Suspects screening to identify transformation products of pharmaceuticals

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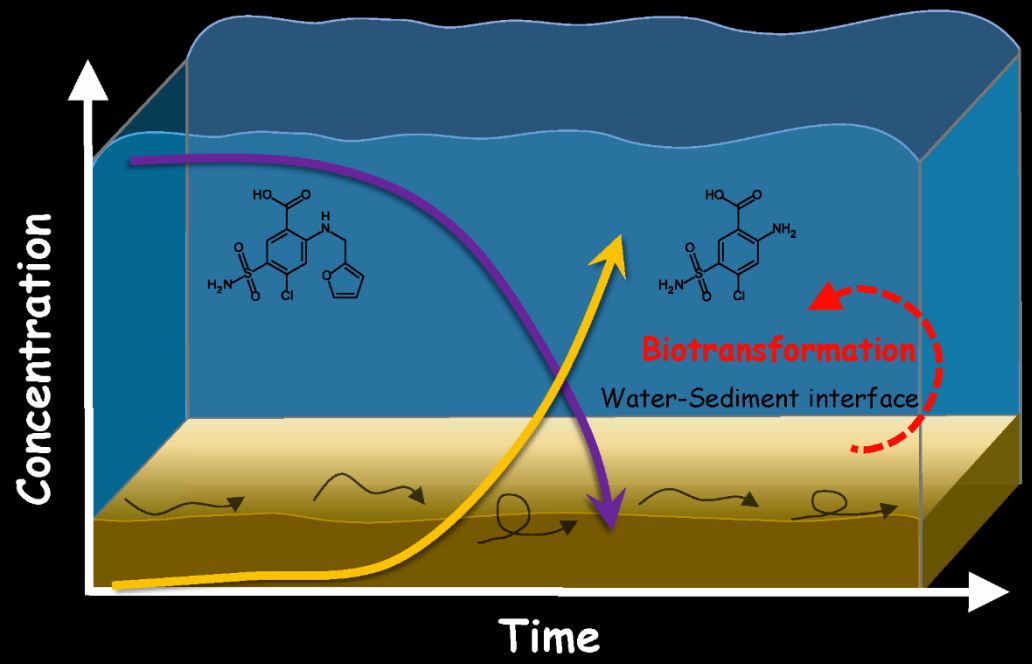


Pharmaceuticals – Emerging Pollutants

Emerging: newly identified or previously unrecognized

Characteristics:

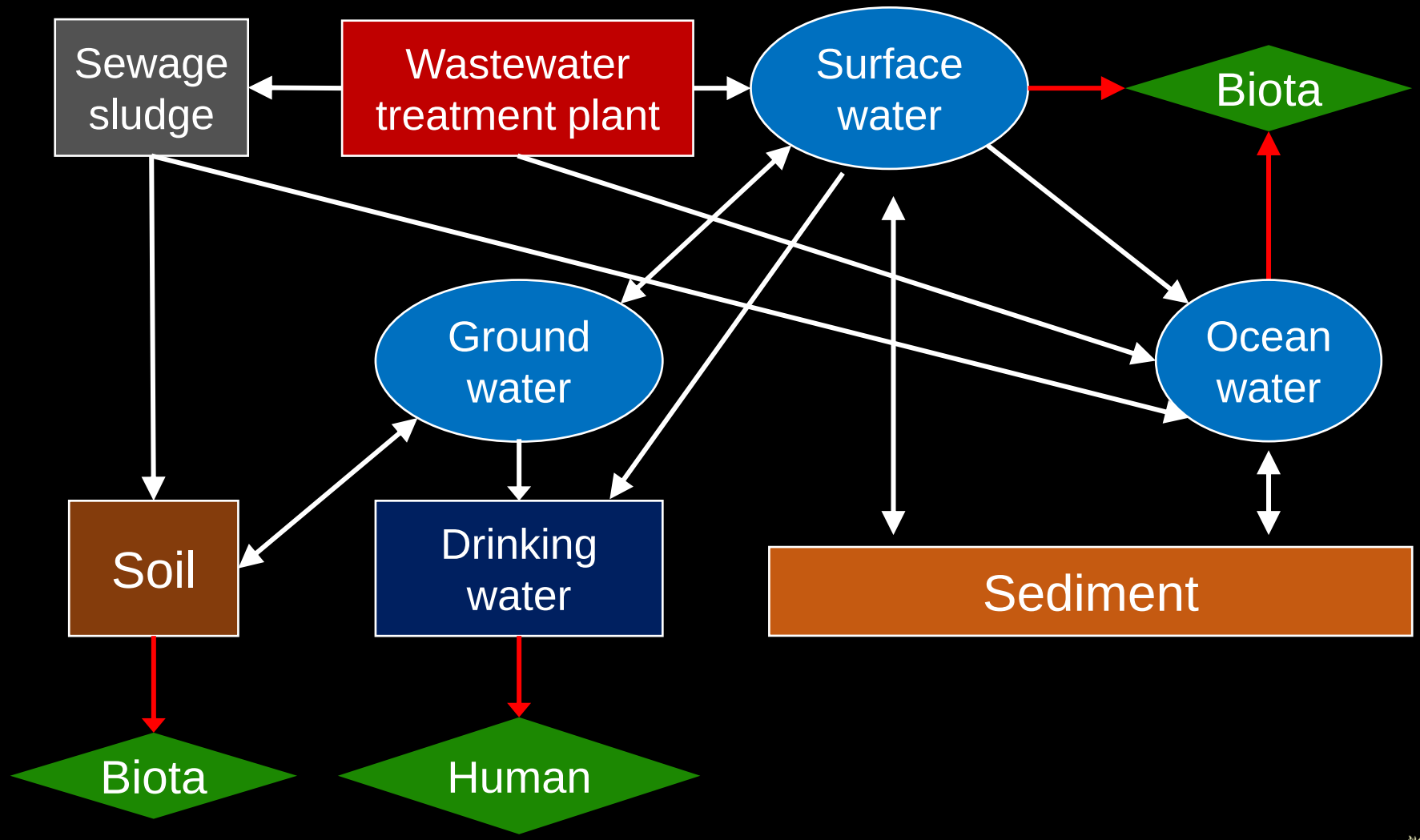
- High water solubility
- Low vapor pressure
- Stable molecules in general

Supposedly safe for the treated patient, **BUT**

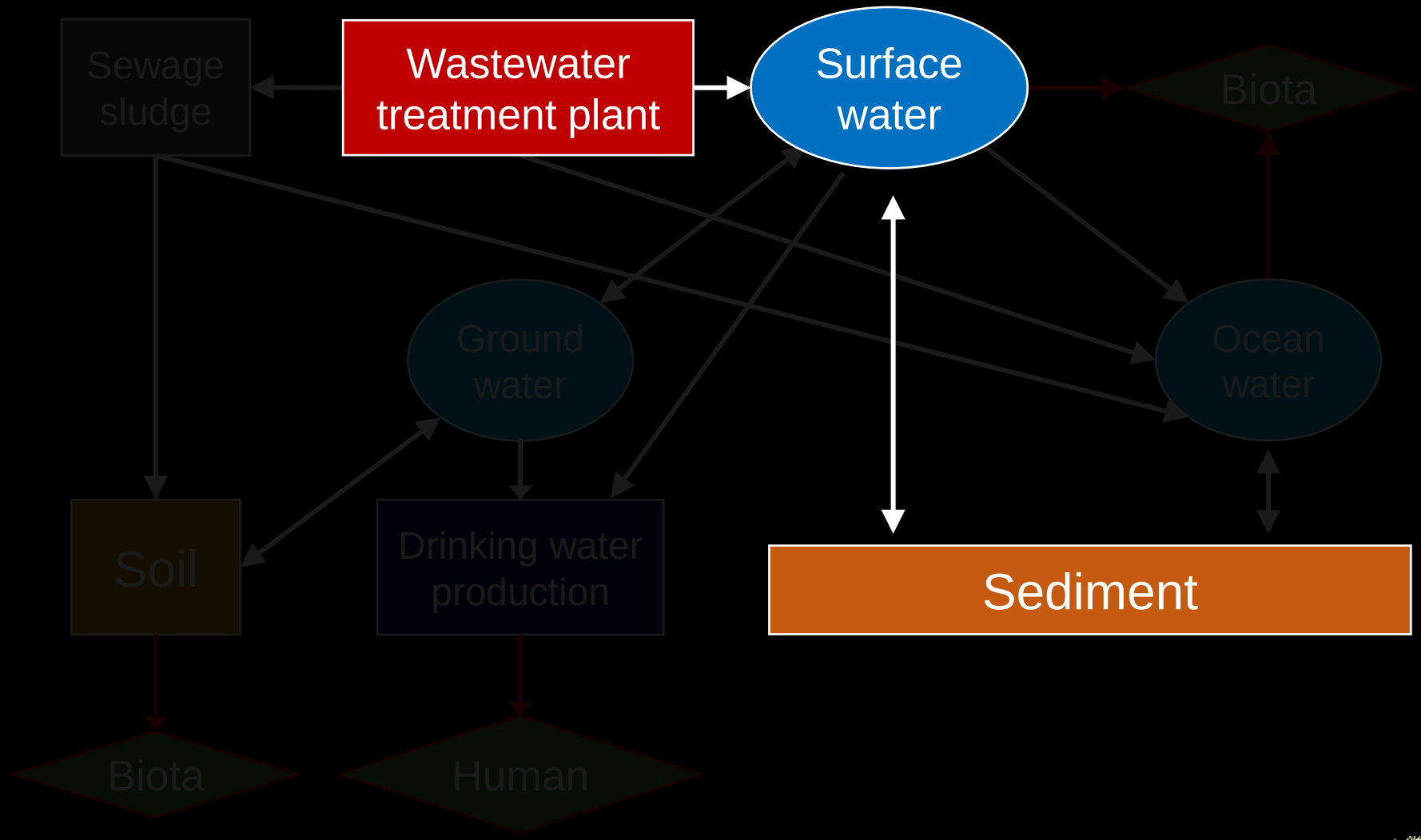
- Designed to interact with biological molecules
- Eco-toxic

Photo source: http://www.thetimes.co.uk/tto/news/world/europe/article3756095.ece

Pharmaceuticals in the Environment



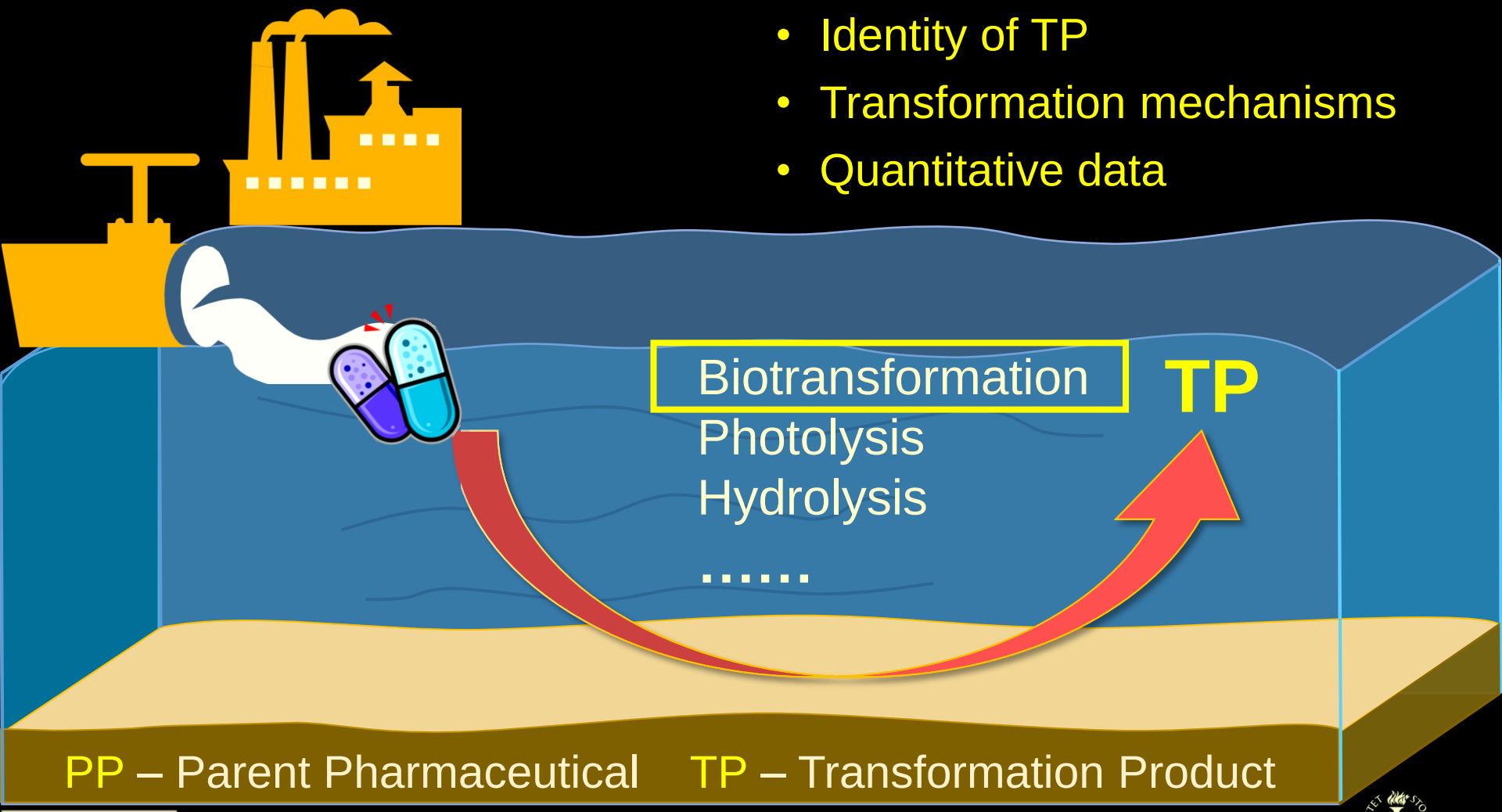
Pharmaceuticals in the Environment



Pharmaceuticals in the Environment

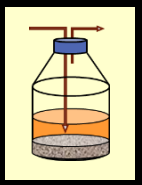
Knowledge gaps:

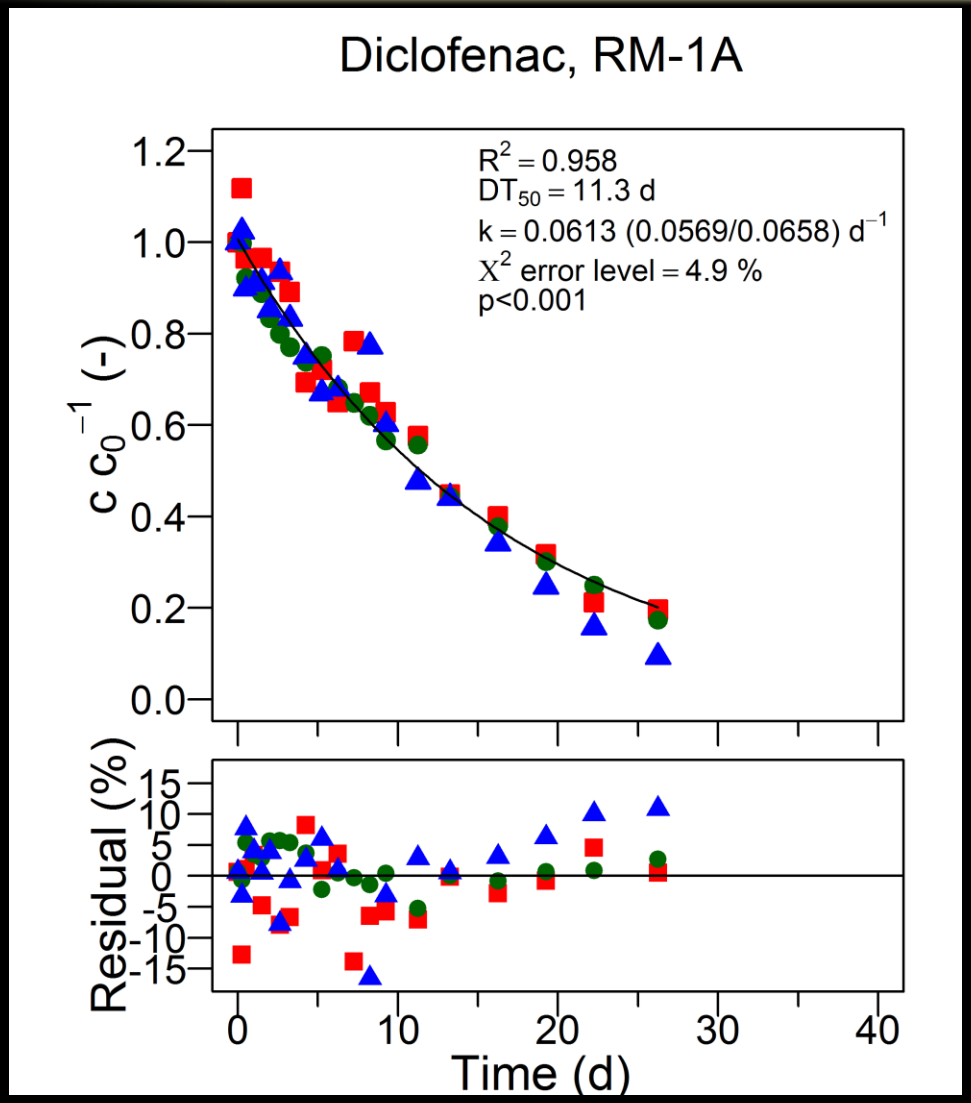
- Identity of TP
- Transformation mechanisms
- Quantitative data



PP – Parent Pharmaceutical TP – Transformation Product

Why study Transformation Products?





Laboratory:

- Dissipation =

  Σ (sorption, transformation)

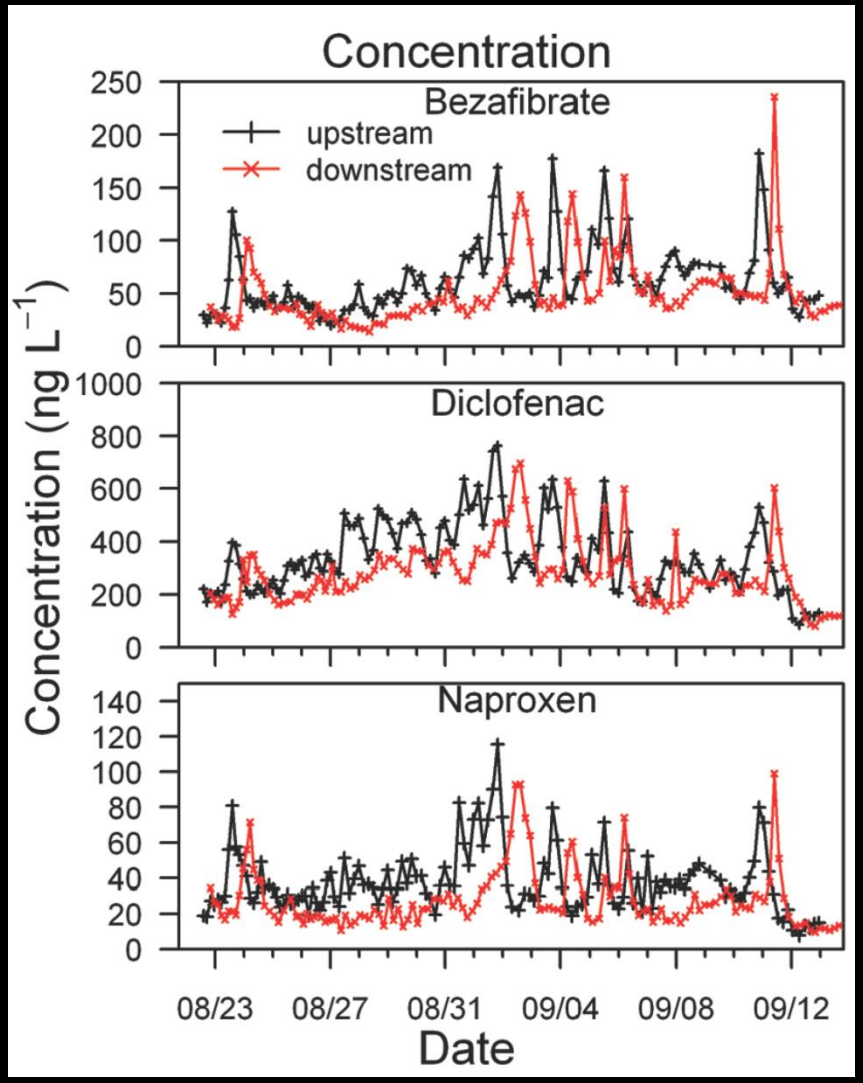
- sterile control

  $\Rightarrow$ sorption

$\Rightarrow$ process identification straightforward

Radke & Maier, Lessons learned from water/sediment-testing of pharmaceuticals, Water Res. 55, 63-73, 2014

Why study Transformation Products?



Radke et al., Environ. Sci. Technol. 44 (8), 2968-2974, 2010

Field studies:

- Dissipation =

  Σ (sorption, transformation, export)
- but: dilution, dispersion, transient storage, etc.

$\Rightarrow$ process identification complex

$\Rightarrow$ reduction of concentration or load $\neq$ transformation

Why study Transformation Products?

Transformation Products

1) as indicators of specific/ongoing environmental transformation
2) but also: as novel contaminants

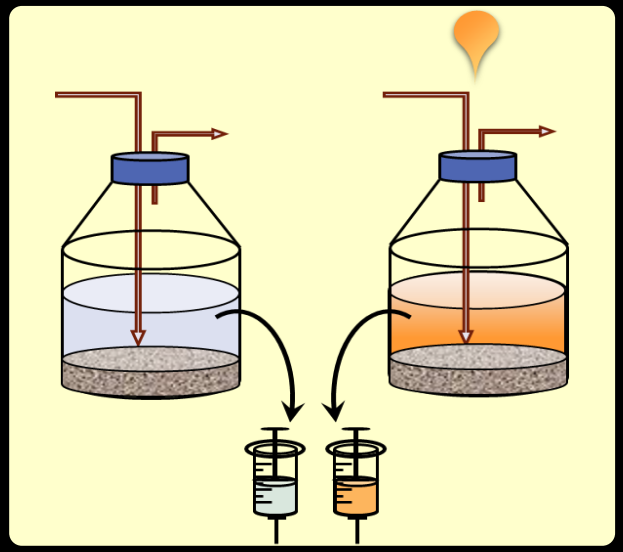
   e.g., 30% of pesticide TPs are more toxic than parent compound

Boxall et al. ., Environ. Sci. Technol. 38 (19), 368A-375A, 2004

TP Formation and Behavior

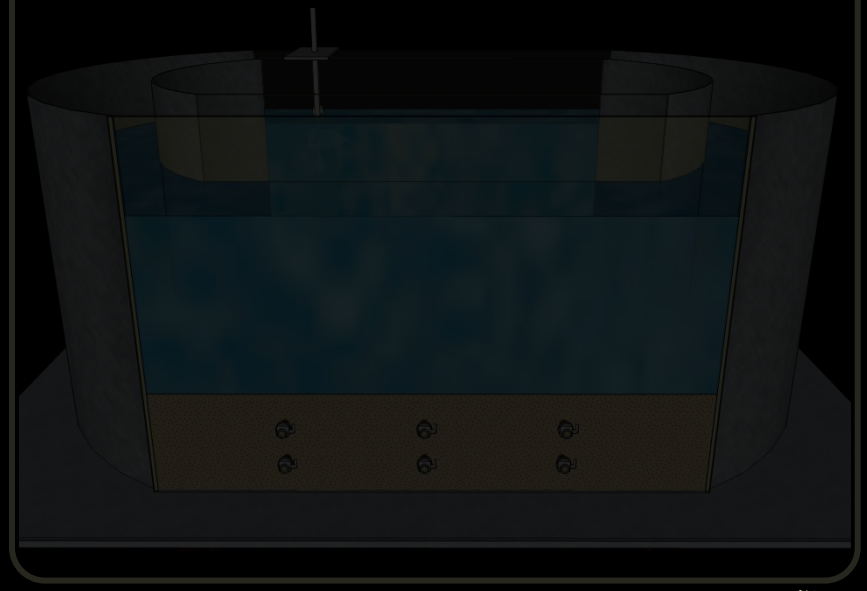
Part I

- Bottle incubation
- Data-processing
- TP identification



Part II

- Flume experiment
- Elimination of PP
- Behavior of TP

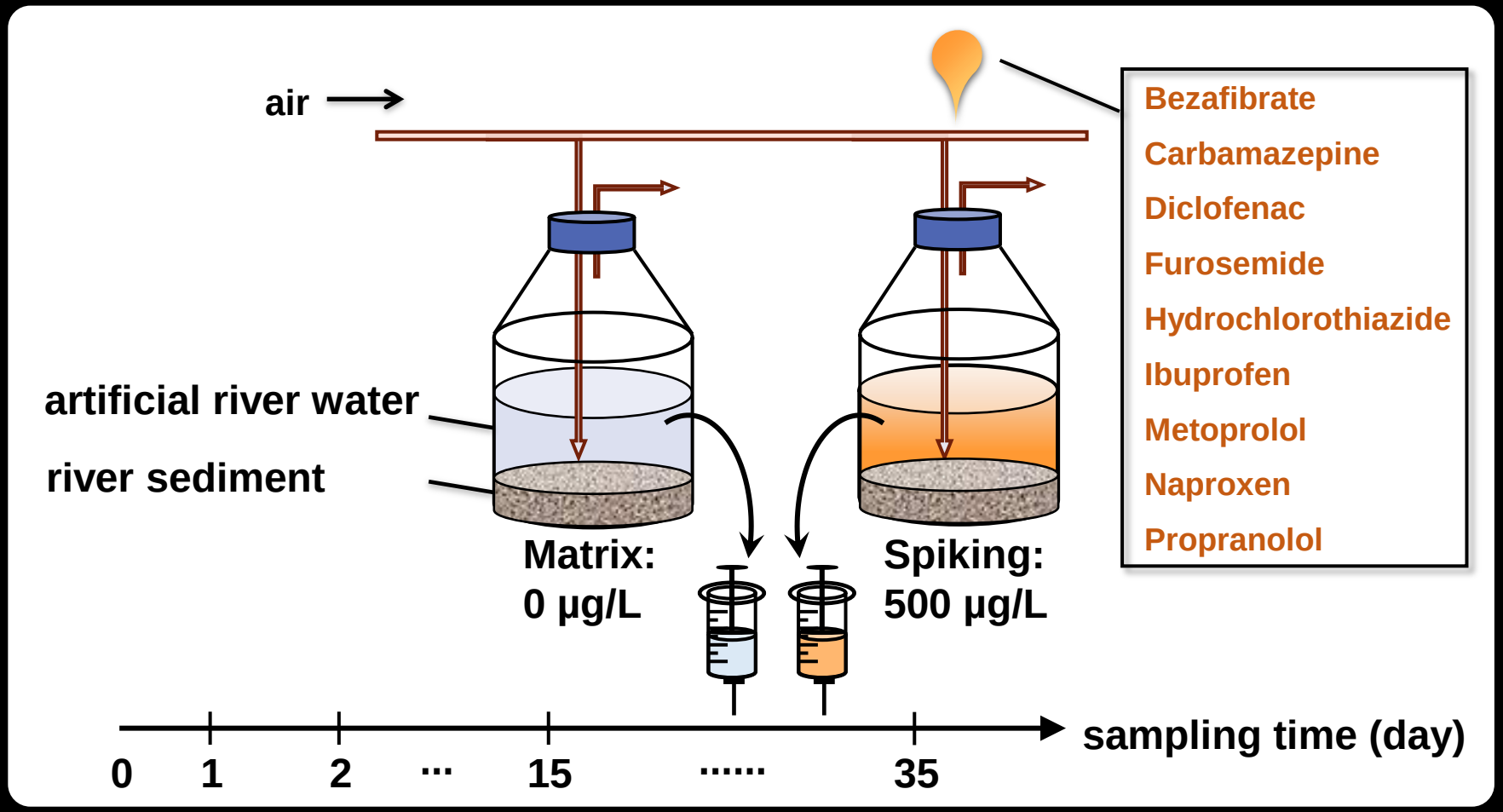


Part I – Identification

incubation

UHPLC / ESI-QToF-MS
full scan acquisition

TP identification



Part I – Identification

incubation

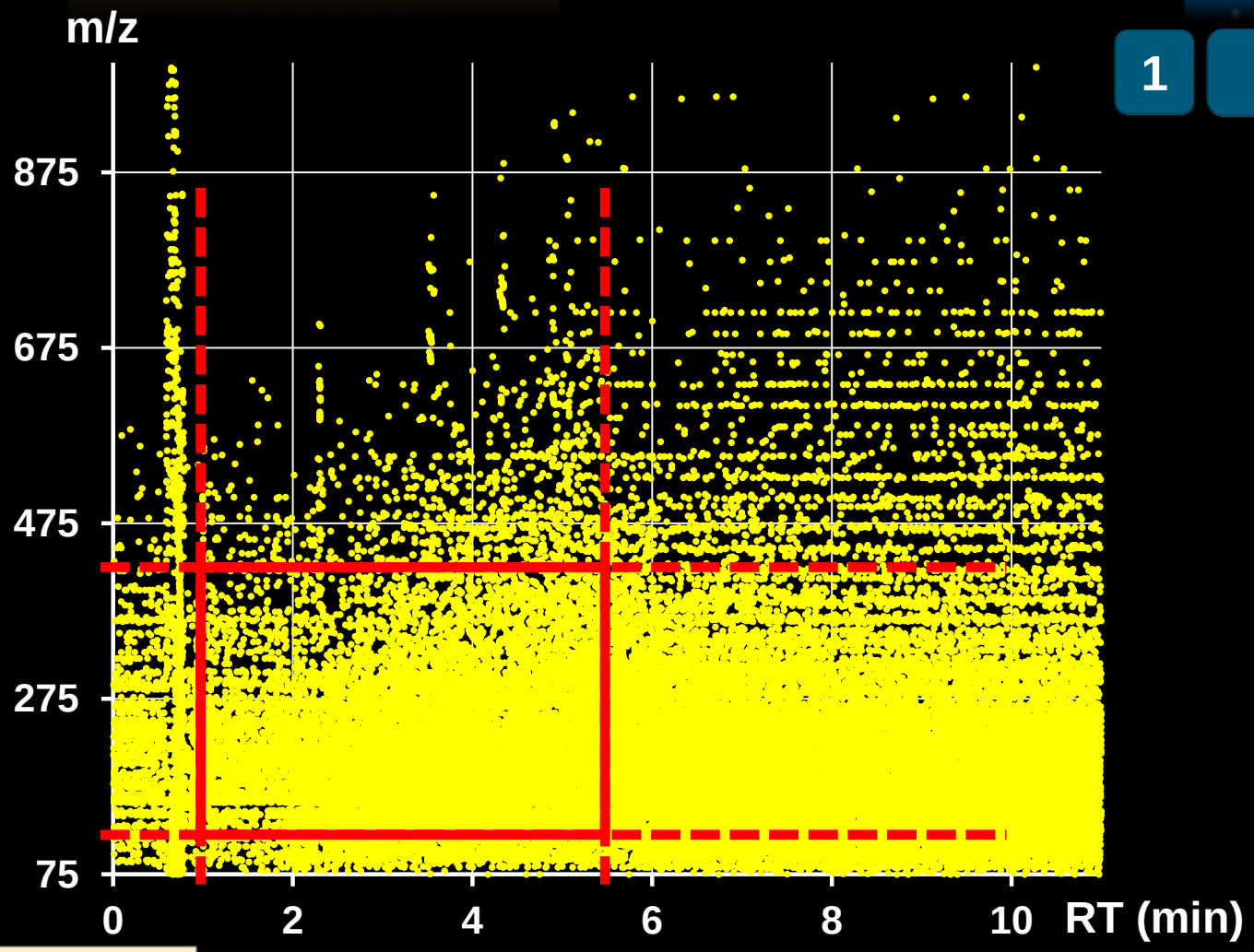
TP identification

1. Peak Detection
2. Time-Trend Filtration
3. Structure Assignment

MZmine, enviMass

Part I – Identification

incubation



TP identification

1 Peak Detection

40 000

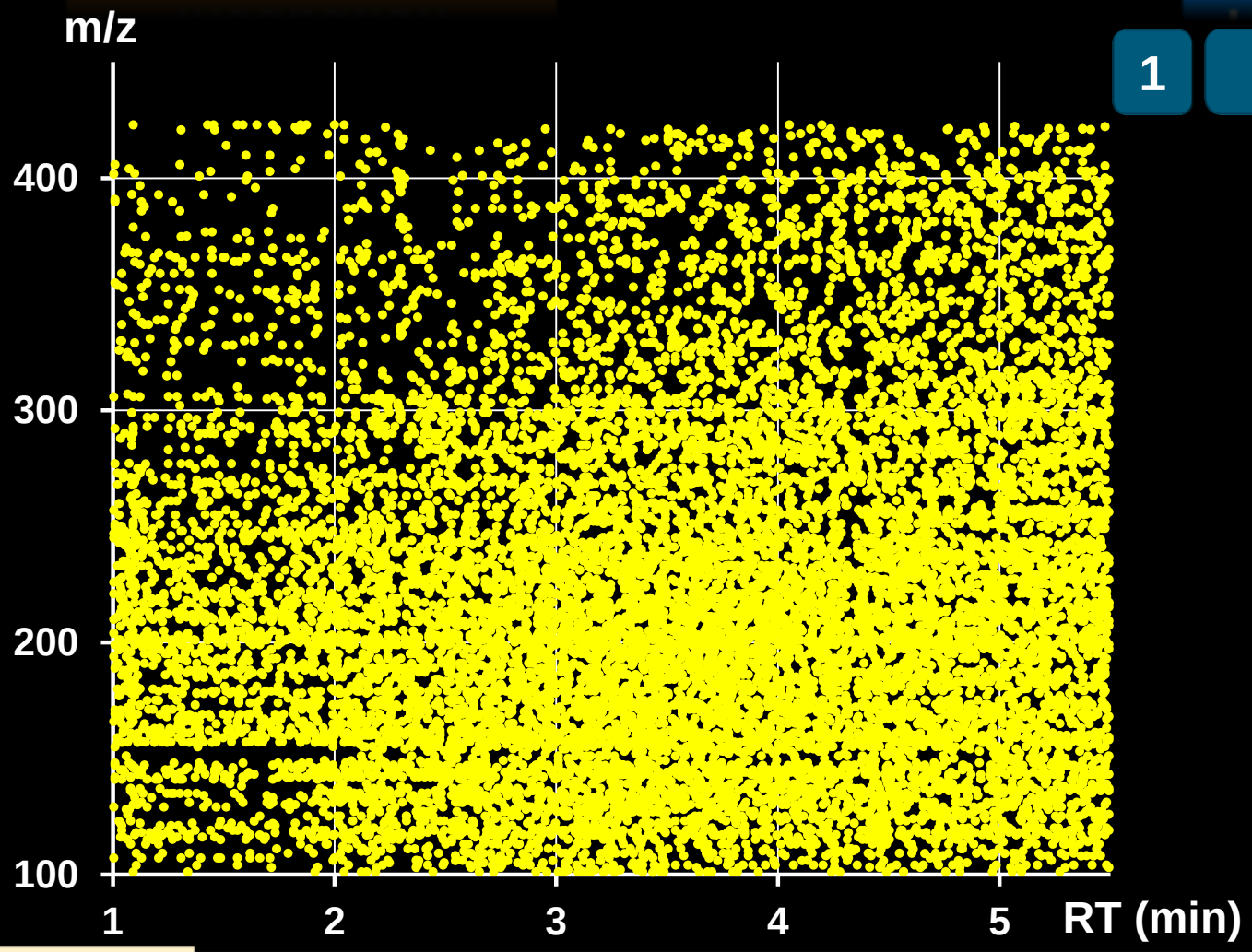
m/z, RT window

10 000

MZmine, enviMass

Part I – Identification

incubation



TP identification

1 Peak Detection

40 000

m/z, RT window

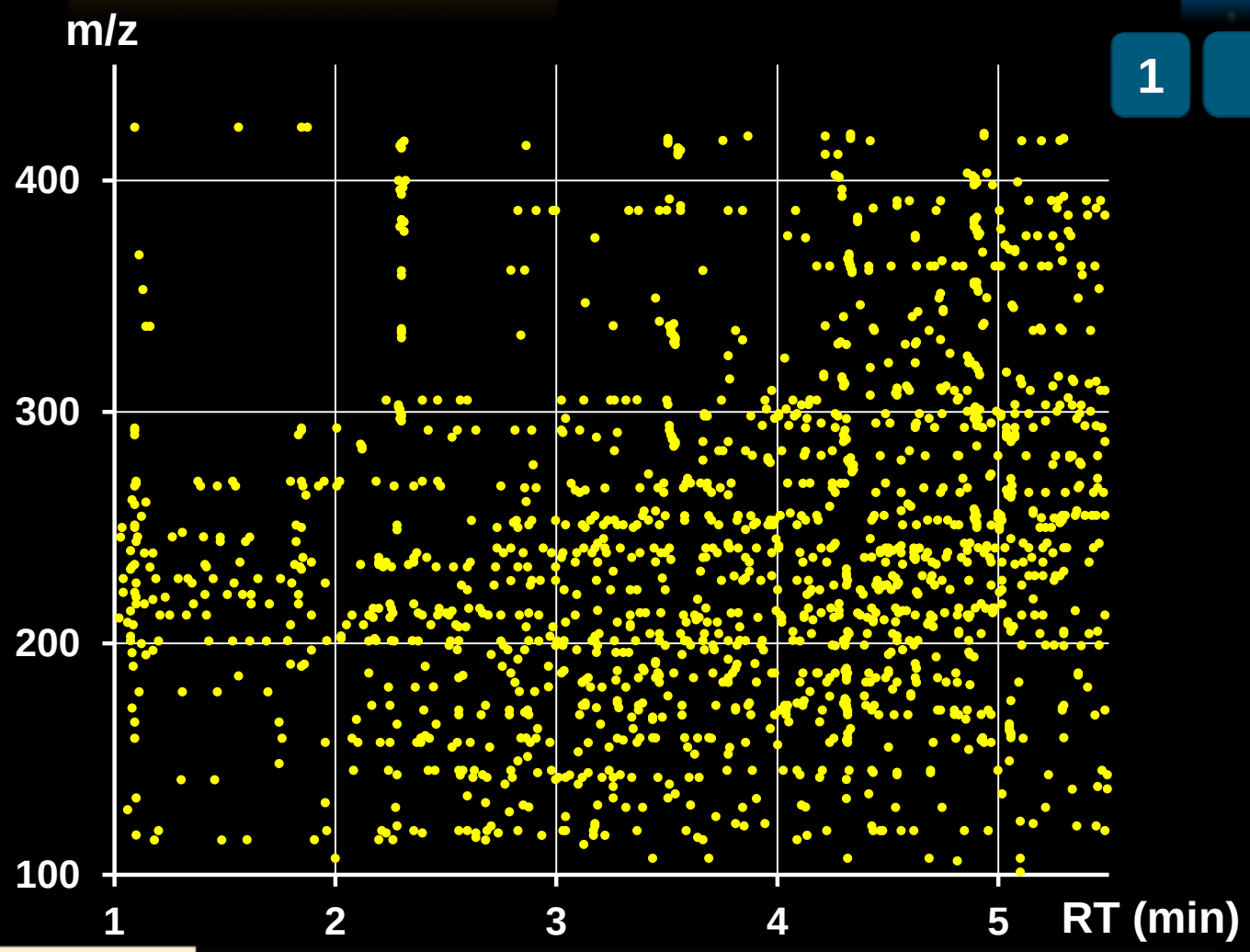
10 000

remove noise

MZmine, enviMass

Part I – Identification

incubation



TP identification

1 Peak Detection

40 000

m/z, RT window

10 000

remove noise

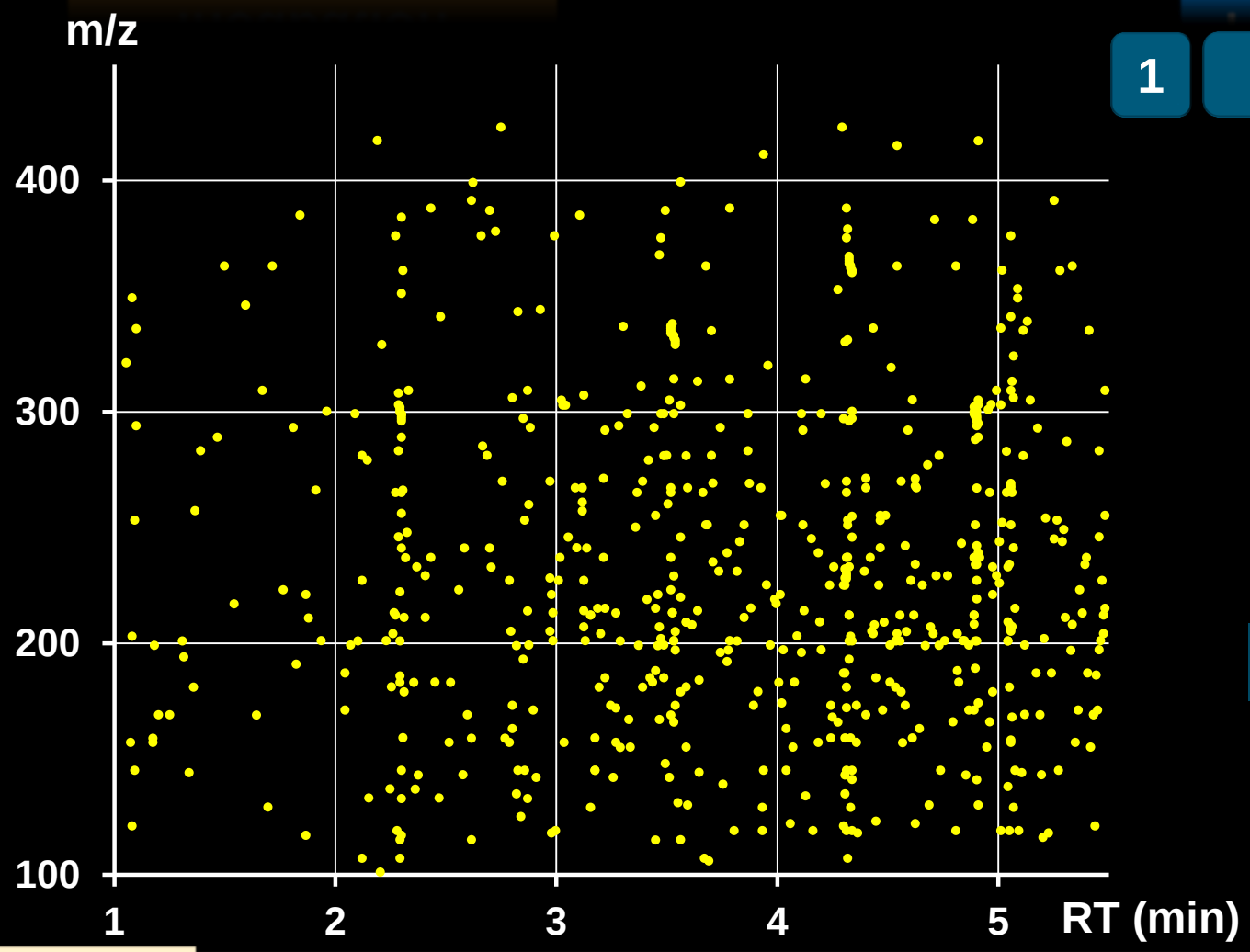
2 000

remove matrix

MZmine, enviMass

Part I – Identification

incubation



TP identification

1 Peak Detection

40 000

m/z, RT window

10 000

remove noise

2 000

remove matrix

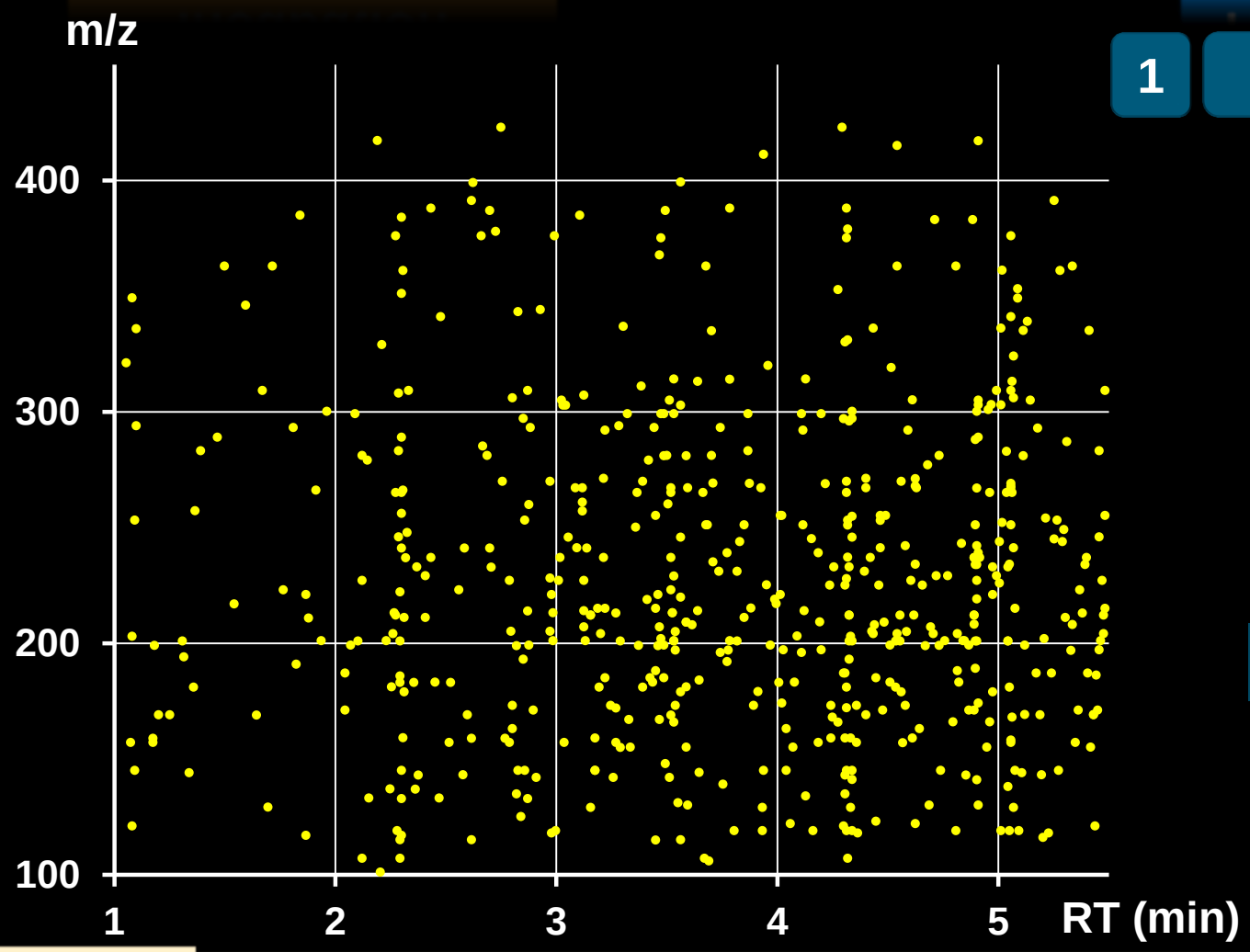
1 400

remove targets

MZmine, enviMass

Part I – Identification

incubation



TP identification

1 Peak Detection

40 000

m/z, RT window

10 000

remove noise

2 000

remove matrix

1 400

remove targets

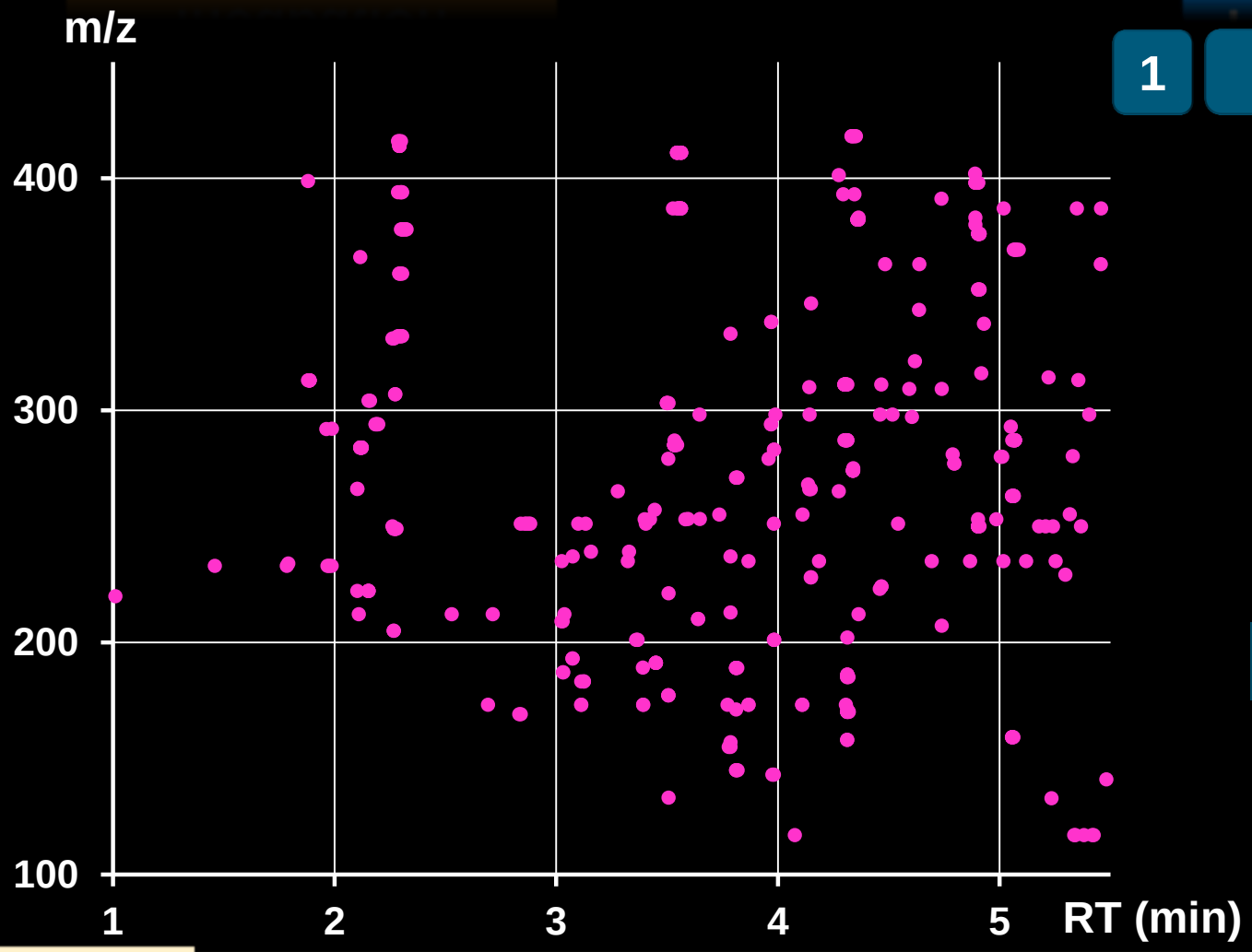
1 300

isotopic peaks

MZmine, enviMass

Part I – Identification

incubation



TP identification

1	Peak Detection
	40 000
	m/z, RT window
	10 000
	remove noise
	2 000
	remove matrix
	1 400
	remove targets
	1 300
	isotopic peaks
	300

MZmine, enviMass

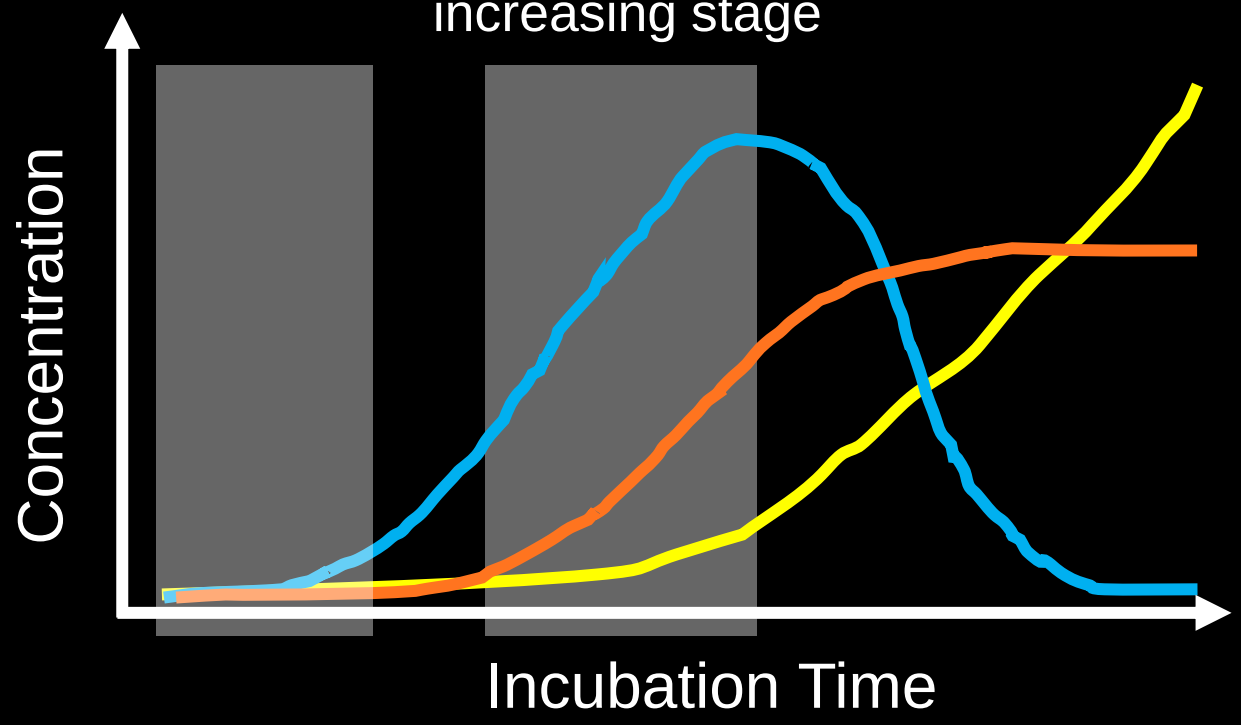
Part I – Identification

incubation

meaningful!

absent stage

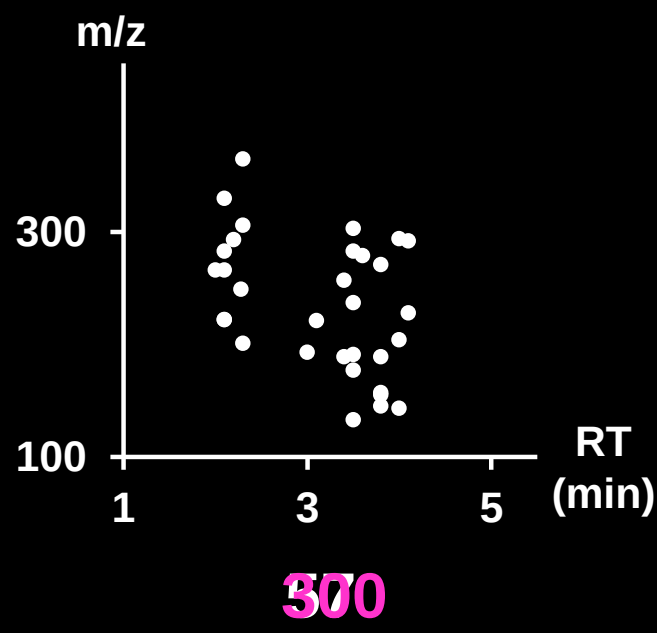
increasing stage



TP identification

1 Peak Detection

2 Time-Trend Filtration



Part I – Identification

incubation

TP identification

1. Peak Detection
2. Time-Trend Filtration
3. Structure Assignment

- Compare m/z with predictions

University of Minnesota
Pathway Prediction System

+

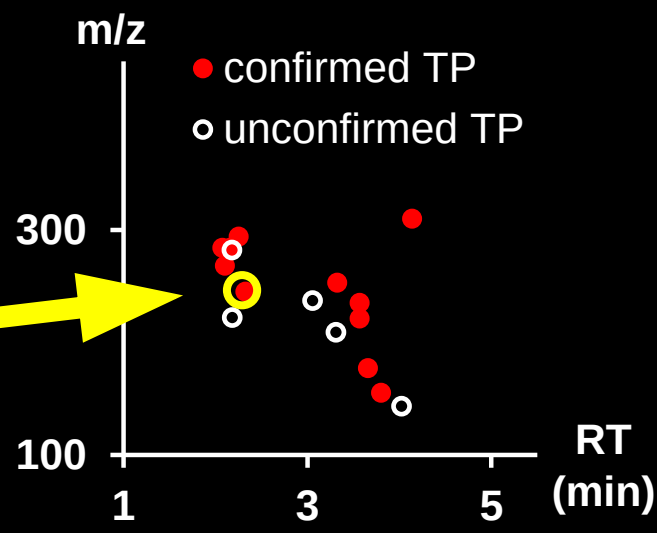
Literature

- Reference standards!

16

Confirmed: 11

Unconfirmed: 5



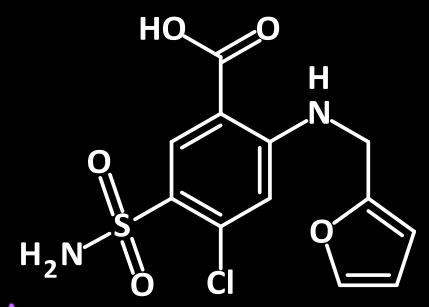
"left-overs": 6

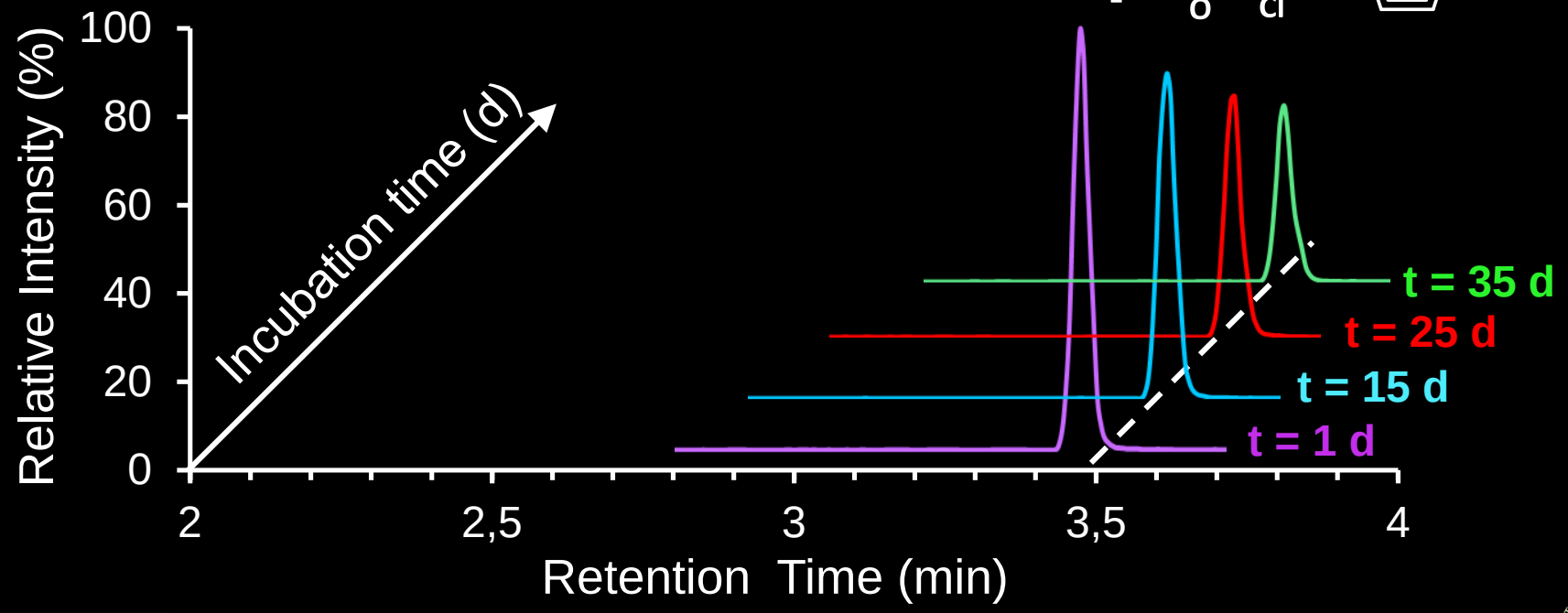
Part I – Identification

incubation

TP identification

PP: furosemide



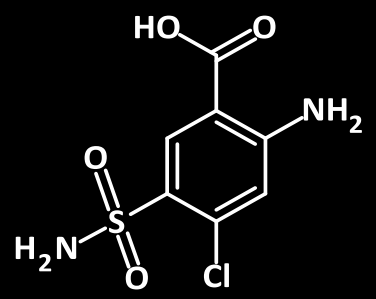


Part I – Identification

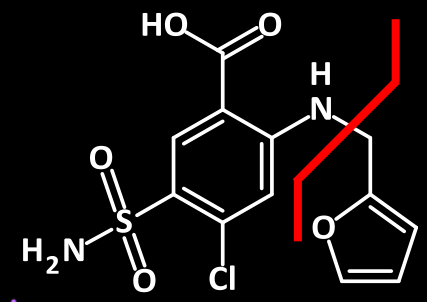
incubation

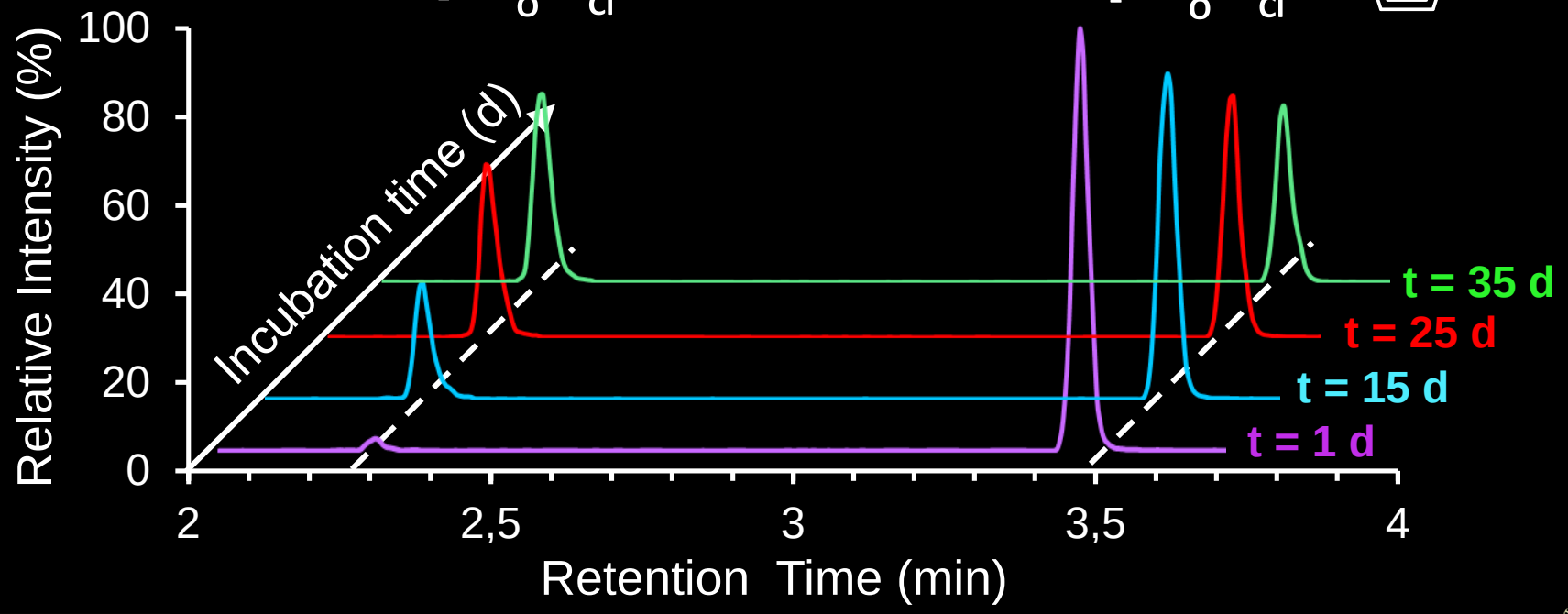
TP identification

TP: saluamine



PP: furosemide

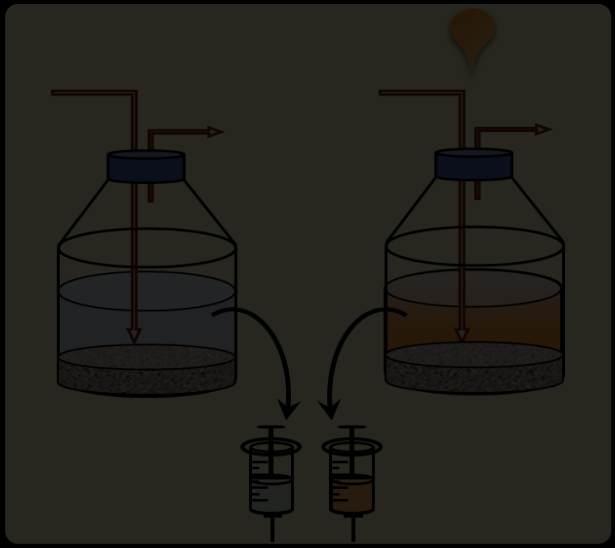




TP Formation and Behavior

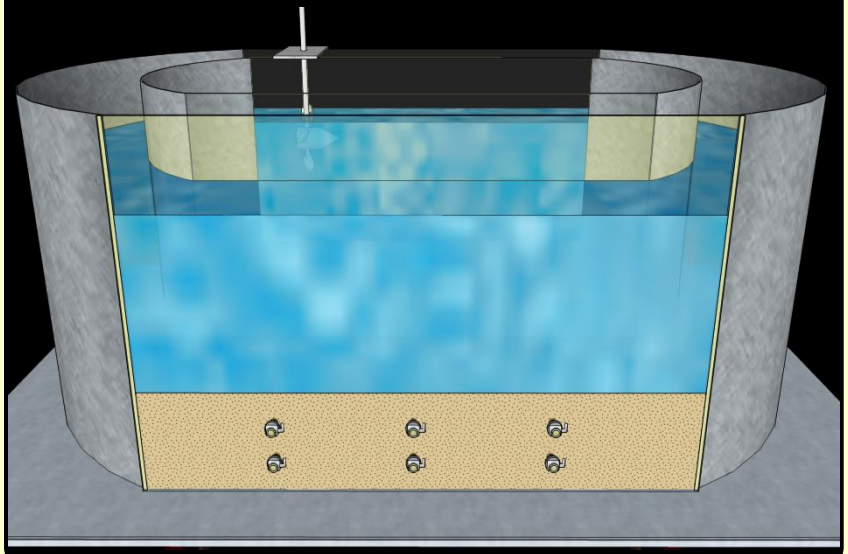
Study I

- Bottle incubation
- Data-processing
- TP identification



Part II

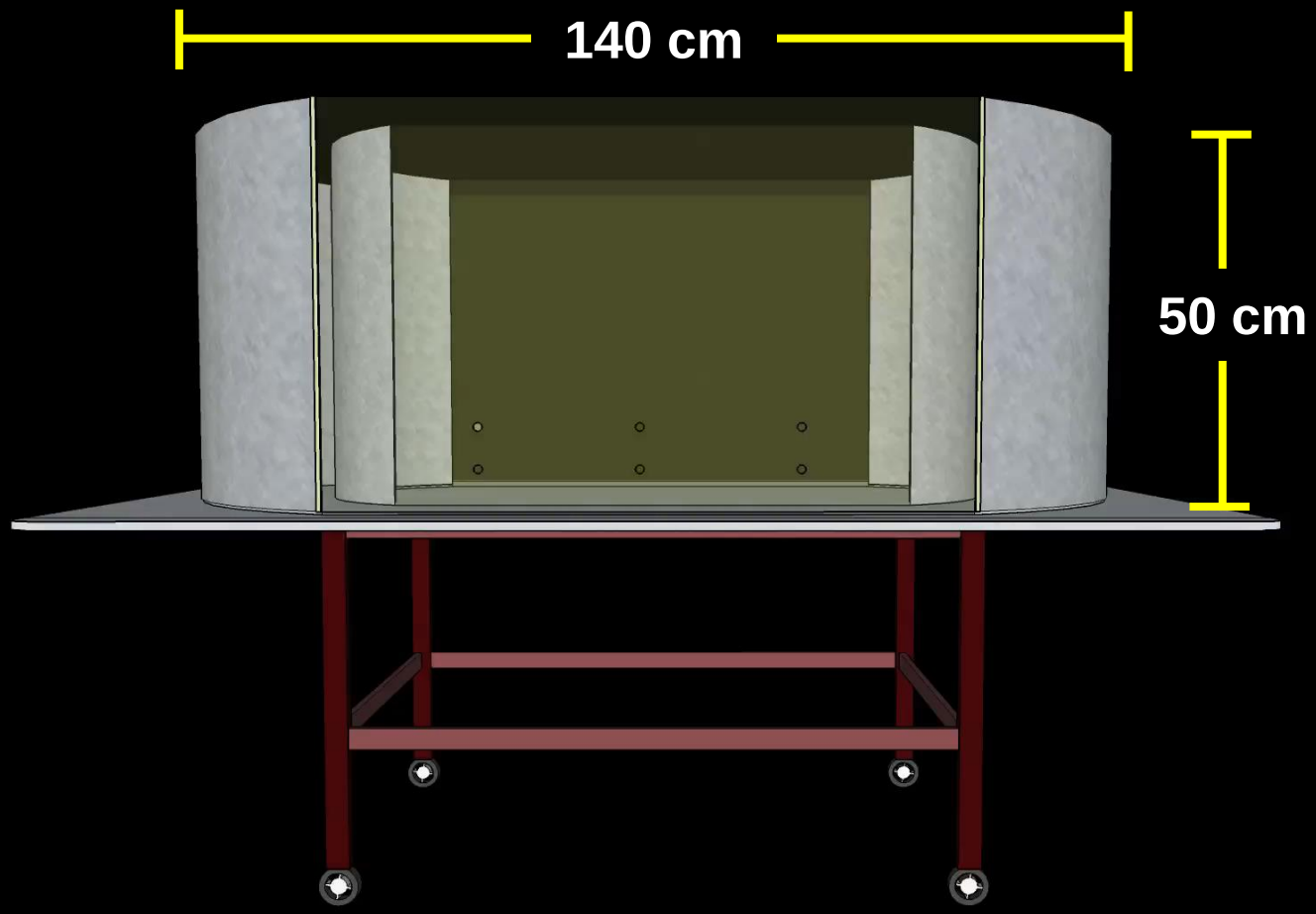
- Flume experiment
- Elimination of PP
- Behavior of TP



Part II – Behavior

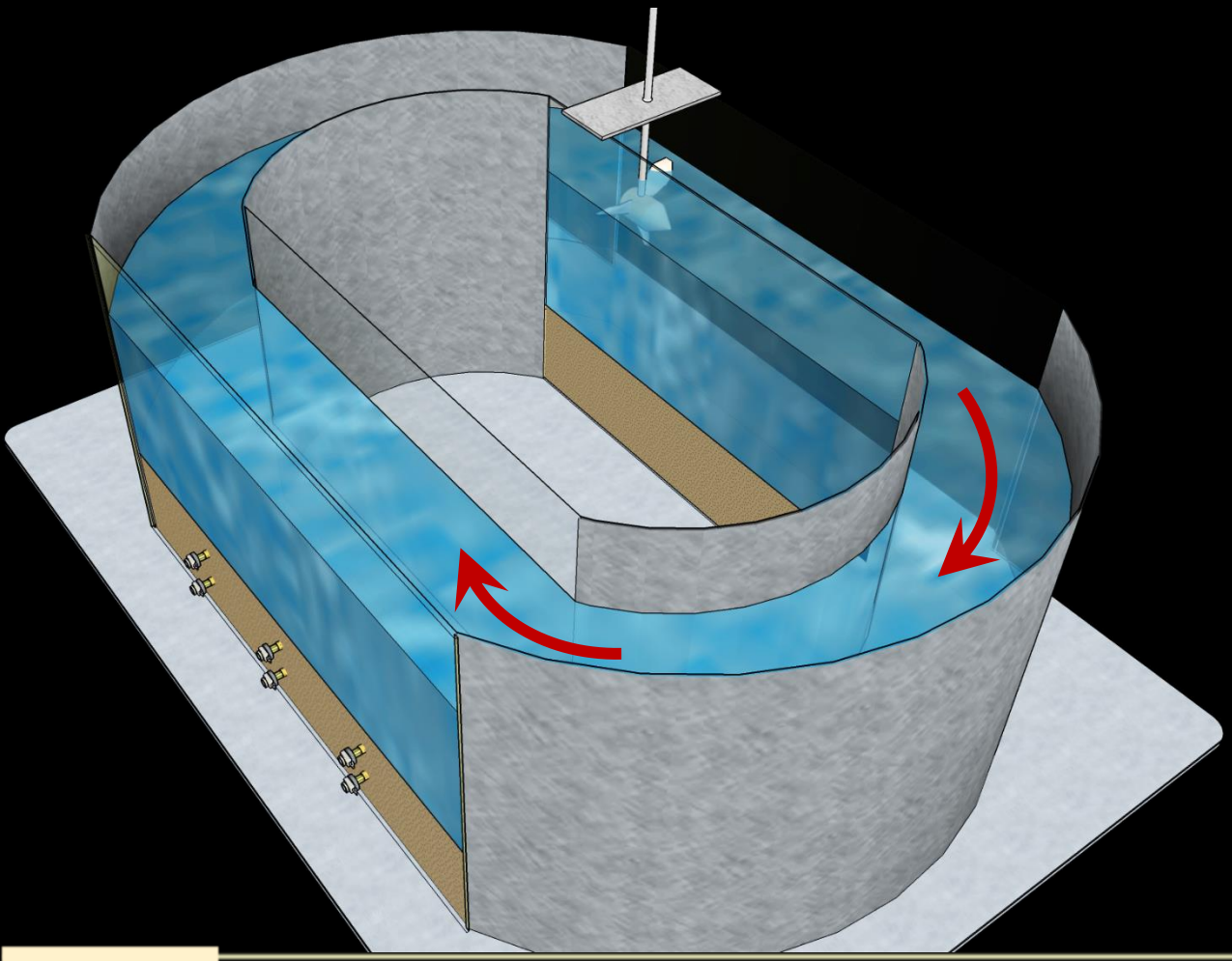
flume experiment

PP / TP behavior



Part II – Behavior

flume experiment



PP / TP behavior

Pharmaceuticals
- 10 µg/L

Lake Largén, Sweden
- 100 L water
- 0.06 m^3 sediment

Surface water
- 24 cm

Sediment
- 13 cm

Flow velocity
- 0.13 m/s

Part II – Behavior

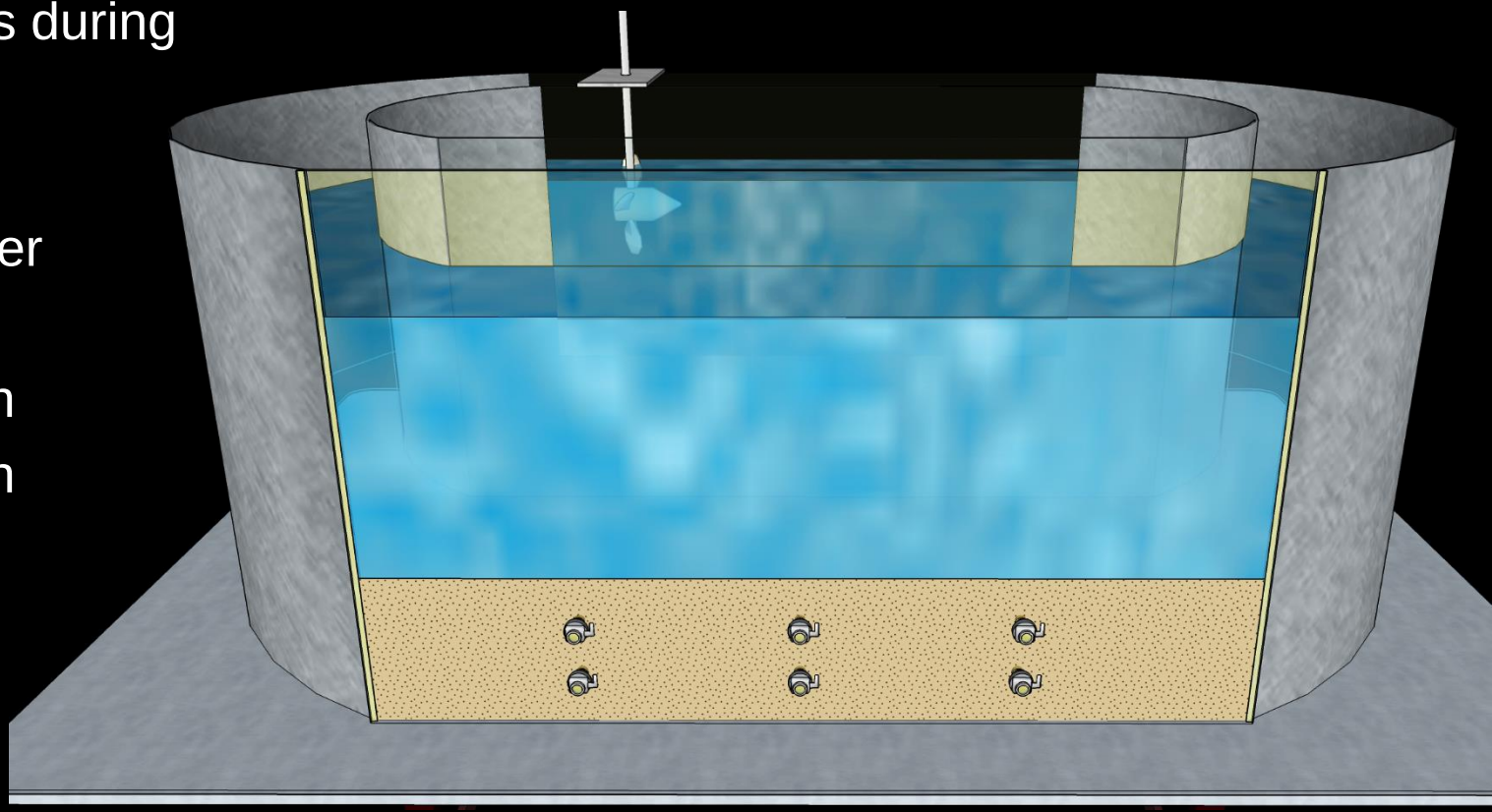
flume experiment | **UHPLC / ESI-TQ-MS** | **PP / TP behavior**

Sampling

- 8 time points during 30 days

Sample

- Surface water
- Pore water:
  - 3 cm depth
  - 8 cm depth



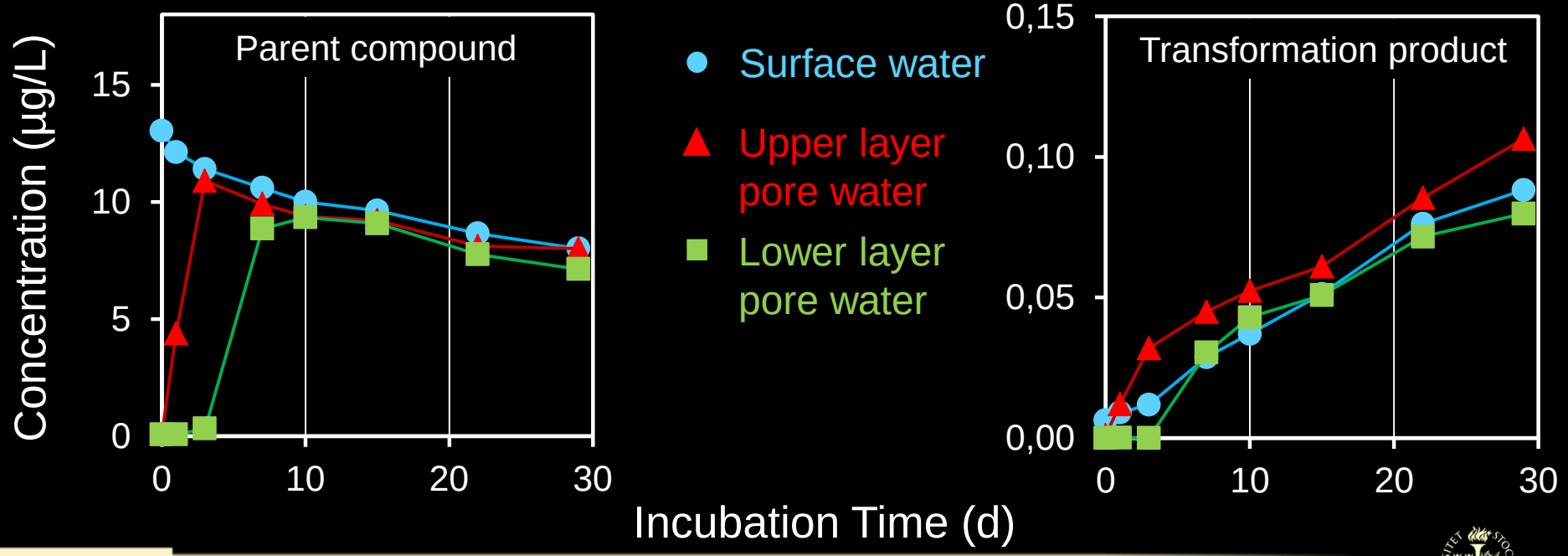
Part II – Behavior

flume experiment

PP / TP behavior

Ex. 1: carbamazepine – carbamazepine-10,11-epoxide

- transformation of PP
- TP behavior agrees with study I
- water exchange > transformation



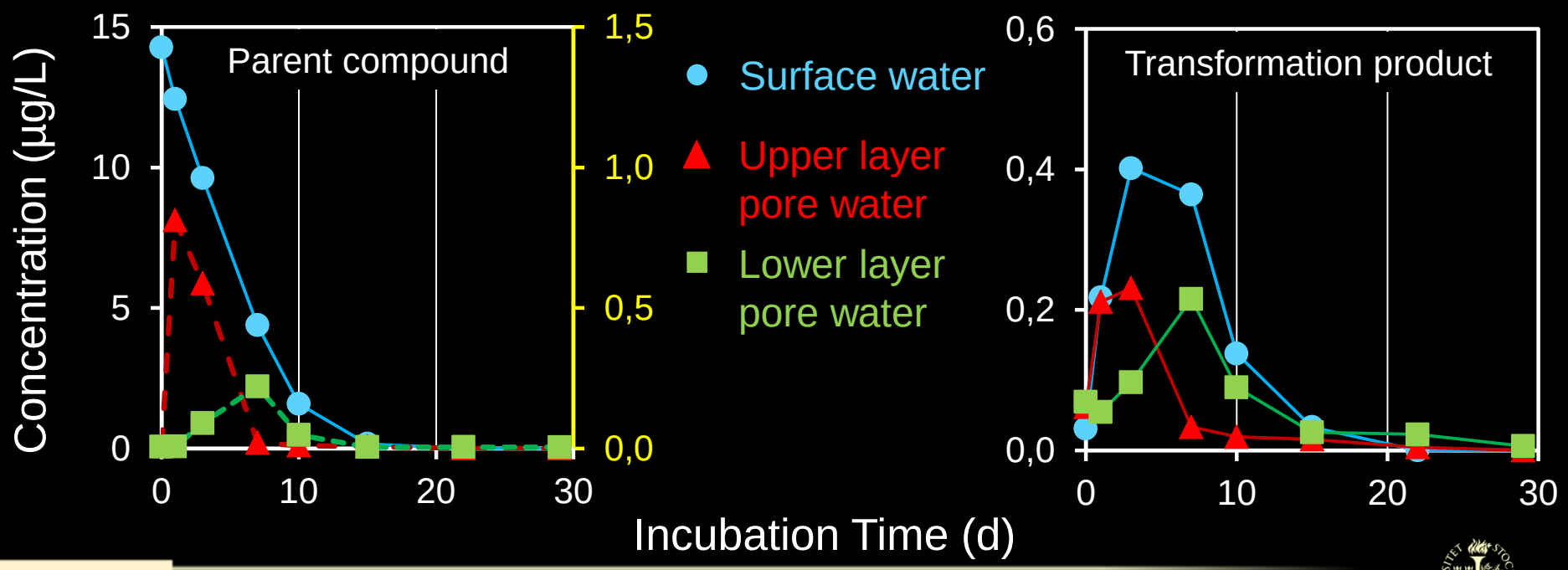
Part II – Behavior

flume experiment

PP / TP behavior

Ex. 2: bezafibrate – 4-chlorobenzoic acid

- quick elimination of both PP and TP
- efficient transport of TP
- water exchange < transformation



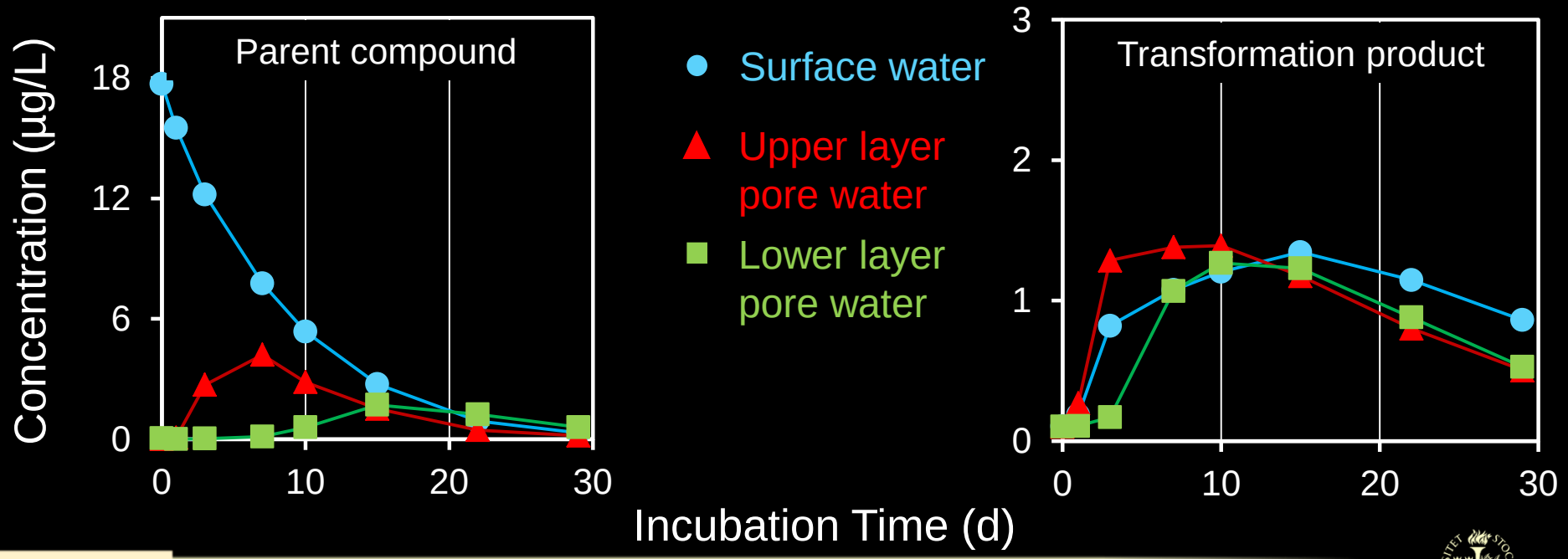
Part II – Behavior

flume experiment

PP / TP behavior

Ex. 3: metoprolol – metoprolol acid

- sorptive to sediment particles
- retarded during the sediment passage
- higher mobility of metoprolol acid



Conclusions

Part I

- 16 TPs were identified, 11 of them were confirmed.
- The data-processing method is efficient and reliable.
- Time-trend filtration helps to focus on persistent TPs.

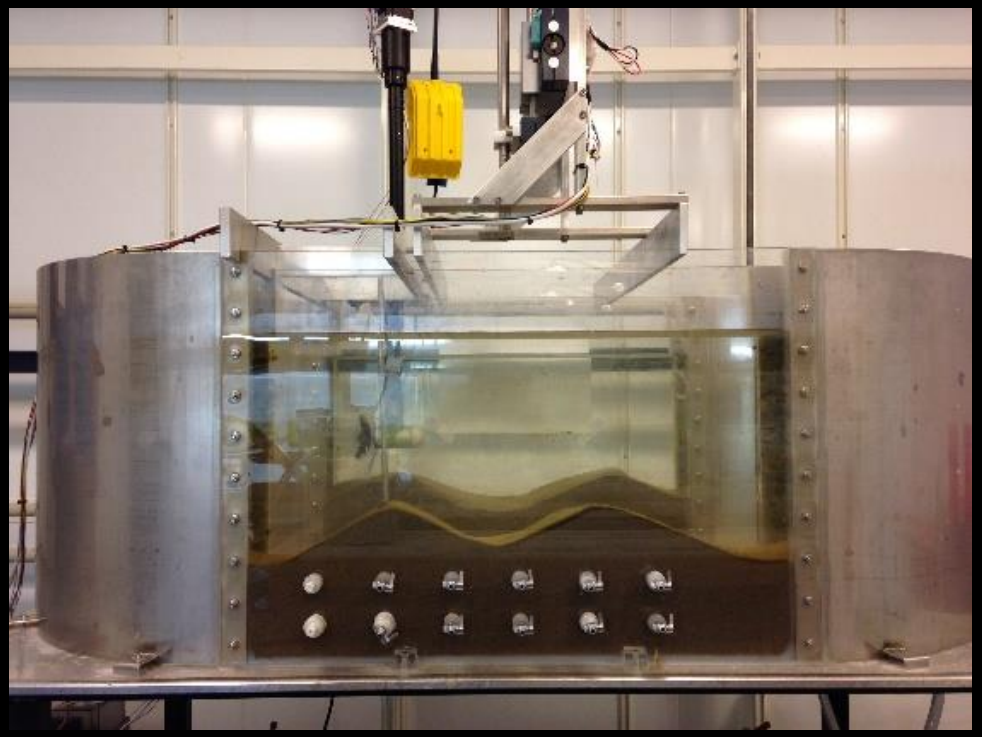
Part II

- 7 out of 11 TPs from study I were detected.
- TPs can be an indicator for transformation of PPs.
- Sediment can be a source of TPs in surface water.

Follow-up studies

Flume:

- Compare different water/sediment interface morphologies.
  e.g. even sediment surface vs. artificial ripples



Follow-up studies

Flume:

- Compare different water/sediment interface morphologies. e.g. even sediment surface vs. artificial ripples

Field:

- Use TPs as indicators for the elimination of PPs.

Application:

- Identify additional TPs in lab experiments
- Explore the applicability to environmental samples.

Thanks for your attention!

Analytica Chimica Acta 810 (2014) 61–70

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journal homepage: www.elsevier.com/locate/aca

Screening for pharmaceutical transformation products formed in river sediment by combining ultrahigh performance liquid chromatography/high resolution mass spectrometry with a rapid data-processing method

Zhe Li [a], Michael P. Maier [b,1], Michael Radke [a,*]

Appendix

Study I – Identified TPs

Parent Pharmaceutical	Transformation Products
bezafibrate	4-chlorobenzoic acid
carbamazepine	carbamazepine-10,11-epoxide
diclofenac	4’-hydroxydiclofenac
furosemide	saluamine
hydrochlorothiazide	chlorothiazide 4-amino-6-chloro-1,3-benzenedisulfonamide
ibuprofen	2-hydroxyibuprofen carboxyibuprofen
metoprolol	metoprolol acid α-hydroxymetoprolol
naproxen	----
propranolol	1-naphthol

Study II – Detected TPs

Parent Pharmaceutical	Transformation Products
bezafibrate	4-chlorobenzoic acid
carbamazepine	carbamazepine-10,11-epoxide
diclofenac	~~4' hydroxydiclofenac~~
furosemide	saluamine
hydrochlorothiazide	chlorothiazide 4-amino-6-chloro-1,3-benzenedisulfonamide
ibuprofen	~~2-hydroxyibuprofen~~ ~~carboxyibuprofen~~
metoprolol	metoprolol acid ~~α-hydroxymetoprolol~~
naproxen	----
propranolol	1-naphthol

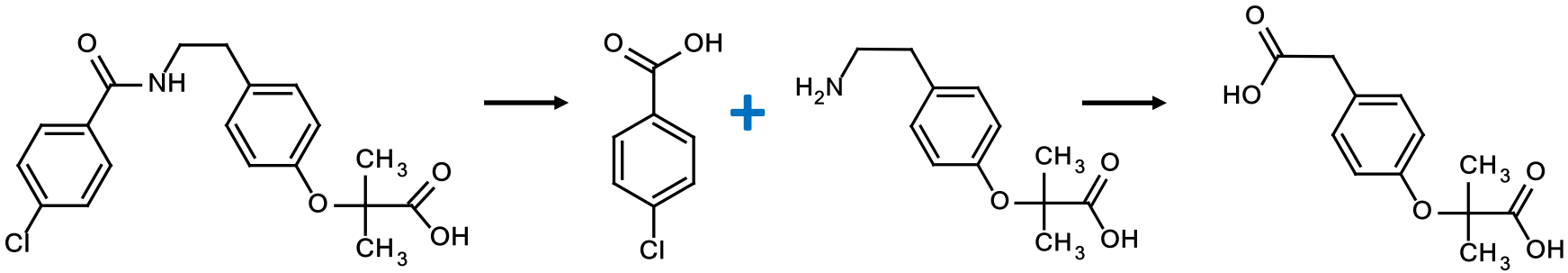
Study II – Behavior

flume experiment | **target analysis** | PP / TP behavior

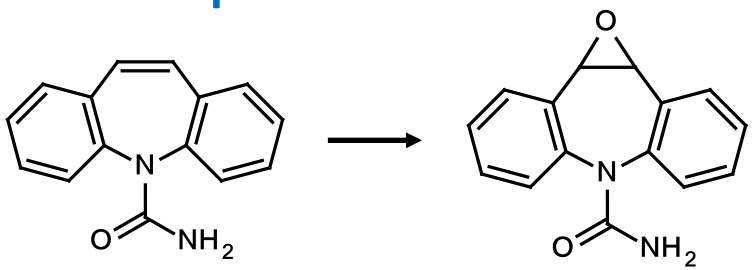
Target compound	18 PPs + 11 TPs
Internal standard	Labelled IS for 18 PPs and 3 TPs
Injection volume	50 µL
Instrument	UHPLC / TQ-MS, ESI +/-
Limit of Quantification (µg/L)	Below 0.01 in general PP: 0.25 for naproxen; 0.5 for ibuprofen TP: 0.1 for 2-hydroxyibuprofen; 2.5 for carboxyibuprofen

Proposed Transformation Pathway

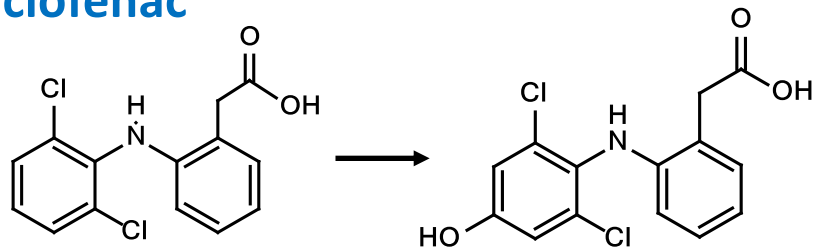
Bezafibrate



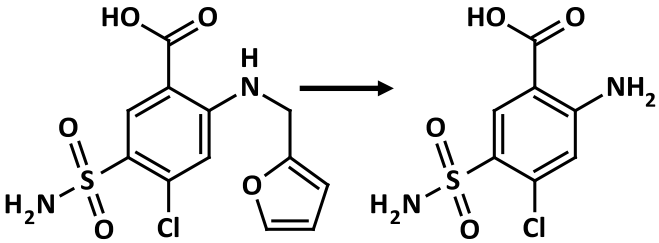
Carbamazepine



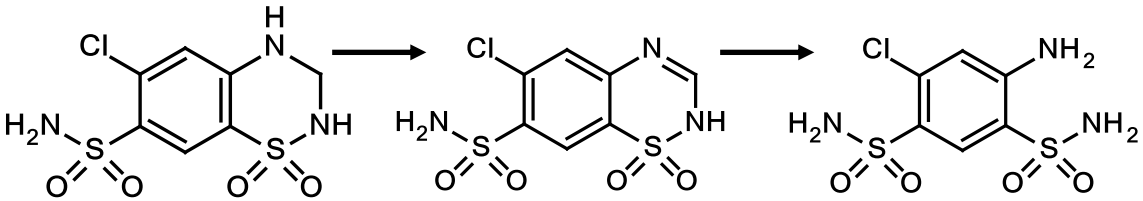
Diclofenac



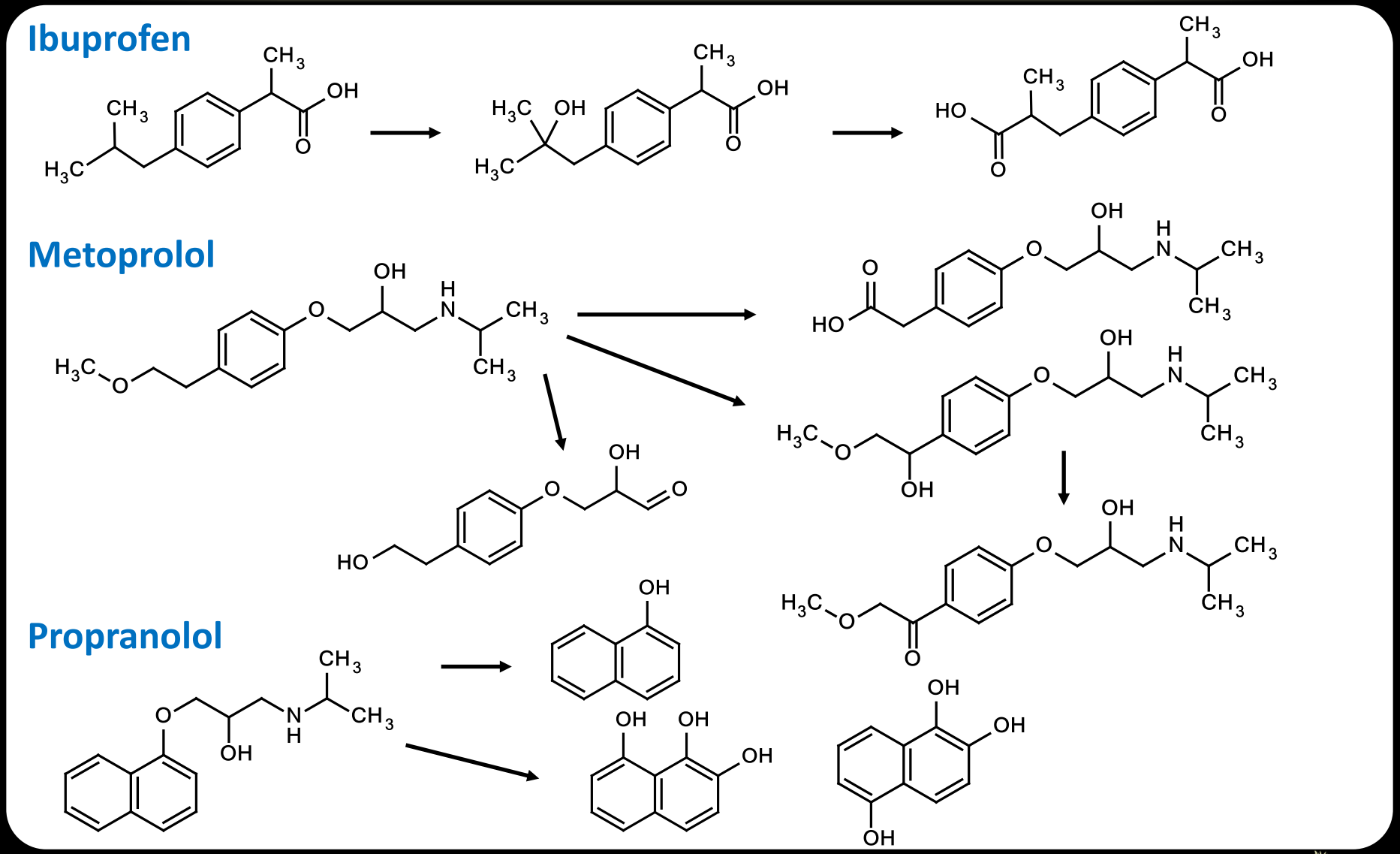
Furosemide



Hydrochlorothiazide



Proposed Transformation Pathway



Uncertainty

False Positive:

- **The tentatively identified TPs which in fact are not related to any parent pharmaceutical.**
- **Hardly can be confirmed without reference standards.**

False Negative:

- **TPs that are formed in the incubation system but were discarded in any of the steps of the screening procedure. Sources:**
- **(1) removal of a monoisotopic peak as noise;**
- **(2) absence of a ^{13}C isotopic peak;**
- **(3) absence of a reasonable time-trend during the incubation period.**

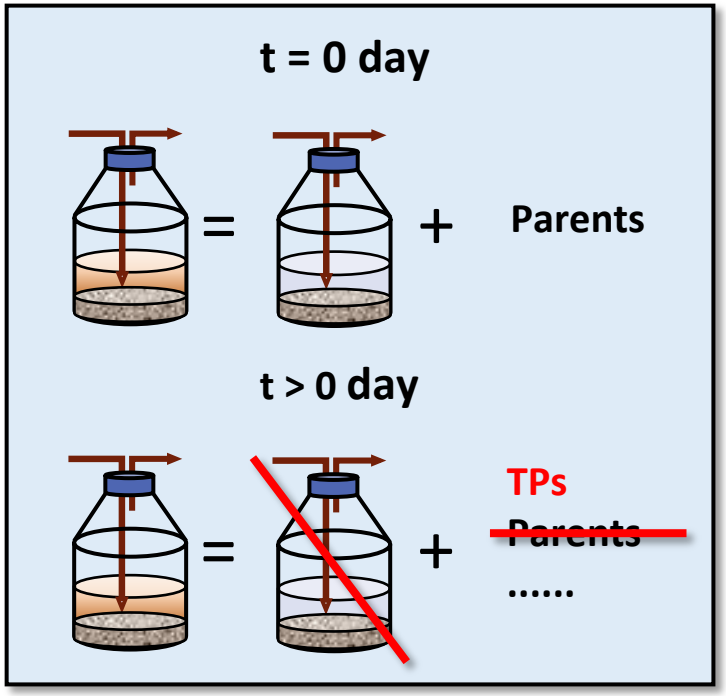
RT relative to Parent

Parent Compound	Transformation Products	RT Relative to Parent
bezafibrate	4-chrolobenzoic acid	0.87 ± 0.003
carbamazepine	carbamazepine-10,11-epoxide	0.88 ± 0.003
diclofenac	4'-hydroxydiclofenac	0.84 ± 0.003
furosemide	saluamine	0.65 ± 0.007
hydrochlorothiazide	chlorothiazide; 4-amino-6-chloro-1,3-benenedisulfonamide	0.96 ± 0.002 0.92 ± 0.002
ibuprofen	2-hydroxyibuprofen; carboxyibuprofen	0.70 ± 0.005 0.69 ± 0.005
metoprolol	metoprolol acid; α-hydroxymetoprolol	0.79 ± 0.003 0.80 ± 0.002
naproxen	---	---
propranolol	1-naphthol	1.32 ± 0.007

Screening Procedure

Computational:

Automated Peak Detection



MZmine™

Noise removal

↓

Peak list generation

↓

enviMass

Blank subtraction

↓

Target elimination

↓

Isotopic peak grouping

(^{12}C / ^{13}C, ^{35}Cl / ^{37}Cl etc.)

Screening Procedure

Computational:

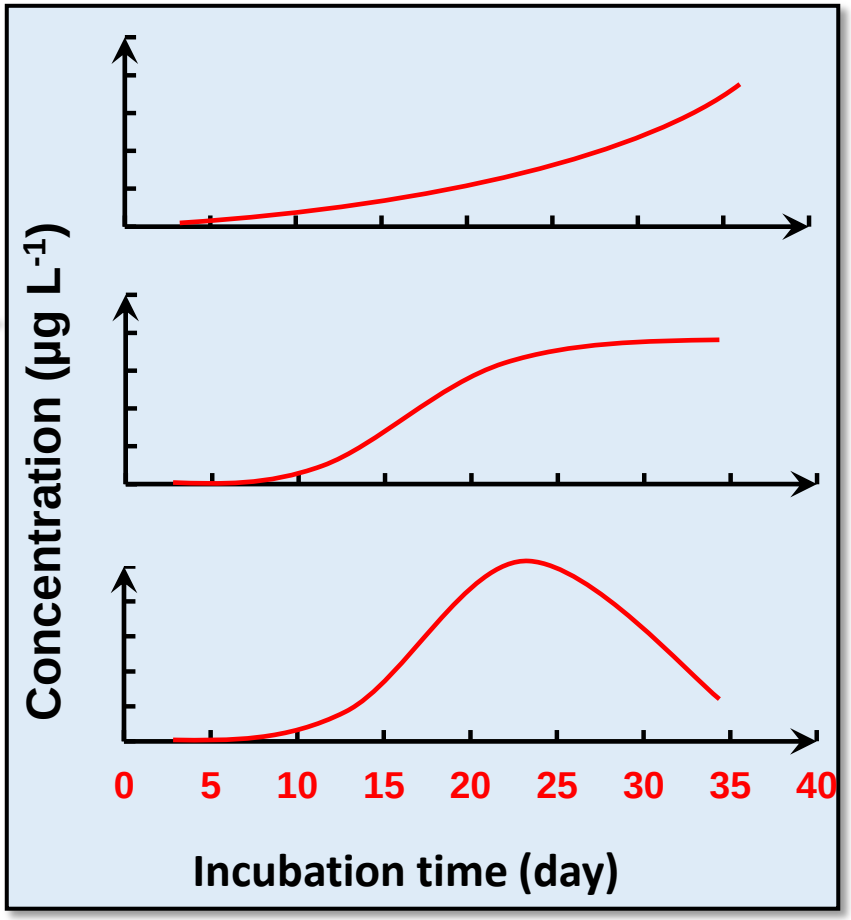
Automated Peak Detection

↓

Meaningful Time-Trend

Structure Assignment

Structure Confirmation



Screening Procedure

Computational:

Automated Peak Detection

↓

Meaningful Time-Trend

↓

Structure Assignment

screening results

Vs

predictions

UM-PPS

Parent

TP 1-1 TP 1-2 TP 1-3 ...

TP 2-1 TP 2-2 TP 2-3 TP 2-4 ...

Literature

- Well-documented TPs

Exact Mass →

Elemental Composition

Screening Procedure

Computational:

Automated Peak Detection

↓

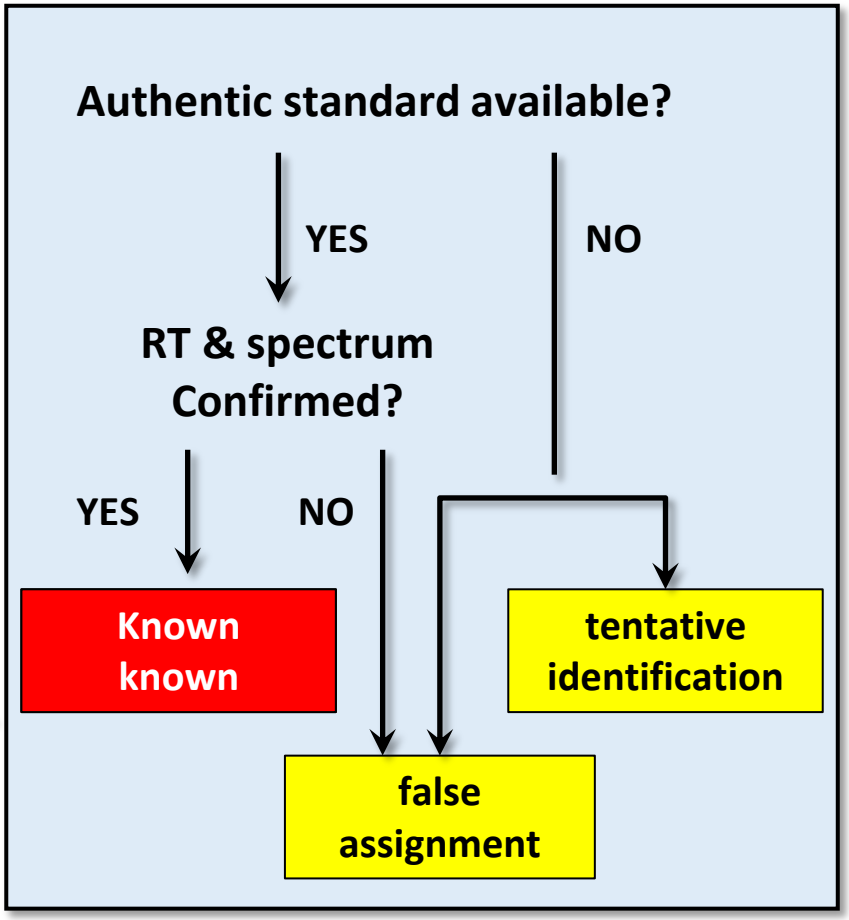
Meaningful Time-Trend

↓

Structure Assignment

↓

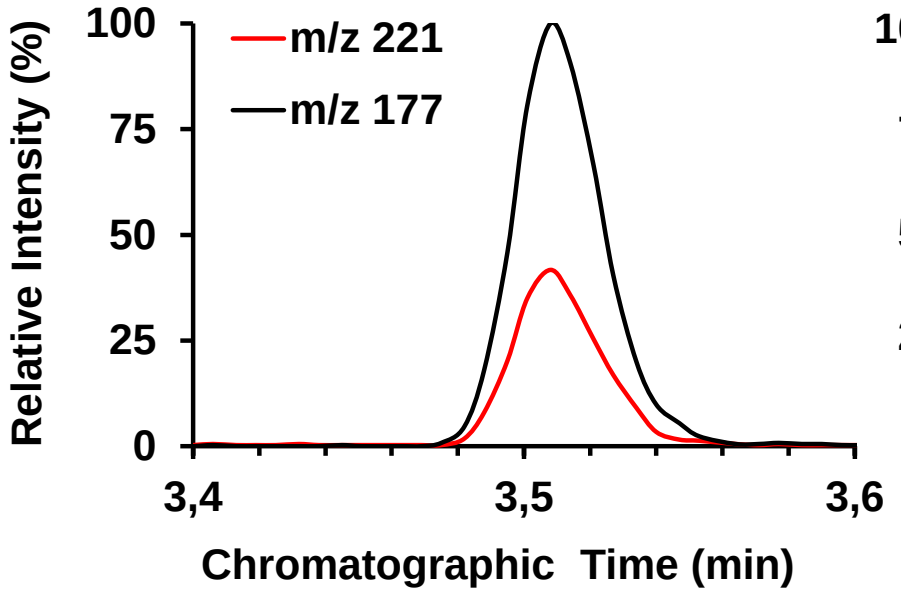
Structure Confirmation

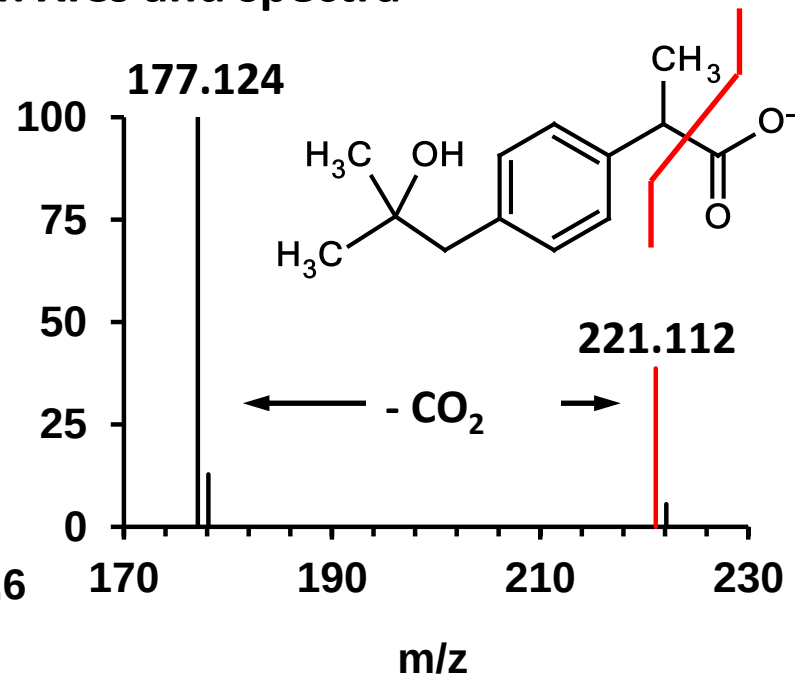


Fragments and Adducts

Characteristic:

- similar time-trend as their molecular ions.
- some were the dominant ions
- needed to be manually examined in XICs and spectra

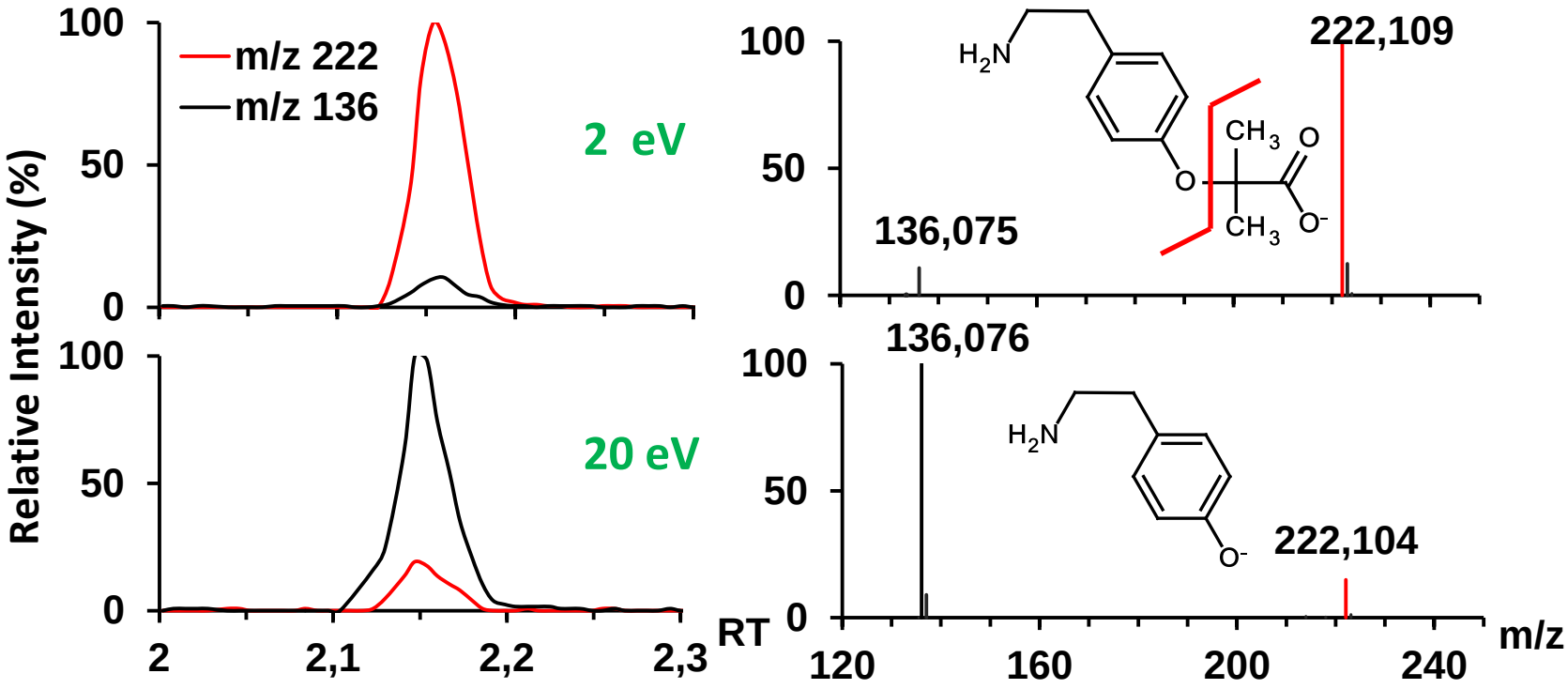




Fragments and Adducts

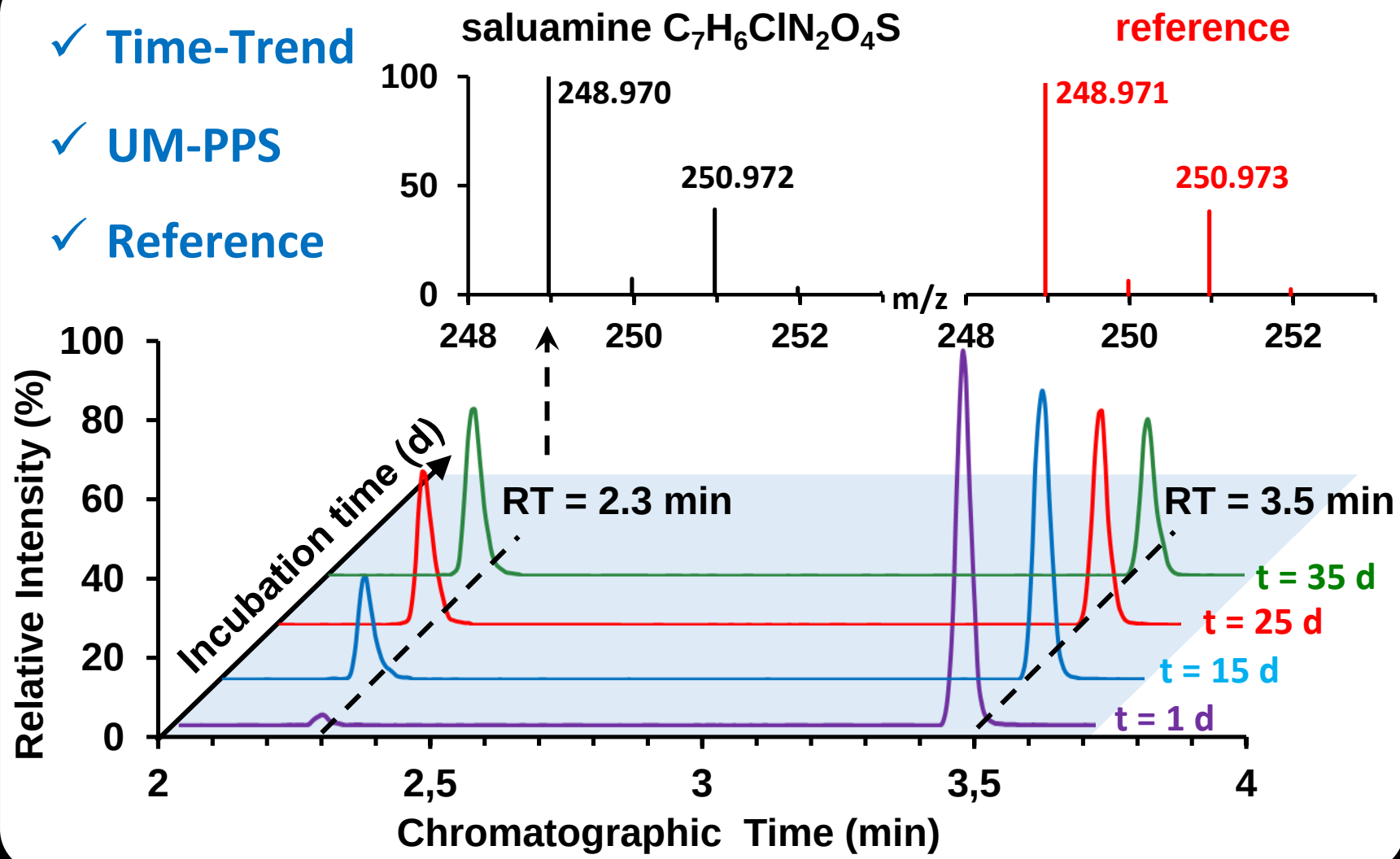
Confirmation:

- reference; or Compare the fragmentation in MS^E.
- Low collision energy: 2 eV; High collision energy: 20 eV.



Known Knowns

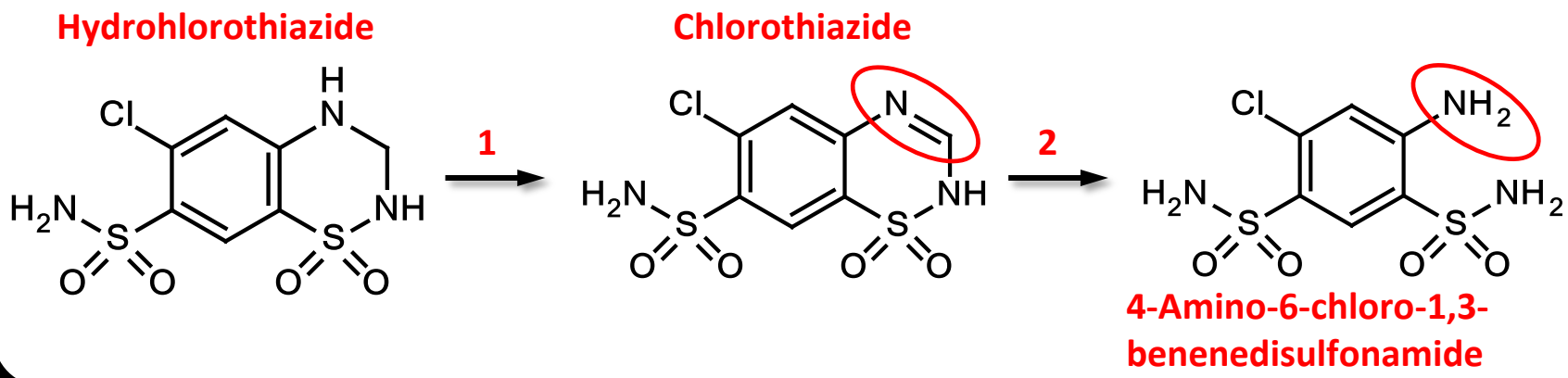
- ✓ Time-Trend
- ✓ UM-PPS
- ✓ Reference



Abiotic Transformation Processes

Control	Water	Sediment	Pharmaceutical	UV light
Sterile	Sterilized	Autoclave + NaN_3	Yes	No
Water-only	Yes	No	Yes	No

- 2 TPs were identified in sterile and water-only control.
- Both were confimed by corresponding authentic standards.
- Most likely formed by abiotic transformation processes (no photolysis).



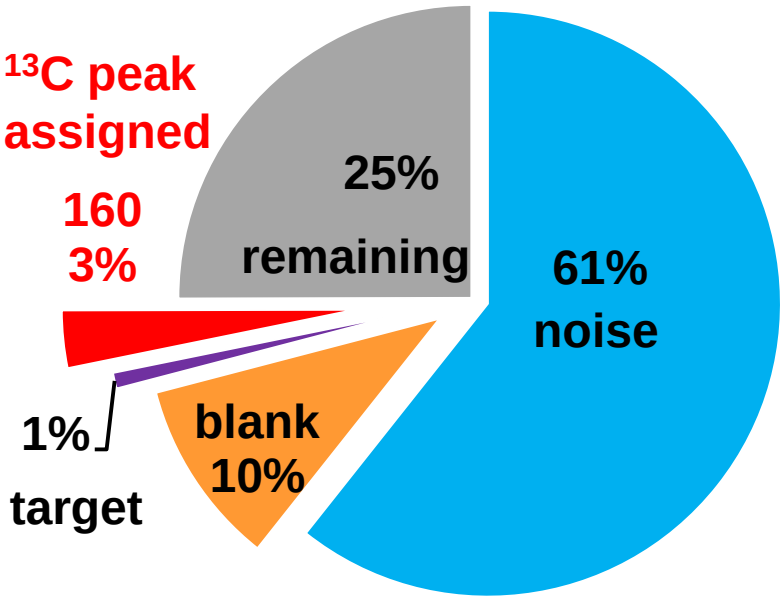
Procedural Performance

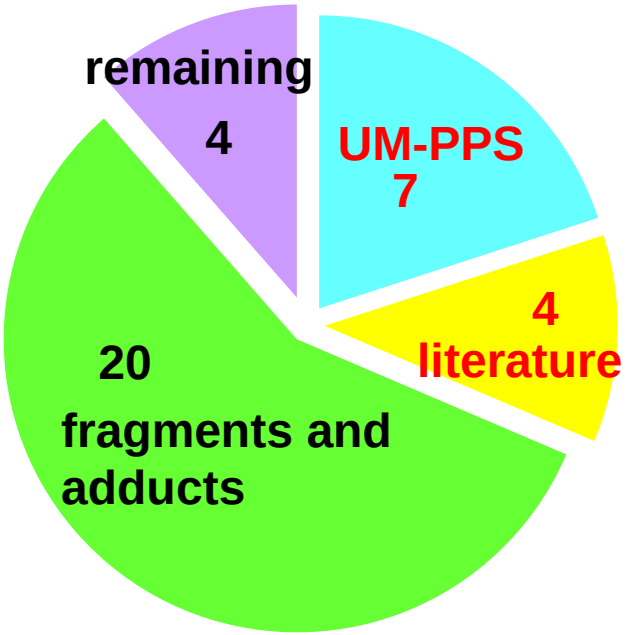
in ESI negative mode: ≈ 5057 peaks per sample

step I

step II
time-trend: 35

step III



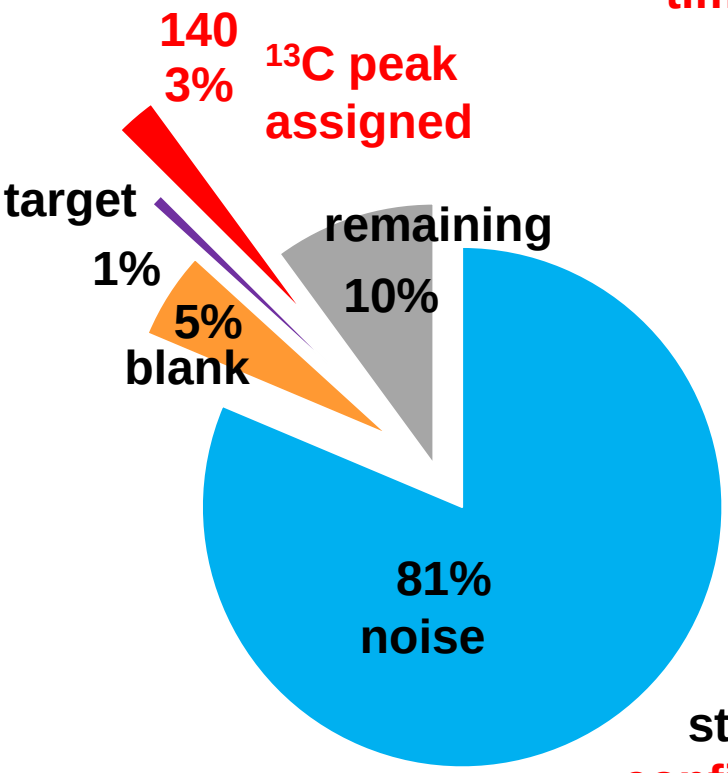


step IV
confirmed: 8

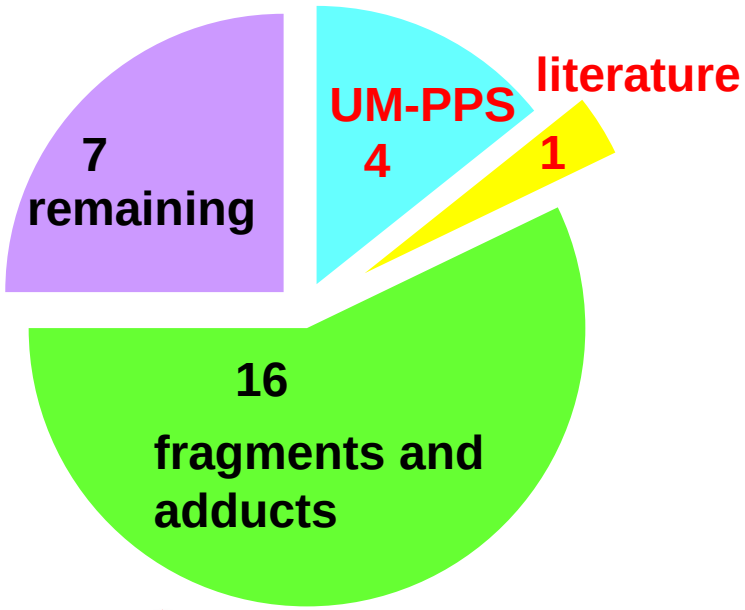
Procedural Performance

in ESI positive mode: ≈ 5678 peaks per sample

step I → step II → step III



step II
time-trend: 28



step IV
confirmed: 3