






UE Metabolomics

Lecture: GC-MS in Metabolomics

Lena Fragner

Molecular Systems Biology, University of Vienna

Mosys

Department Molecular Systems Biology

universität wien

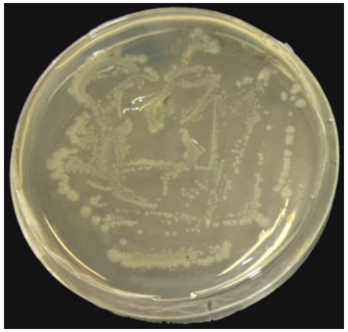
University of Vienna | Faculty of Life Sciences | Althanstrasse 14 | 1090 Vienna | Austria

Overview

- Introduction
- Technical aspects
  - Chromatography
  - Ionisation techniques
  - Mass analyzer
- Data validation

Introduction

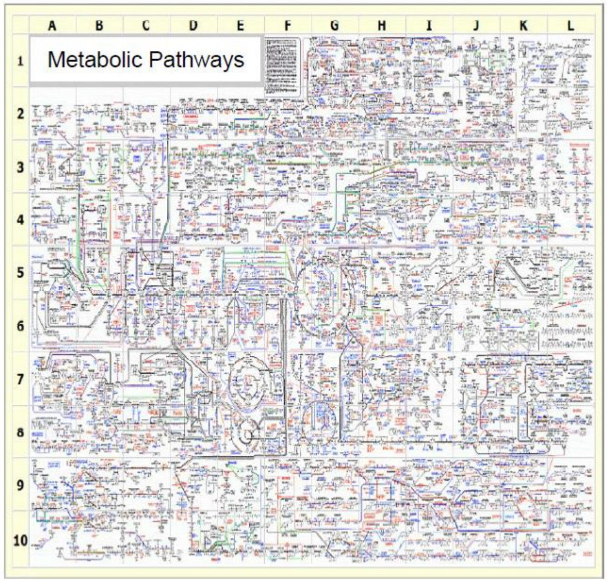
metabolomics

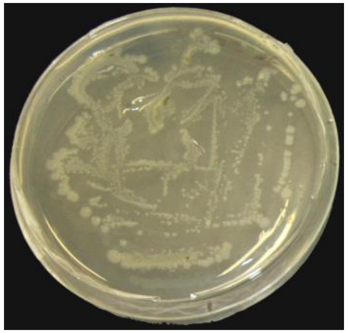


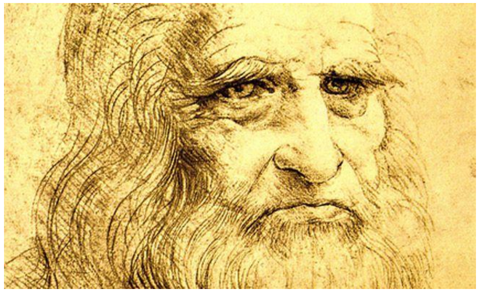
highly complex biological systems

physico-chemical diversity of metabolites

variable biological matrix







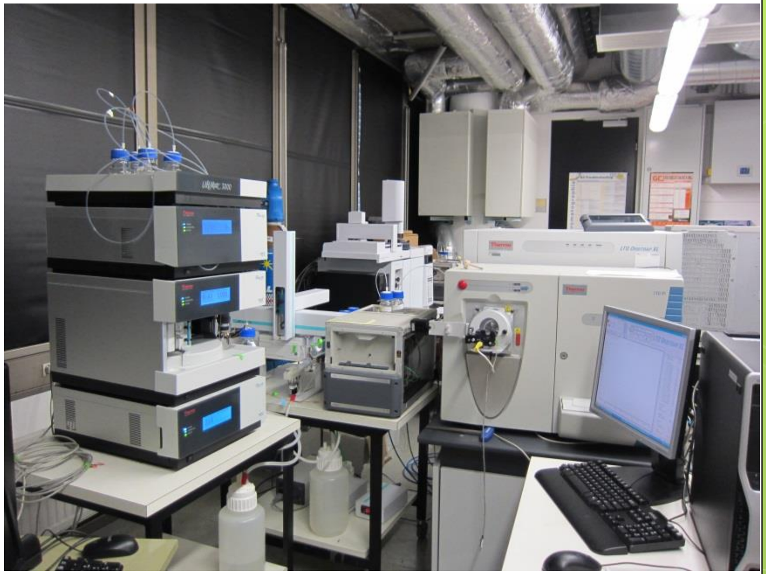
> 200.000 - 1 million putative structures occuring in plant kingdom

high dynamic range of analytes

Metabolomics platform

GC-MS

LC-MS

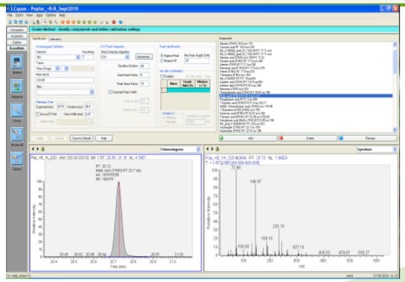


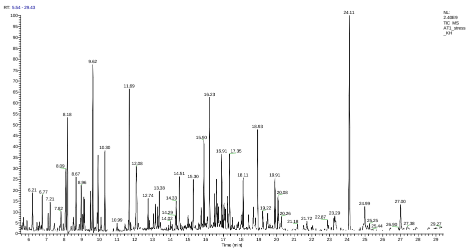
IC-(MS)

sample pathway

from biological material to interpretable data

sample pathway





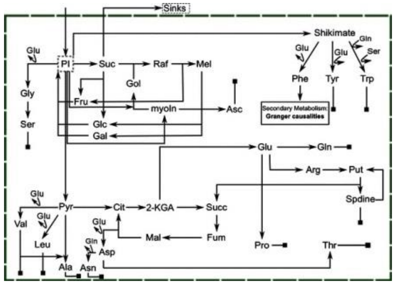
Experimental design

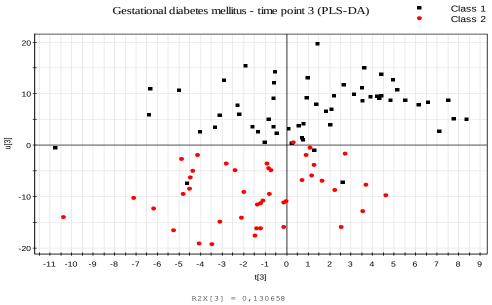
sample preparation & extraction

derivatisation

MS analysis

data processing & interpretation





Technical aspects

Components of a mass spectrometer

Sample introduction → Chromatography → Ion source → Mass analyzer → Ion detector → Data acquisition

Components of a mass spectrometer

Chromatography

GC or LC

Sample introduction

Autosampler

Ion source

Mass analyzer

Ion detector

EI electron ionisation

ESI Electrospray ionisation

TSQ Triple Stage Quadrupole

TOF Time Of Flight

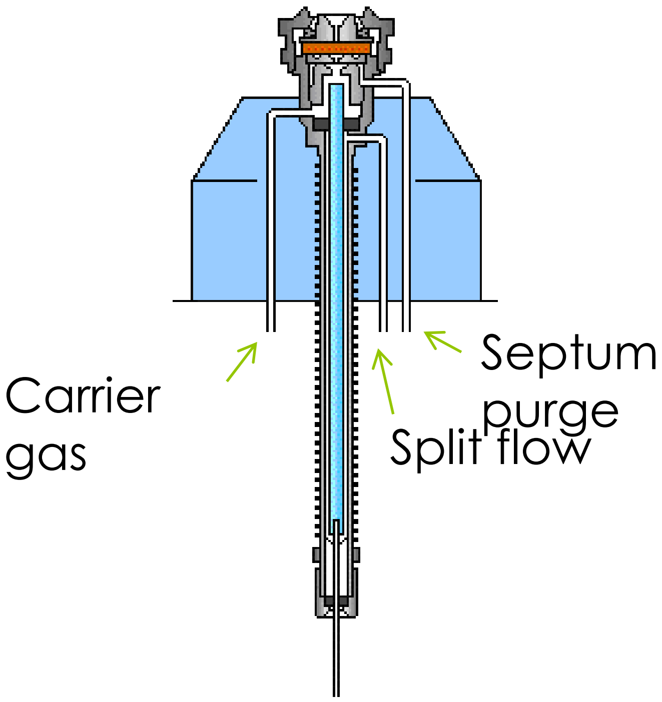
LTQ Orbitrap

Data acquisition

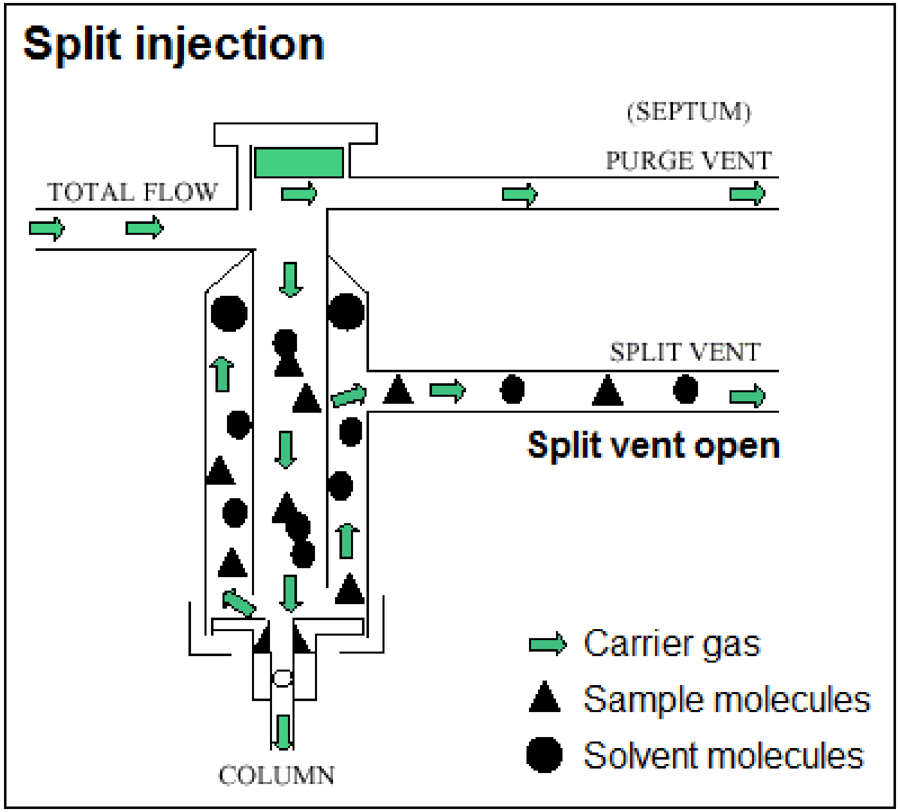
Computer

instruments

Injector (GC) -



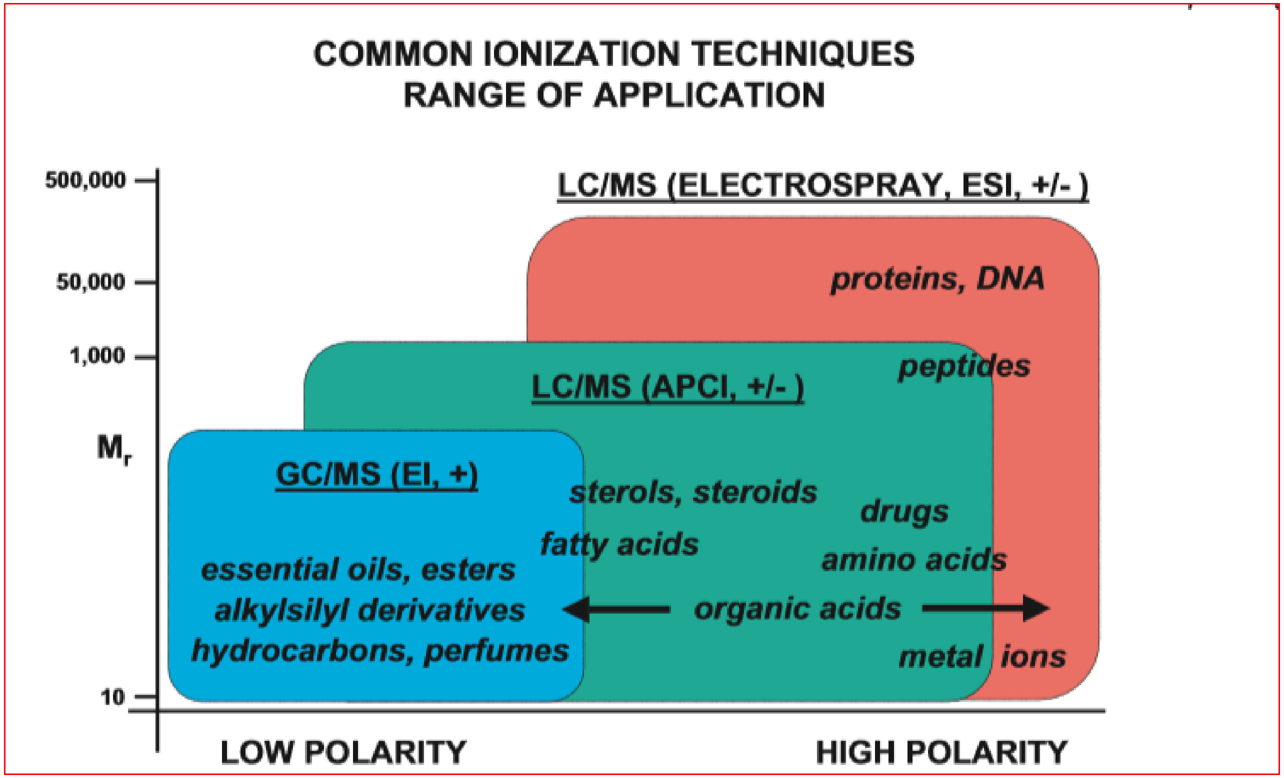
- Liquid sample introduction
- Vaporisation in liner
- S/SL
- PTV



https://www.trajanscimed.com/pages/training-gc-injection

Chemical derivatisation

- increase of volatiliy
- Deacrease polarity
- Reduce formed peaks in chromatogram

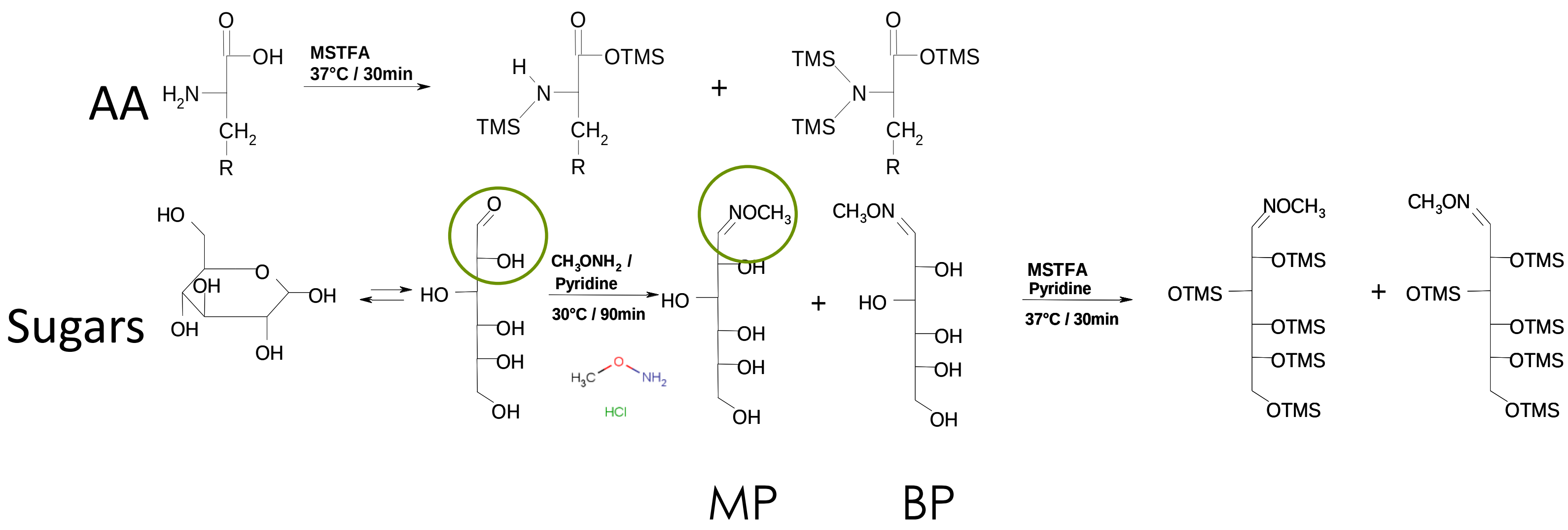


Halket *et. al.*, 2005, J. of. Exp. Bot.

Chemical derivatisation

Two steps

1. Methoxymation
2. Silylation



Components of a mass spectrometer

Chromatography

GC or LC

Sample introduction

Autosampler (Injector)

Ion source

EI **e**lectron **i**onisation

ESI **E**lectro**s**pray **i**onisation

Mass analyzer

TSQ **T**riple **S**tage **Q**uadrupole

TOF **T**ime **O**f **F**light

LTQ Orbitrap

Ion detector

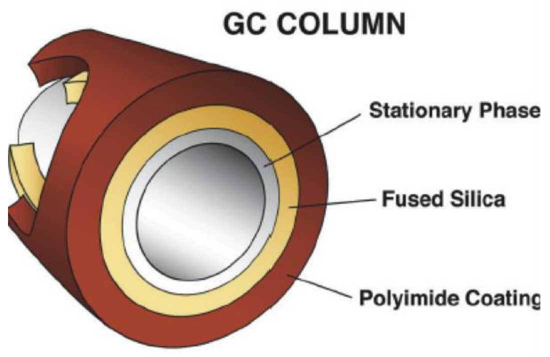
Data acquisition

Computer

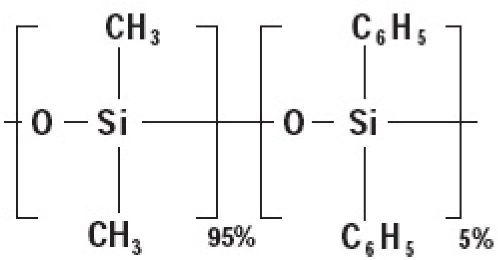
Chromatography

- **LC** → **H**igh-**P**erformance **L**iquid **C**hromatography (HPLC)
  mobile phase: liquid (solvents), stationary phase: hydrophobic (RP) or hydrophilic (NP) columns
- **GC** → **G**as **C**hromatography (Helium mobile phase, hydrophobic-coated capillary column)
- **CE** → **C**apillary **e**lectrophoresis (ions migrate in an electrical field)
- **IC** → **I**on **C**hromatography
  (ions are seperated according the affinity to the ion exchanger)

Chromatography - GC



- Optional uncoated precolumn
- Analytical column
  e.g. HP-5ms (siloxanes substituted with 5% phenyl and 95% methyl groups)
  - Seperation according to boiling point (volatility) (adsorption and desorption)
  - And interaction with active groups of column (polarity)
    - Apolar analytes: Van-der-Waals-forces
    - Polar analytes: hydrogen bonds



5% phenyl (HP-5, DB-5, etc.)	Nonpolar
35% phenyl (HP-35, DB-35, etc.)	Mid-polar
50% phenyl (HP-50+, DB-17, etc.)	Mid-polar

Chromatography - LC

HPLC

Normal phase column

-- stationary phase: high polar rigid silica, or silica-based compositions

-- mobile phases: relatively nonpolar solvent, hexane, methylene chloride, or mixtures of these

-- more polar solvent has higher eluent strength

-- the least polar component is eluted first

HPLC

Reverse phase column

-- stationary phases: nonpolar hydrocarbons, waxy liquids, or bonded hydrocarbons (such as C18, C8, etc.)

-- mobile phase: polar solvents or mixtures such as methanol-water or acetonitrile-water

-- the most polar component is eluted first

-- less polar solvent has higher eluent strength

-- less sensitive to polar impurities

∇ Avoid to measure a sample that pH value is greater than 7.5 in a reversed –phase column, because of hydrolysis of the siloxane.

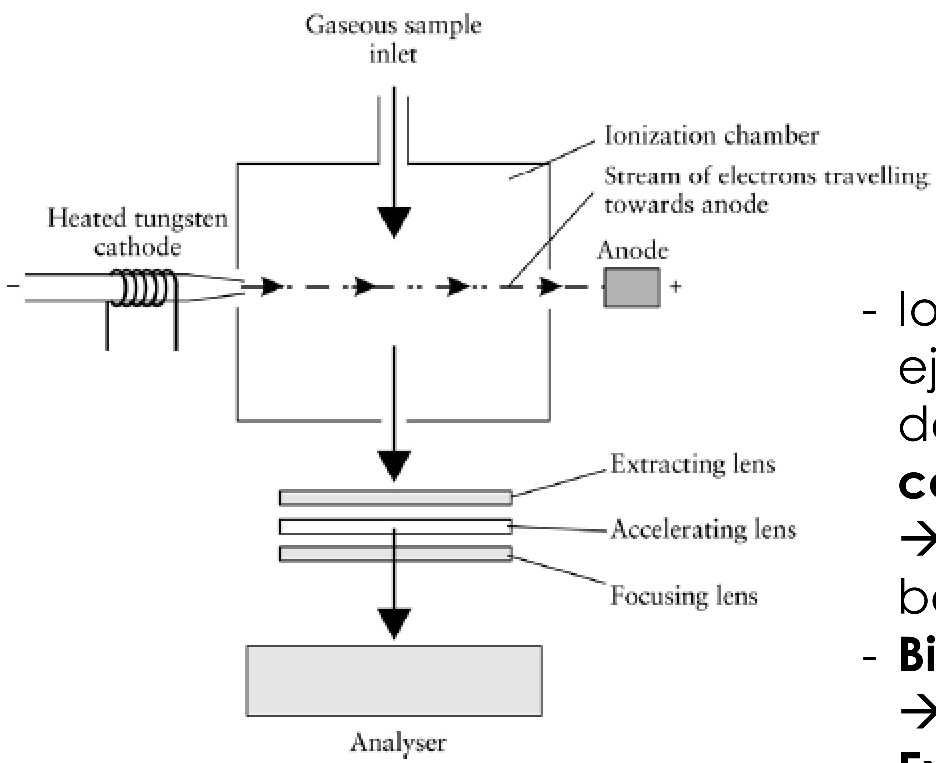
Ion Source

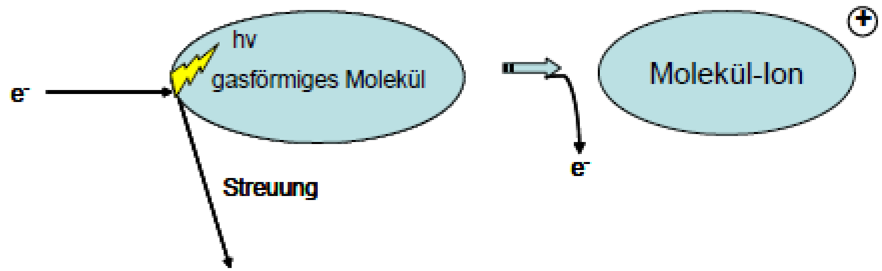
Ionisation

Ionisation

- Ionisation essential („hard“ and „soft“)
- EI = electron ionisation (electron impact)
- CI = chemical ionization
- API (atmospheric pressure ionization): ESI, APCI
- MALDI = Matrix-assisted Laser Desorption Ionisation

EI - Electron Impact

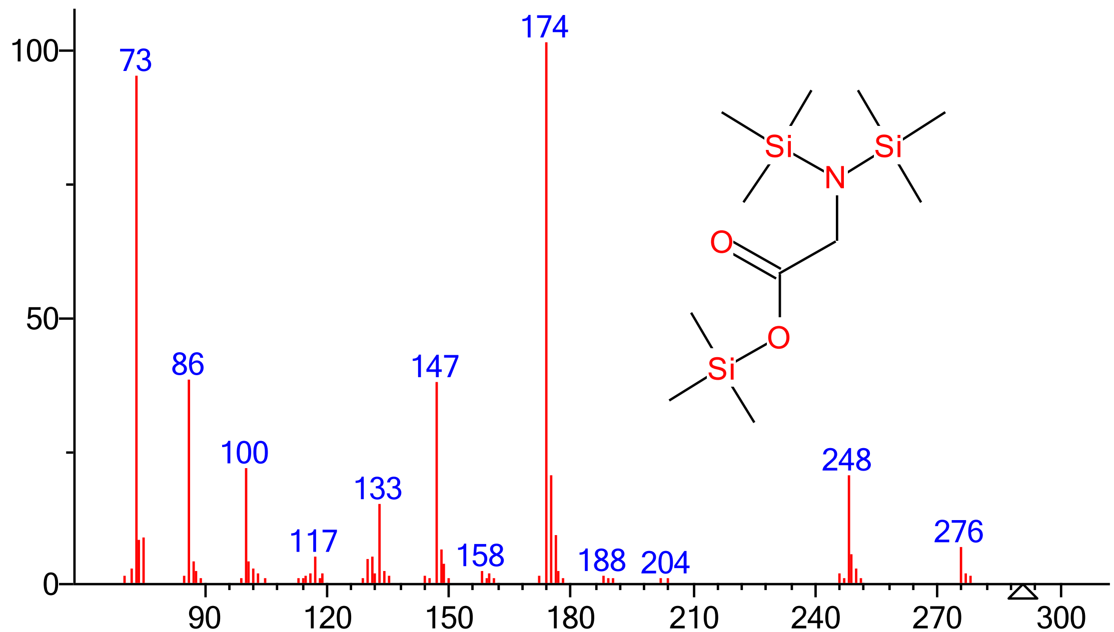




$$M + e^- \rightarrow M^{+\bullet} + 2e^-,$$

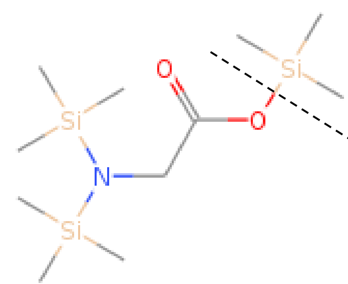
- Ionisation is caused by electron ejection from analyte or by analyte decomposition, **not by direct collision**
  → energy transfer from electron beam to analyte
- **Binding energy** of organic molecules
  → 2-8eV/mol
- **Excitation energy of 70eV** gets converted into Oscillation energy
  → leading to fragmentation

EI spectrum



(gmd_pred_mosys-ri) Glycine (3TMS)_M000031_A133001-101-xxx_NA_1302,5_

Glycine (3TMS)



Sum formular:
$C_{11}H_{29}NO_2Si_3$

$M^{+\bullet}$ 291.15061

$$M + e^- \rightarrow M^{+\bullet} + 2e^-,$$

Single charged fragment ions

ESI – Electrospray Ionisation

John Benne
Fenn

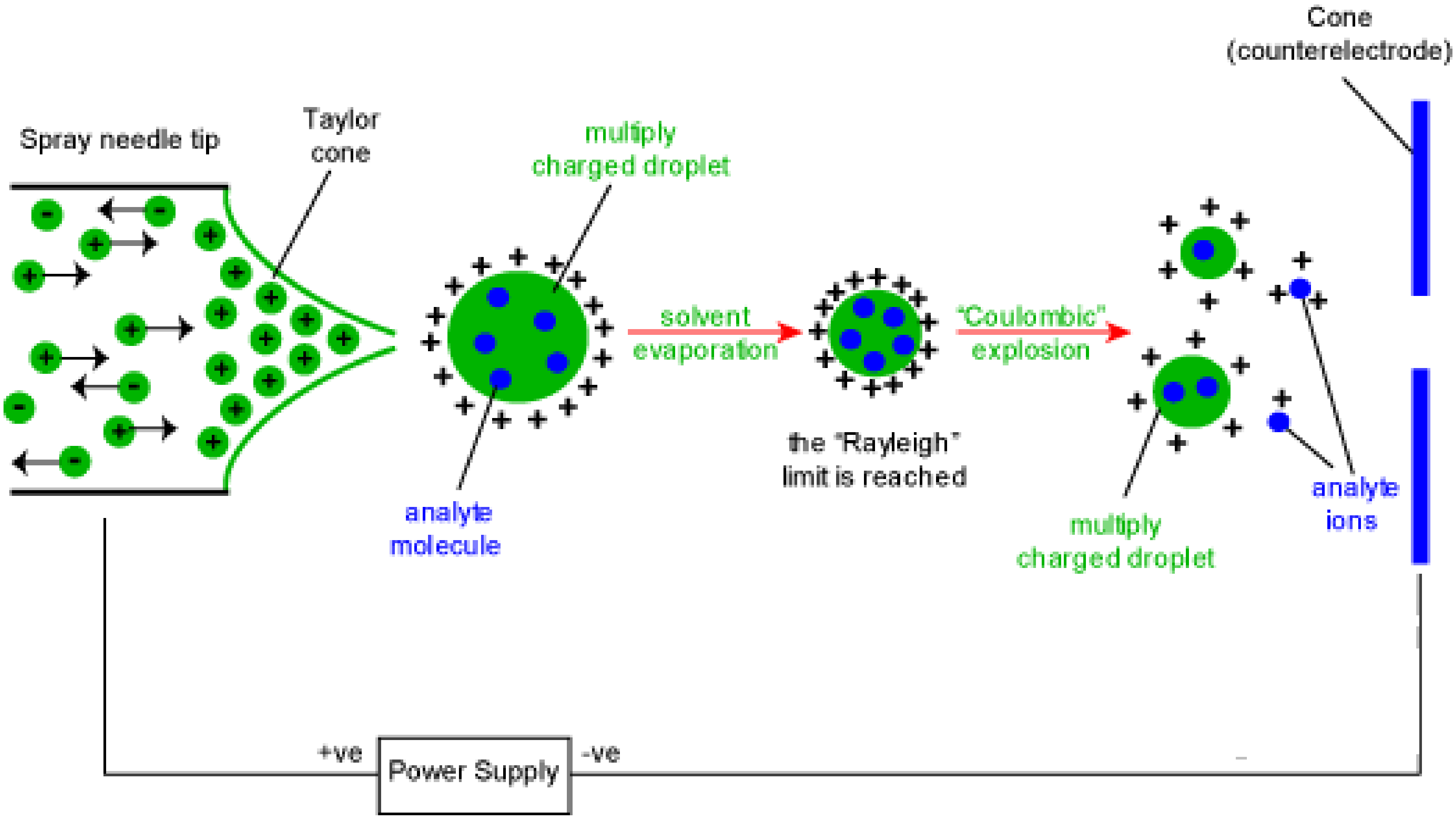
Koichi Tanak

Nobelprice for chemistry
2002

„Of course, proteins can fly!“

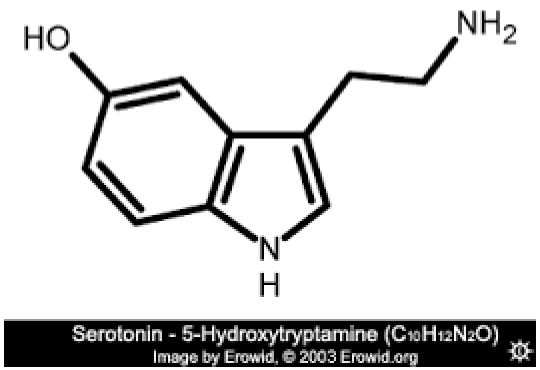
http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2002/illpres/mass.html

ESI – Electrospray Ionisation



http://www.bris.ac.uk/nerclsmsf/techniques/hplcms.html

ESI – spectrum



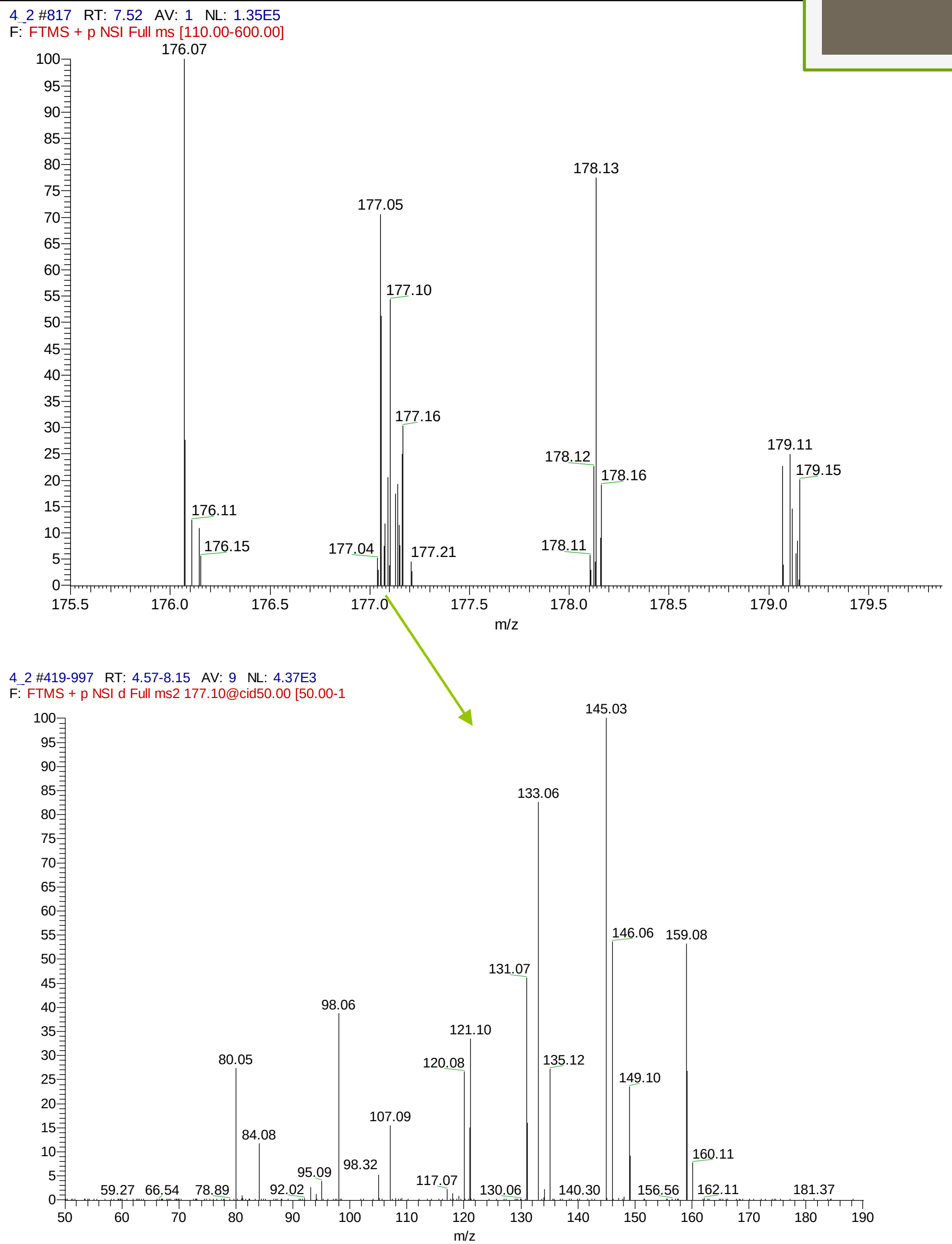
Exact mass of molecular ion -->
mass accuracy <5ppm
→ sum formular calculation

Still structure = ?????

→ MS^n fragmentation pattern
→ Comparison with standards

Monoisotopic mass C10H12N2O: 176.094955 Da
Monoisotopic mass H: 1.007276 Da

$[M+H]^+$ 177.102231 Da



Full Scan MS1

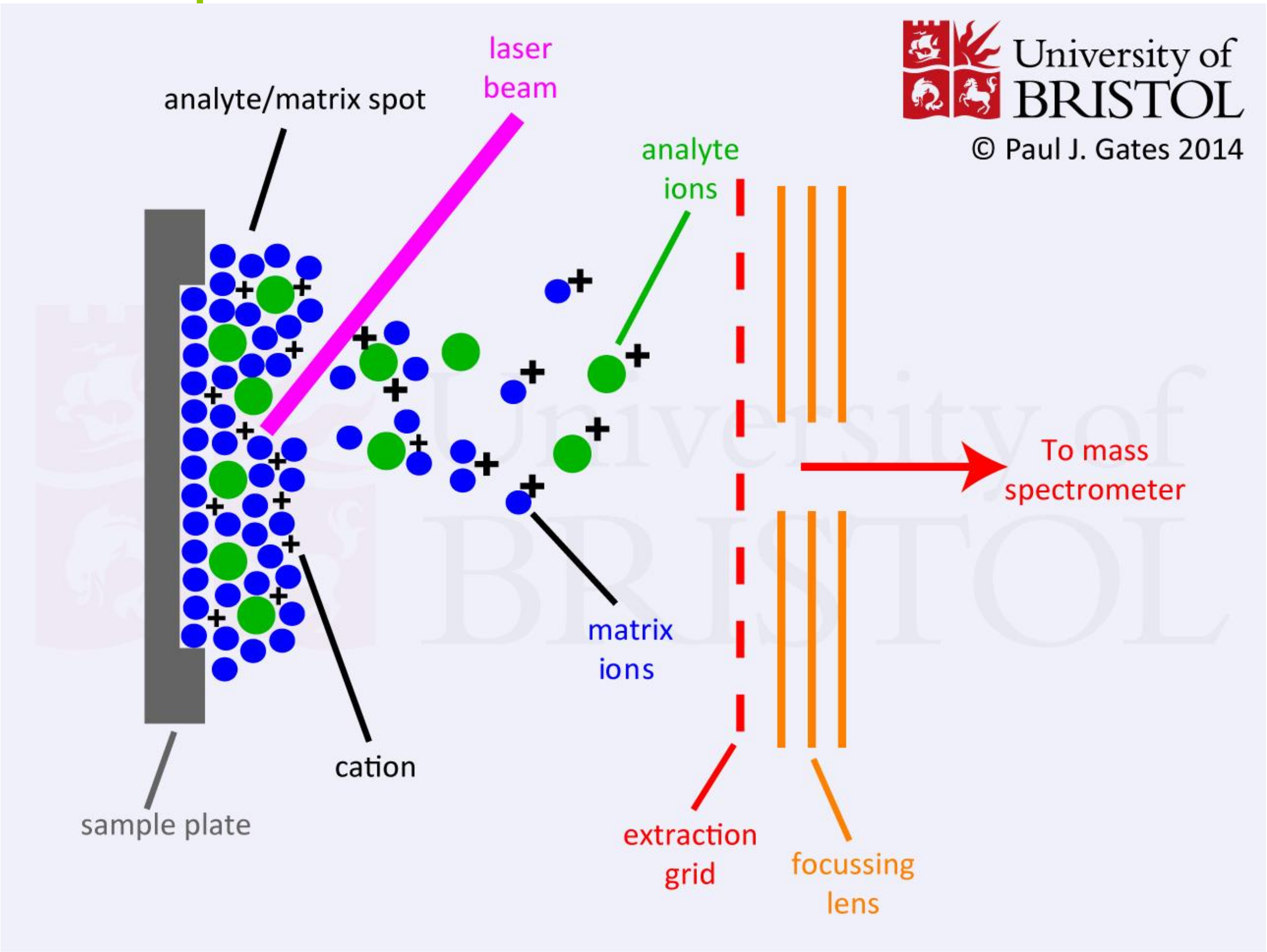
Precursor: $[M+H]^+$ 177.102231

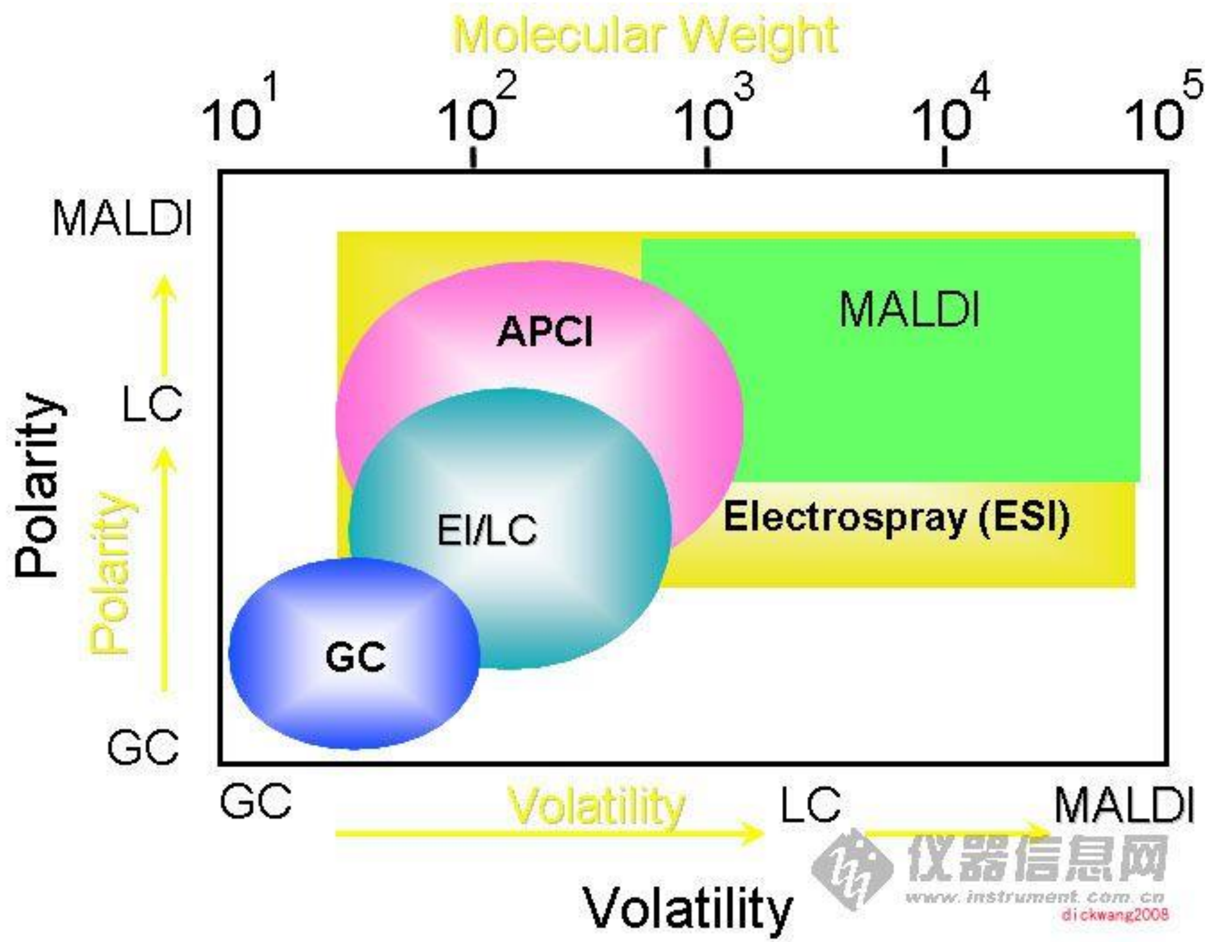
Multiple charges possible

Full Scan MS2

Only for personal use!!!

MALDI – Matrix-assisted Laser Desorption Ionisation

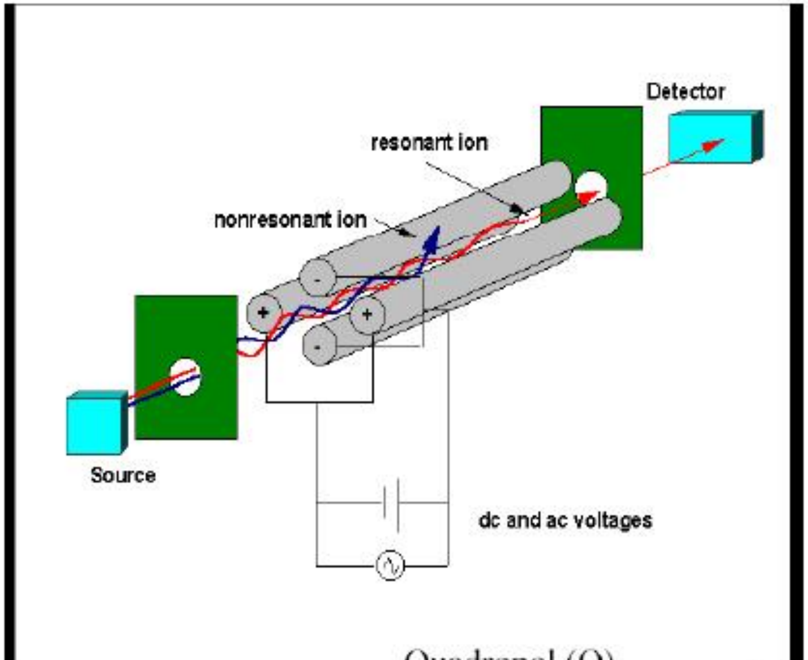




http://www.antpedia.com/viewspace-59127

Mass analyzer

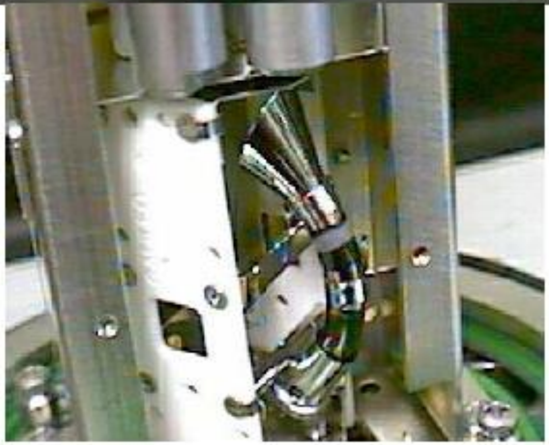
Quadrupole

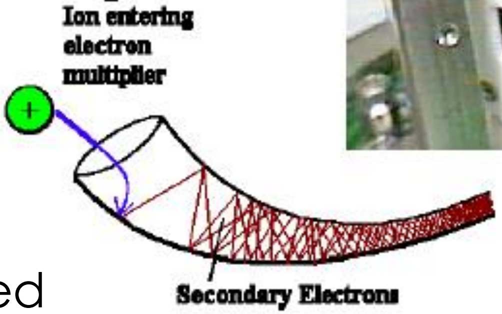


Quadropol (Q)

$m/z = 5.7U/\omega 2r2$

ω: Frequenzanteil, r: ½ Stababstand
U: Beschleunigungsspannung (10-20 V)





oszillating electric fields are used to select or scan specific ions

TOF – Time-of-flight

Figure 2.

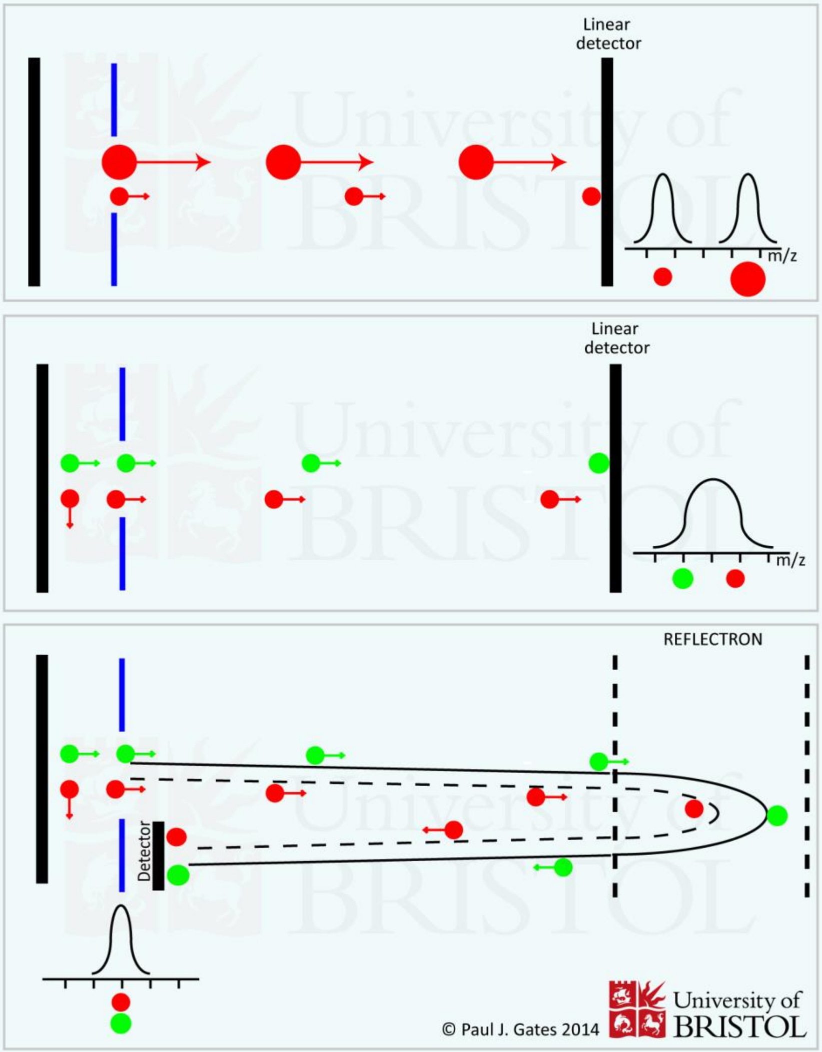
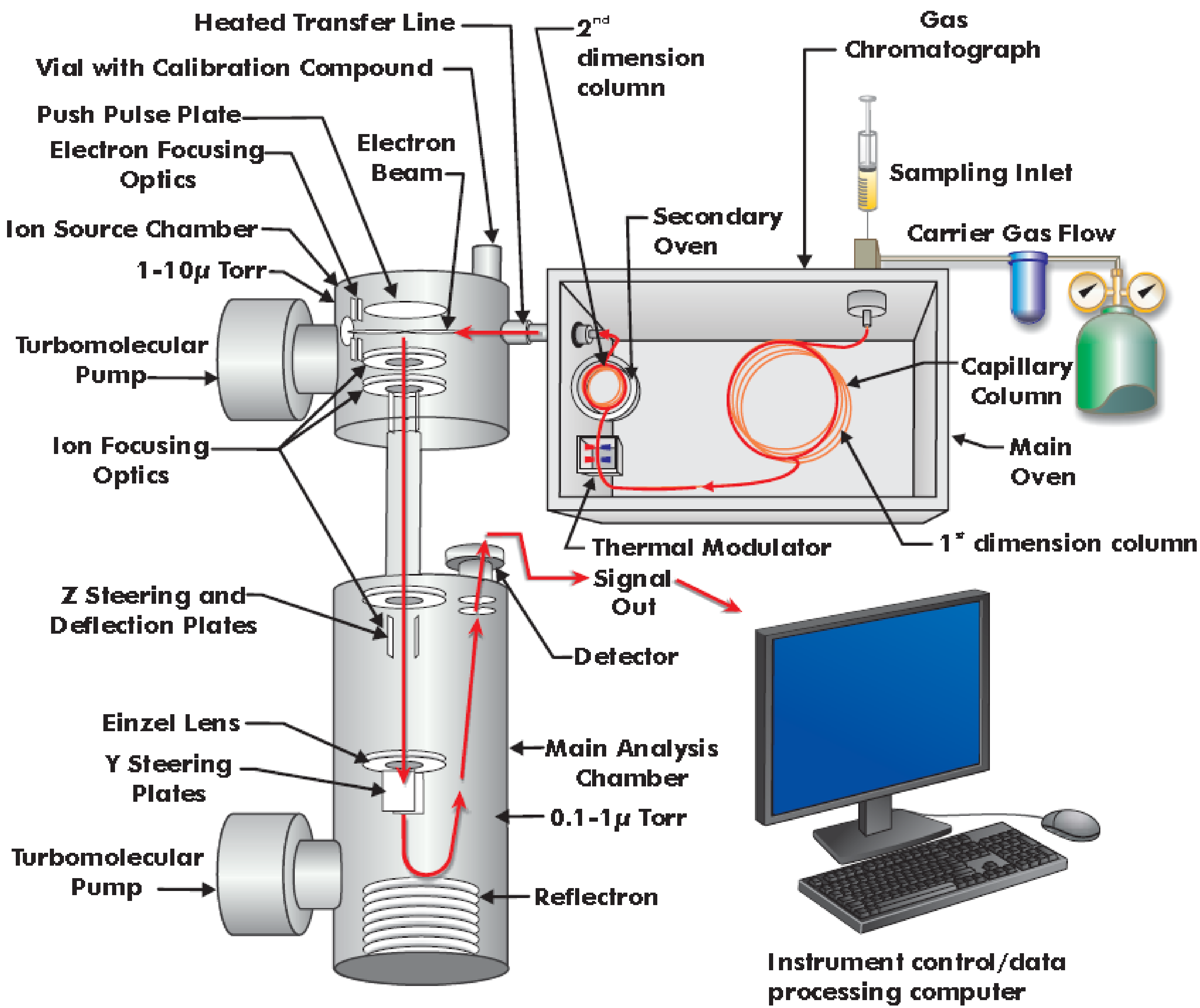
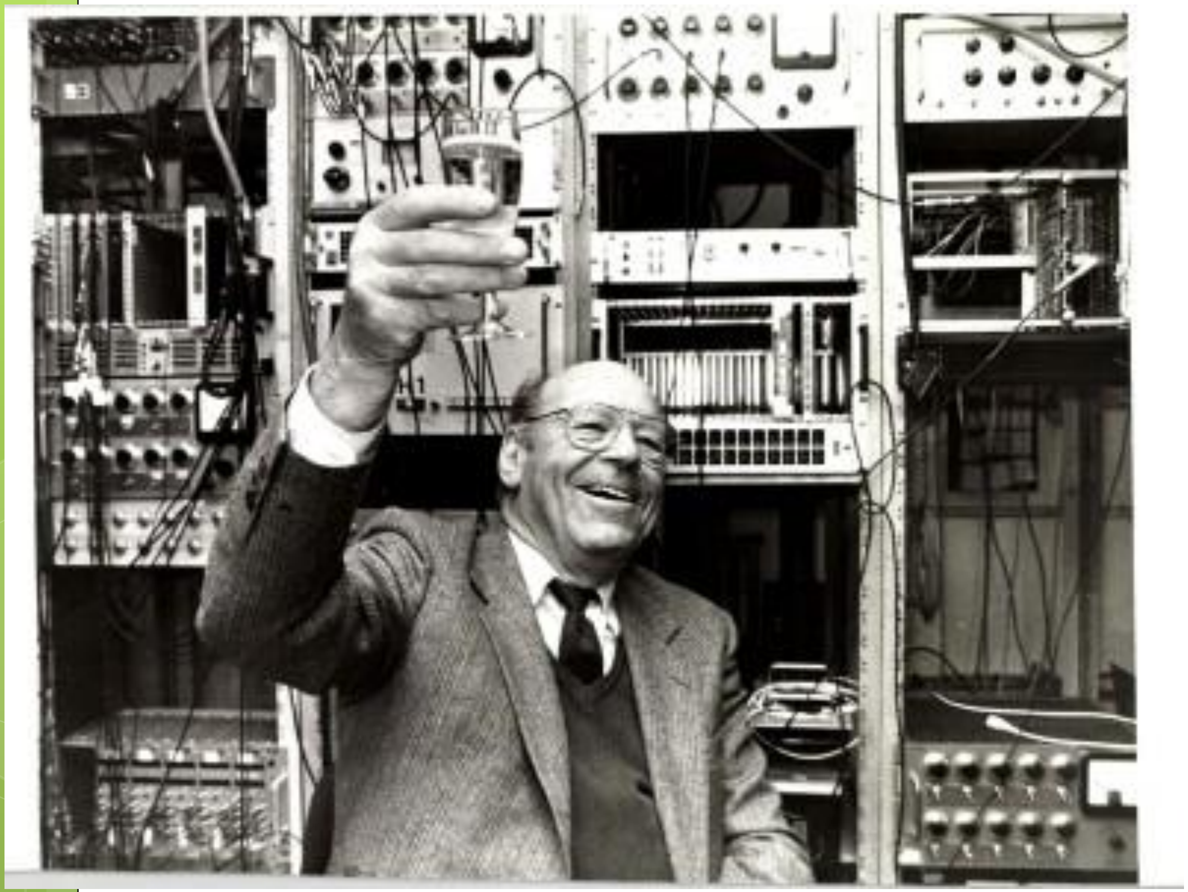


Diagram of GCxGC-TOFMS Instrument



Only for personal use!!!

Ion Trap



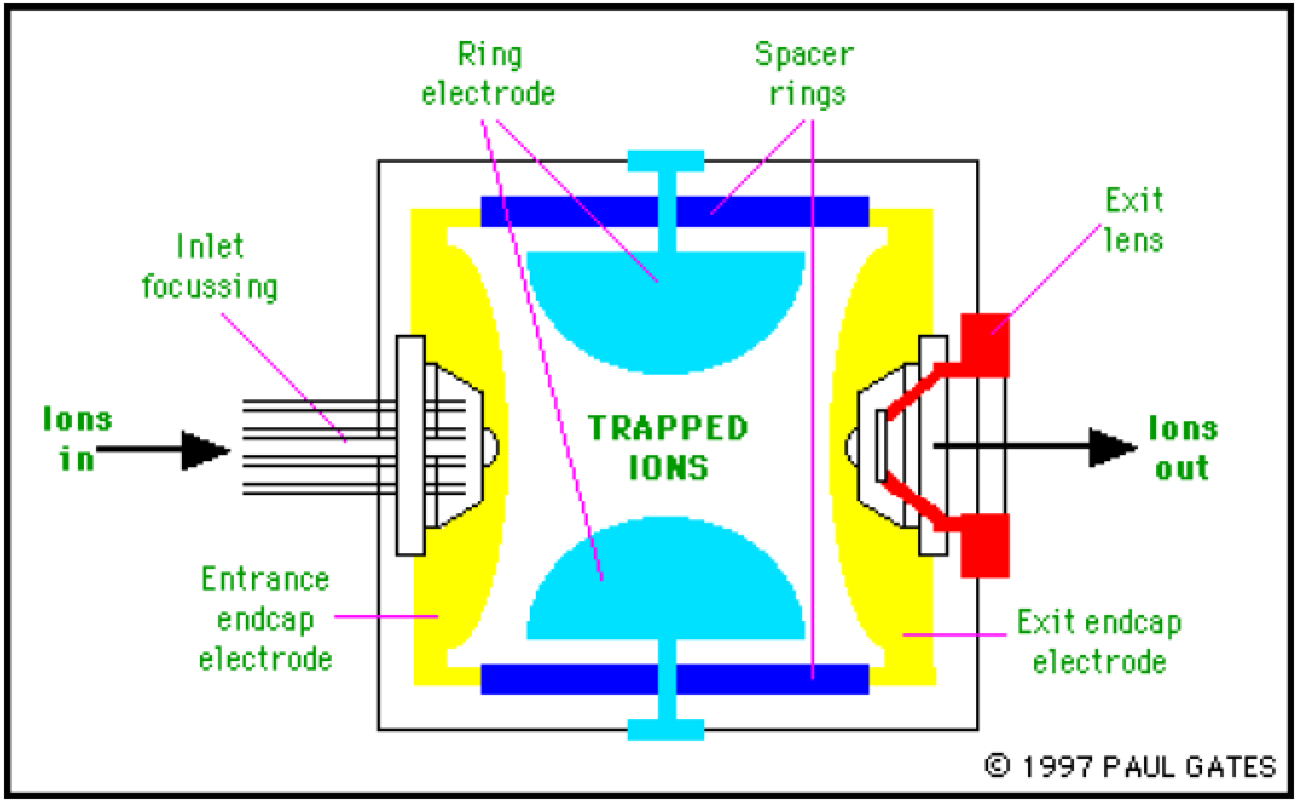
Prof. Wolfgang Paul
Nobel price in physics 1989

1950ies: Quadrupole Ion Trap (QIT) was developed together with Quadrupole

http://www.general-anzeiger-bonn.de/bonn/wisse

Ion Trap

e.g. Quadrupole Ion Trap (Paul trap)

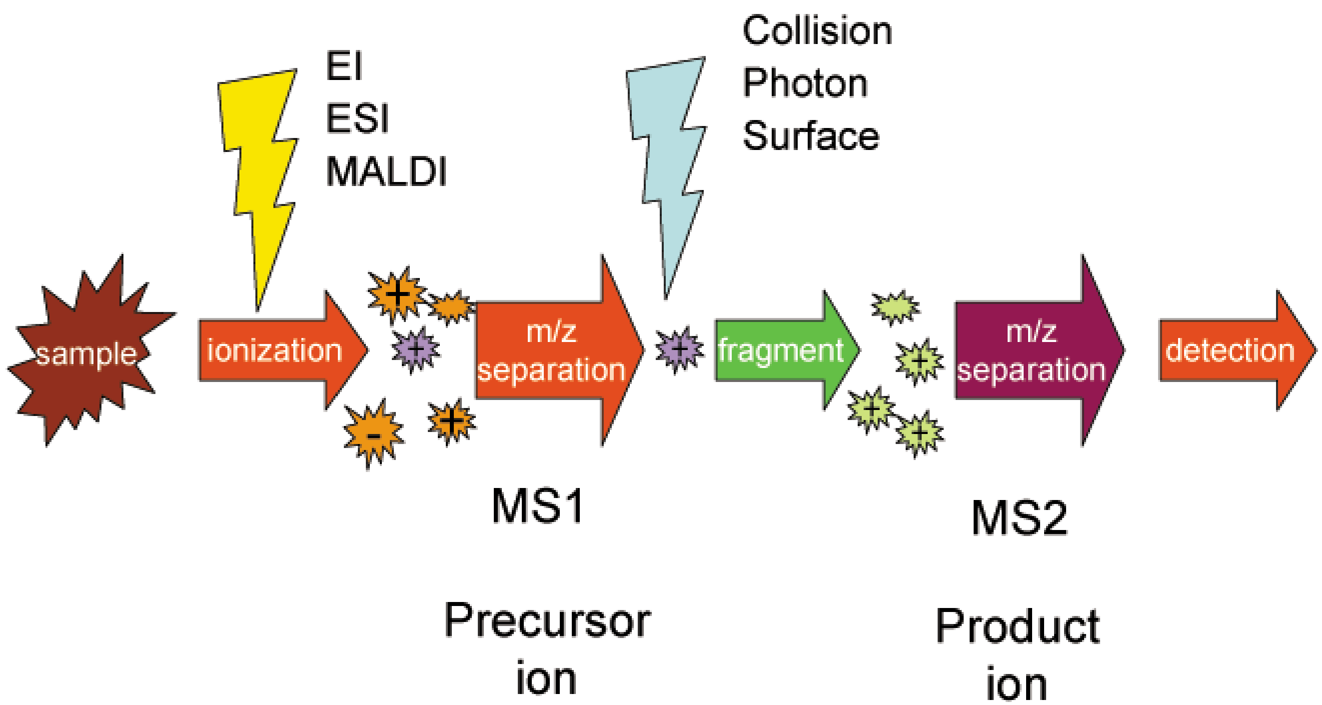


Trapping of ions by

static direct current and (DC)

radio frequency oscillating electric fields (AC)

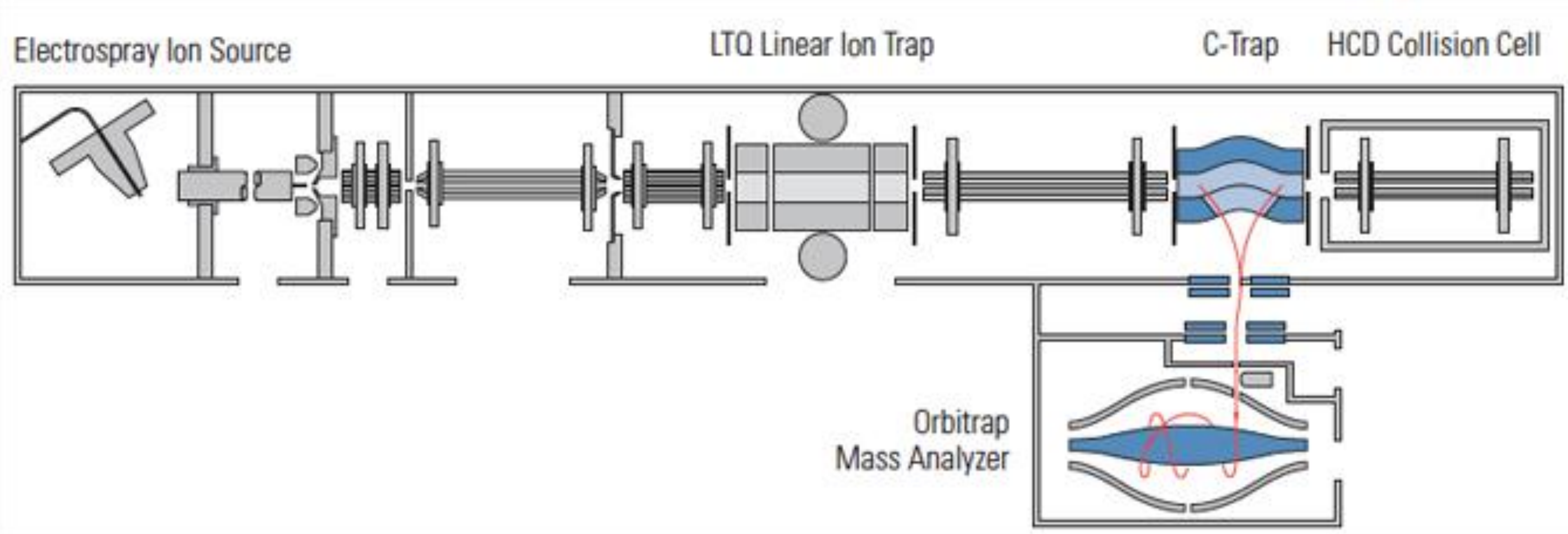
Tandem mass spectrometry (MS/MS)



By K. Murray (Kkmurray) - Own work, CC BY-SA 3.0,
https://commons.wikimedia.org/w/index.php?curid=1943319

Tandem mass spectrometry

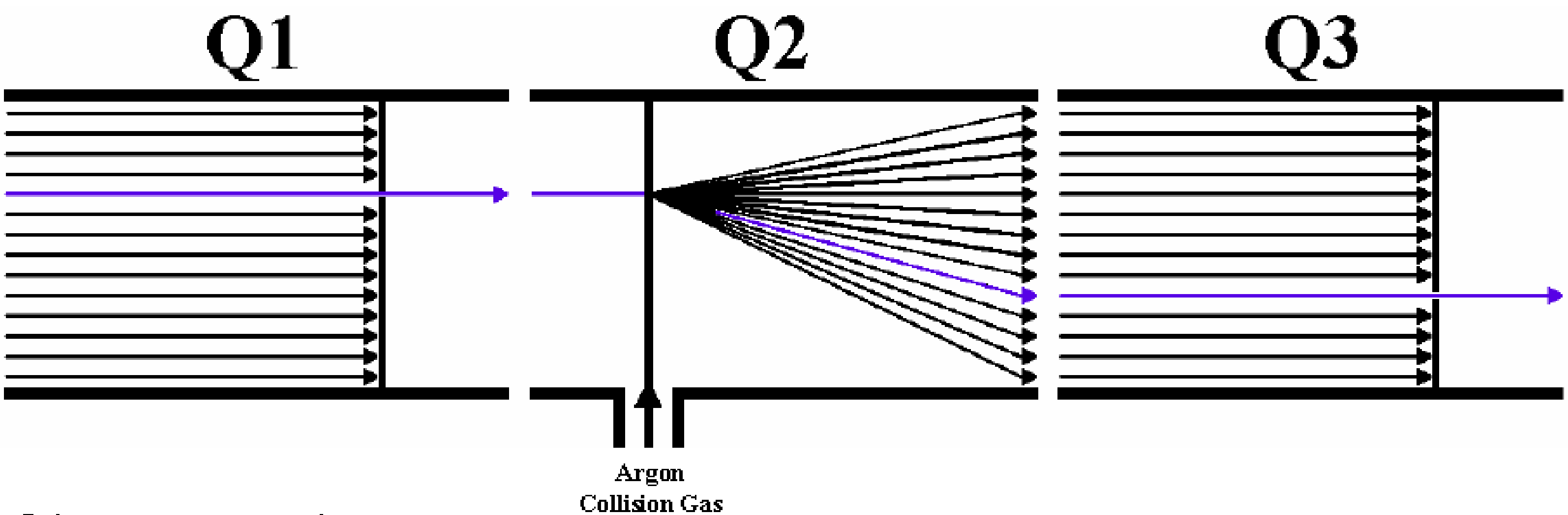
LTQ Orbitrap



http://planetorbitrap.com/ltq-orbitrap-xl#.VrYYEfnhDq4

Selected Reaction Monitoring

"Monitor a Transition"
from *Precursor in Q1* to *Product Ion in Q3*



Q1 on precursor ion
***m/z* 272**

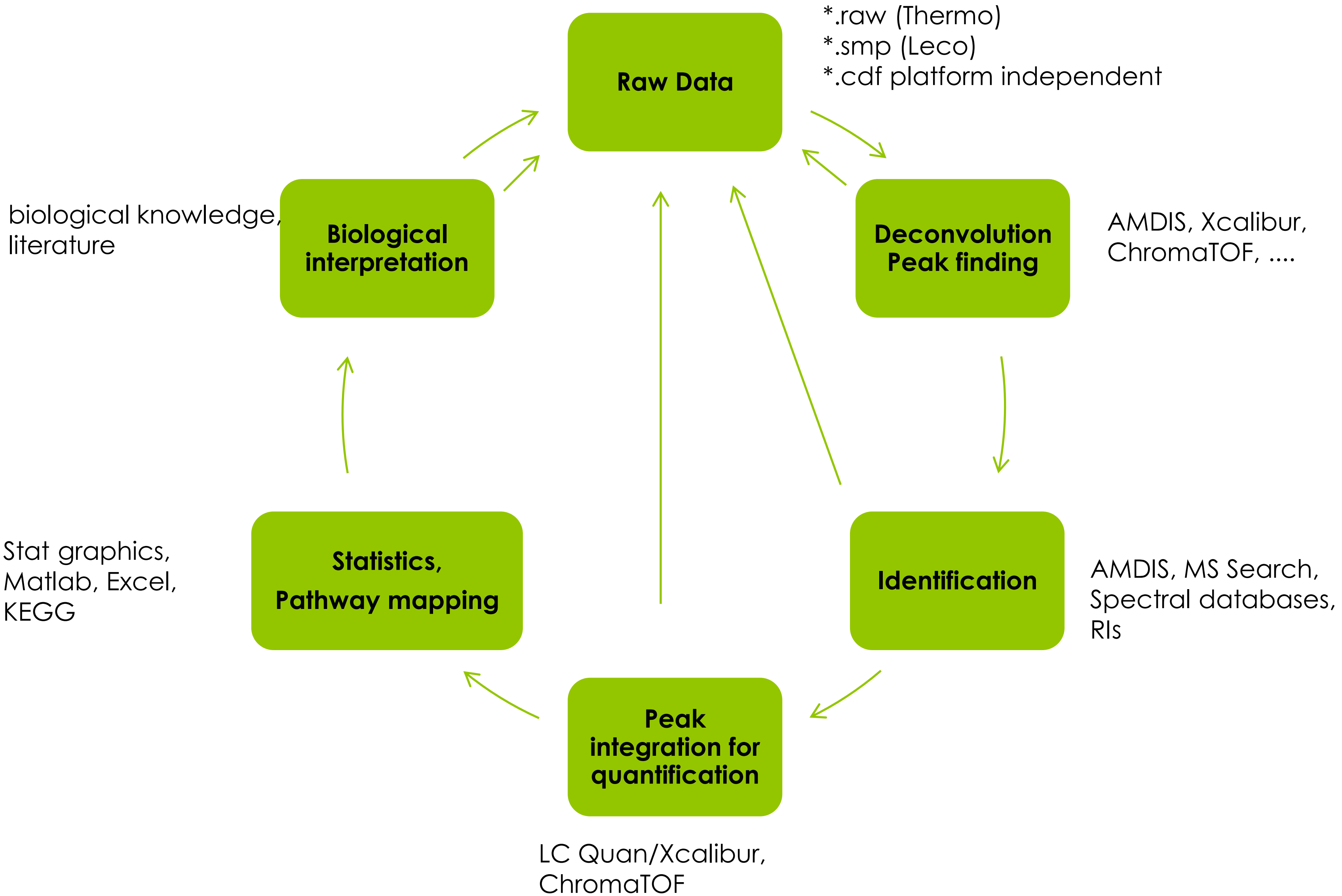
Q3 on product ion
SRM on *m/z* 237

Q1	**Q2**	**Q3**
mass filter	collision cell 2nd fragmentation	scanning of selected product ion

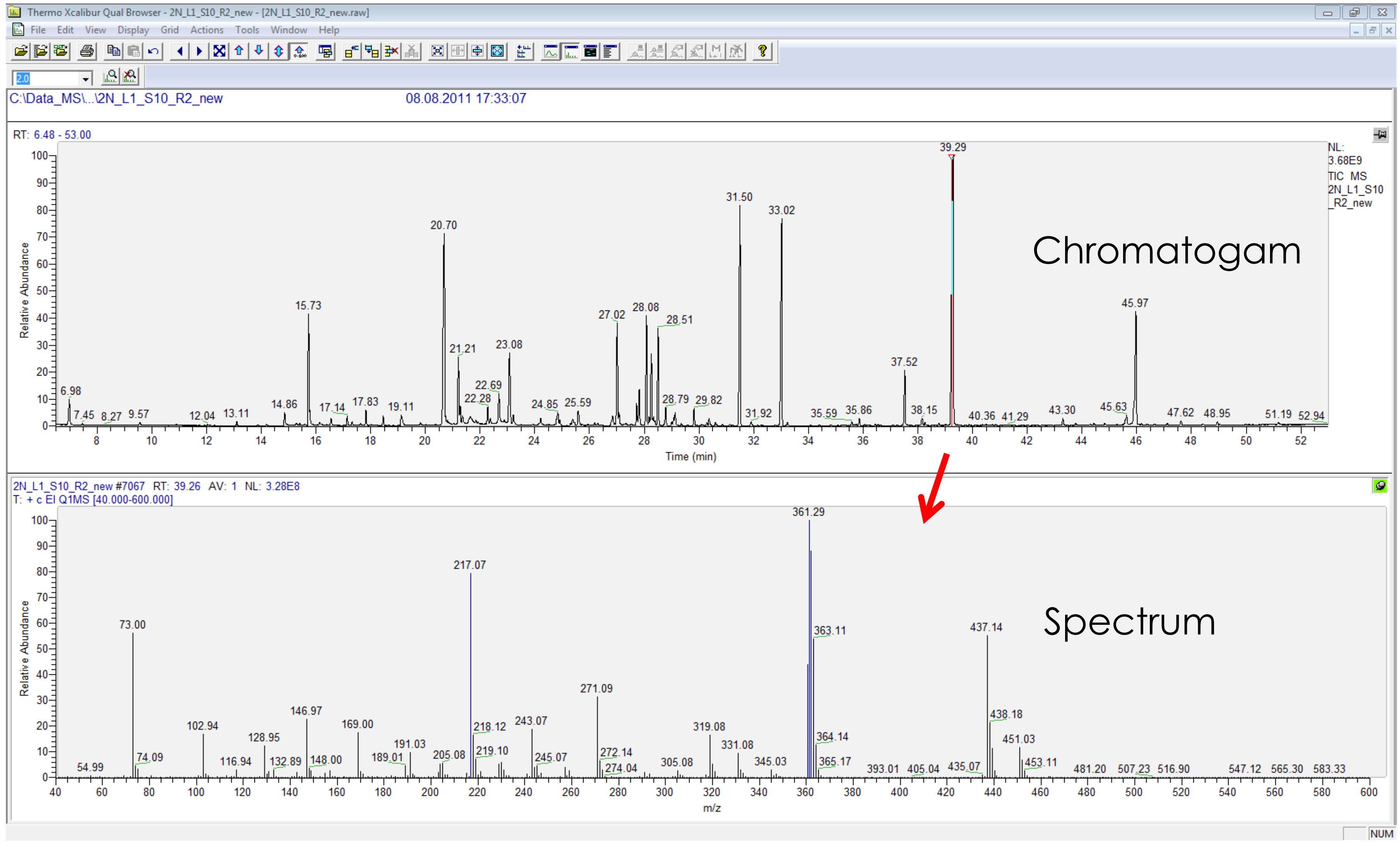
Data validation

workflow

data processing

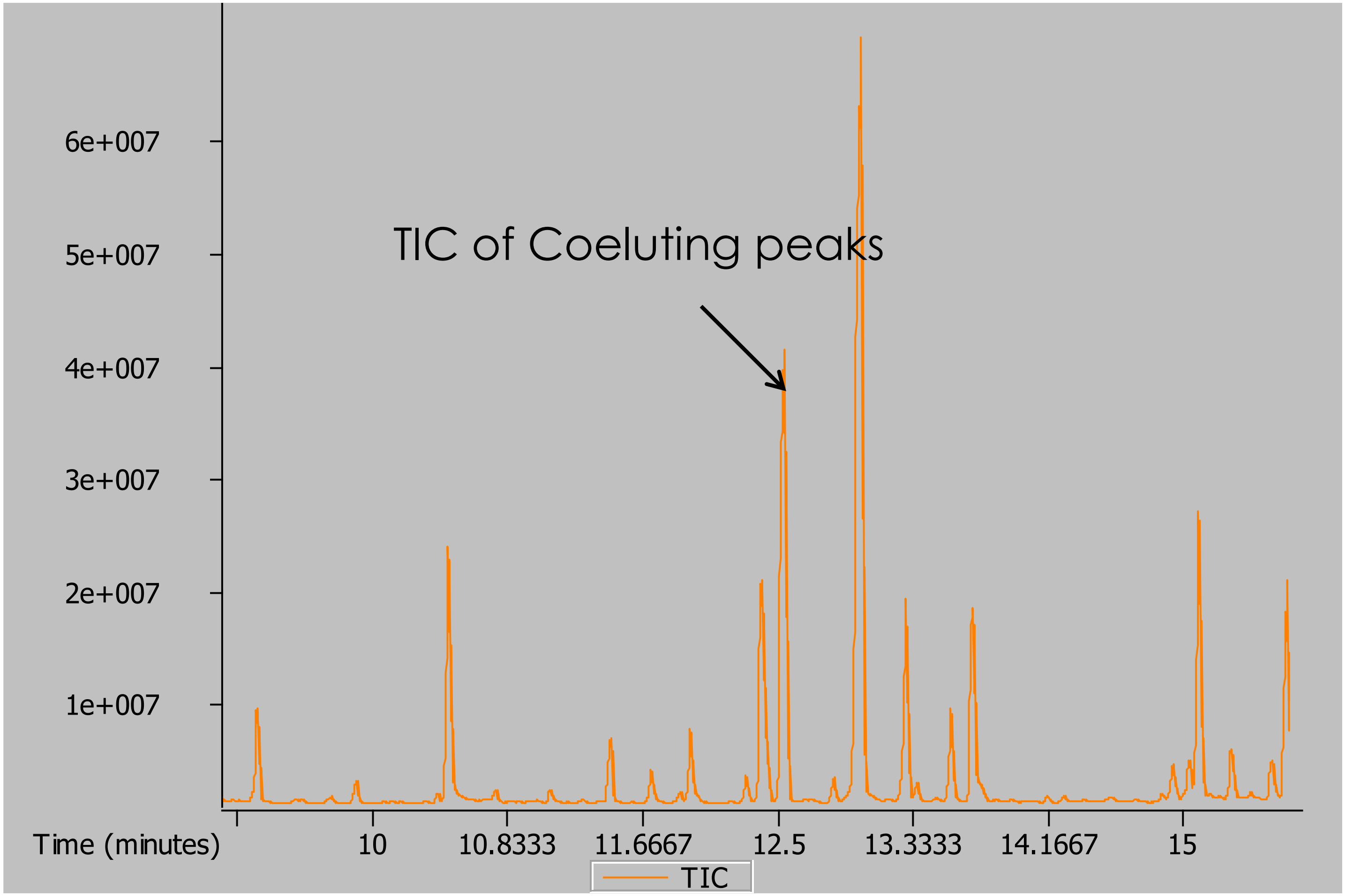


Spectrum and Chromatogram

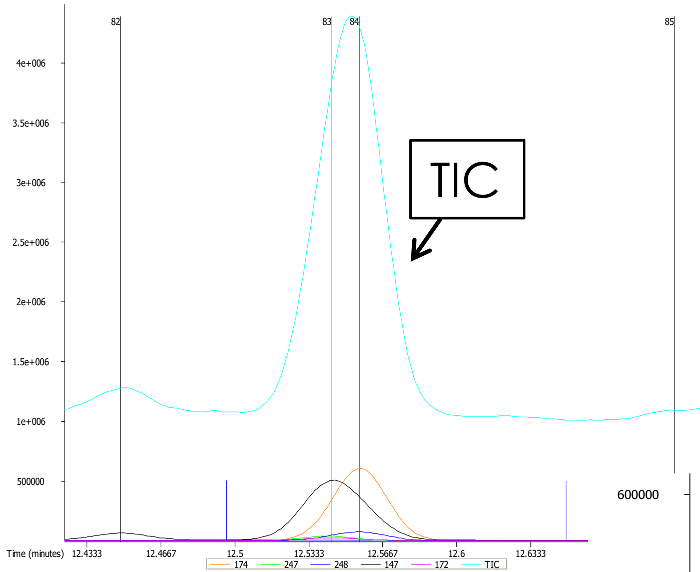


Only for personal use!!!

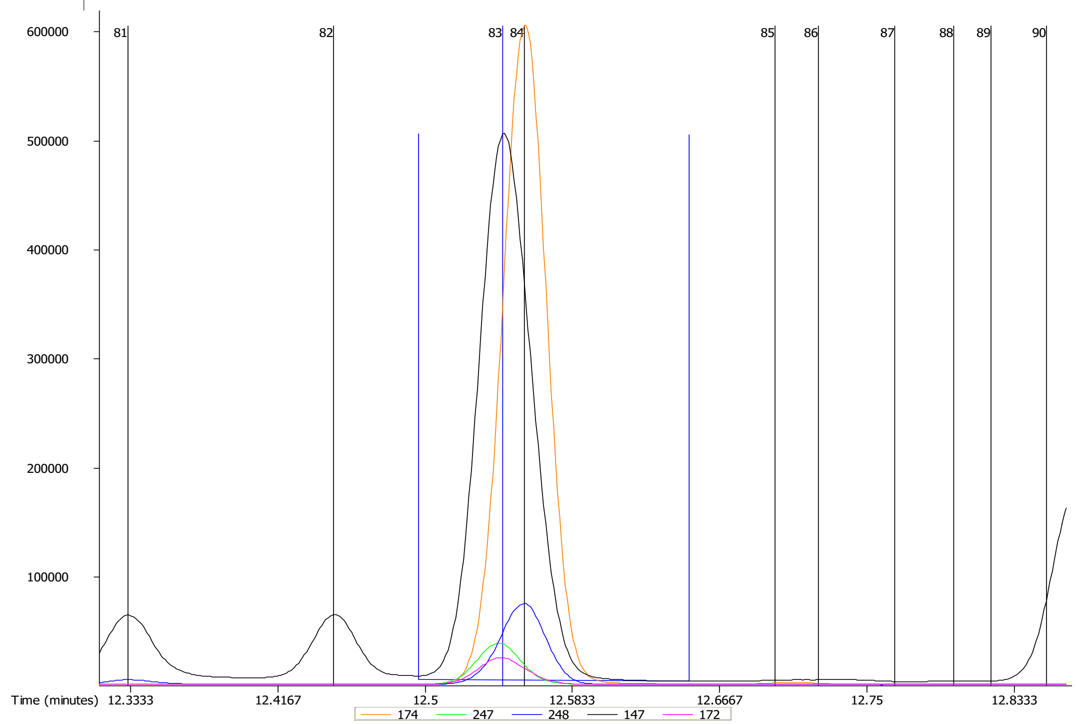
chromatographic and mass spectral separation



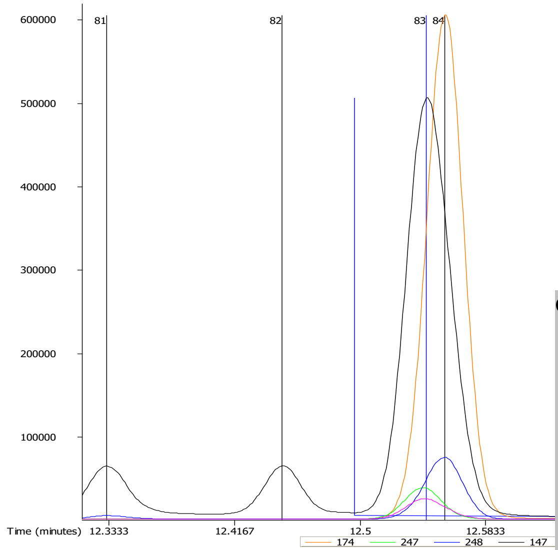
deconvolution



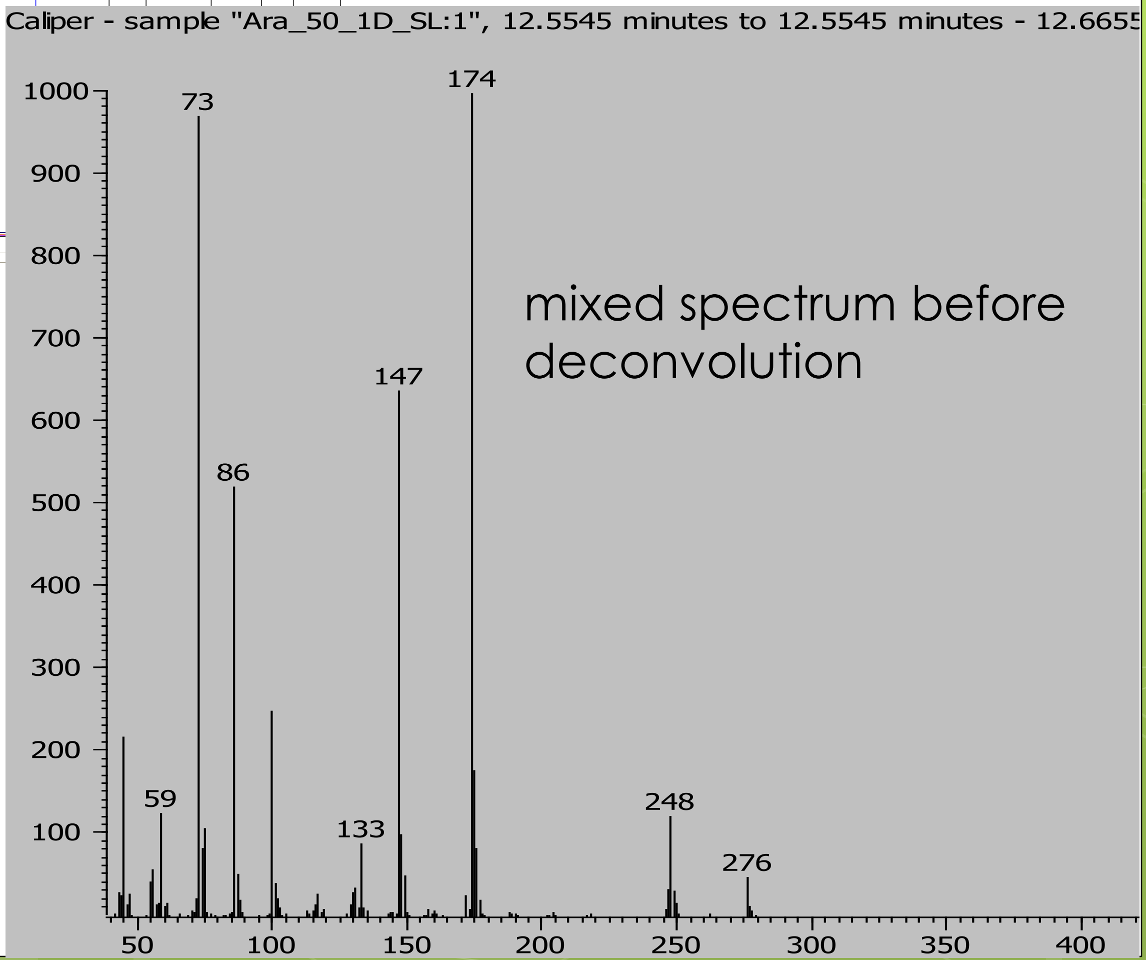
EIC m/z 174, 247, 248, 147, 172



deconvolution



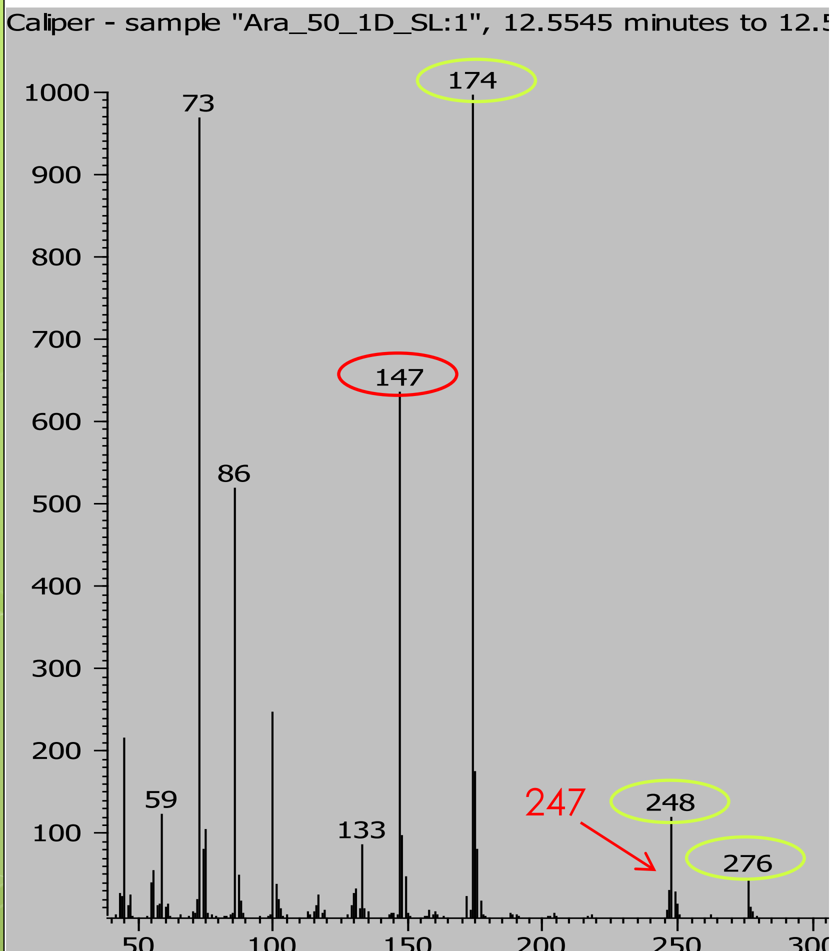
m/z 174, 247, 248, 147, 172

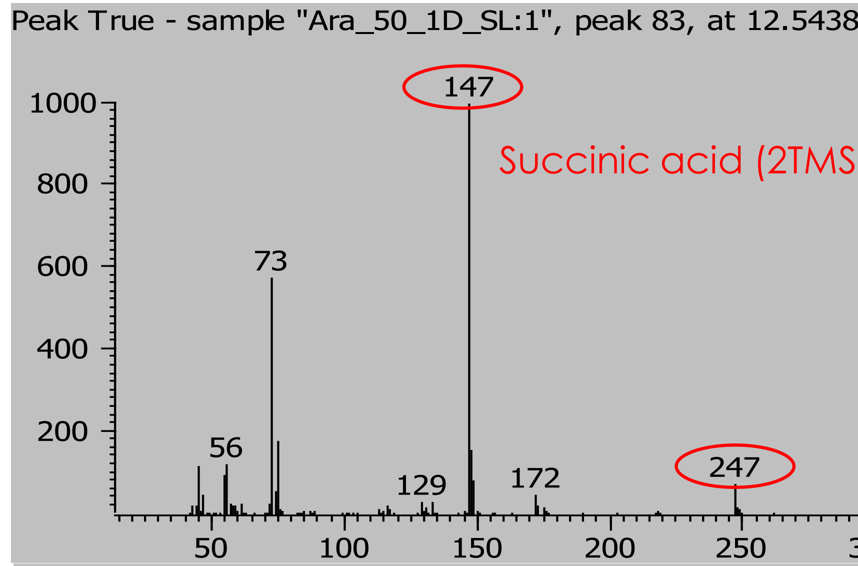


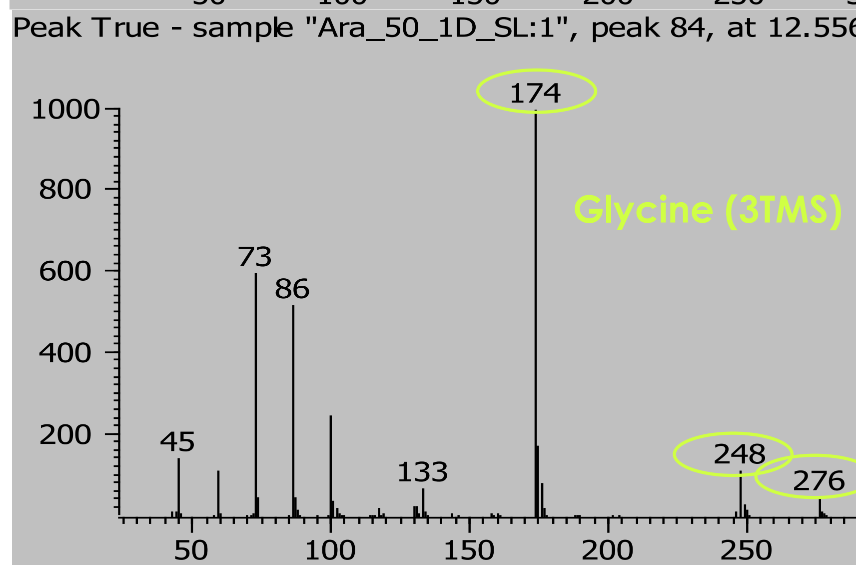
Only for personal use!!!

deconvolution

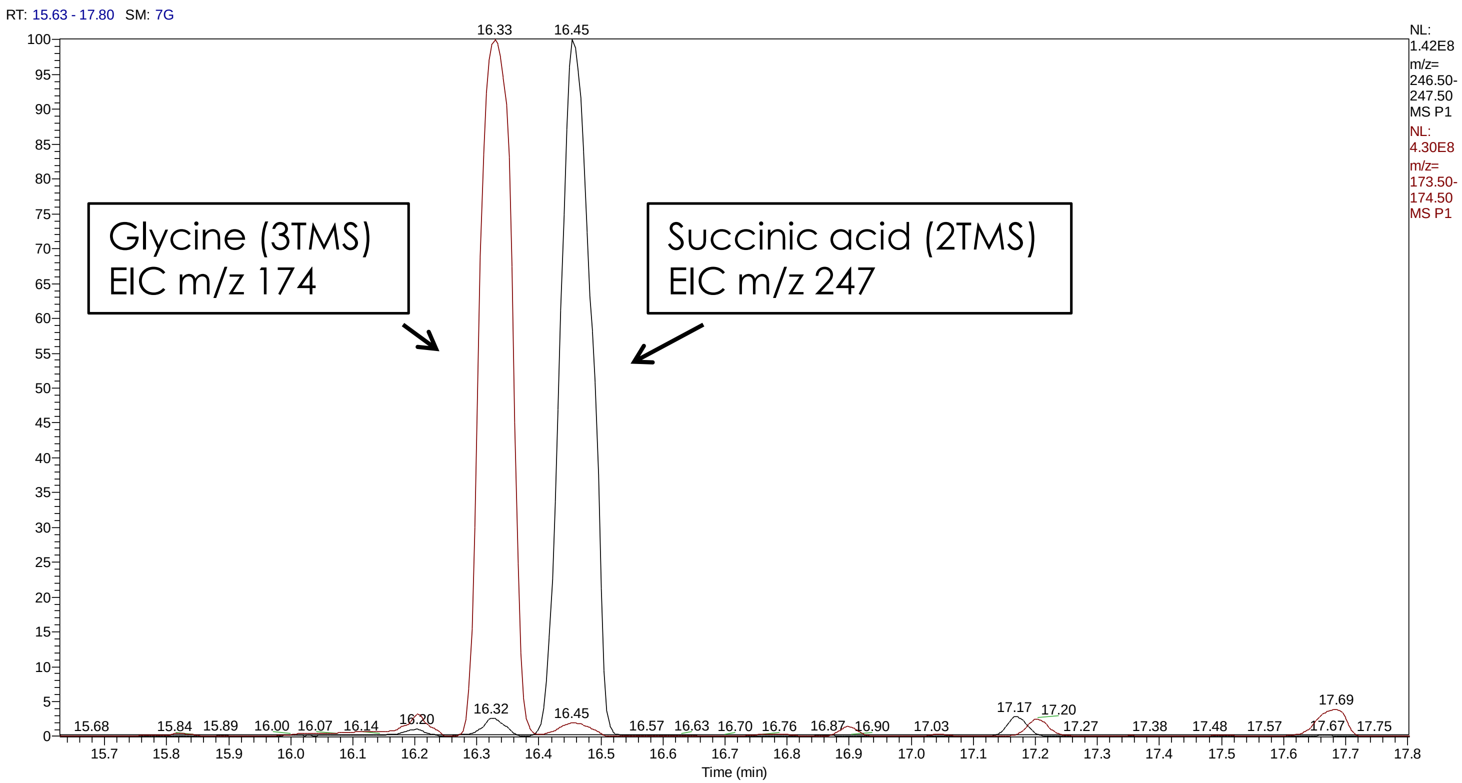
mixed spectrum before deconvolution





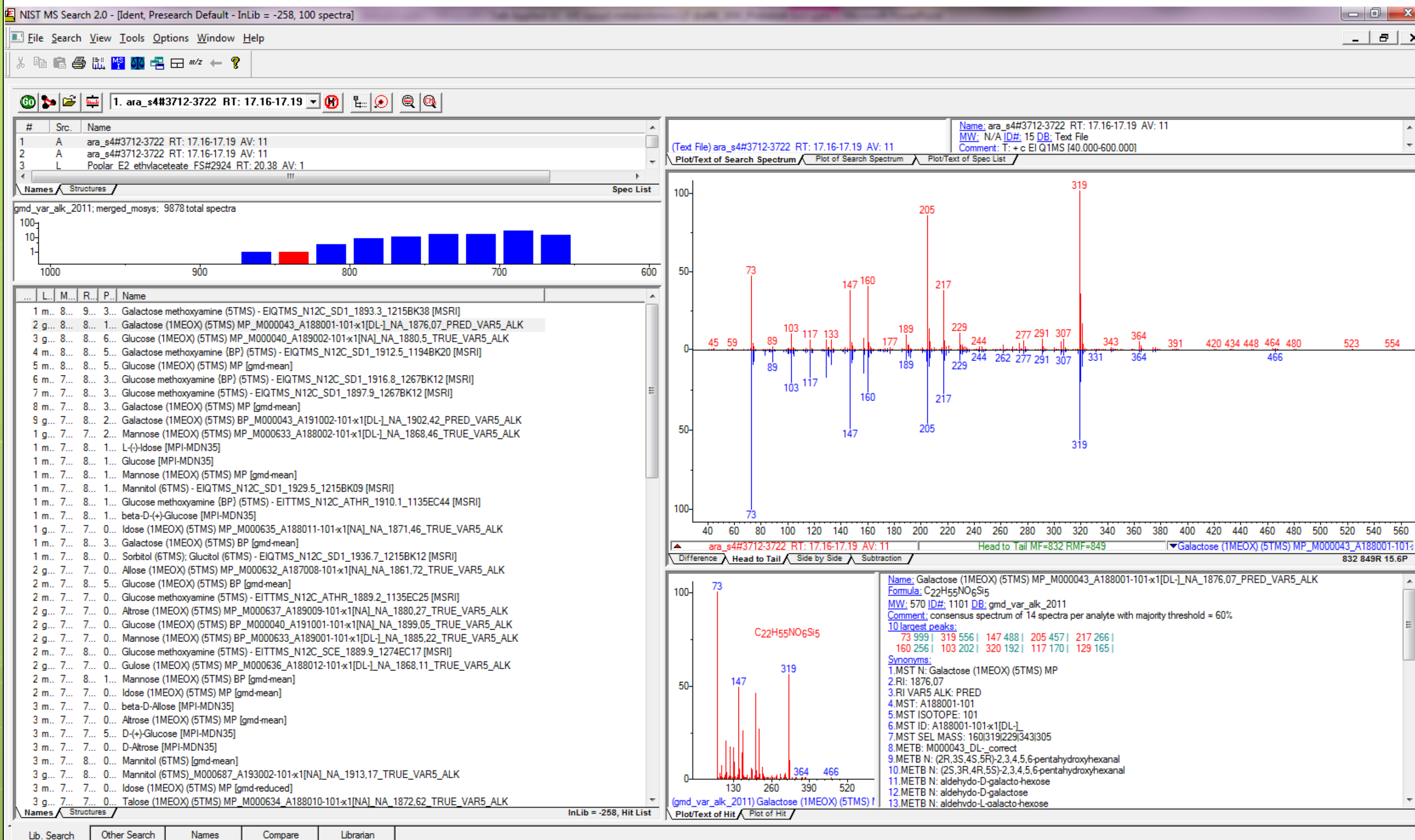


chromatography

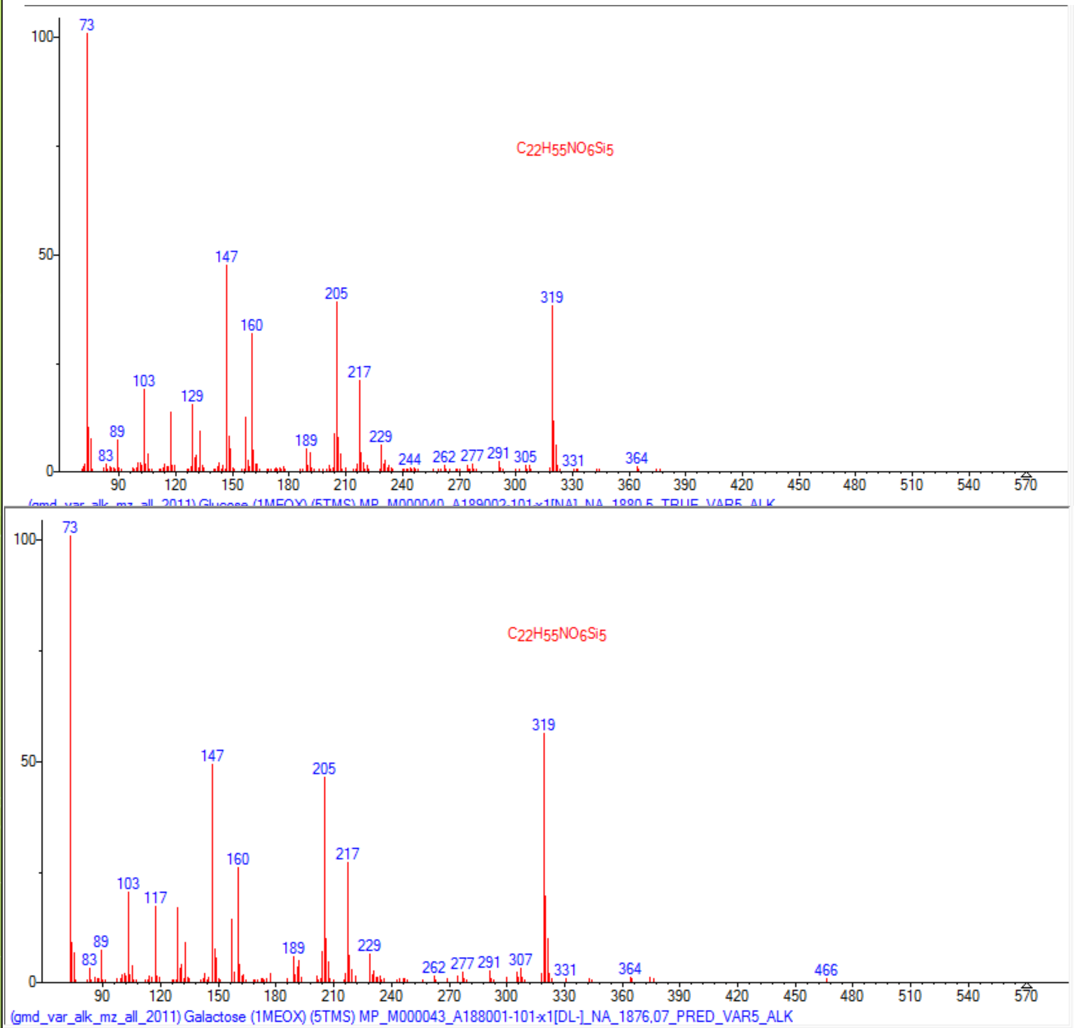


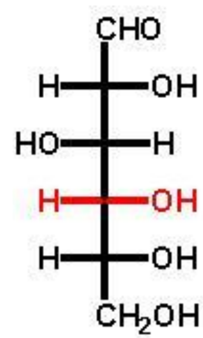
baseline seperation using a longer temperature gradient

identification – spectral match

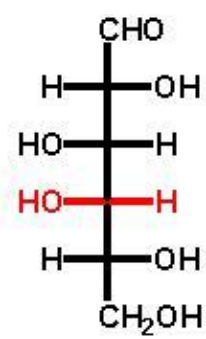


identification – spectral match



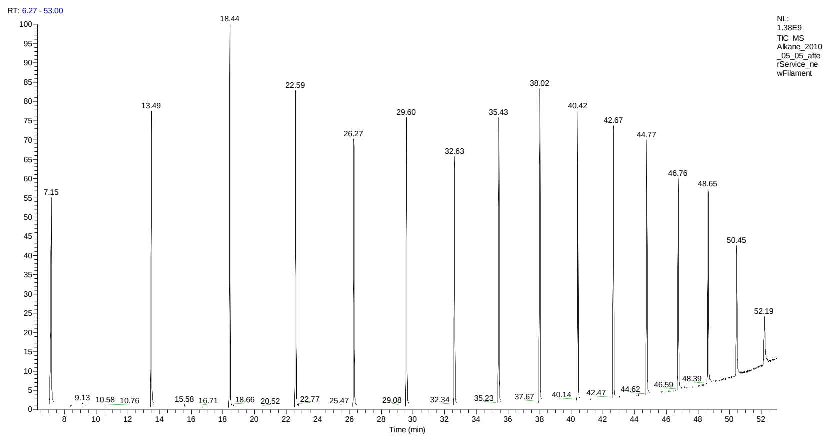


D-glucose

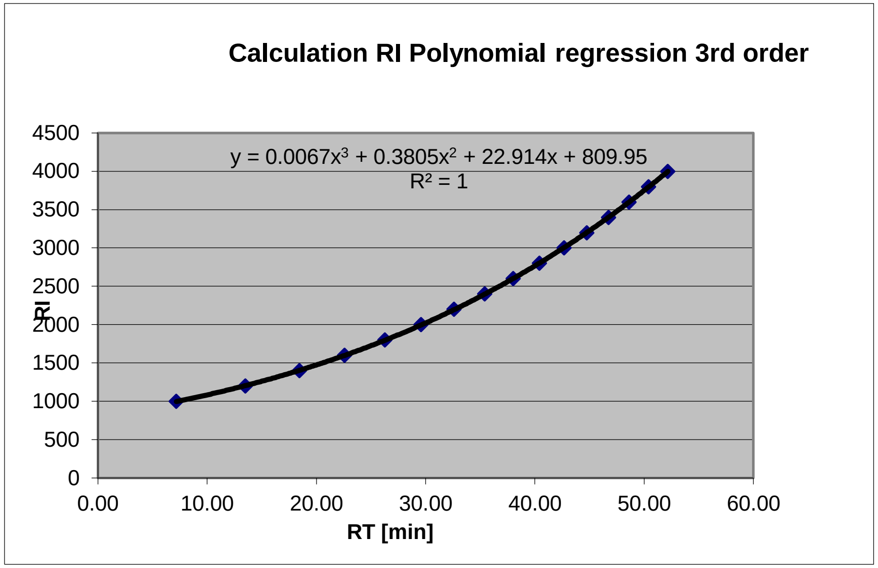


D-galactose

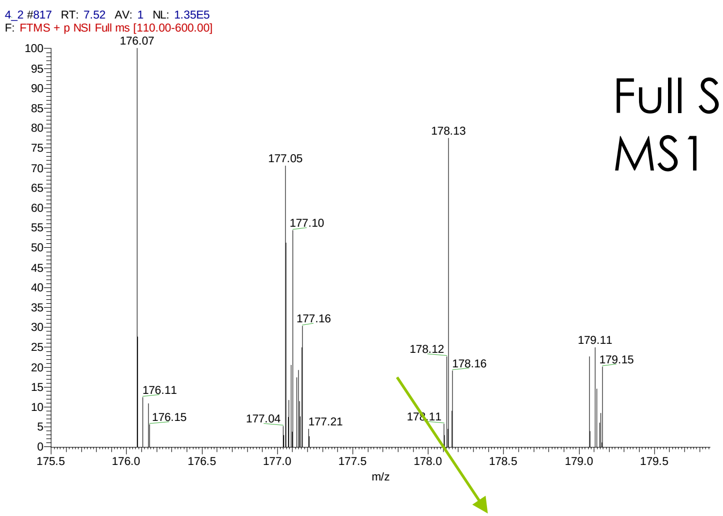
identification – retention index



	RT	RI
Decane ($C_{10}H_{22}$)	7.31	1000
Dodecane ($C_{12}H_{26}$)	12.96	1200
Tetradecane ($C_{14}H_{30}$)	17.74	1400
…	…	…



„identification“ – exact mass

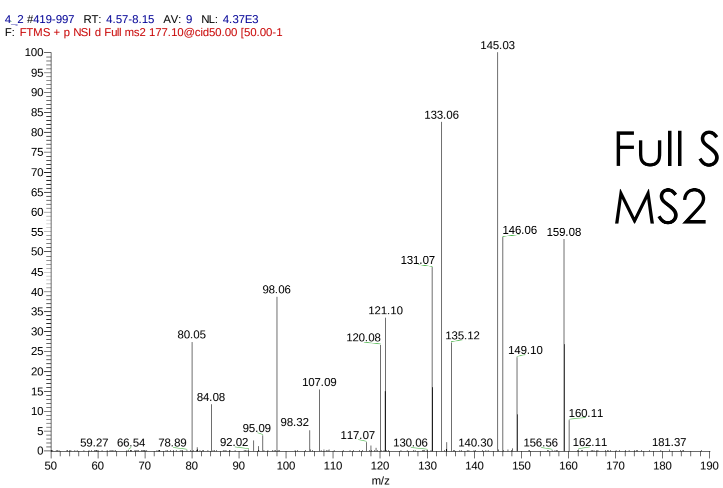


Full Scan MS1

Sum formular annotation by exact mass

Precursor:
$[M+H]^+$ 177.102231

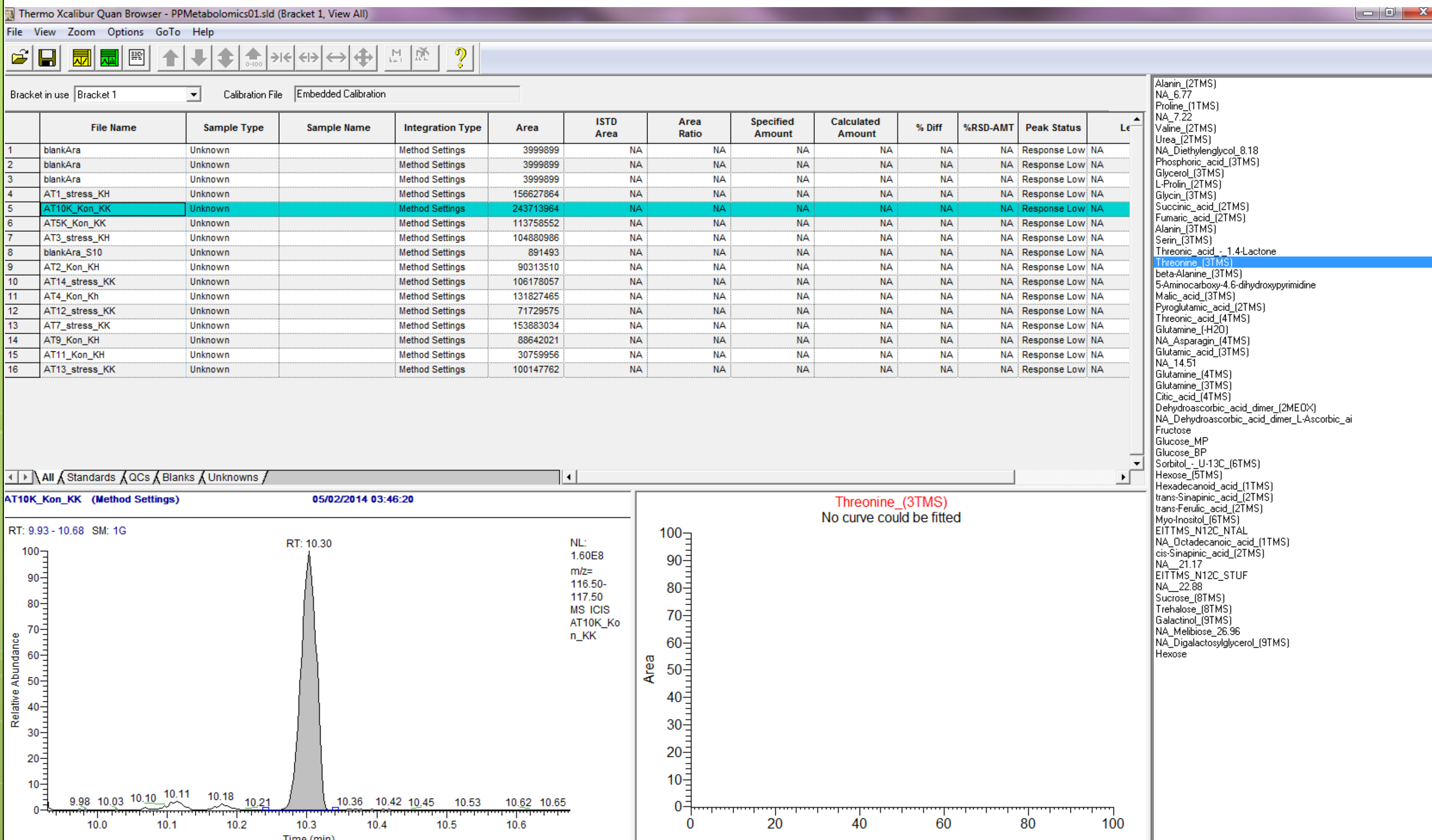
Multiple charges possible



Full Scan MS2

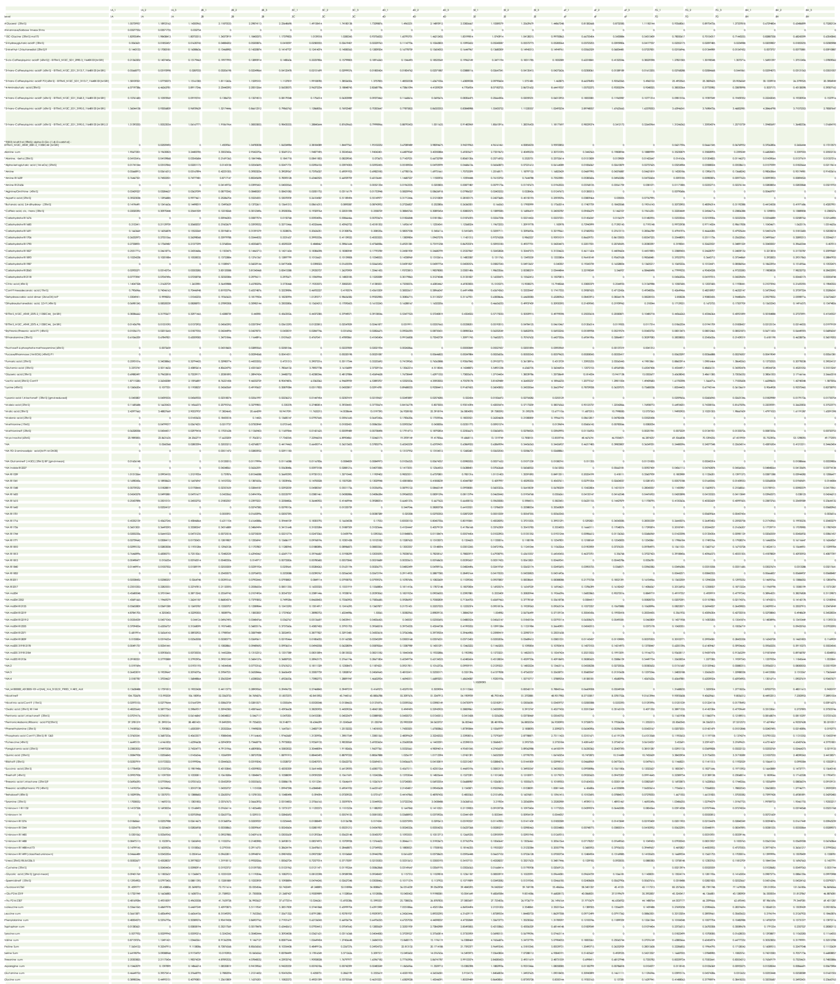
Structure information by comparing MSn spectra to libraries

alignment & peak integration



	File Name	Sample Type	Sample Name	Integration Type	Area	ISTD Area	Area Ratio	Specified Amount	Calculated Amount	% Diff	%RSD-AMT	Peak Status	Le
1	blankAra	Unknown		Method Settings	3999899	NA	NA	NA	NA	NA	NA	Response Low	NA
2	blankAra	Unknown		Method Settings	3999899	NA	NA	NA	NA	NA	NA	Response Low	NA
3	blankAra	Unknown		Method Settings	3999899	NA	NA	NA	NA	NA	NA	Response Low	NA
4	AT1_stress_KH	Unknown		Method Settings	156627864	NA	NA	NA	NA	NA	NA	Response Low	NA
5	AT10K_Kon_KK	Unknown		Method Settings	243713964	NA	NA	NA	NA	NA	NA	Response Low	NA
6	AT5K_Kon_KK	Unknown		Method Settings	113758552	NA	NA	NA	NA	NA	NA	Response Low	NA
7	AT3_stress_KH	Unknown		Method Settings	104880986	NA	NA	NA	NA	NA	NA	Response Low	NA
8	blankAra_S10	Unknown		Method Settings	891493	NA	NA	NA	NA	NA	NA	Response Low	NA
9	AT2_Kon_KH	Unknown		Method Settings	90313510	NA	NA	NA	NA	NA	NA	Response Low	NA
10	AT14_stress_KK	Unknown		Method Settings	106178057	NA	NA	NA	NA	NA	NA	Response Low	NA
11	AT4_Kon_Kh	Unknown		Method Settings	131827465	NA	NA	NA	NA	NA	NA	Response Low	NA
12	AT12_stress_KK	Unknown		Method Settings	71729575	NA	NA	NA	NA	NA	NA	Response Low	NA
13	AT7_stress_KK	Unknown		Method Settings	153883034	NA	NA	NA	NA	NA	NA	Response Low	NA
14	AT9_Kon_KH	Unknown		Method Settings	88642021	NA	NA	NA	NA	NA	NA	Response Low	NA
15	AT11_Kon_KH	Unknown		Method Settings	30759956	NA	NA	NA	NA	NA	NA	Response Low	NA
16	AT13_stress_KK	Unknown		Method Settings	100147762	NA	NA	NA	NA	NA	NA	Response Low	NA

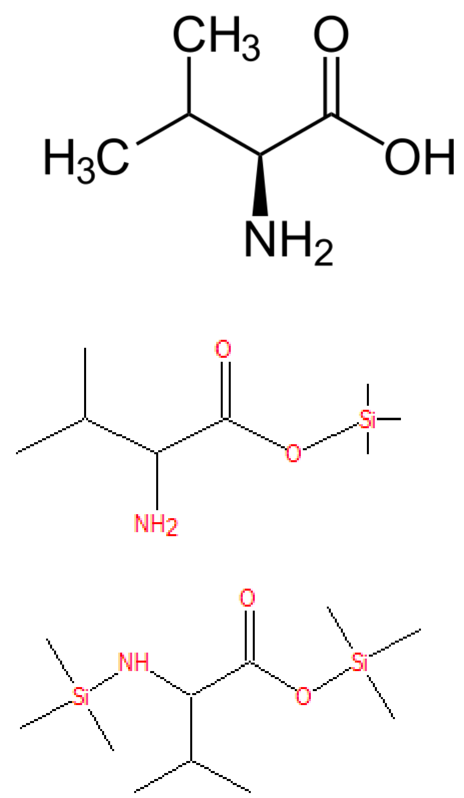
raw data matrix



	S1	S2	S3
M1_D1	680518	22042	5913
M1_D2	2218715500	1177147637	114881670
M2	12090493	1674566	2551986
M3	38668521	16650636	4641401
….	…	….	….

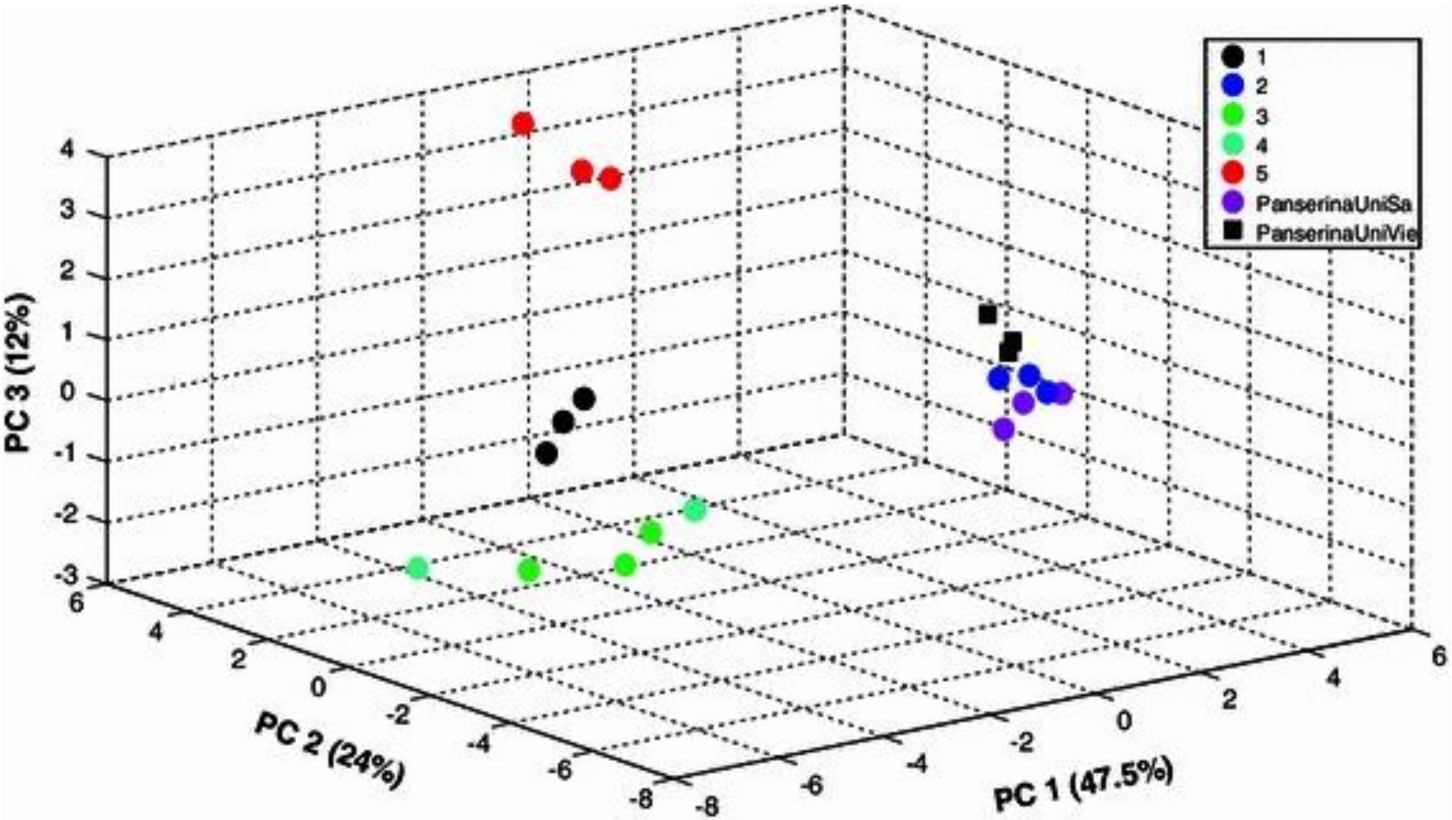
curated data matrix

	S1	S2	S3
Valine (1+2TMS)	56856630	3066789	2316542
Malic acid	1010149883	232986113	999818170
Glucose (MP)	3230720402	2316640517	1818410005
....	....	...	...



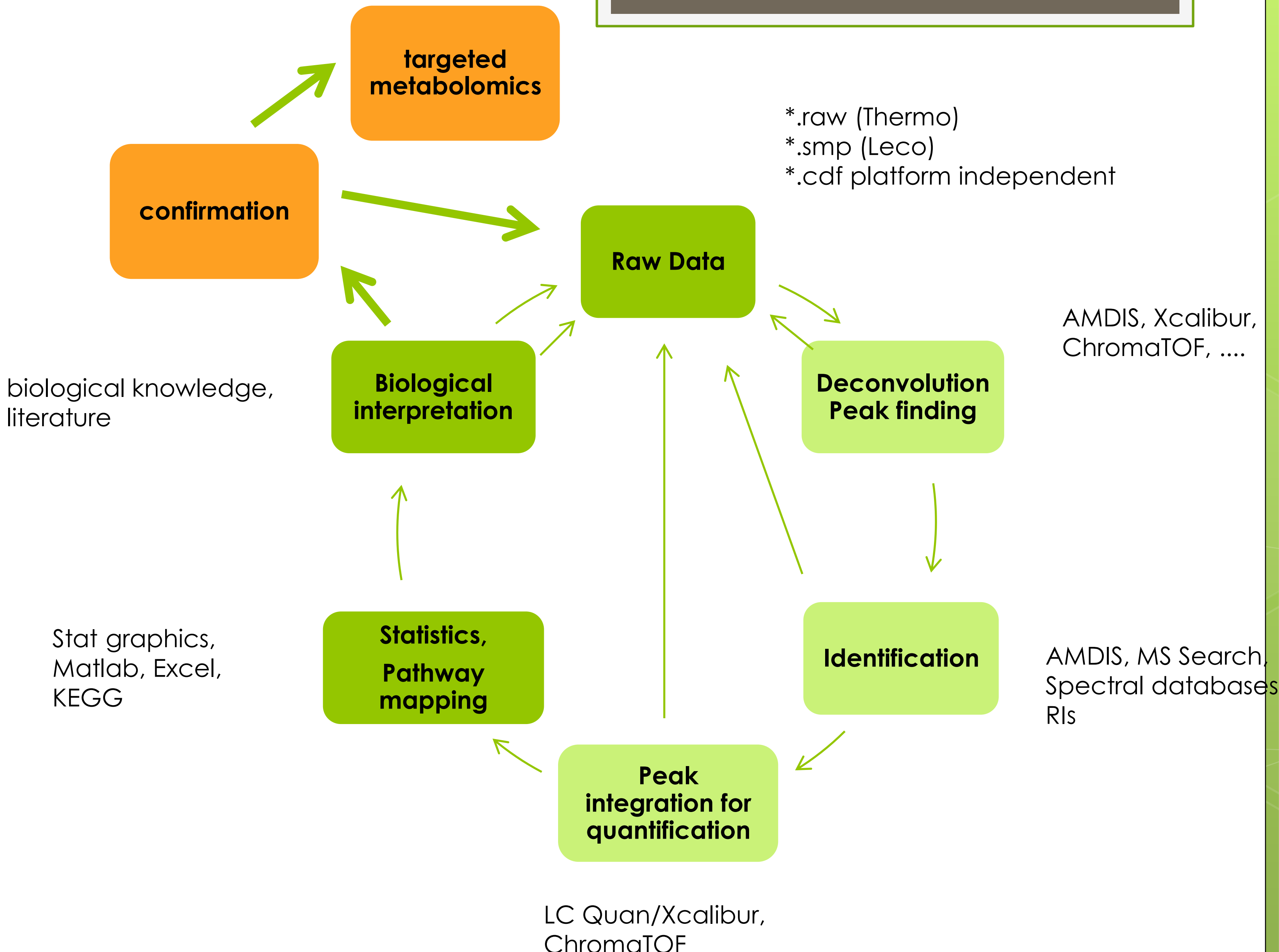
summed derivatives
normalised to sample amount (weight, volume, protein content)
checked for 0-values, contaminants, ...
Absolute quantification using calibration curves of standard mix

statistics - PCA



Mari et al. (2013), Metabolomics 9(3): 564–574

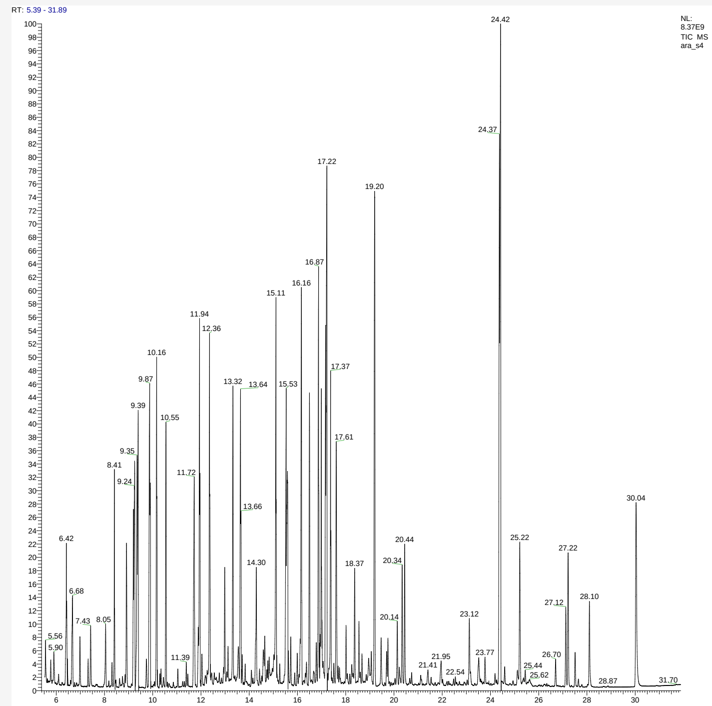
data processing





Questions?



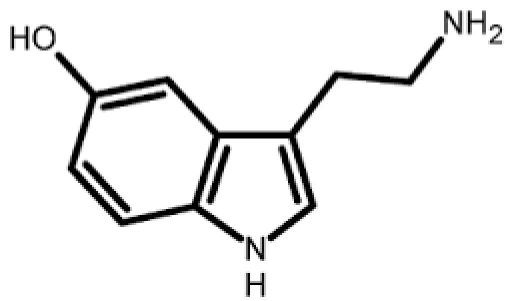




Mosys
Department
Molecular Systems Biology
universität wien
University of Vienna | Faculty of Life Sciences | Althanstrasse 14 | 1090 Vienna | Austria

Nachtrag

ESI – spectrum



Serotonin - 5-Hydroxytryptamine ($C_{10}H_{12}N_2O$)
Image by Erowid, © 2003 Erowid.org

Exact mass of molecular ion -->
mass accuracy <5ppm
→ sum formular calculation

Still structure = ?????

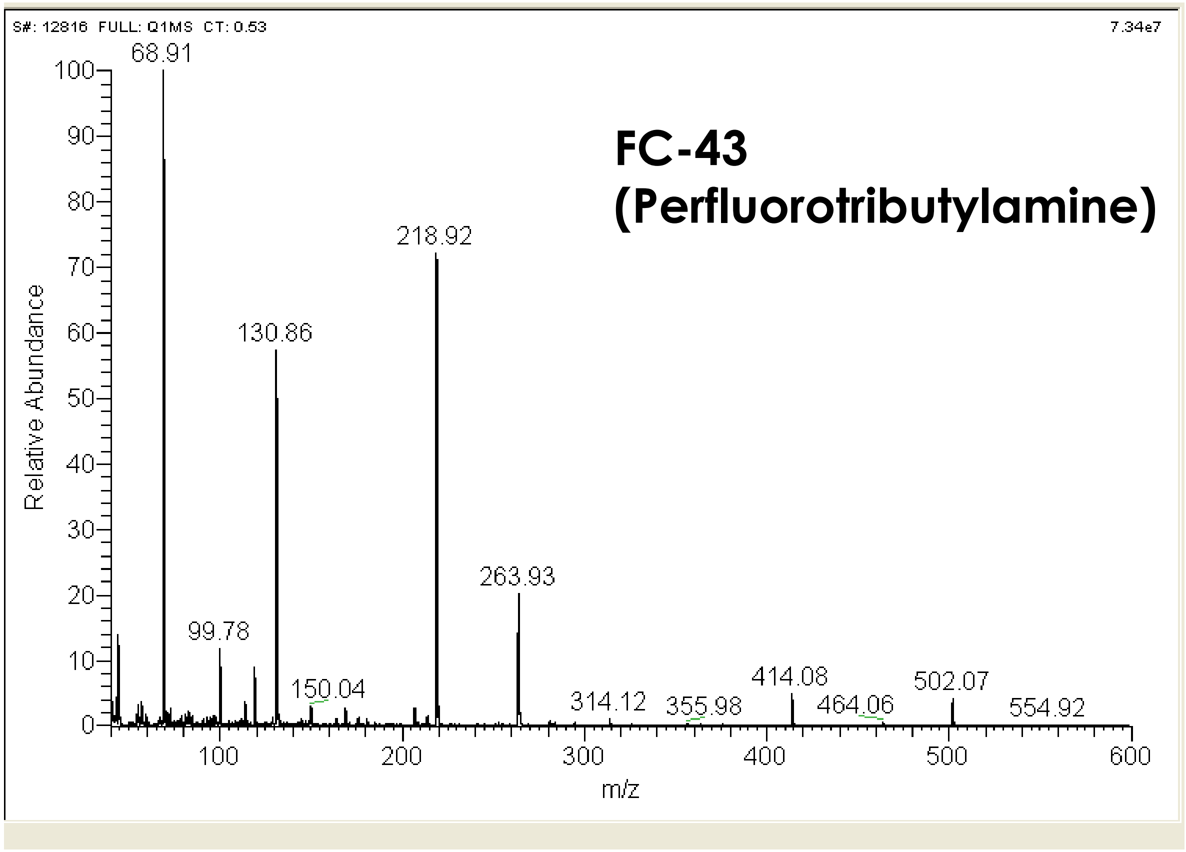
→ MS^n fragmentation pattern
→ Comparison with standards

Monoisotopic mass C10H12N2O: 176.094955 Da
Monoisotopic mass H: 1.007276 Da

$[M+H]^+$ =177.102231 Da

Mass error calculation [ppm]:
(theoretical mass-experimental m/z)/
Theoretical mass * 10^6

calibration gas



Thank you!