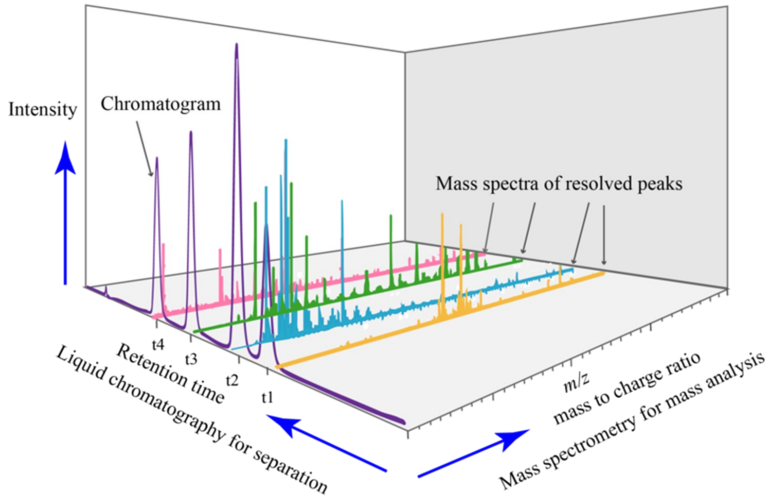


Part 1 : Mass spectrometry

Chapter 5 : Separation & Quantification

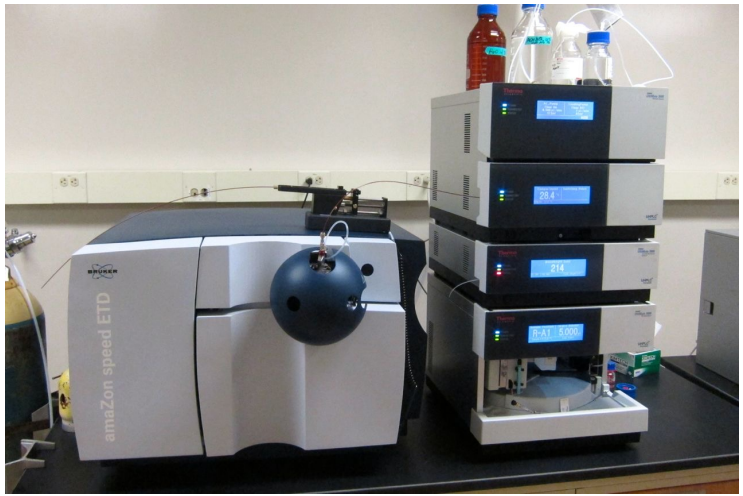
Dr MER Pascal Miéville
Assistant : Leander Choudhury

Separation & Quantification

Introduction

- The coupling of separation techniques with mass spectrometers has become a ubiquitous part of modern mass spectrometry.
  - Gas chromatography
  - Liquid chromatography
  - Electrophoresis
  - Ion mobility
- All can each be combined with a mass spectrometer to form an integrated system.
- These so-called **'hyphenated techniques'** can enable more selective and comprehensive analysis of complex mixtures,
- As well as provide a platform for quantitative analysis of individual analytes.

Why couple separation techniques to mass spectrometry?



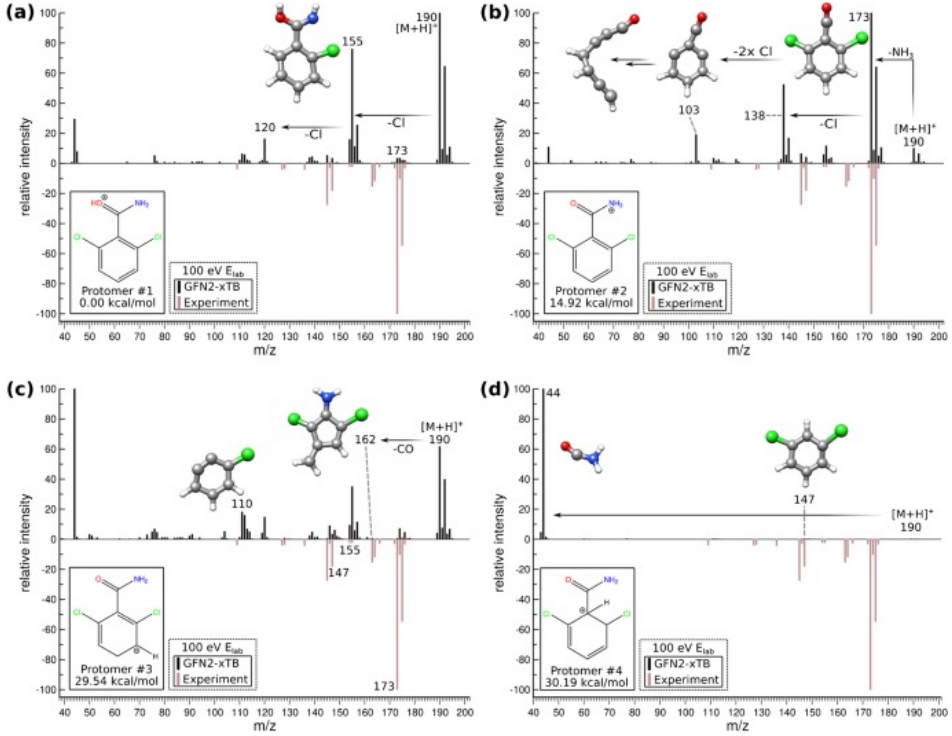
Why couple separation techniques to mass spectrometry?

- The signal response from a mass spectrometer is the **ion abundance (intensity) associated with specific m/z values**.
- This **ion abundance** is **related** to the **concentration of sample molecules or atoms being analysed.**
- But it is also affected by a number of additional variables:
  - The type of ionization source and ionization mechanisms
  - The molecular structure of the compound being ionized
  - The mass and charge state
  - Whether other compounds are present in the sample (Matrix effect)

Why couple separation techniques to mass spectrometry?

- **In addition, in analysis of complex mixtures**
  - Preotomics,
  - Genomics
  - Lipidomics,
  - Metabolomics
  - Forensic
  - Toxicology
  - Environnemtal analysis
  - Food analysis
  - Reaction mixture analysis
  - Kinetic analysis

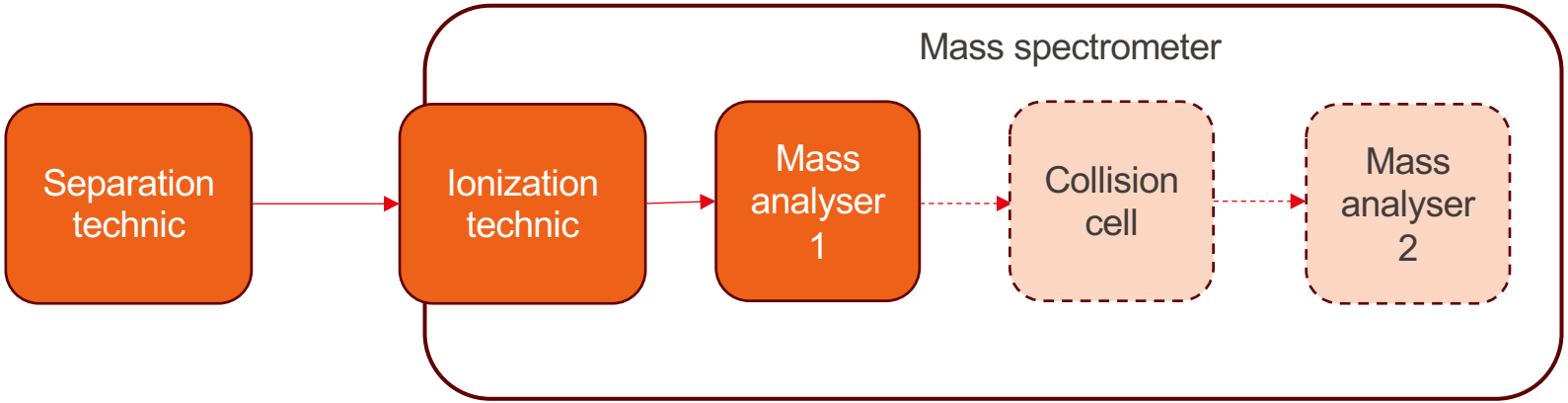
- **Molecules can be :**
  - Isobaric
  - Isomers
  - Isotopologues
  - Protomers



Fu, D., Habtegabir, S.G., Wang, H. *et al.* Understanding of protomers/deprotomers by combining mass spectrometry and computation. *Anal Bioanal Chem* **415**, 3847–3862 (2023). https://doi.org/10.1007/s00216-023-04574-1

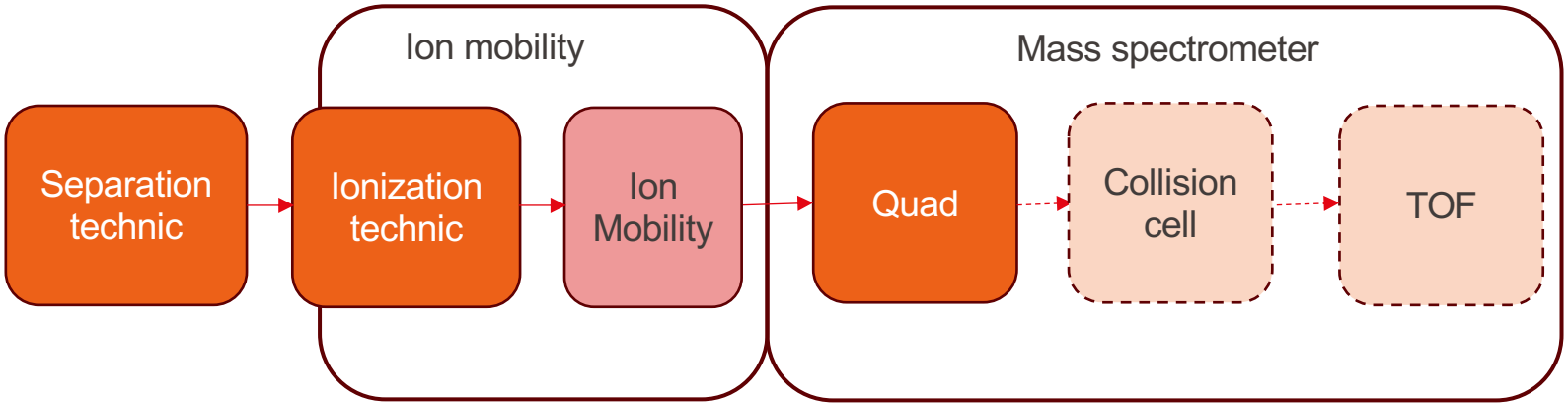
Why couple separation techniques to mass spectrometry?

- **Coupling separation technics to MS :**
  - Introduces a supplementary separation/purification dimension
    => improve mixture analysis
  - Introduce a control through calibration on the amount of injected molecules
    => improve quantification



Why couple separation techniques to mass spectrometry?

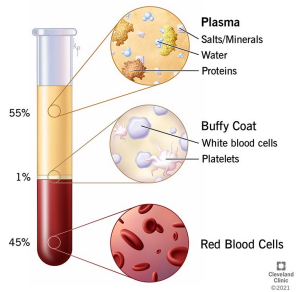
- **Couplings can be more complex** with complementary separation methods such as **Ion Mobility (IM)**
- IM provides information about **molecular volume** or **collisional cross section (CCS, Å²)**



Why couple separation techniques to mass spectrometry?

Matrix effects and ion suppression

- **Another good reason** to couple chromatography with MS is **to limit matrix effects and ions suppressions due to ions interferences**.
- **Common in complex mixtures analysis.**
- **They can also be caused by vials (plasticizers) components.**



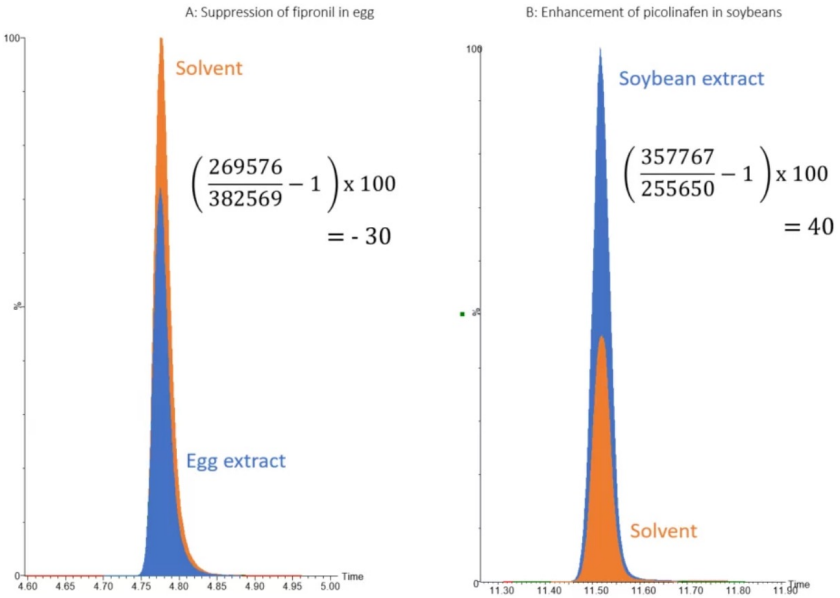
Why couple separation techniques to mass spectrometry?

Matrix effects and ion suppression

- **Matrix effects often reduce (but can occasionally enhance) ionization efficiency**.
- When the analyte signal is attenuated, this is often referred to **as 'ion suppression**'
- **Ion suppression has negative effect** on :
  - sensitivity
  - quantitativity
- **The mechanism of ion suppression is not well understood and complex**, but is associated with
  - samples of increased chemical complexity,
  - basicity or acidity,
  - the concentration of the analyte,
  - the presence of non-volatile compounds, in particular, salts.

Why couple separation techniques to mass spectrometry?

Matrix effects and ion suppression

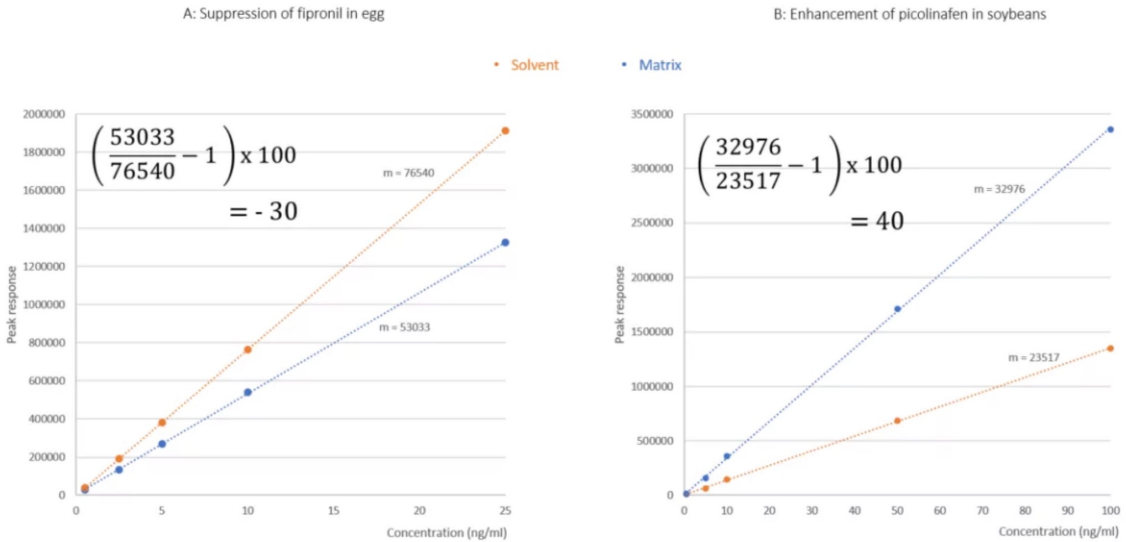


Chromatographic peaks overlaid for 5ng/ml spiked in solvent (orange) and food matrices (blue) of A. fipronil and B. picolinafen, showing 30% suppression of fipronil by egg matrix and 40% enhancement of picolinafen in soybean, respectively

https://www.waters.com/blog/understanding-sample-complexity-determining-matrix-effects-in-complex-food-samples

Why couple separation techniques to mass spectrometry?

Matrix effects and ion suppression



Overlay of calibration series in solvent and food matrices for A. fipronil and B. picolinafen, showing matrix suppression (31%) and enhancement (40%), respectively

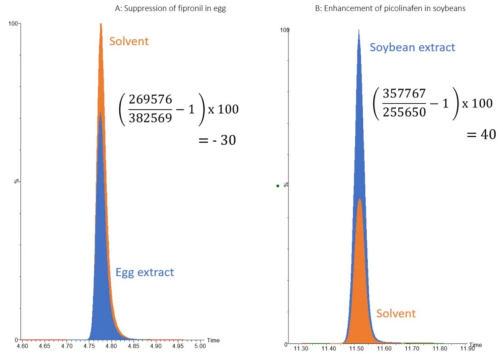
https://www.waters.com/blog/understanding-sample-complexity-determining-matrix-effects-in-complex-food-samples

Why couple separation techniques to mass spectrometry?

Matrix effects and ion suppression

Matrix Effect by Peak Area Comparison (Direct Method)

$$ME_{Area}(\%) = \left(\frac{Area\ Peak_B}{Area\ Peak_A} - 1\right) \cdot 100$$



Chromatogrpahic peaks overlaid for 5ng/ml spiked in solvent (orange) and food matrices (blue) of A. fipronil and B. picoliafen, showing 30% suppression of fipronil by egg matrix and 40% enhancement of picolinafen in soybean extract respectively.

- **Peak B : Post-extraction matrix -** Blank matrix extracted → spiked with analyte after extraction
- **Peak A : Pure solvent** - Analyte standard prepared in neat solution (no matrix)

Interpretation:
- ME ≈ 0 → no matrix effect
- ME < 1%→ ion suppression
- ME > 1% → ion enhancement

Pros:
- Quick and easy
- Good for screening ionization issues

Limitations:
- Gives information at one concentration point only
- Does not capture calibration curve behavior (non-linearity)

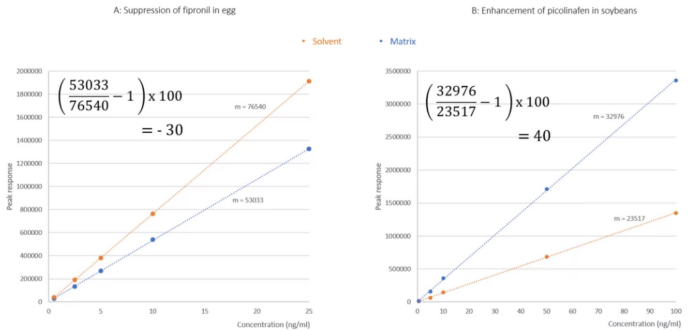
Matuszewski et al., 2003, Anal. Chem. 75, 3019
https://www.waters.com/blog/understanding-sample-complexity-determining-matrix-effects-in-complex-food-samples

Why couple separation techniques to mass spectrometry?

Matrix effects and ion suppression

Matrix Effect by Peak Area Comparison (Direct Method)

$$ME_{slope}(\%) = \left(\frac{Slope\ Calibration.Line_B}{Slope\ Calibration.Line_A} - 1\right) \cdot 100$$



Overlay of calibration series in solvent and food matrices for A. fipronil and B. picolinafen, showing matrix suppression (30%) and enhancement (40%), respectively.

- **Calibration.Line B : Post-extraction matrix -** Blank matrix extracted → spiked with different analyte concentration after extraction
- **Calibration.Line A : Pure solvent** – Various concentration of Analyte standard prepared in neat solution (no matrix)

Interpretation:
Same as for area

Pros:
- Recommended by FDA, official agencies
- Reflects matrix effect across the whole dynamic range
- Accounts for potential non-linearity
- More representative for quantitative work

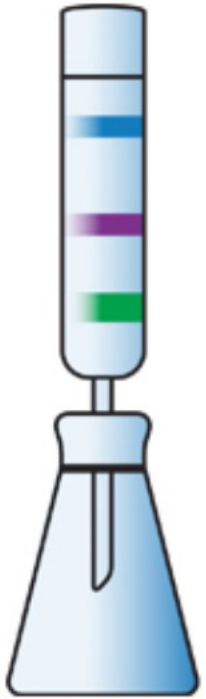
Limitations:
- Work intensive

Matuszewski et al., 2003, Anal. Chem. 75, 3019
https://www.waters.com/blog/understanding-sample-complexity-determining-matrix-effects-in-complex-food-samples

Chromatography coupled to mass spectrometry

Chromatography coupled to mass spectrometry

- **As discussed before – coupling separation technics with MS bring multiple advantages.**
- **Chromatography is one of the most common separation technic.**
  - 1) Measurement of time-resolved mass spectra for multiple compounds in a sample.
  - 2) A straightforward/automatable approach to introduce samples into a mass spectrometer.
  - 3) A way to analyse complex mixtures by reducing matrix effects and ion suppression.
  - 4) A technique to distinguish structural and stereo isomers.
  - 5) A method to combine relative and absolute quantitation with analytical mass spectrometry.



Chromatography coupled to mass spectrometry

Terminology alert

There is a lack of consensus in the literature on the appropriate way to indicate coupling of chromatography and mass spectrometry. Should it be LC/MS, LC-MS, LCMS, or LC MS?

Exactly the same question can be asked of gas chromatography coupled to mass spectrometry. All these acronyms can be found in contemporary scientific literature, and all refer to the same thing: the coupling of mass spectrometry and the particular form of chromatography indicated.

***As in the reference book, we use the hyphenated form (LC-MS and GC-MS)** in accordance with 2013 IUPAC recommendations and commensurate with the often-used term 'hyphenated technique'.*

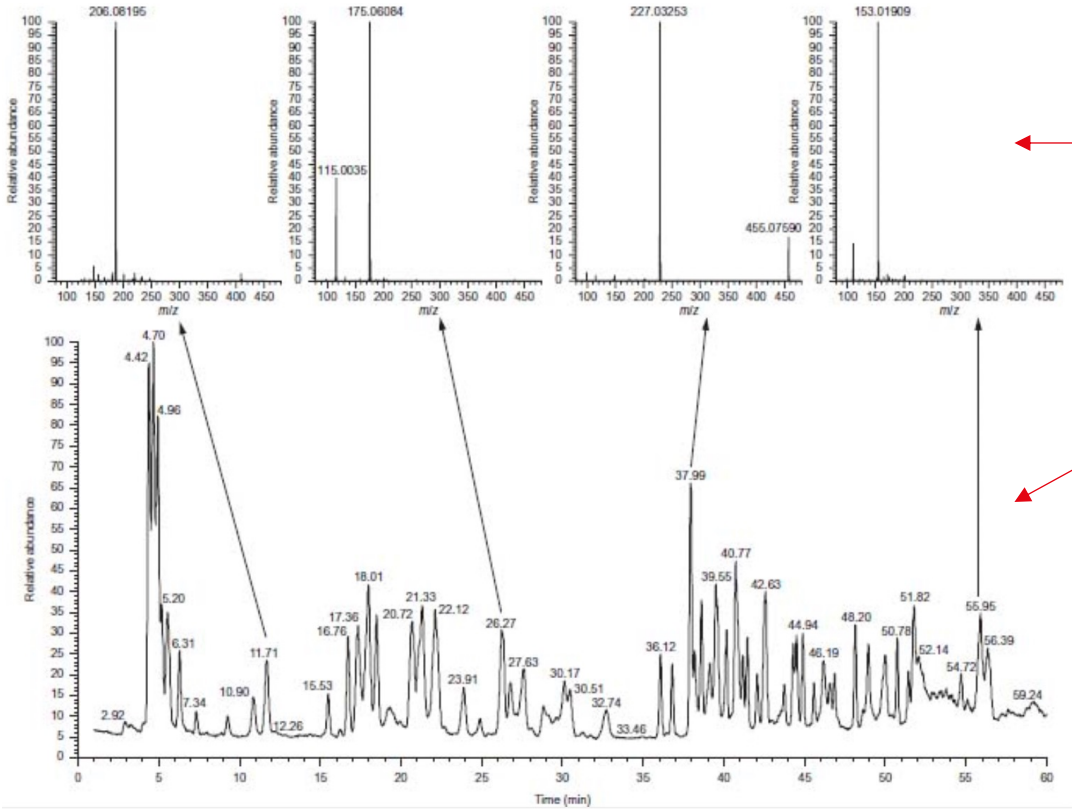
Chromatography coupled to mass spectrometry

Time-resolved mass spectra

- When chromatography is coupled to mass spectrometry, the data collected **have an additional time-dimension.**
- **Chromatographic separation of analytes is a much slower process than subsequent analysis by mass spectrometry.**
- Instead of a static mass spectrum being produced across the data acquisition period (as is the case for analysis of a sample directly infused into an ion source), chromatographic coupling leads to a **dynamic series of spectra where the presence of ions and their abundance changes with time.**
- **For every 'scan' or 'packet' of ions** processed by the mass analyser**, a spectrum is recorded,** and there can be many scans, across a designated mass range, per second depending on the **mass analyser duty-cycle**
- **Mass spectra therefore record the changes in the abundance of ions over time as they elute from the chromatograph.**

Chromatography coupled to mass spectrometry

Time-resolved mass spectra



Extracted MS spectra

TIC

A total ion chromatogram (TIC) showing the change in total ion abundance (vertical axis) over the duration of the 60-minute (horizontal axis) LC-MS run.

Chromatography coupled to mass spectrometry

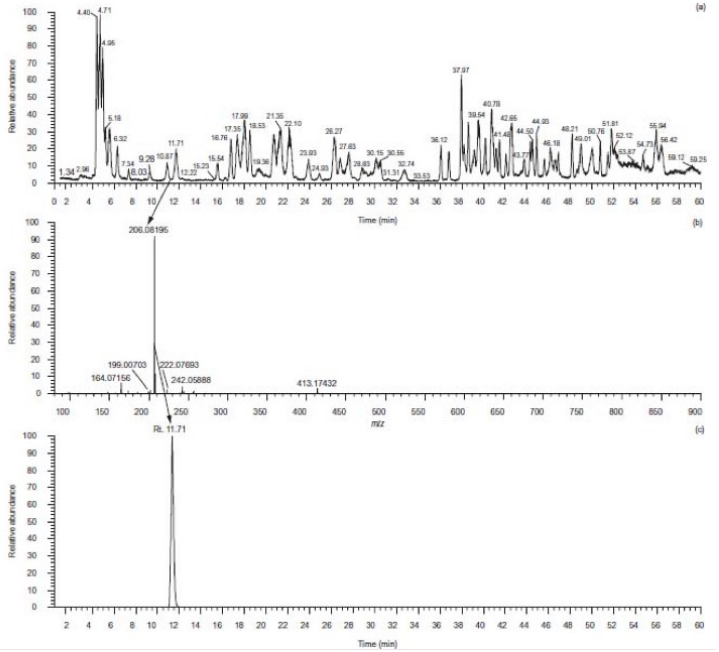
Time-resolved mass spectra

TIC & EIC

- Mass spectra and total ion chromatograms are simply **two ways of displaying the ion abundance data collected by the mass spectrometer over time**.

- **It is possible to interconvert** between a mass spectrum and a mass chromatogram display.
  - A **mass spectrum** can be generated at any point in time from the total ion chromatogram **(TIC)**,
  - Conversely an **extracted ion chromatogram (EIC)** can be generated for a specific m/z value or wider m/z range.

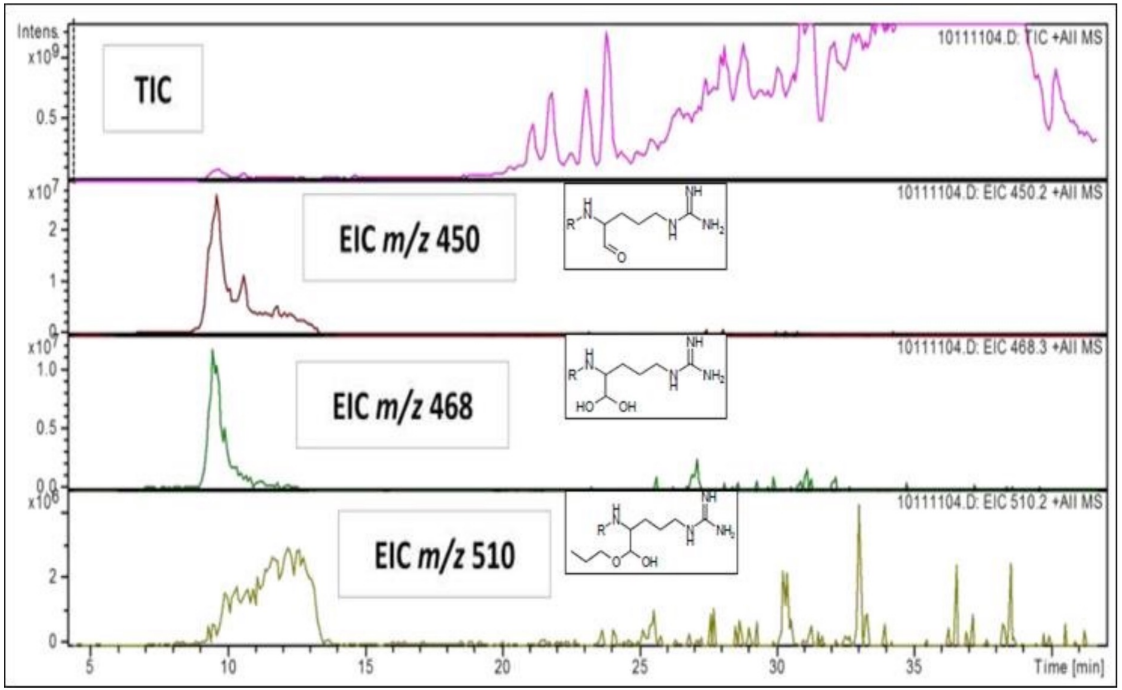
- The EIC for a particular m/z value is created by plotting its ion abundance as a function of retention time.



(a) shows TIC for the LC-MS analysis of a complex mixture of compounds, (b) shows the extracted mass spectrum at retention time (Rt) 11.71 min where a compound elutes represented by ions at *m/z* value of 206.08195, and (c) shows the EIC associated with the *m/z* value of 206.08195 (5 ppm mass accuracy).

Chromatography coupled to mass spectrometry

Time-resolved mass spectra



Total ion chromatogram (**TIC**) and extracted ion chromatogram (**EIC**) of the protonated [MH +] new peptide aldehyde compound (450.2 m/z), [M + H2O + H +] (468 m/z) and [M + IPA + H +] (510.2 m/z). The x-axis represents retention time (min), and the y-axis represents signal intensity. Intensity is measured in counts per second (cps). N15 labeling experiment was performed to confirm the subunit structure of the new peptide aldehyde compound. By comparing the LC-MS results of the labeled with that of the unlabeled extract of the ASN biomass, a 5-Dalton shift was observed, and 5 nitrogen

Nowruzi, Bahareh & Sattari, Taher & Jokela, Juoni. (2019). A Report on Finding a New Peptide Aldehyde from Cyanobacterium Nostoc sp. Bahar M by LC-MS and Marfey's Analysis. Iranian Journal of Biotechnology. 17. 71-78. 10.21859/ijb.1853.

Chromatography coupled to mass spectrometry

Time-resolved mass spectra

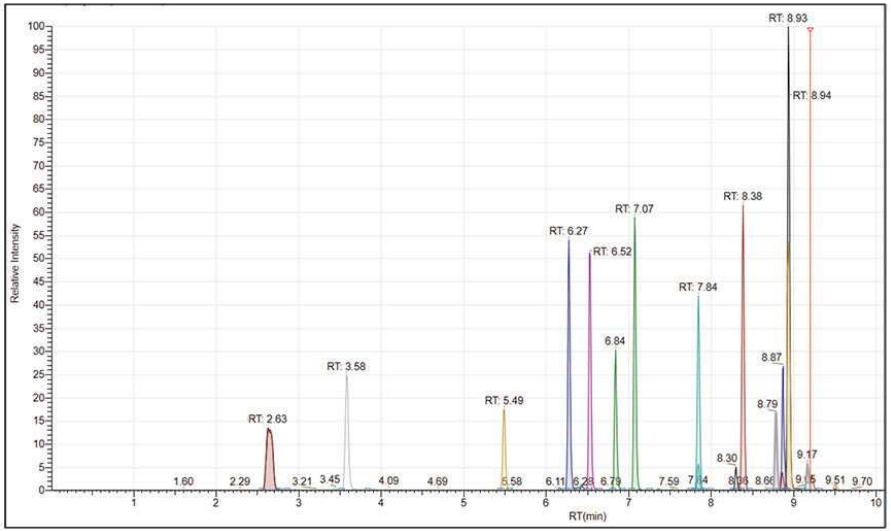
Terminology alert

- In a **mass spectrum the peaks are referred to as mass spectral peaks**,
- In a **chromatogram the peaks are referred to as chromatographic peaks**.
- Both types of peak are separate entities that represent quite different information.
  - MS peaks : Number of ions per m/z
  - Chromato peaks : Number of ions per RT

=> be careful to be precise

Chromatography coupled to mass spectrometry

Chromatographic peak area and concentration



Relative intensity (a.u.)

- Analyte concentration
- Separation efficiency
- Flow splitting
  - Ionization efficiency → Analyser sensitivity
  - Molecular sensitivity → Analyser sensitivity

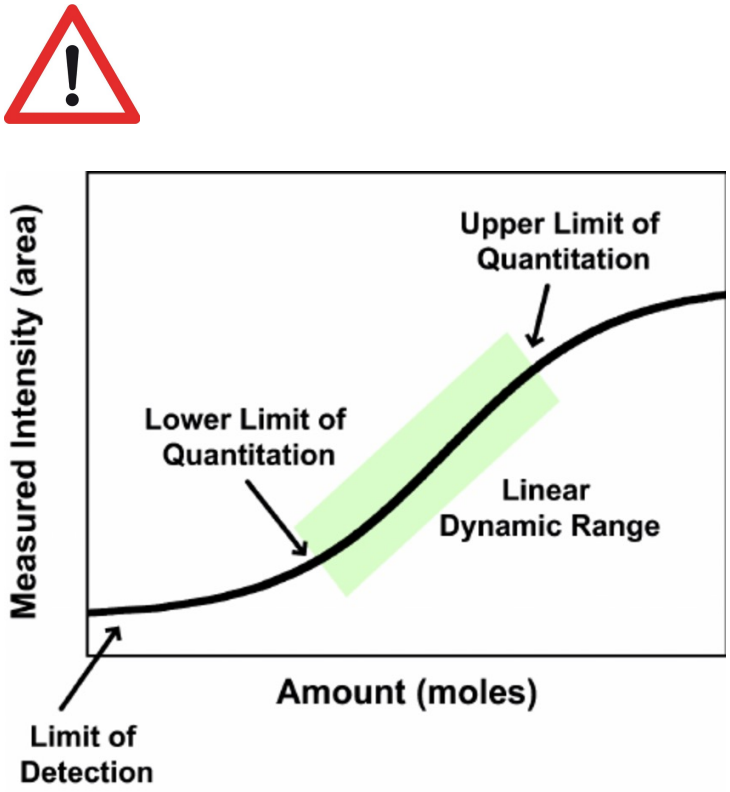
Retention time (RT)

- Injection time
- Elution time
- Transfer time
- MS analysis time

Chromatography coupled to mass spectrometry

Chromatographic peak area and concentration

- **Important to note** that chromatographic **peak area alone does not tell us the absolute amount of an analyte present**; the peak area **is not in concentration units.**
  - **Area comparison is valid** for relative intensities of same m/z
  - **Absolute quantification** requires **an external or internal calibration**
  - In both cases, the concentration must be in the linear region of the mass analyser
    - **Above LOQ (LLOQ)**
    - **Below saturation (ULOQ)**

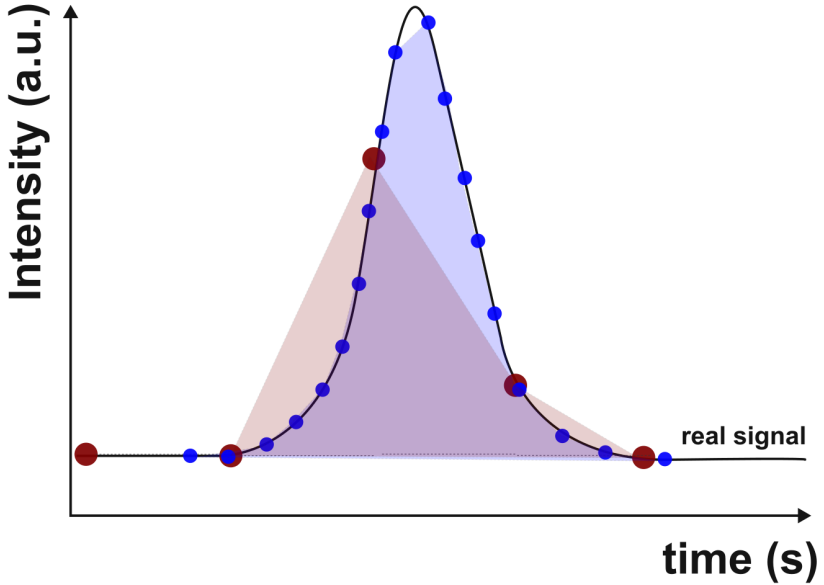


Kristjansdottir, Kolbrun & Takahashi, Satoe & Volchenboum, Samuel & Kron, Stephen. (2012). Strategies and Challenges in Measuring Protein Abundance Using Stable Isotope Labeling and Tandem Mass Spectrometry. 10.5772/33421.

Chromatography coupled to mass spectrometry

Scan speed, mass range, and sensitivity

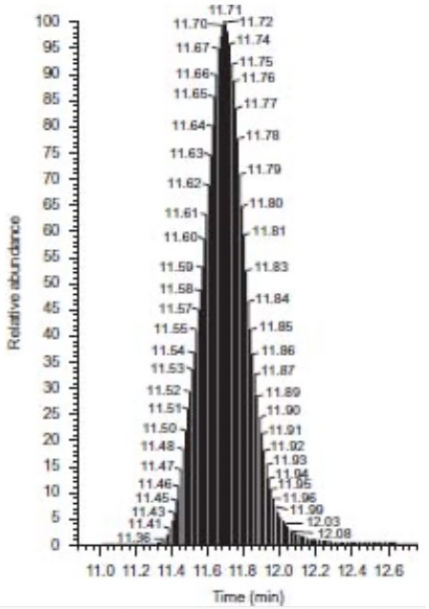
- The fact **that a mass spectrometer has a finite scan time** means that there are only a **finite number of data points per chromatographic peak.**
- Usually, a **minimum of 10 data points** are required to ensure that the chromatographic peak can be produced accurately.
- Note that for a fixed scan time the chromatographic peak width will have a direct impact on the number of data points collected.



Chromatography coupled to mass spectrometry

Scan speed, mass range, and sensitivity

Chromatographic peak width (s)	Necessary acquisition rate for quantification (Hz)
1	10 (10 points per peak)
0.5	20
0.2	50
0.1	100
0.05	200



Analyser type	Typical scan per second
Magnetic sector	0.5–2
Quadrupole	0.1–1
Quadrupole ion trap	0.01–1
Linear ion trap	0.01–1
Time-of-flight	0.1
Orbitrap FTMS	0.1–1
FT-ICR-MS	0.5–10

?

Be careful with table 7.1 in the book ! Given values are outdated (wrong ?) and do not reflect the current machine capabilities :
e.g. TOF 50-200 Hz (scans/s)
Orbitraps 1-15 Hz

Chromatography coupled to mass spectrometry

Scan speed, mass range, and sensitivity - low to high resolution - updated acquisition frequency range

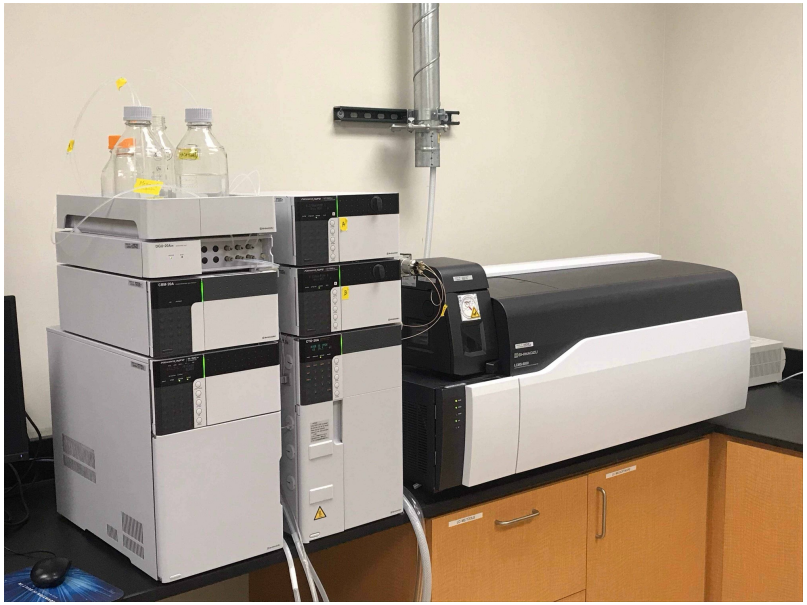
Mass Analyzer	Typical Scan Speed	Resolution / Accuracy	Advantages	Typical Applications
Quadrupole (SQ and TQ)	~10,000–30,000 Da/s (~100–500 ms for full scan) **2-10 spectra/s (2-10 Hz)** **Much faster in SIM/MRM mode**	Unit resolution	Fast, robust, inexpensive, **good for targeted SIM, SRM, MRM**	Routine quantitation, LC–MS/MS for drugs, metabolites, environmental analysis
Time-of-Flight (TOF)	**50–200 Hz**	High resolution (10k–60k)	Very fast, parallel detection, wide m/z range	LC–TOF profiling, high-throughput omics, MALDI imaging
Ion Trap (3D / Linear)	~10,000–40,000 Da/s (**a few Hz in MS/MS mode**)	Unit to moderate resolution	MS^n capability (multi-stage fragmentation), sensitive	Structural elucidation, proteomics (older platforms), method development

Chromatography coupled to mass spectrometry

Scan speed, mass range, and sensitivity – ultrahigh resolution

Mass Analyzer	Typical Scan Speed	Resolution / Accuracy	Advantages	Typical Applications
Orbitrap	1–15 spectra/s (depends on resolution: **15k ≈ 15 Hz, 240k ≈ 1 Hz**)	Very high resolution (up to 500k), ppm accuracy	Excellent resolution & accuracy, good dynamic range	Proteomics, metabolomics, complex mixture analysis
FT-ICR	0.2–2 spectra/s (long transient acquisition) **0.2-2 Hz**	Ultra-high resolution (>1,000,000), sub-ppm	Highest resolving power and accuracy, ideal for complex mixtures	Top-down proteomics, petroleomics, high-precision isotope analysis

Liquid chromatography mass spectrometry (LC-MS)



Liquid chromatography mass spectrometry (LC-MS)

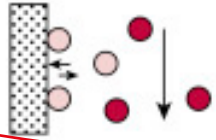
Ion dissociation

- **LC-MS is now the most common** hyphenated mass spectrometry technique.
- Historically, it **was difficult to interface efficiently**.
- One of the **major hurdles was to couple the LC system directly with the ionization source** of the mass spectrometer.
- This was **problematic for high vacuum sources** because it required **continuous removal of large volumes of eluting solvent**.
- The advent of **atmospheric pressure ionization (API)** sources in the 1980s and 1990s and the development of the **ESI in the 1990s - 2000s** made it possible.
- In particular there are two aspects of electrospray ionization (ESI) that made it an ideal ionization source for LC-MS.
  - **Rapid evaporation of the solvent** is an integral part of the ionization process,
  - **Gas-phase charged molecules are formed from non-volatile compounds at atmospheric pressure.**

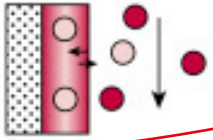
Liquid chromatography mass spectrometry (LC-MS)

Liquid Chromatography - mechanisms

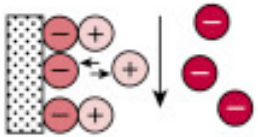
Adsorption chromatography
Separation based on adsorption of chemicals to the surface of a support



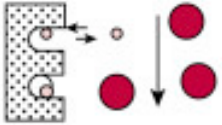
Partition chromatography
Separation based on partitioning of chemicals into a layer of the stationary phase



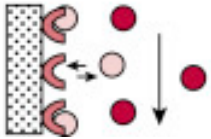
Ion-exchange chromatography
Separation of ions based on their binding to fixed charges on a support



Size-exclusion chromatography
Separation of chemicals based on their size and ability to enter a porous support



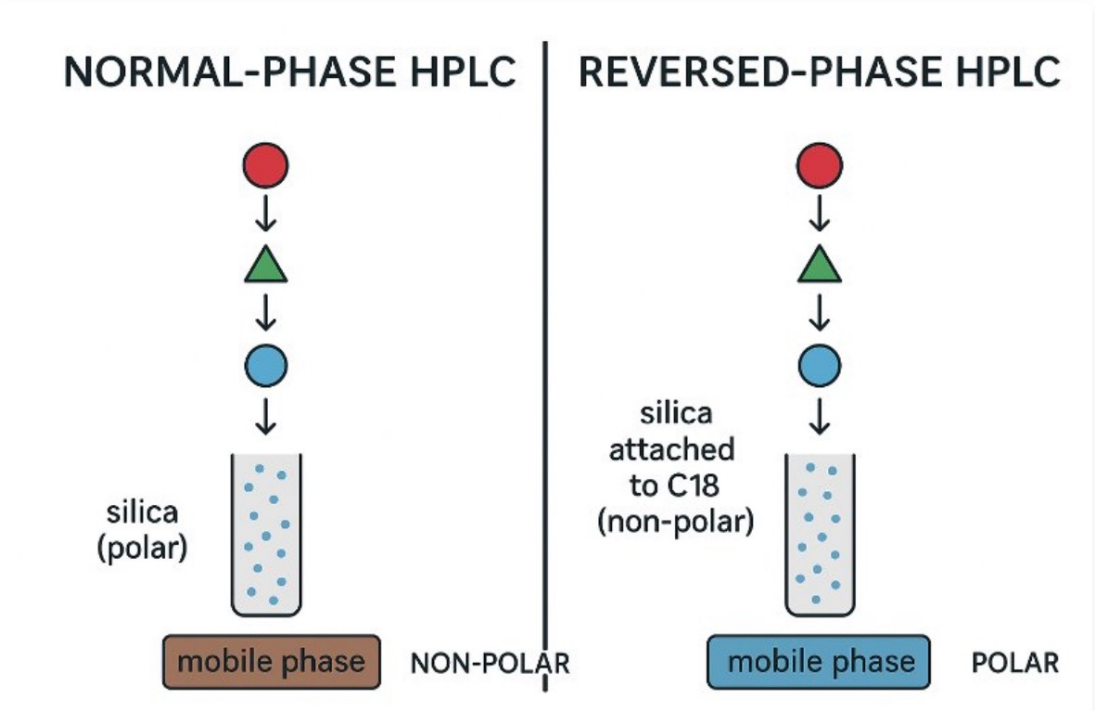
Affinity chromatography
Separation of chemicals based on their interactions with a biologically related binding agent



M. Kele, P.C. Iraneta, CHROMATOGRAPHY: LIQUID | Column Testing and Evaluation, Encyclopedia of Separation Science, Academic Press, 2007, https://doi.org/10.1016/B0-12-226770-2/00291-X.

Liquid chromatography mass spectrometry (LC-MS)

Liquid Chromatography – type of columns



https://hplccalculator.khemit.net/?tag=analyticalchemistry

Liquid chromatography mass spectrometry (LC-MS)

Liquid Chromatography – type of columns

Liquid chromatography separation mechanism	Acronym	Retention mechanism	Types of analyte	Suitability for MS detection
Reversed phase	RP-LC	Hydrophobic interactions	Medium to high polarity compounds	Very compatible
Ion exchange	IEC	Ionic interactions	Ionic and highly polar compounds	Not generally compatible unless ion suppression used
Ion pairing	IP-LC	Ion-pair formation and hydrophobic interactions	Medium to highly polar compounds	Use with caution IP reagents can lead to ion suppression and contamination
Hydrophilic interaction	HILIC	Hydrophilic and polar interactions	Medium to highly polar compounds	Very compatible
Mixed-mode	MM-LC	Hydrophobic, polar and ionic interactions	Low to highly polar compounds	Can be suitable depending on the pK_a of the embedded groups
Normal phase	NP-LC	Hydrophilic interactions	Low to medium polarity compounds	Not generally suitable

Liquid chromatography mass spectrometry (LC-MS)

Liquid Chromatography – configuration & performance

Liquid chromatography system	Abbrev.	Typical column ID (mm)	Typical flow rates (μL/min)
High-performance liquid chromatography	HPLC	2.1–4.6	100–1000
Ultra-high-performance liquid chromatography	UHPLC	1–2.1	100–400
Capillary liquid chromatography	Cap-LC	0.3–0.5	5–50
Nano-liquid chromatography	Nano-LC	0.1	< 1

The ionization source and the frequency of mass analysis must be adapted to the flow rate and the peaks duration.

Liquid chromatography mass spectrometry (LC-MS)

Liquid Chromatography – challenges and compromises

- **Mobile phase additives (e.g. trifluoroacetic acid TFA, in % range)** can have significant enhancing effect on the chromatographic retention time and peak shape for certain analytes**.**
  - TFA (or other additives) can **lead to large, non-analyte peaks in the MS spectrum.**
- **Inorganic buffers such as potassium phosphate** have traditionally been used in LC **to control solvent pH.**
  - but with ESI especially, these produce **large background signals**, **lead to ion suppression**, and can **coat the inside of the ion source with residue.**
- To avoid these effects, **in general only volatile buffers are recommended for LC-MS,** but these are not always as effective in controlling chromatographic conditions.

Liquid chromatography mass spectrometry (LC-MS)

Liquid Chromatography – challenges and compromises

- Common buffers and additives used in HPLC, their pH range, and their suitability for LC-MS.

Volatile buffer/additive	pH range	Suitability of LC-MS
Ammonium formate	2.7–4.7	Yes
Ammonium acetate	3.6–5.6	Yes
Acetic acid	4.3–5.3	Yes
Formic acid	2.7–4.7	Yes
Ammonium bicarbonate	6.5–8.5	Yes
Trifluoroacetic acid	2	Not recommended
Boric acid	8–10	Yes
Ammonia	11.6	Yes
Ammonium carbonate	6–7	Yes
Pyrrolidine	10.3–12.3	Yes

Liquid chromatography mass spectrometry (LC-MS)

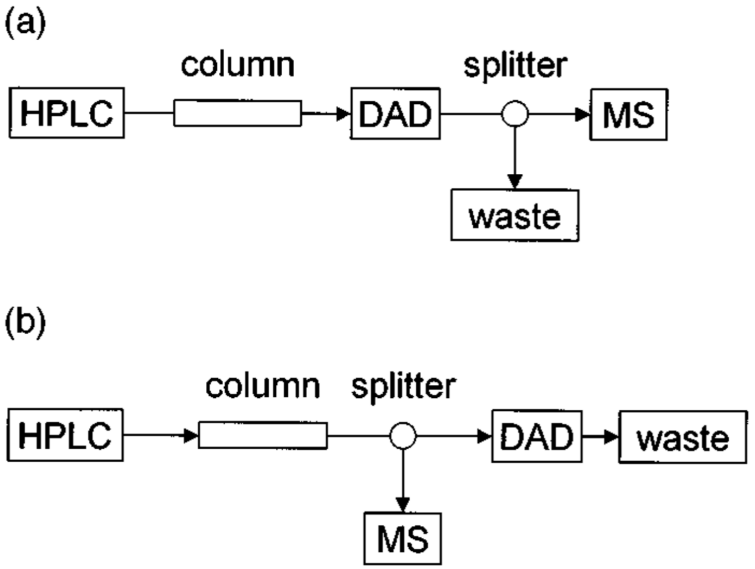
Liquid Chromatography – challenges and compromises

Pyrrolidine	10.3–12.3	Yes
Ammonium hydroxide	8.3–10.3	Yes
Ammonium bicarbonate	6.7–11.3	Yes
Triethylamine	9.7–11.7	Yes
Tributylamine	N/A	Yes
TRIS	7–9	No
Phosphoric acid	< 1	No
Phosphate (pK1)	1–3	No
Phosphate (pK2)	6–8	No
Phosphate (pK3)	11–13	No

Liquid chromatography mass spectrometry (LC-MS)

Liquid Chromatography – coupling with multiple detectors amd waste

- MS is highly sensitive and also destructive
- It is connected to the LC through a splitter that extract the required amount of eluant + analyte.



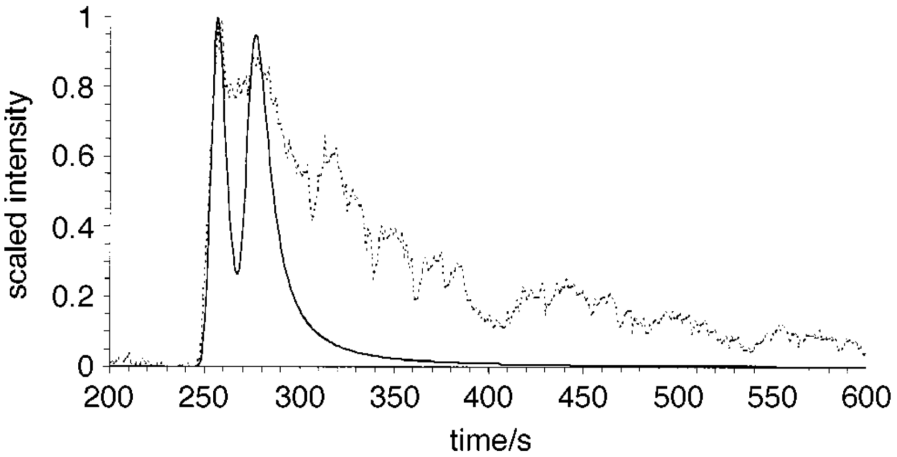
Coupling arrangements for the HPLC-DAD and the MS systems with the splitting device between (a) the DAD and the waste and (b) the column and the DAD

Dunkerley, Samantha & Crosby, John & Brereton, Richard & Zissis, Konstantinos & Escott, Richard. (1998). Chemometric analysis of high performance liquid chromatography diode array detection electrospray mass spectrometry of 2- and 3-hydroxypyridine. The Analyst. 123. 2021-33. 10.1039/A804345K.

Liquid chromatography mass spectrometry (LC-MS)

Liquid Chromatography – coupling with multiple detectors amd waste

- In coupling MS with other detectors e.g. DAD, ELSD, the respective flow pathway lengths must be taken in account and corrected in the software to ensure a corrected spectrum alignment.



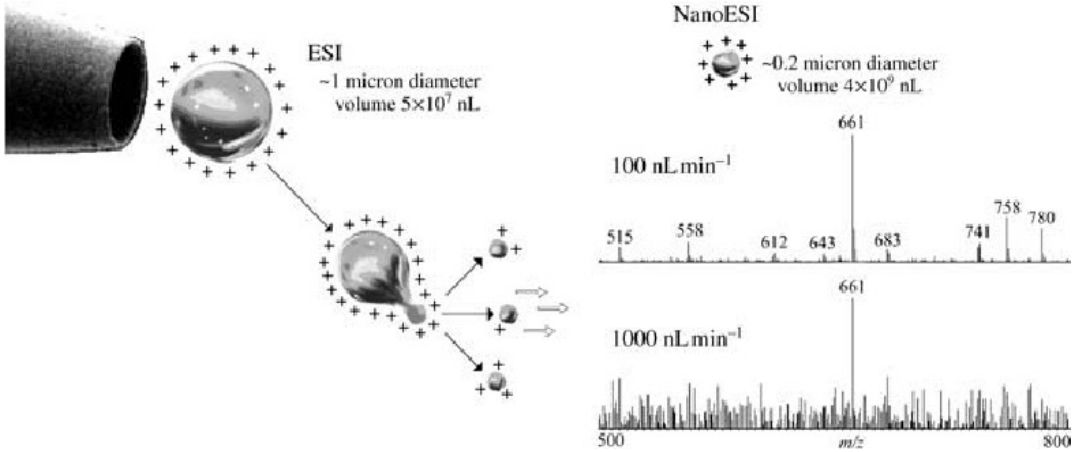
Comparison of the summed DAD profile (solid line) and the TIC for the 20 most significant masses (dotted line).

Dunkerley, Samantha & Crosby, John & Brereton, Richard & Zissis, Konstantinos & Escott, Richard. (1998). Chemometric analysis of high performance liquid chromatography diode array detection electrospray mass spectrometry of 2- and 3-hydroxypyridine. The Analyst. 123. 2021-33. 10.1039/A804345K.

Liquid chromatography mass spectrometry (LC-MS)

Liquid Chromatography – adaptation of the machine configuration to the analysis

- **Nano-electrospray (nano-ESI)** is one of the most efficient and sensitive ionization methods available for coupling with mass spectrometry.
- The small droplet size in nanoESI leads to less signal suppression and greater coverage of a solution's metabolites.
- Due to the low flow rates, narrow flow path, and micro-unions it is much slower to perform an equivalent chromatographic separation compared with HPLC or UHPLC, and is generally less robust for routine use.

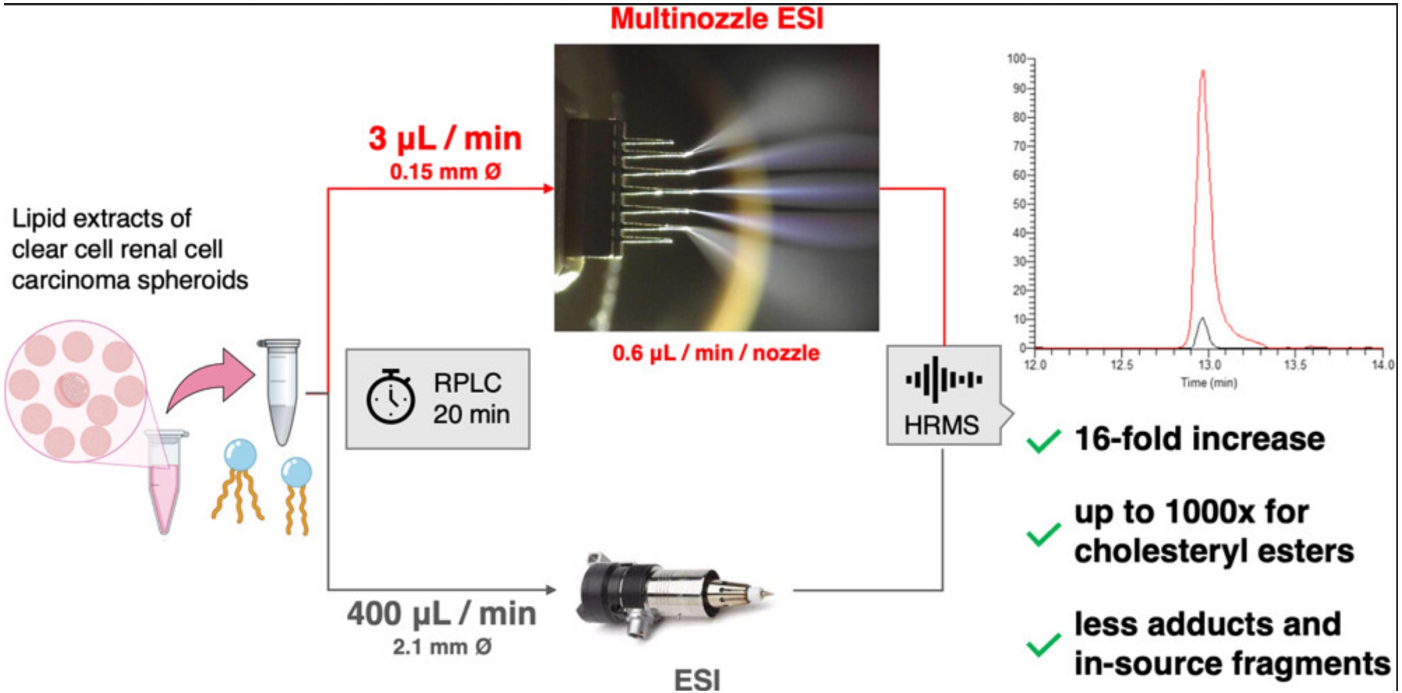


LC/MS spectra of methanol-extracted serum samples at flow rates of 100 and 1000 nL min À 1 show a significant improvement in the signal-to-noise ratio at the lower flow rate

Want, Elizabeth & Cravatt, Benjamin & Siuzdak, Gary. (2015). 112 art.

Liquid chromatography mass spectrometry (LC-MS)

Liquid Chromatography – adaptation of the machine configuration to the analysis

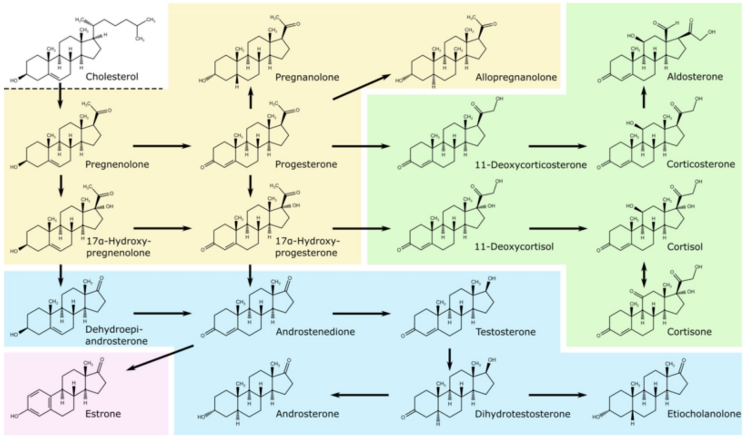


Sergey Girelm Mathis Fiault, Valentin Mieville, Patrycja, Nowak-Sliwinska, Serge Rudaz, Isabel Meister, Anal. Chem. 2025, 97, 9, 5109–5117
https://pubs.acs.org/doi/10.1021/acs.analchem.4c06337

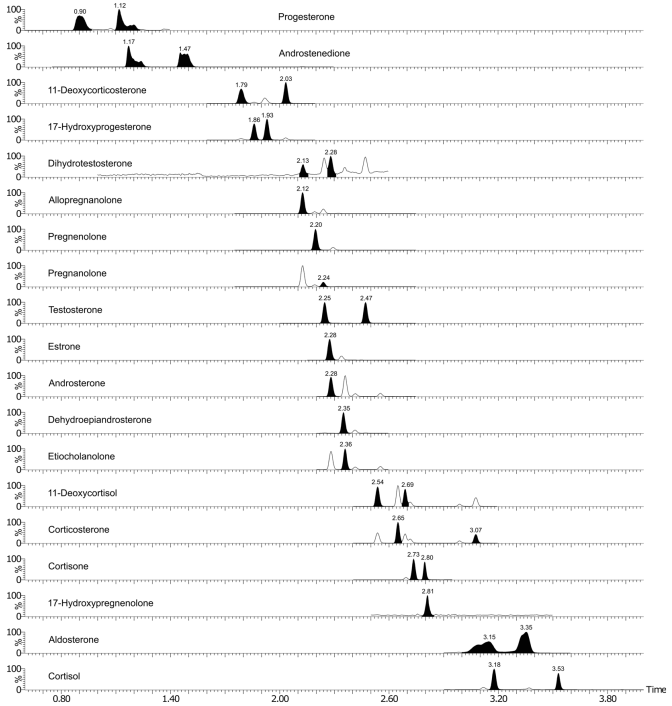
Liquid chromatography mass spectrometry (LC-MS)

Liquid Chromatography – Structure elucidation in complex mixtures

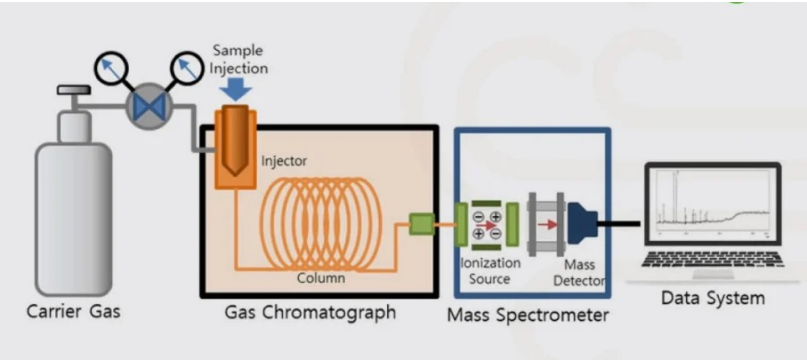
- **Couple LC with HR-MS**



de Kock, N., Acharya, S.R., Ubhayasekera, S.J.K.A. *et al.* A Novel Targeted Analysis of Peripheral Steroids by Ultra-Performance Supercritical Fluid Chromatography Hyphenated to Tandem Mass Spectrometry. *Sci Rep* **8**, 16993 (2018). https://doi.org/10.1038/s41598-018-35007-0



Gas chromatography mass spectrometry (GC-MS)



Gas chromatography mass spectrometry (GC-MS)

General principle

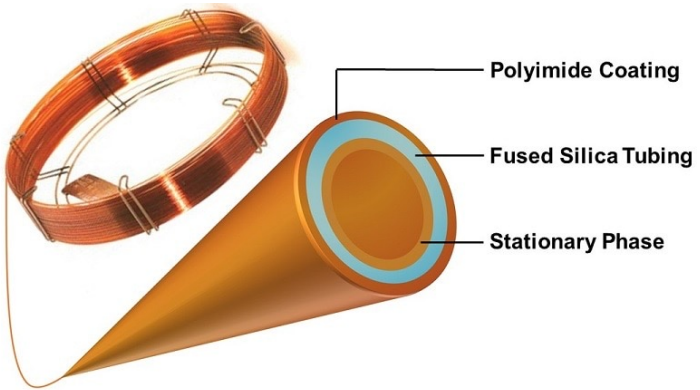
- **Gas chromatography** was first coupled with mass spectrometry (**GC-MS**) in the 1960s.
- In GC the mobile phase is a gas (usually helium, but sometimes nitrogen or and more and more hydrogen).
- There are **three main types of interaction** that take place leading to separation of analytes:
  - dispersion interactions (van der Waals),
  - permanent dipole–dipole interactions,
  - hydrogen bonding (where applicable).
- All three can play a role in the retention of molecules on a GC column.

GC Carlo Erba (1990)

Gas chromatography mass spectrometry (GC-MS)

Ion dissociation

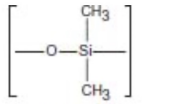
- **Modern GC-MS is performed using capillary columns** that have **polysiloxanes** bonded to the inside surface, and these tend to have high separation efficiencies.
- Retention on a **GC column works on the principle that stationary phase functional groups are of a similar polarity to the analytes** they retain.
- By definition, gas-phase analytes tend to be relatively **small and non-polar molecules**.



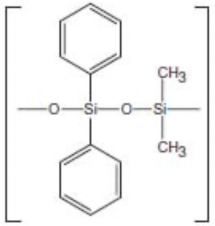
Gas chromatography mass spectrometry (GC-MS)

Ion dissociation

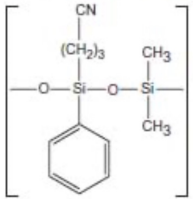
GC stationary phase	Polarity
Dimethylpolysiloxane	+
Diphenyl dimethylpolysiloxane	++
Cyanopropylphenyl dimethylpolysiloxane	+++
Trifluoropropyldimethylpolysiloxane	++++
Polyethylene glycol (PEG) or Wax	+++++



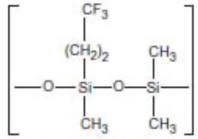
Dimethylpolysiloxane



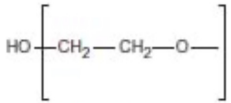
Diphenyl dimethylpolysiloxane



Cyanopropylphenyl dimethylpolysiloxane



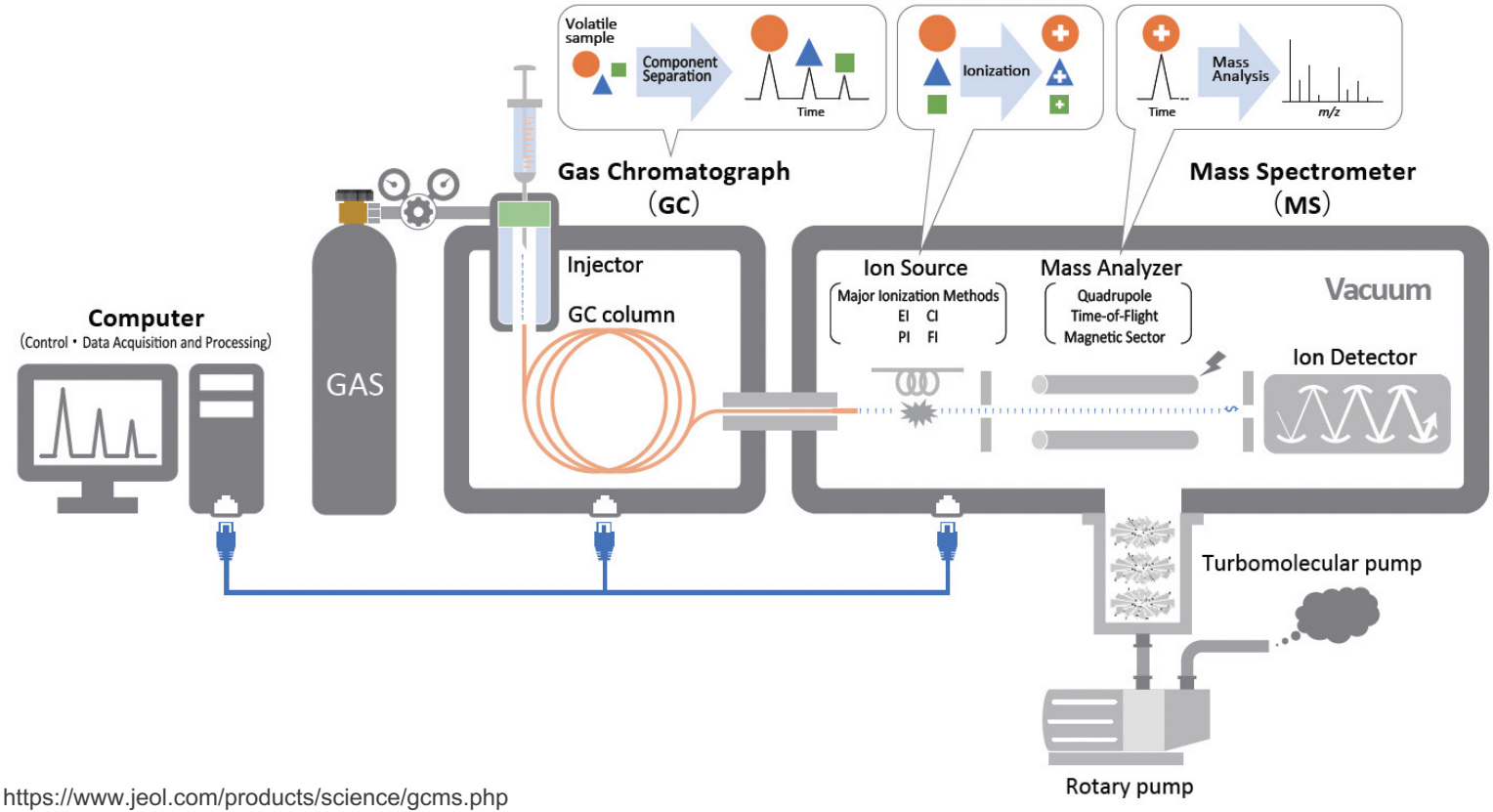
Trifluoropropyldimethylpolysiloxane



Polyethylene glycol (PEG) or Wax

Gas chromatography mass spectrometry (GC-MS)

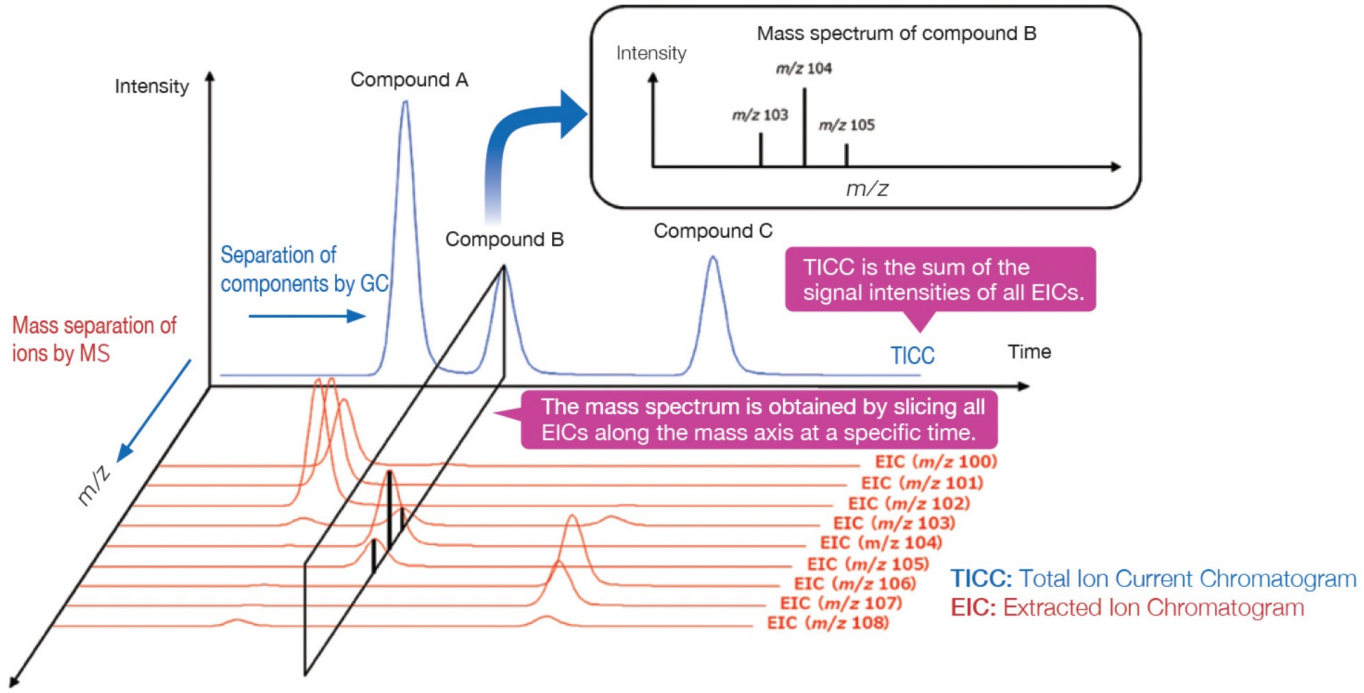
Equipment



https://www.jeol.com/products/science/gcms.php

Gas chromatography mass spectrometry (GC-MS)

Typical spectrum

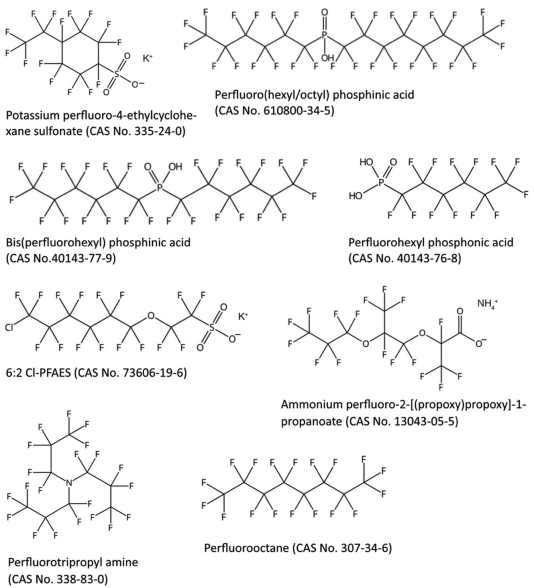


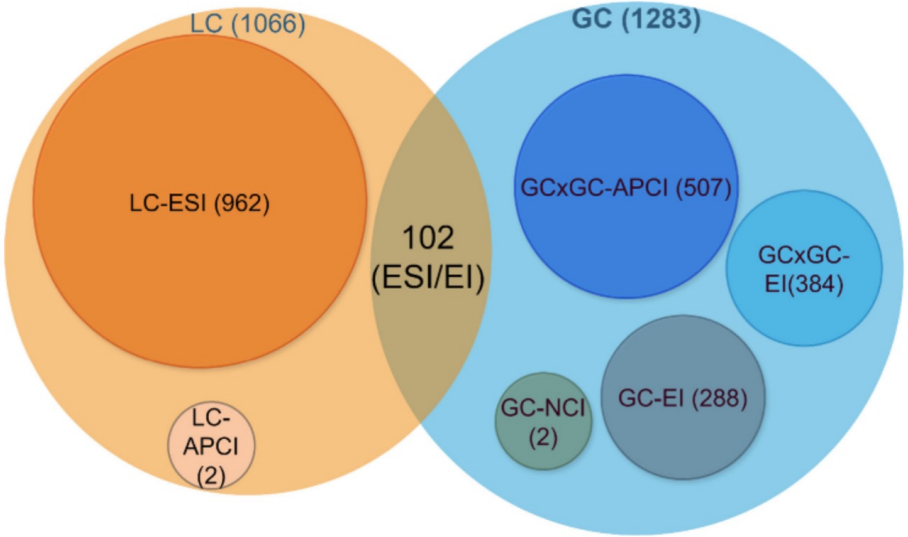
https://www.jeol.com/products/science/gcms.php

Gas chromatography mass spectrometry (GC-MS)

Example of application

- PFAS analysis





Strategies for grouping per- and polyfluoroalkyl substances (PFAS) to protect human and environmental health, Environ. Sci.: Processes Impacts, 2020, **22**, 1444-1460, 0.1039/D0EM00147C

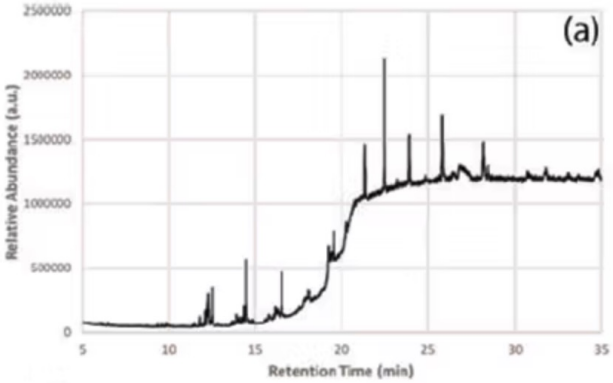
Filling the Gaps in PFAS Detection: Integrating GC-MS Non-Targeted Analysis for Comprehensive Environmental Monitoring and Exposure Assessment Environ. Sci. Technol. Lett. 2025, 12, 2, 104–112

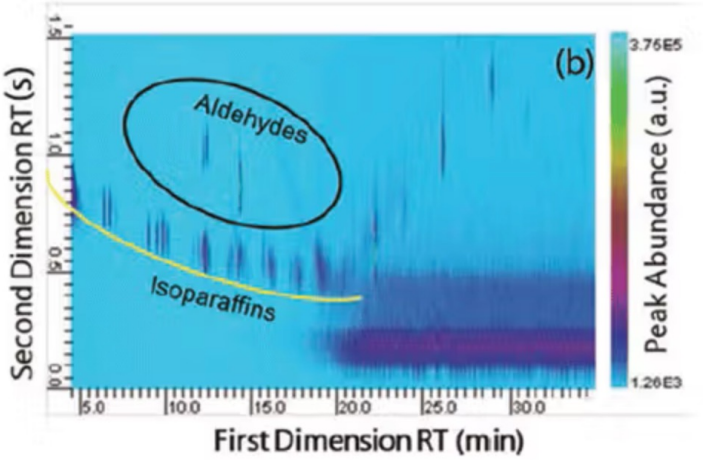
Gas chromatography mass spectrometry (GC-MS)

Example of application

- Forensic analysis (GC-GC-MS)

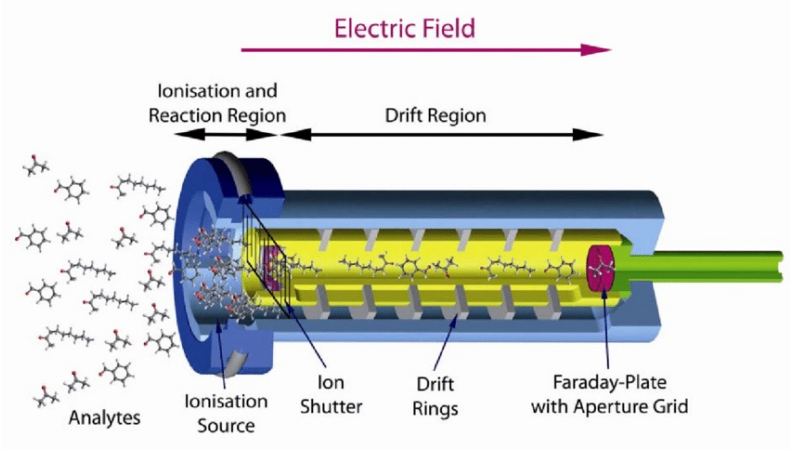
  Identification of the presence of a lubricants after a sexual aggression.





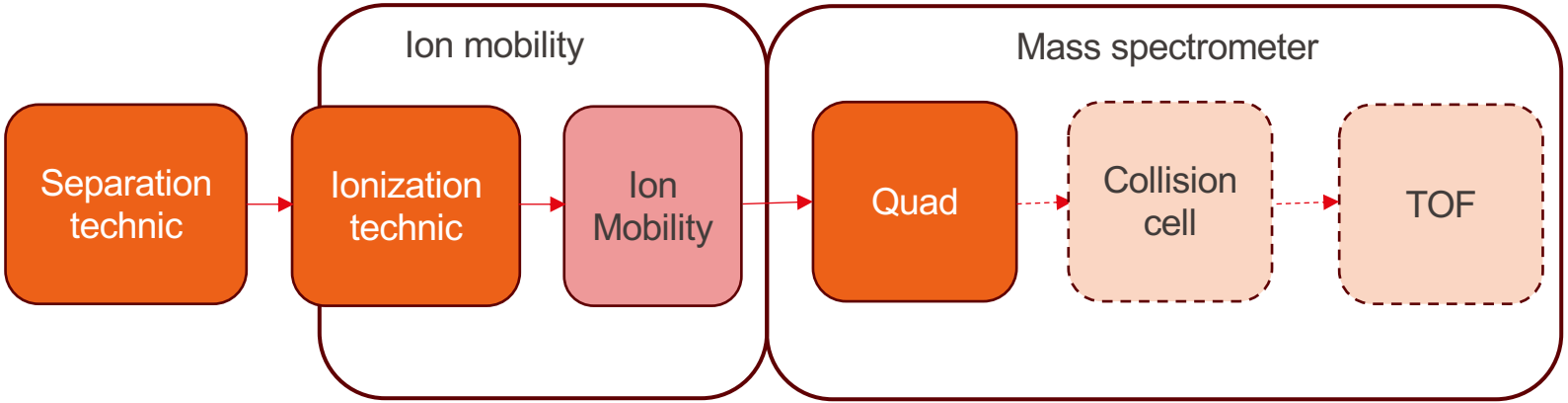
Candice M. Bridge, Kaitlin Jones, **GC×GC–MS for Forensic Analysis,** Spectroscopy Supplements, Special Issues-03-01-2019, Volume34, 3, Pages: 22–25

Ion mobility separations and mass spectrometry

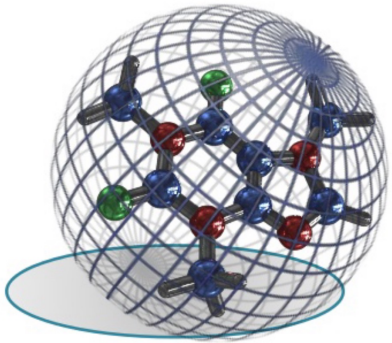


Ion mobility separations and mass spectrometry

General principle



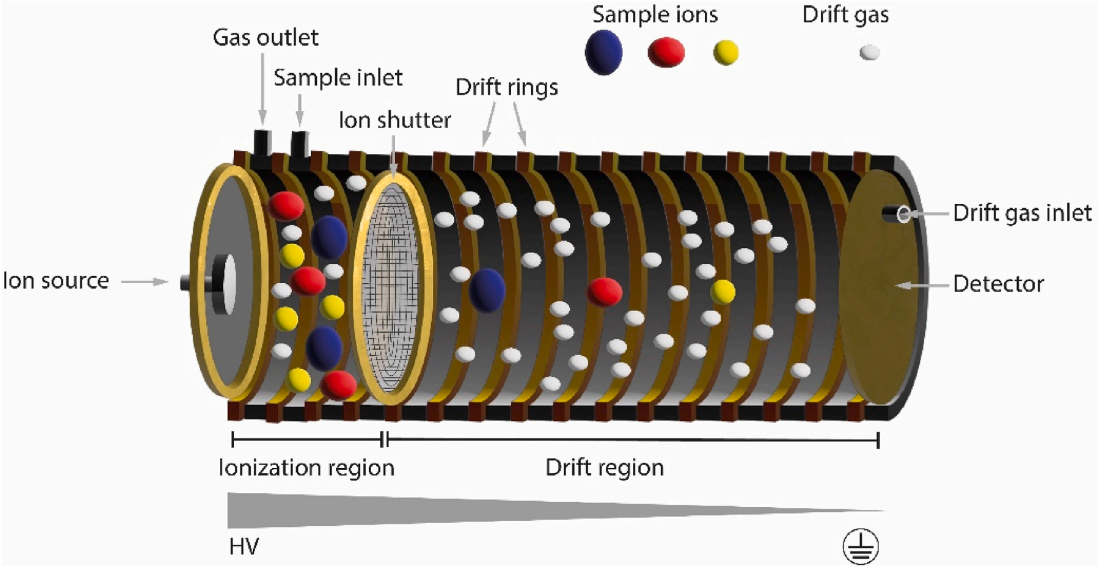
- Ion mobility add an additional dimension of separation with respect to the mass spectrometry
- Ion mobility sperates ions with respect to their respective molecular volume or collisional cross section (**CCS, Å²**)



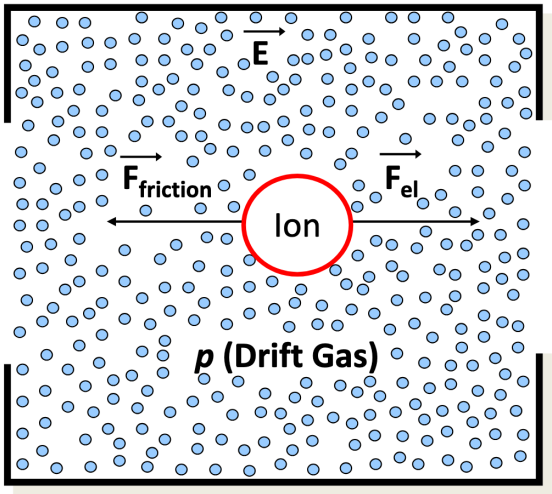
Ion mobility separations and mass spectrometry

Drift tube

- Drift tube



https://www.uab.edu/proteomics/metabolomics/workshop/2017/day5/ASMS_IMS_Tutorial_060417_present.pdf



Drift Cell

Drift velocity : $v_d = KE$, with ion mobility factor K, electric field $E = V/L$ in Volt/m and drift length L.

Ion mobility separations and mass spectrometry

Drift tube

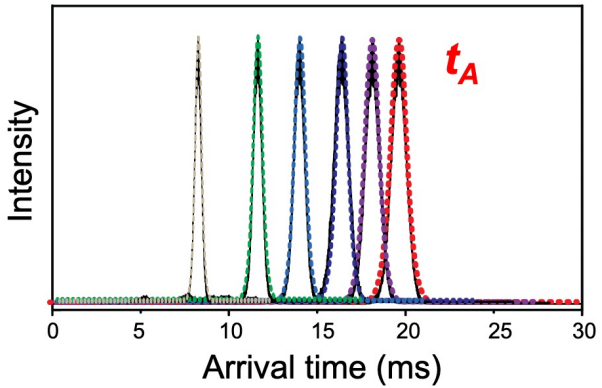
Drift velocity : $v_d = KE$, with ion mobility factor K, electric field $E = V/L$ in Volt/m and drift length L.

Drift time : $t_d = \frac{L}{v_d} = \frac{L}{KE} = \frac{L}{K(\frac{V}{L})} = \frac{L^2}{KV}$

Total drift time or arrival time t_A : we must add the non-drift delays t_0 (ion gating, transfers, electronics) $t_A = \frac{L^2}{KV} + t_0$

For a specific ion with K = cst, at increasing voltages, we get a proportional increase of t_A.

Drift Voltage	t_A
50 V	19 ms
55 V	18 ms
60 V	16 ms
70 V	14 ms
100 V	11 ms
150 V	8 ms



Ion mobility separations and mass spectrometry

Drift tube

Arrival time t_A : $t_A = \frac{L^2}{KV} + t_0$

K (ion mobility) : in the low-field limit, **Mason-Schamp** gives

$$K = \frac{3ze}{16N}\frac{1}{\Omega}\sqrt{\frac{2\pi}{\mu k_B T}}$$

Replacing K in arrival time equation t_A gives

$$t_A = \frac{L^2}{\frac{3ze}{16N}\frac{1}{\Omega}\sqrt{\frac{2\pi}{\mu k_B T}}V} + t_0$$

where

- z = charge state, e = elementary charge,
- N = gas number density,
- T = gas temperature, k_B = Boltzmann constant,
- $\mu = \frac{m_{\text{ion}}\, m_{\text{gas}}}{m_{\text{ion}}+m_{\text{gas}}}$ = ion–neutral reduced mass,
- Ω = rotationally averaged **collision cross section (CCS)**.

Ion mobility separations and mass spectrometry

Drift tube

Using the perfect gas law $N = \frac{P}{k_B T}$, we get an equivalent pressure form

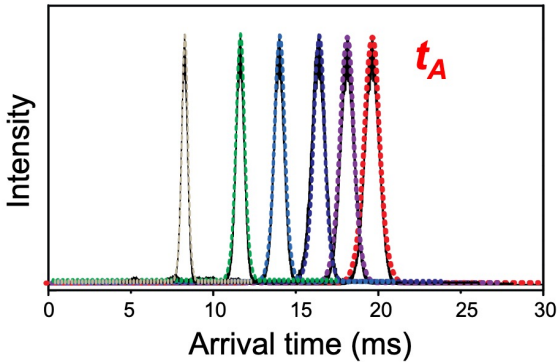
$$t_A = \frac{L^2}{\frac{3ze}{16P}\frac{1}{\Omega}\sqrt{\frac{2\pi k_B T}{\mu}}V} + t_0$$

$$\Rightarrow \frac{L^2}{V}\left(\frac{16P\Omega}{3ze}\right)\sqrt{\frac{\mu}{2\pi k_B T}} + t_0$$

$$\Rightarrow \frac{P}{V}\left(\frac{L^2 16\Omega}{3ze}\right)\sqrt{\frac{\mu}{2\pi k_B T}} + t_0$$

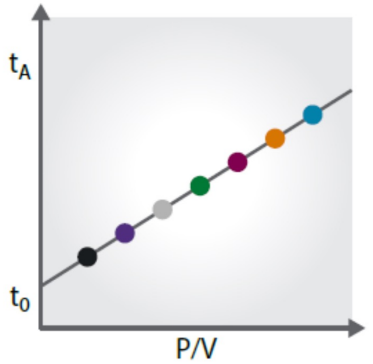
For a specific ion with K = cst, at increasing voltages, we get a proportional increase of t_A.

Drift Voltage	t_A
50 V	19 ms
55 V	18 ms
60 V	16 ms
70 V	14 ms
100 V	11 ms
150 V	8 ms



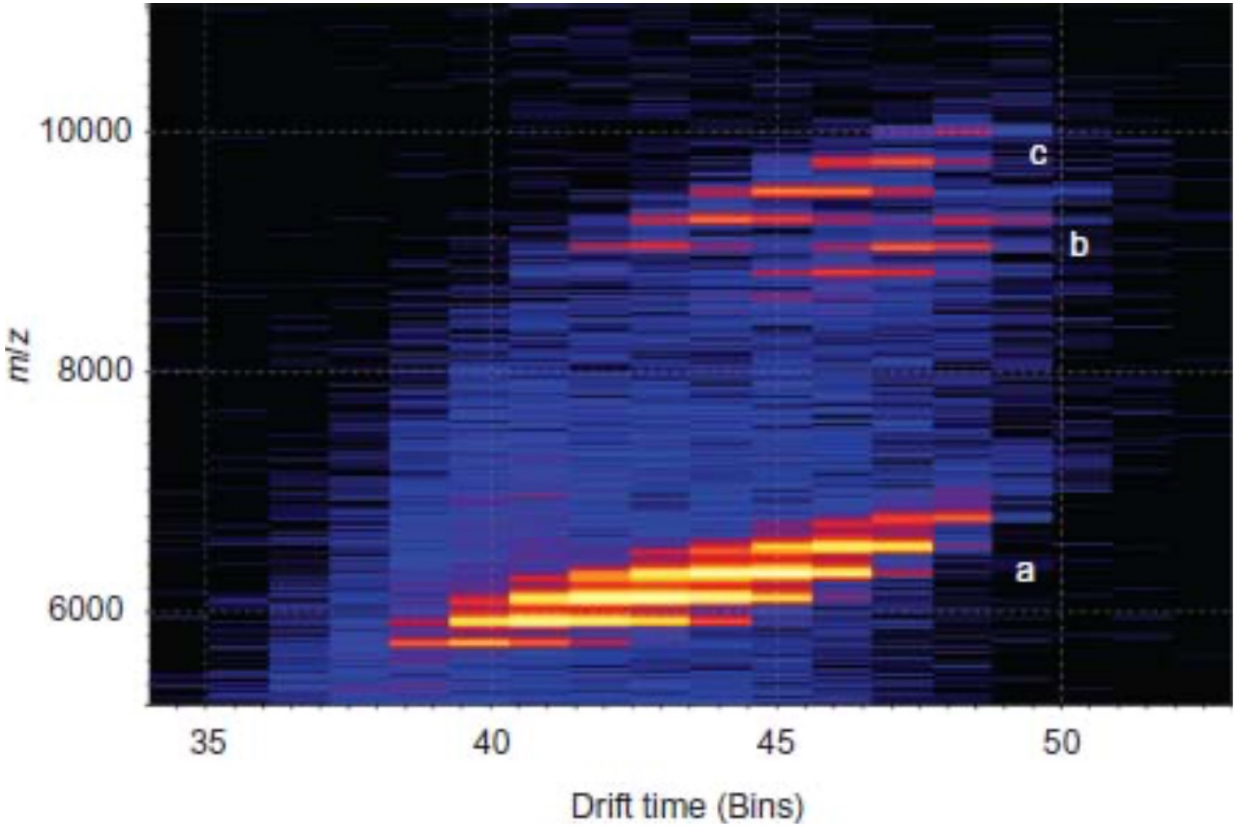
And plot t_A w.r.t to P/V -> all other parameters being cst, we can extract Ω (CCS) from the slope.

=> Consequence, the machine measure t_A, to get CCS, a calibration is systematically required.



Ion mobility separations and mass spectrometry

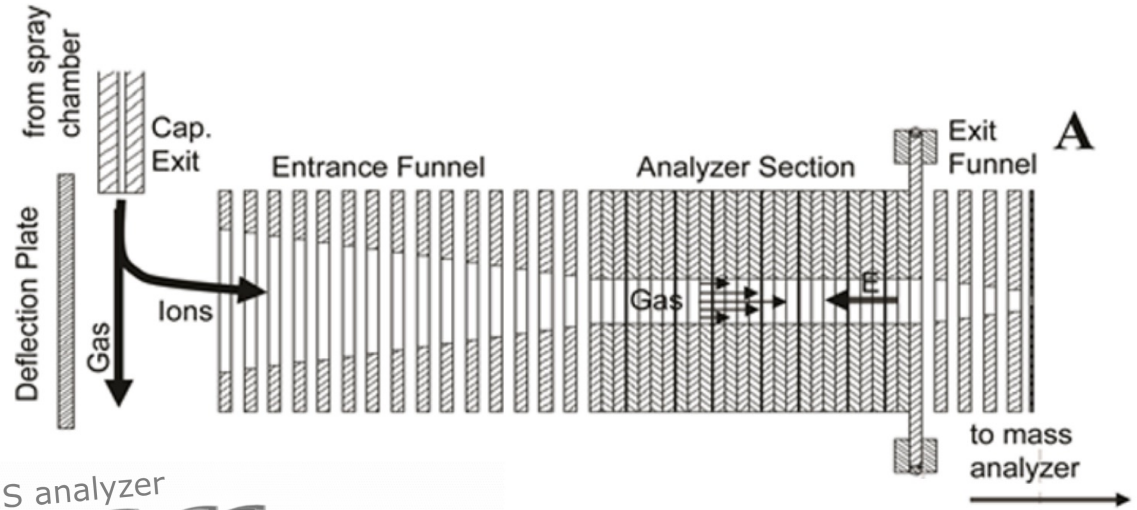
Typical result

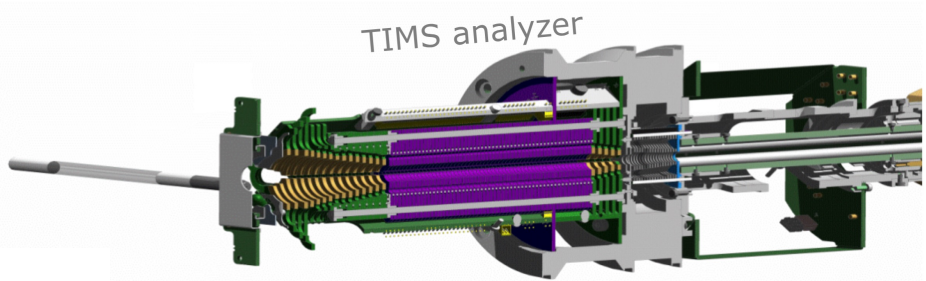


Ion mobility separations and mass spectrometry

Other type of IM device

- TIMS (trap-ion)



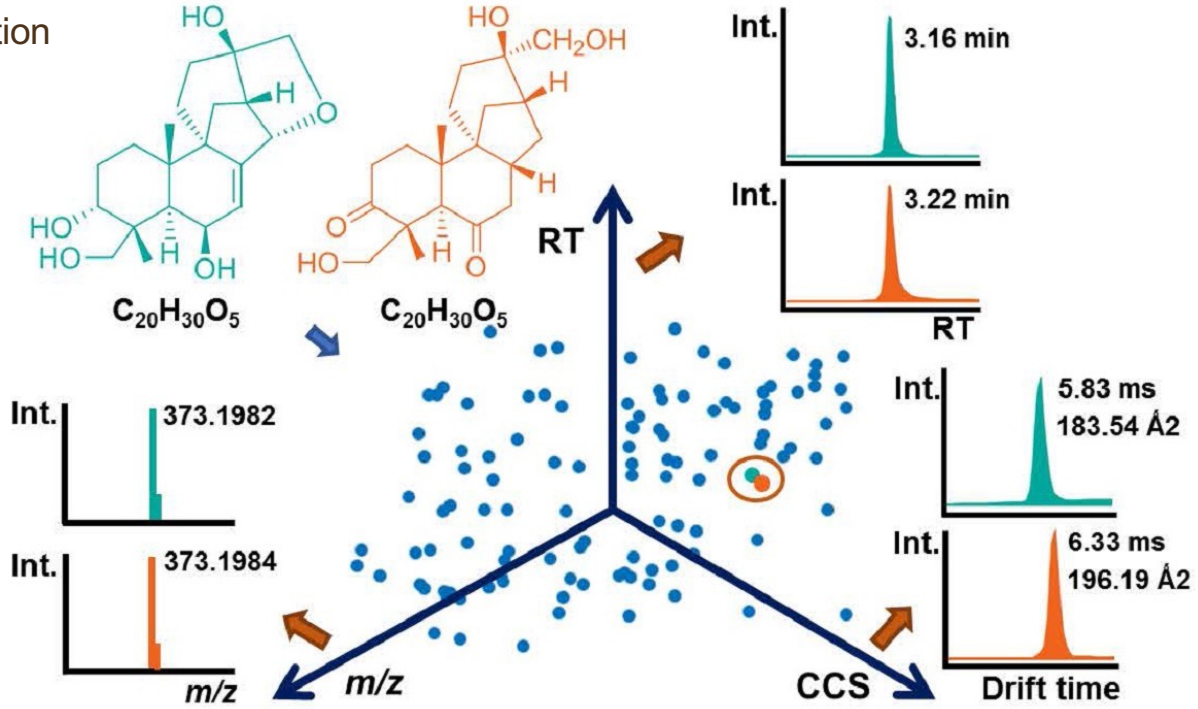


M.E. Ridgeway, J.J. Wolff, J.A. Silveira, C. Lin, C.E. Costello, M.A. Park, Int. J. Ion Mobil. Spectrom. 2016, 1-9.

Ion mobility separations and mass spectrometry

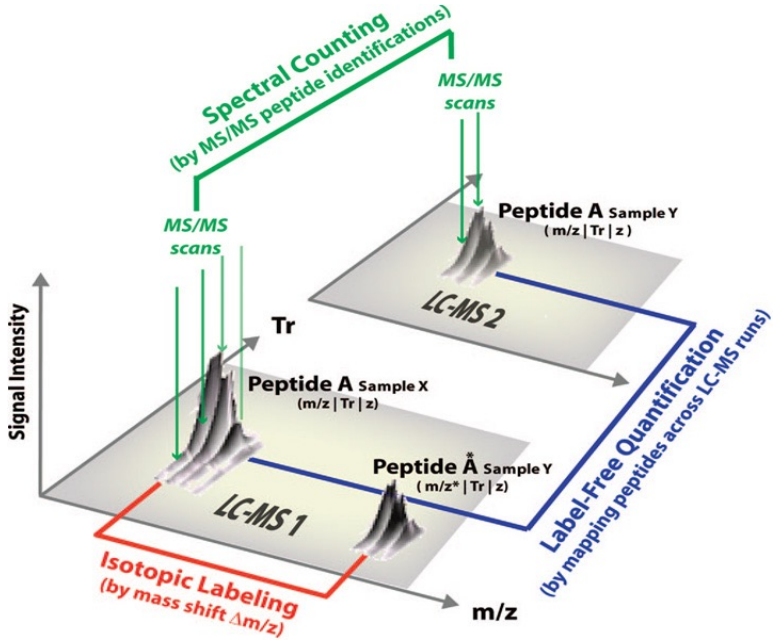
Example of application in structure elucidation

- Isomer identification



Mar. Drugs **2022**, *20*(9), 541; https://doi.org/10.3390/md20090541

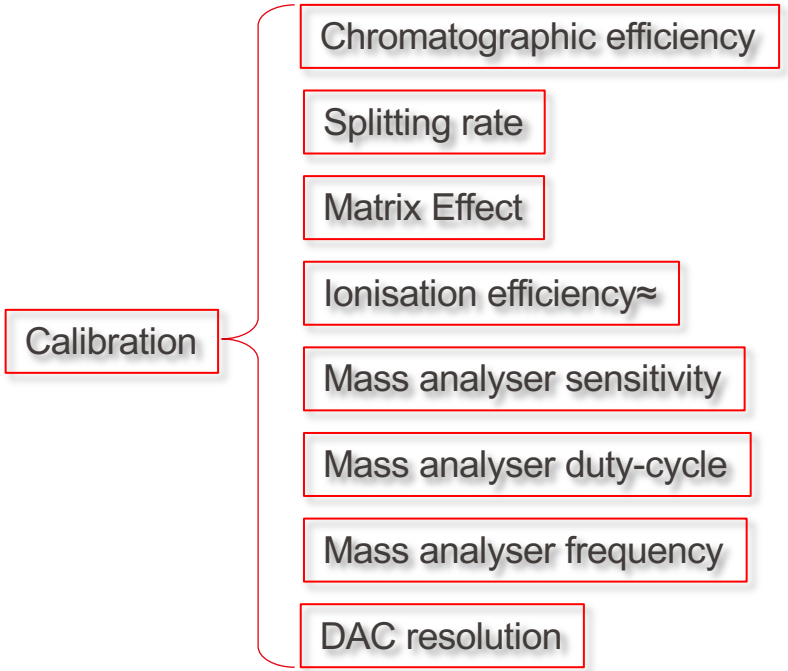
Quantitation by mass spectrometry



Quantitation by mass spectrometry

Relative vs absolute quantification

- **Relative quantification** informs us (in principle) about the **ratio between** different samples of the same analyte acquired with the same equipment and in the same matrix.
- **Absolute quantification** informs us about the **effective concentration** of an analyte in a sample and (in principle) independent on the equipment and matrix.
- **Absolute quantification requires a calibration** of the equipment and of the method taking in account the matrix.



Quantitation by mass spectrometry

Calibration

External standard :

1. Prepare a series of analyte standard solutions of increasing concentration C
2. Measure the signal intensity $S(C)$
3. Plot $S(C) = a \cdot C + b$ with a is the slope of the function and b its y-intercept

The quality of the calibration is defined by the correlation coefficient R^2

- **Different matrix**
- **No sample dilution**

Internal standard by isotope dilution :

1. Spike the sample to be analysed with an isotope labelled (^{13}C or ^{15}N) version of the analyte.
   - Same RT
   - Same ionisation
   - Slightly different m/z then
   - Similar m/z range
   - Known concentration
   - **Same matrix**
   - **Labelled molecule required**

Calibration

Monitor and correct sample preparation and analysis

Internal standard by standard addition :

1. Spike the sample with an analyte standard with increasing amounts to form a calibration curve
   - Sample concentration $C = -\frac{b}{a}$,
   - **Same matrix**
   - **No labelled molecule required**