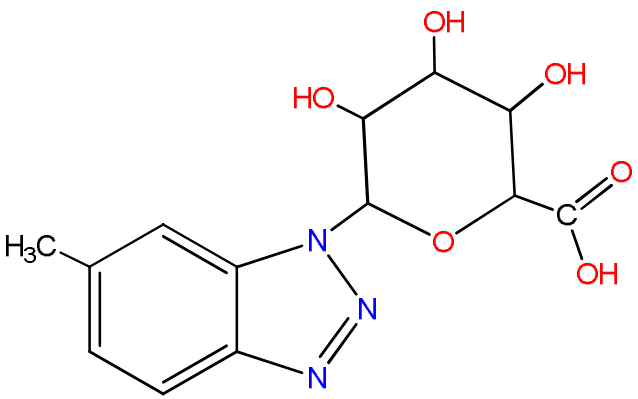
5-Me-BTR-N-glucuronide (2 isomers)

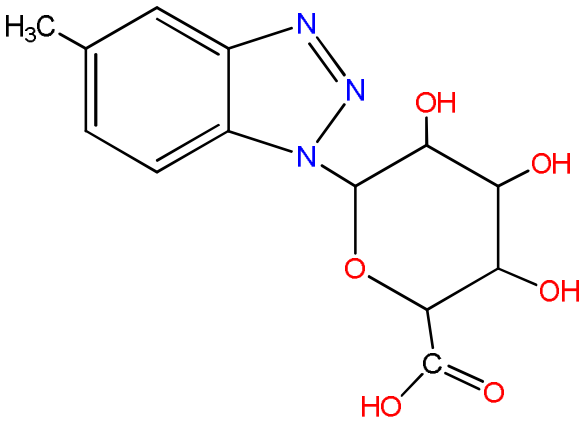




By HILIC mode

- ✓ Smaller peak width
- ✓ Greater sensitivity
- ✓ Reversed elution order

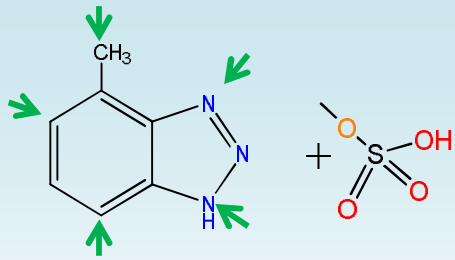




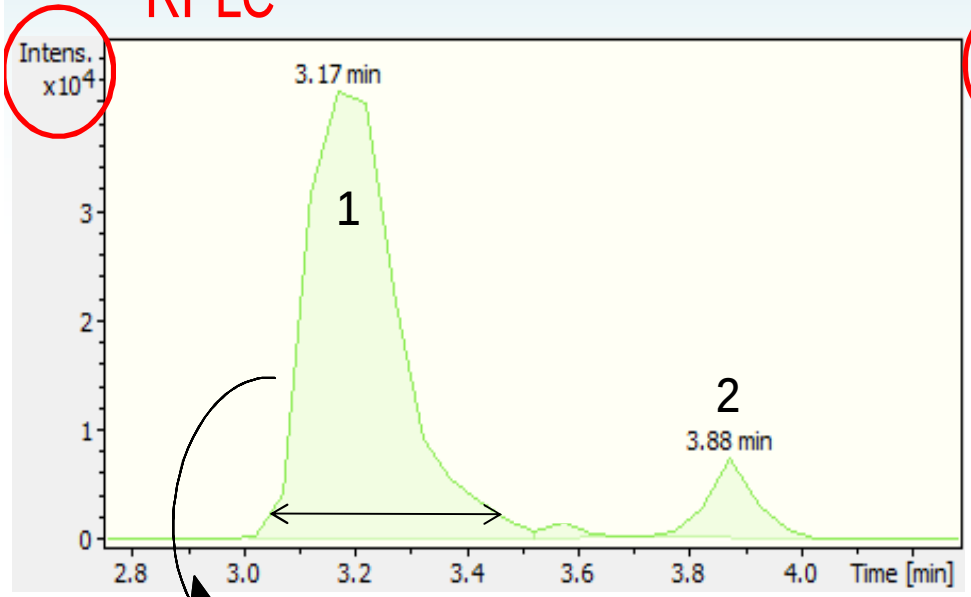
Examples (bio-TPs identification)

4-Me-BTR-O-sulfate (5 possible isomers)

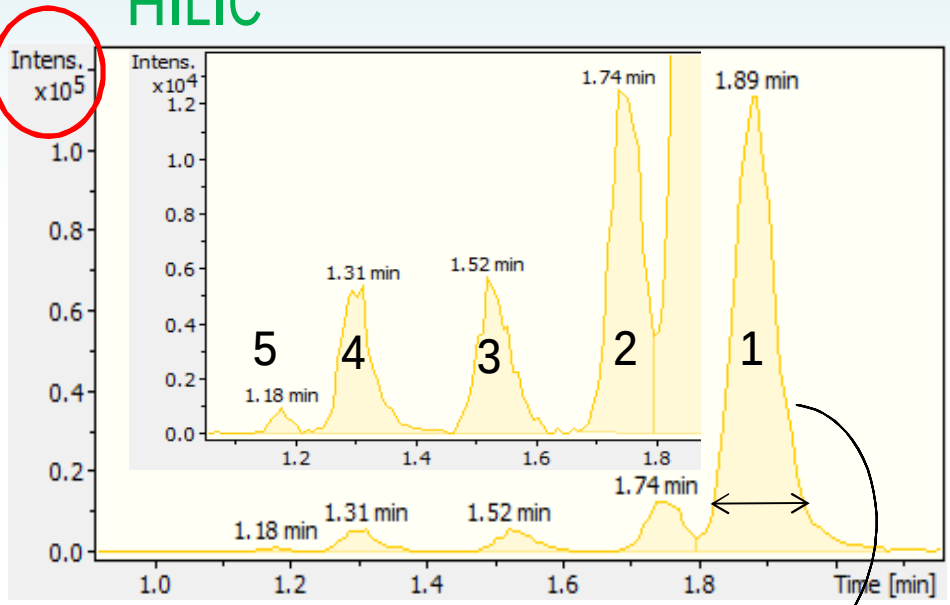
Negative

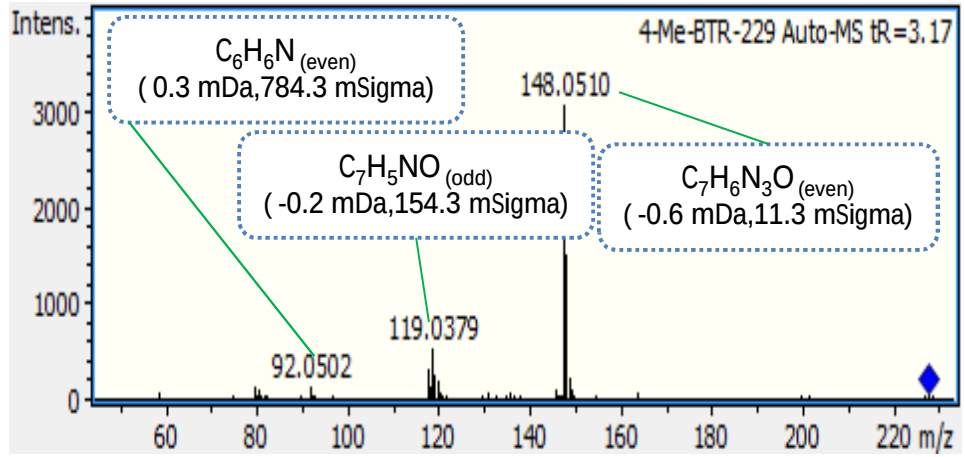


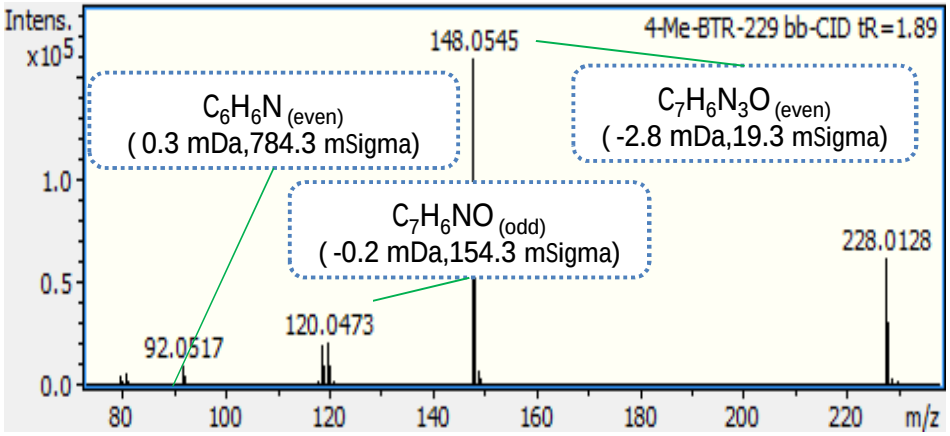
RPLC



HILIC







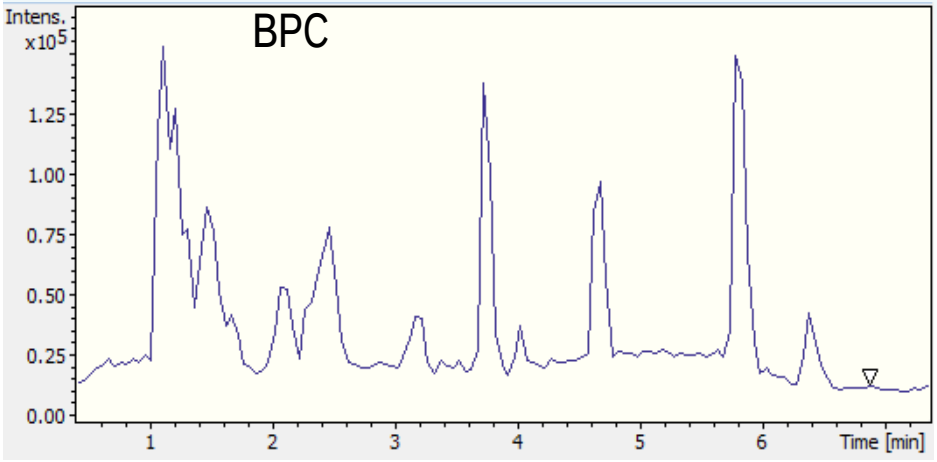
Examples (Targeted metabolic profiling)

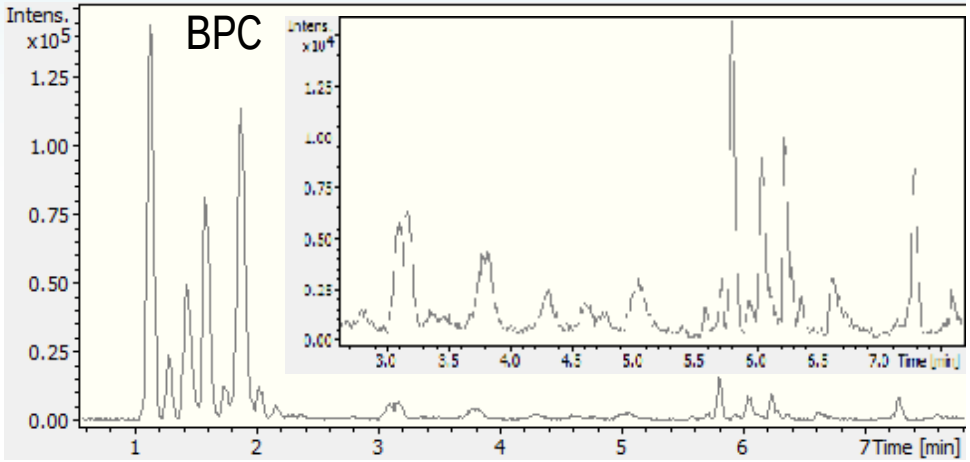
ZFE treated with 4-Me-BTR

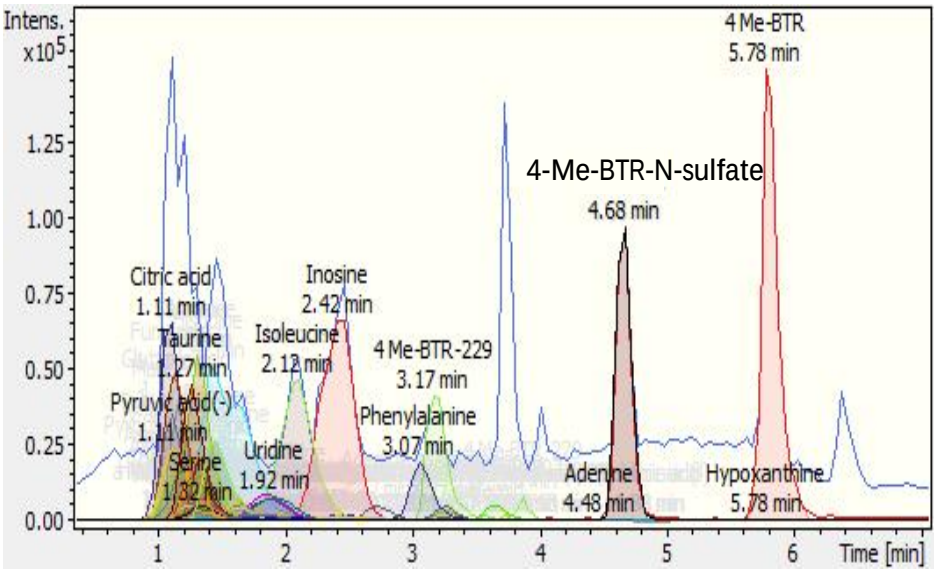
Negative

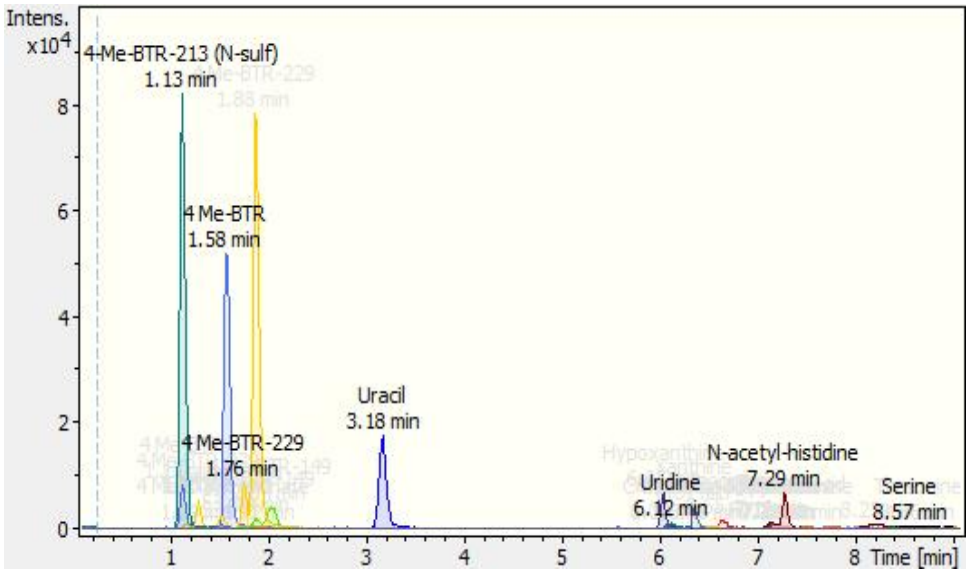
RPLC

HILIC





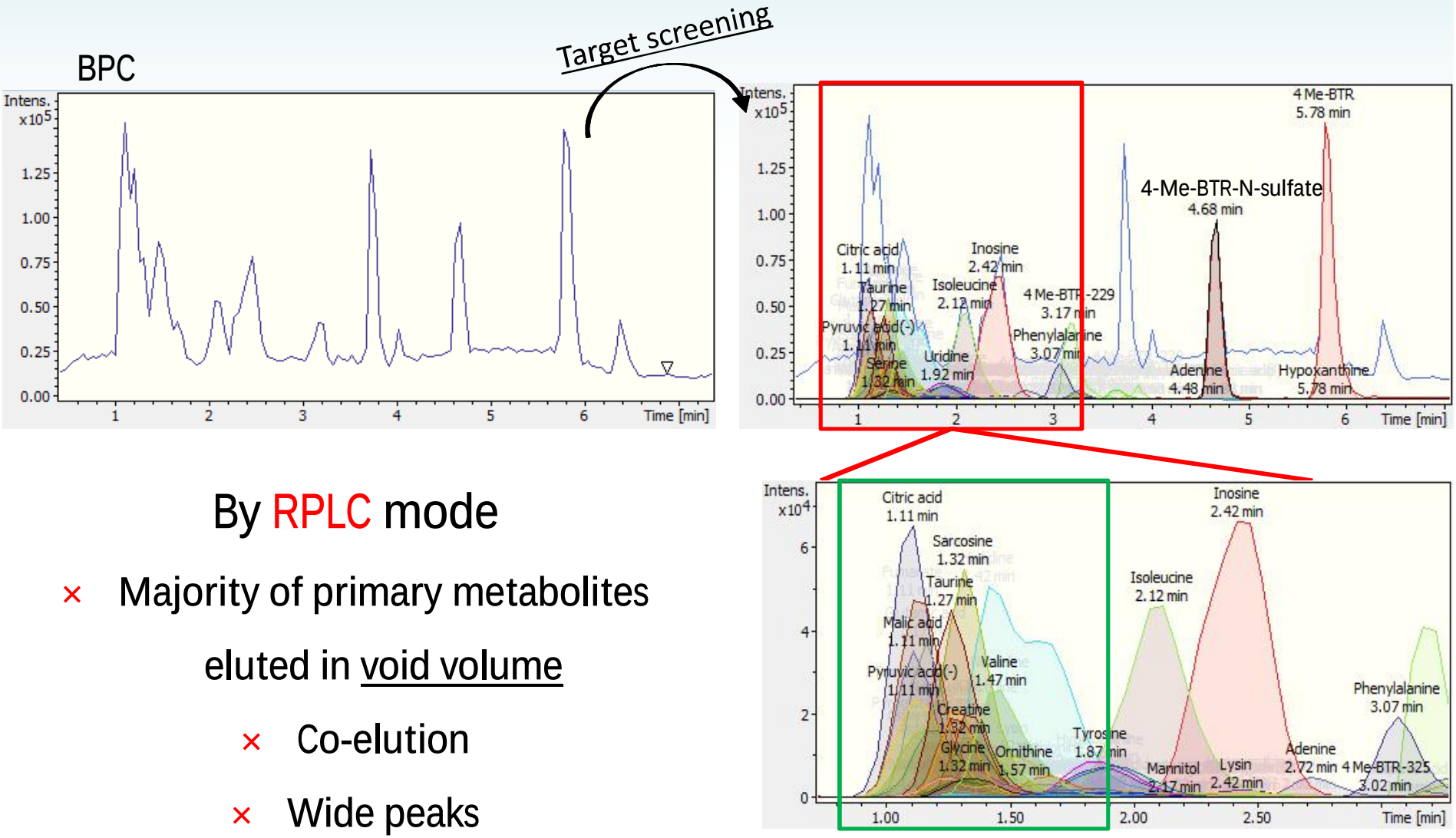




Examples (Targeted metabolic profiling)

ZFE treated with 4-Me-BTR

RPLC - Negative



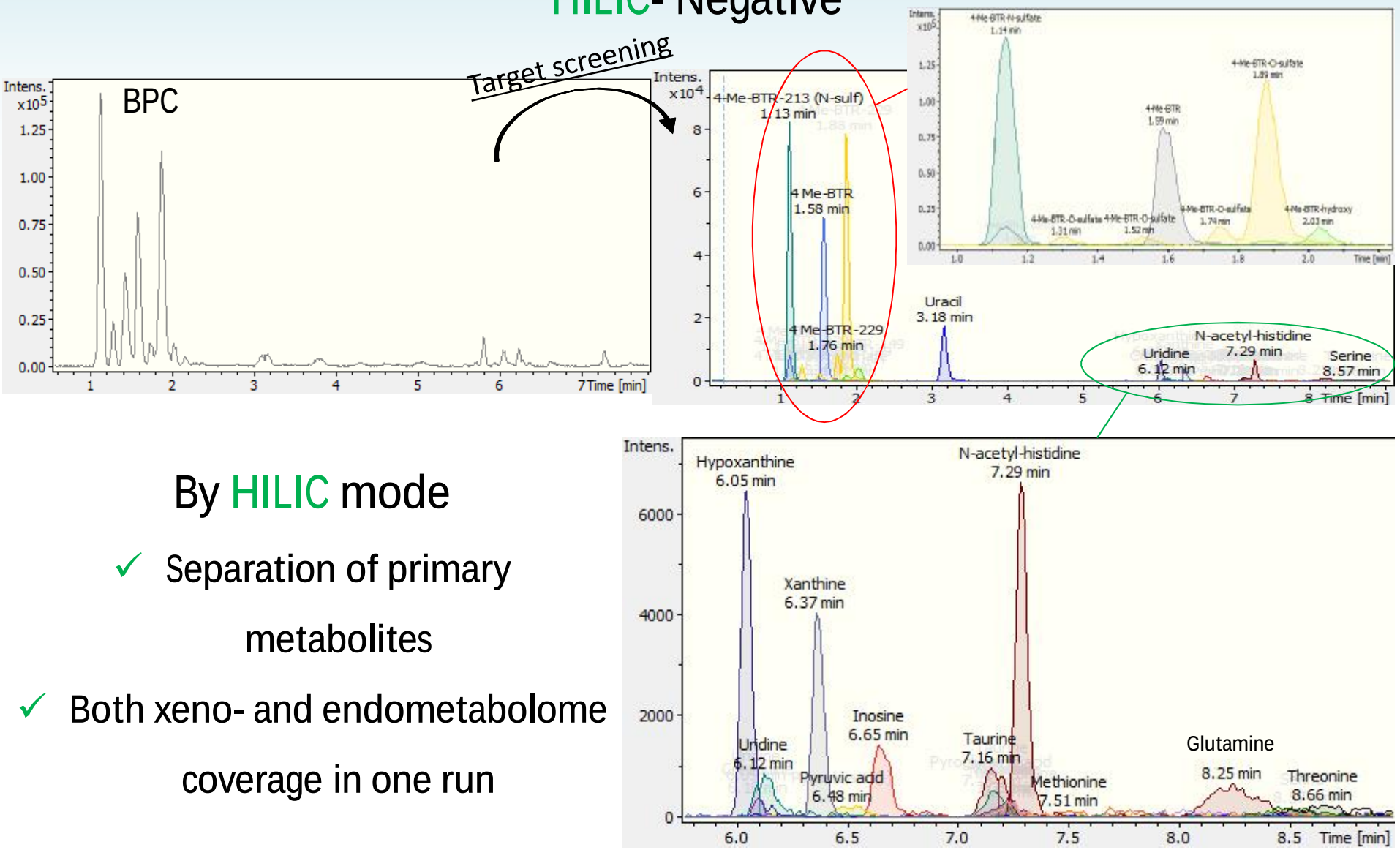
By RPLC mode

- × Majority of primary metabolites eluted in <u>void volume</u>
  - × Co-elution
  - × Wide peaks

Examples (Targeted metabolic profiling)

ZFE treated with 4-Me-BTR

HILIC- Negative



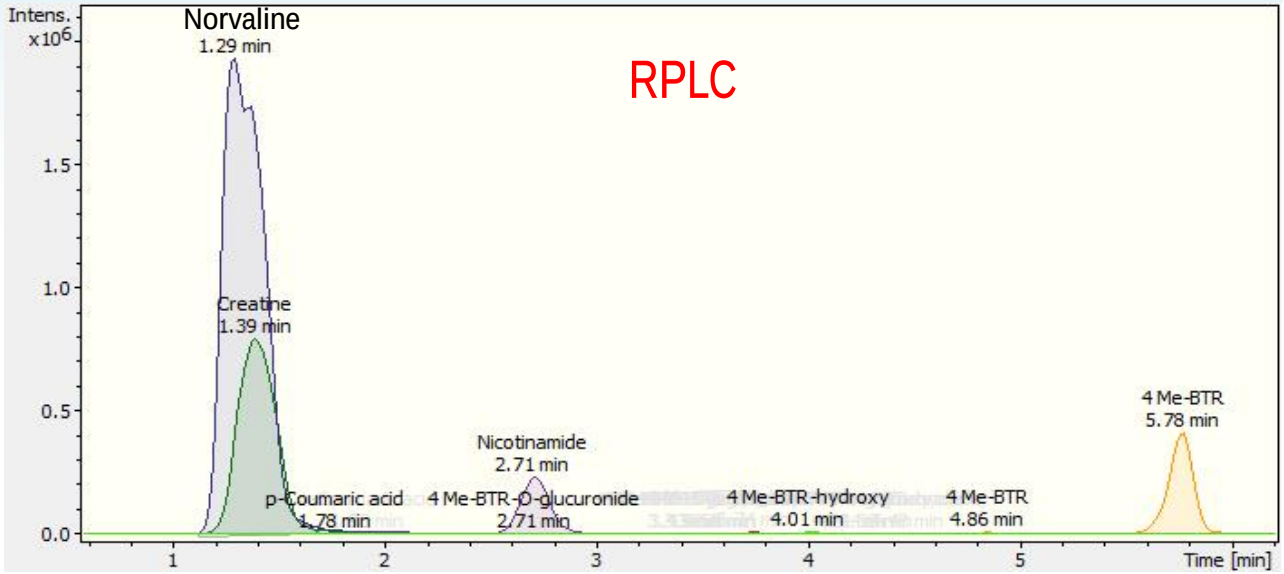
By HILIC mode

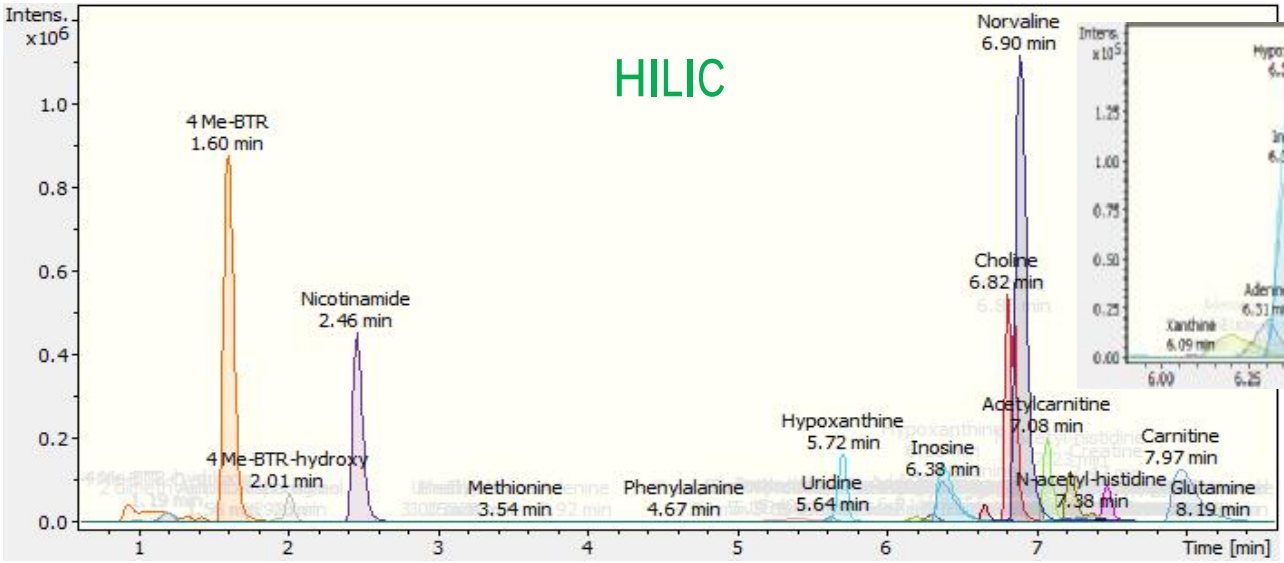
- ✓ Separation of primary metabolites
- ✓ Both xeno- and endometabolome coverage in one run

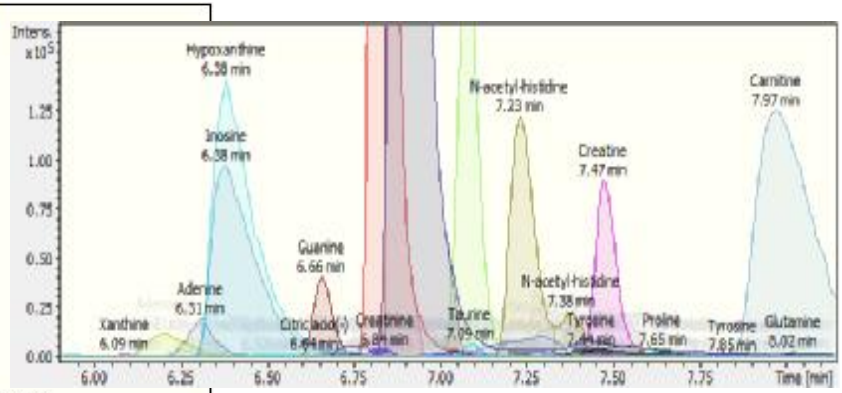
Examples (Targeted metabolic profiling)

ZFE treated with 4-Me-BTR

Positive







Conclusion

❑ Complementarity of HILIC mode to RPLC

✓ Improved separation efficiency and detection sensitivity

✓ Orthogonality to RPLC

❑ Use in suspect and non-target for additional information

❑ Adequate retention of primary metabolites

❑ Identification of both endogenous and exogenous metabolites with a single separation mode

Acknowledgments

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