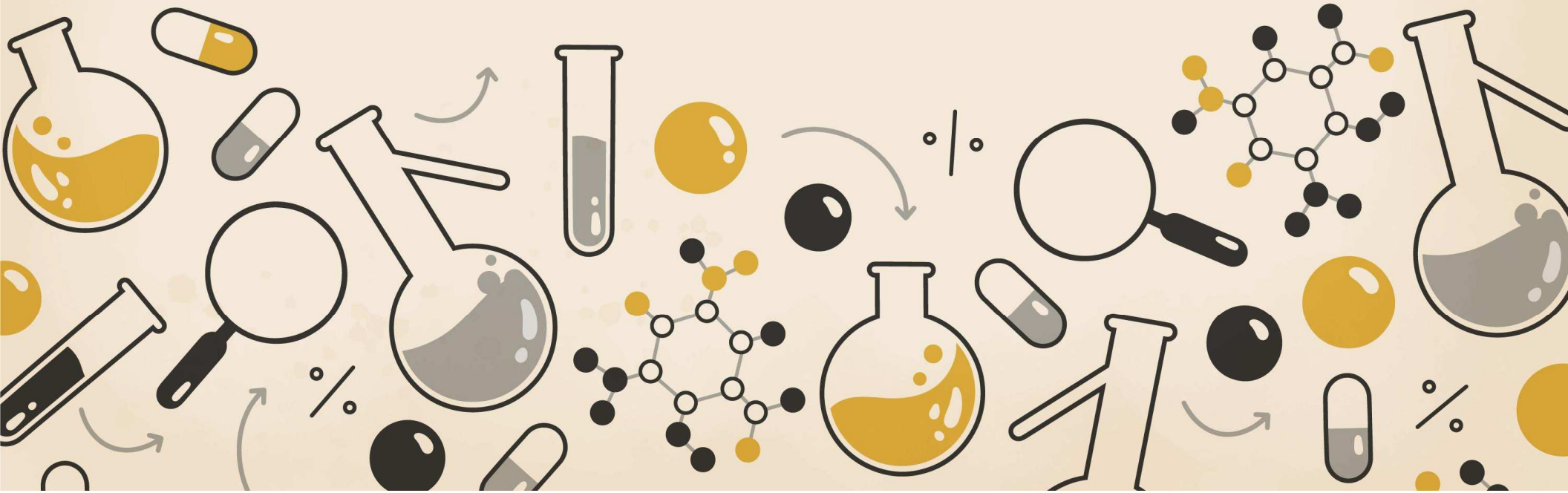
QEL, Spices Board

In-house Training – 1:
Analysis of Pesticide Residues

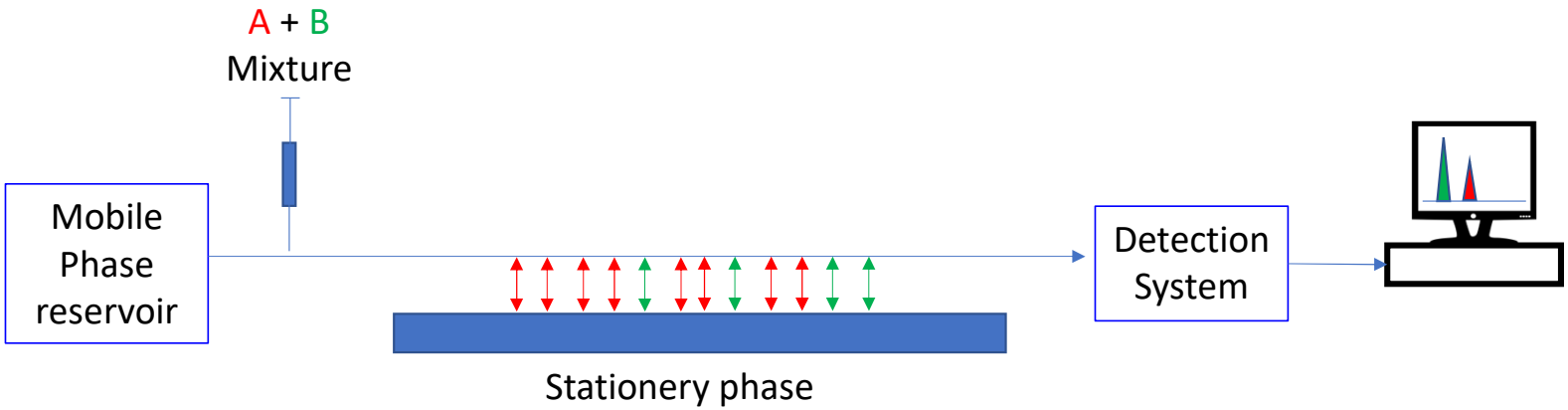
Lecture - 1
Introduction to instrumentation:
LC-MS/MS
03-Jul-23

Dr. Ramesh BN
Spices Board



Chromatography

HPLC, GC…



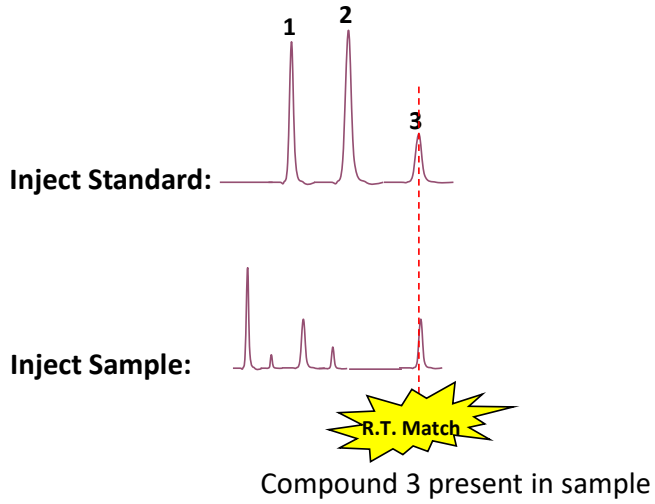
HPLC Analysis

Limitations

When conventional detectors are used, identification of unknowns by retention time matching

Possibility of errors

To avoid these errors, we use mass spectrometry along with chromatography



Problems:

- ↳ Two compounds with same Retention Time: FALSE POSITIVE
- ↳ Retention Time shift for one compound: FALSE NEGATIVE

Mass Spectrometry

Why?

A mass spectrometer (MS) looks at the masses of the analytes being tested

- ↳ Uses information derived from these masses for identification
- ↳ High degree of specificity
- ↳ Retention time can be used for additional confirmation

MS as a detector

Hyphenated techniques

An MS can function as a detector to a chromatographic system, replacing conventional detectors

- ↳ Gas chromatography (GC-MS), liquid chromatography (LC-MS)
- ↳ Can provide very specific and sensitive analysis
- ↳ Currently, MS is the default detector in trace analysis work
- ↳ 'Tandem' MS is possible, which gives even more specificity and sensitivity (MS/MS)

Mass Spectrometry

§ General principles

Mass Spectrometry

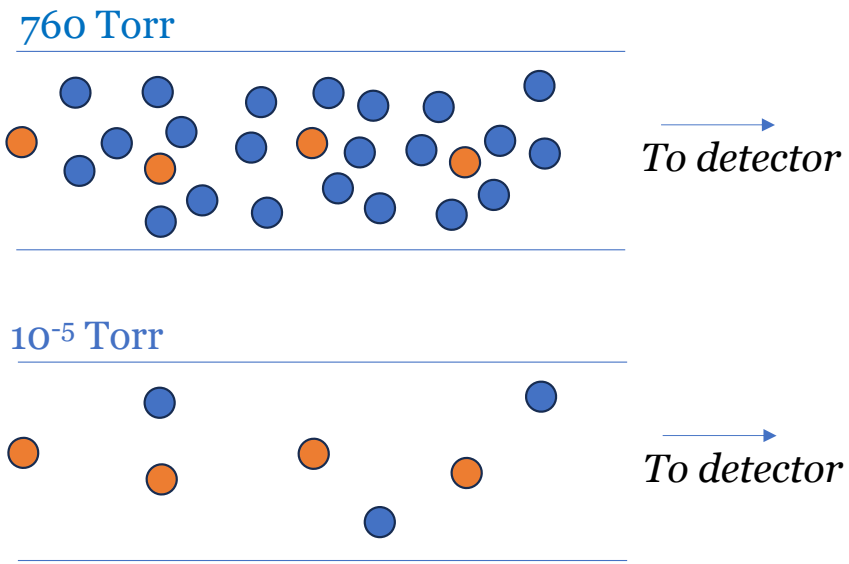
Principles - 1

A mass spectrometer can 'see' only charged entities (ions)

Typically functions under high vacuum

- ↳ The ions need a stable path through the mass spectrometer, without colliding with air molecules or with one another

The 'mean free path': average distance between collisions for a gaseous ion – must be high



Mass Spectrometry

Principles - 2

In MS the vacuum is achieved in two stages:

- The rouging pump (outside the instrument): from 760 Torr to 10^{-3} Torr
- The turbo molecular pump (inside the instrument): from 10^{-3} Torr to 10^{-5} Torr

Mass Spectrometry

Principles - 3

The connection between LC and MS is called the 'Interface':

- From HPLC the mobile phase flows out
- MS cannot handle this – it is a vacuum technique
- The liquid mobile phase has to be evaporated and taken into the MS
- Loss of analytes should be kept to a minimum
- In LC-MS, ionization also happens in the interface

Mass Spectrometry

Principles - 4

MS can operate in two ways.

First way:

- ↳ Molecule of interest is ionized
- ↳ Typically called 'parent' ion.
- ↳ Number of parent ions can be counted for quantification (LC-MS).

Second way:

- ↳ Molecule of interest is ionized, to get the 'parent' ion
- ↳ Parent ion is broken down into multiple 'daughter' ions
- ↳ Selected 'daughter' ions are counted for quantification (LC-MS/MS)

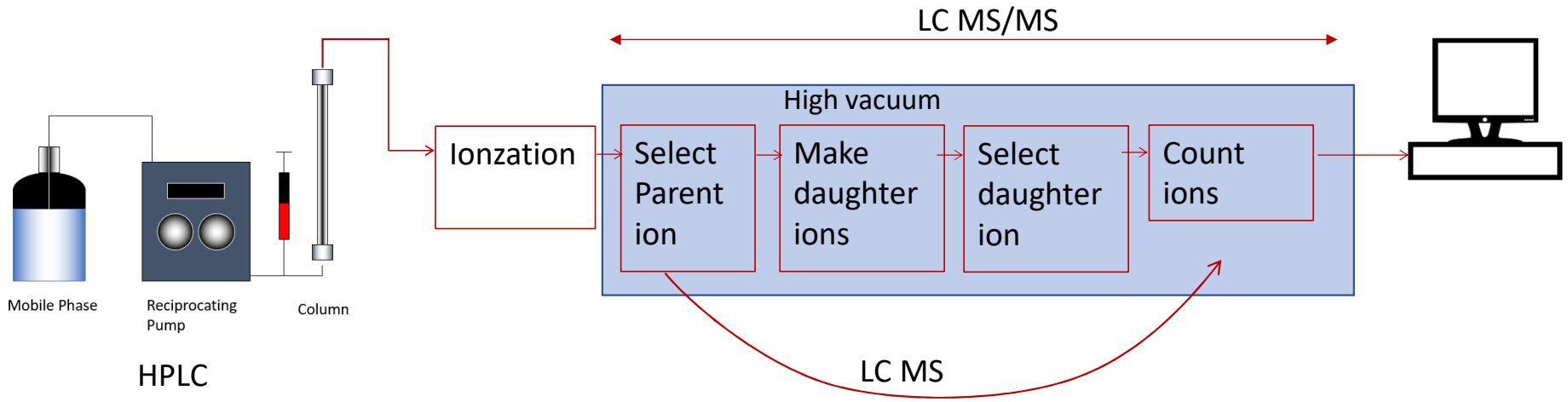
Why is this useful?

Mass Spectrometry

§ Components and operation

Mass Spectrometry

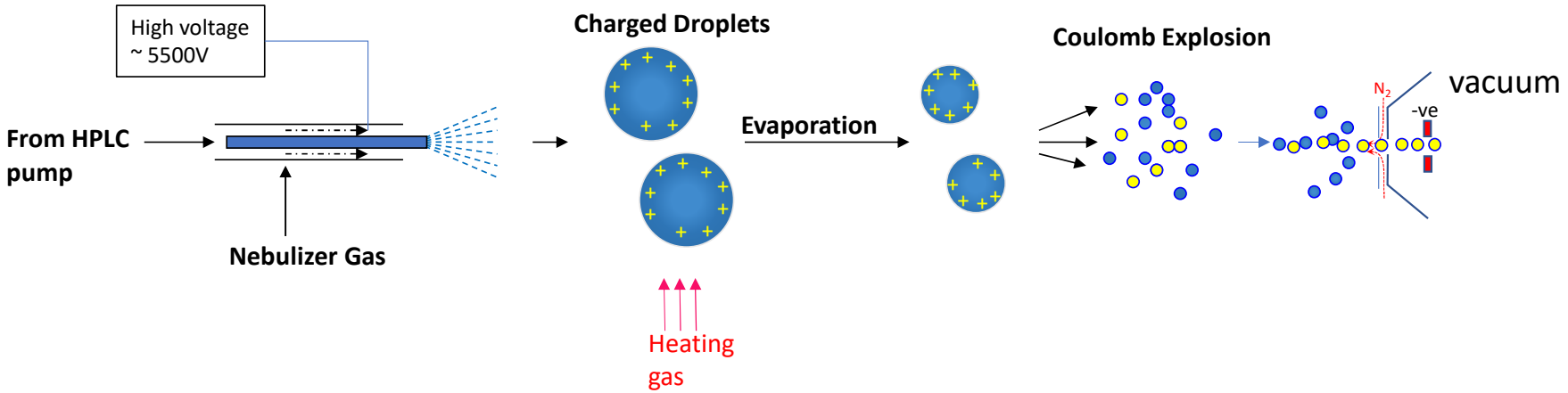
Components - overview



In LC MS/MS, ionization happens in atmospheric pressure
Ionization is soft: MH^+ ion is formed
Standard spectrum libraries not possible

Mass Spectrometry

Ionization



From solvent

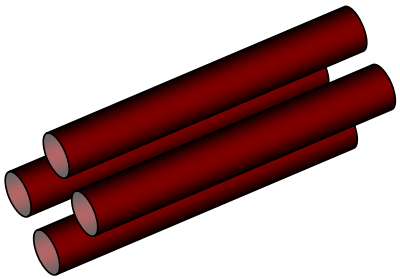
Parent ion

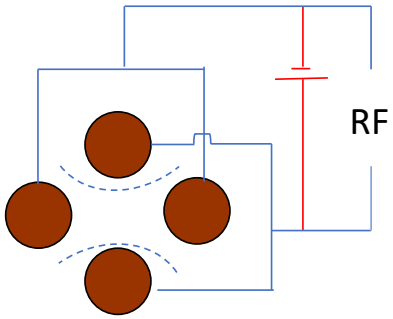
$$M + H^+ \xrightarrow{High\ potential} [MH]^+$$

Used for MS/MS

Mass Spectrometry

Quadrupoles (1)

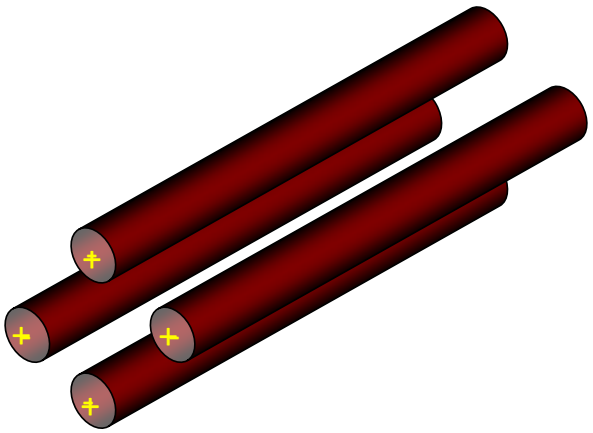




- Four electrodes arranged in a hyperboloid fashion
- Two Voltages:
  - Radio Frequency - RF (alternating)
  - Direct Current - DC
  - Applied simultaneously, the two voltages will trap ions within the quadrupole area
  - Scanning the voltages will filter the ions out by mass:
    - For one RF:DC value, only one mass can have a stable trajectory through the quadrupole
    - When RF:DC is progressively increased (scanned) Ions pass through in the order of masses

Mass Spectrometry

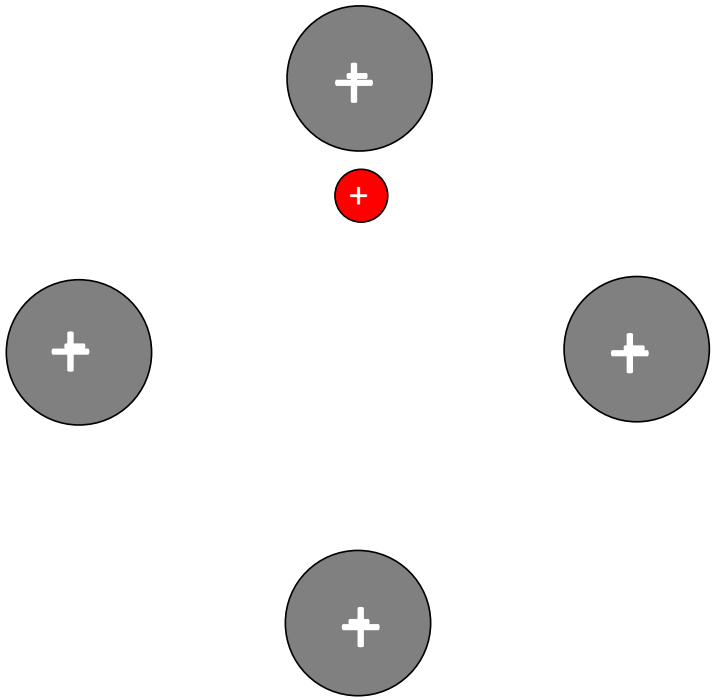
Quadrupoles (2)



- Opposite electrodes of the Quadrupole have the same potential
- The potential alternates with the same frequency as the RF Voltage

Mass Spectrometry

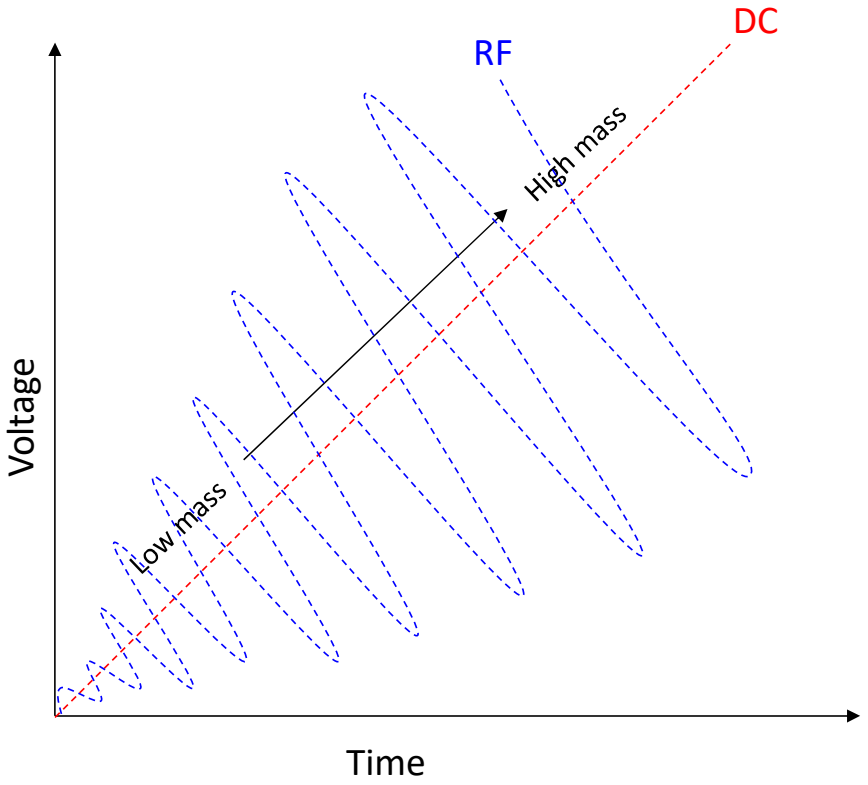
Quadrupoles (3)



- The ion oscillates in the volume between the quadrupoles
- A negative potential at the exit end of the quadrupole can the be used to pull the ion through the quadrupole

Mass Spectrometry

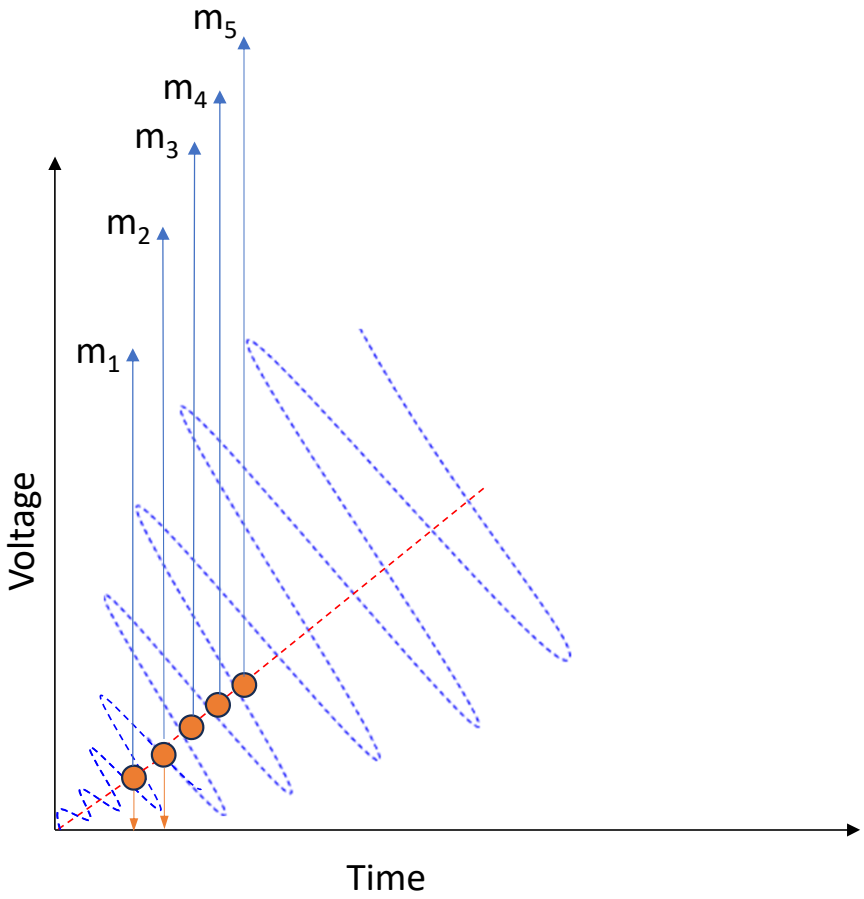
Mass analysis by the quadrupoles (1)



- When RF and DC are applied together, Quadrupoles function as a mass analyzer.
- The RF-DC voltages can be scanned
  - DC voltage increases with time
  - RF amplitude increases with time, but frequency remains constant
- For one RF-DC combination, only an ion of one particular mass will have a stable trajectory through the quadrupoles.
- Technically, it is the mass/charge (m/z) that is affected

Mass Spectrometry

Mass analysis by the quadrupoles (2)



RF-DC combinations in the quadrupole can be used to filter ions by mass:

- ↳ Low RF-DC combination, ions of low mass transmitted.
- ↳ High RF-DC combination, ions of high mass is transmitted

Mass Spectrometry

Mass analysis by the quadrupoles (3)

Quadrupoles can function in two modes

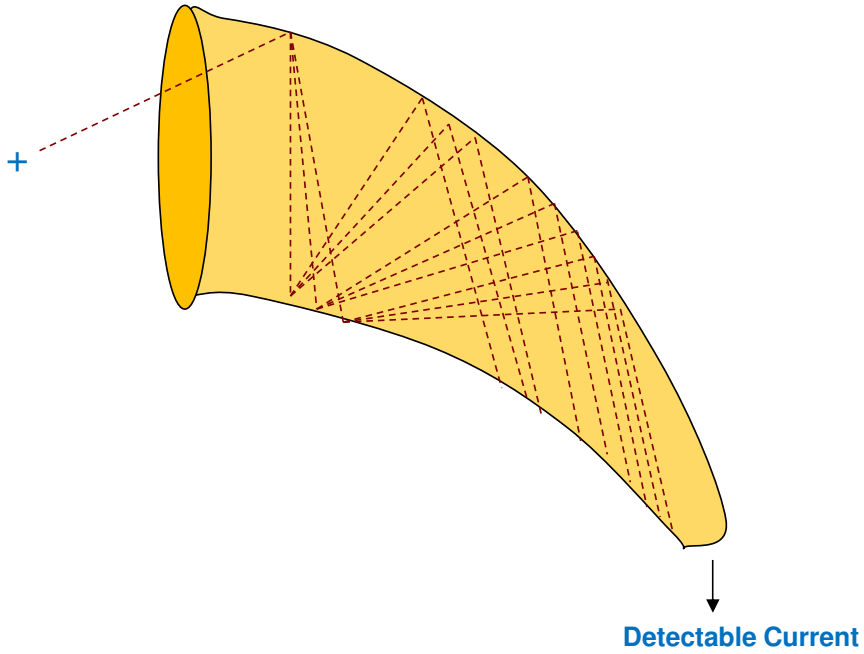
- Fixed RF-DC : Only ions with a fixed mass will be transmitted
- Switching DC off, i.e. RF only:
  - All ions oscillate within the quadrupole
  - By keeping a potential at the other end of the quadrupole, all ions can be transmitted together through the quadrupole

Mass Spectrometry

Detection of ions

Electron Multiplier

- The sides of the detector are coated with material that produces electrons on impact by charged particles



Mass Spectrometry

Putting everything together

- Multiple Reaction Monitoring

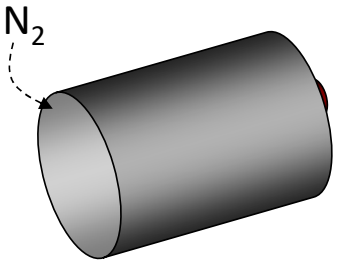
- Based on the theory that, under the same conditions,
  - An ion from an analyte will always fragment in the same way
  - Fragmentation pattern wIll be unique

- Highly selective and sensitive
  - We can choose to look at only molecules of interest
  - Quantification in ppb levels

Mass Spectrometry

Putting everything together (2)

- Three Quadrupoles:
  - First Quadrupole will select the parent ion MH^+
  - Second Quadrupole functions as the collision cell:
    - N_2 is infused into the collision cell
    - Daughter ions produced by Collision Induced Dissociation (CID)
  - Third Quadrupole scans the daughter ions

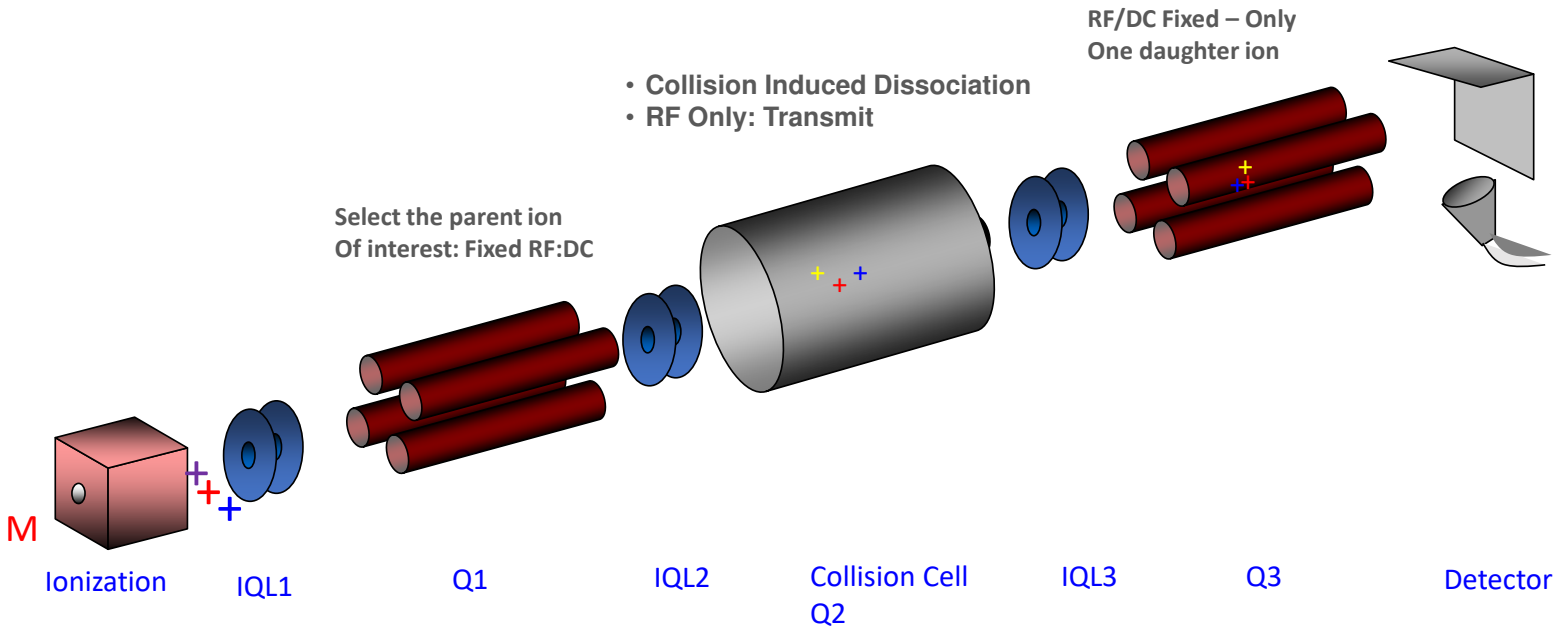


- Linear ion trap:
  - Second quadrupole ejects all ions except the most intense ion
  - Can study how this ion behaves with time
  - Trapped ion can be fragmented further by CID

- We have AB Sciex Qtrap® instrument at QEL Kochi but we are not using it in routine analysis

Mass Spectrometry

Putting everything together (3)



Mass Spectrometry

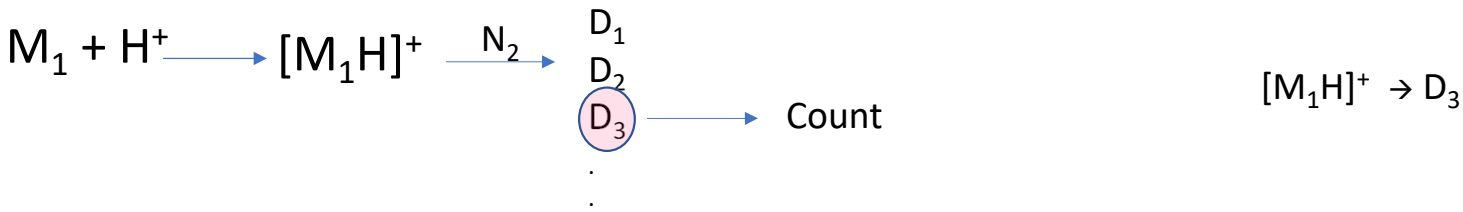
§ in practice

Multiple Reaction Monitoring

Quantitative analysis using MS/MS

"Transitions"

LC MS/MS



Multiple such reactions, or transitions, can be anaysed in a single run – hence the name 'MRM'

Multiple Reaction Monitoring

Quantitative analysis using MS/MS (2)

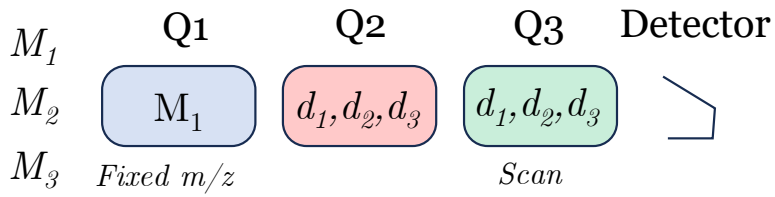
In MRM, we choose 2 transitions per analyte

- Higher intensity transition is called the quantifier, and is used for quantification
- Lower intensity transition is called the qualifier
- Quantifier : Qualifier ratio should match between standard and sample

	Compound	Parent Ion	Daughter Ion	Type	Ratio
LC MS/MS	Ethion	385	199	Quantifier	28.6
		385	171	Qualifier	

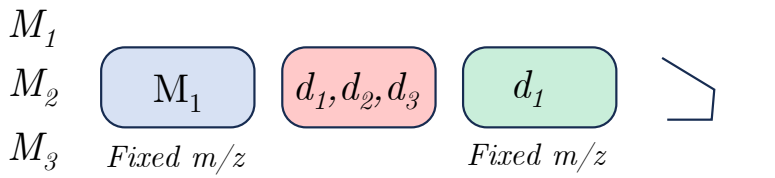
MS/MS scan modes

Not just MRMs



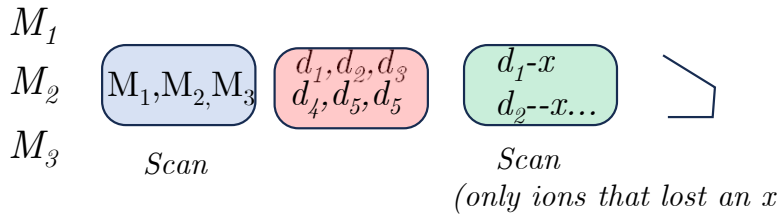
Product ion scan

To understand the ways in which an ion can fragment. First step in MS method development



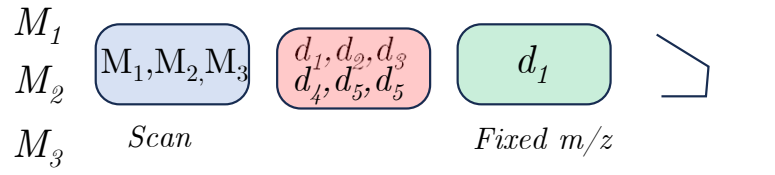
Selected ion monitoring

MRM mode,
Mainly used for quantitation work



Neutral loss Scan

To study reaction mechanisms



Precursor ion Scan

To find which ions (precursors) produce a given daughter ion

Topics that remain to be covered
in LC-MS/MS

Mass accuracy and resolution, calibration of mass-response, MS tuning

↳ Will be covered in the method validation class

Basic troubleshooting in LC-MS/MS

↳ There will be a separate class on this

More to learn..

Next session 10th June 2023

- ☑ (1) Introduction to instrumentation: LC-MS/MS, 03-Jul-23
- 🕒 (2) Introduction to instrumentation: GC-MS/MS, 10-Jul-23 ⟸
- 🕒 (3) Pesticide residue analysis - Introduction, 17-Jul-23
- 🕒 (4) Advanced pesticide residue analysis, 24-Jul-23
- 🕒 (5) Method validation: requirements and practice, 31-Jul-23
- 🕒 (6) Introduction to measurement uncertainty calculation, 7-Aug-23

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Thank you!

§ Questions?