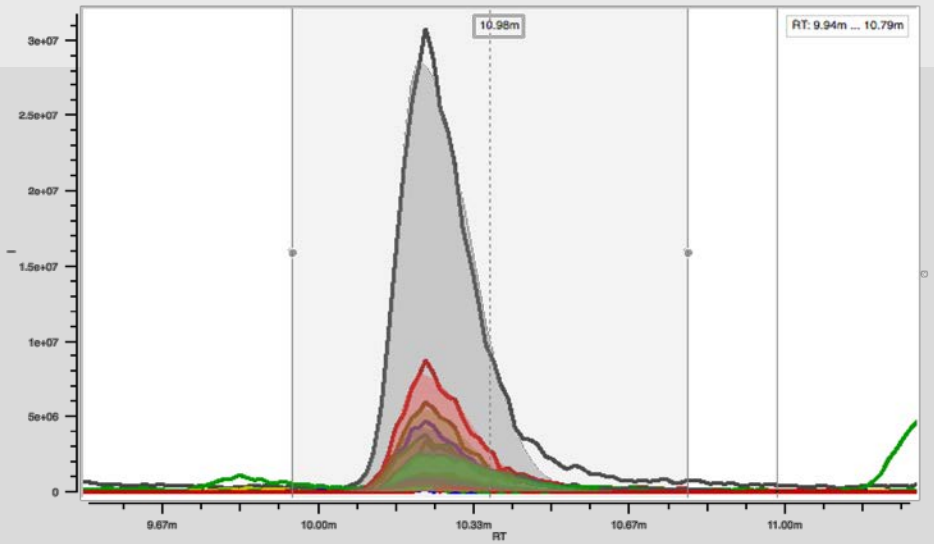
Suspect and non-target screening approaches for LC coupled to high resolution MS

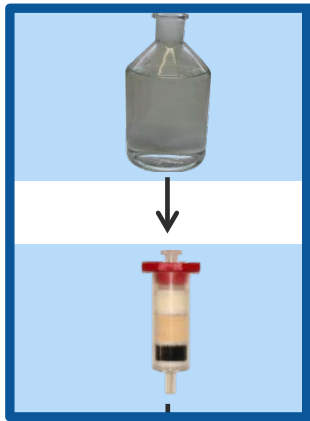
recent method developments

Heinz Singer



Matthias Ruff
Martin Loos
Michele Stravs
Philipp Longree
Christoph Moschet
Juliane Hollender

Current screening methods



Sample preparation

Liquid chromatography

Mass spectrometry

Target screening	Suspect screening	Non-Target screening
526	394 + libraries	∞

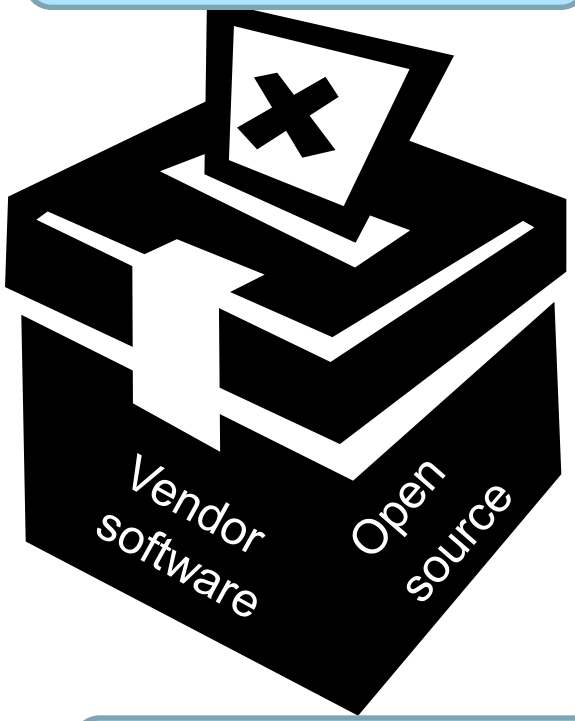
Data evaluation – Pandora's box

Fragmentation prediction

Retention time prediction

Chromatogram alignment

MS/MS Library x,y,z



Peak peaking

Structure generation

Background subtraction

Structure search

Mass re-calibration

Non-target search

Molecular formula fit

Metabolite and homologue search

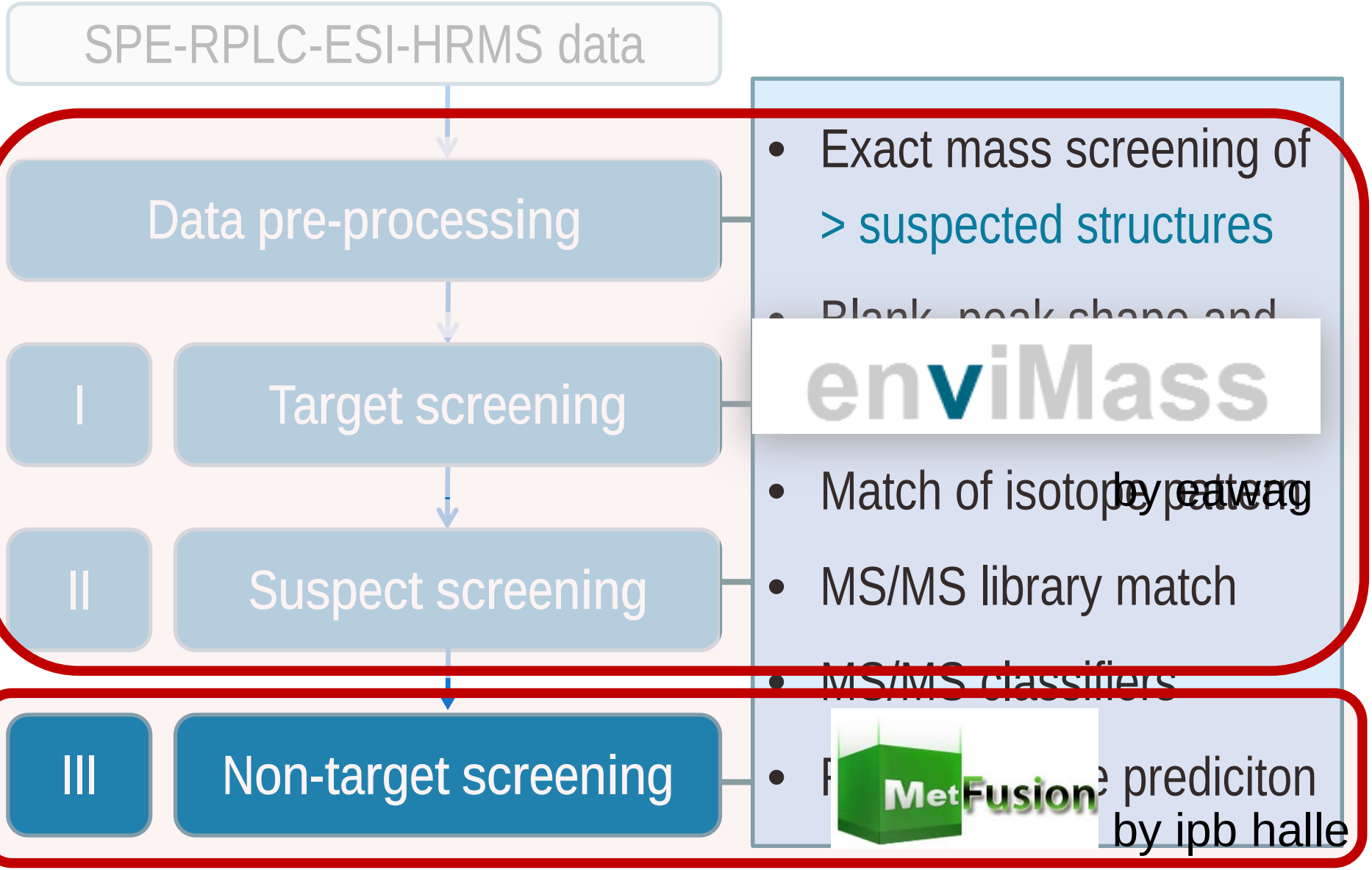
Adduct search

Target quantitation

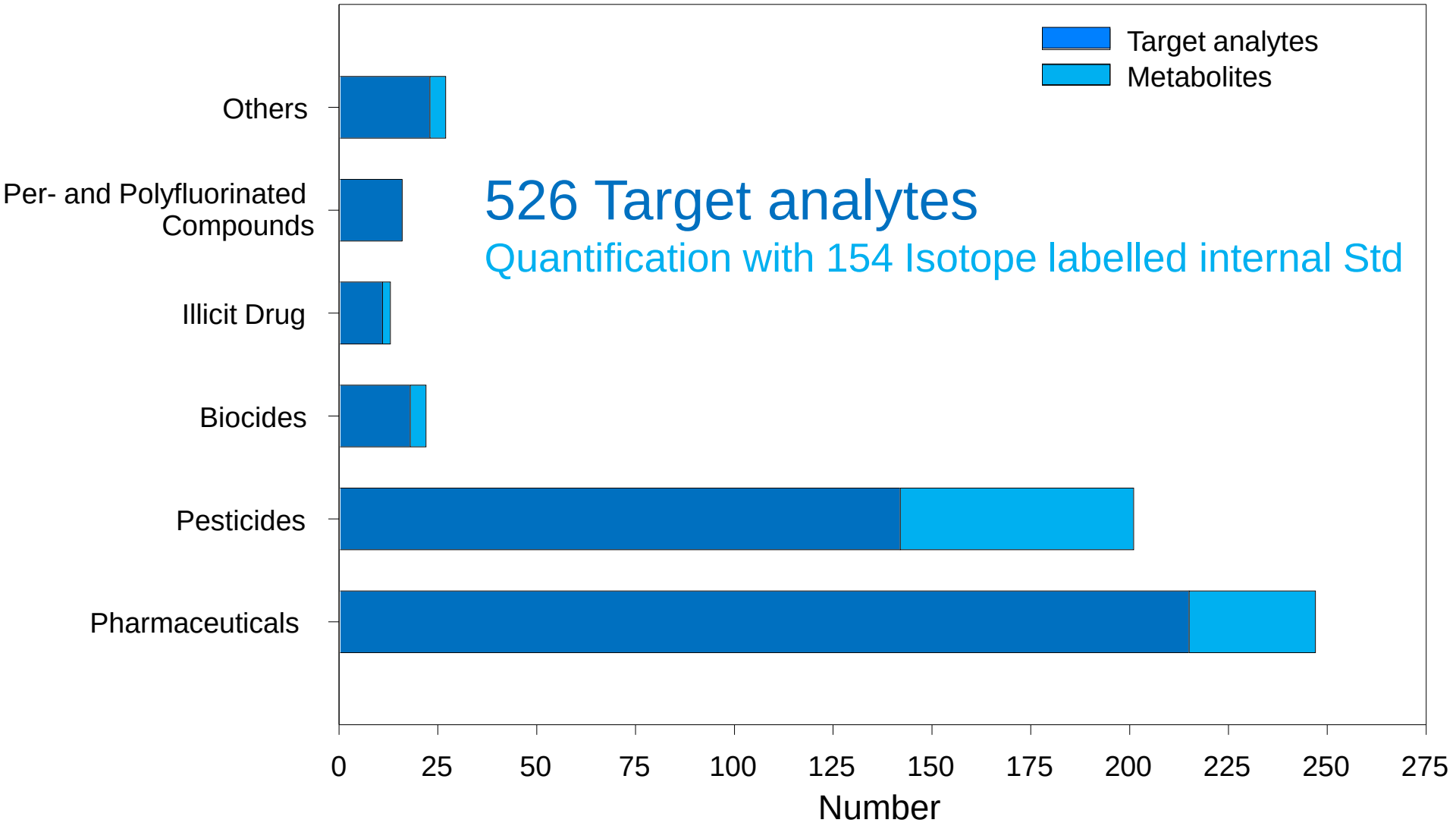
Isotope grouping

Suspect screening

Workflow – target , suspect, non-target

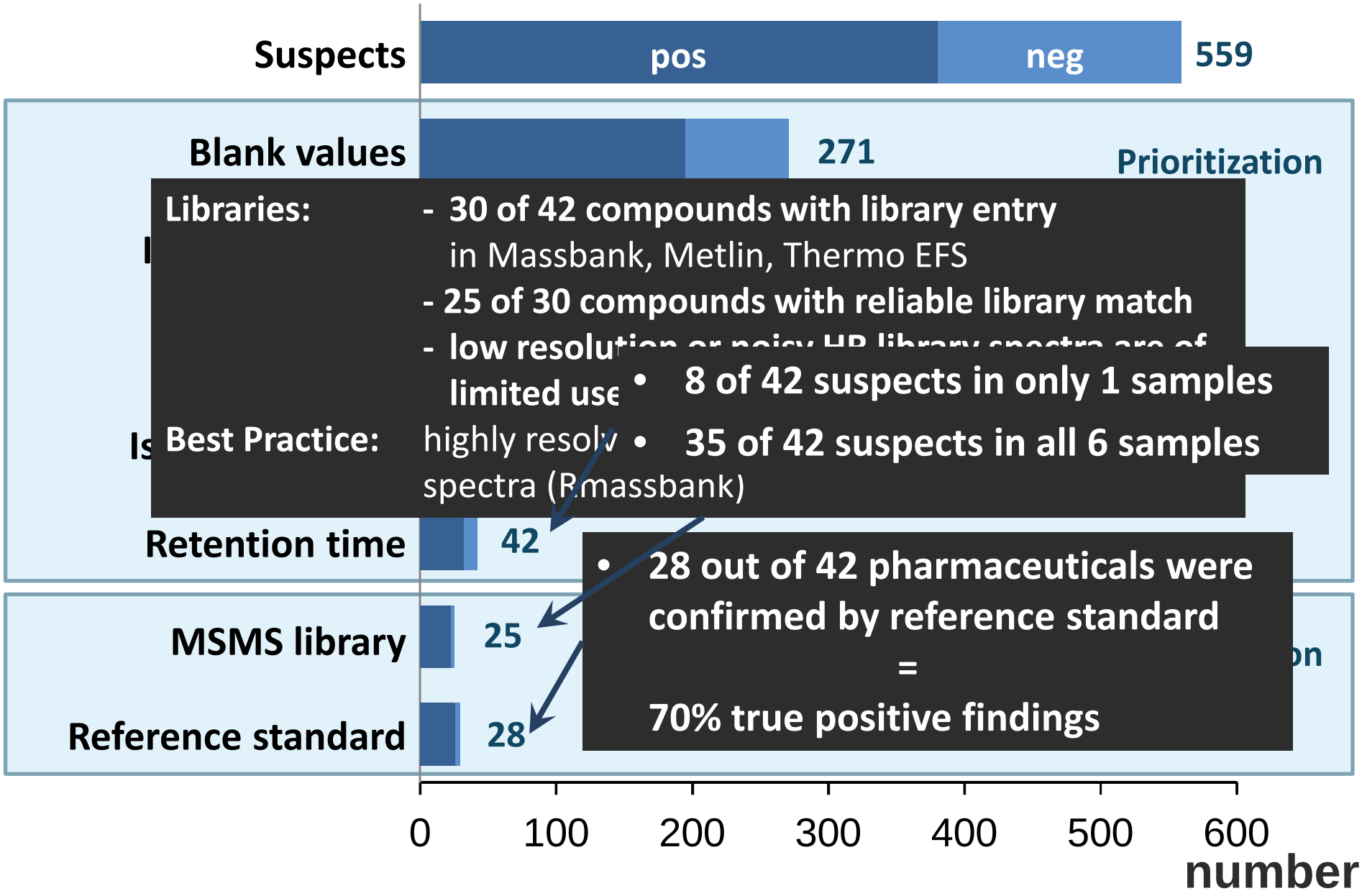


Target Screening – legacy of the past



- **Collection of water relevant compounds from monitoring, literature, and research data**

Suspect screening - 886 drugs in waste water



Peak inventory - waste water samples

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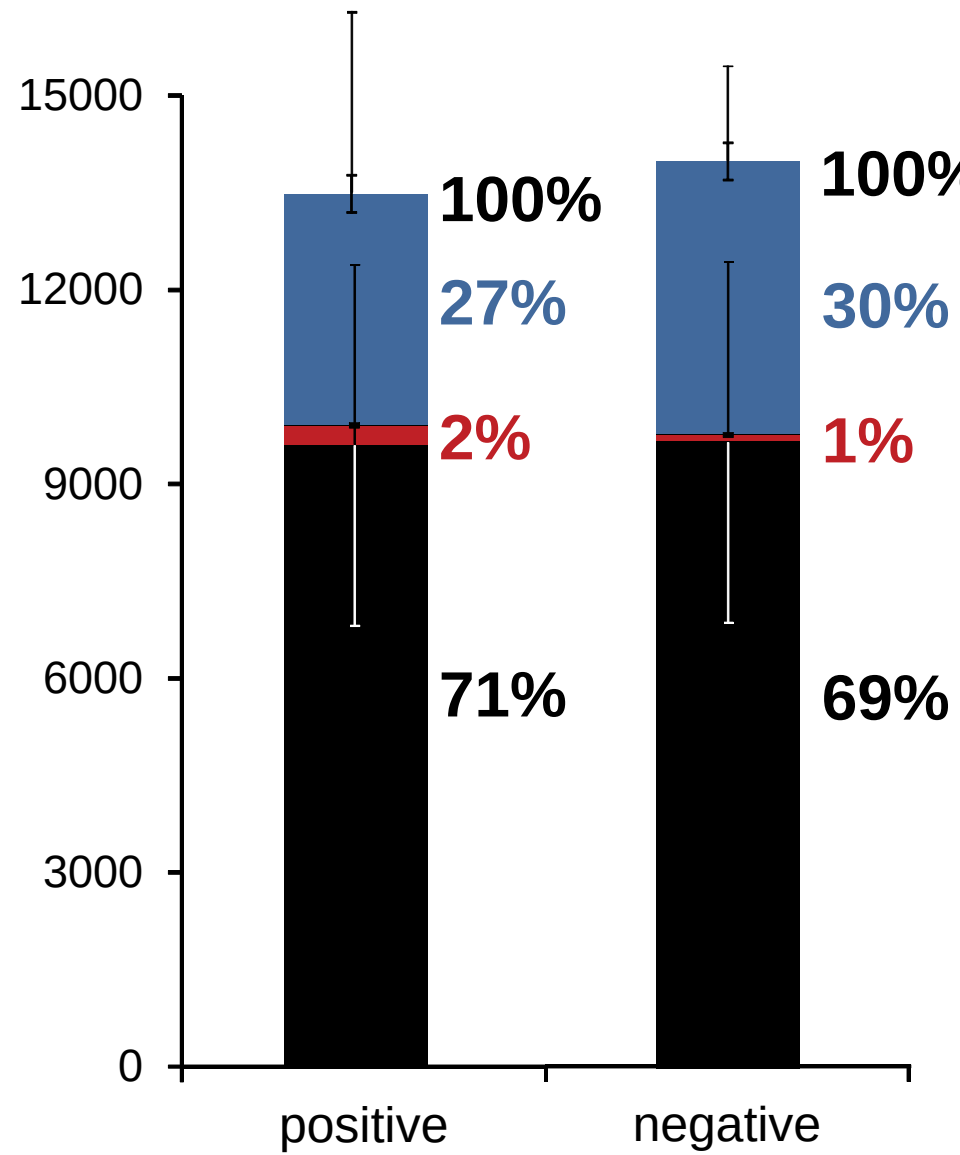
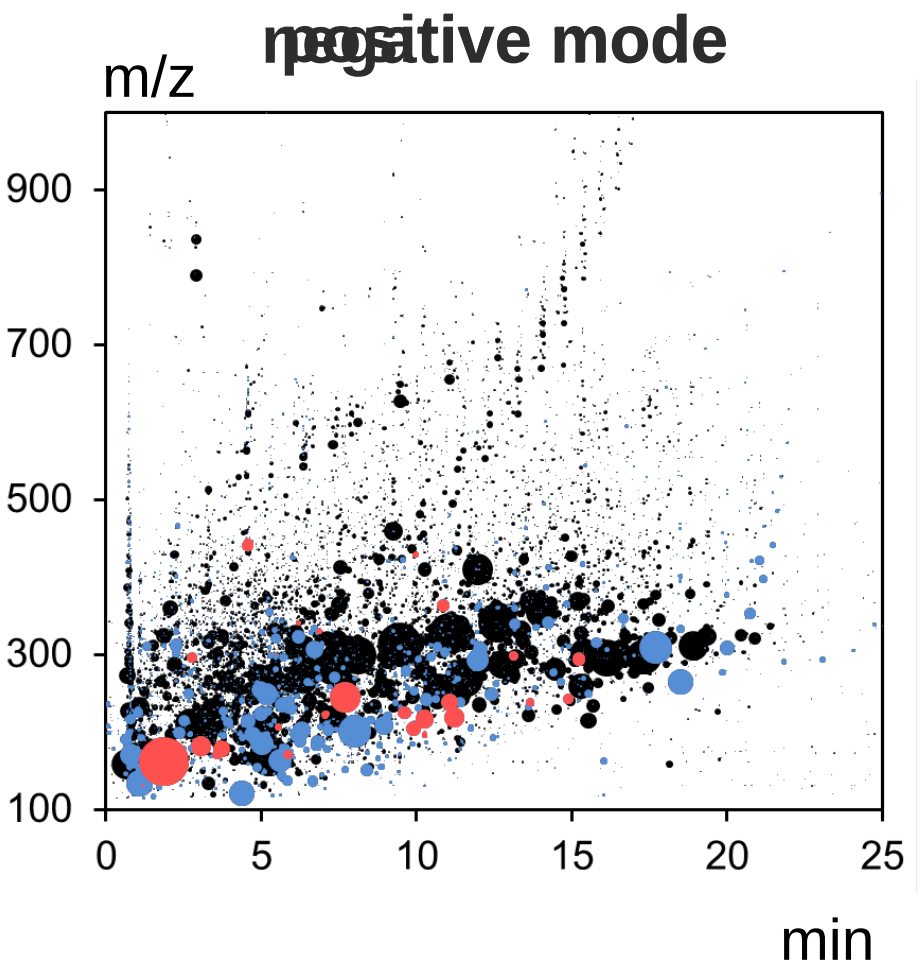
Remaining non-target peaks after intensive base peak framing:

-> 5 ppm x 1 min frame





Homologous Series – surfactants

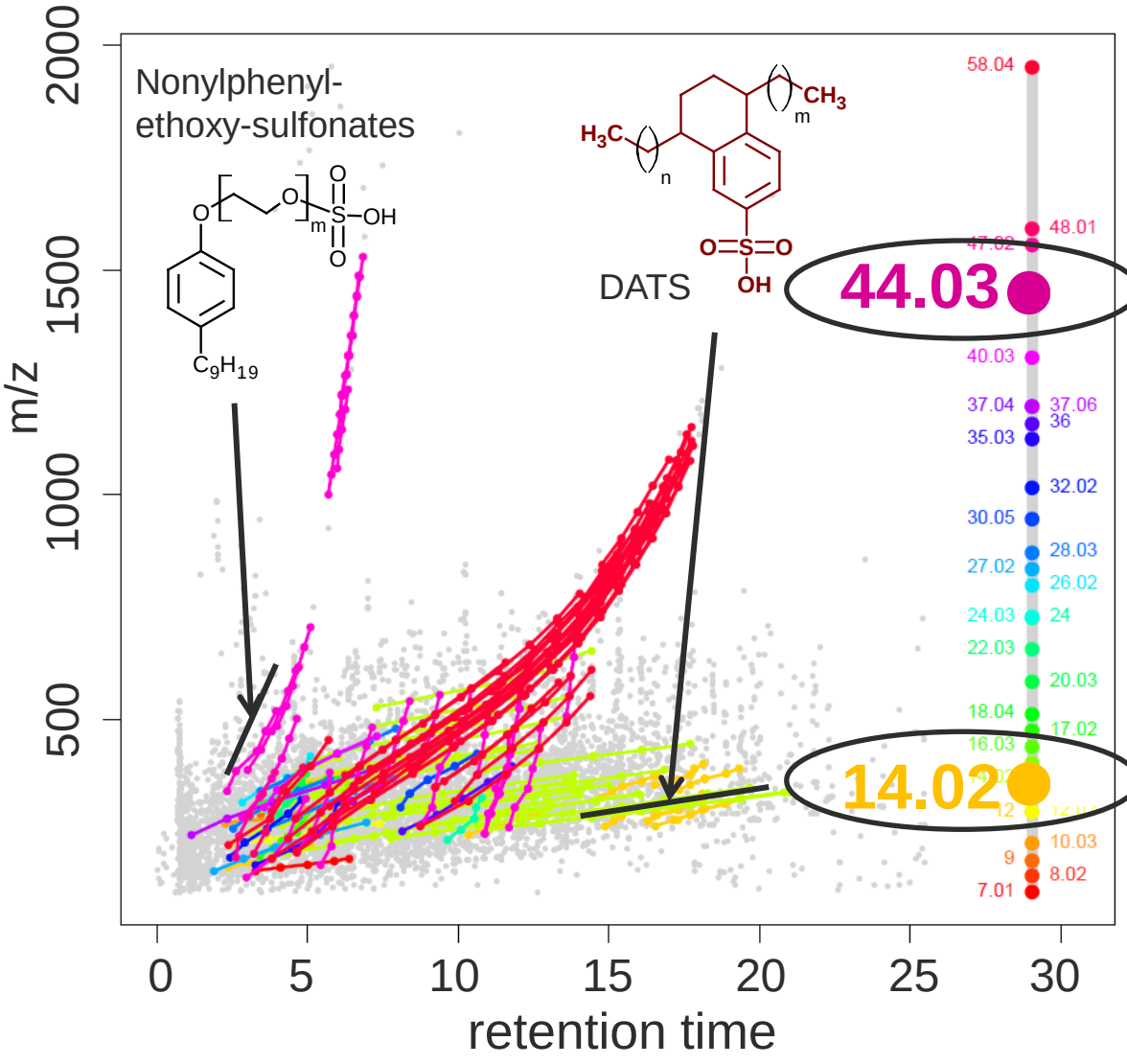
R-Package “nontarget”

Search for consistent mass differences across sample

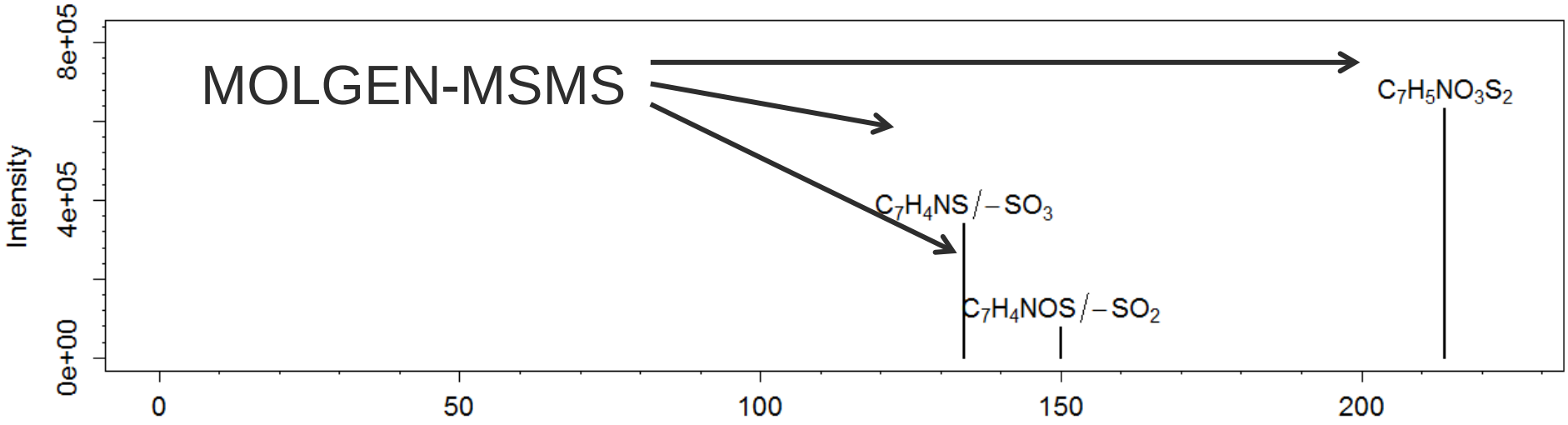
- o mz_{diff}: 6 - 60
- o Rt_{diff}: 0 - 1.8 min
- o homologoues length: ≥ 5

Results

- o 184 homologous series on average (incl. isotope, adduct, multiple charged series)
- o Mass differences 14.02 (CH_2), 44.03 (C_2H_4O) and 58.04 (CH_2+C_2H_4O) very common



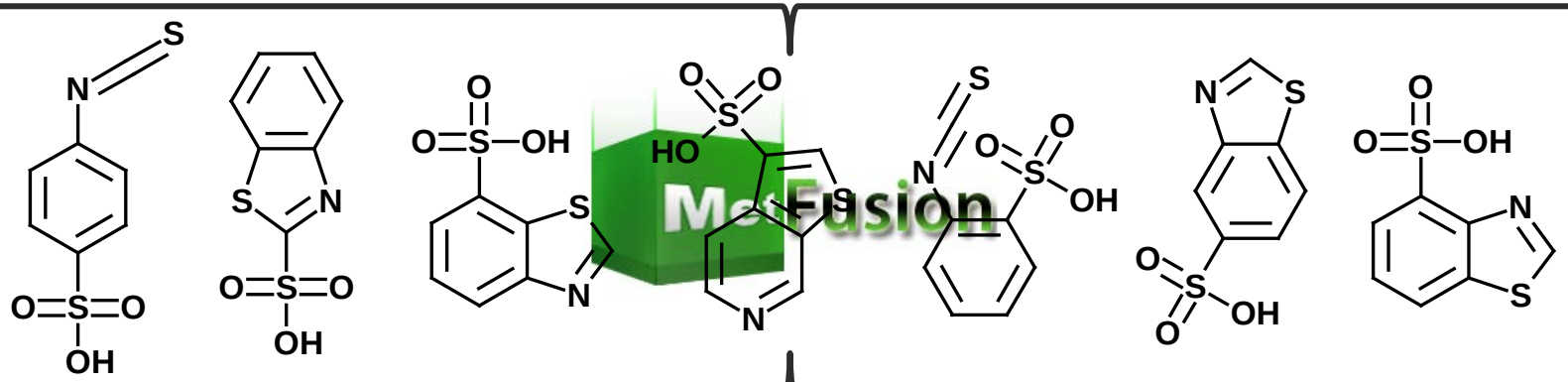
Non-target - $[M-H]^-$ = 213.9634, 4.54 min



MassBank

ChemSpider
The free chemical database

MetFrag



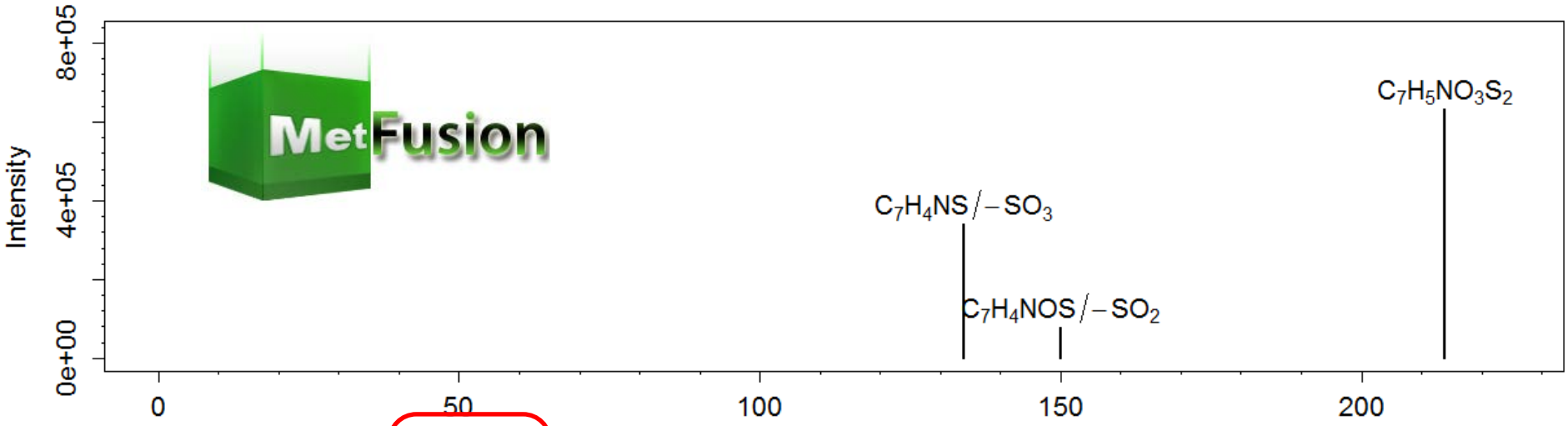
MASS FRONTIER
Confident Path from Spectra to Structure

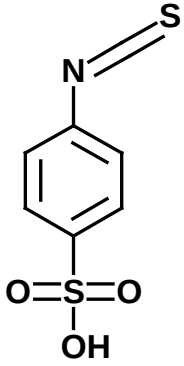
log K_{ow} & RT check

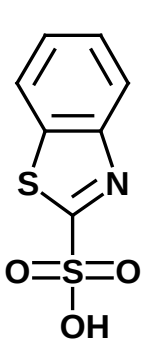
Number of References

Standard Available

Non-target - [M-H]$^-$ = 213.9634, 4.54 min



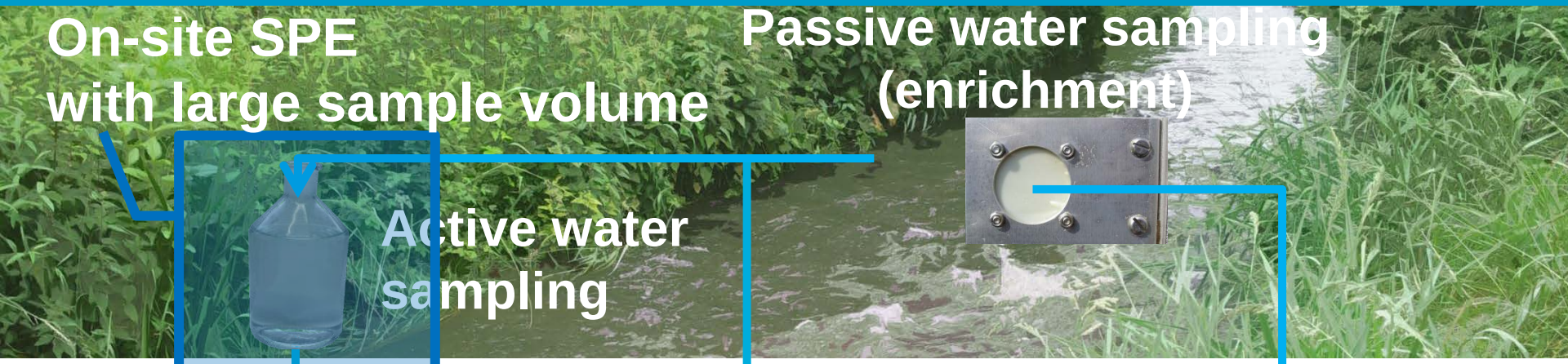




References	38	29
Standard	No	Yes
Match?		MSMS, RT

- 1,3-benzothiazole-2-sulfonic acid
- Vulcanisation accelerator
- Corrosion Inhibitor
- Antifreeze
- TP of 2-mercaptobenzothiazole (high volume rubber production)
  - De Wever et al. 2001

Sampling and enrichment



extraction

Enrichment

direct

evaporation

evaporation

extraction

large volume
injection

LC-HRMS

extract
injection

extract
injection

2 x MS^{1}ddMS^2

1 x ±MS^{1}ddMS^2

2 x MS^{1}diaMS^2

Sampling and enrichment

Active water sampling		Passive water sampling
SPE	Direct injection	
+ sensitive	- less sensitive	+ sensitive
+ quantitative	+ quantitative	- qualitative
- slow	+ fast	+ fast

Data dependent vs data independent acquisition

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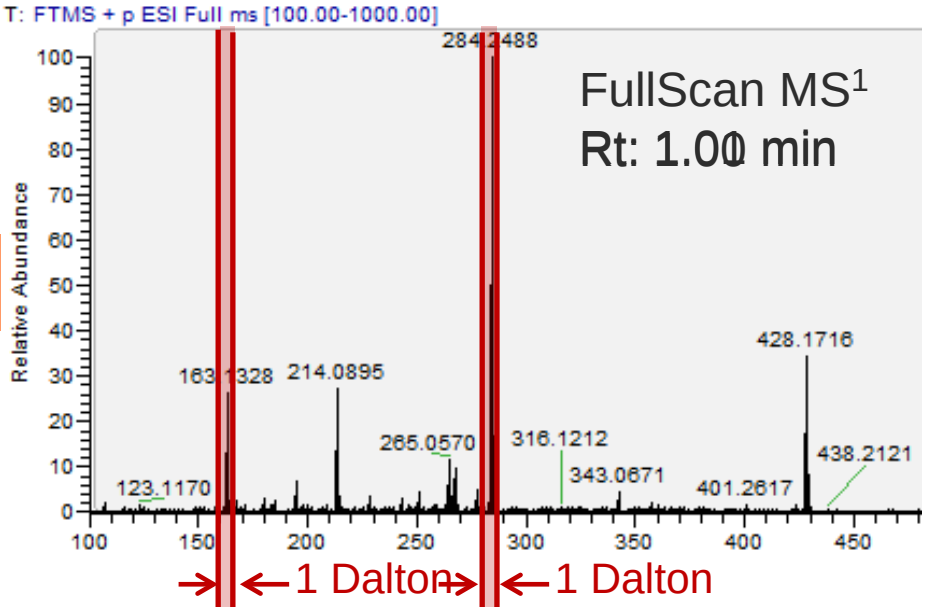
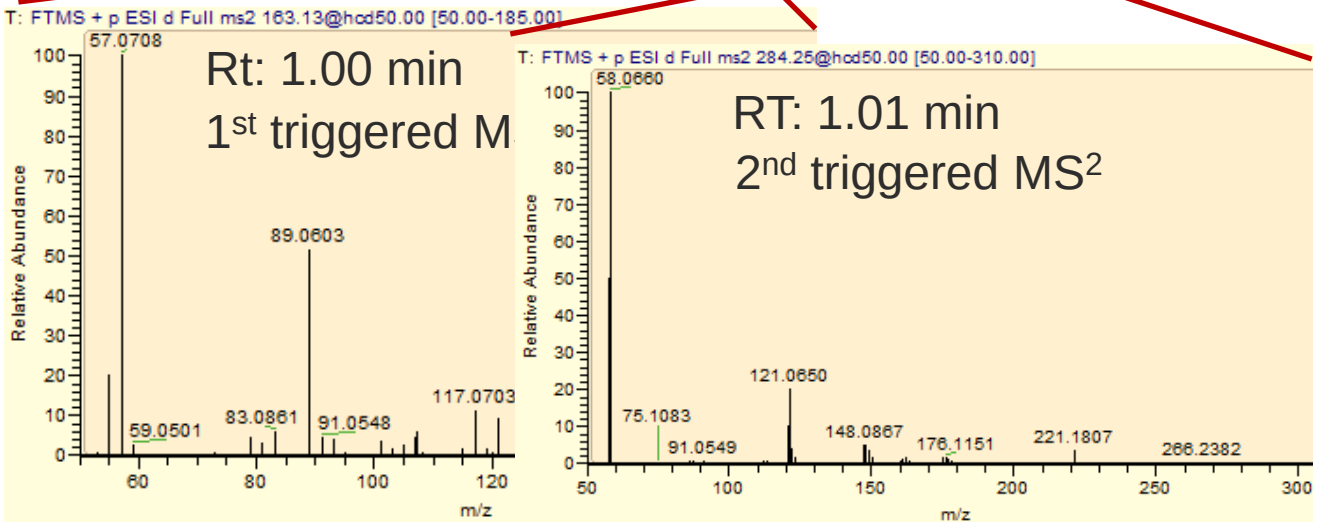
QExactive

Data-dependent MS^2

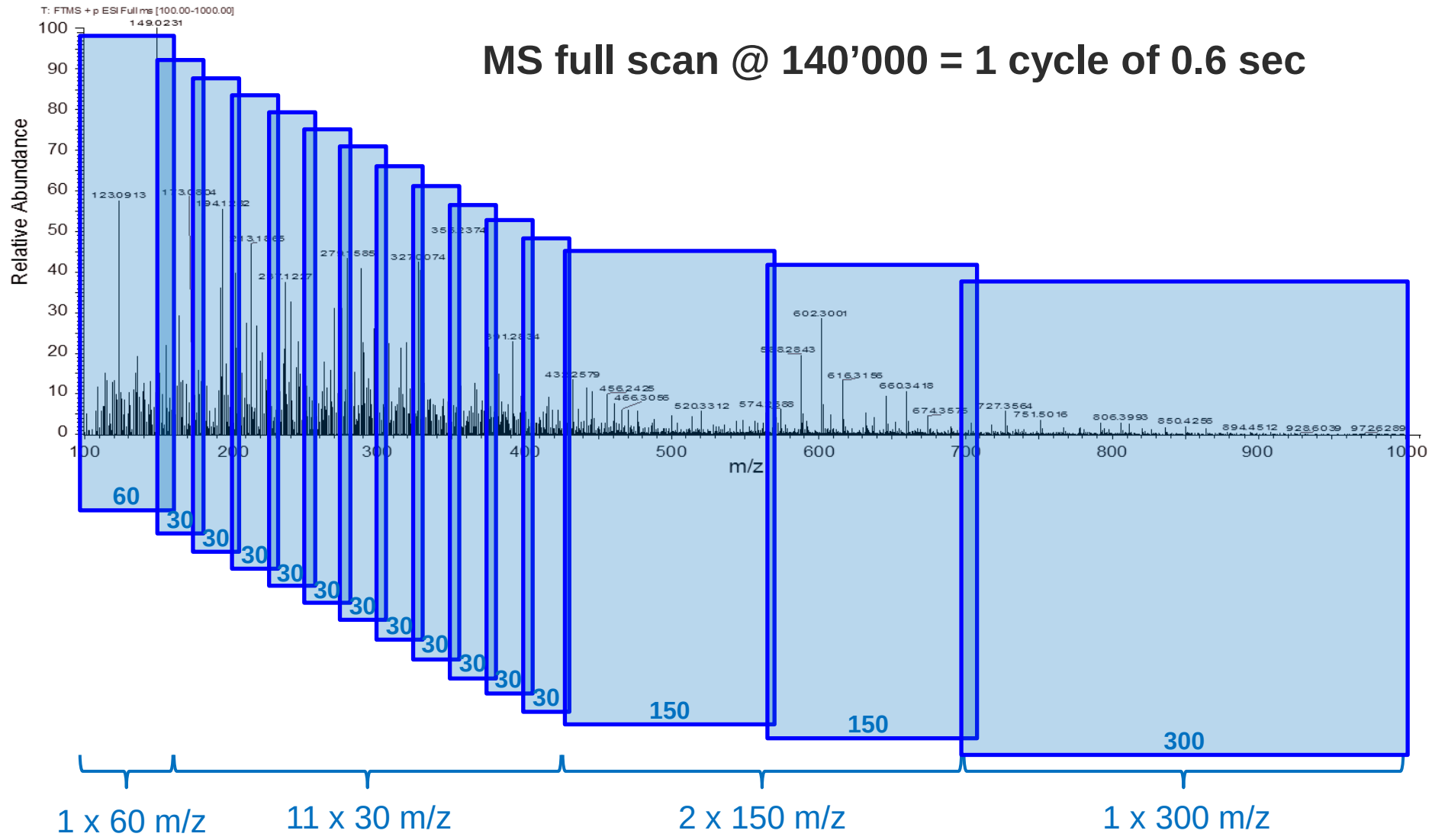
- Trigger list for MS^1 ions
- trigger of most intense MS^1 ions
- Narrow isolation window of MS^1 ions

⇨ MS^2 is acquired dependent on MS^1 data



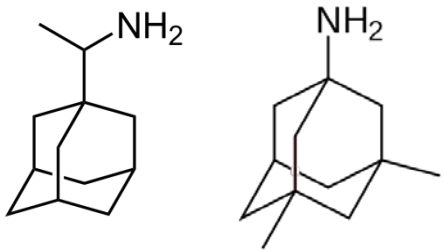


More sensitivity – more isolation windows



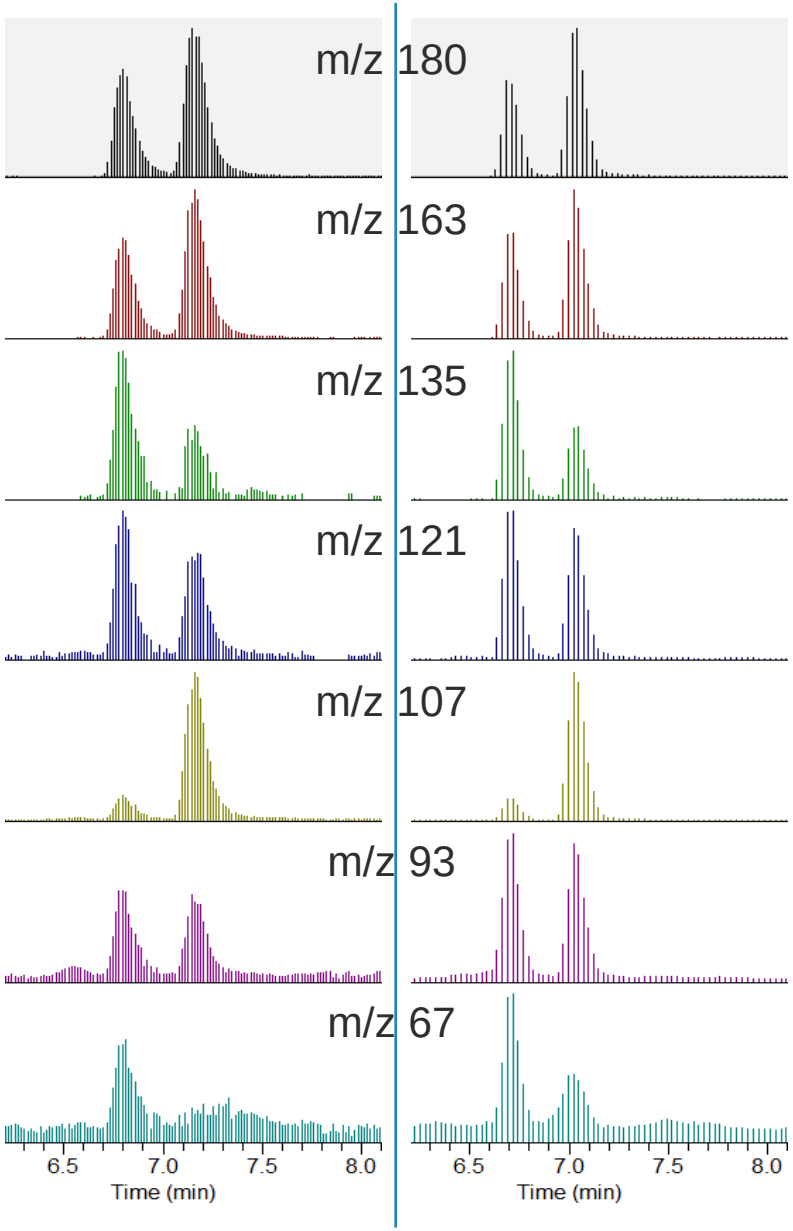
Data-independent = Swath = MS^E = wideband isolation = all ion fragmentation

Rimantadine and Memantine: 100 ng/mL



Method:

5 isolation windows



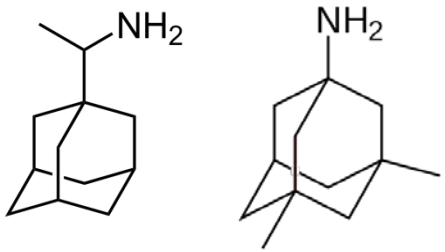
Precursor

Fragments 1-6

Method:

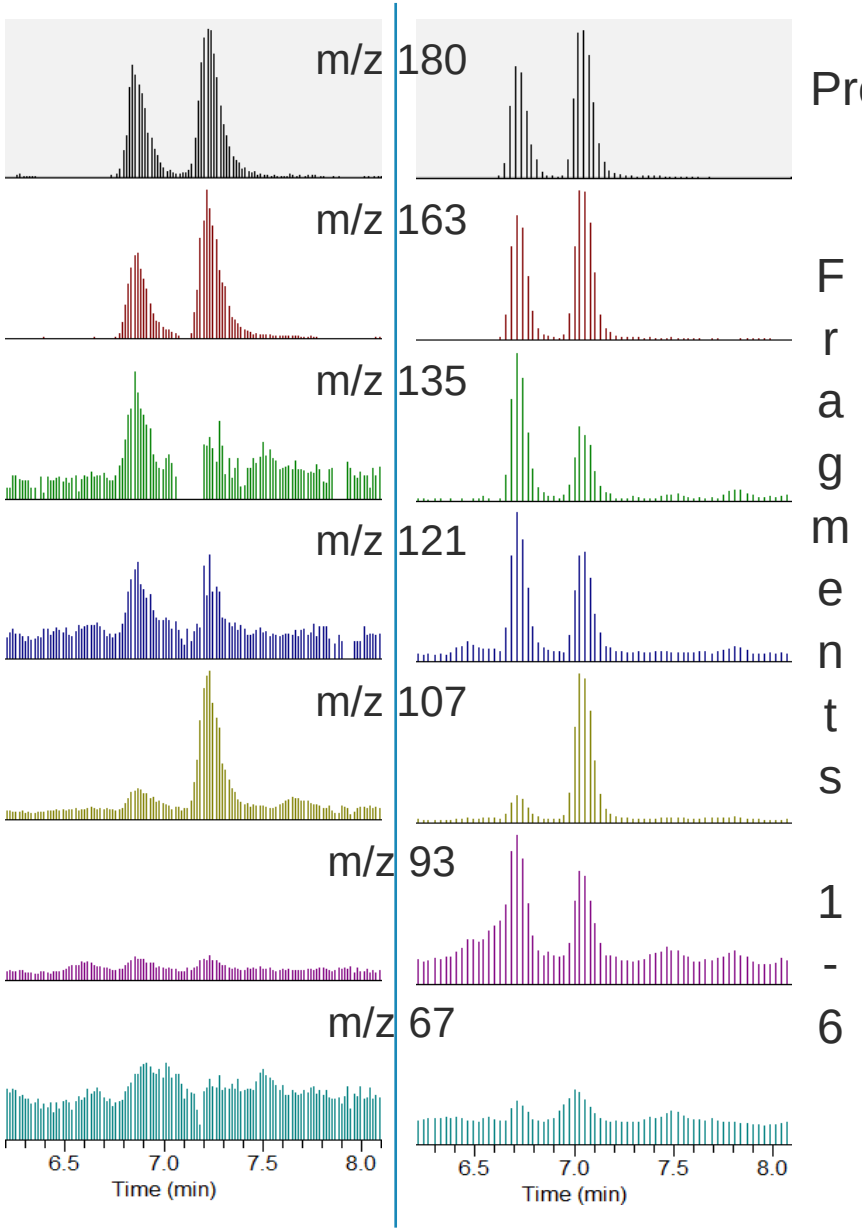
15 isolation windows

Rimantadine and Memantine: 10 ng/mL



Method:

5 isolation windows



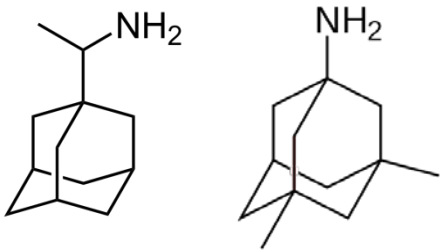
Precursor

Fragments 1-6

Method:

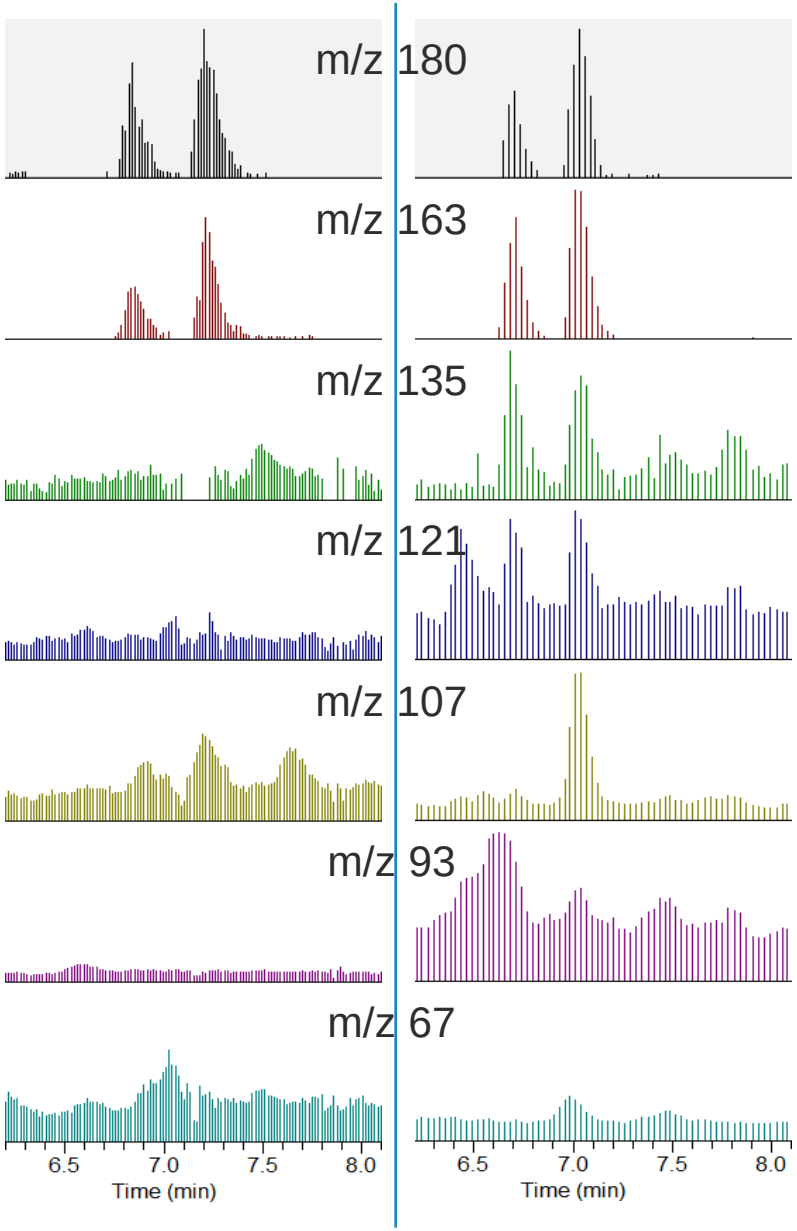
15 isolation windows

Rimantadine and Memantine: 1 ng/mL



Method:

5 isolation windows



Precursor

Fragments 1-6

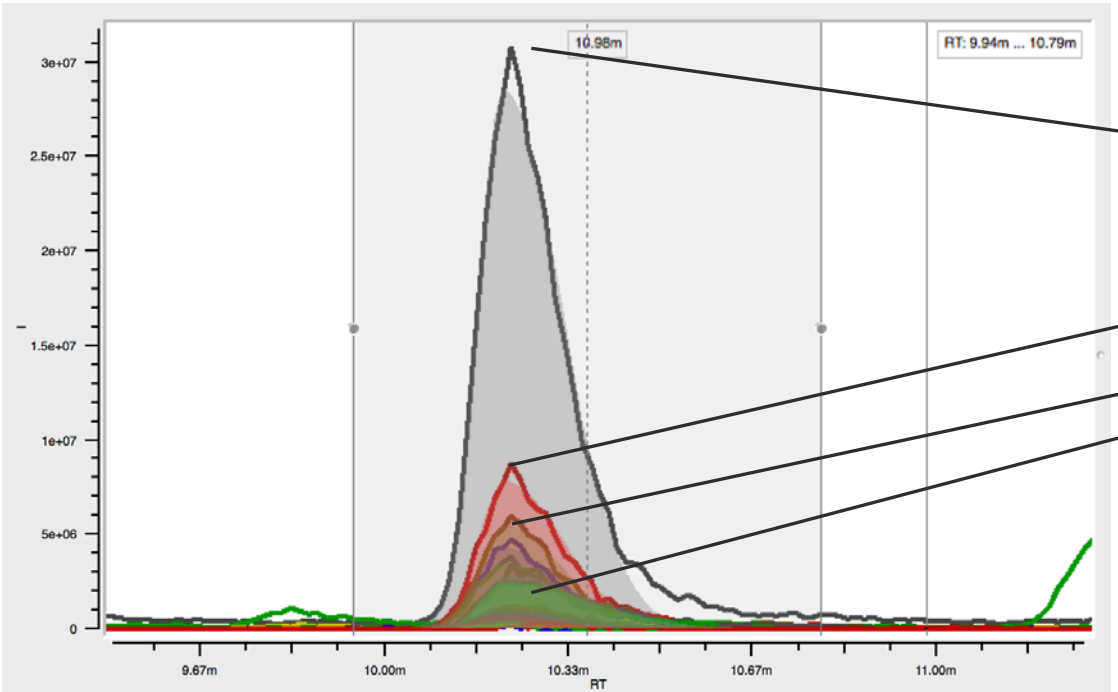
Method:

15 isolation windows

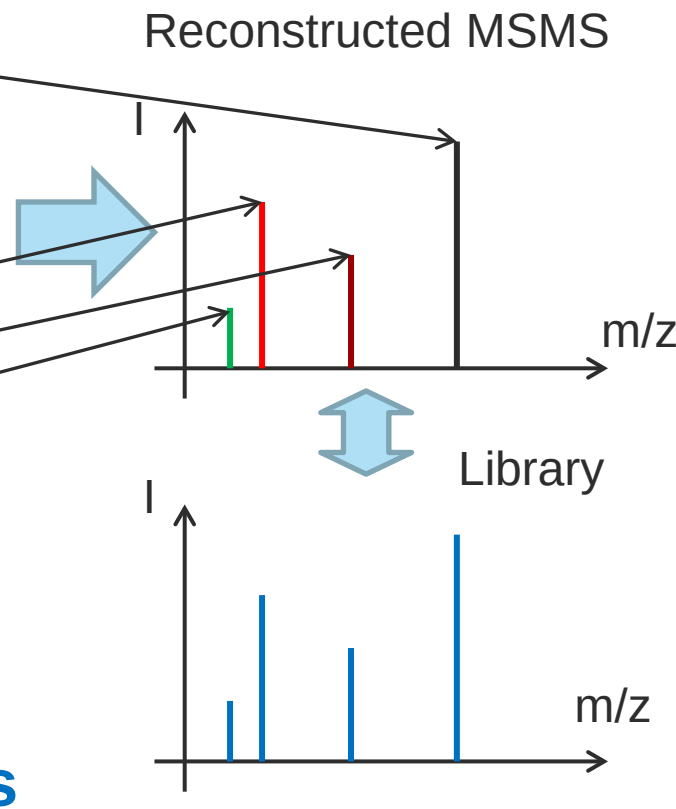
Reconstruct MS2 from DIA chromatograms

Coelution plot of precursor and fragments from DIA experiments

- reassigning precursor and fragment ions by peak shape fitting
- MS^2 reconstruction for library search

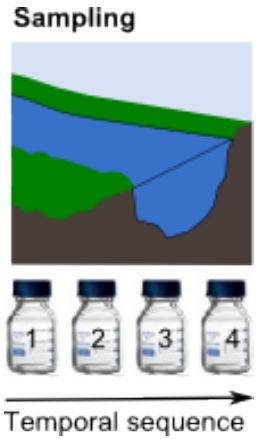


Martin Loos, Eawag und Uwe Schmitt, Scientific IT Services, ETH Zürich

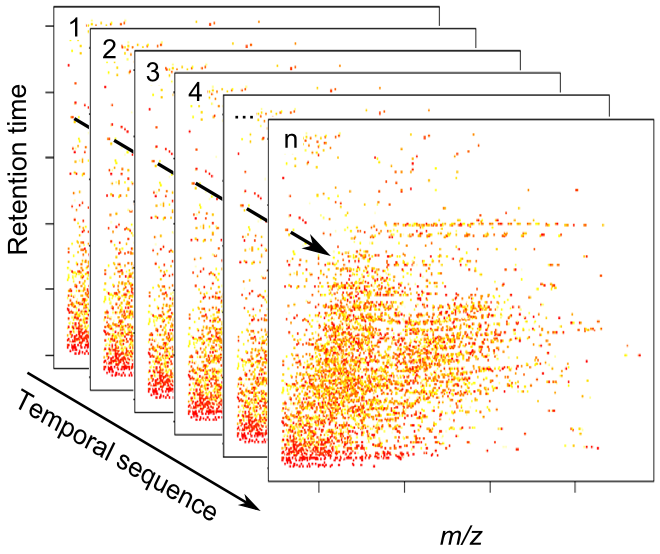


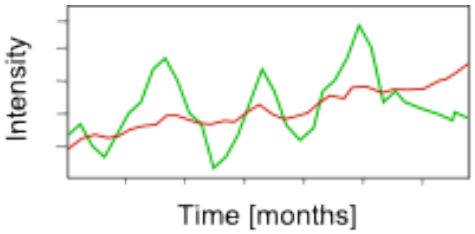
⇨ **many, narrow DIA isolation windows enhance selectivity and sensitivity**

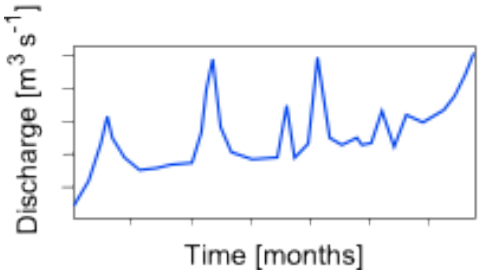
River monitoring – time series extraction

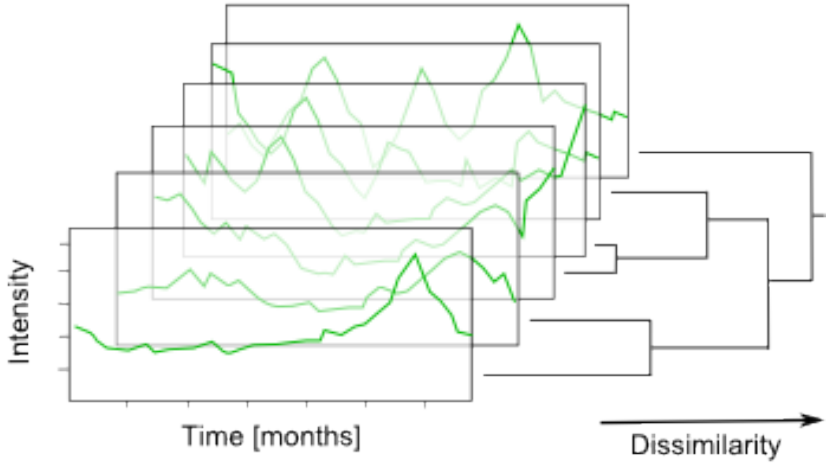


SPE (mixed layered cartridge)
LC (C18 reversed phase)
Orbitrap (MS 70k, 5dd MSMS 12k)









enviMass 2

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Project data | LC-HRMS files | Target, IS compounds | Parameters

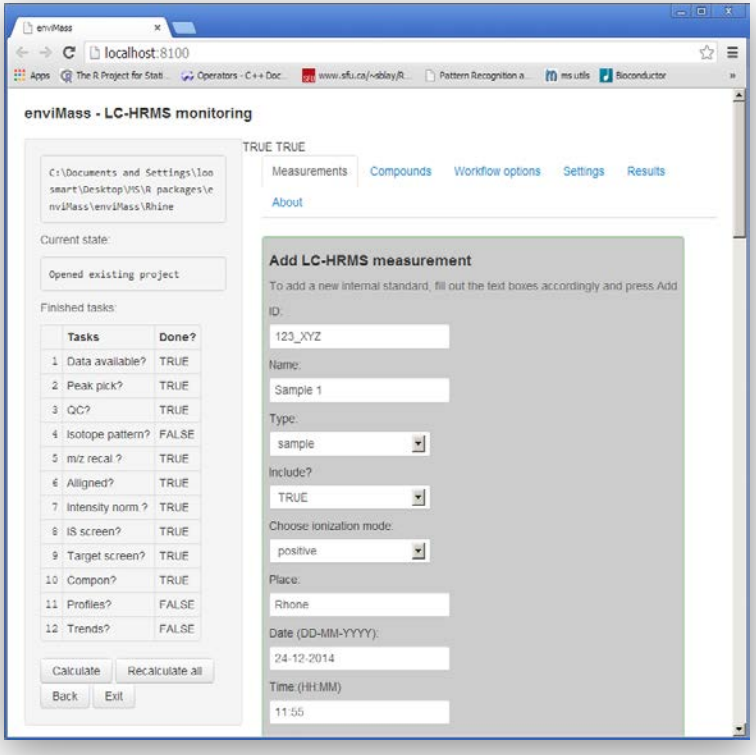
R Packages

- enviMass
- enviPick
- enviPat
- nontarget
- nontargetData
- shiny
- dtw
- …

C/C++

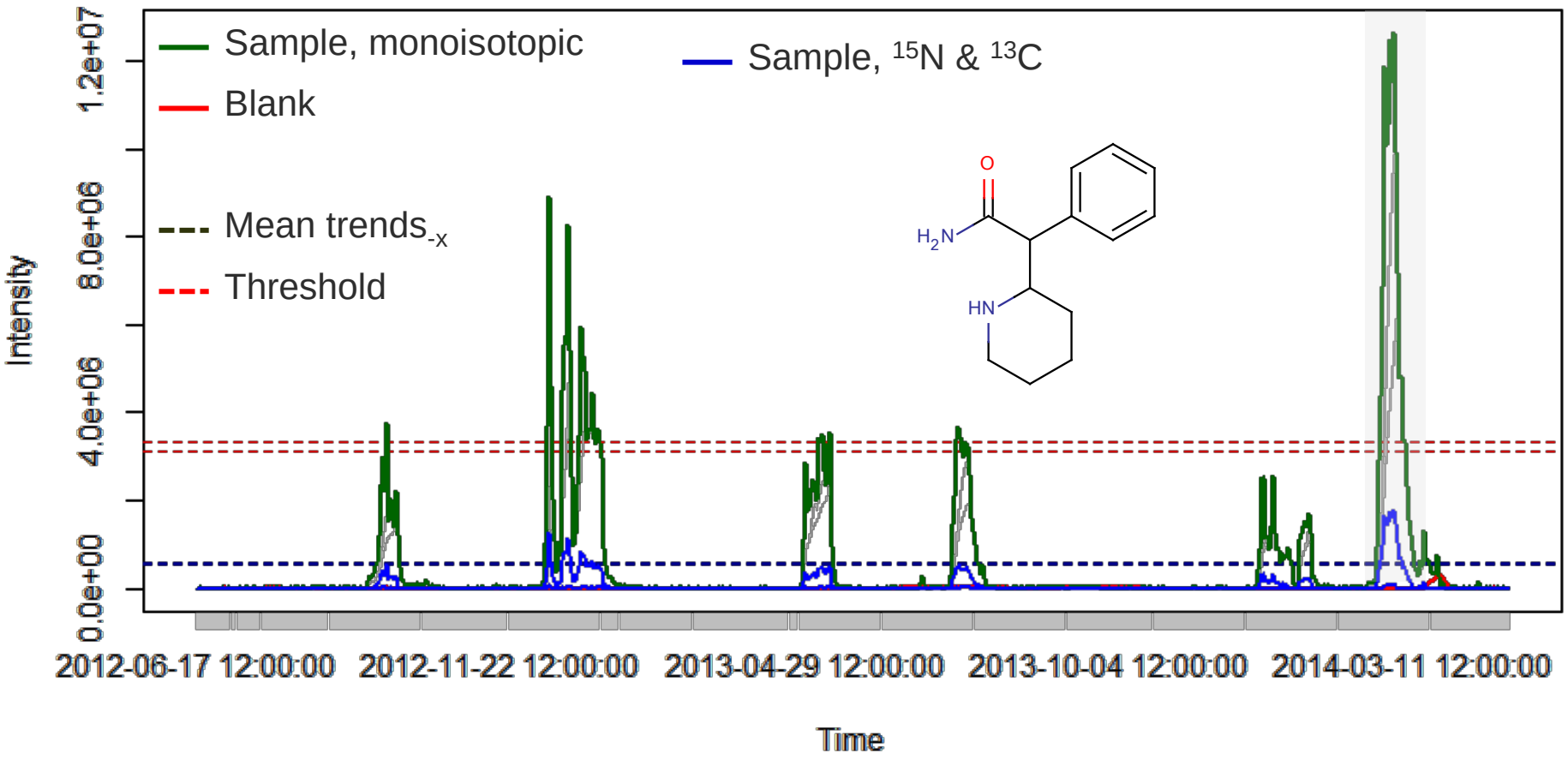
PW .mzXML

Browser: user interface



Package data | Adducts | Instrument resolution | nontargetData

2-Phenyl-2-(2-piperidin)acetamid



- Intense temporal trend March 2014 at *m/z*=219.1419
- Industrial emission source located, structure identified, quantification
- $C_{13}H_{18}N_2O$: temporal ^{15}N- and ^{13}C-profiles found

Ideas, gaps and future challenges

Experimental approaches for structure elucidation:

- Multi fragmentation mode: DD and DIA, CID and HCD
- Different ionization techniques for the identification of functional groups: ESI, APCI, APPI, APGC, derivatization
- Advanced chromatographic resolution: LCxLC, UHPLC, micro/nano LC, multi-modal LC (IC, HILIC,RP)
- Orthogonal analytical techniques: ion mobility, NMR
- H/D exchange experiments: post-column infusion of D_2O

Software approaches:

- storage and processing ‘non-target’ pipeline in the cloud with standardized/comprehensive target and suspect lists (library) tailored to environmental needs: Norman cloud server

Thanks for your attention!

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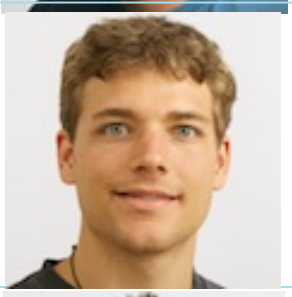
River Rhine,
Non-target

Martin Loos
R package developer,
enviMass

Philipp Longree
Target Screening
Quantification

Emma Schymanski
RMassbank,
structure generation



Ch. Moschet
Passive sampler
Suspect screening

Juliane Hollender
Department head,
biotransformation

Michele Stravs
NanoLC,
Non-target

FOEN – Financial support
Thermo – Software support

Free Isotope Pattern Calculator: http://www.envipat.eawag.ch