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Zurich^{UZH}

Zurich Institute of Forensic Medicine

New trends in mass spectrometry in forensic pharmacology and toxicology

II Международная научно-практическая конференция
«Современная химико-токсикологическая экспертиза»

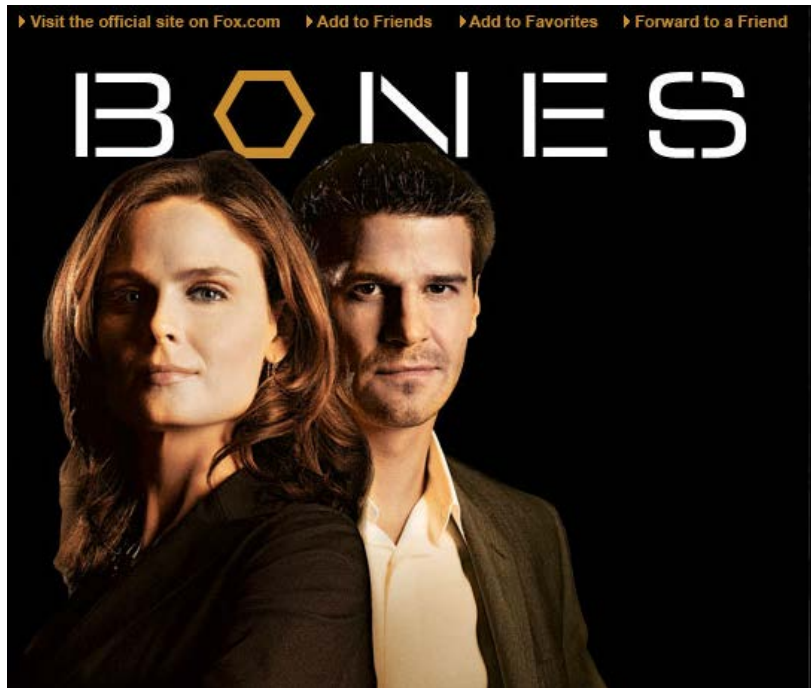
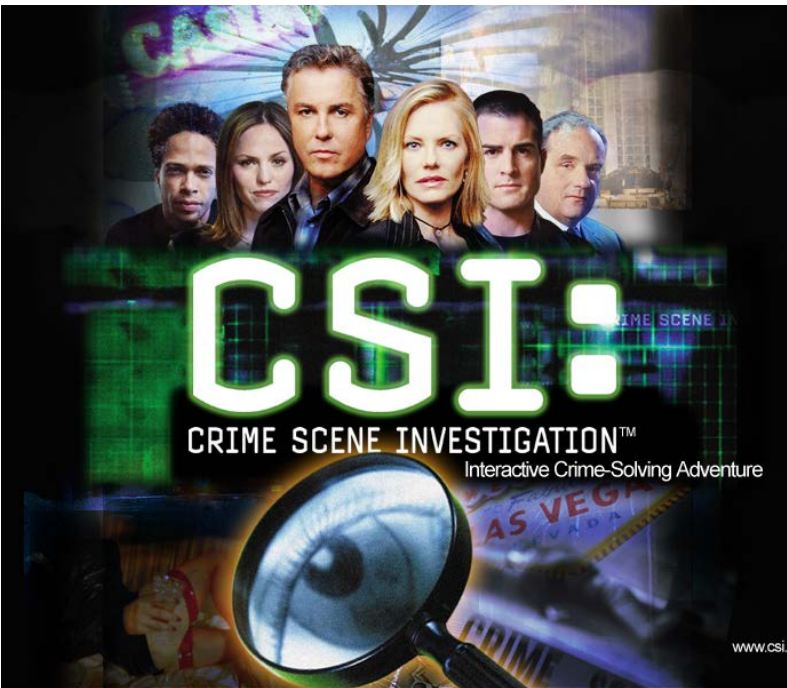
II International scientific conference АСТЕ'2015

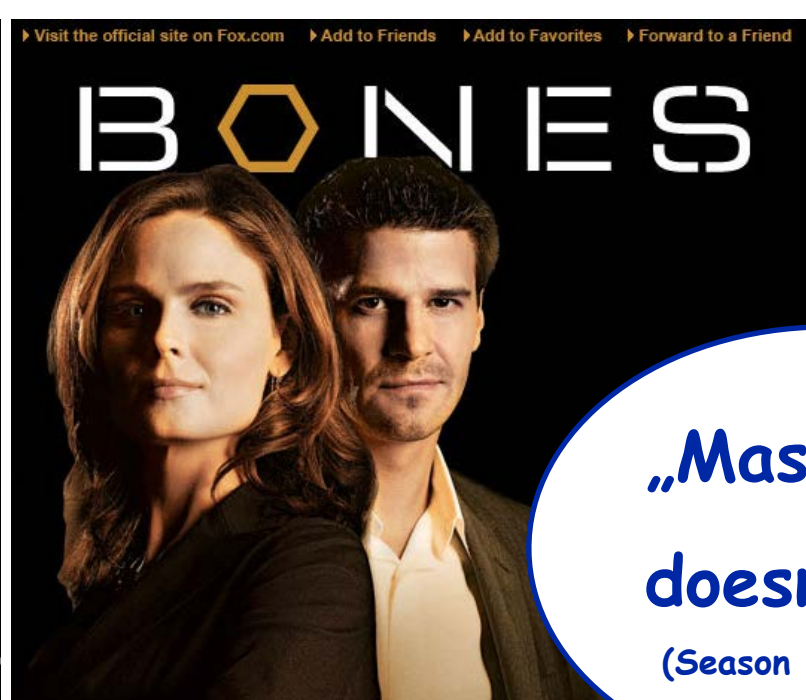
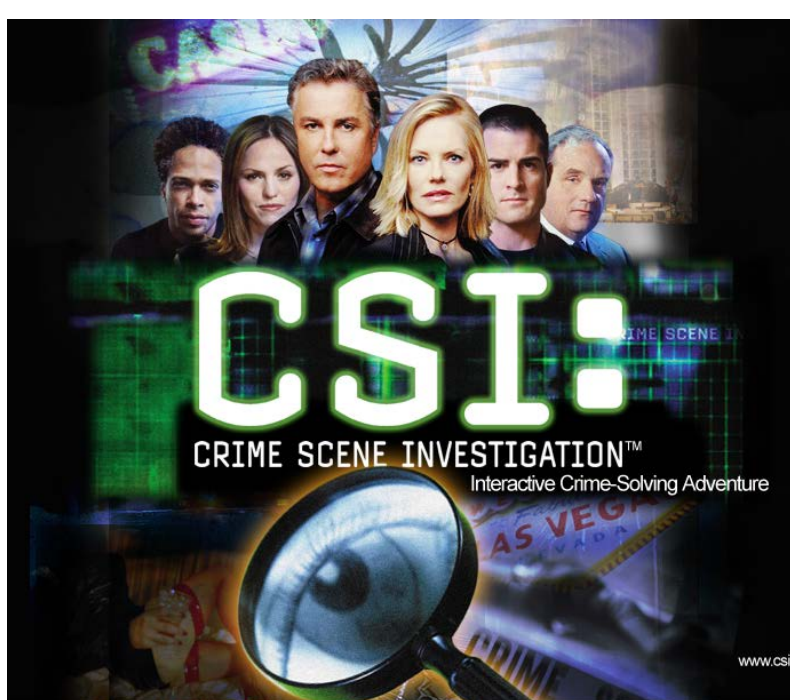
06 - 07 октября 2015 г., Москва

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„Mass Spec
doesn't lie!“
(Season 1 Episode 1)





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Mass Spectrometry is Everywhere





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Finding the Needle in the Haystack



Finding the Needle in the Haystack

.... is pretty easy:
use a big magnet ...



.... or simply burn it down!



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Finding the Needle in the Haystack

.... is pretty difficult
(in forensic toxicology)



blood/urine



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Finding the Needle in the Haystack

.... is pretty difficult
(in forensic toxicology)



blood/urine

hair



oral fluid





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Finding the Needle in the Haystack

.... is pretty difficult
(in forensic toxicology)



blood/urine

hair



oral fluid



nails



(rotten) tissues





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Finding the Needle in the Haystack

.... is pretty difficult
(in forensic toxicology)



blood/urine

hair



oral fluid



nails



(rotten) tissues



exhaled air



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Finding the Needle in the Haystack



**Holy Grail in Forensic Toxicology:
General Unknown Analysis (GUA)
or
Systematic Toxicological Analysis (STA)**



LC- MS Screenings in Forensic Toxicology

LC- MS/MS Screening

Targeted Approach

Multiple Reaction Monitoring

Q_1 window = 0.2-1 Da



LC- MS Screenings in Forensic Toxicology

LC- MS/MS Screening

Targeted Approach

Multiple Reaction Monitoring

Q_1 window = 0.2-1 Da

Not a general unknown screening



LC- MS Screenings in Forensic Toxicology

LC- MS/MS Screening

Targeted Approach

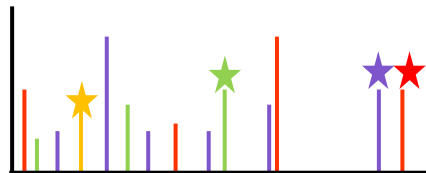
Multiple Reaction Monitoring

Q_1 window = 0.2-1 Da

Untargeted Approach

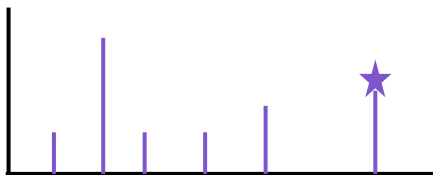
Fullscan, MS^{ALL}

Q_1 window = total transmission



Information Dependent Acquisition (IDA)

Q_1 window = 0.2-1 Da





LC- MS Screenings in Forensic Toxicology

LC- MS/MS Screening

Targeted Approach

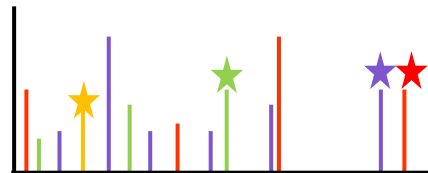
Multiple Reaction Monitoring

Q_1 window = 0.2-1 Da

Untargeted Approach

Fullscan, MS^{ALL}

Q_1 window = total transmission



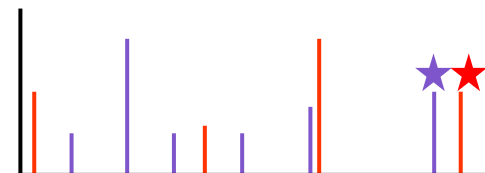
Information Dependent
Acquisition (IDA)

Q_1 window = 0.2-1 Da



Sequential Window Acquisition of
all Theoretical Ions (SWATH)

Q_1 window = 20 Da





LC- MS Screenings in Forensic Toxicology

LC- MS/MS Screening

Targeted Approach

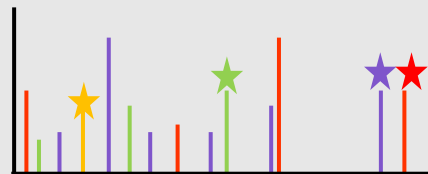
Multiple Reaction Monitoring

Q_1 window = 0.2-1 Da

Untargeted Approach

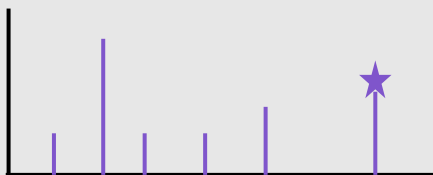
Fullscan, MS^{ALL}

Q_1 window = total transmission



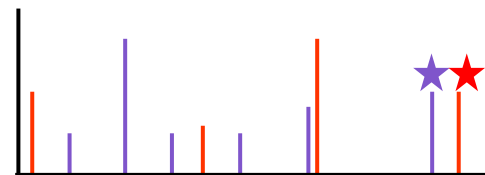
Information Dependent
Acquisition (IDA)

Q_1 window = 0.2-1 Da



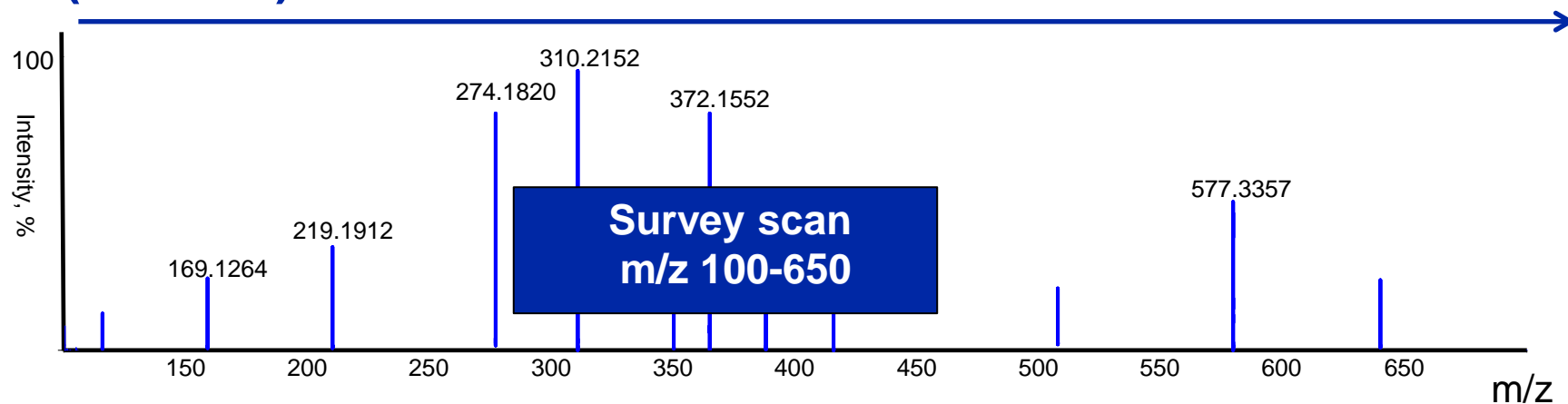
Sequential Window Acquisition of
all Theoretical Ions (SWATH)

Q_1 window = 20 Da

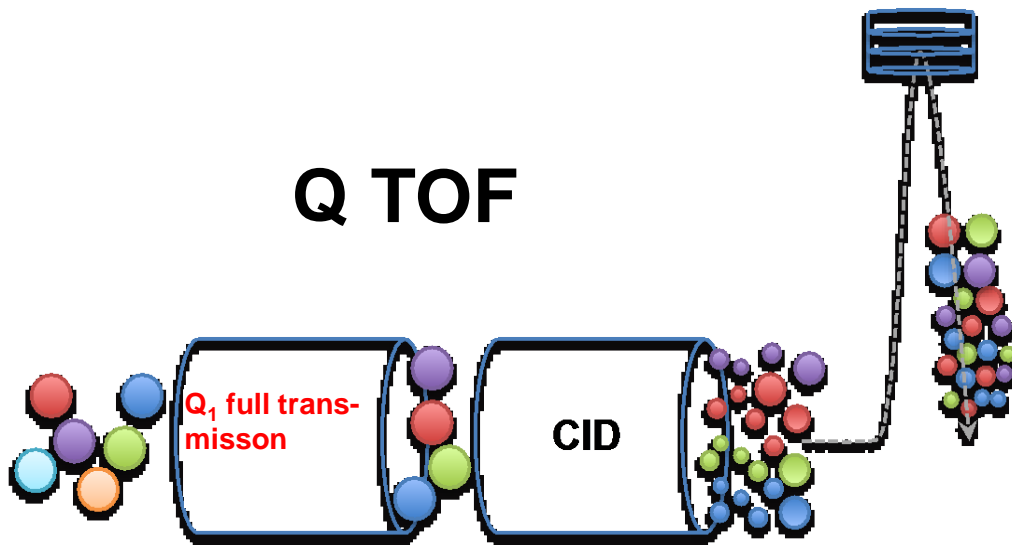




Sequential Window Acquisition of All Theoretical Ions (SWATH)

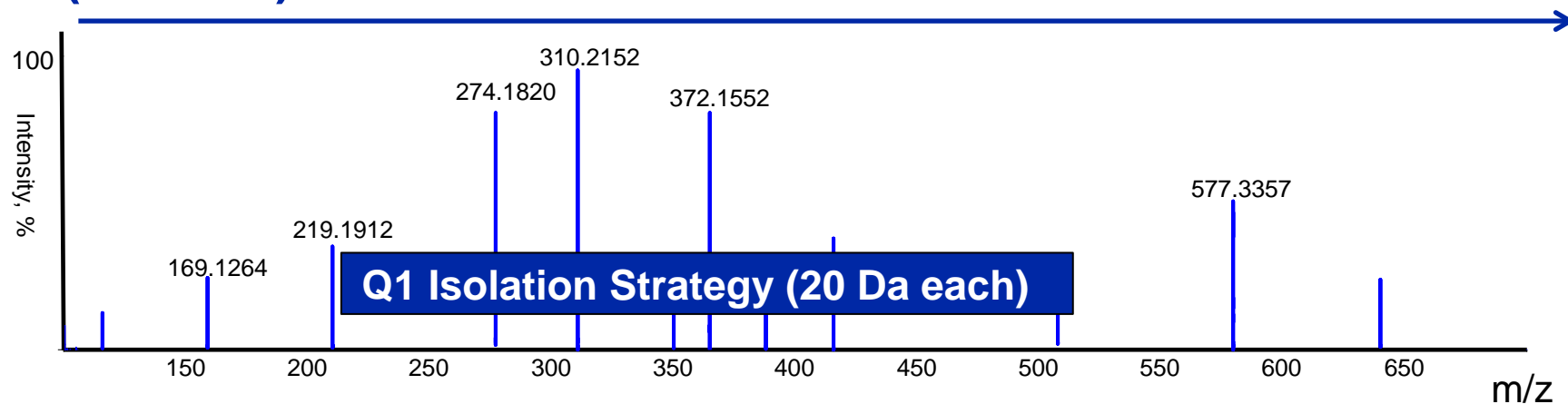


Q TOF

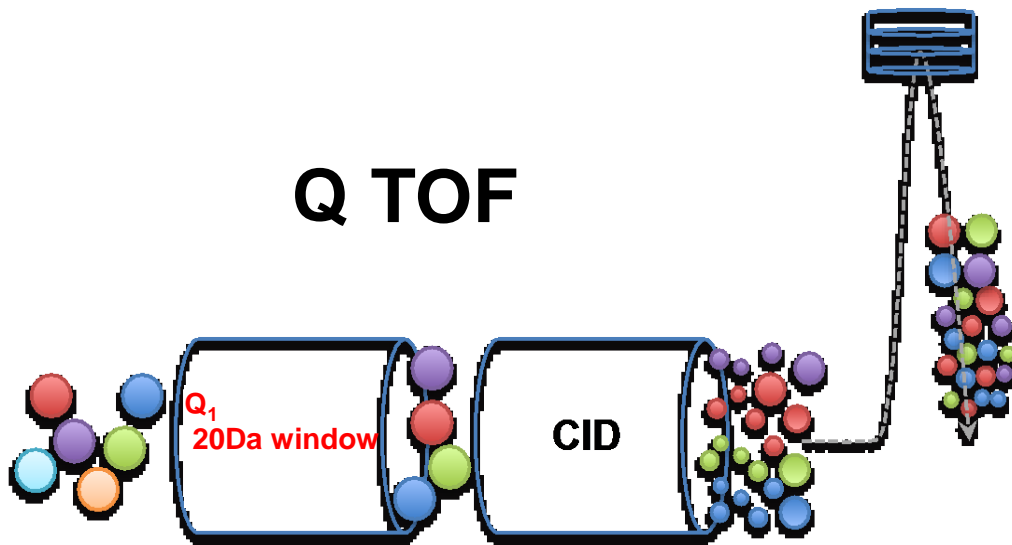




Sequential Window Acquisition of All Theoretical Ions (SWATH)

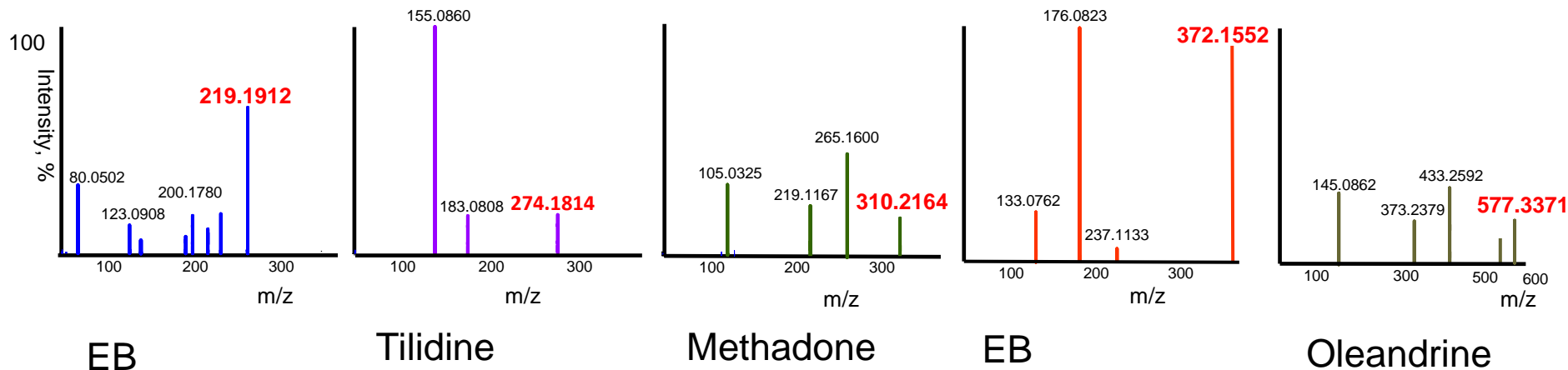
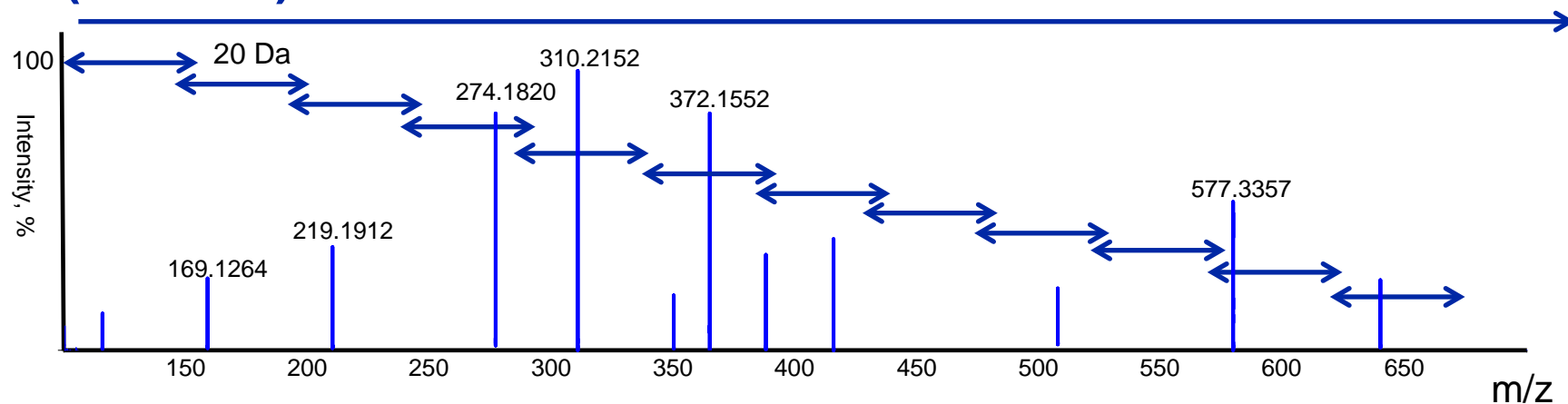


Q TOF



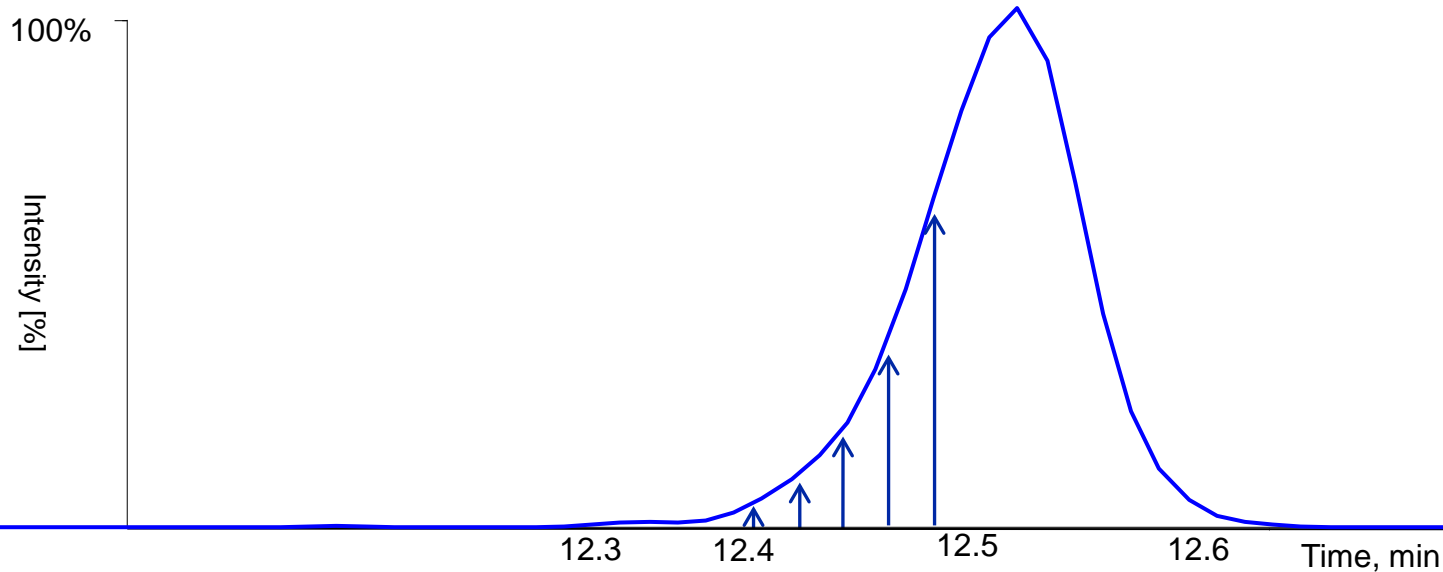
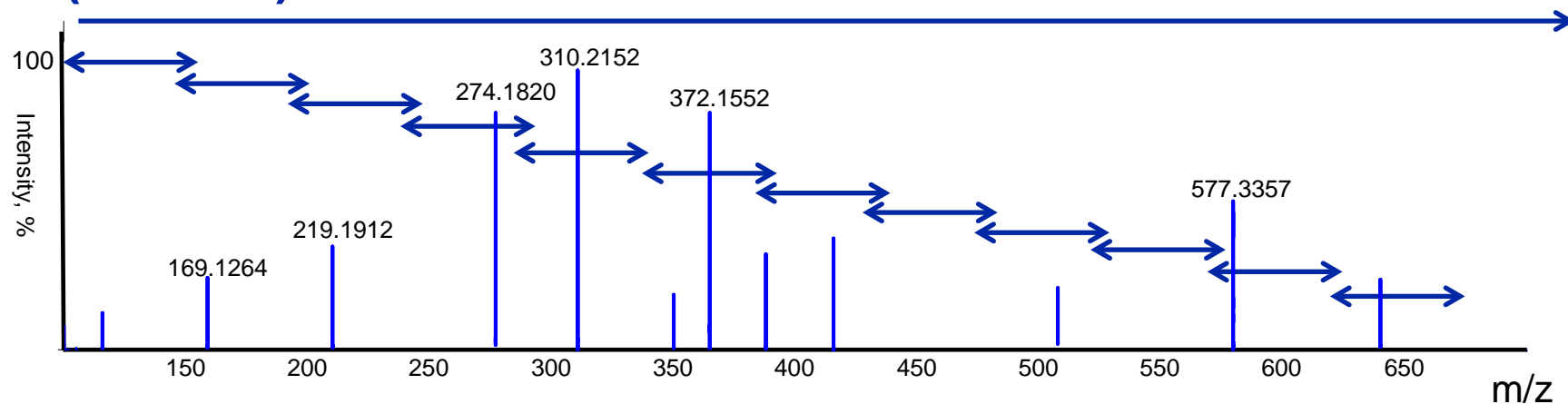


Sequential Window Acquisition of All Theoretical Ions (SWATH)



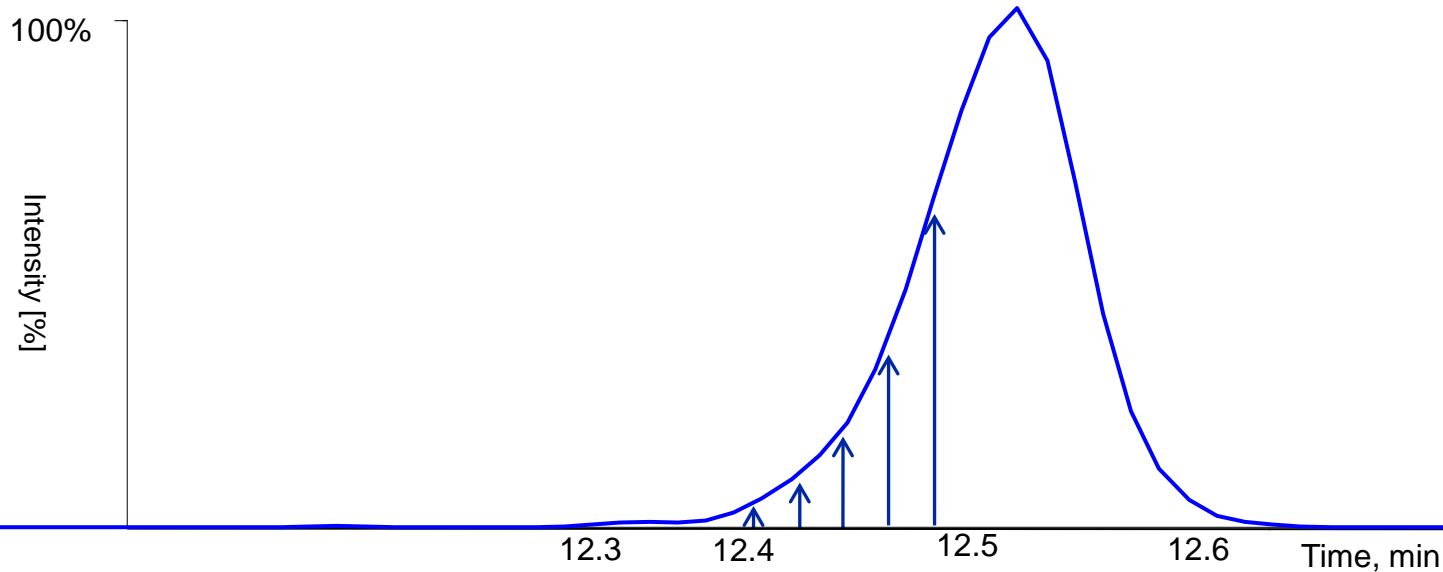
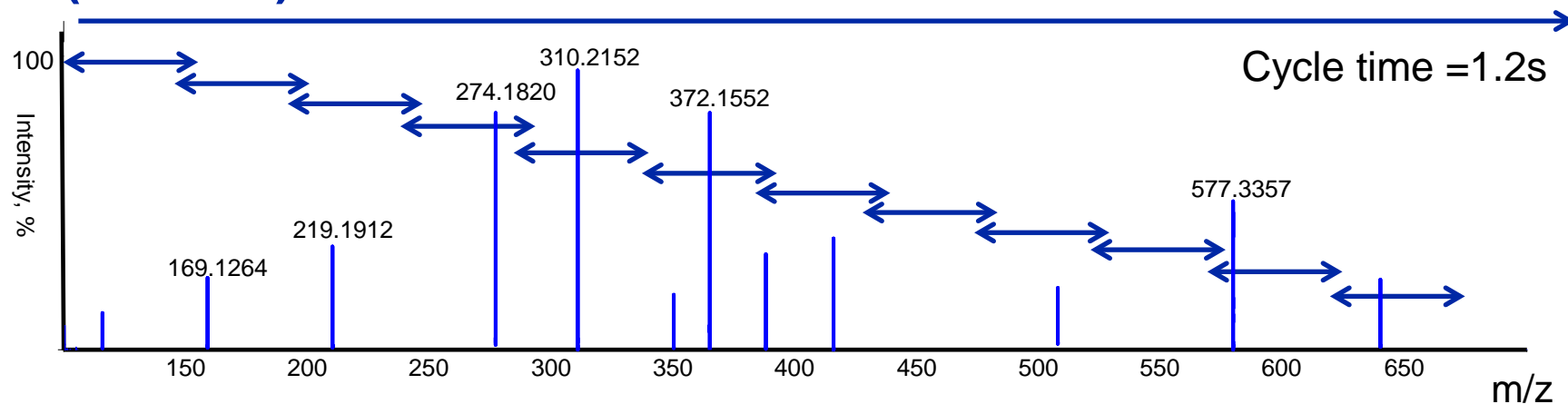


Sequential Window Acquisition of All Theoretical Ions (SWATH)



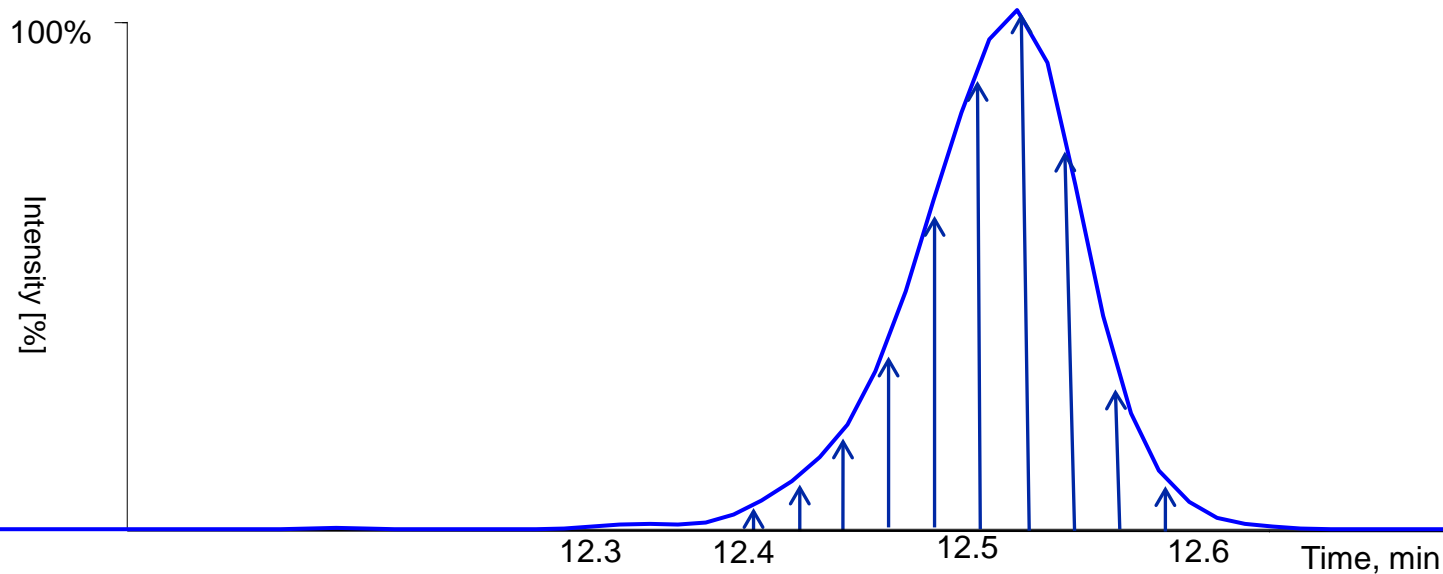
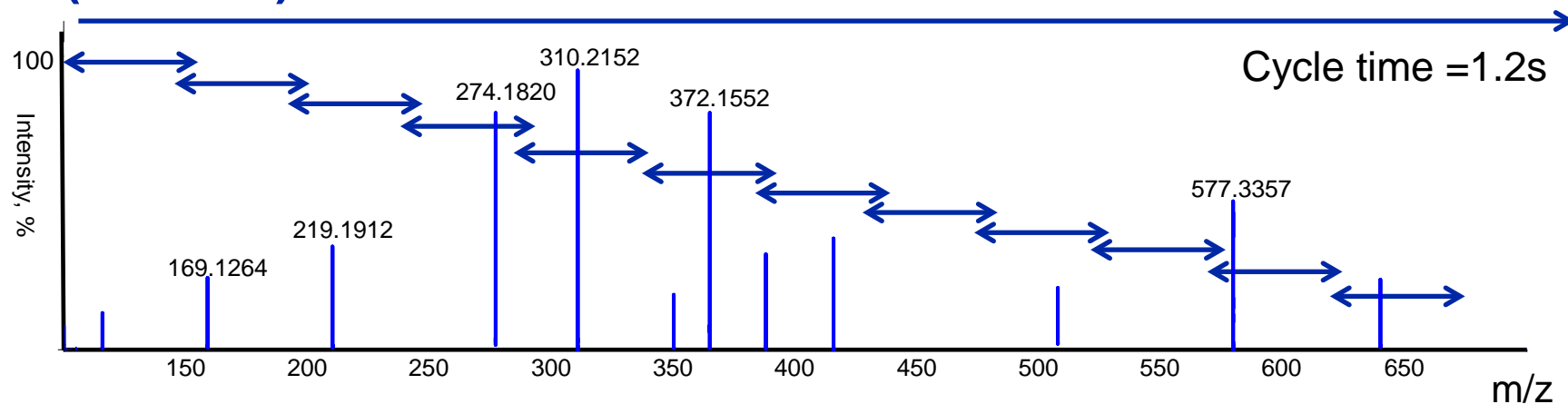


Sequential Window Acquisition of All Theoretical Ions (SWATH)



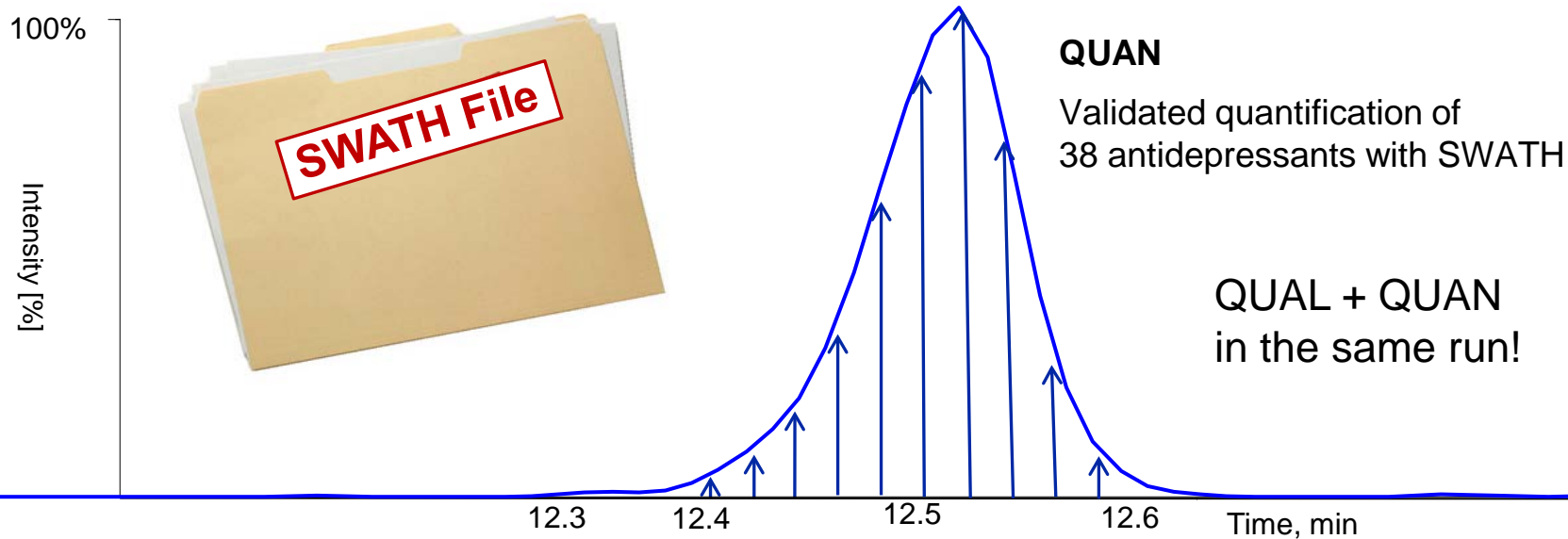
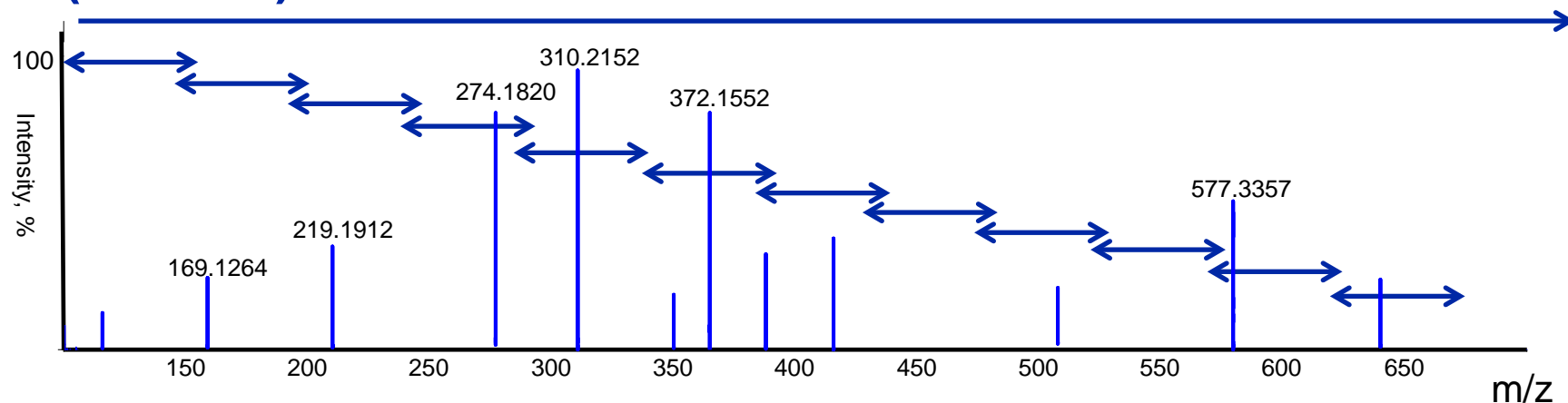


Sequential Window Acquisition of All Theoretical Ions (SWATH)



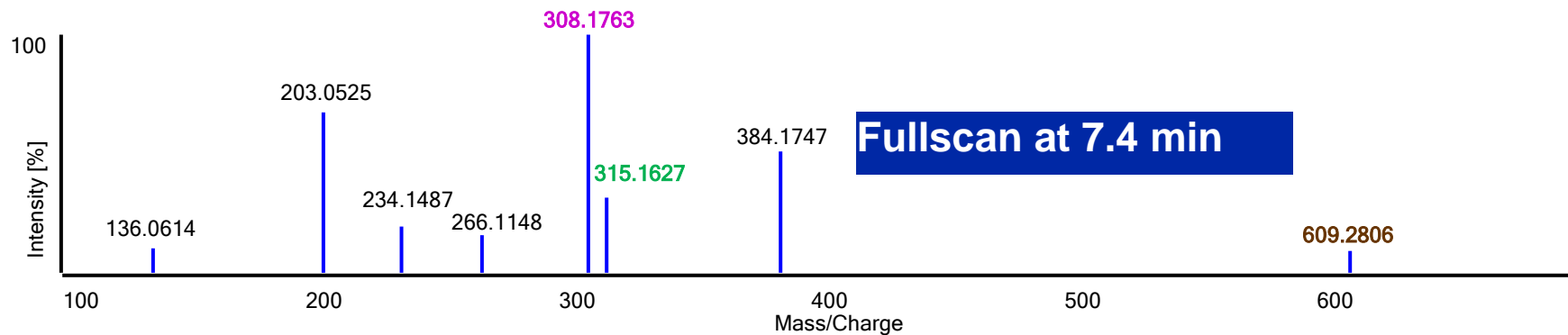
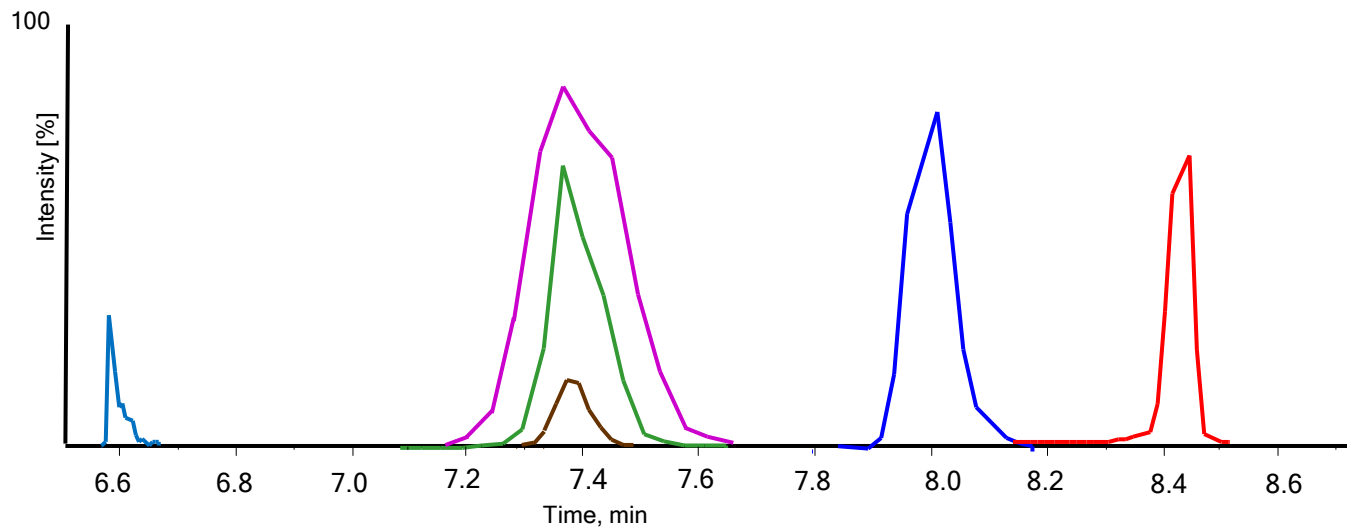


Sequential Window Acquisition of All Theoretical Ions (SWATH)



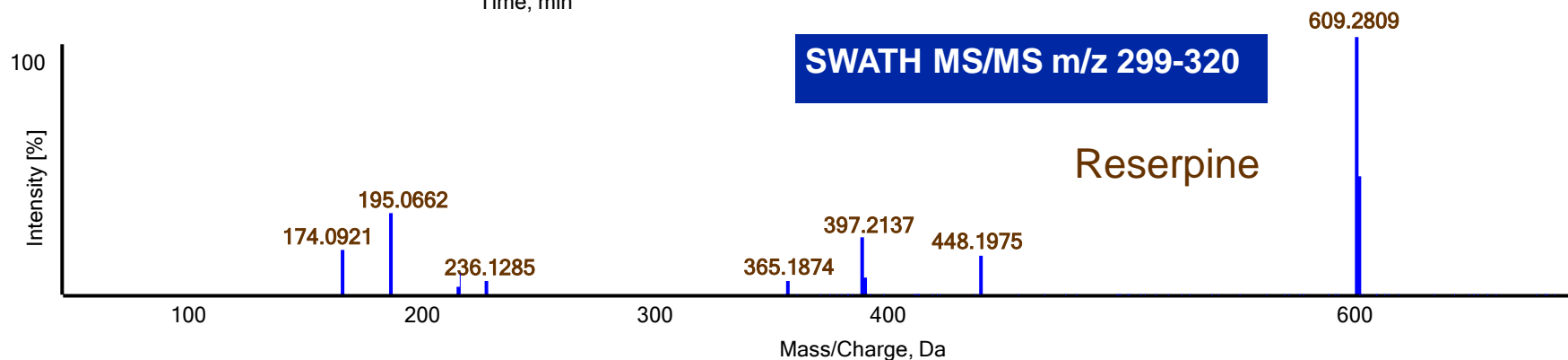
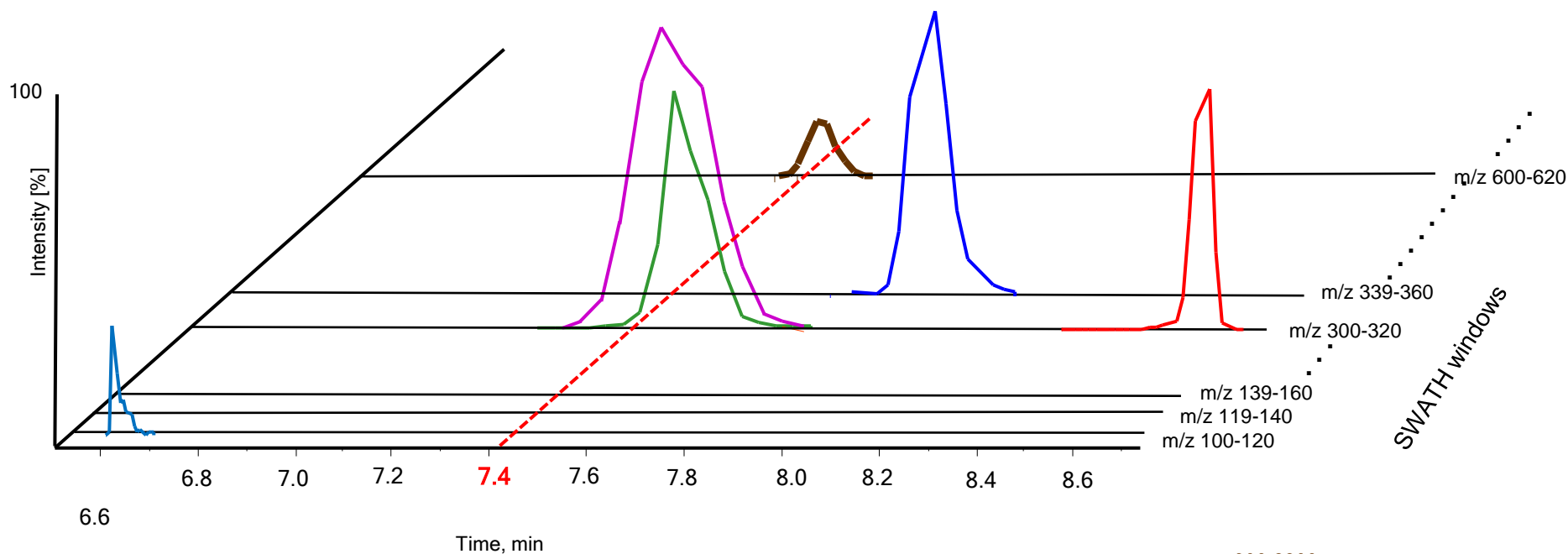


SWATH Application



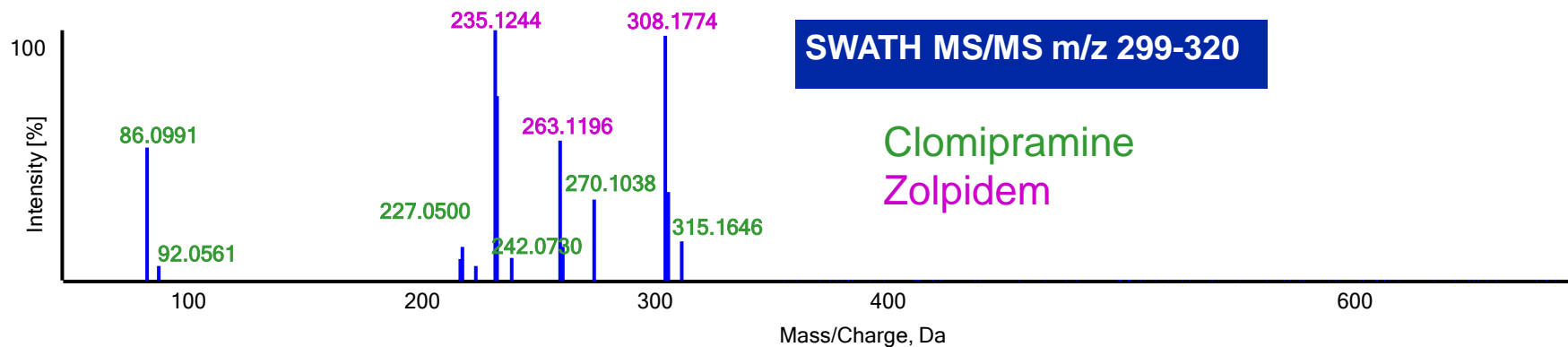
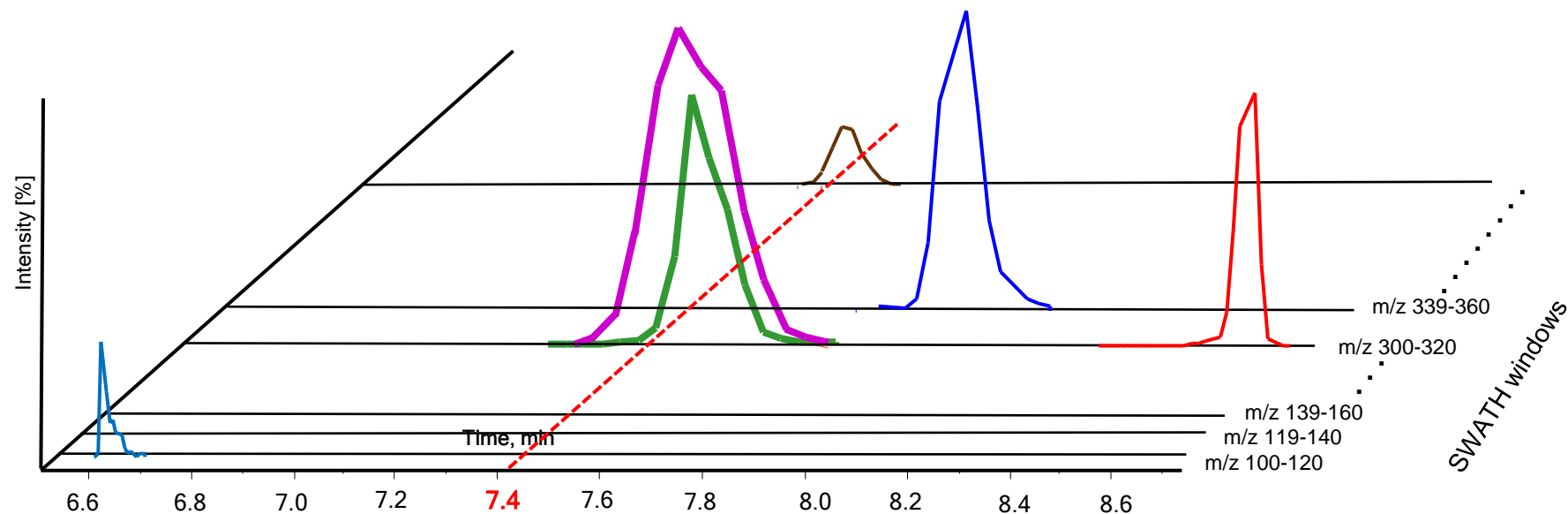


SWATH Application



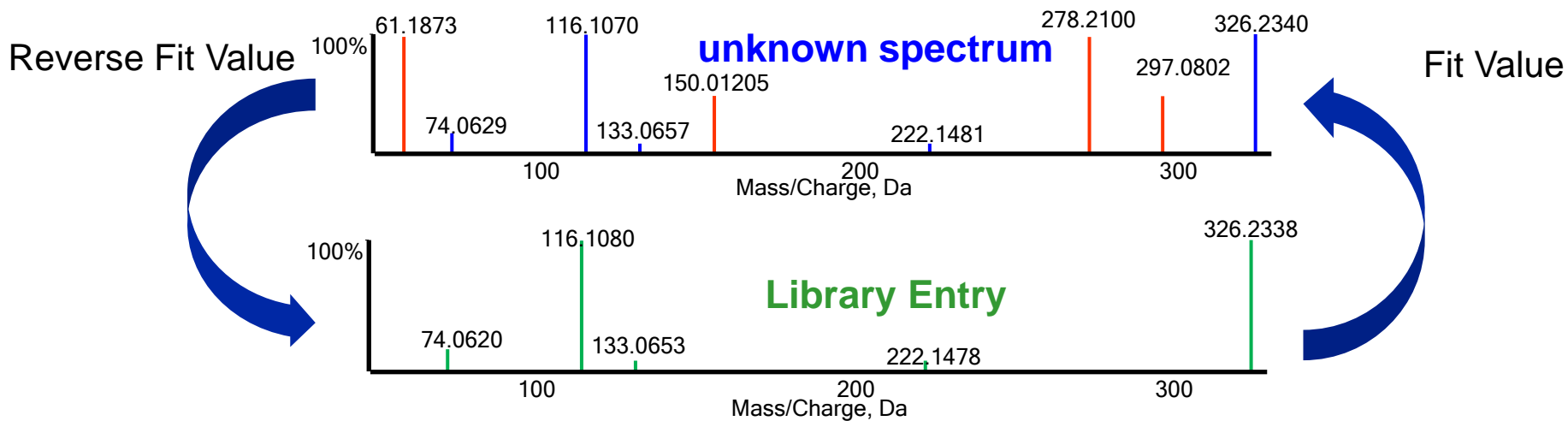


SWATH Application

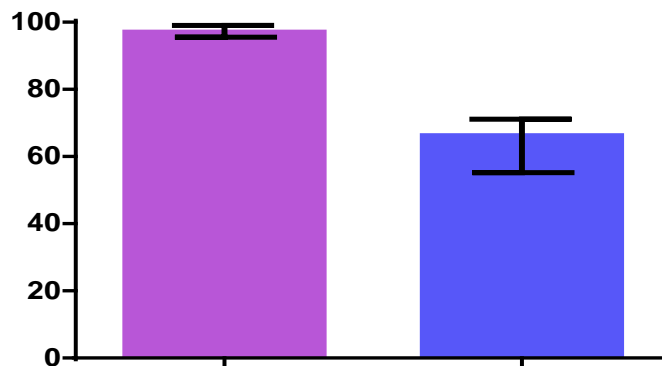




Library Match Quality



Diazepam M (Nor-)



fit

reverse fit

fit

reverse fit

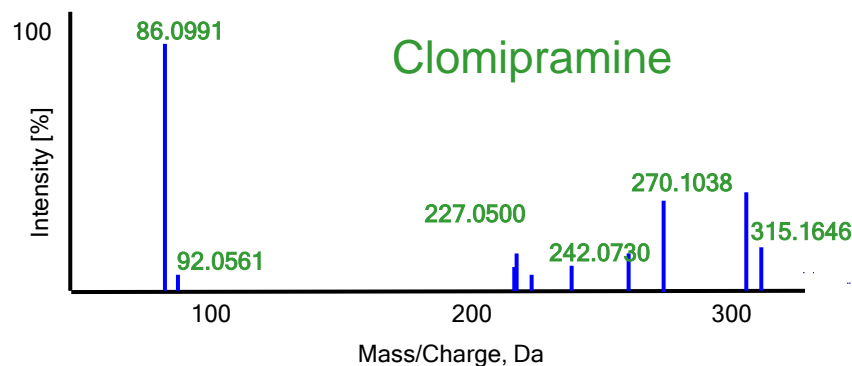
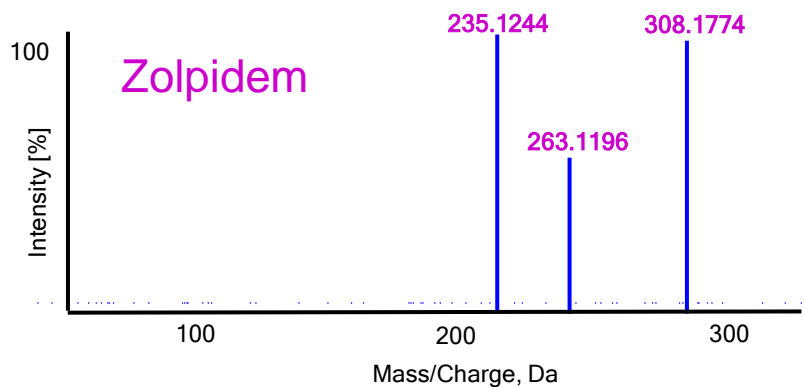
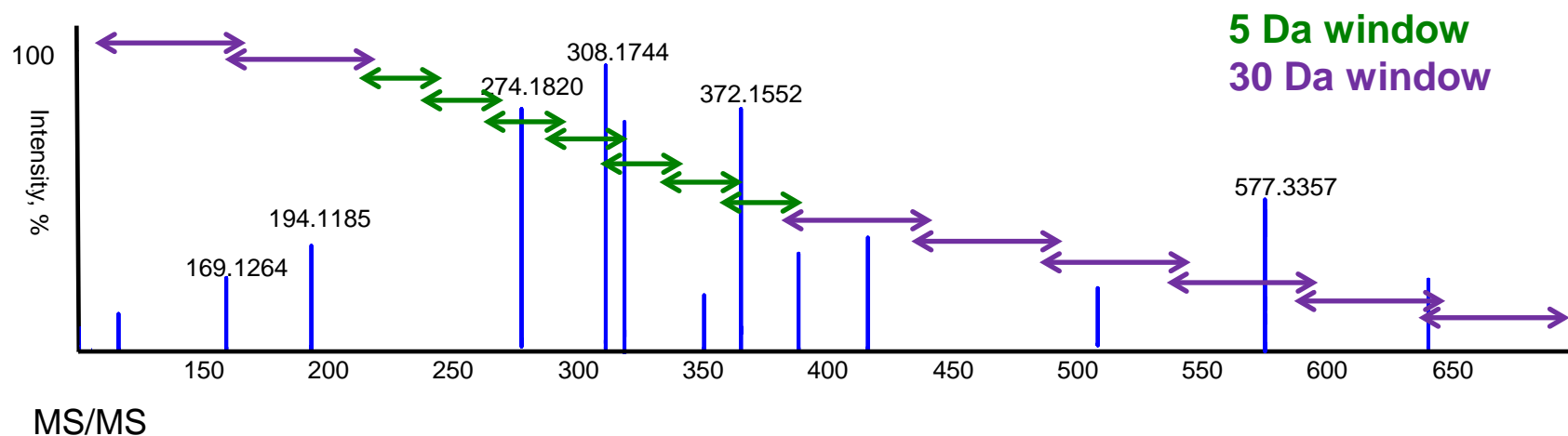
fit

reverse fit

match [%]

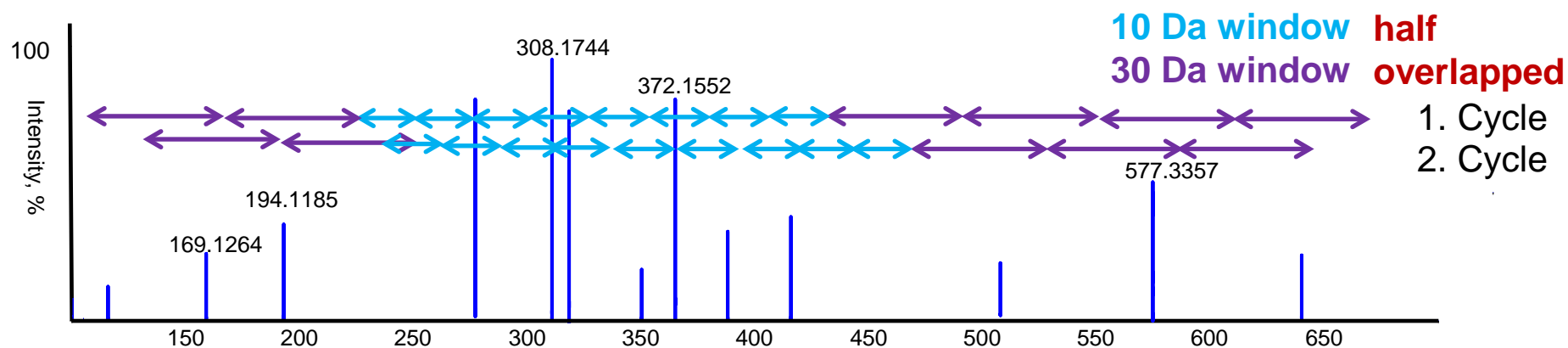


Variable windows in SWATH MS/MS



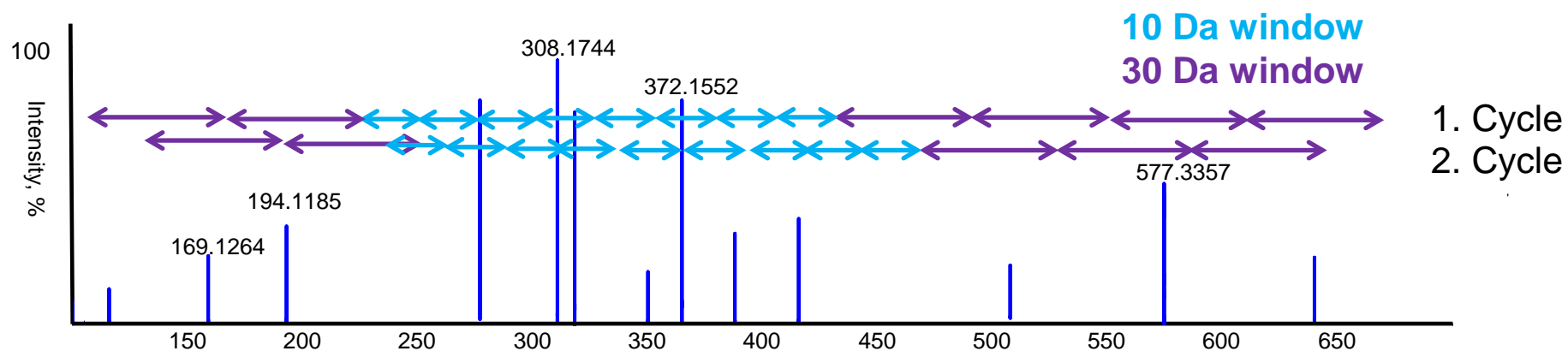


Multiplexed windows in SWATH MS/MS

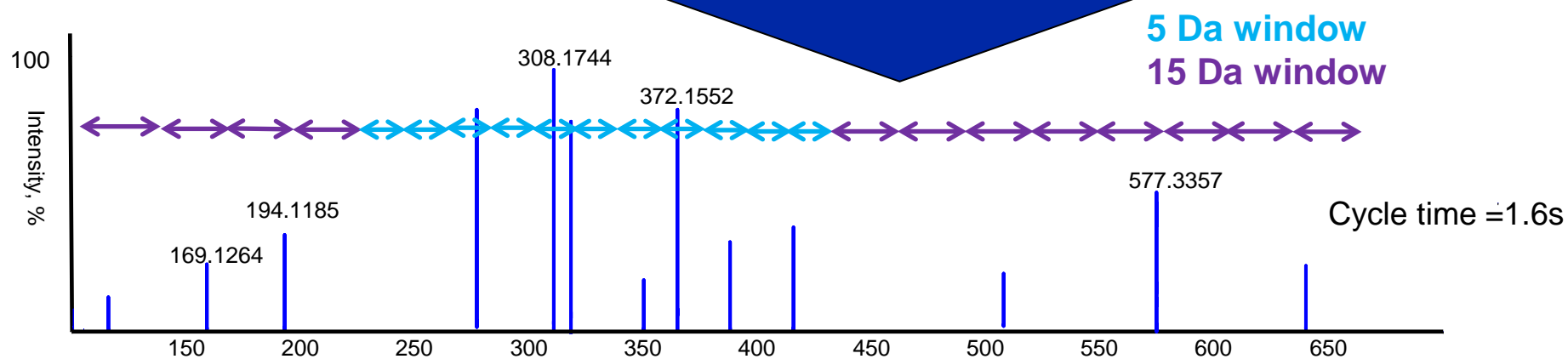




Multiplexed windows in SWATH MS/MS

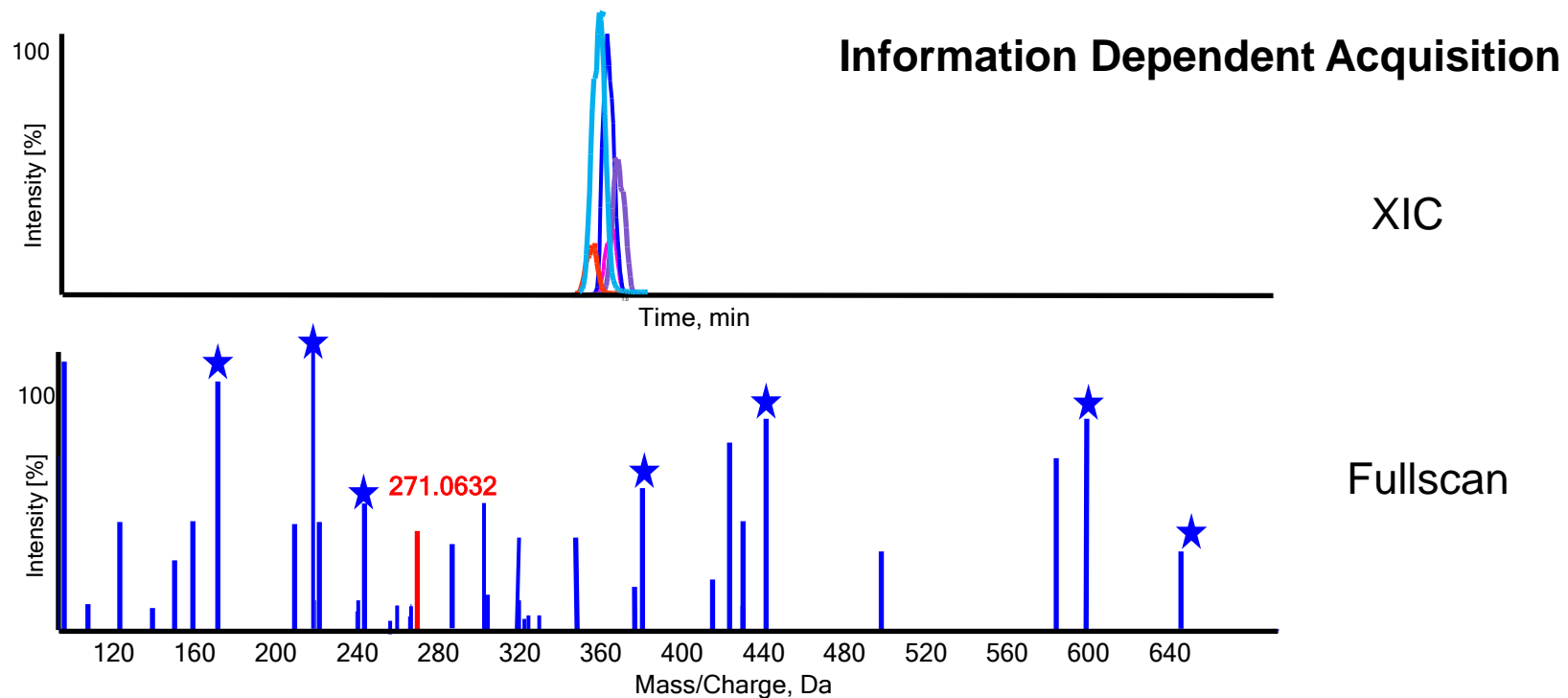


MS/MS



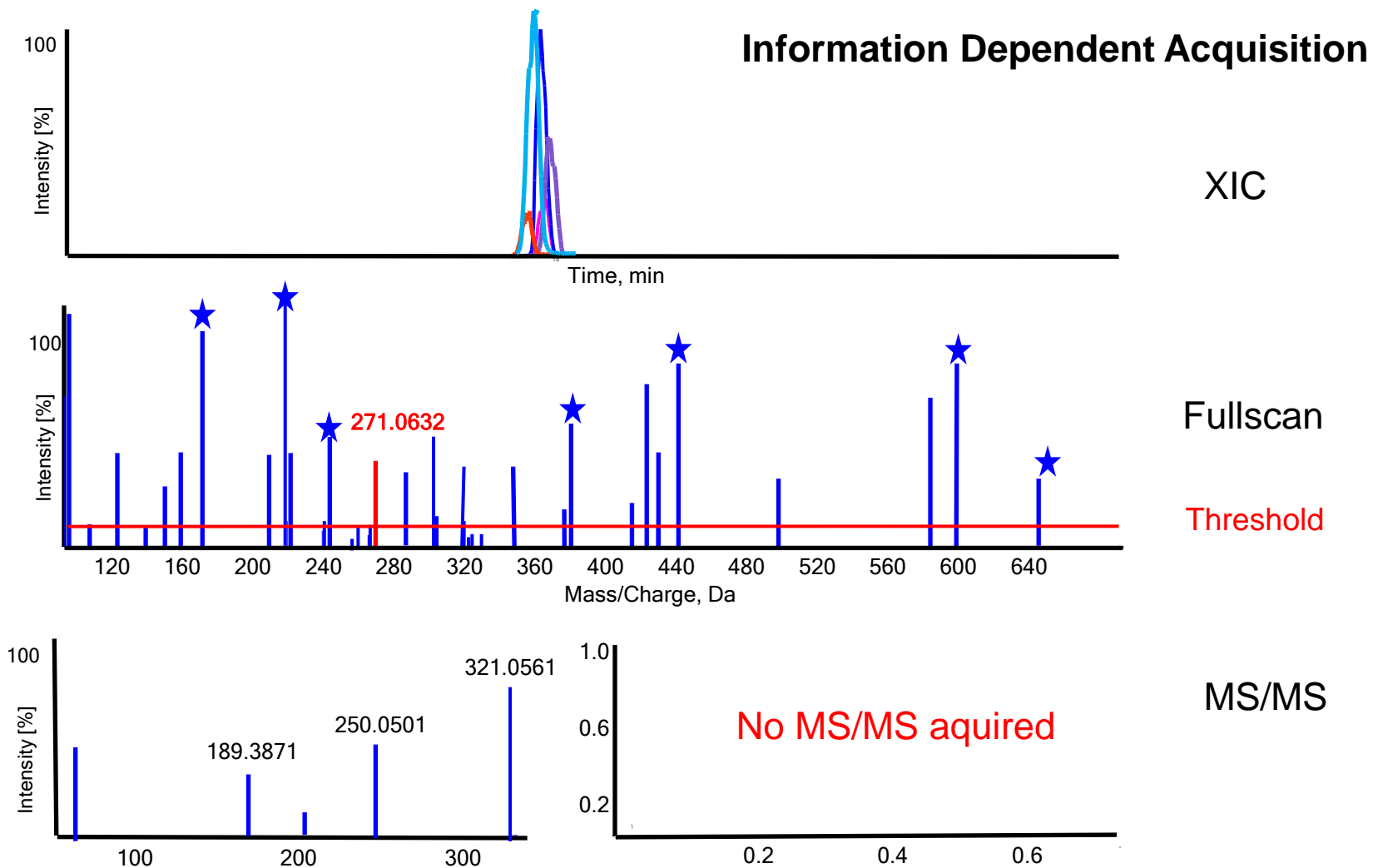


SWATH vs. IDA Acquisition





SWATH vs. IDA Acquisition





SWATH vs. IDA Acquisition

samples



HPLC



**MS
settings**

IDA

TOF MS 100 ms
IDA 75 ms
exclusion time: 6s
min. intensity: 100 cps
isotope exclusion: off

7 IDA experiments
10 IDA experiments
20 IDA experiments

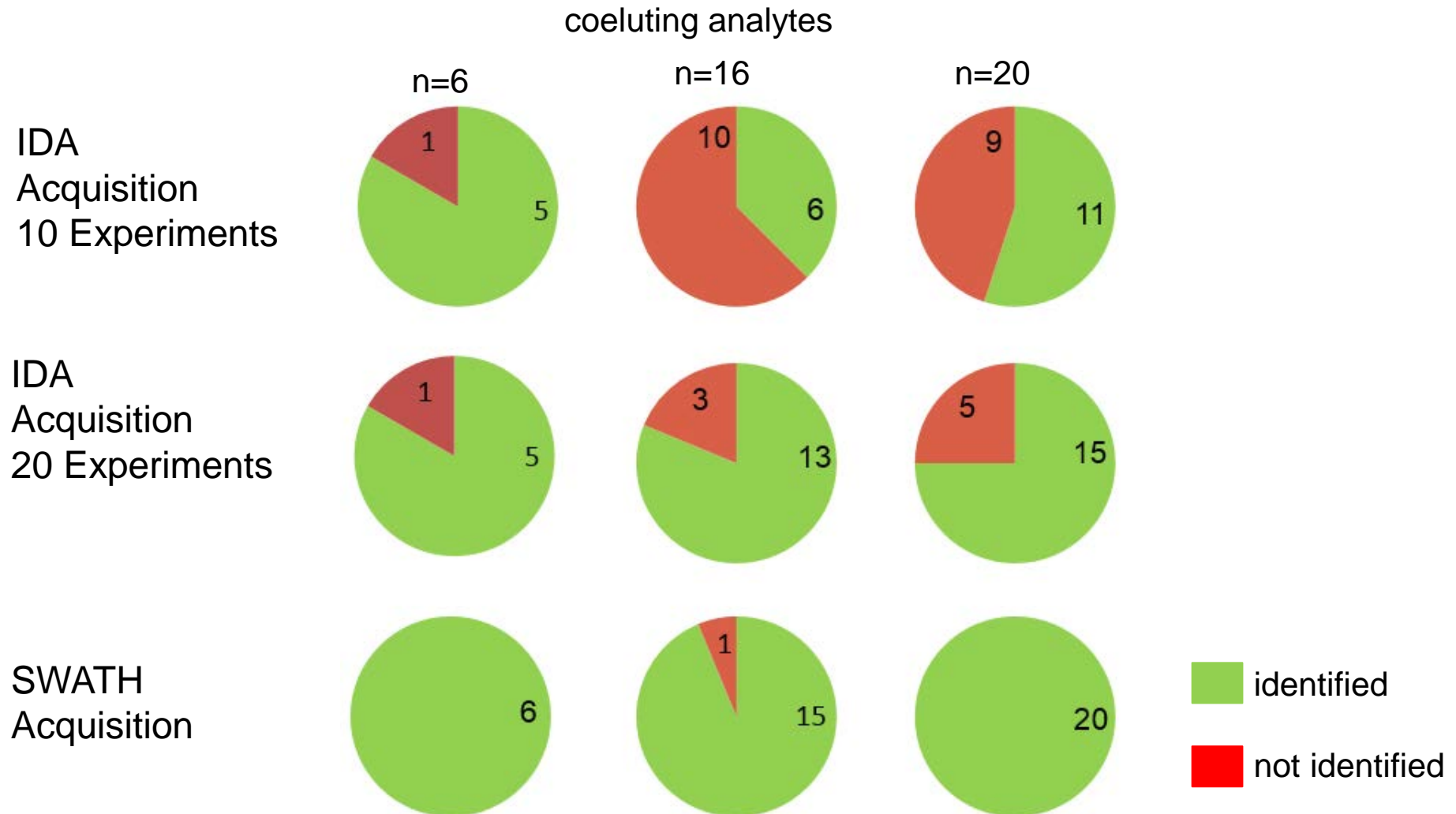
SWATH

TOF MS 50 ms
SWATH 40 ms

SWATH width: 20 Da



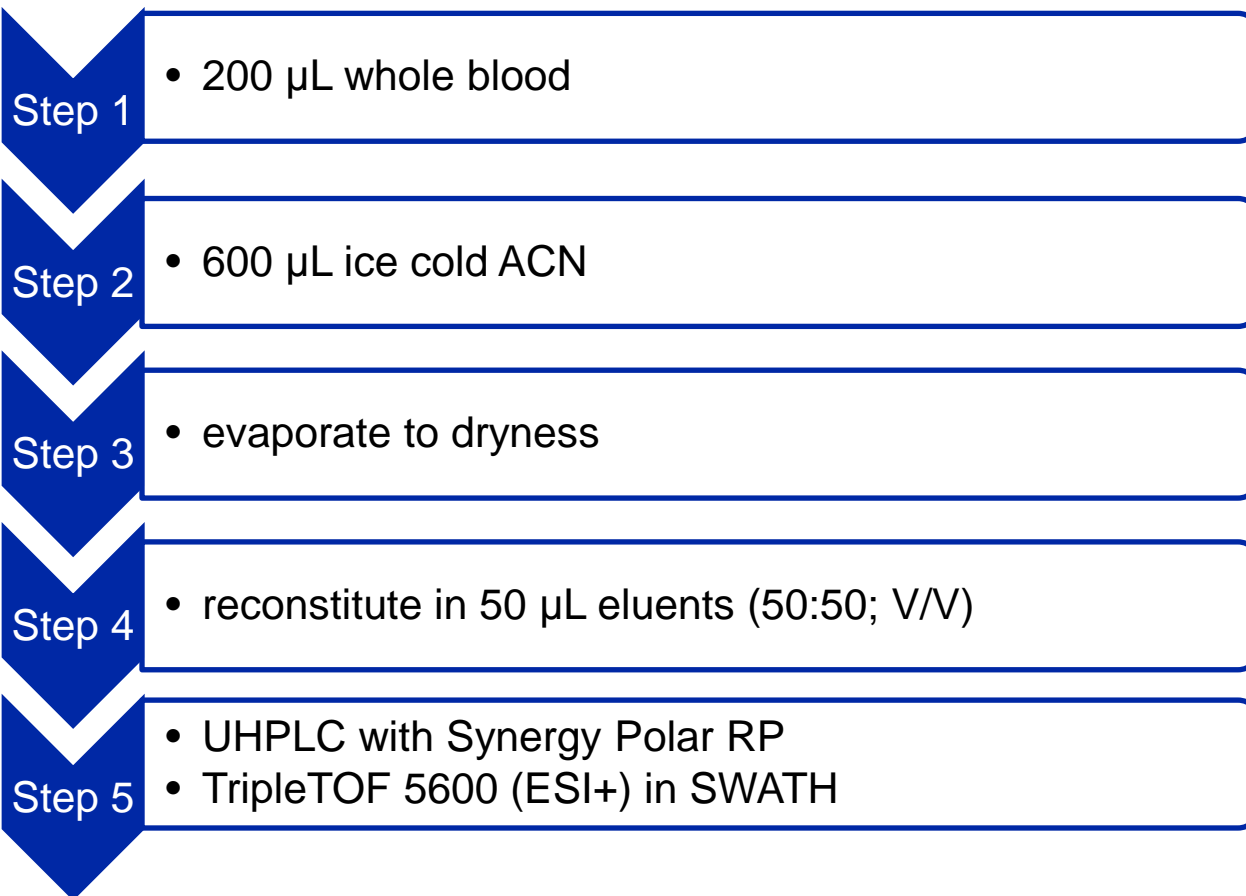
SWATH vs. IDA Acquisition





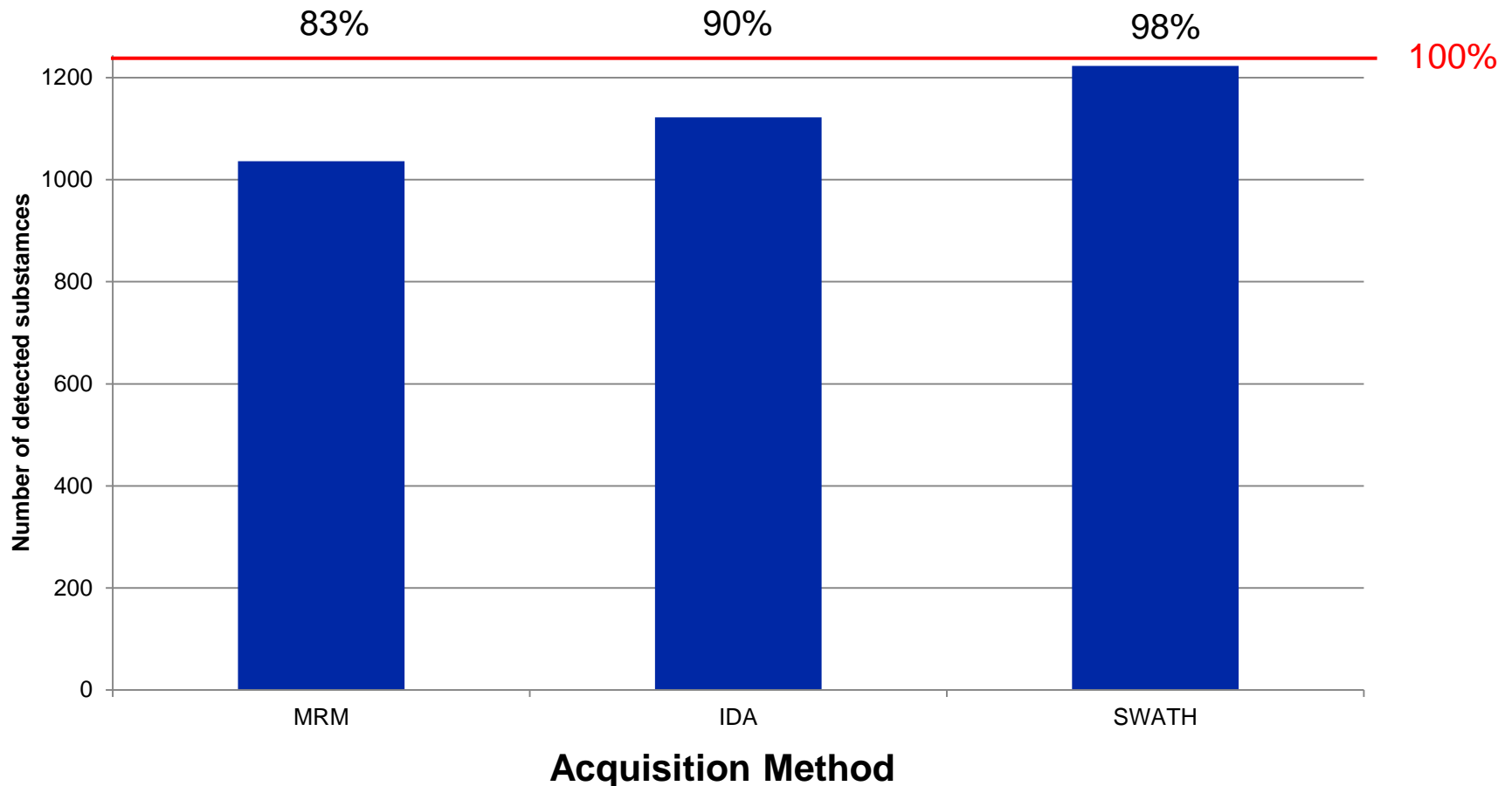
SWATH vs. IDA Acquisition - 382 Authentic Cases

Sample Preparation





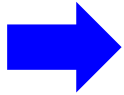
SWATH vs. IDA Acquisition-382 Authentic Cases



~10% of relevant MS/MS spectra were missed by IDA



Conclusions



SWATH closest to an ideal screening

MS^{ALL} with SWATH acquisition

- no considerations about instrument settings
- SWATH detected most analytes
- all sample information in one data file
- > QUAN accurate and reproducible
- > QUAL targeted and untargeted screening
- post run experiments of the data file (neutral loss, mass defect filter etc.)
- **Combines advantages of untargeted screening with targeted data extraction**



Postmortem Toxicology: A Very Special Problem in Forensic Toxicology

How to measure concentrations?

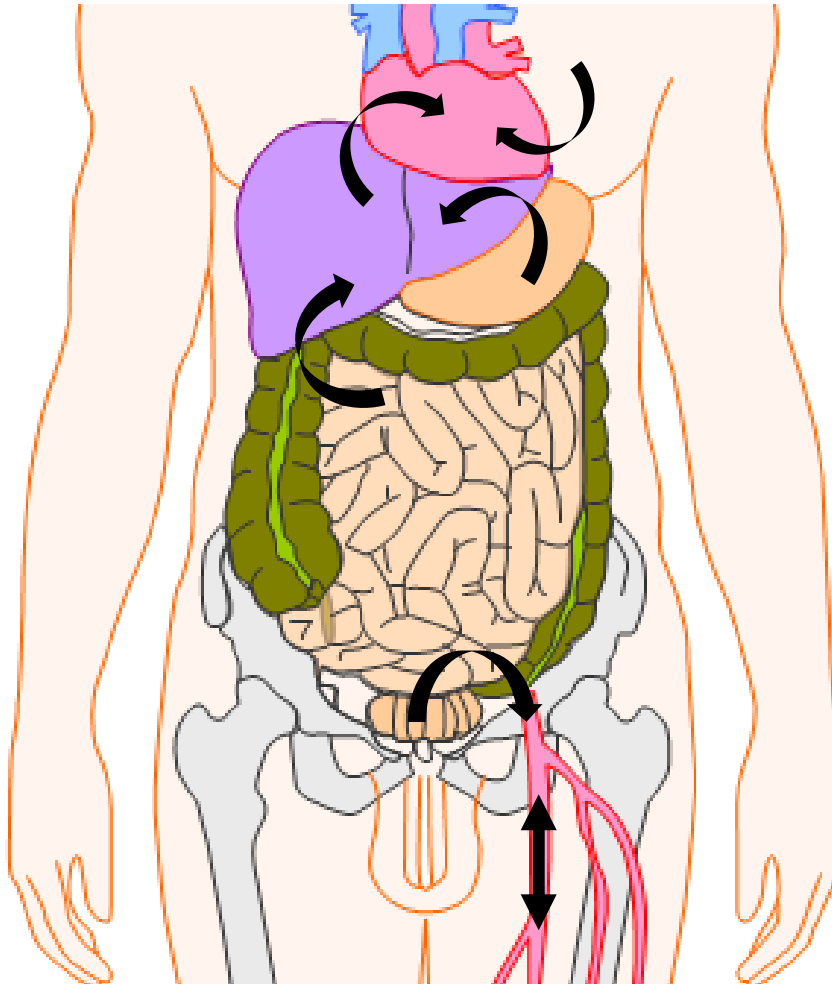
- e.g. rotten blood or tissues
huge concentration differences
- Huge dynamic range necessary

Interpretation of measured concentrations?

- Data usually from the living
- Postmortem degradation and formation
- Postmortem redistribution

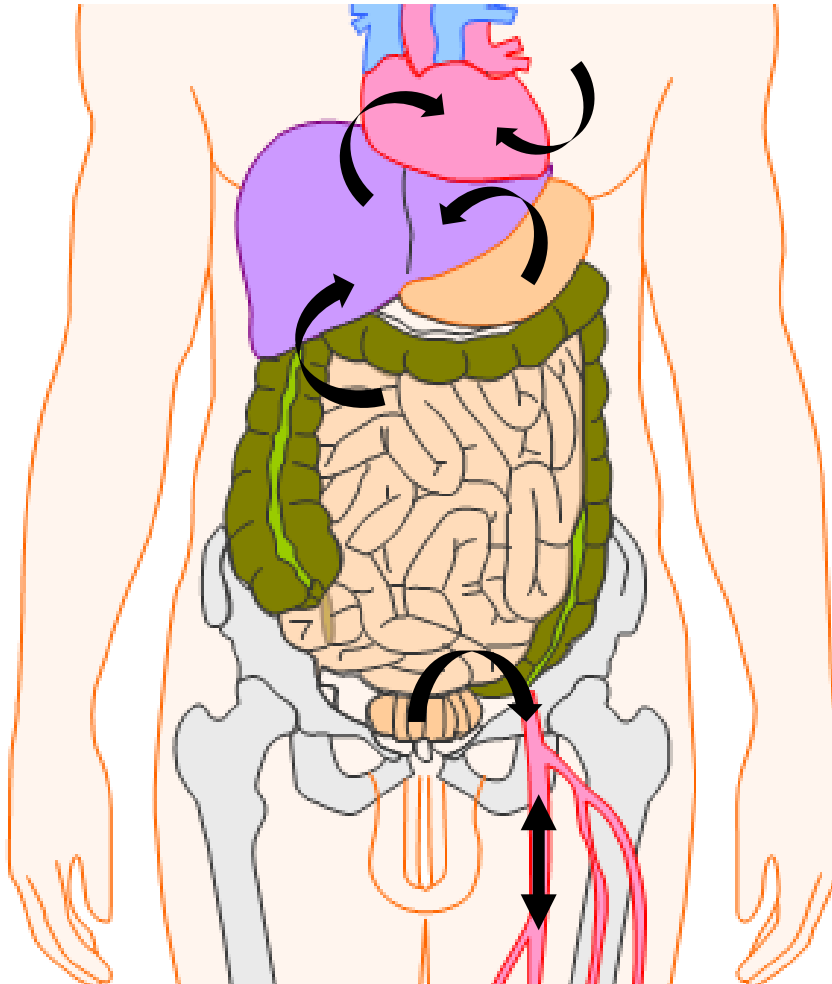


Postmortem redistribution – To date



- Chemical properties
- Central/peripheral concentration ratios
- Animal experiments
- Human data
 - 2-3 time points
 - blood

Aims



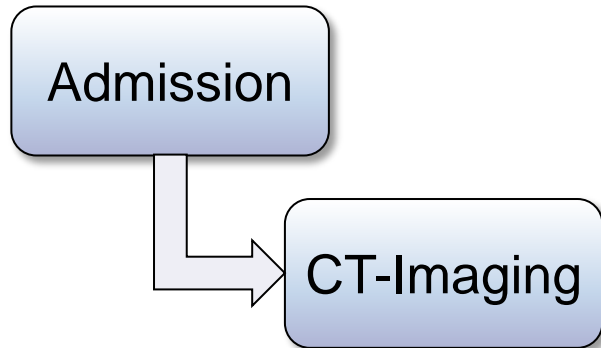
- Alternative matrices
 - Organs
 - Tissues
 - Body fluids
- Time-dependence
 - 2 time points



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Standard workflow in postmortem cases



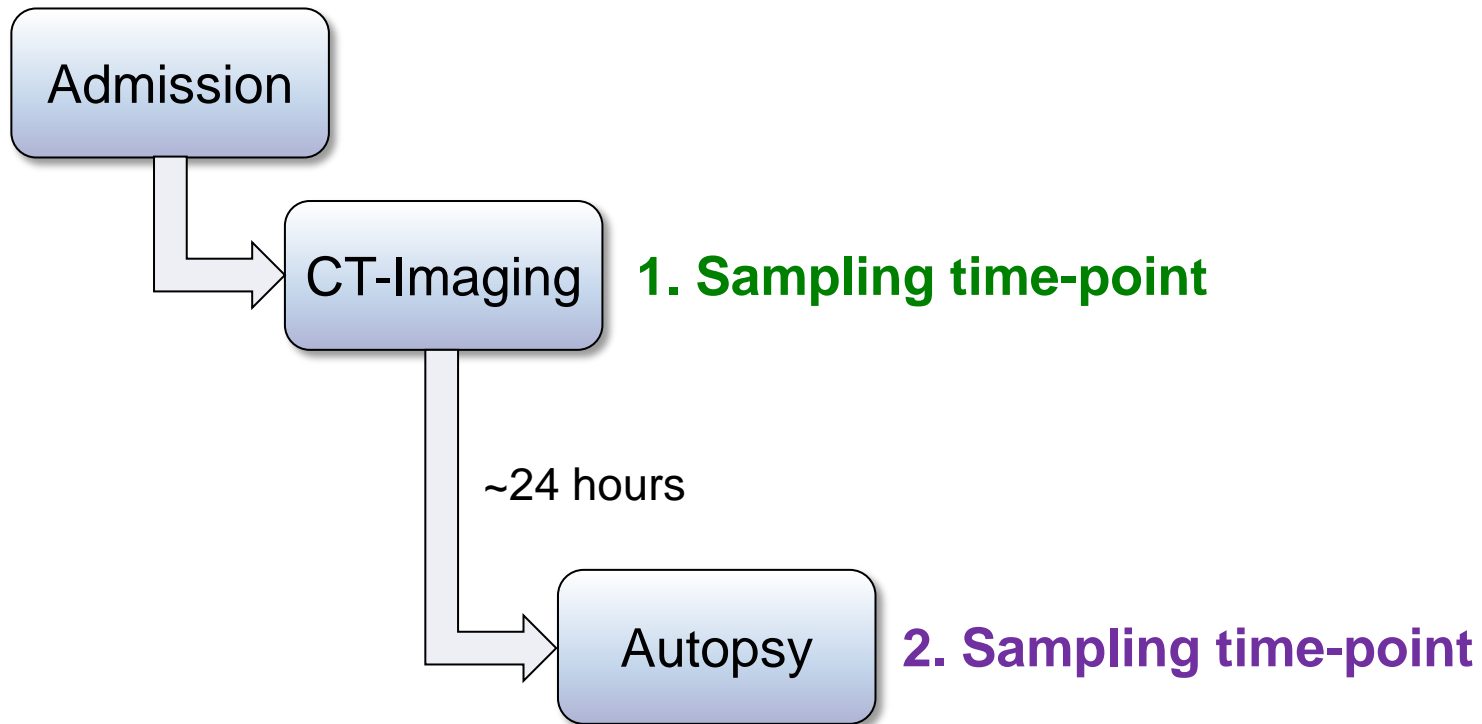


Standard workflow in postmortem cases





Standard workflow in postmortem cases

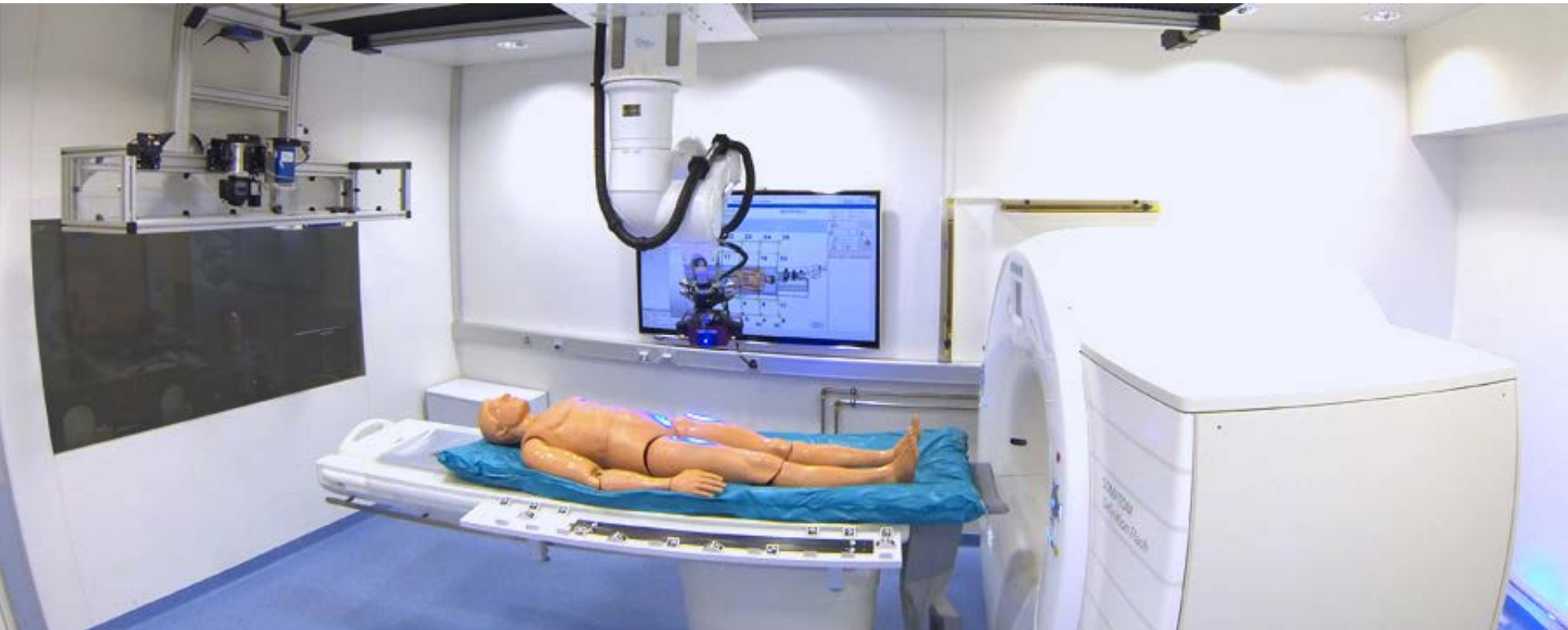




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Virtobot system

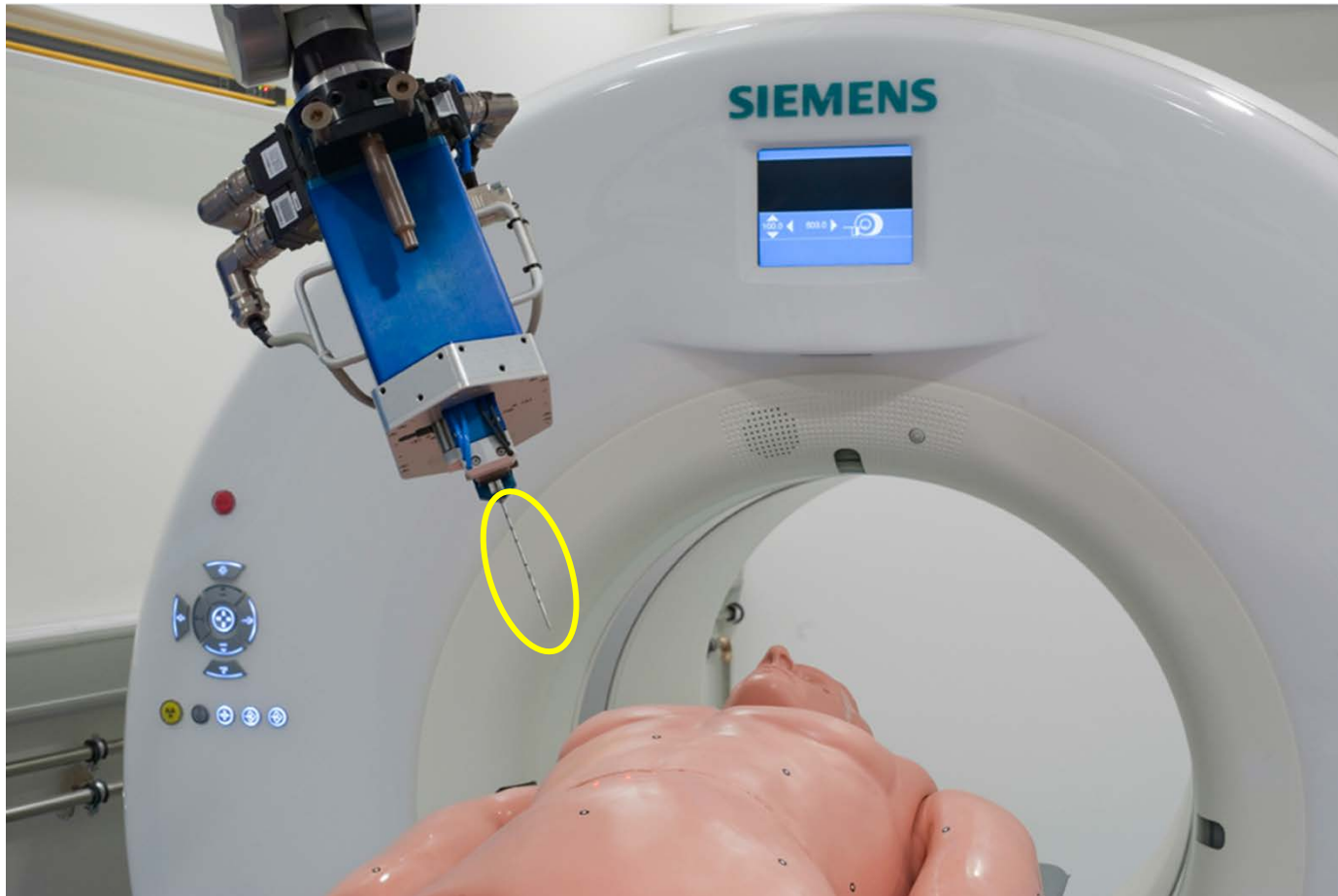




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Biopsy tool

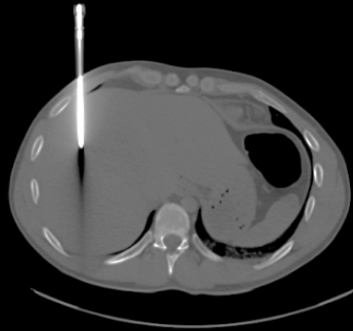




Biopsy sampling



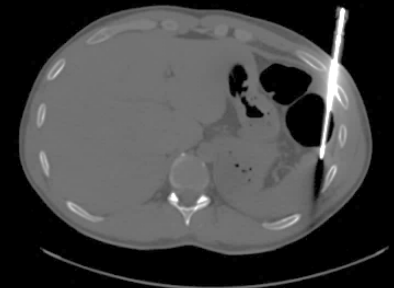
Heart



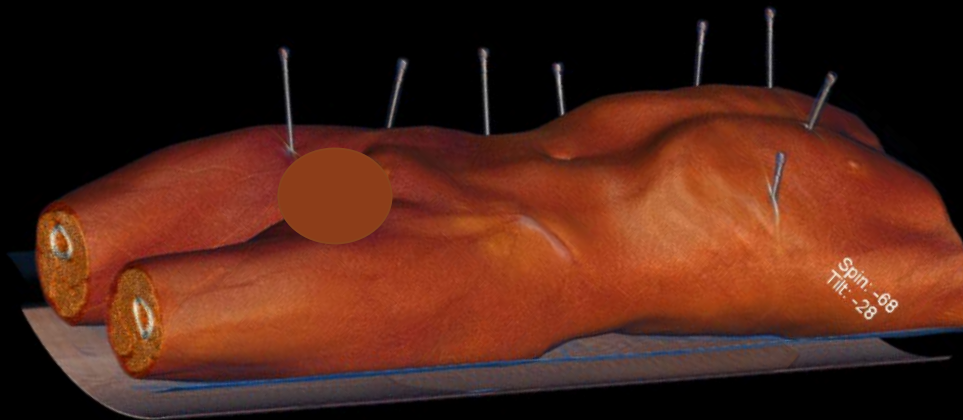
Liver



Lung



Spleen

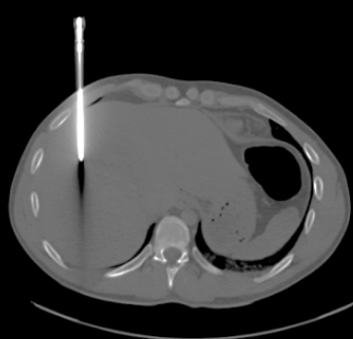




Biopsy sampling



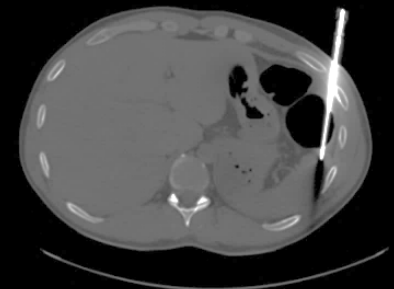
Heart



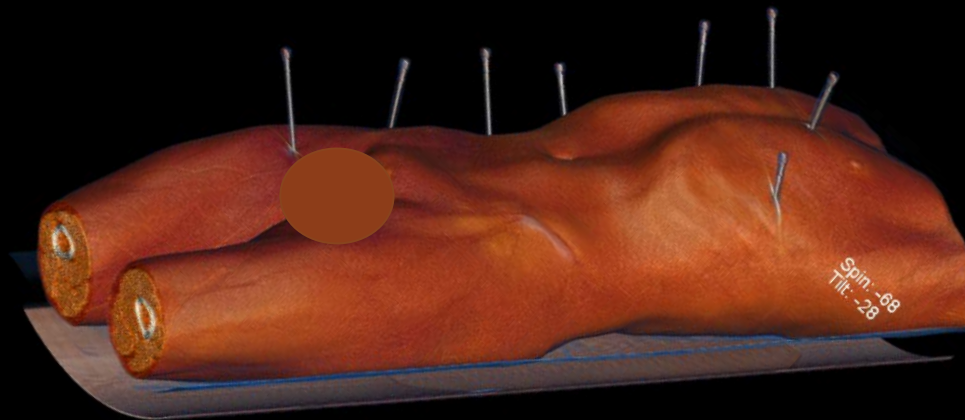
Liver



Lung



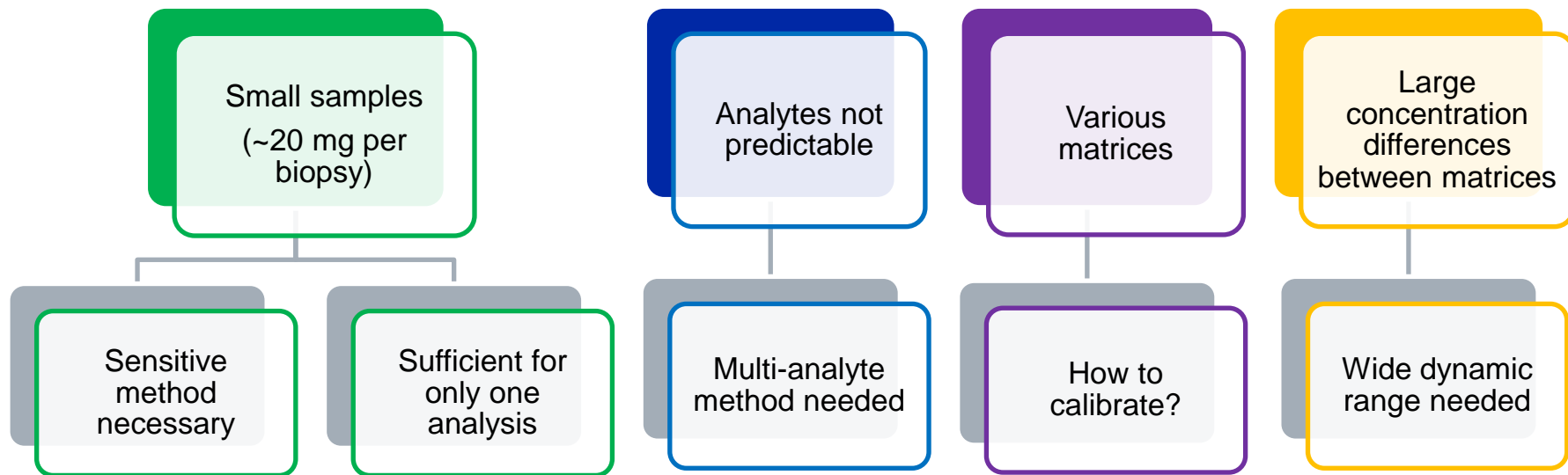
Spleen



20 mg per biopsy

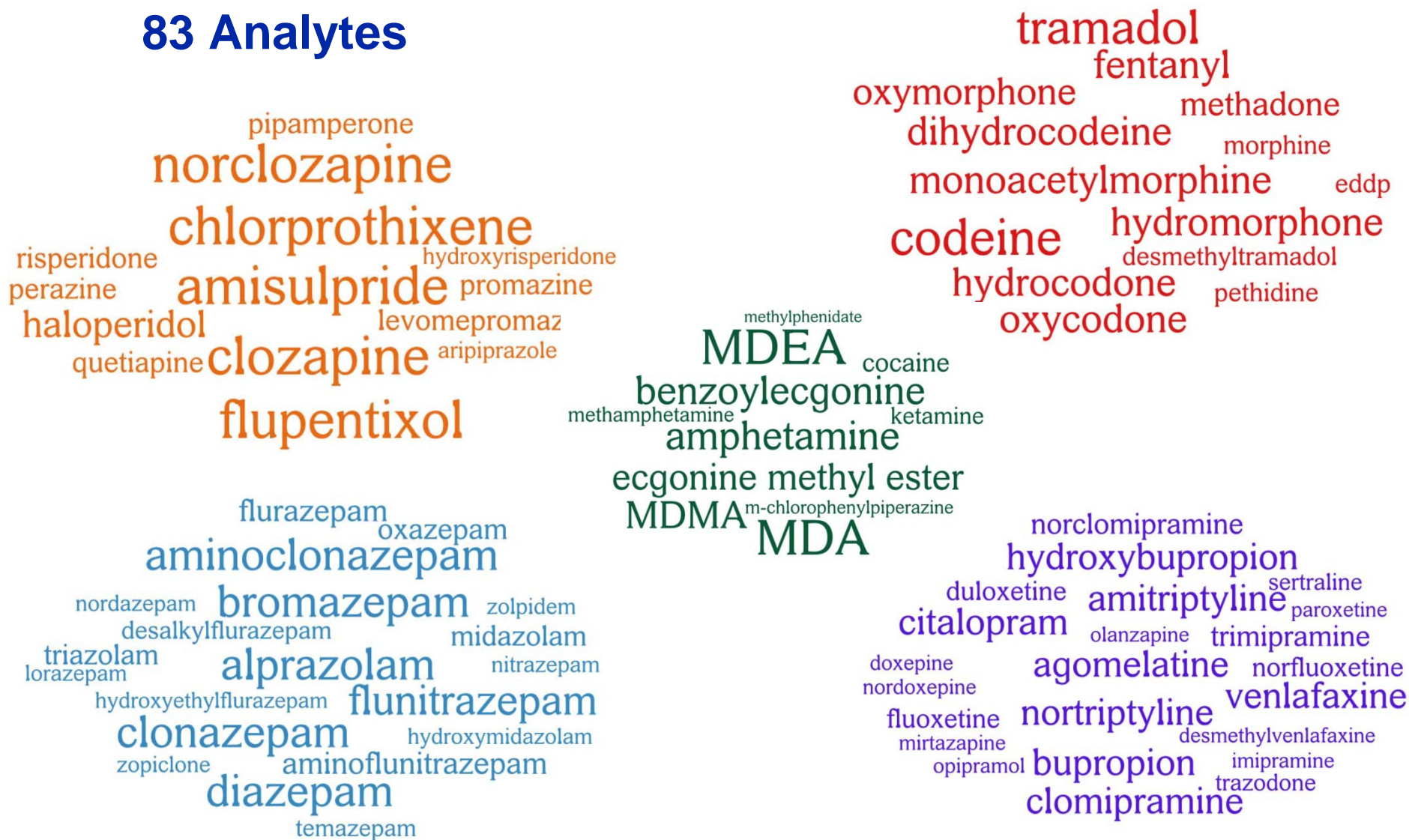


Challenges





83 Analytes





Sample preparation

Sample

- 20 mg tissue
- 20 µl body fluid

Neutral extraction

- EtAc/BuAc 1:1 at pH 7.4

Alkaline extraction

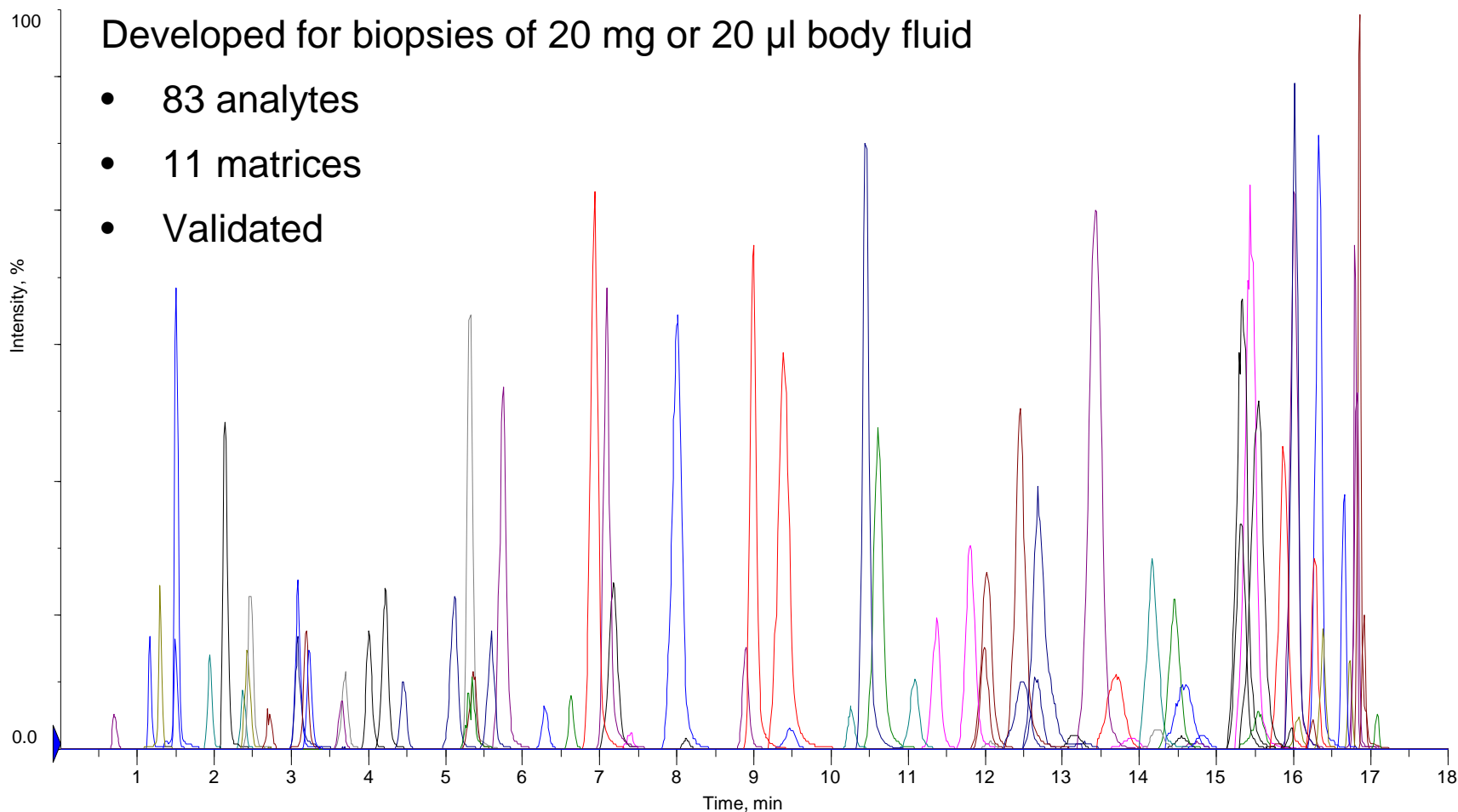
- EtAc/BuAc 1:1 at pH 13

LC-MS/MS

- Column: Phenomenex Synergi Polar-RP
- LC: Thermo Fischer Ultimate 3000 UHPLC
- MS: ABSciex 5500 QTrap linear ion trap

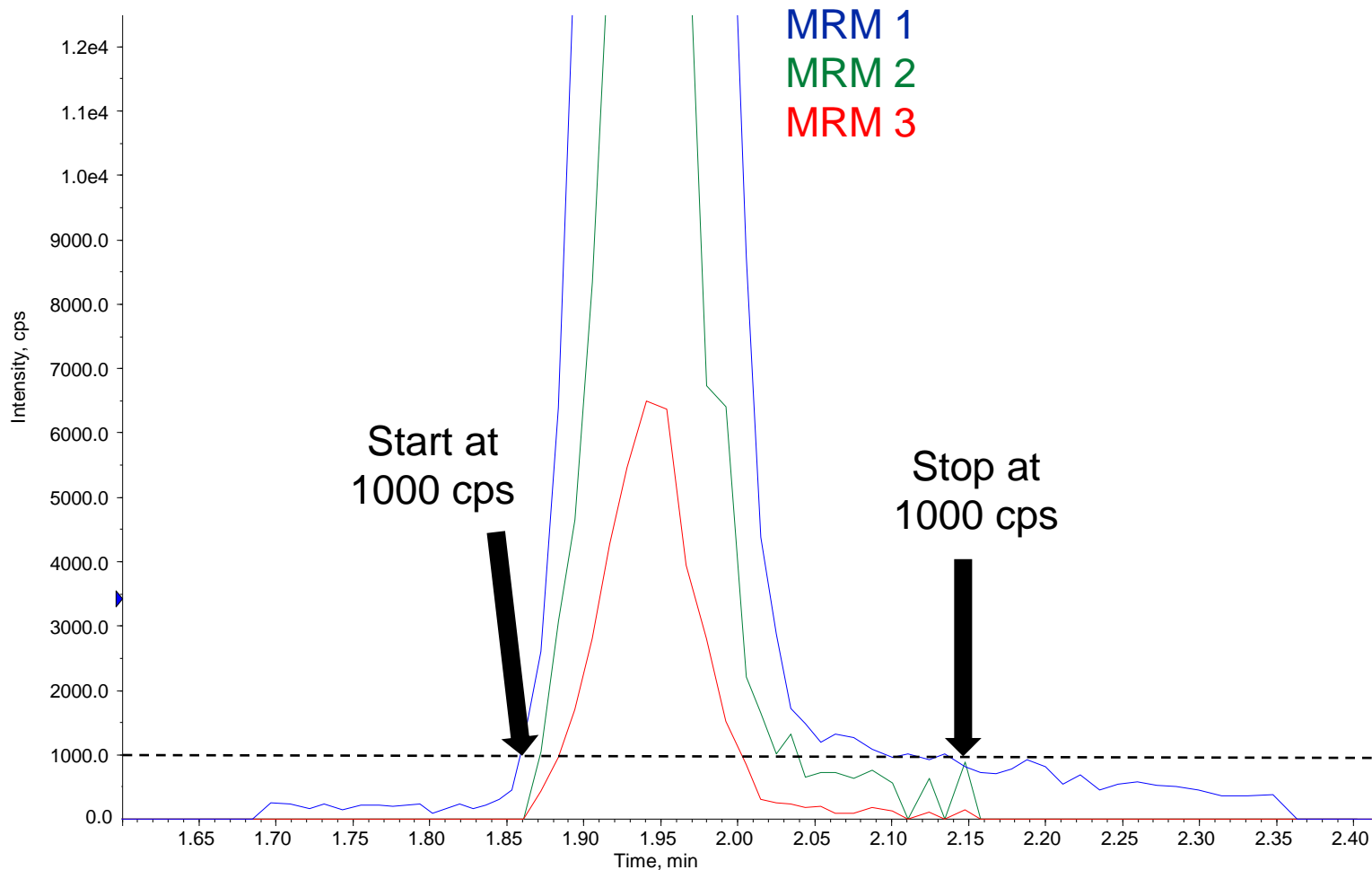


LC-MS/MS method



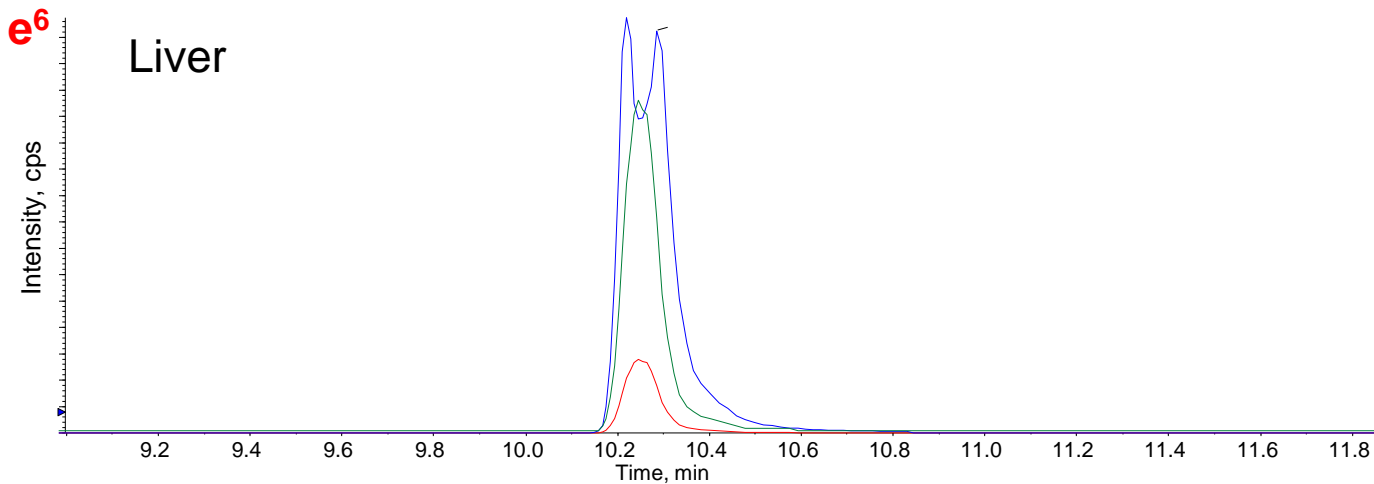
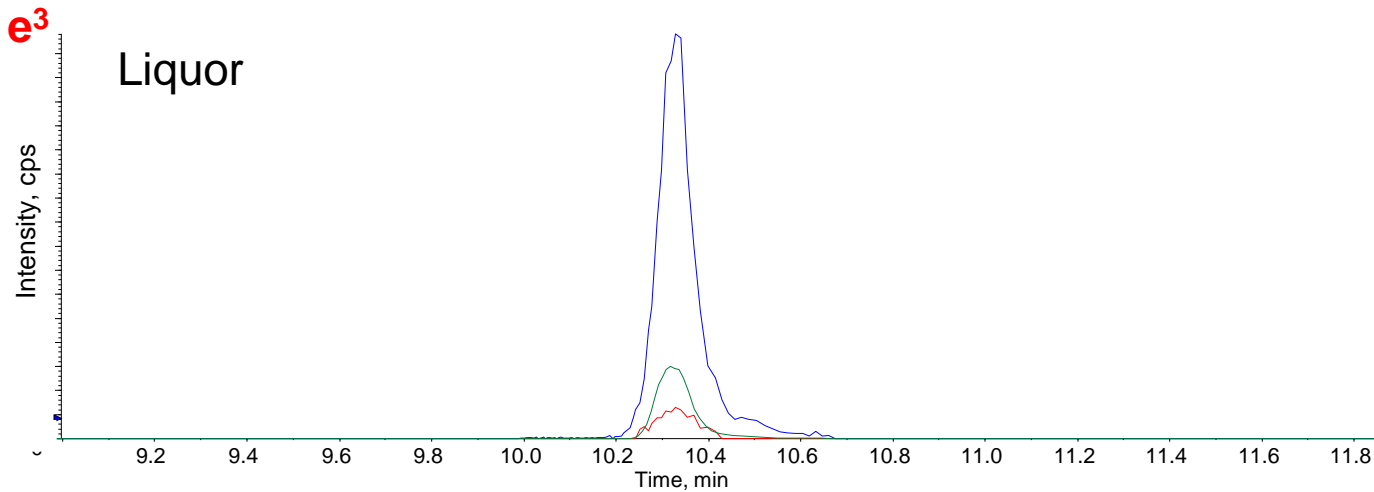


Advanced scheduled MRM method





Challenge: concentration differences

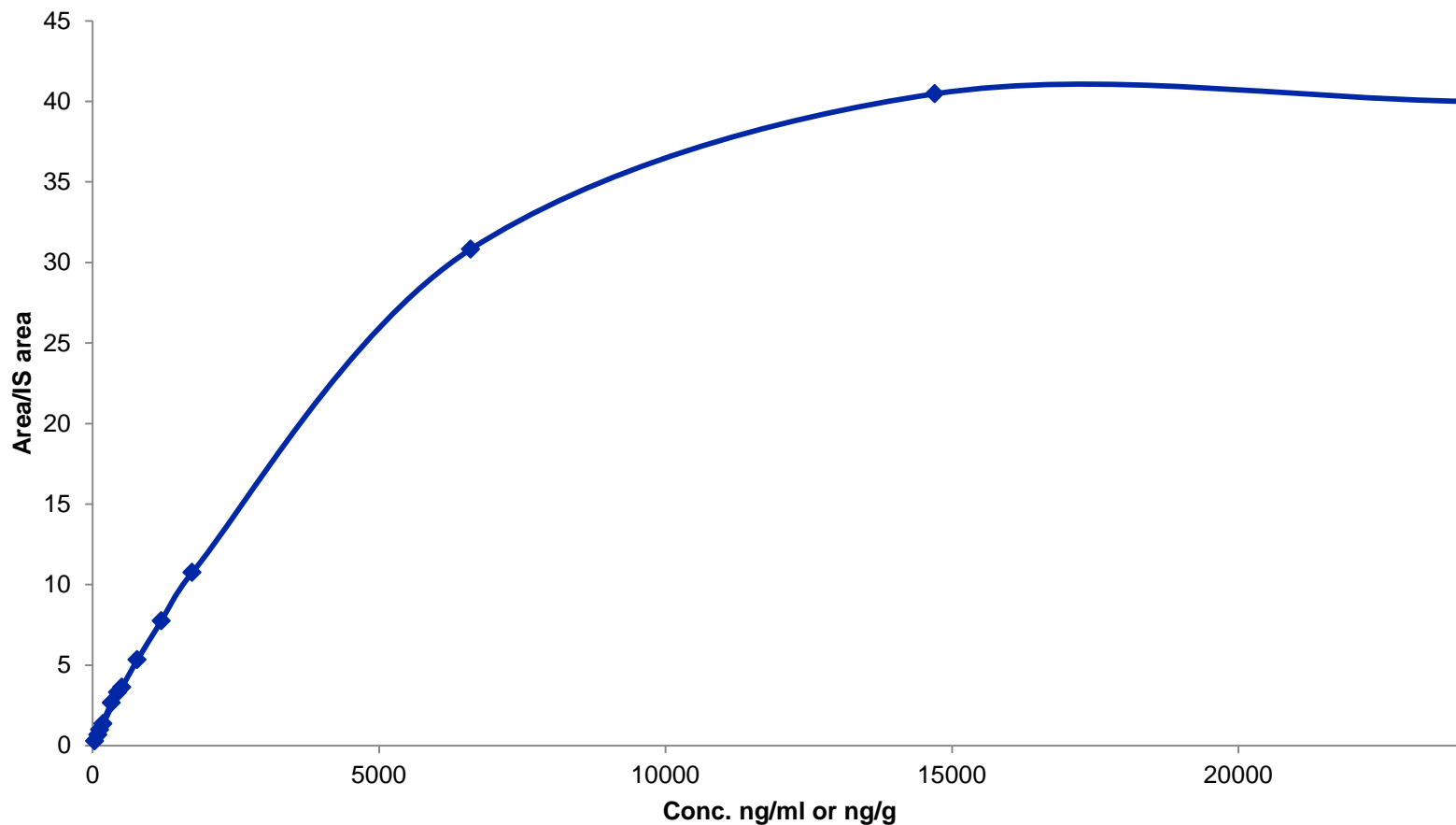




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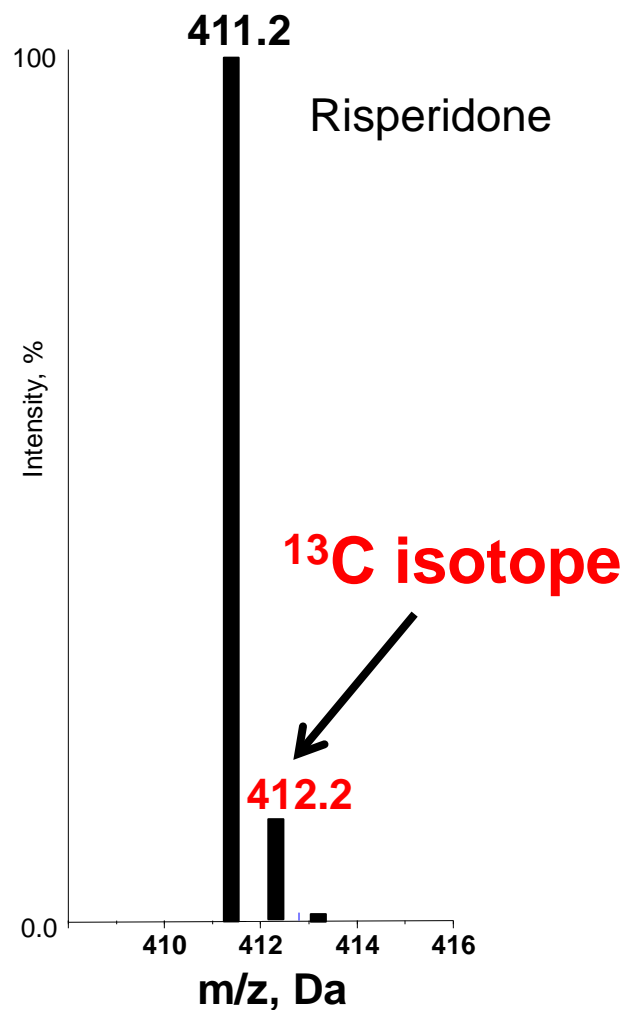
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What if dynamic range of your MS is insufficient?



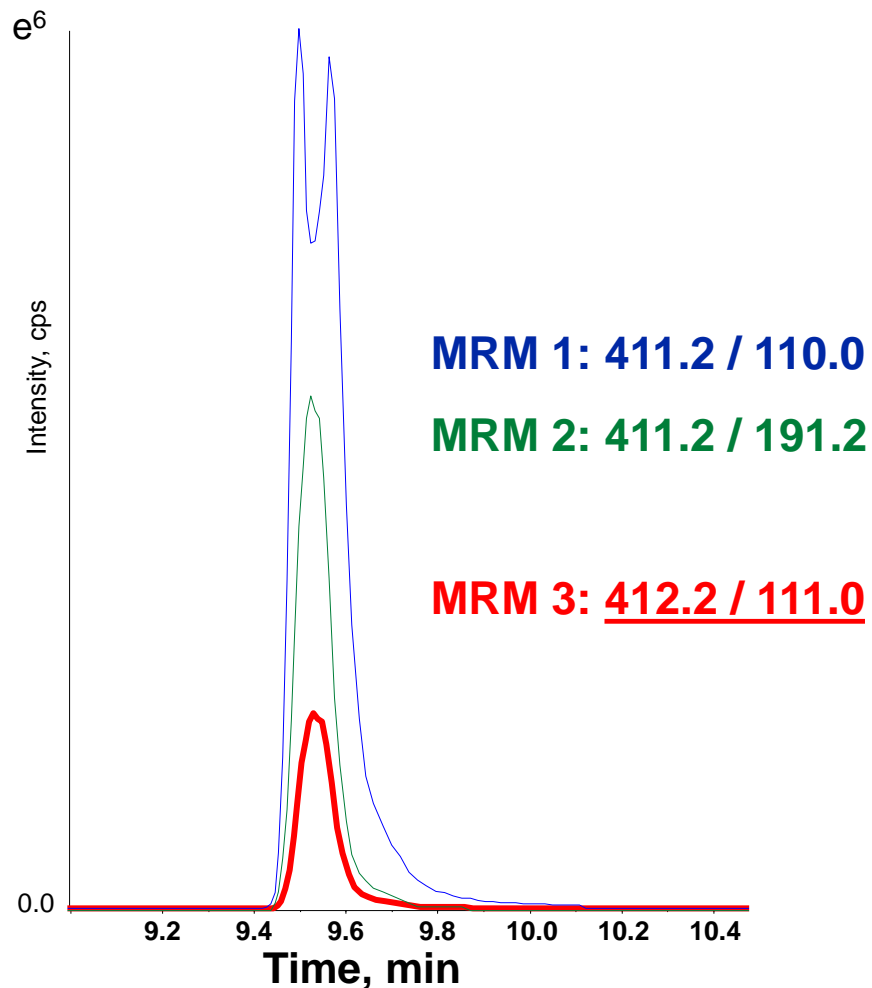
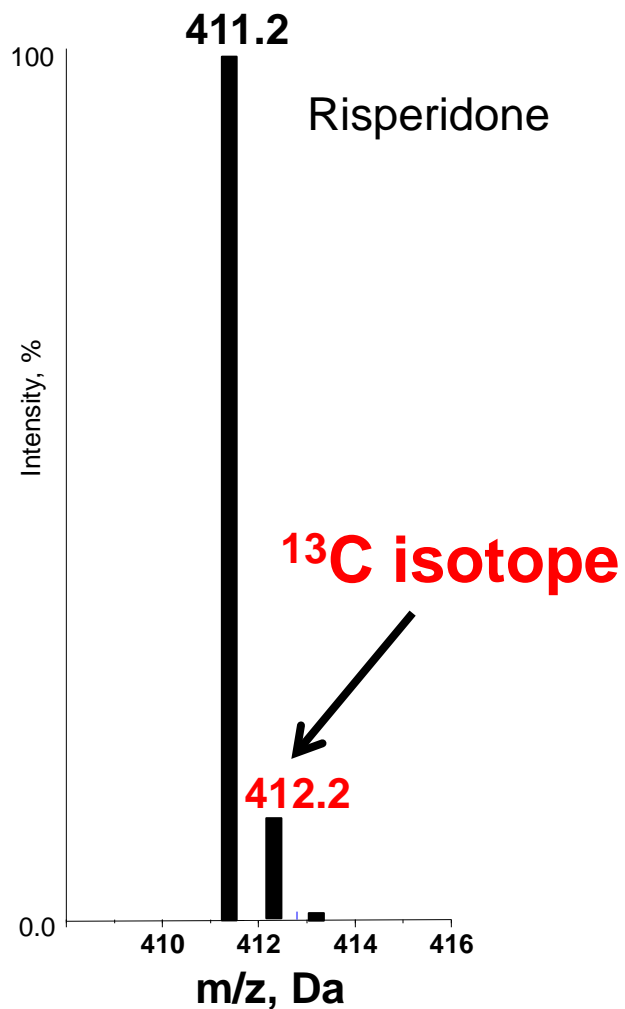


Solution: ^{13}C isotope as quantifier



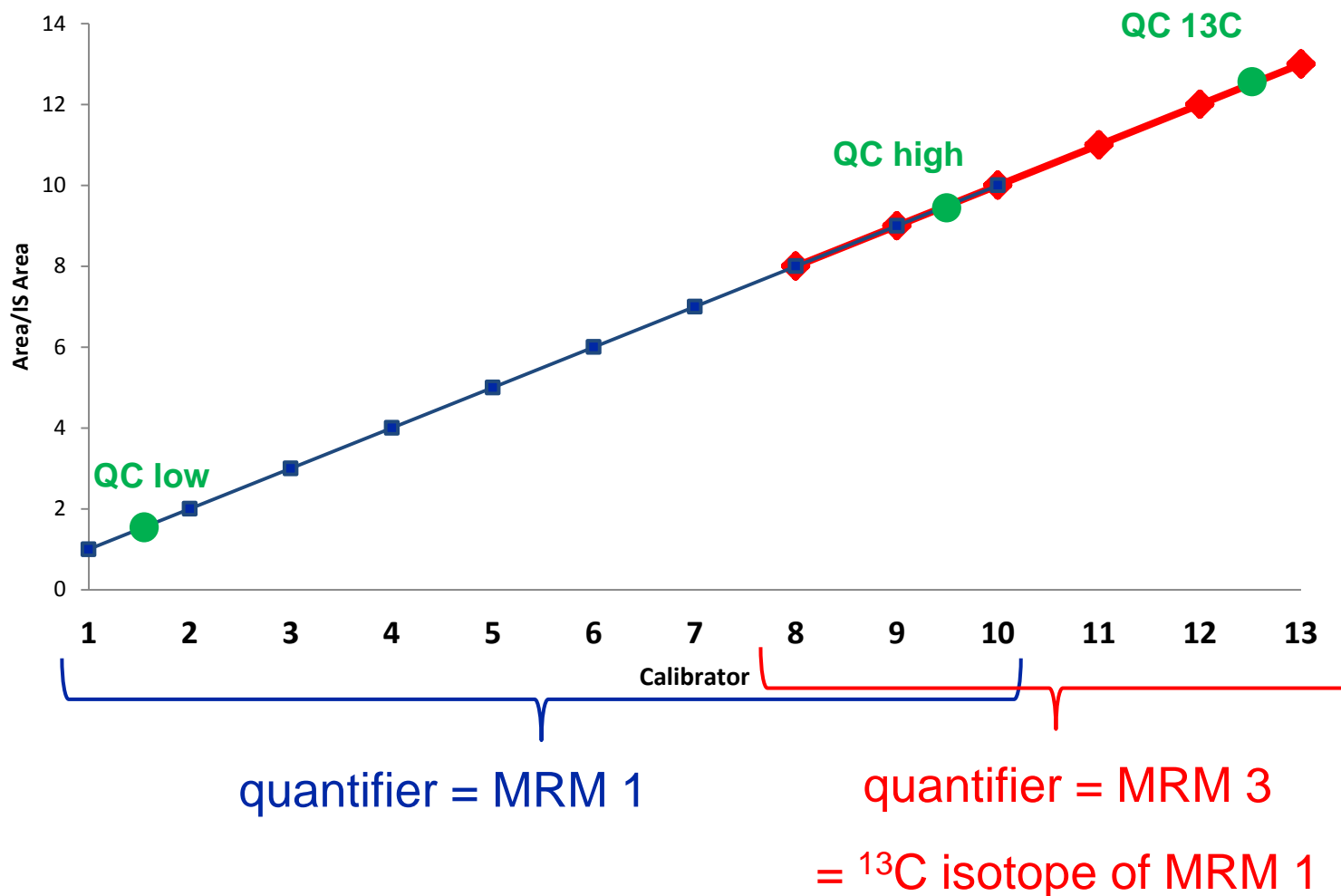


Solution: ^{13}C isotope as quantifier





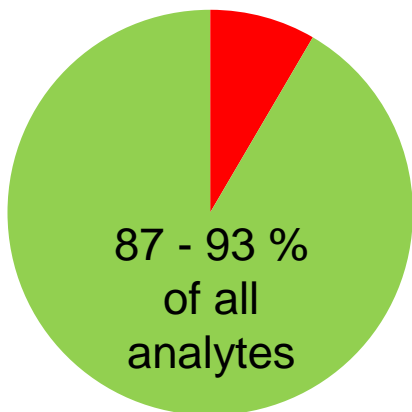
Problem solved! Extremely wide calibration range



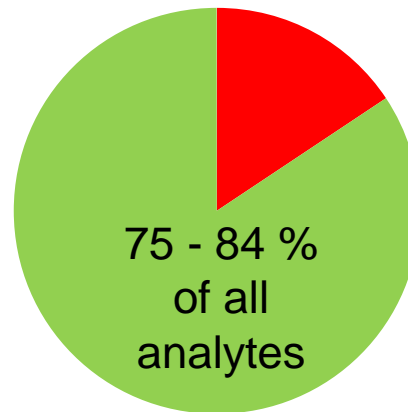


Validation

Intra-day imprecision < 20 %



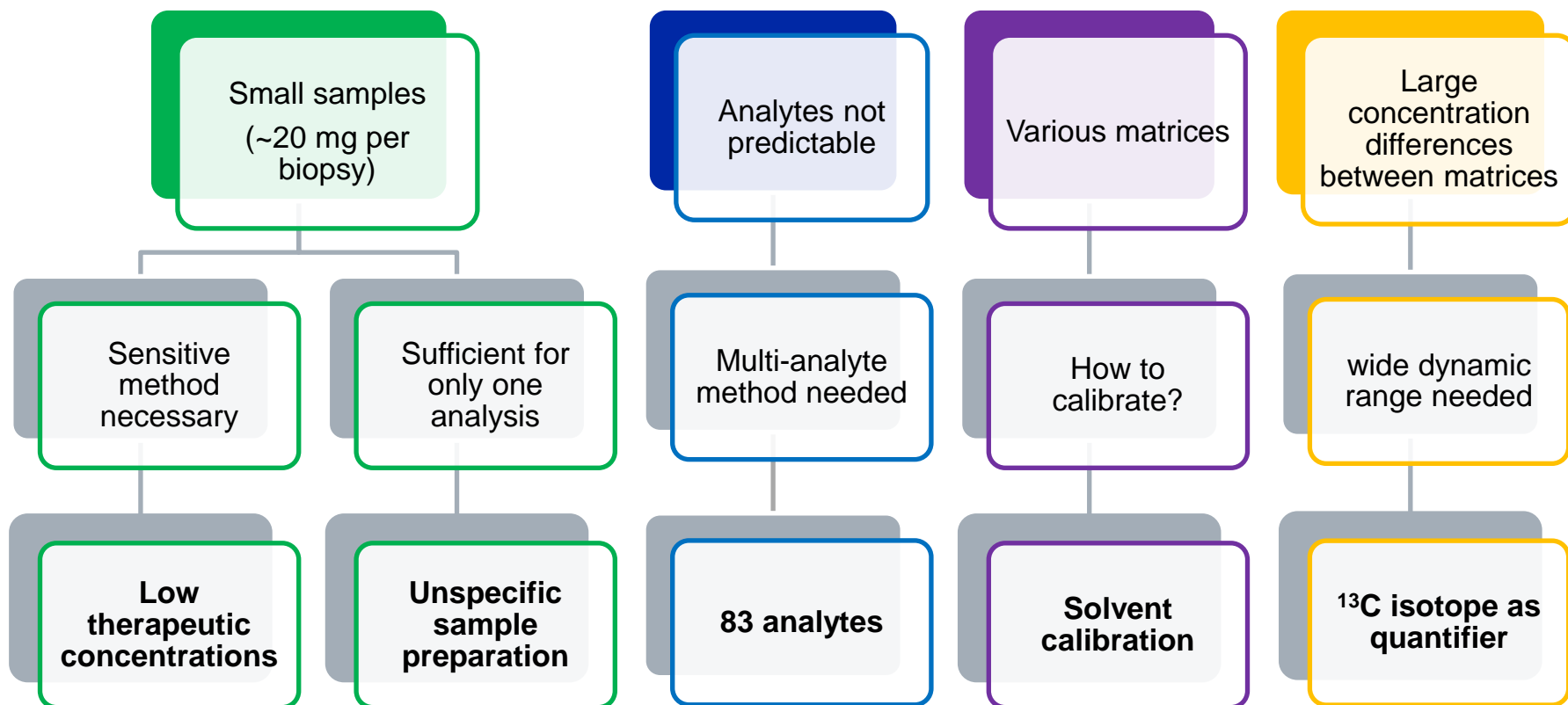
- pB
- HB
- CSF
- Urine
- Muscle
- Kidney
- Spleen
- Lung



- Liver
- Adipose tissue
- Brain

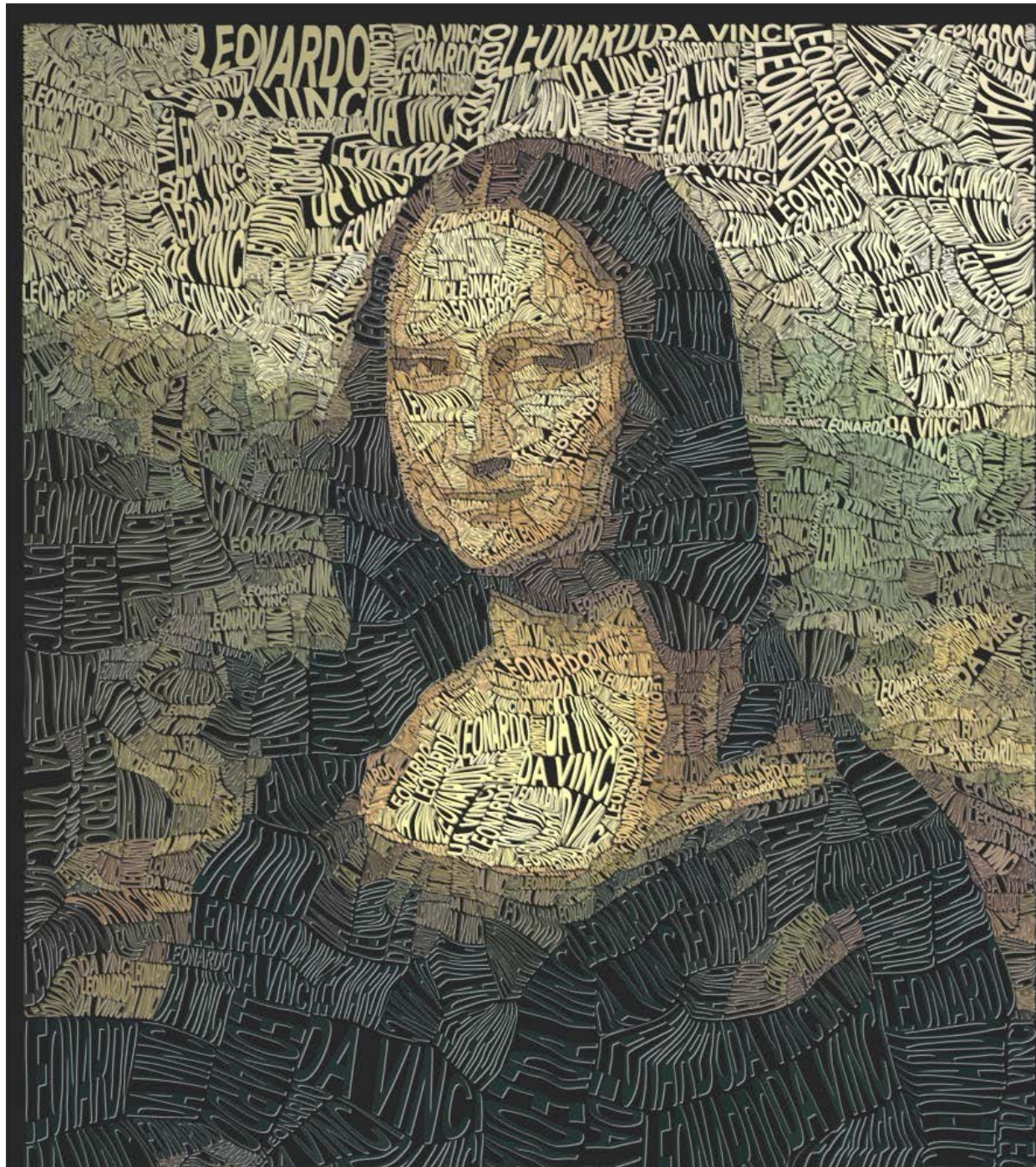


Conclusion: Postmortem Biopsy-Project



**A picture
is worth a
thousand
words ...**

**... but it uses up three
thousand times the memory**





Imaging? That's old hat in forensics!

The Virtopsy[®] approach:

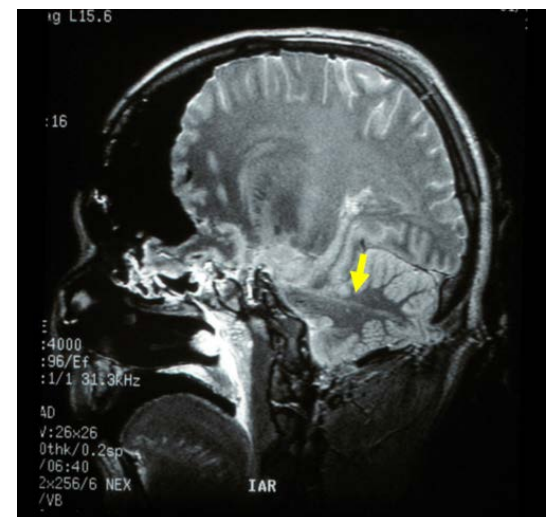
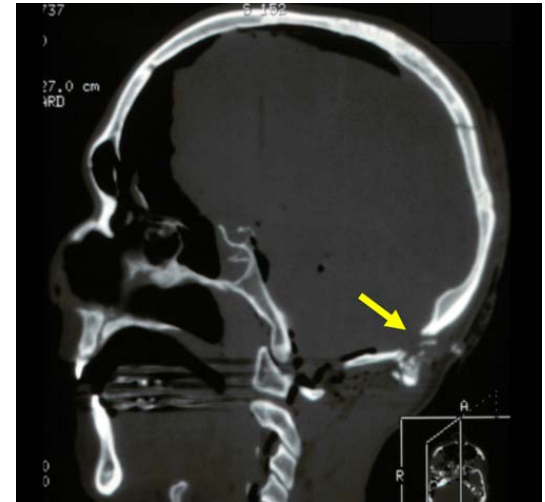
- 3D photogrammetry-based optical surface scanning
- Computed tomography (CT) and magnetic resonance imaging (MRI)
- Postmortem biopsy
- Postmortem angiography



Imaging? That's old hat in forensics!

The Virtopsy[®] approach:

- 3D photogrammetry-based optical surface scanning
- Computed tomography (CT) and magnetic resonance imaging (MRI)
- Postmortem angiography
- Postmortem biopsy





Imaging? That's old hat in forensics!

The Virtopsy[®] approach:

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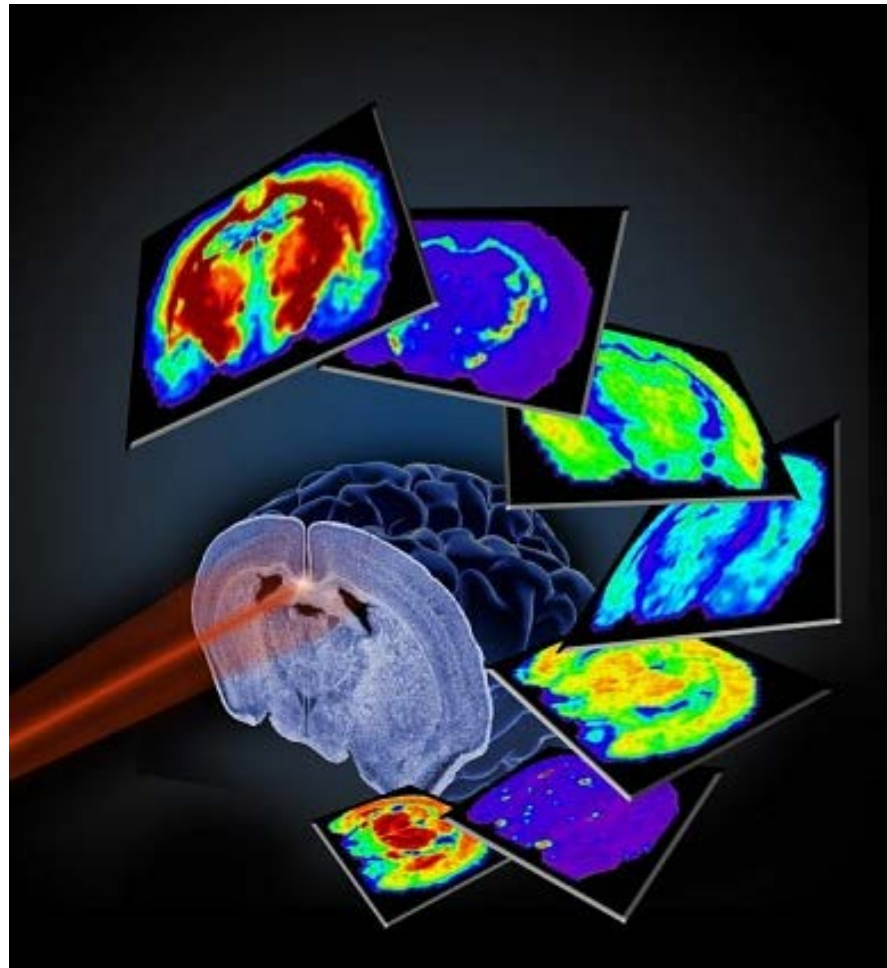




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Imaging? MALDI mass spectrometric imaging



Toxicological Analysis in Postmortem Tissue Samples

Oh No,
it's a
tissue sample!



Sample preparation:

- tedious
- time consuming
- unpleasant

GC/LC-MS Analysis

- strong matrix signals
- time consuming
- unpleasant

→ **Better solutions?**



Sample Preparation for MALDI-MS Imaging

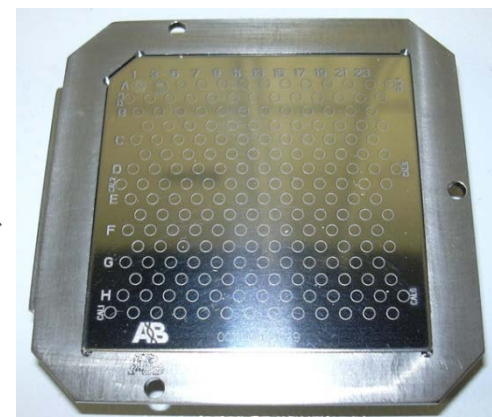
deep freezing



Cryosectioning (12 µm)



transfer to plate

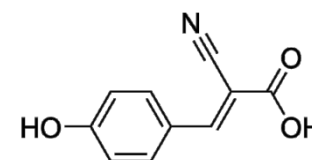


MALDI-MS



matrix coating

α -cyano-4-hydroxy-
cinnamic acid (CHCA)





MALDI-MSI Data Acquisition

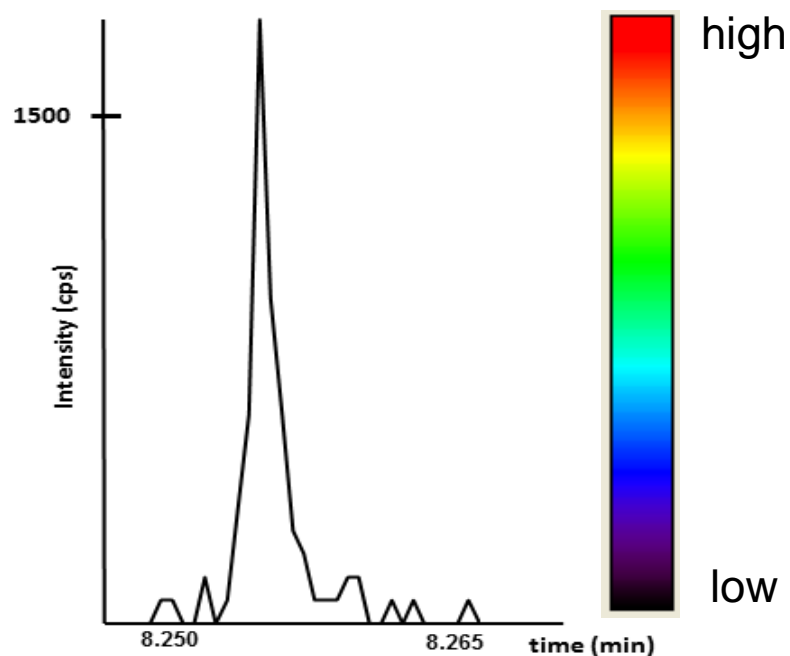
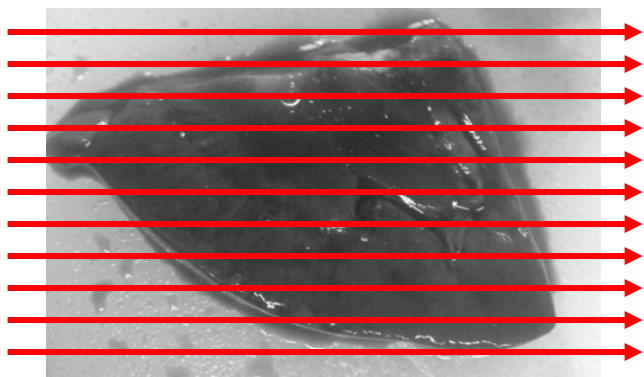


MALDI-QqQ:

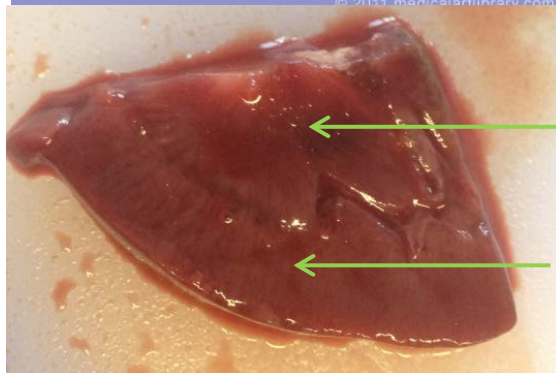
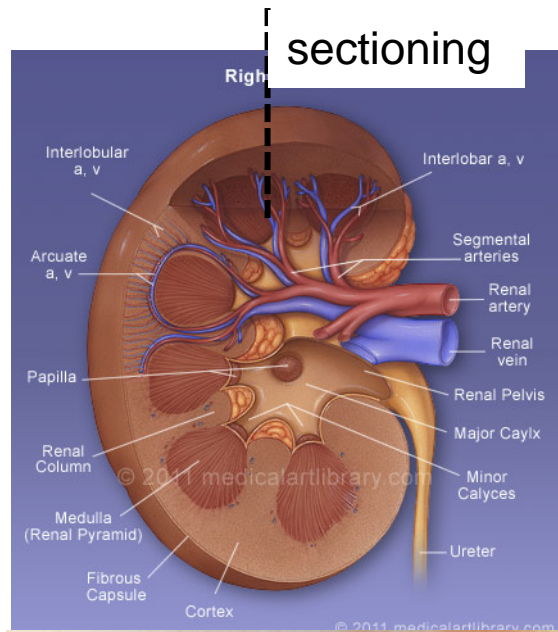
AB Sciex
Flash Quant Workstation 4000 Qtrap

Data Acquisition:

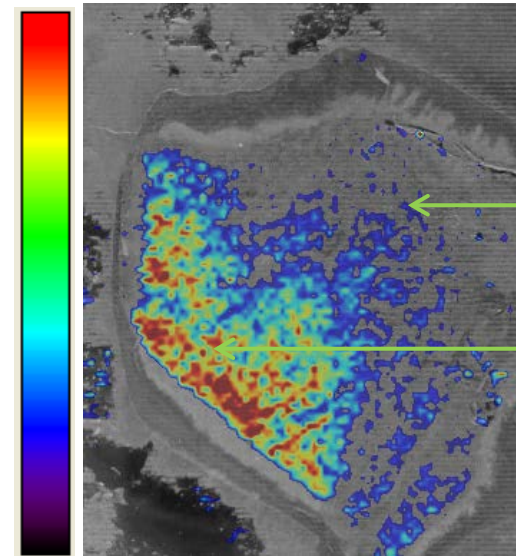
- MRM mode
- Spectra (EPI; for confirmation)



Post Mortem Drug Distribution in Kidney Sections Using MALDI-MSI



high



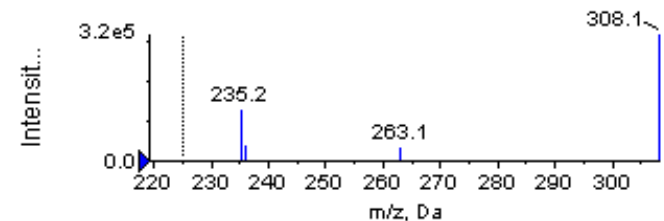
Medulla

Cortex

low

Zolpidem EII/OI image overlay

■ + Zolpidem Prec(308.0) CE(35... Max. 3.2e5 cps



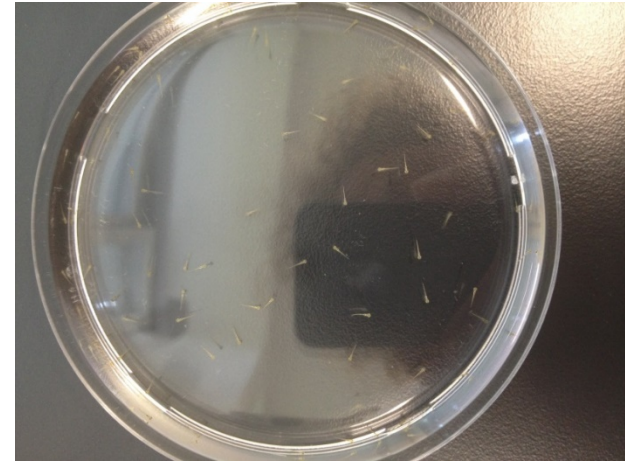
EPI Spectrum from positive areas



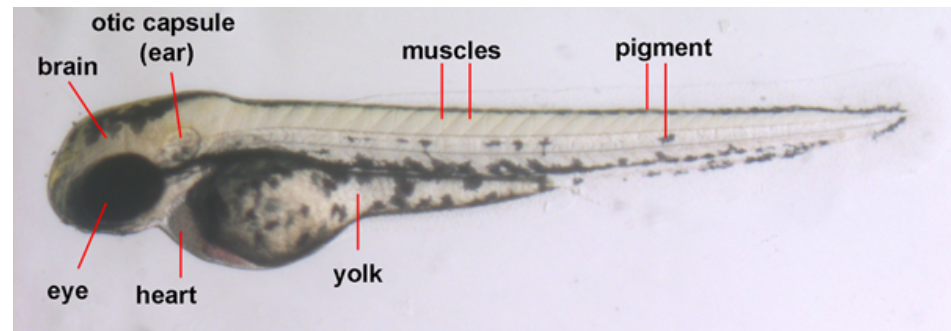
Zebrafish as Toxicity Test System



Organisation for Economic Co-operation and Development (OECD)



Zebrafish larvae at day 5



OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Fish Embryo Acute Aquatic Toxicity (FET) Test



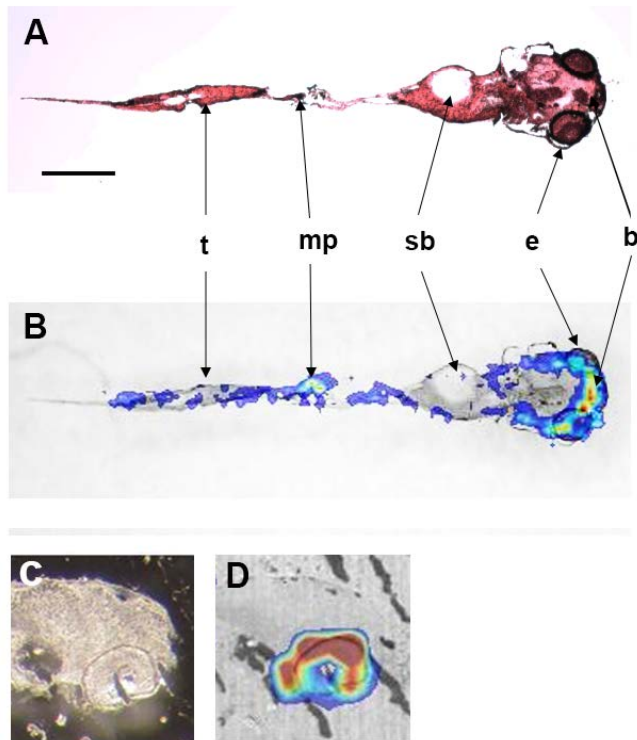
New Psychoactive Substances (NPS) Spice and RCs



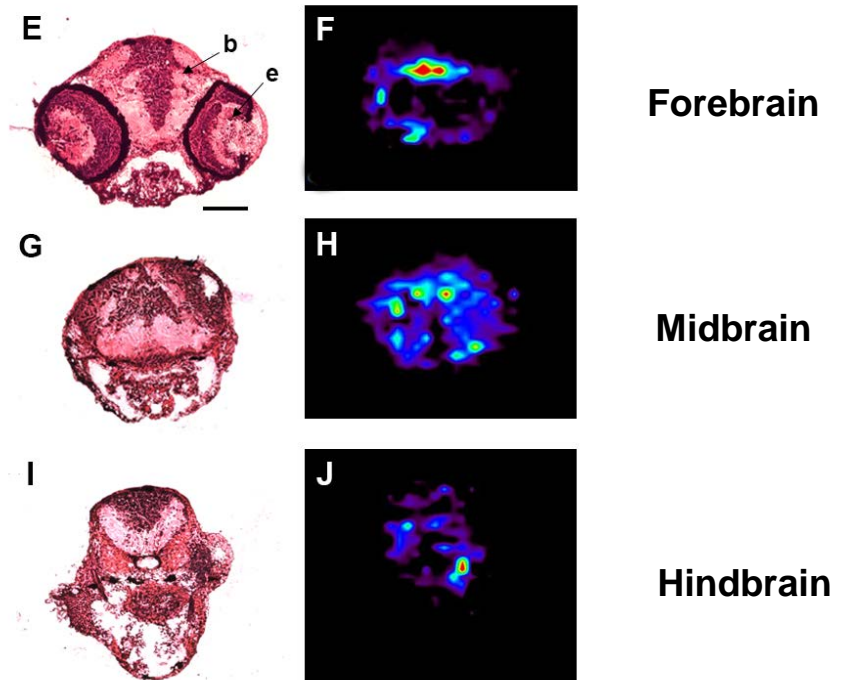
Spice group
(synthetic cannabinoids)

Bath salts, Research-Chemicals





H & E stained and overlaid coronal section (A, B) and sagittal section (C, D)

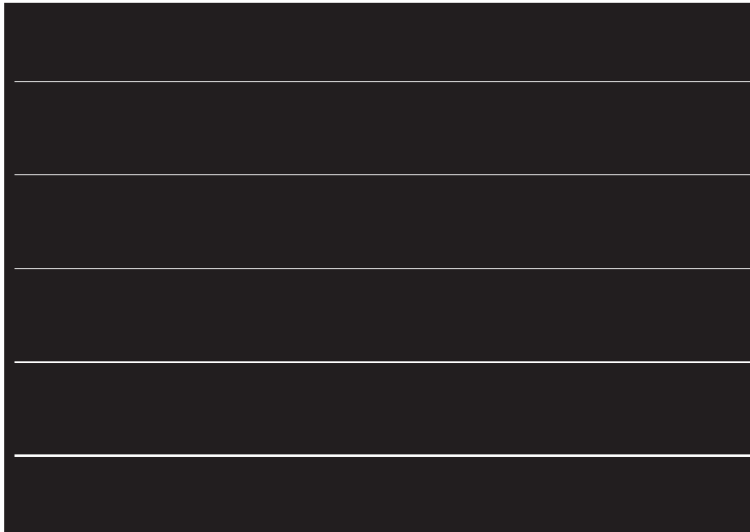


H & E stained transverse sections



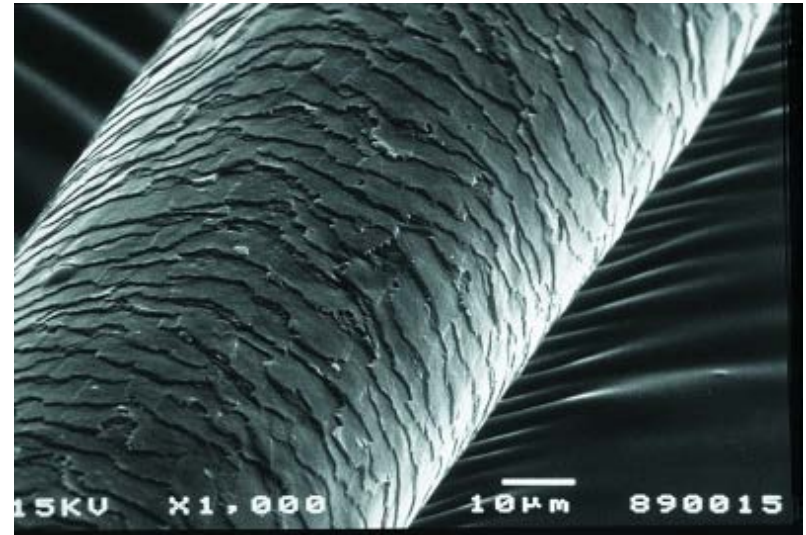
Other Matrices?

fine



medium

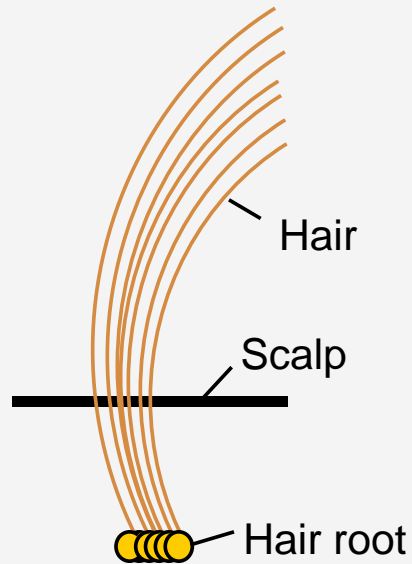
thick



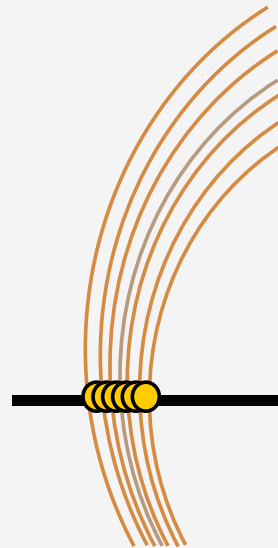
Hair: 40 – 120 μm



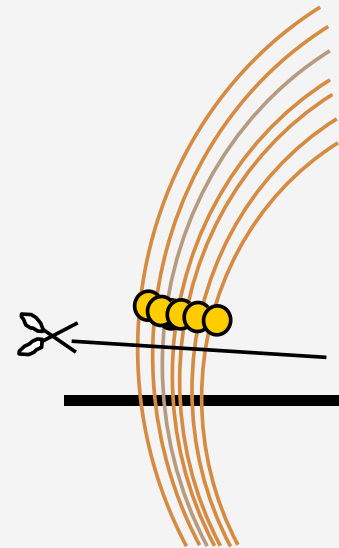
Incorporation and Time Schedule



**Incorporation into the hair
root, fixation**



10 days later



Weeks later



MALDI-MSI Sample Preparation

- washing hair samples (SINGLE HAIR !)



- transfer to steel-MALDI plate
fixation with double adhesive tape



- manual MALDI-matrix application (α -CHCA)



- data acquisition





MALDI-MSI Data Acquisition

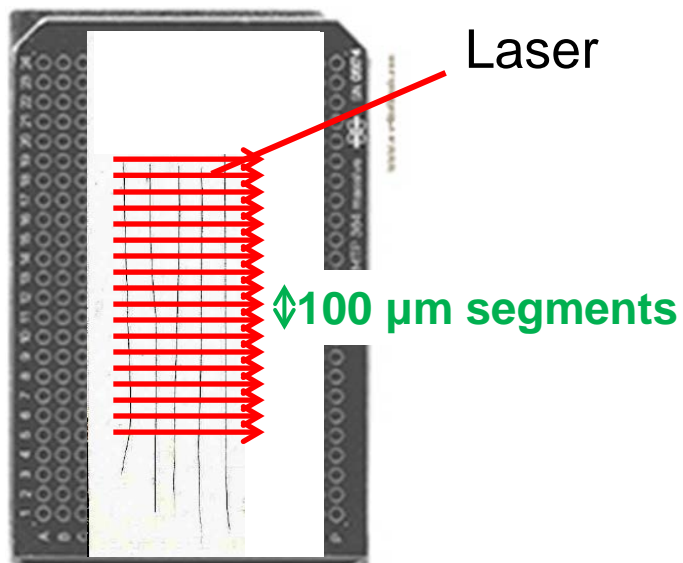


MALDI-QqQ:

AB Sciex
Flash Quant Workstation 4000 Qtrap

Data Acquisition:

- MRM mode
- Spectra (EPI)

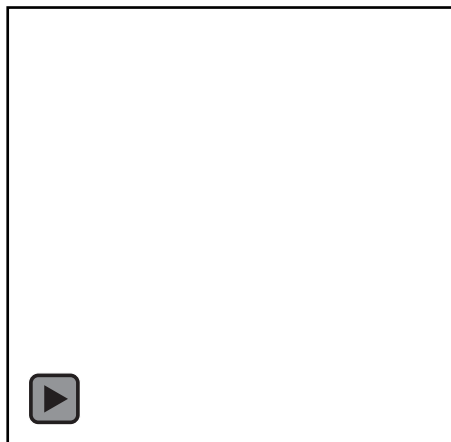




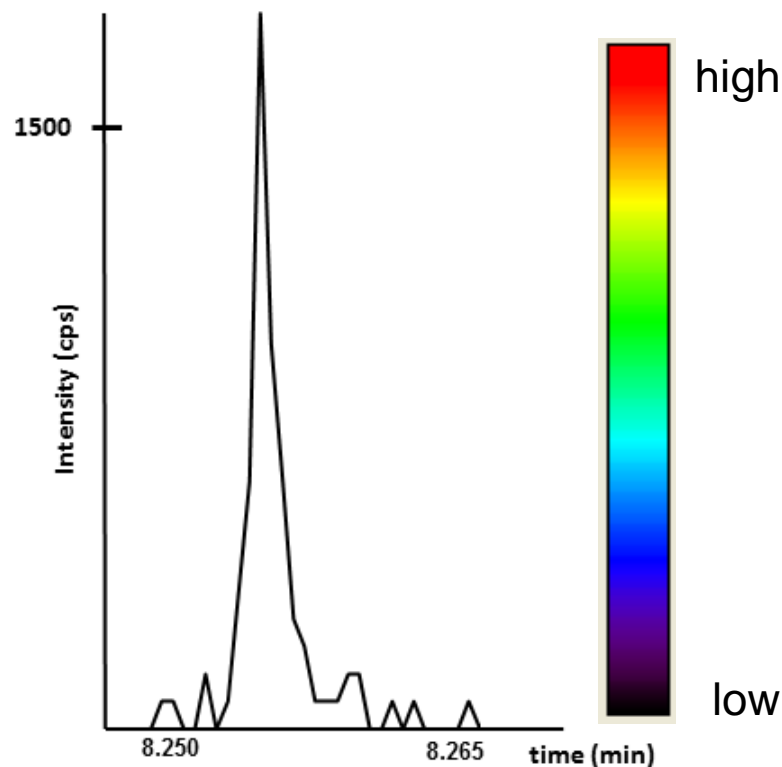
MALDI-MSI Data Acquisition



AB Sciex
Flash Quant Workstation 4000 Qtrap

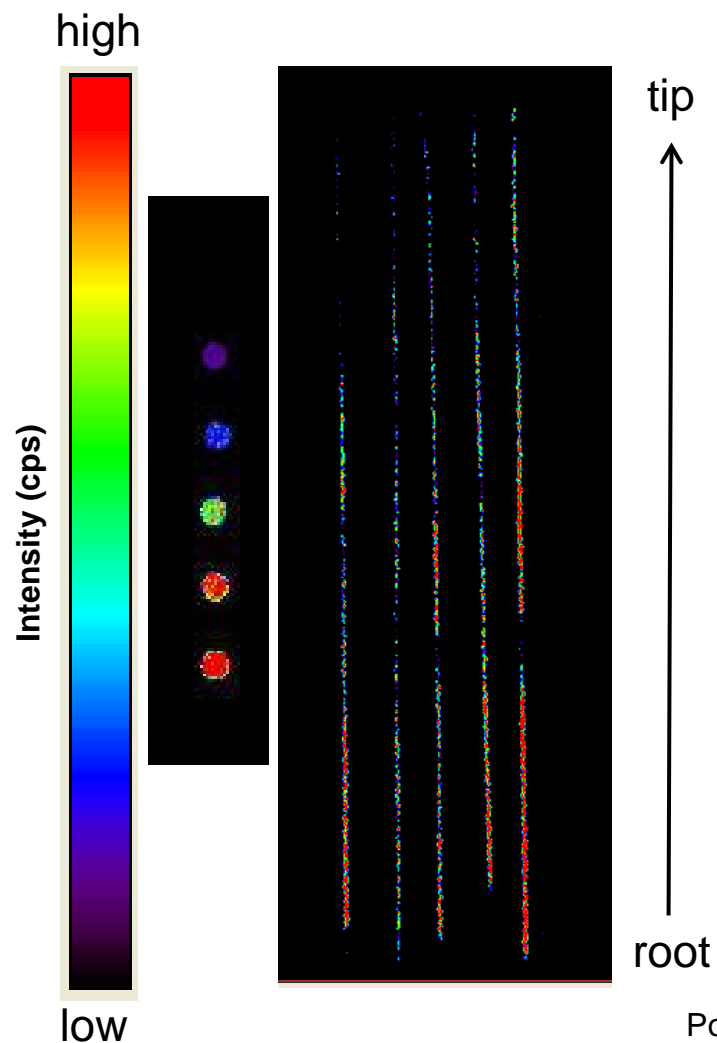


- MRM mode
- Spectra (EPI)



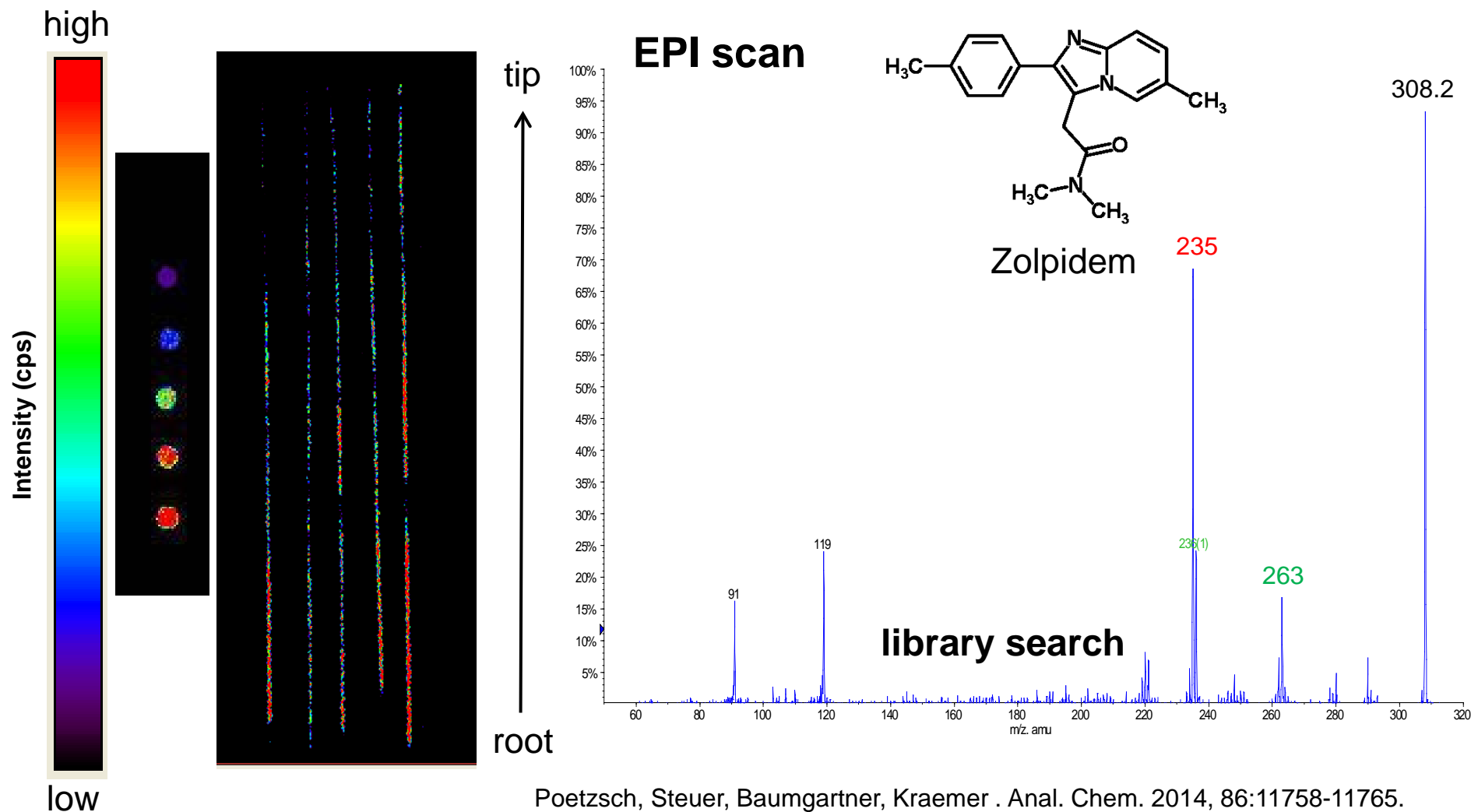


MALDI-MSI: DOA Screening in Single Hair



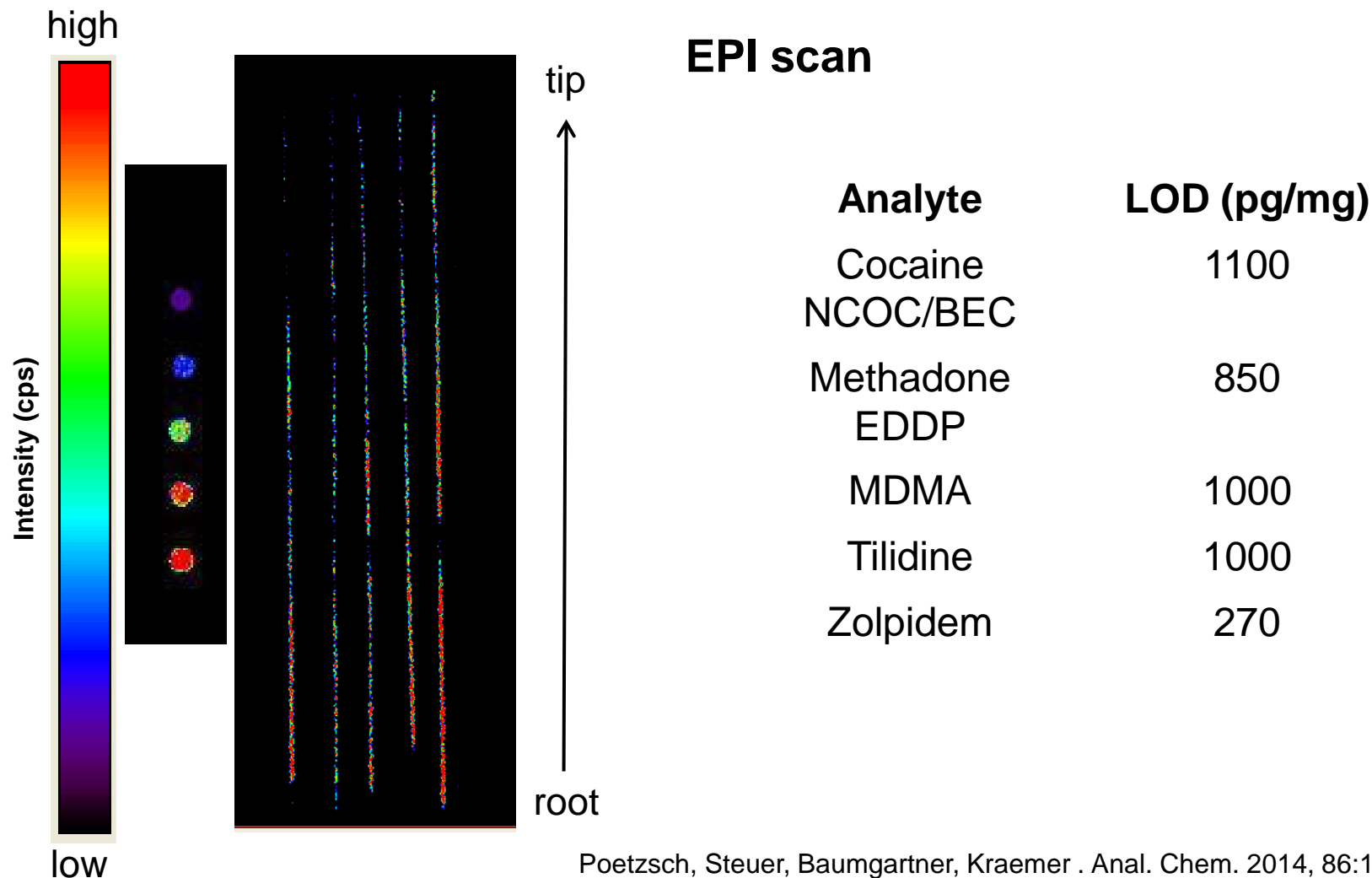


MALDI-MSI: DOA Screening in Single Hair

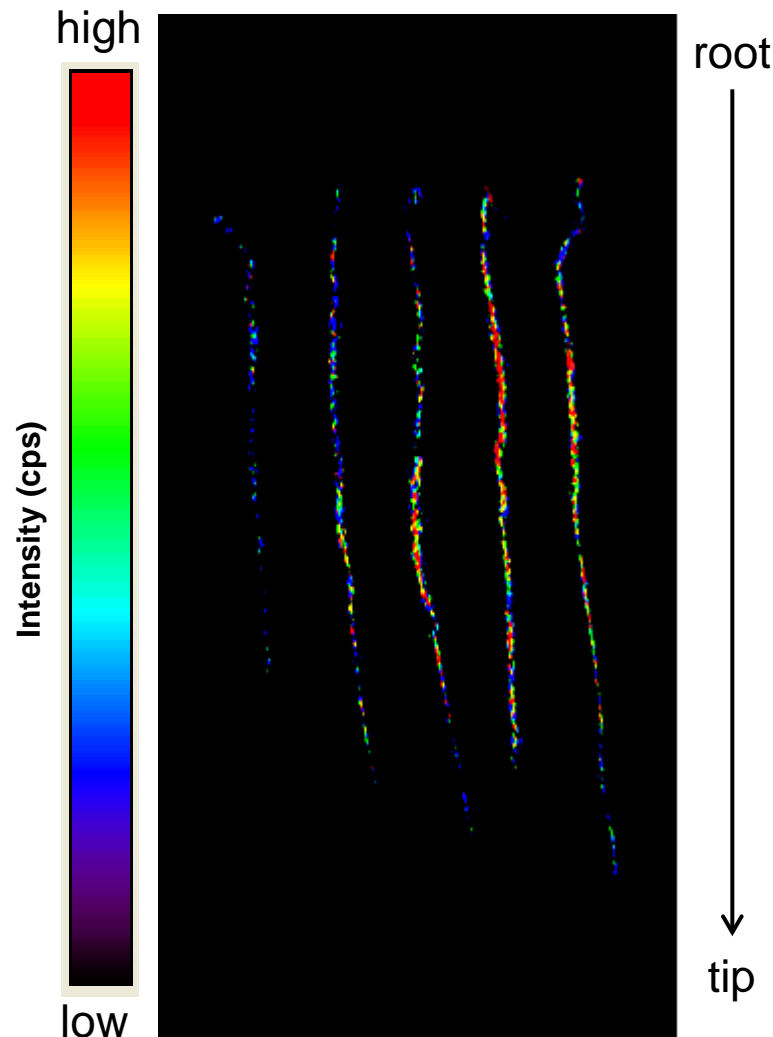




MALDI-MSI: DOA Screening in Single Hair



Imaging of Tilidine in Hair in a Forensic Case



Munchhausen by proxy



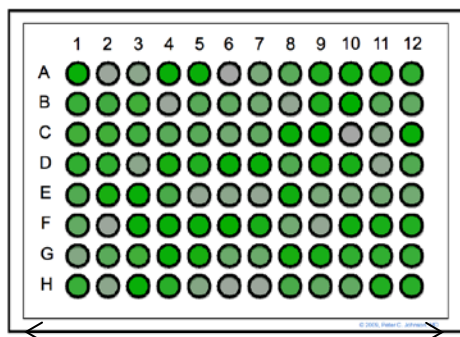
- tilidine identified over whole hair
- no indication for single / multiple application
- no pattern in single hair

MALDI-MS High Throughput Quantification

MALDI-QqQ:



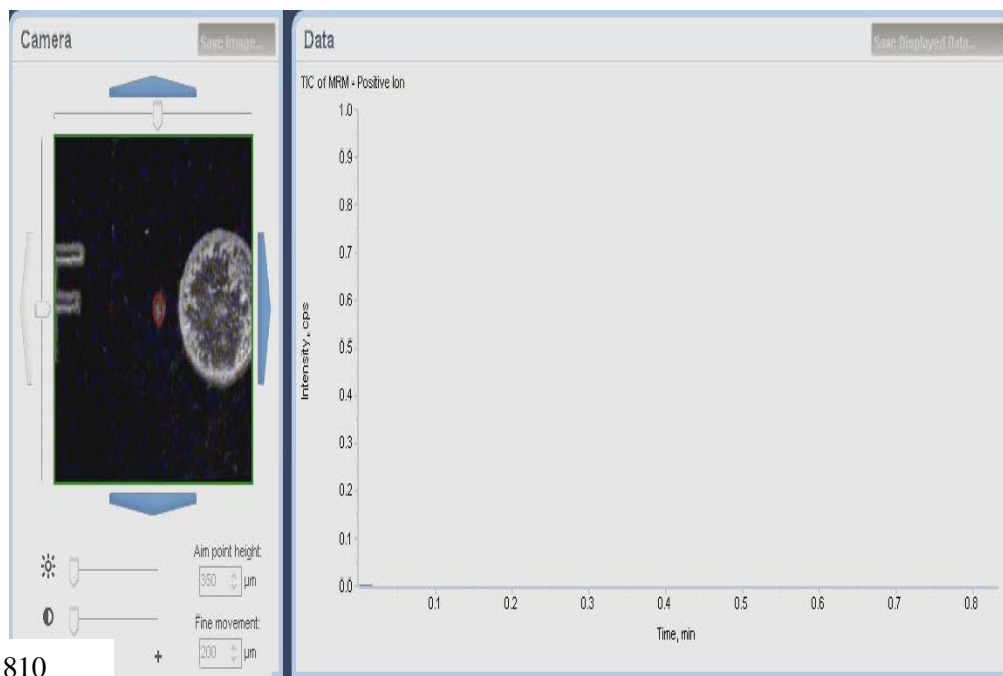
AB Sciex
Flash Quant Workstation 4000 Qtrap



stainless steel 96/384 well plate

Data Acquisition:

- MRM Mode
- 3 Seconds per well
- **384 measurements in 20 min!**
- **Vs. LC-MS 1 measurement /20 min**





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Dactyloscopy

.... is pretty easy:

Sherlock Holmes could do it ...





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Dactyloscopy

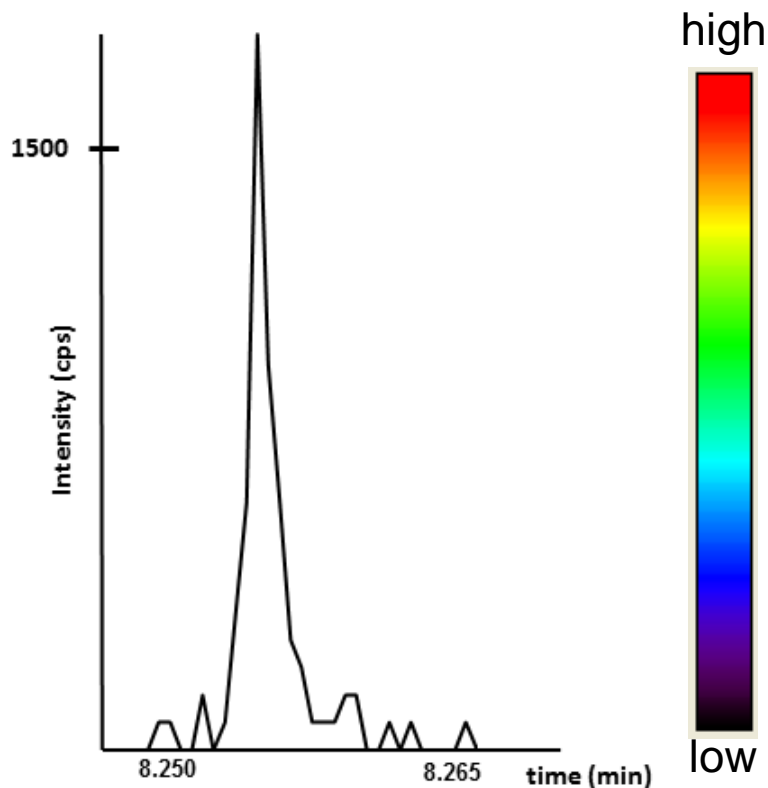
.... is pretty easy:

there is an app for that ...

Touch ID.
Your fingerprint. Your iPhone.



MALDI-MSI in Dactyloscopy



- Imaging of endogenous substances
- Imaging and identification of contaminants

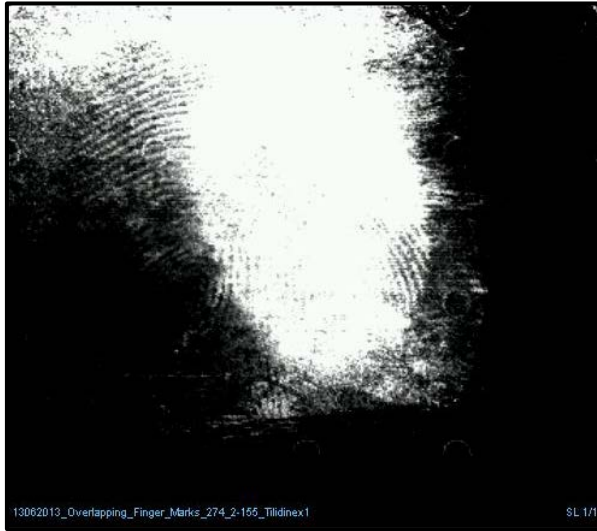


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Substance Specific Isolation of Finger Marks

**Overlapping
Fingermarks**



13062013_Overlapping_Finger_Marks_274_2-165_Tilidnex1

SL 1/1



Substance Specific Isolation of Finger Marks

Overlapping
Fingermarks



Substance Specific
MRM Transitions
(Zolpidem)



Substance Specific Isolation of Finger Marks

Overlapping
Fingermarks



Isolation of
substance
specific
transition



B/W conversion
database search

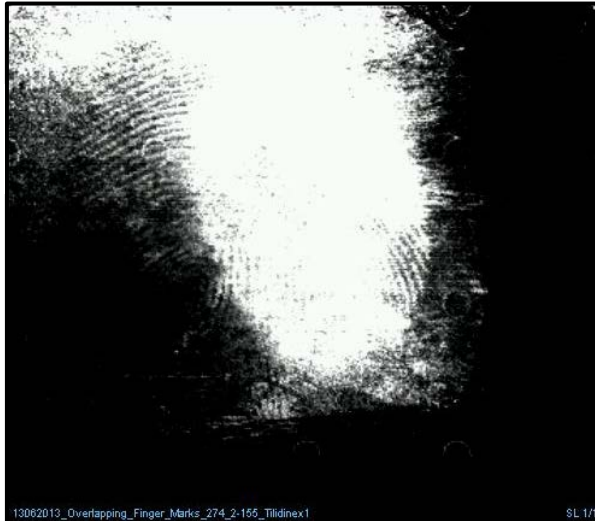
Substance Specific
MRM Transitions
(Zolpidem)





Substance Specific Isolation of Finger Marks

Overlapping
Fingermarks



13062013_Overlapping_Finger_Marks_274_2-155_Tilidnex1

SL 1/1



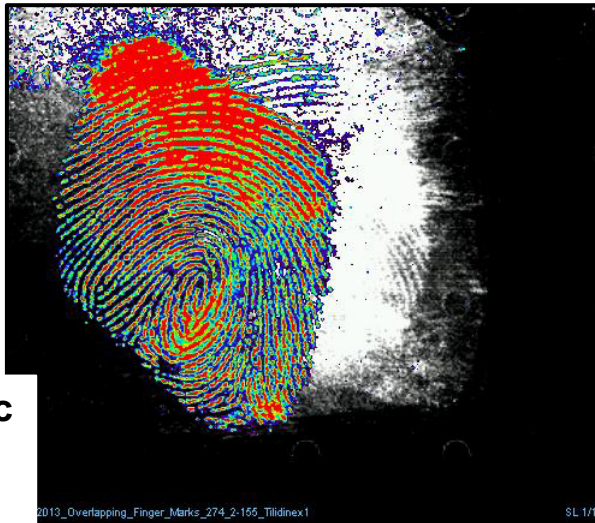
13062013_Overlapping_Finger_Marks_308_2-235_Zolpidem1_x1

SL 1/1

Isolation of
substance
specific
transition



Substance Specific
MRM Transitions
(Zolpidem)



2013_Overlapping_Finger_Marks_274_2-155_Tilidnex1

SL 1/1



13062013_Overlapping_Finger_Marks_308_2-235_Zolpidem1_x1

SL 1/1

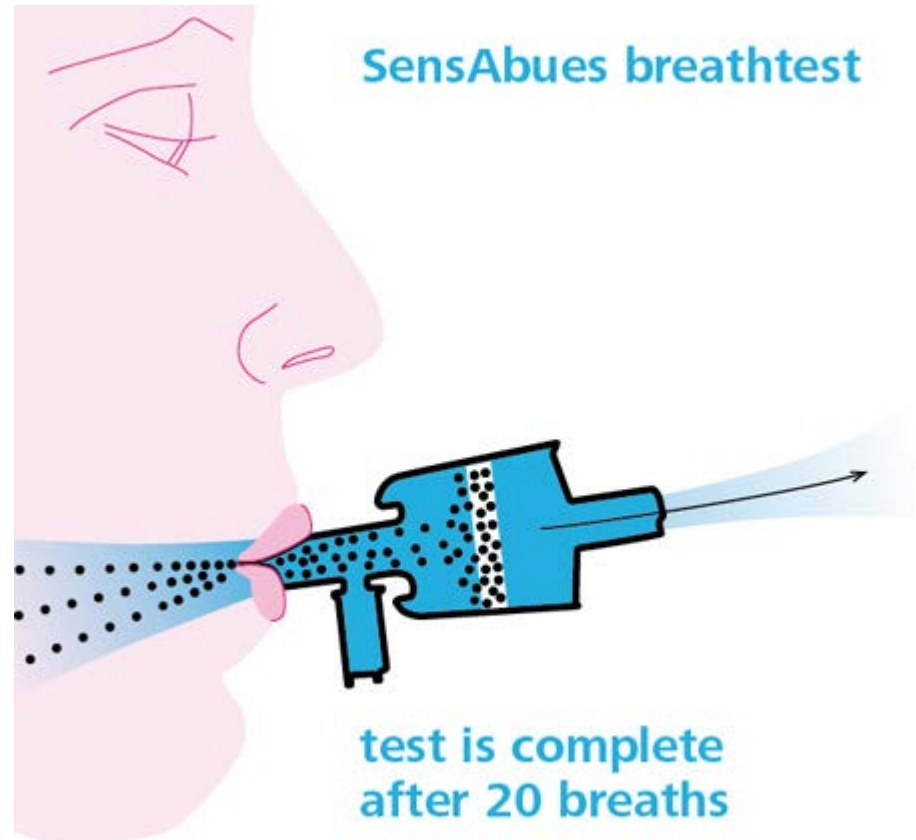
B/W conversion
database search



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Detection of Drugs/Medicaments in Exhaled Air





Drugs of Abuse Breath Testing

Step 1

- 20 µl deuterated standard solution on filter

Step 2

- preconditioning with 2 ml MeOH, extraction 5 ml MeOH

Step 3

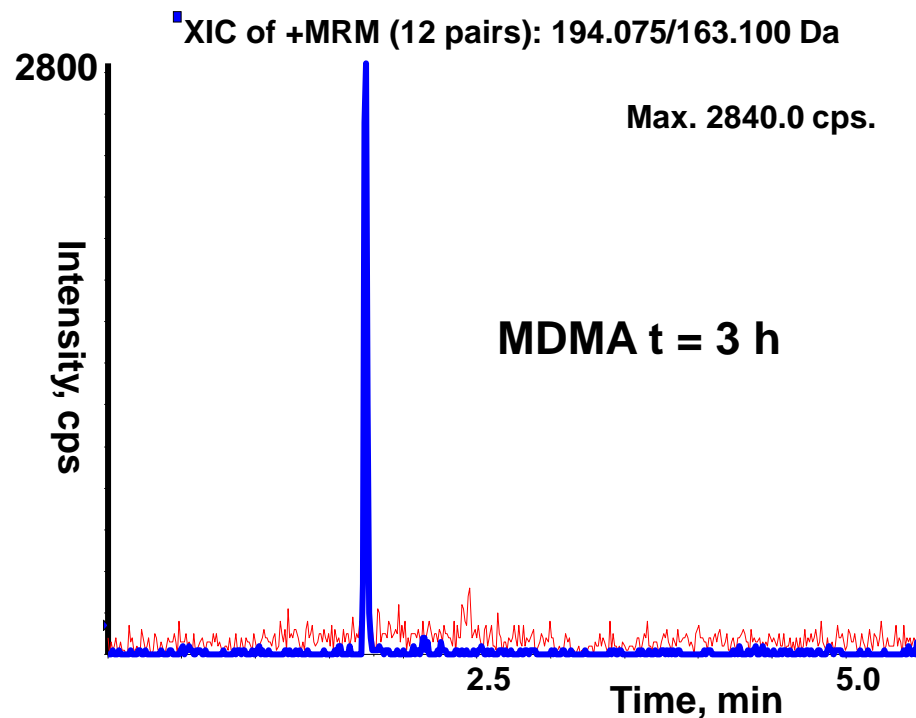
- adding 20 µl formic acid
- evaporation to dryness 40 °C, N₂

Step 4

- reconstitution in eluents

Step 5

- Dionex UHPLC, Kinetex[®] PFP column
- AB Sciex 5500, 3 MRMs





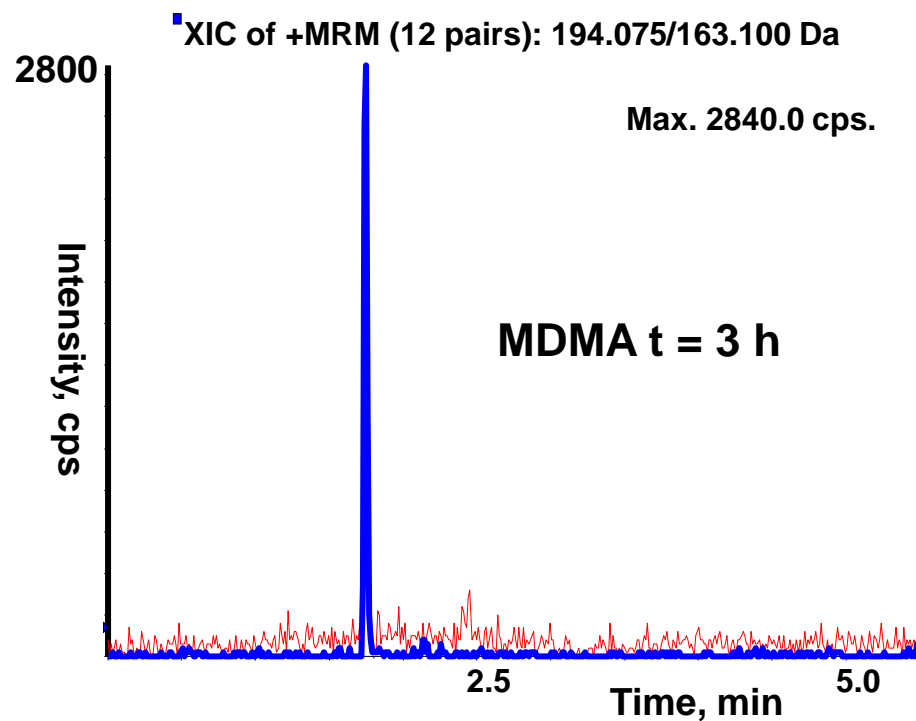
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DOA Breath Testing



**Stop breathing!
Or they might catch you ...**





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Mass Spectrometry is Everywhere





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Thanks to:



Working group FPT, Zurich

Beat Keller from

Forensisches Institut Zürich

Eine Organisation von Kantonspolizei und Stadtpolizei Zürich



Olof Beck



Gérard Hopfgartner



Emmanuel Varesio



Tiffany Porta



Matthias Liechi
Working group
Clinical Pharmacology



Kristin Schirmer



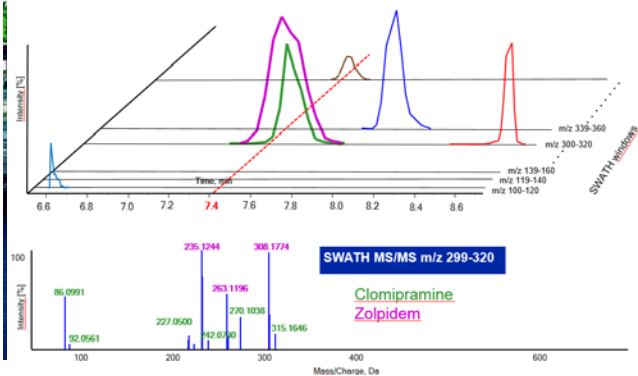
Ksenia Groh

..... and to all criminals who make sure that we have all the interesting cases !

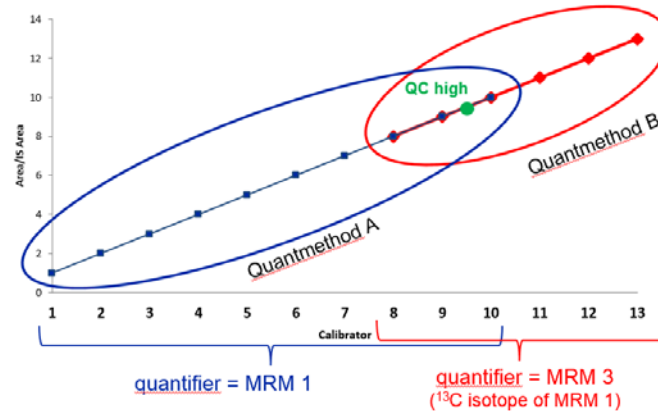
Thank you for your attention!



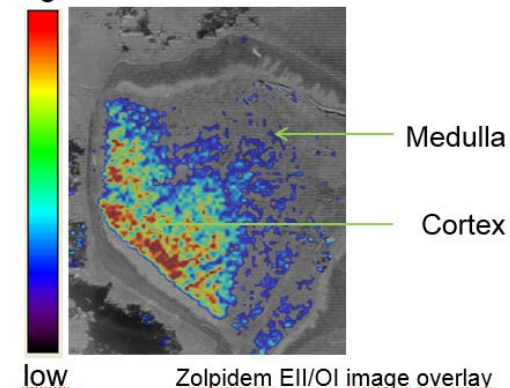
SWATH Application



Dynamic Range Extension by ^{13}C Calibration



high





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