The usefulness of Ion Mobility-Mass Spectrometry for Small Molecules Analysis

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Plan

- Breve introduction to Ion-mobility Spectroscopy
- IMS for small molecules (low molecular weight)
  - Metallomics (selenometabolomics – proof of concept) and *de-novo* structural assignation
  - Pesticides screening (S. Goscinny *et al*.)
  - Disulfide bridge assignation in oligopeptide for venomics (proof of concept; J. Echterbille *et al*.)
- conclusions

Concept of Ion Mobility

The Drift Tube Ion Mobility Mass Spectrometry

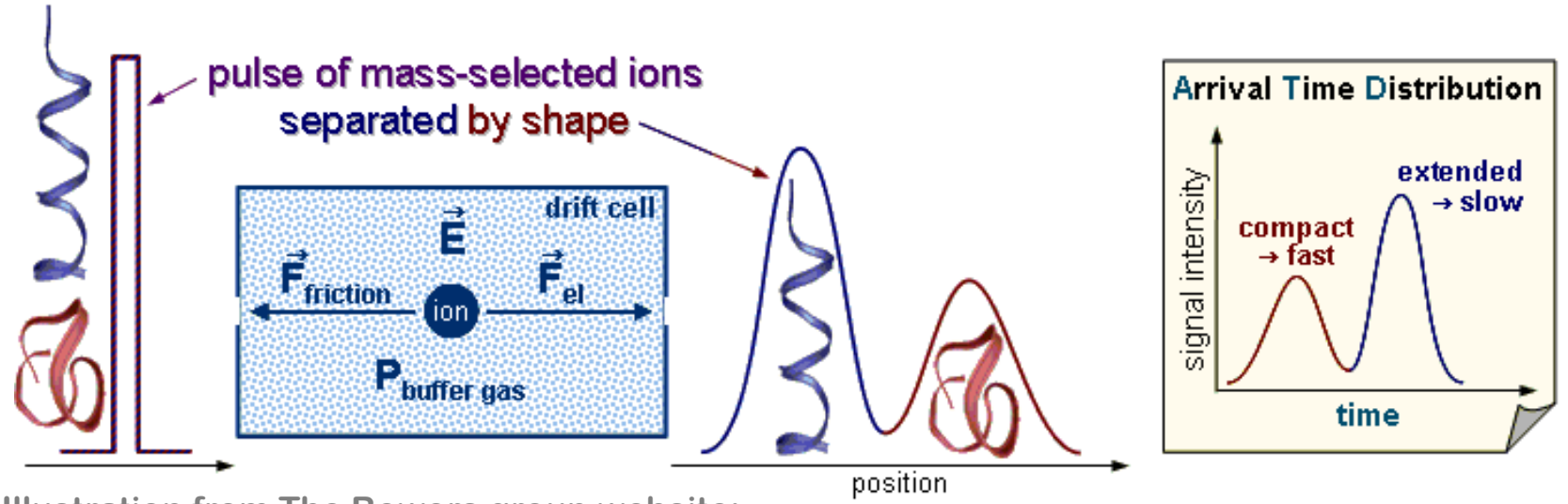


Illustration from The Bowers group website:
www.bowers.chem.ucsb.edu/theory_analysis/ion-mobility/index.shtml

Ion Mobility Spectrometry is usually the technique of choice of (bio)macromolecules like proteins and DNA

- **G. Gabellica *et al.*, G-quadruplex structure determination**

V. Gabellica *et al.*, J. Am. Chem. Soc., 2007, 129: 895-904

- **Proteins structures analysis (e.g. prions)**

Hilton *et al.*, J. Mass Spectrom, 2010, 21 (5): 845-854

Different design of IMS

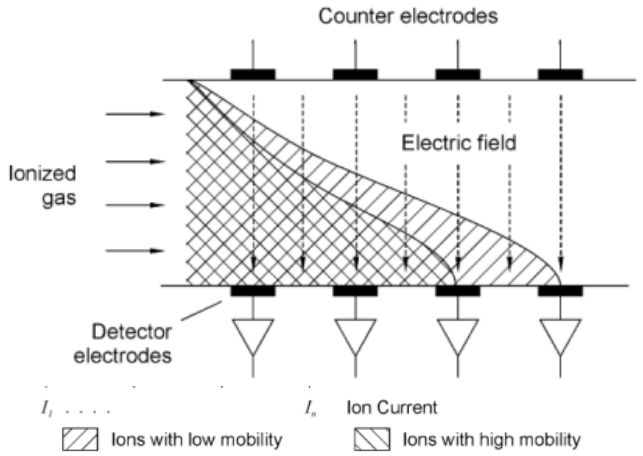
- **DTIMS (previous slide)**

Drift-Time Ion Mobility Spectrometry

- **AIMS**

aspiration ion mobility spectrometry

Zimmermann and coworkers, Sensors and Actuators B, 2007: 428-434



- **DMS / FAIMS**

Differential-Mobility Spectrometry

Field-Asymmetric waveform Ion Mobility Spectrometry

Kolakowski and Mester, Analyst, 2007 132: 842-854

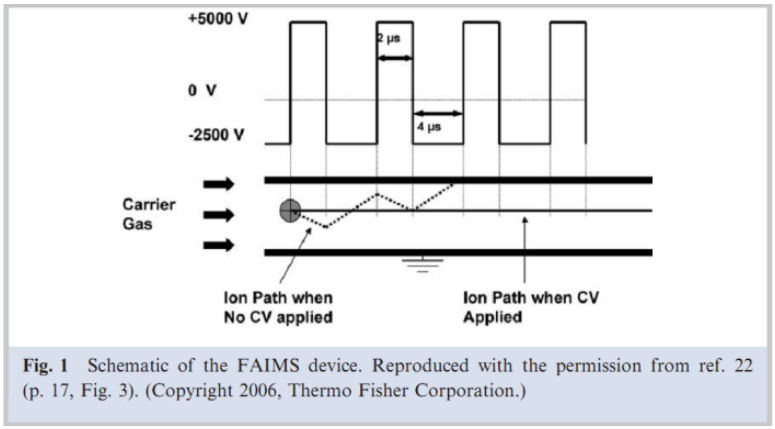


Fig. 1 Schematic of the FAIMS device. Reproduced with the permission from ref. 22 (p. 17, Fig. 3). (Copyright 2006, Thermo Fisher Corporation.)

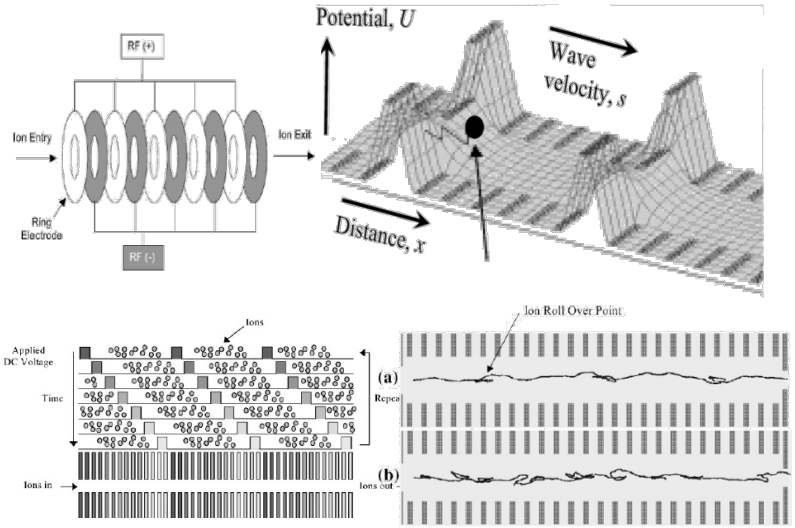
- **TWIMS (next slide)**

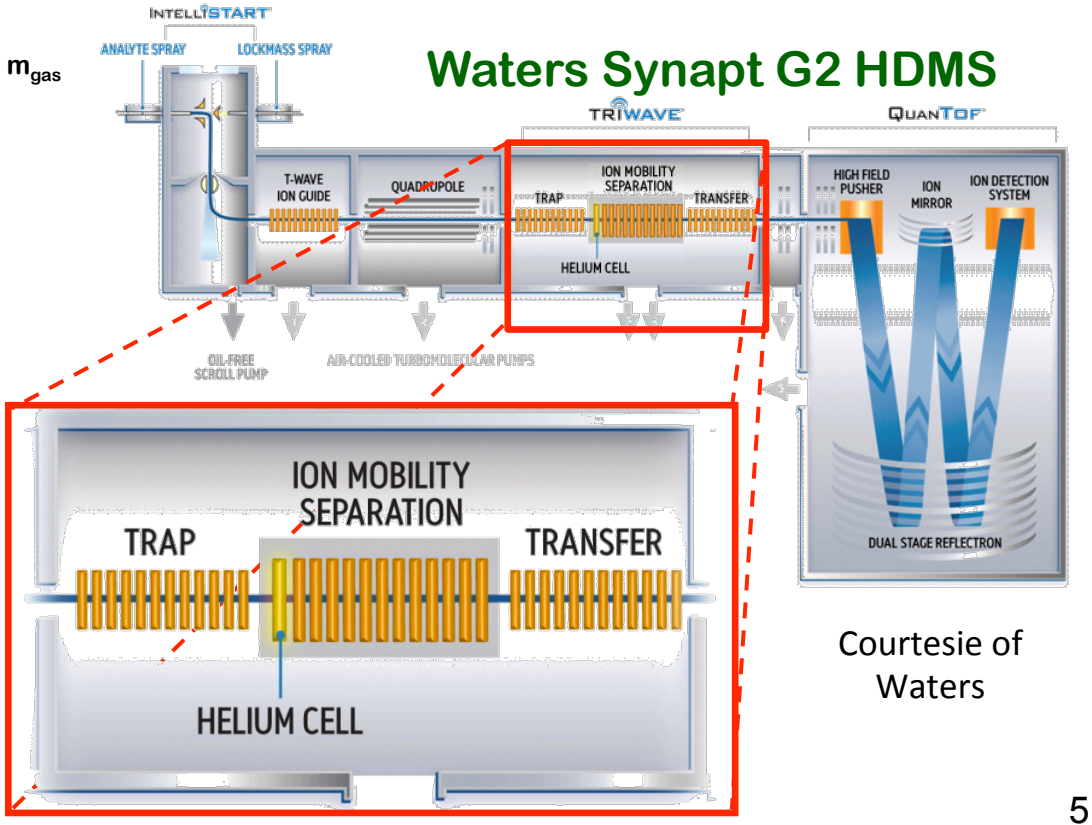
Ion Mobility Mass Spectrometry

- **General principles of Travelling Wave Ion Mobility Spectroscopy:**

$$K=(3q/16N)(2\pi/kT)^{1/2}(m+M/mM)^{1/2}(1/\Omega)$$

- K: ion mobility constant — q: charge of ion (Coulomb)
- N: density of buffer gas — k: Boltzmann's constant
- T: temperature (Kelvin)
- m: mass of gas, M mass of ion → reduced mass ≈ m_{gas}
- Ω: collision cross-section (A^2)

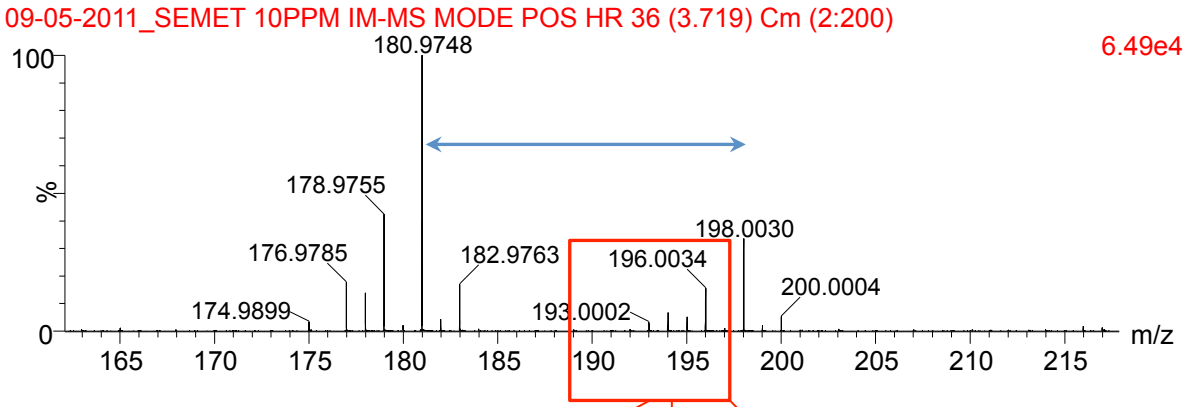




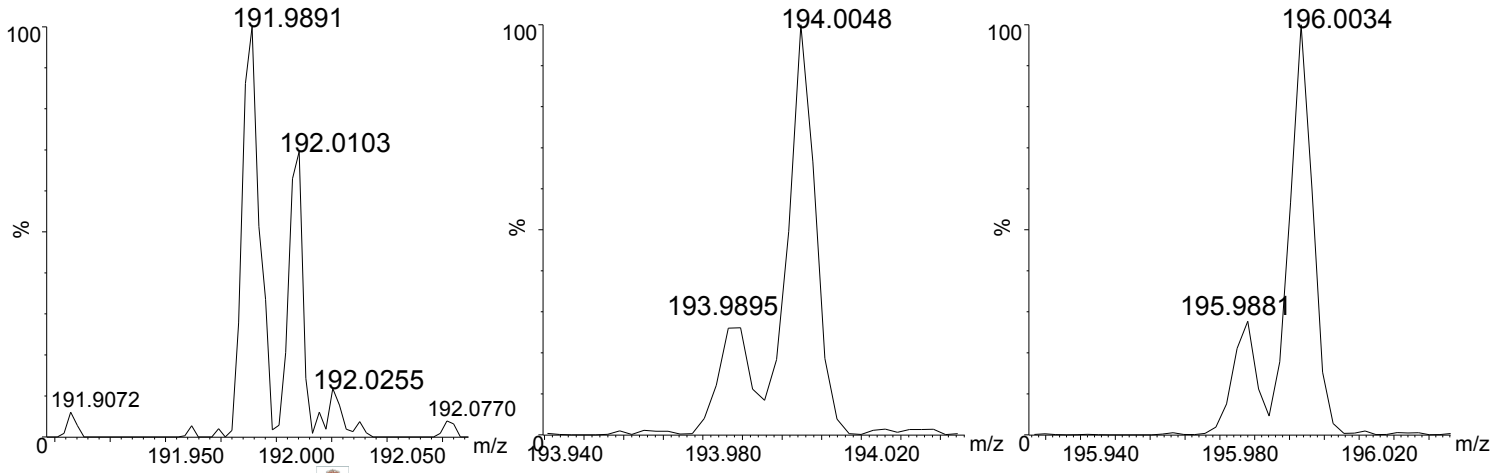
Courtesie of Waters

Structural assignation of an isobar compound in a L and D,L- SeMet standard by ESI IM MS and MS^E

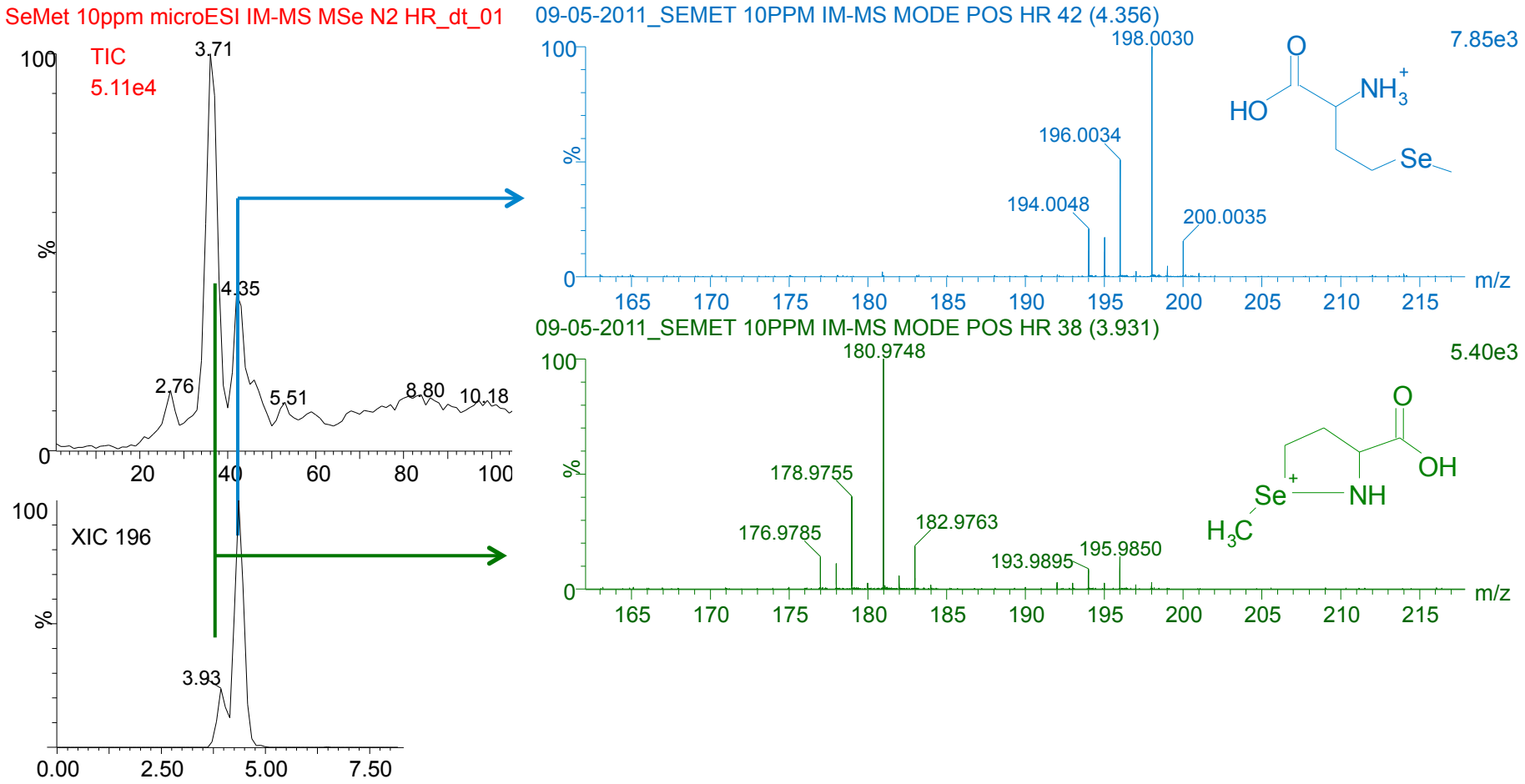
- ESI MS SeMet and reflectron in V optic mode:
- Δm = -2.02 ppm,
- $R_{FWHM} \approx 12\,000$
- Loss of 17,0282 (NH_3?)
  (198.0030-180.9748)
- NH3 = 17.0265
  - Δm = 98.8 ppm !!!



- ESI MS SeMet and reflectron in W optic mode :
- Δm SeMet = -2.02 ppm, $R_{FWHM} \approx 25\,000$

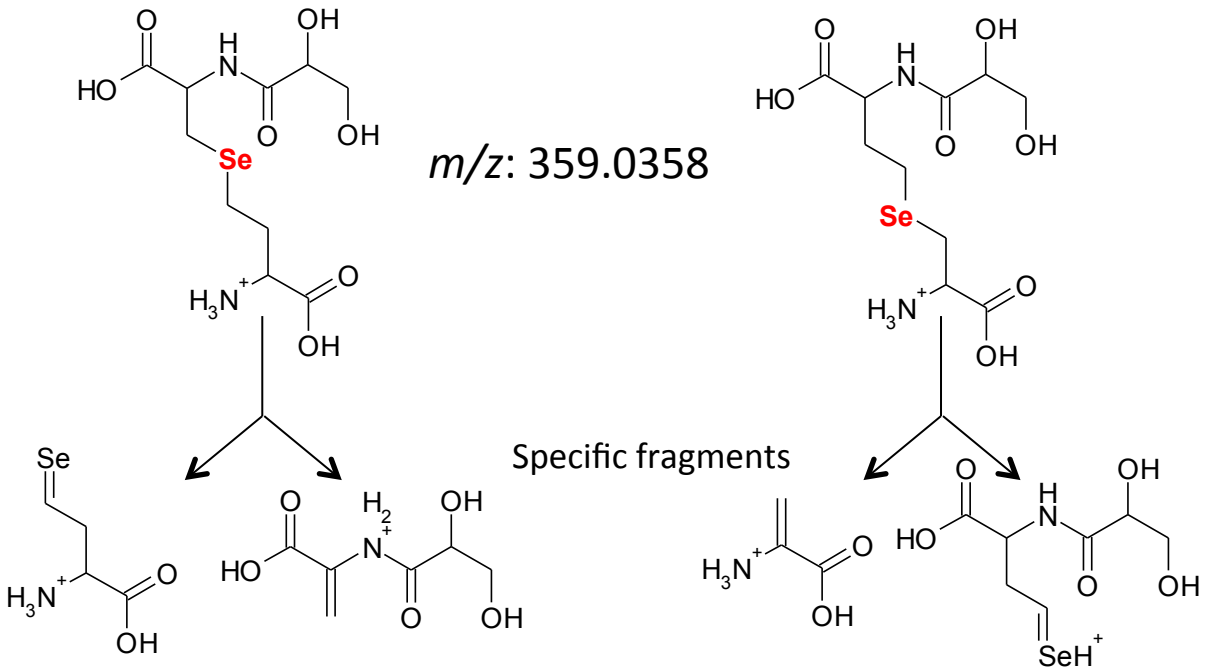


Structural assignation of an isobar compound in a L or D,L- SeMet standard by ESI IM MS



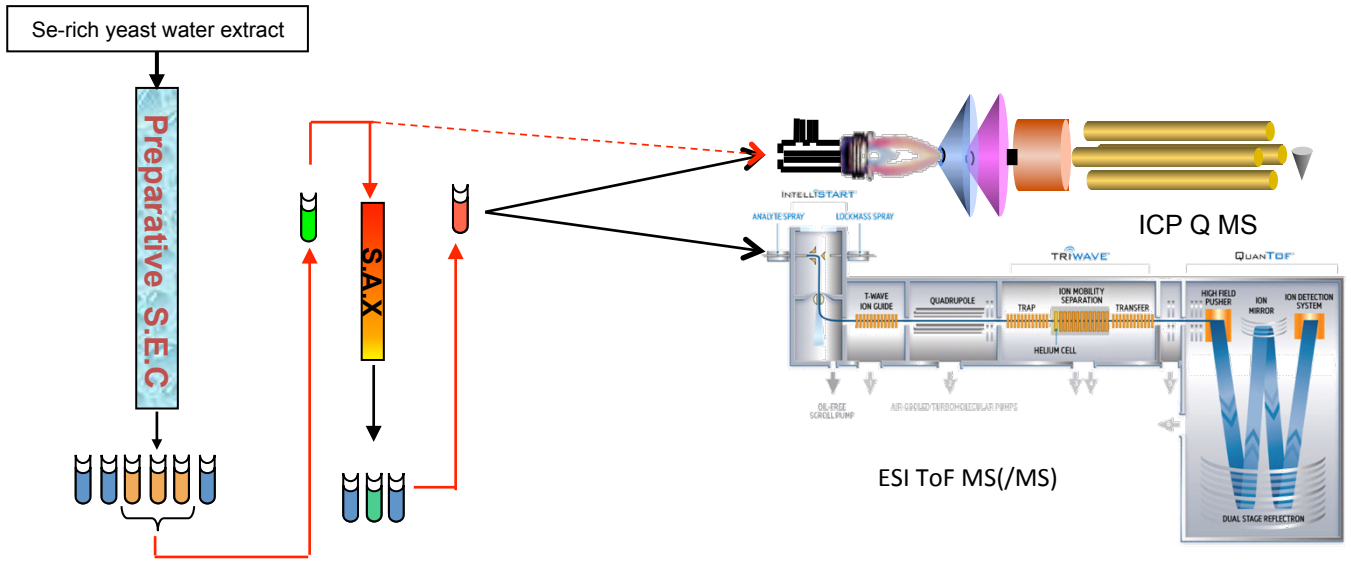
N-2,3-dihydroxypropionyl-selenocystathionine

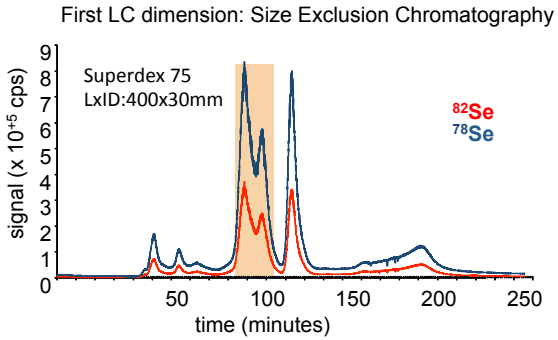
- **Major selenocompound in the low molecular weight water extract from Se-rich yeast**
- **[$C_{10}H_{18}N_2O_7Se + H^+$] *m/z* : 359.0358**
- **Present in various batches of commercial Se-rich yeast [Gil-Casal and coworkers Metallomics (2010)] and Lab-made Se-rich yeast [Rao and coworker Anal. Chem. (2010)]**
- **Metabolic origin or by-product ? (No sulfur analogue describe in the literature)**
- **Two coeluted isomers, confirmation on the basis of (MS^2), MS^3 and MS^4 data**

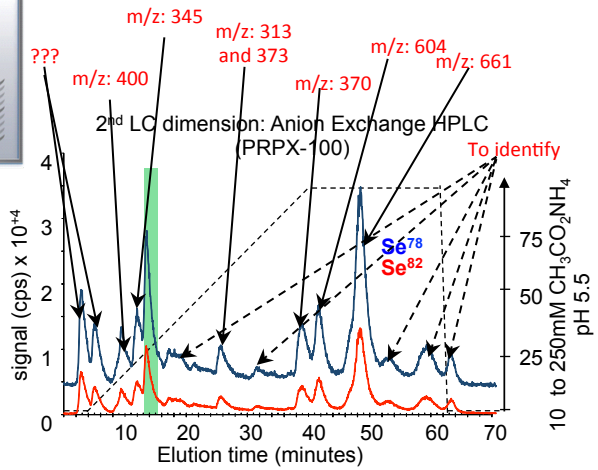


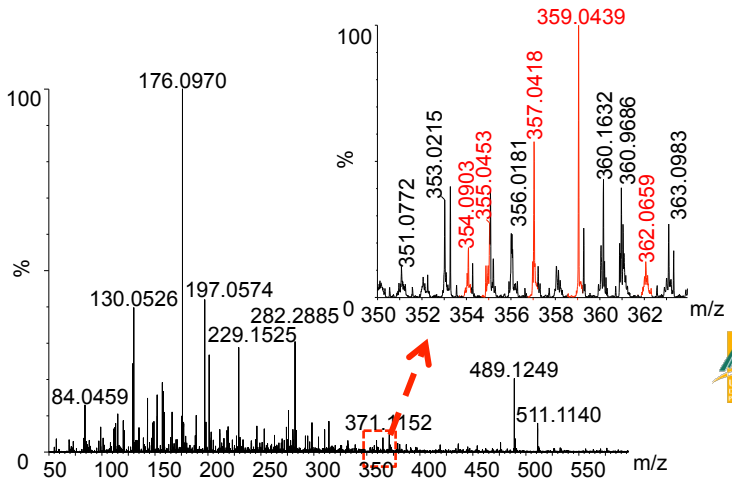
2D-LC purification with of selenometabolites using ICP/MS and ESI ToF MS(/MS) detection

- **Purification of *m/z*: 359 according to Dernovics *and coworkers*, (2009) metallomics, with modifications**









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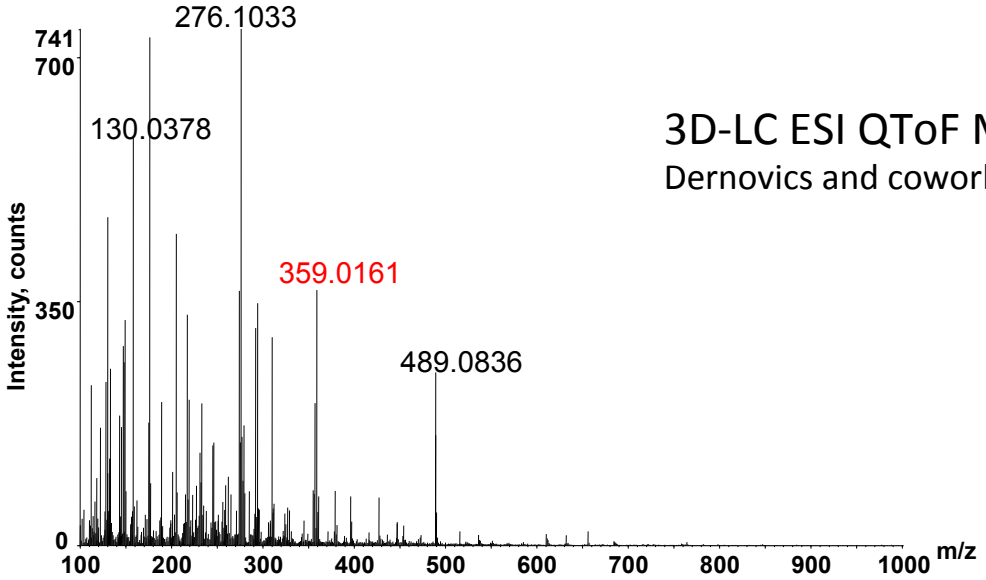
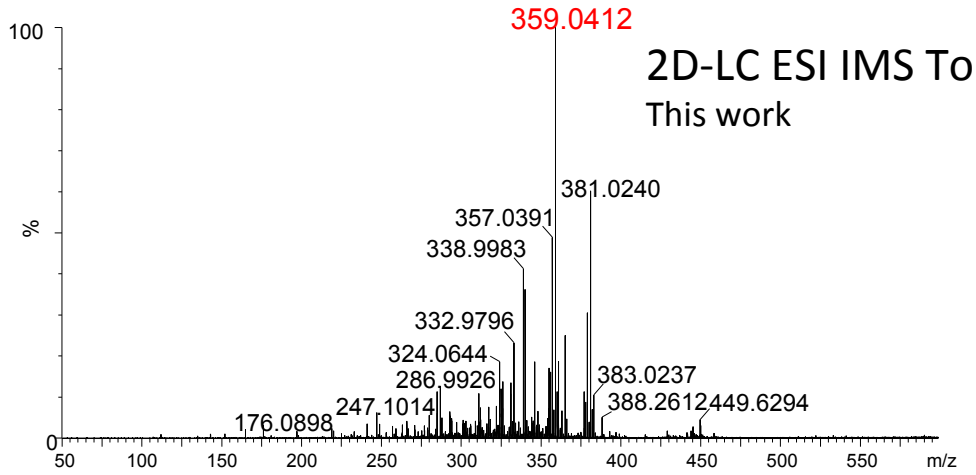
Clean-up of mass spectra by IM-MS:



3D-LC ESI QToF MS

Dernovics and coworkers, Metallomics (2009) **1**, 317–239



2D-LC ESI IMS ToF MS

This work

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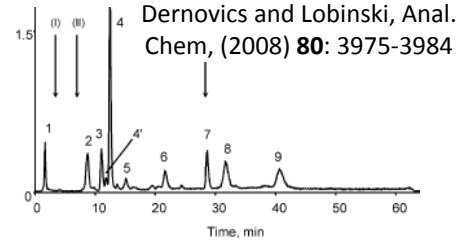
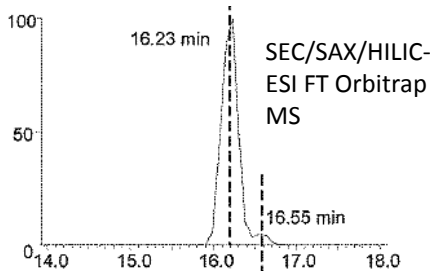
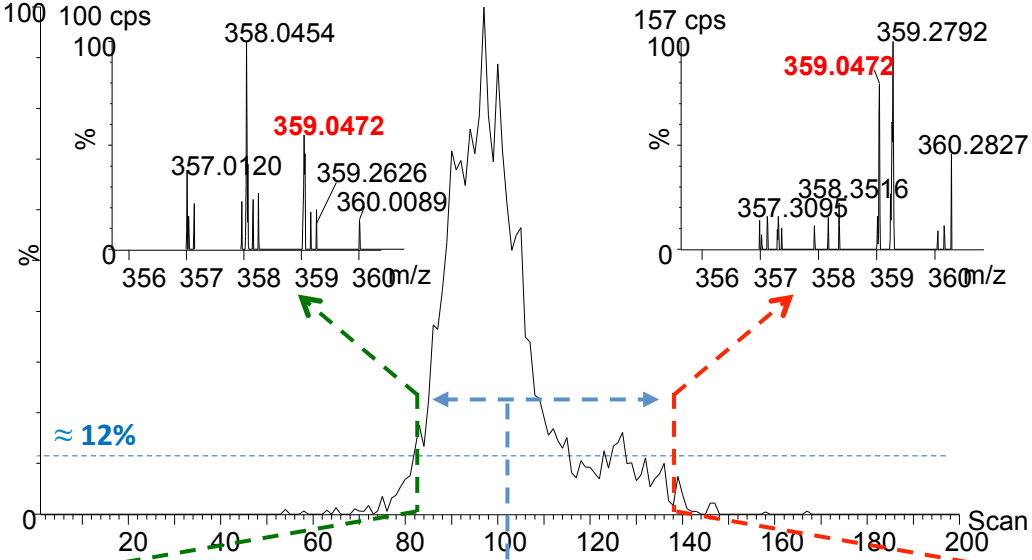
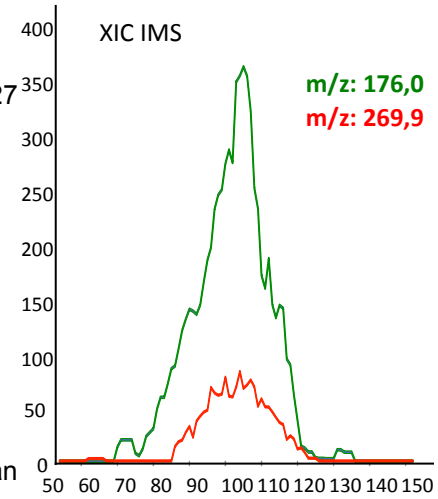
Separation of *N*-2,3-DiHydroxyPropionyl-SelenoCystathionine by ESI IM-MS and estimation of isomer ratio

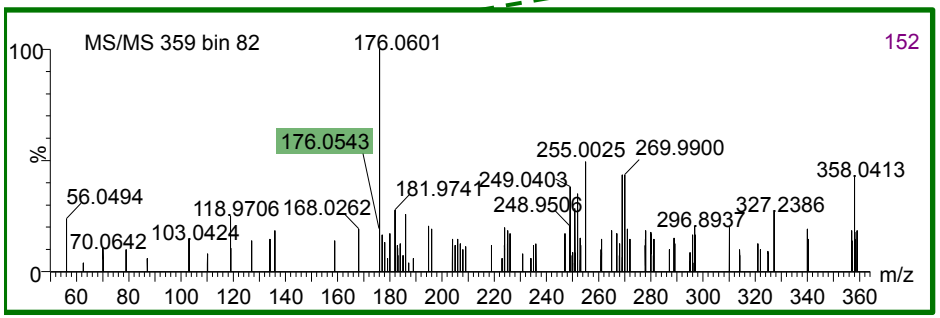
Selection of *m/z*: 357 and 359 on quad, then IMS and MS spectra

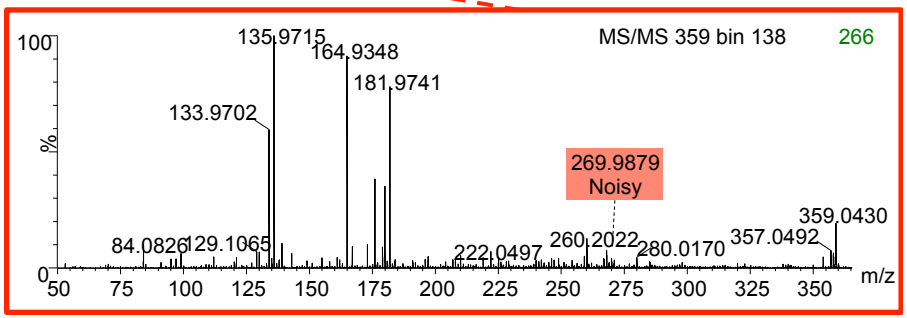


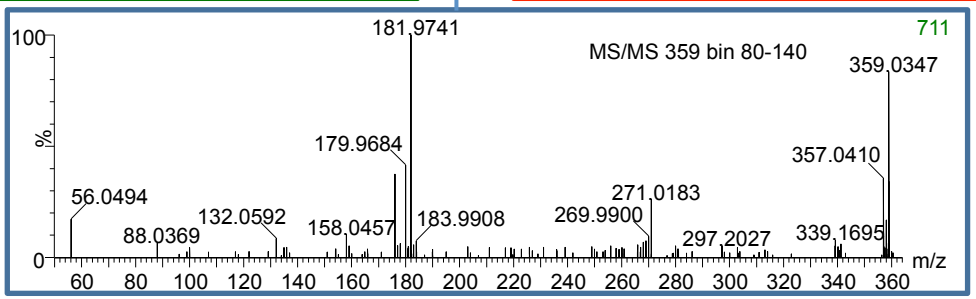












Confirmation experiments in progress

Ion Mobility Mass Spectrometry for pesticides screening

- Pesticides are rich in diversity:
  - chemical structure,
  - solubility, volatility,
  - Persistence in organisms and environment

- in number: More than 1200 molecules
  - around 740 are allowed to be used in the EU
  - around 500 compounds sought/sample

- LC based separation is the major used analytical method
- Pesticides screening: Multiresidues methods are mandatory

isp WIV
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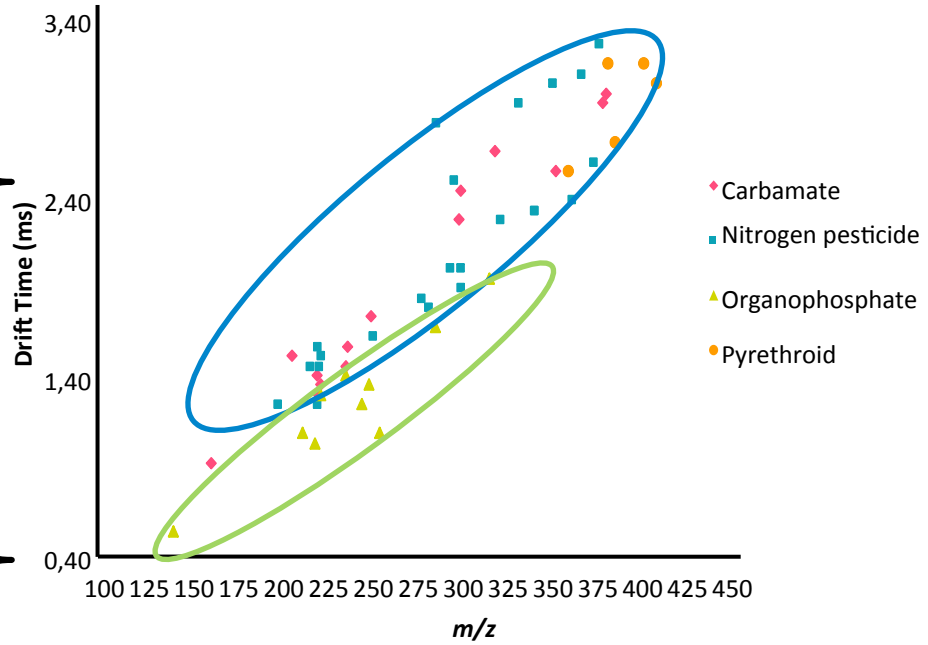
Plackett-Burman design

4 representative groups of pesticides

construction of the design is done with 15 runs

- 5 parameters
  - IMS T-Wave velocity ($m.s^{-1}$)
  - IMS T-Wave Height (V)
  - Gas Pressure (mbar)
  - Helium Cell Pressure (mbar)
  - Biais (V)
- 3 responses
  - Intensity (cps, aera)
  - Resolution
  - Relative drift time (ms)



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IM-MS analyses of pesticides: spectrum clean-up

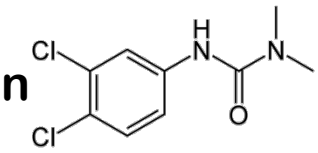
- Pesticides screening:
  - solubility, volatility: LC retention time
  - chemical structure: *m/z* in MS
  - Discrimination using drift time of isobar and coeluted compounds

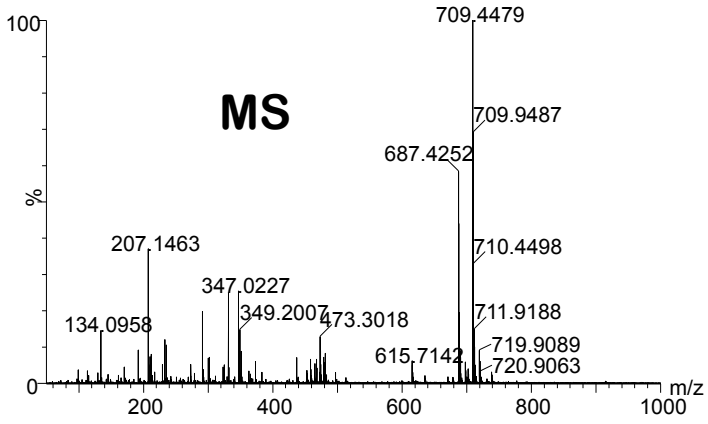
Water / Methanol extraction

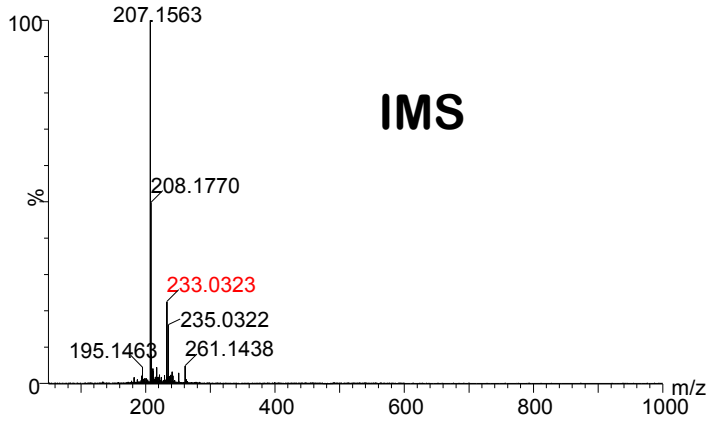
RP-UPLC ESI MS screening of Diuron

RT: 6.38 min

$[M+H]^+$: 233.0248







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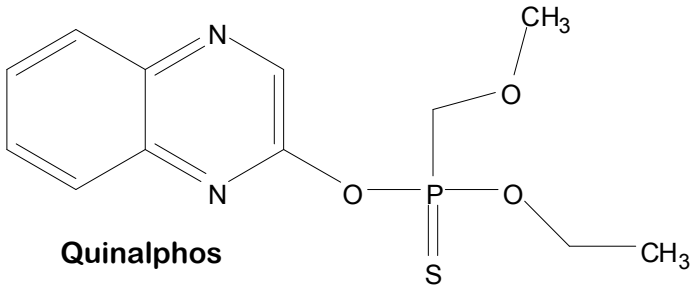
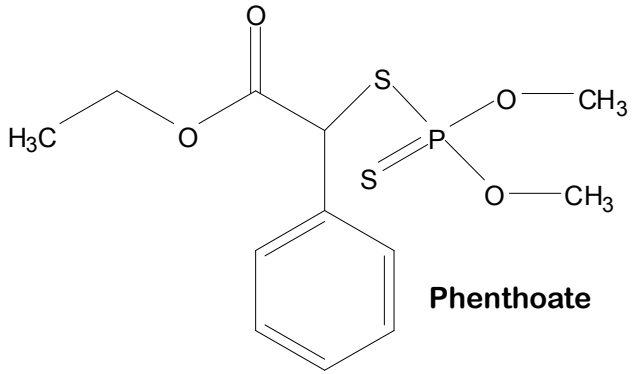
Discrimination of co-eluted molecules by IM-MS during pesticides screening

Pesticide:	*m/z*	drift time (ms)
Quinalphos	$[M+H]^+$: 299.0619	3,81
Quinalphos	$[M+Na]^+$:321.0439	4,97
Phenthoate	$[M+H]^+$: 321.0384	4,22



Quinalphos



Phenthoate

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IMS for peptides structure elucidation in venomics field

- Toxins have many disulfide brigdes and are highly structured

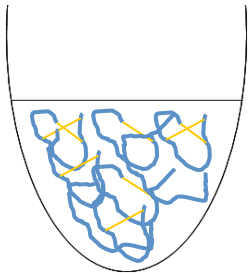
- These bonds fulfill multiple functions :
  - Provide the toxin proper conformation to bind to the targeted receptor
  - Reduce the immunogenicity
  - Provide a high stability in time

- But…
  - SS bonds imply low fragmentation ratio during MS/MS (CID) experiments ➔ Sequencing ☹
  - Disulfide bonds assignment is very complex and time-consuming!!!

Cytotoxin-1 from Naja oxiana venom- ~7KDa / 5 SS

Conotoxin MVIIA from Conus magus - ~3KDa / 3 SS

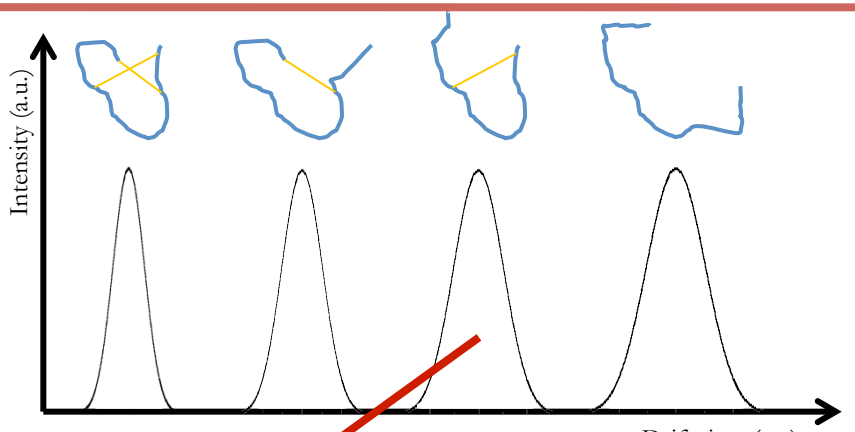
Methodology: IMS for peptides structure elucidation in venomics field



Mix of native, partially reduced and reduced forms of a 2 disulfide bridge-containing peptide

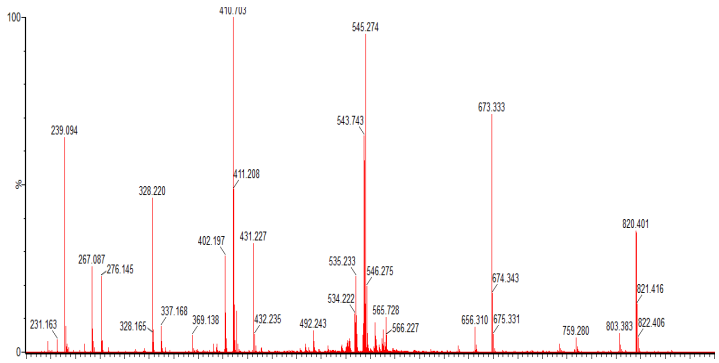
Nano-ESI infusion

Mobility separation



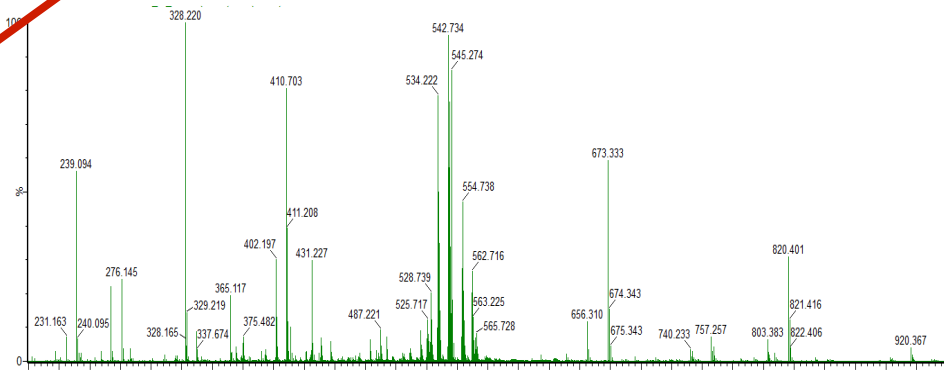
Four contributions to the arrival time distribution

CID in the Transfer module



Sequencing of each toxin's form

Extraction of CID spectrum



Recording of a total CID spectrum

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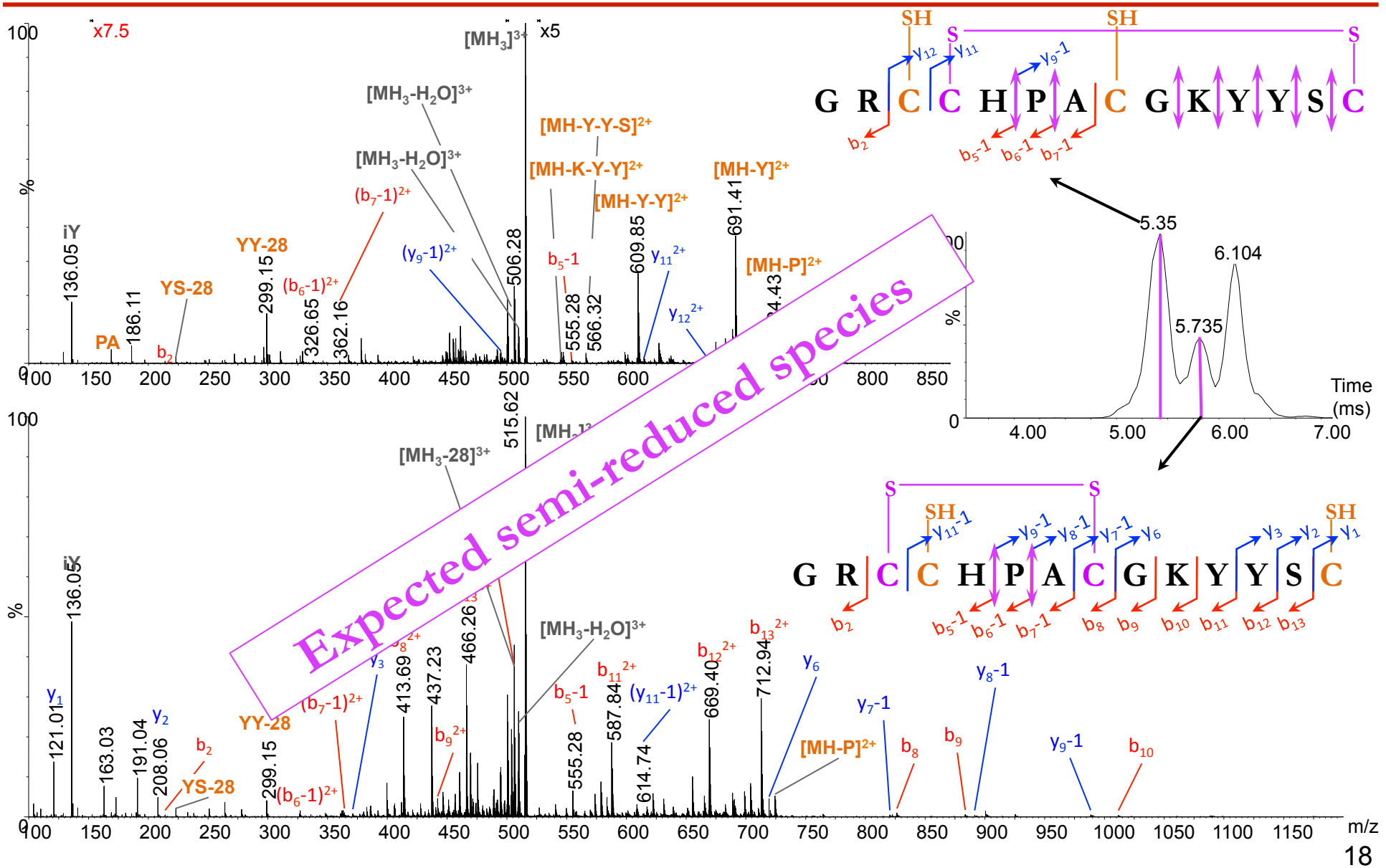
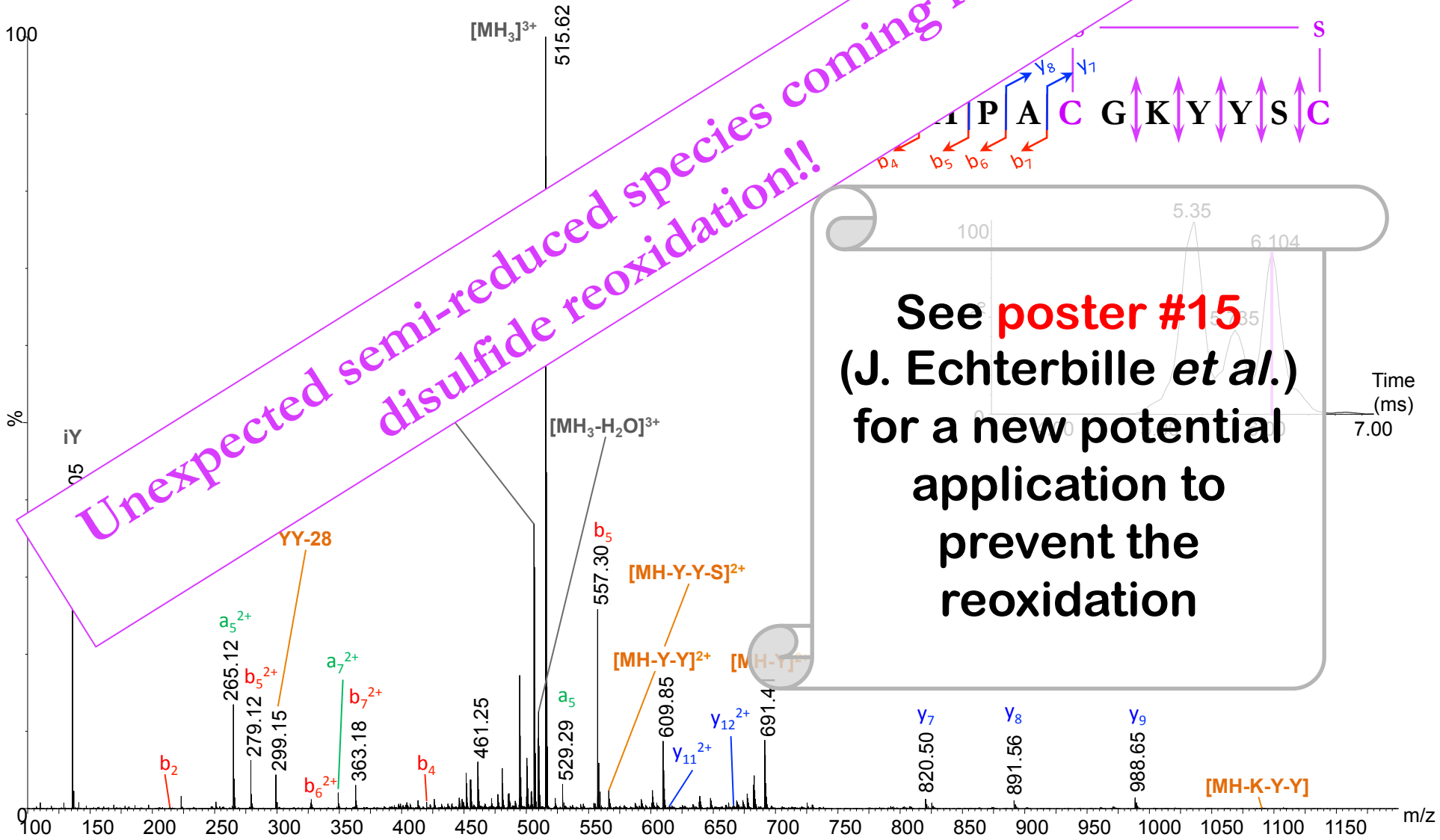
Poster 15

Fragmentation spectrum of the semi-reduced forms (arrival time : 5.371 & 5.735 ms)



Fragmentation spectrum of the semi-reduced form (arrival time : 6.104 m



Conclusions and perspectives

- **Ion Mobility is a valuable tool for small molecules analyses because of:**
  - **Spectra cleaning of trace residues (addition of a new orthogonal separation of ion, removing the matrix background)**
  - **Provide a new potential identification criterion for screening and identification for analytical purposes: the drift time**
  - **Get deeper insight into structural information compare to "regular" MS**

- **Instrument can offer additional potentialities that are under investigations to propose new strategies with IM-MS**

Acknowledgments:

I would like to thanks:

- **The respective authors for providing figures (pesticide, venomics)**
- **Romain Touilloux for his work during the pesticide screening project by IMS**
- **Members of the LSM lab for valuable discussions**
- **ANALIS SA/NV for my current financial support**
- **The organizers to give us the opportunity to present these works**

Thank you for your attention