THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Knowledge that will change your world

Choosing the metabolomics platform

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Targeted Metabolomics & Proteomics Laboratory

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Synopsis

- **History of metabolomics**
- **Guide to the choice**
  - NMR
  - GC – transition from packed to wall-coated columns
  - LC
- **Mass spectrometers**
  - Mass accuracy
  - Targeted vs untargeted
  - Other techniques
- **The mass spectrum**
- **The mass of an ion**
  - Homework

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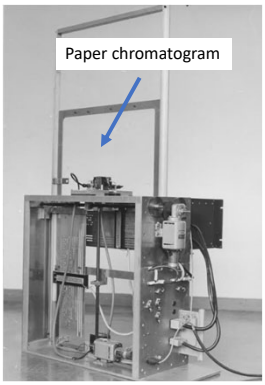
Early beginnings of metabolomics in London

- Sir Ernst Chain (1945 Nobel Laureate – the biochemist who characterized penicillin)
  - Also renown for his work on microanalysis
- Used 2D-paper chromatography to resolve glycolytic, Krebs cycle and amino acids derived from ^{14}C-glucose
  - Geiger counter mounted on a typewriter frame
  - Digitized the collected data and prepared computer-generated figures

METABOLOMICS

Keith Mansford



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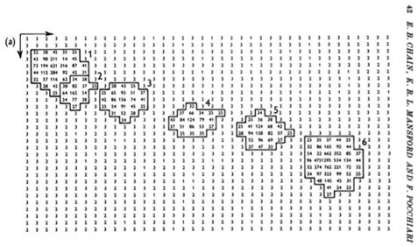
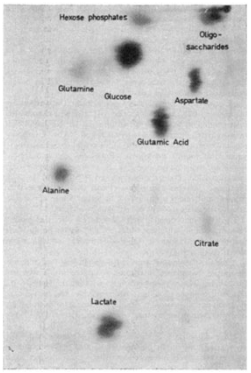
Radiochromatography examples



J Physiol (1960) 154:39
E.B. Chain, K.R.L. Mansford and F. Pocchiari



Biochem. J. (1969) 115, 537
E.B. Chain, K.R.L. Mansford and L.H. Opie

Autoradiogram of ^{14}C-glucose metabolites from an isolated perfused Langendorff rat heart preparation. The metabolites were separated by 2D-paper chromatography.

The conditions were:
1st dimension: butan-l-ol-acetic acid-water (40:11:25, by vol.) for 16hr.;
2nd dimension: (-) phenol-aq. NH_3 (sp.gr. 0.88)-water (80:1:20, by vol.) for 24hr.

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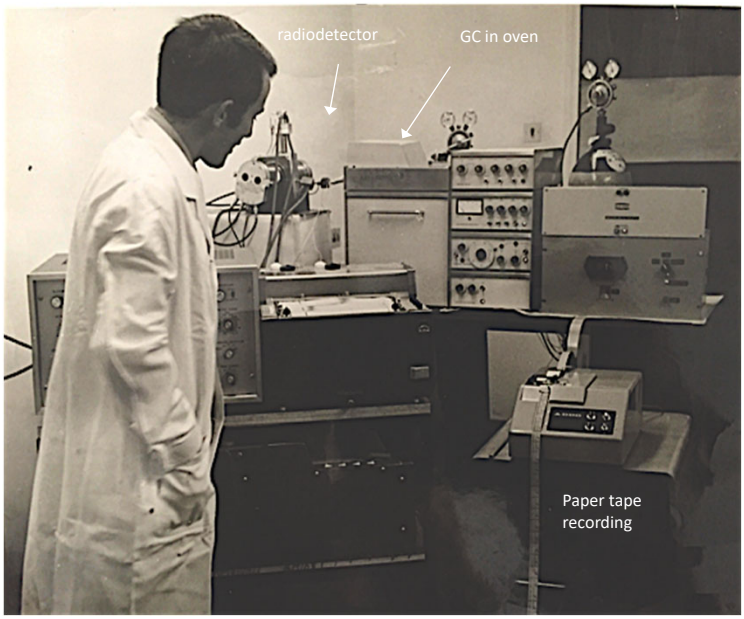

Radio-GC analysis

metabolomics in its infancy

Radio gas-liquid chromatography with digitization of collected data

Developed this for my PhD work (1967-1970) to study glucose metabolism in acellular slime mold, *Physarum polycephalum*

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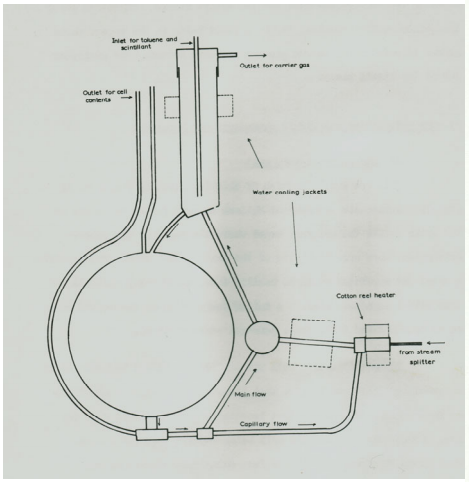
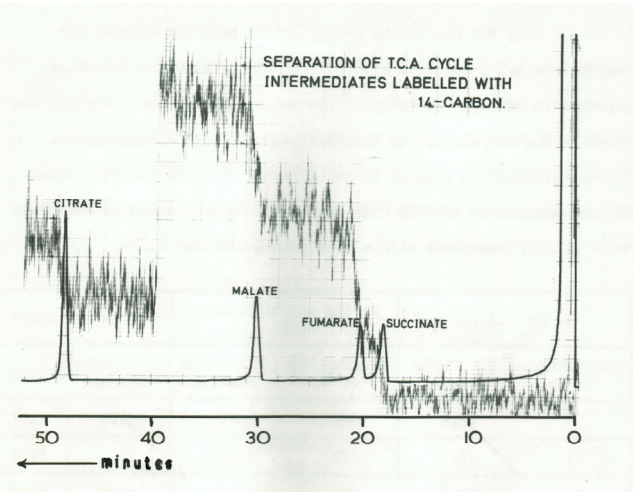
Radio-GC of Krebs cycle intermediates



Popjak scintillation cell



Stephen Barnes, PhD thesis

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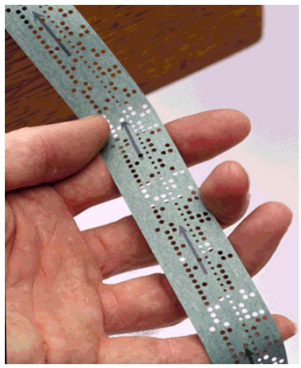
Software for data analysis on a PDP9 computer

```
DIMENSION IBUFF(1000), IDATA(725,2)
COMMON IBUFF,IDATA,ITIME,INT,ISIG
5       ITIME=0
7       CALL TAPE(ISIG,INTA)
8       IWRITE(1,1001)INTA
        INT=INTA/100
10      NPOINT=1
11      CALL TAPE(ISG,IDATA(NPOINT,1))
        IF (ISIG.EQ.1) GO TO 16
        IF (ISIG.EQ.2) GO TO 13
        WRITE(1,1001)NPOINT
GO TO 11
13      IF (IDATA(1,1).GT.940) GO TO 11
        IF (IDATA(NPOINT,1).EQ.0) GO TO 11
        IF (NPOINT.EQ.725) GO TO 16
15      NPOINT=NPOINT+1
        GO TO 11
```



Punched tape data
1 data point/sec

Digital PDP computer
Had a screen

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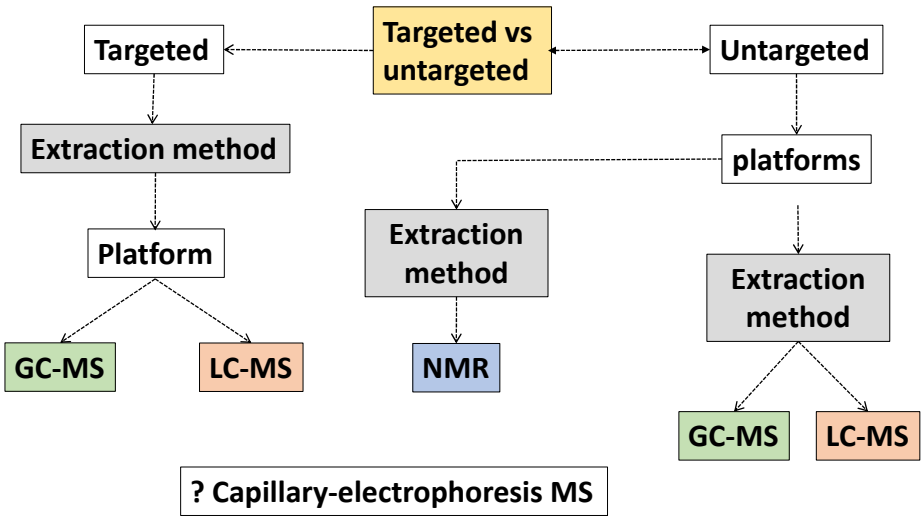
Decision tree



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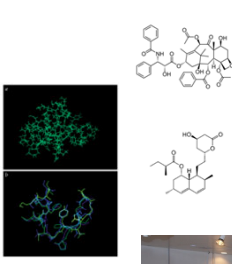
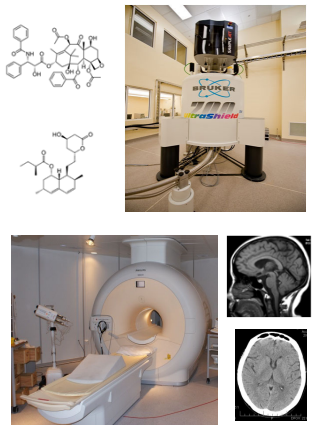
Nuclear Magnetic Resonance (NMR) Spectroscopy

- Detects NMR active nuclei
- Robust and highly reproducible
- Non-destructive
- Quantitative
- Used in
  - Structure elucidation
    - Small molecules
    - Macromolecules (DNA, RNA, Proteins)
  - A number of techniques
    - 1D , 2D, 3D
  - Molecular motion and dynamics
- Similar method used in medical Imaging (MRI, fMRI)





from Wimal Pathmasiri

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NMR considerations

- **Sample amount:**
  - Typical 600 MHz instrument requires 0.5 ml plasma/serum
  - Higher field instruments and micro coil detector allows use of 0.1 ml
- **Quality control:**
  - In the UK Phenome Center, all samples are analyzed by NMR
    - This allows for detection of outliers
    - Also found that there is a correlation between the NMR spectrum and whether problems occur in LC-MS analysis
    - NMR analysis used to filter out these samples

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Hyperpolarization NMR

- **The NMR signal comes from non-equilibrium of the two or more energy states a nucleus experiences in a strong magnetic field**
  - However, the natural excess population of the higher energy states is no more than 0.01%
  - This accounts for the low sensitivity of NMR
- **By hyperpolarizing the compound, the excess population can be increased by 10^4-10^5.**
  - Much increased sensitivity
- **Carbonaceous materials (metabolites) can be hyperpolarized by cooling to 1°K in a strong magnetic field (3 T or larger)**
  - However, the lifetime of the hyperpolarized state is quite short (10-30 s) making metabolomics experiments quite difficult

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Gas-liquid Partition Chromatography: the Separation and Micro-estimation of Volatile Fatty Acids from Formic Acid to Dodecanoic Acid

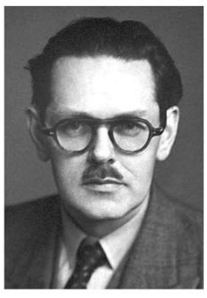
By A. T. JAMES and A. J. P. MARTIN
National Institute for Medical Research, Mill Hill, London, N.W. 7

(*Received* 5 *June* 1951)

Martin and Synge's 1941 paper contained the thought "The mobile phase need not be a liquid but may be a vapour. By means of this, refined separations may be carried out."

Also, "Very refined separations of volatile substances should be possible in a column in which permanent gas is made to flow over gel impregnated with a non-volatile solvent..."

First presented at a Biochemical Society Meeting on October 20, 1950



Martin with Richard Synge received the Nobel Prize in Chemistry in 1952

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Transition from packed columns to open tubular columns

- **In the first GC columns, the "liquid phase" was coated onto an inert support (firebrick and then diatomataceous earth – the silica shells of sea creatures)**
- **However, these particles create a significant back pressure, limiting the (glass) column lengths to 2 meters**
  - The disadvantage of using a gas as the mobile phase is that it is compressible
  - For a 2-meter column, the head gas pressure is twice atmospheric
  - This slows linear gas velocity and decreases separation power
- **A capillary column where the liquid is coated onto the wall of the column was patented in 1955, but not commercialized until 1975**
  - First capillaries were borosilicate glass – broke easily
  - Column lengths were up to 100 meters
  - Fiberoptic quartz capillaries with polysulfone coating are now standard
  - Resolution went from 5,000 to 100,000

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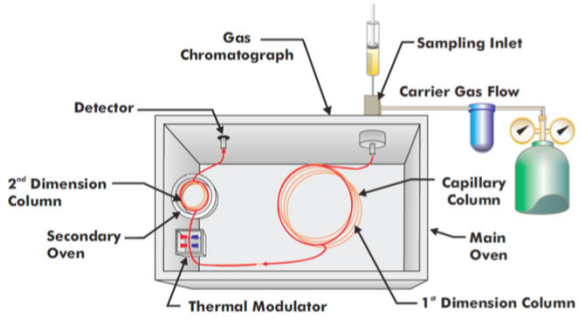
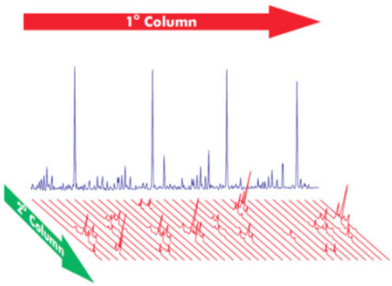
Two dimensional GC to resolve metabolites



As compounds elute from column 1, they are passed to (cooler) column 2 where they condense. After a period of collection, column 2 is heated so as to separate and elute the compounds.

Leco Corp.



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Metabolomics and GC-MS

- **PROS**
  - Capillary columns can achieve very high chromatographic resolution
  - Retention times are reproducible
  - Mass spectral libraries are well developed
- **CONS**
  - Not all compounds can be analyzed by GC-MS
  - Although amino acids, sugars, fatty acids, amines and organic acids can be derivatized, complex polyphenol glycosides and polar lipids are too unstable, even when derivatized, at the temperatures used to elute them
  - Approximate mass limit of 400 Da

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LC considerations

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Liquid chromatography-Mass Spectrometry

- **PROS**
  - **Almost all compounds can be analyzed by LC-MS**
    - Exceptions - hydrocarbons do not ionize due to soft ionization of electrospray
    - New technology may overcome this limitation
  - **Several orders of magnitude increased sensitivity compared to NMR**
  - **Can collect MS, MSMS and ion mobility data**
- **CONS**
  - **Unlike NMR, not uniformally quantitative**
  - **Mass spectral libraries are not well enough developed**
  - **Chromatographic separation not adequate**
  - **Retention time reproducibility not as good as GC-MS**

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LC flow rate

- **MS Sensitivity is inversely related to flow rate**
  - Slower flow gives more sensitivity

normal flow (0.2-0.4 ml/min)

microflow/capillary (5-50 µl/min)

nanoflow (0.3-5 µl/min)

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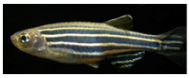
Optimizing nanoLC for metabolomics

- Objective is to develop metabolomics for small animal model systems
  - *D. melanogaster*
    - We have done analyses on single muscles isolated from fruit flies

  - *C. elegans*

  - *D. rerio*
    - A single zebrafish yields about 1 µl of plasma



- Need to move down to the nanoscale
- Important to maintain consistency and quantitation
  - Reproducible columns and temperature

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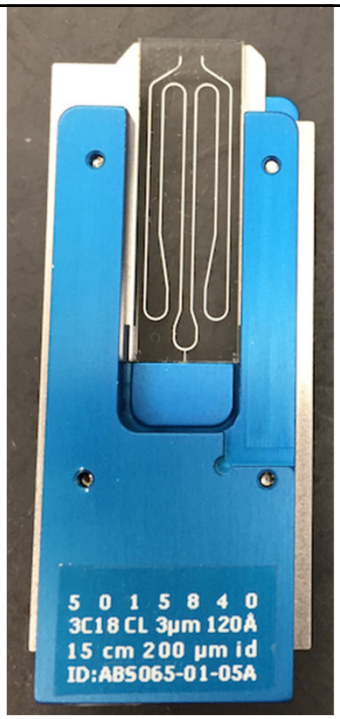

Close up of a nanochipLC cartridge (15 cm x 0.2 mm ID).

- **Each long section of the column is ~2.5 cm (1 inch).**
- **Can be machined to a better tolerance.**
- **Simpler connections to the liquid stream.**
- **Can be placed in a temperature-controlled environment**

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HPLC and UHPLC

- **In LC, the chemical groups are covalently attached to the silica beads**
  - First columns had 7 μm beads
  - Filtered to be homogeneous – it improves laminar flow
- **Bead size reduced to 5 μm, then to 3 μm**
  - As for GC, the back pressure increases, but liquids are not compressible
  - The increase is inversely proportional to the square of the particle diameter
  - For 7 μm -> 3 μm, this is 7/3 squared – 49/9 (5.44 fold)
- **Waters introduced the 1.7 μm UPLC column**
  - Increased chromatographic resolution – faster runs
  - But another (3/1.7) squared - 9/2.89 (3.11-fold) increase in back pressure
  - Required new pump engineering since the back pressure was 15,000 psi
  - Particles had to resist this pressure

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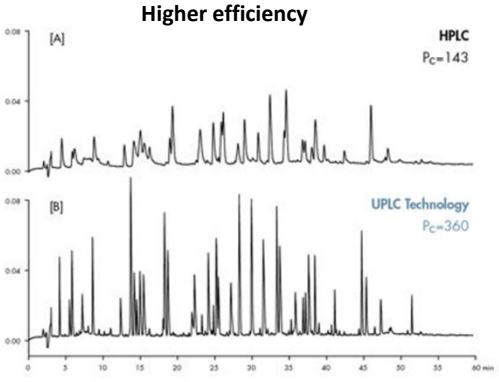
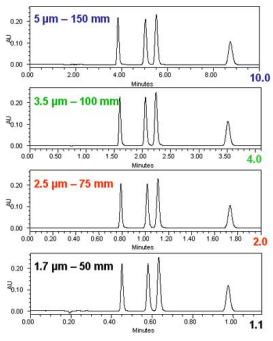
The LC

- **1D-approach**
  - Use of reverse-phase, normal phase and HILIC phase
  - particle size – smaller is more efficient, but back pressure is a problem

Higher efficiency



Same efficiency, but shorter run times



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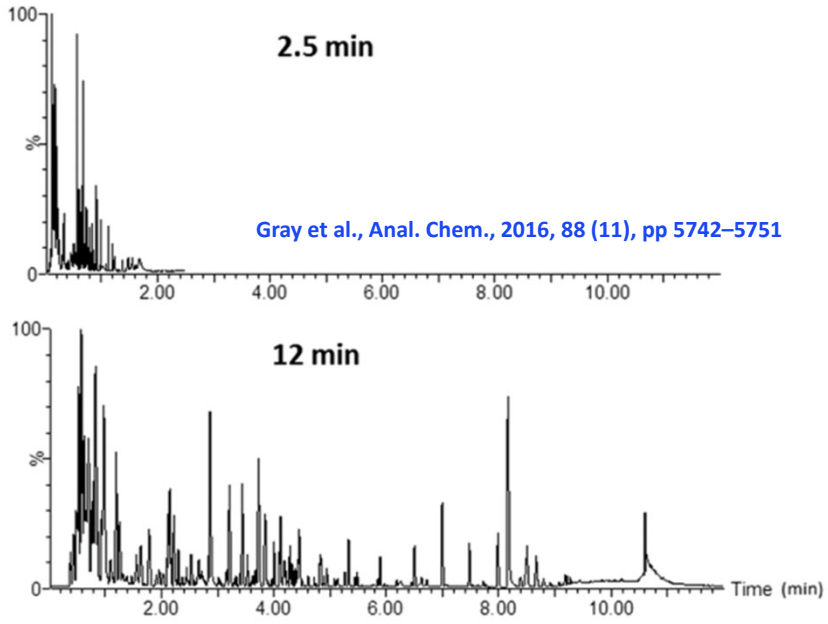
RAMMP, speeding up metabolomics



Gray et al., Anal. Chem., 2016, 88 (11), pp 5742–5751

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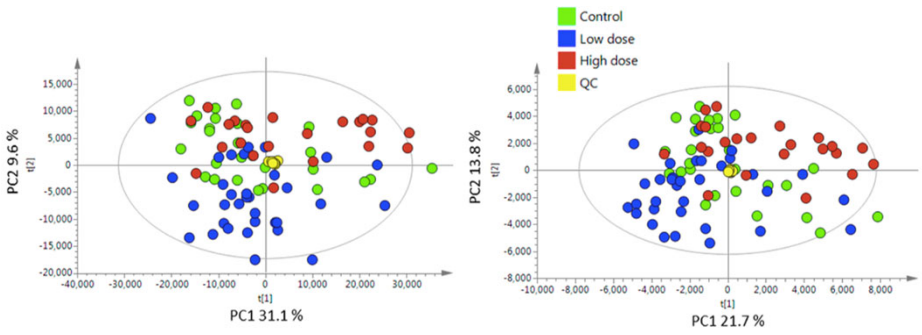
RAMMP

- **There was a reduction in independent features**
  - 19,000 by conventional method
  - 6,000 by RAMMP



Conventional method **RAMMP**

Gray et al., Anal. Chem., 2016, 88 (11), pp 5742–5751

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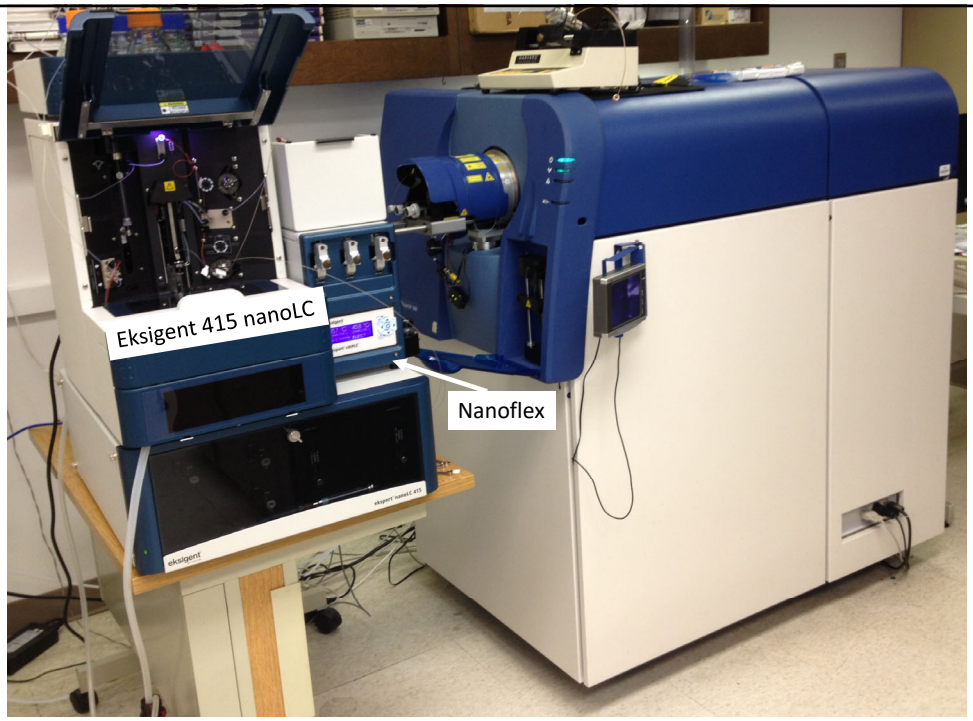

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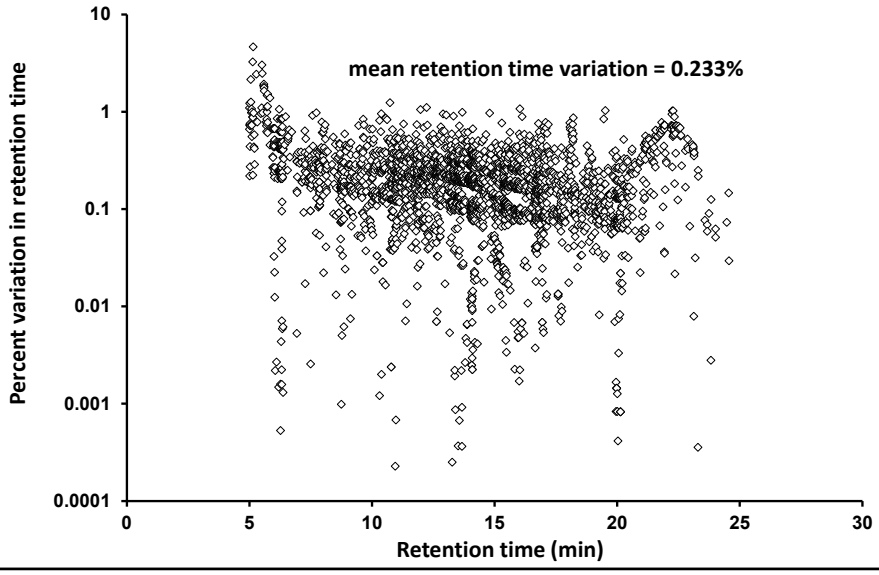
Coefficient of variation of retention time for the three separate extracts by nanoLC-MS



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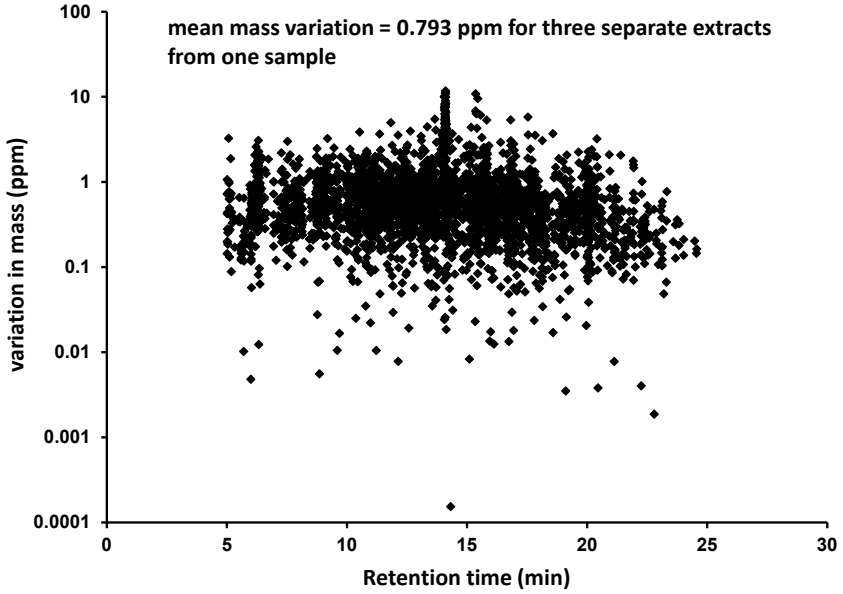
Coefficient of variation of the mass of peaks identified by XCMS using nanoLC-MS



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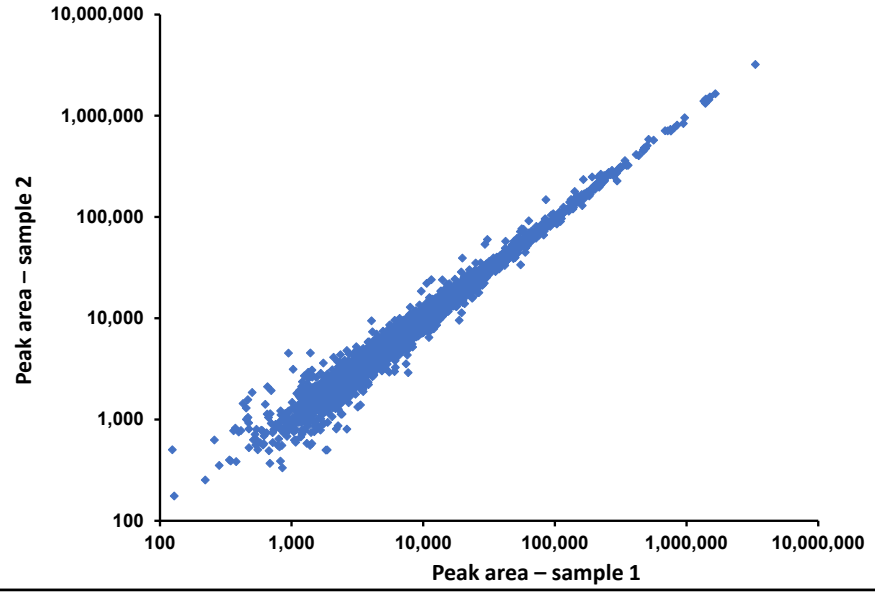
Reproducibility of peak areas using the nano chipLC approach



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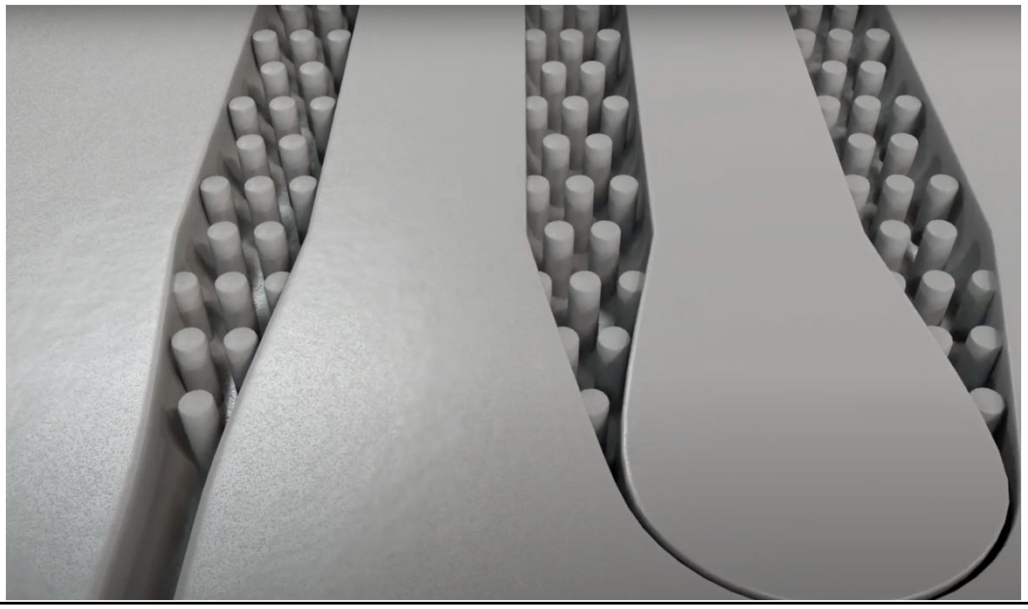
Towards open tubular LC



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Mass and resolution

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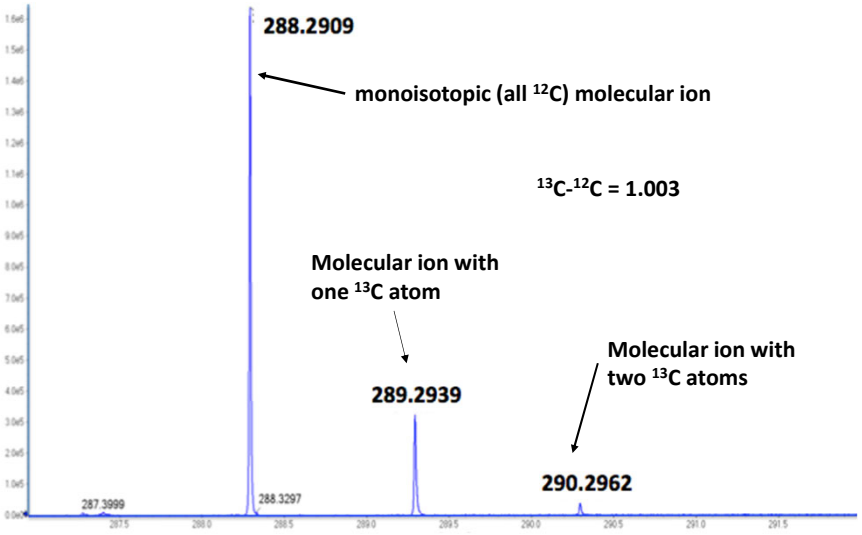
Mass spectrum of a compound



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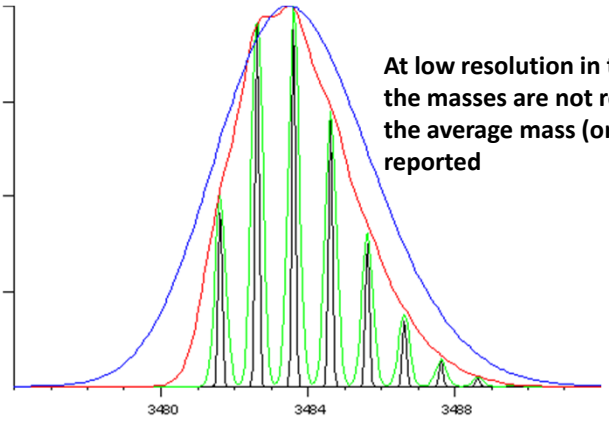
Mass resolution



At low resolution in the blue and red, the masses are not resolvable. Instead, the average mass (or centroid) is reported

Blue = ion trap, Red = quadrupole, Green = Quadrupole TOF, Purple = FT-ICR

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Selecting the mass spectrometer

- **It is necessary to use an instrument to measure:**
  - The mass of the metabolites accurately
  - To provide sufficient mass resolution to distinguish the isotopes associated with each metabolite
- **There are several types of MS detectors**
  - Quadrupole
  - ion trap
  - time-of-flight (TOF)
  - Orbitrap
  - Fourier Transform-Ion Cyclotron Resonance (FT-ICR)

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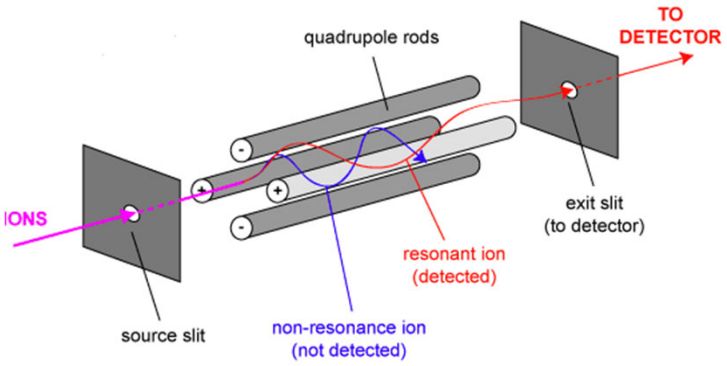
Quadrupole mass filter



Consists of four parallel rods. Each opposing rod pair is connected together electrically, and a radio frequency (RF) voltage with a DC offset voltage is applied between one pair of rods and the other. This causes the ions to rotate in spirals as they go through the quadrupole. For a given voltage, only ions of a specific *m/z* can pass through. The voltage can be scanned to generate a mass spectrum or held constant to allow one ion to pass through.

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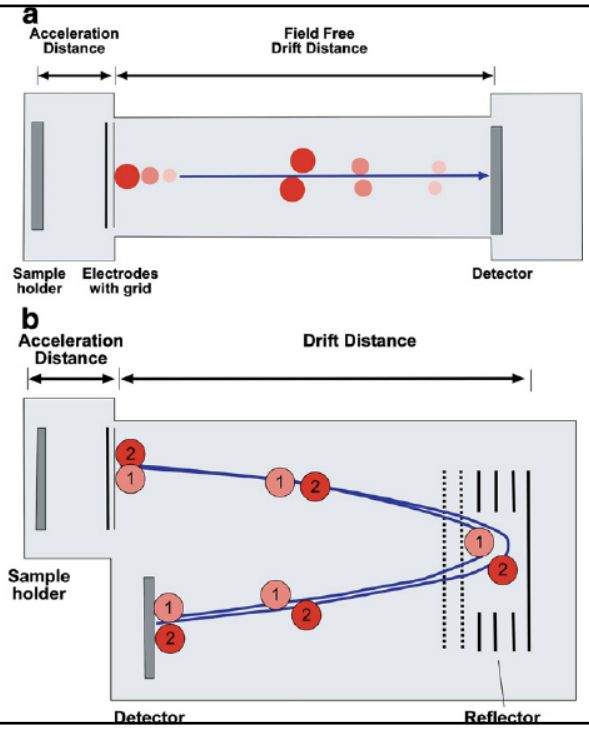

Time-of-flight (TOF) analyzer

Ions can come from a static position (MALDI plate or frozen tissue section) or those passing through a quadrupole mass selector.

The ions pass down the flight tube in ~1 μs

Using the reflectron process, ions can be more effectively focused. Over-energized ions of the same mass dig deeper into the reflectron allowing the less energetic ions to catch up so they both arrive at the detector at the same time

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The mass spectrometer

- **For untargeted analysis it is important to have high mass resolution, accuracy and speed**
  - Initial data analysis is performed on the molecular ions
  - Each metabolite has a unique mass (*m/z*)
    - Nonetheless, a particular mass, however exact, is not necessarily a unique metabolite
- **Fourier transform-ion cyclotron resonance and Orbitrap instruments have the greatest mass accuracy**
  - However, their performance is time-dependent and is degraded significantly using short acquisition times (<100 ms)
  - They are best used for follow up experiments

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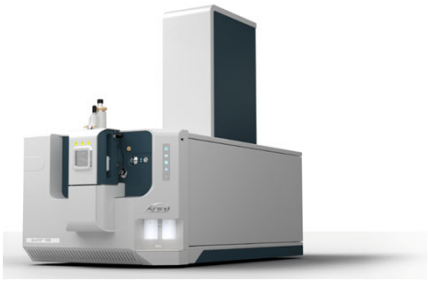
TOF is the mass analyzer of choice for untargeted metabolomics

- **Quadrupole-orthogonal time-of-flight (Q-tof)**

Agilent 6500

Waters Synapt G2/HMDS

Bruker timsTOF



Sciex XenoTOF 7600

Current models have 30-80,000 mass resolution and 1 ppm or better mass accuracy

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Links to the different Q-TOFs

- **Agilent - 6546 Q-TOF LC/MS**
  - **https://www.agilent.com/en/product/liquid-chromatography-mass-spectrometry-lc-ms/lc-ms-instruments/quadrupole-time-of-flight-lc-ms/6546-lc-q-tof**
- **Bruker timsTOF-**
  - https://www.bruker.com/en/products-and-solutions/mass-spectrometry/timstof/timstof-pro.html
- **Waters – Synapt G2Si**
  - https://www.waters.com/waters/en_US/SYNAPT-G2-Si-Mass-Spectrometry/nav.htm?cid=134740653&locale=-
- **SCIEX – ZenoTOF 7600 with electron activation dissociation**
  - https://sciex.com/products/mass-spectrometers/qtof-systems/zenotof-7600-system

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Novel Hybrid Quadrupole-Multireflecting Time-of-Flight Mass Spectrometry System

Dale A. Cooper-Shepherd, Jason Wildgoose, Boris Kozlov, William J. Johnson, Richard Tyldesley-Worster, Martin E. Palmer, John B. Hoyes, Michael McCullagh, Emrys Jones, Robert Tonge, Emma Marsden-Edwards, Peter Nixon, Anatoly Verenchikov, and James I. Langridge*

J Am Soc Mass Spectrom. 2023 Jan 5. doi: 10.1021/jasms.2c00281. Online ahead of print.

A Miniature Multilevel Structures for Lossless Ion Manipulations Ion Mobility Spectrometer with Wide Mobility Range Separation Capabilities

Adam L. Hollerbach, Randolph V. Norheim, Pearl Kwantwi-Barima, Richard D. Smith, and Yehia M. Ibrahim*

Anal Chem. 2022 Feb 1;94(4):2180-2188.

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Selected ion monitoring

- **The summation of all the ions collected in a GC or LC analysis is called the total ion current (TIC) and produces a total ion chromatogram**
- **By selecting a particular mass-to-charge ratio (*m/z)* value, one can see where a metabolite's molecular ion elutes from the column**
  - This produces a selected ion chromatogram (SIC or XIC)
  - The quality of the SIC depends on the mass accuracy and resolution of the collected data

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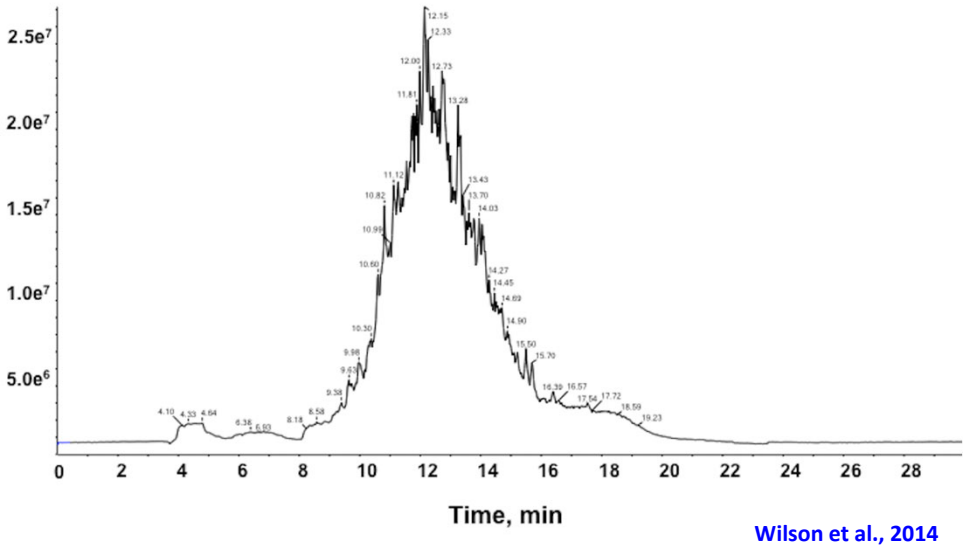
Example of a TIC of human urine



Wilson et al., 2014

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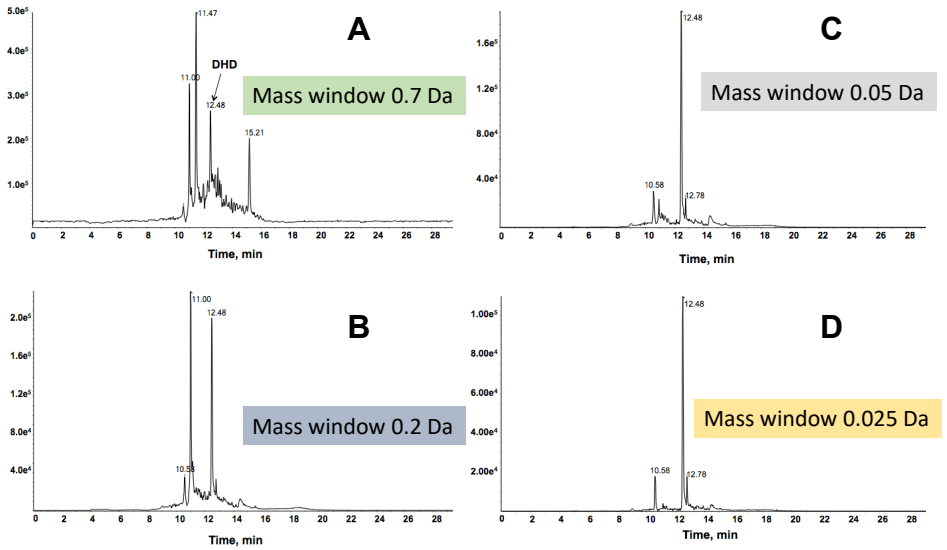
Selected ion chromatograms from TIC

Dihydrodaidzein



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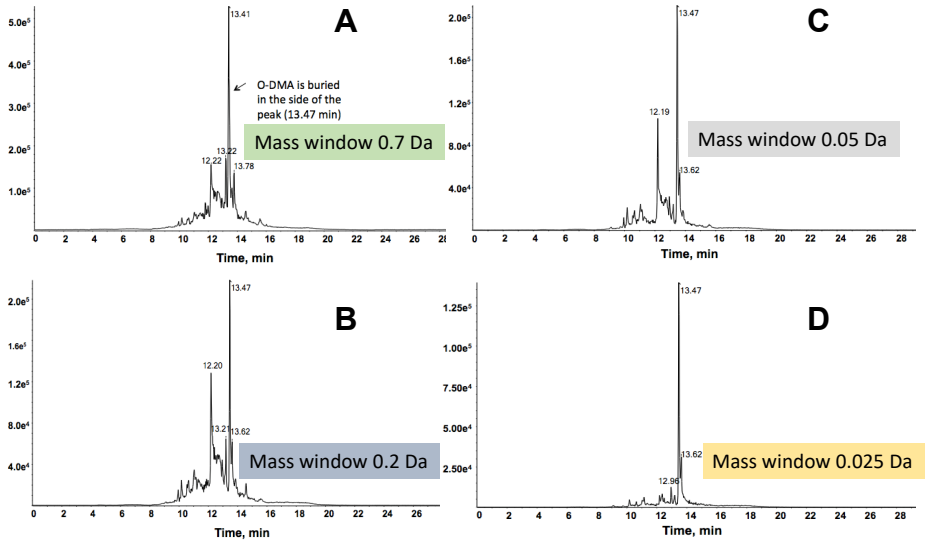
Selected ion chromatograms from TIC

O-desmethylangolensin



43

MS/MS

- **A second mass spectrum (MSMS) that is informative arises from isolating the molecular ion**
- **The molecular ion is heated, either by collision with neutral gas (quadrupole, ion traps) or by using IR radiation (FT-ICR)**
  - The extra energy increases the stretching of critical bonds, leading to dissociation of the molecular precursor ion into charged product ions
  - These generate the MS/MS spectrum for a metabolite
  - Ion traps can also isolate a product ion and create MS^n spectra

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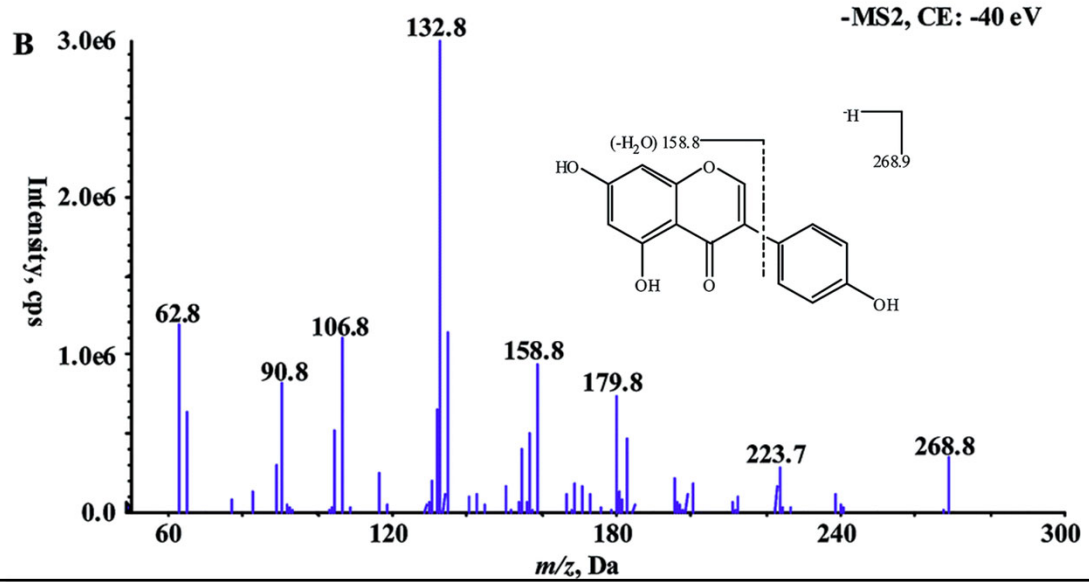
MS/MS spectrum of genistein



45

Measuring a mass transition

- Instead of measuring the full MS/MS spectrum, ions from the MS/MS can be individually measured
- This is referred to as a mass transition from the molecular or precursor ion to a specific product ion
- It is also known as reaction ion monitoring

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Targeted vs untargeted methods

- If we know what the metabolites to be measured are (from previous untargeted analyses, or prior knowledge), then a multiple reaction monitoring (MRM) approach is the best way to go since allows quantitative analysis of possibly 100s of metabolites
- If there is no hypothesis, but instead you want to generate hypotheses, then the untargeted approach is better.

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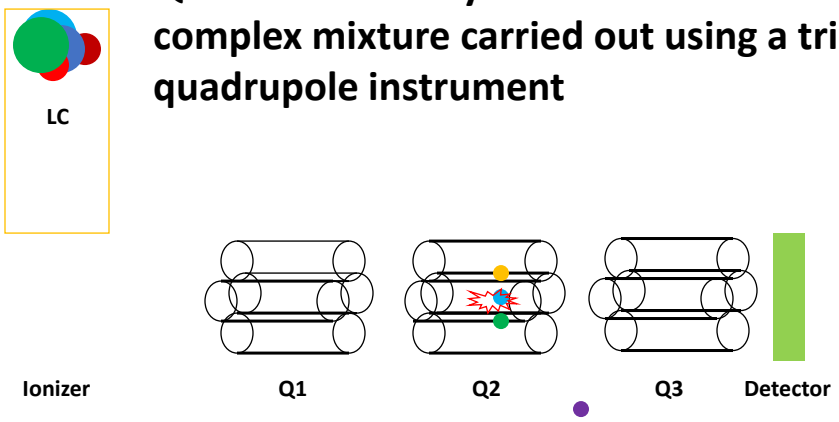
Multiple reaction ion monitoring

Quantitative analysis of metabolites in a complex mixture carried out using a triple quadrupole instrument



Based on precursor ion/product ion pair(s)

Courtesy, John Cutts

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How many MRM transitions?

- Acquisition can be as little as 2 msec, but acquisition time determines sensitivity
- Fast switching electronics can measure as many as 500 different transitions per second
- Since measuring the area under a peak requires 10 data points, the number of transitions measured has to be matched against the shape and width of the chromatographic peaks – to be discussed in more detail later

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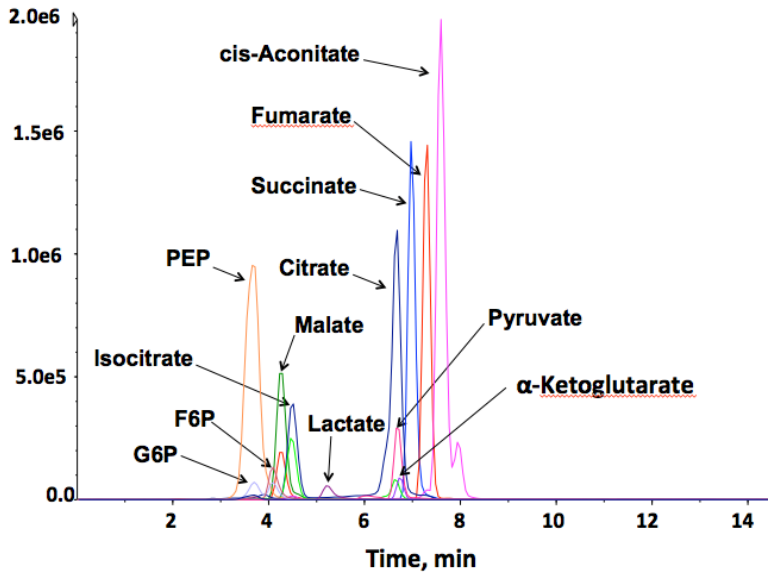
Combined channels for Krebs cycle



50

Ion mobility – another parameter to characterize a component

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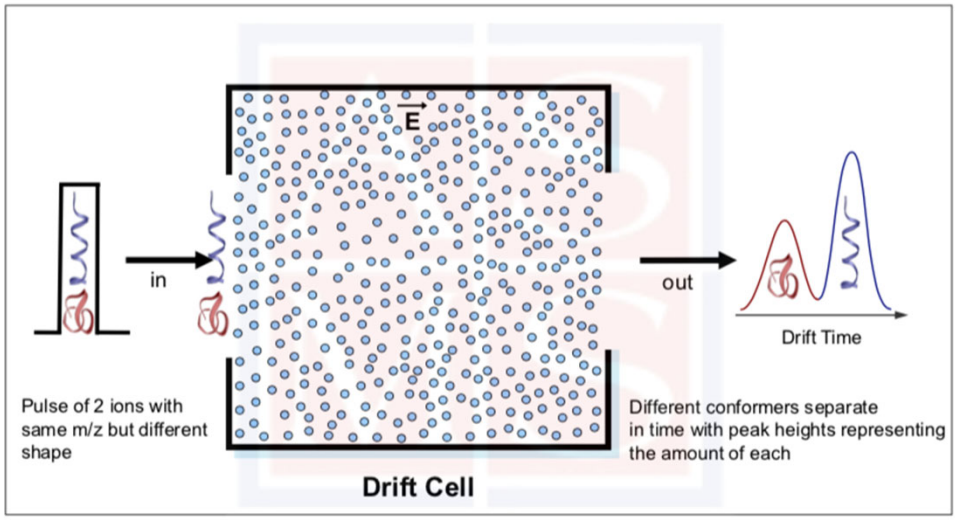
Ion Mobility Concept



velocity is constant

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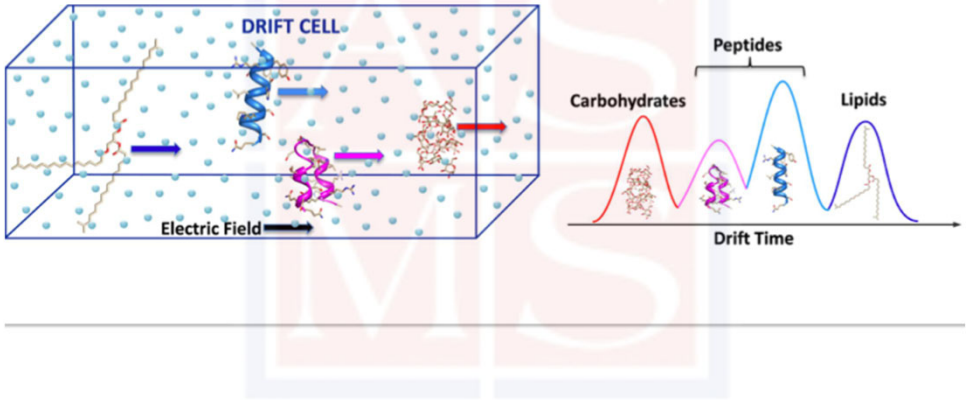
Ion Mobility Concept



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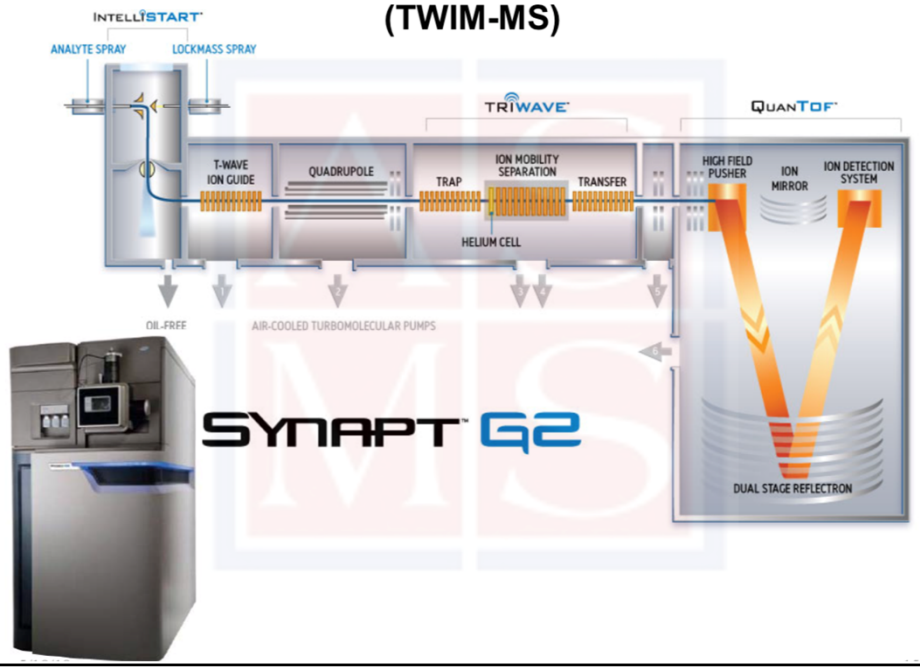
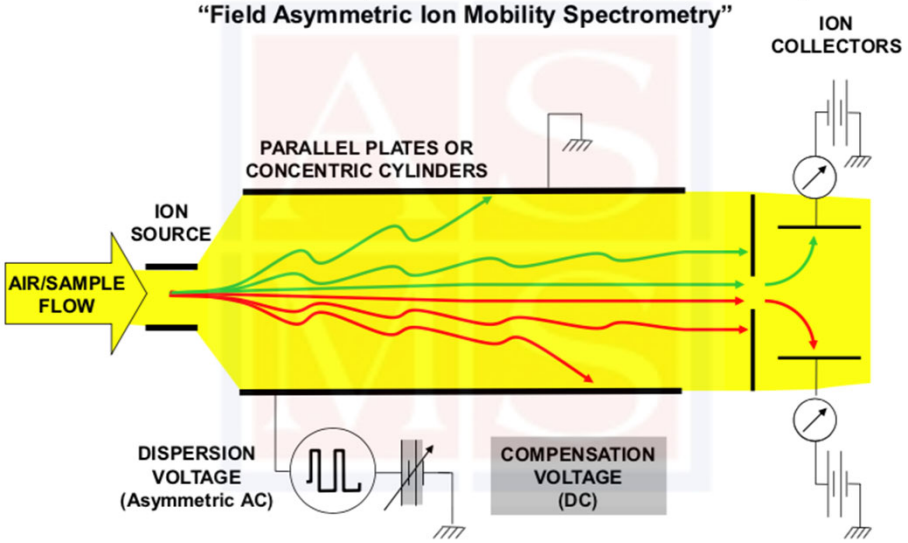
Electrodynamic time-dispersive IM-MS (TWIM-MS)



54

RF-DC Ion Mobility Spectrometry

"Field Ion Spectrometry" "Differential Mobility Spectrometry"
"Field Asymmetric Ion Mobility Spectrometry"



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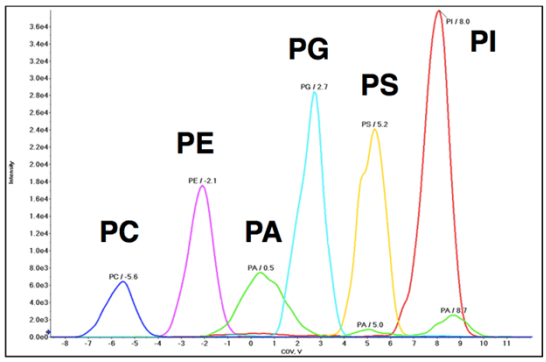
Ion mobility mass spectrometry

Another method of separating classes of compounds as well as compounds with the same molecular mass

Experiment: MRM scan of 6 phospholipid standards with COV ramp



This is a gas-phase separation of these phospholipids, i.e., no chromatography.

SCIEX use a differential mobility process.

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Other mass spectrometry applications

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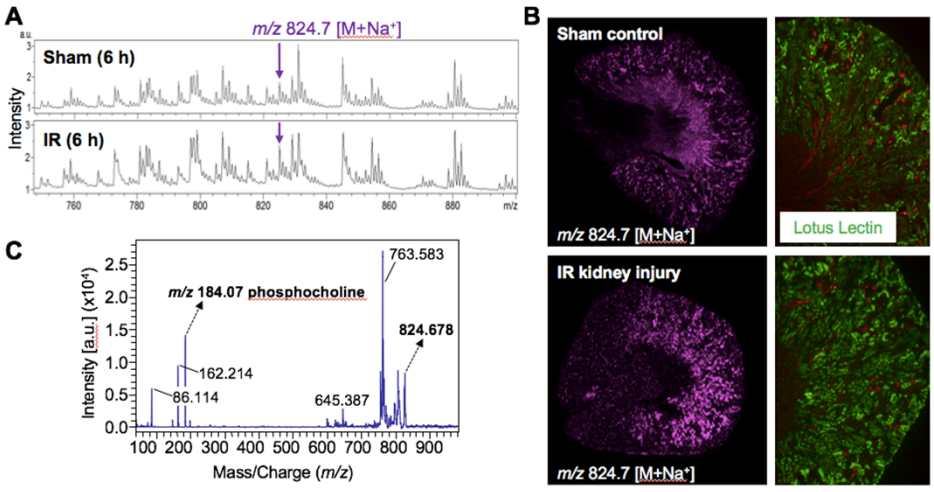
Imaging mass spectrometry



Generated by Janusz Kabarowski and Kelly Waters using MALDI-MS

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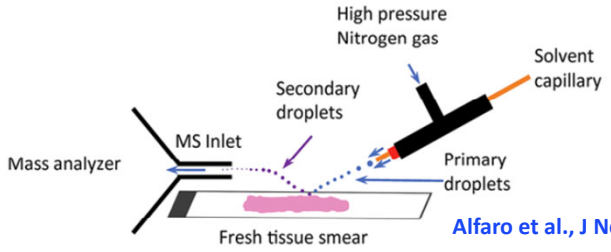
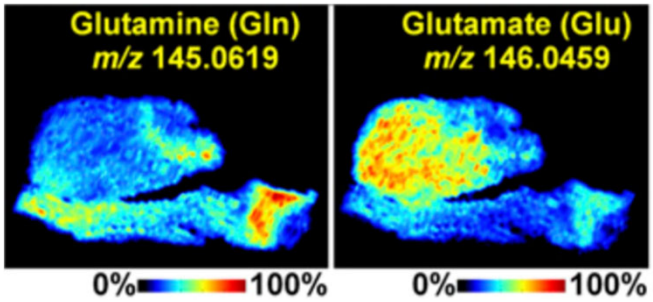
Desorption electrospray ionization (DESI)



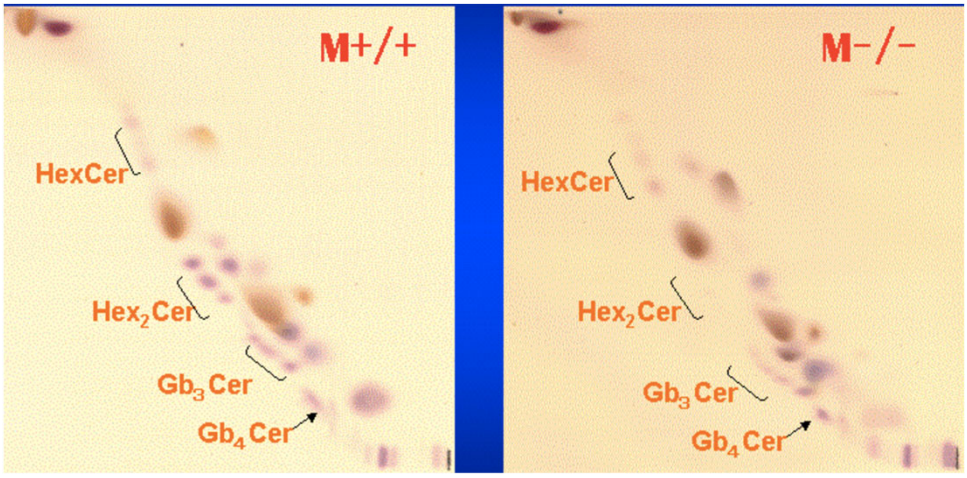
Alfaro et al., J Neurosurg 2019)



Sun et al., PNAS US 116:52, 2019

59

2D-Thin layer chromatography of lipids
KO of cerebroside sulfatase in kidney



These days, TLC plates can be studied by direct electrospray ionization (DESI)

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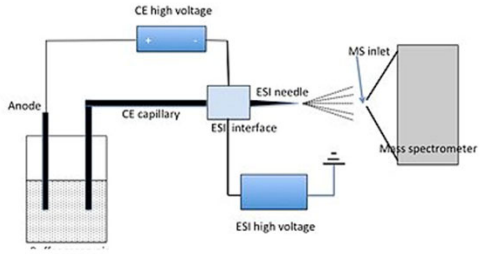
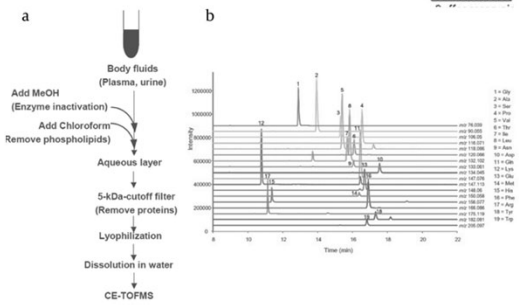
Capillary electrophoresis in metabolomics

- Capillary electrophoresis (CE-MS)





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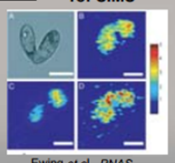
Single-cell MS for Metabolomics

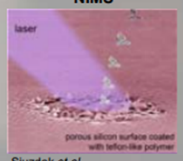
In Vacuo

Tof-SIMS



Ewing *et al.*, *PNAS* **2010**, 107, 2751

NIMS

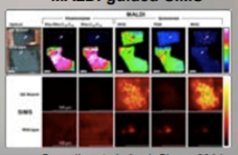


Siuzdak *et al.*, *Anal. Chem.*,**2011**, 83, 2

NAPA

Vertes, A. *et al., Phys., Chem., Chem., Phys.* **2011**,13, 9146

MALDI-guided SIMS

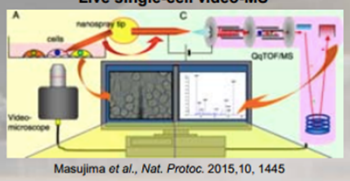


Sweedler *et al.*, Anal. Chem. **2014**, 86, 9139

At Ambient Conditions

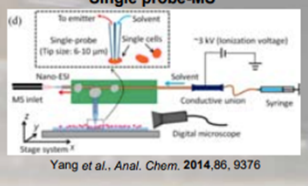
- Fast analysis
- *In situ* studies

Live single-cell video-MS



Masujima *et al., Nat. Protoc.* 2015,10, 1445

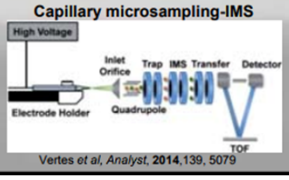
Single probe-MS



Yang *et al., Anal. Chem.* **2014**,86, 9376

- Reduce sample complexity
  - Differentiate isobaric ions
  - Aid identifications

Capillary microsampling-IMS



Vertes *et al, Analyst*, **2014**,139, 5079

- **CE-ESI-MS**
  - Minimize matrix effects
  - Aid Identifications

Courtesy of Peter Nemes

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Imaging metabolites in real time

- **In an ideal world, we want to measure metabolites without their degradation, spatially (preferably sub-cellularly) and with regard to time**
  - MS has high qualitative mass resolution and sensitivity, but it is destructive and not subcellular. Has poor time resolution
  - NMR is non-destructive and quantitative, but is not sensitive and not subcellular. Poor time resolution
- **Correlated anti-Stokes Raman Spectroscopy**
  - https://www.pnas.org/content/pnas/102/46/16807.full.pdf
  - Is nondestructive, has high sensitivity and spatial and time resolution, but poor qualitative resolution (distinguishing metabolites)

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Where is metabolomics headed?

- **Better LC chromatography**
  - Micropillar columns allow the use of much longer, highly reproducible columns
  - Suitable for Precision Medicine samples
    - Often single samples to be mapped against pooled “normal”samples
    - Can take time to get the best resolution
- **Perhaps no chromatography?**

Acoustic ejection mass spectrometry

SCIEX

Samples at 3/sec

https://sciex.com/content/SCIEX/na/us/en/products/integrated-solutions/Echo-ms.html

Lossless ion mobility
Path length extended from 24 cm to >10 m

https://availabletechnologies.pnnl.gov/technology.asp?id=396

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Calculating the mass of a metabolite

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Masses of elements and their isotopes

- Mass is defined using the mass of carbon-12 being 12.0000 (exactly) – the others have non-integer mass defects
- On this scale,
  - ^{1}H is 1.007825 and ^{2}H is 2.014102 (extra neutron)
  - ^{14}N is 14.003074 and ^{15}N is 15.000108 (extra neutron)
  - ^{16}O is 15.994915, ^{17}O is 16.999132 and ^{18}O is 17.999161
  - ^{31}P is 30.973761
  - ^{32}S is 31.972071 and ^{34}S is 33.967867 (4%)
- You can find the mass of every element and its isotopes and their natural abundances at

  http://www.nist.gov/pml/data/comp.cfm
- The mass of a proton is 1.0072766 and that of an electron is 0.000548597

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Predicted mass defects for $C_xH_nO_m$

	Oxygen atoms										
H atoms	O=0	O=1	O=2	O=3	O=4	O=5	O=6	O=7	O=8	O=9	O=10
1	0.0078	0.0027	-0.0023	-0.0074	-0.0125	-0.0176	-0.0227	-0.0278	-0.0329	-0.0379	-0.0430
2	0.0157	0.0106	0.0055	0.0004	-0.0047	-0.0098	-0.0149	-0.0199	-0.0250	-0.0301	-0.0352
3	0.0235	0.0184	0.0133	0.0082	0.0031	-0.0020	-0.0070	-0.0121	-0.0172	-0.0223	-0.0274
4	0.0313	0.0262	0.0211	0.0160	0.0110	0.0059	0.0008	-0.0043	-0.0094	-0.0145	-0.0196
5	0.0391	0.0340	0.0290	0.0239	0.0188	0.0137	0.0086	0.0035	-0.0016	-0.0066	-0.0117
6	0.0470	0.0419	0.0368	0.0317	0.0266	0.0215	0.0164	0.0114	0.0063	0.0012	-0.0039
7	0.0548	0.0497	0.0446	0.0395	0.0344	0.0294	0.0243	0.0192	0.0141	0.0090	0.0039
8	0.0626	0.0575	0.0524	0.0473	0.0423	0.0372	0.0321	0.0270	0.0219	0.0168	0.0117
9	0.0704	0.0653	0.0603	0.0552	0.0501	0.0450	0.0399	0.0348	0.0297	0.0247	0.0196
10	0.0783	0.0732	0.0681	0.0630	0.0579	0.0528	0.0477	0.0427	0.0376	0.0325	0.0274
11	0.0861	0.0810	0.0759	0.0708	0.0657	0.0607	0.0556	0.0505	0.0454	0.0403	0.0352
12	0.0939	0.0888	0.0837	0.0786	0.0736	0.0685	0.0634	0.0583	0.0532	0.0481	0.0430
13	0.1017	0.0966	0.0916	0.0865	0.0814	0.0763	0.0712	0.0661	0.0610	0.0560	0.0509
14	0.1096	0.1045	0.0994	0.0943	0.0892	0.0841	0.0790	0.0740	0.0689	0.0638	0.0587
15	0.1174	0.1123	0.1072	0.1021	0.0970	0.0920	0.0869	0.0818	0.0767	0.0716	0.0665
16	0.1252	0.1201	0.1150	0.1099	0.1049	0.0998	0.0947	0.0896	0.0845	0.0794	0.0743
17	0.1330	0.1279	0.1229	0.1178	0.1127	0.1076	0.1025	0.0974	0.0923	0.0873	0.0822
18	0.1409	0.1358	0.1307	0.1256	0.1205	0.1154	0.1103	0.1053	0.1002	0.0951	0.0900
19	0.1487	0.1436	0.1385	0.1334	0.1283	0.1233	0.1182	0.1131	0.1080	0.1029	0.0978
20	0.1565	0.1514	0.1463	0.1412	0.1362	0.1311	0.1260	0.1209	0.1158	0.1107	0.1057

For positively charged ions, add **1.007276** to the overall *m/z* value
For negatively charged ions, subtract **1.007276** from the overall *m/z* value

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Empirical formula

If the mass of an ion is known accurately enough, then it is possible to write down its empirical formula

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What is the neutral monoisotopic mass of a metabolite?

- **Hexanol**

$C_6H_{14}O$ = 6*12.0 + 14*1.007825 + 15.994915
= 102.1044651

- **Glucose**

$C_6H_{12}O_6$ = 6*12.0 + 12*1.007825 + 6*15.994915
= 180.063388

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Masses of genistein's ions

- **Genistein, $C_{15}H_{10}O_5$**

Mass = 15*12.0 + 10*1.007825 + 5*15.994915

$[M+H]^+$ = M + 1.00727638 = 271.060073

$[M-H]^-$ = M - 1.00727638 = 269.045547

- **If glucose is joined to genistein and water (H_2O) is eliminated, what are the values of the $[M+H]^+$ ion and the $[M-H]^-$ ion?**

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Determining the mass of bile acid-amino acid conjugates

- **Let's take cholic acid which has the empirical formula $C_{24}H_{40}O_5$**
- **For registered students,**
  - Calculate its neutral, monoisotopic mass, and the mass-to-charge ratio (*m/z*) of its $[M+H]^+$ molecular ion and its $[M-H]^-$ molecular ion
- **Cholic acid in the liver is converted to glycine ($C_2H_5NO_2$) and taurine ($C_2H_7NSO_3$) conjugates – hint they lose water during conjugation**
  - Determine the *m/z* values of their $[M+H]^+$ and $[M-H]^-$ molecular ions
- **BONUS – calculate the *m/z* values of the $[M+H]^+$ and $[M-H]^-$ molecular ions for cholic acid conjugated with leucine, phenylalanine and tyrosine**

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