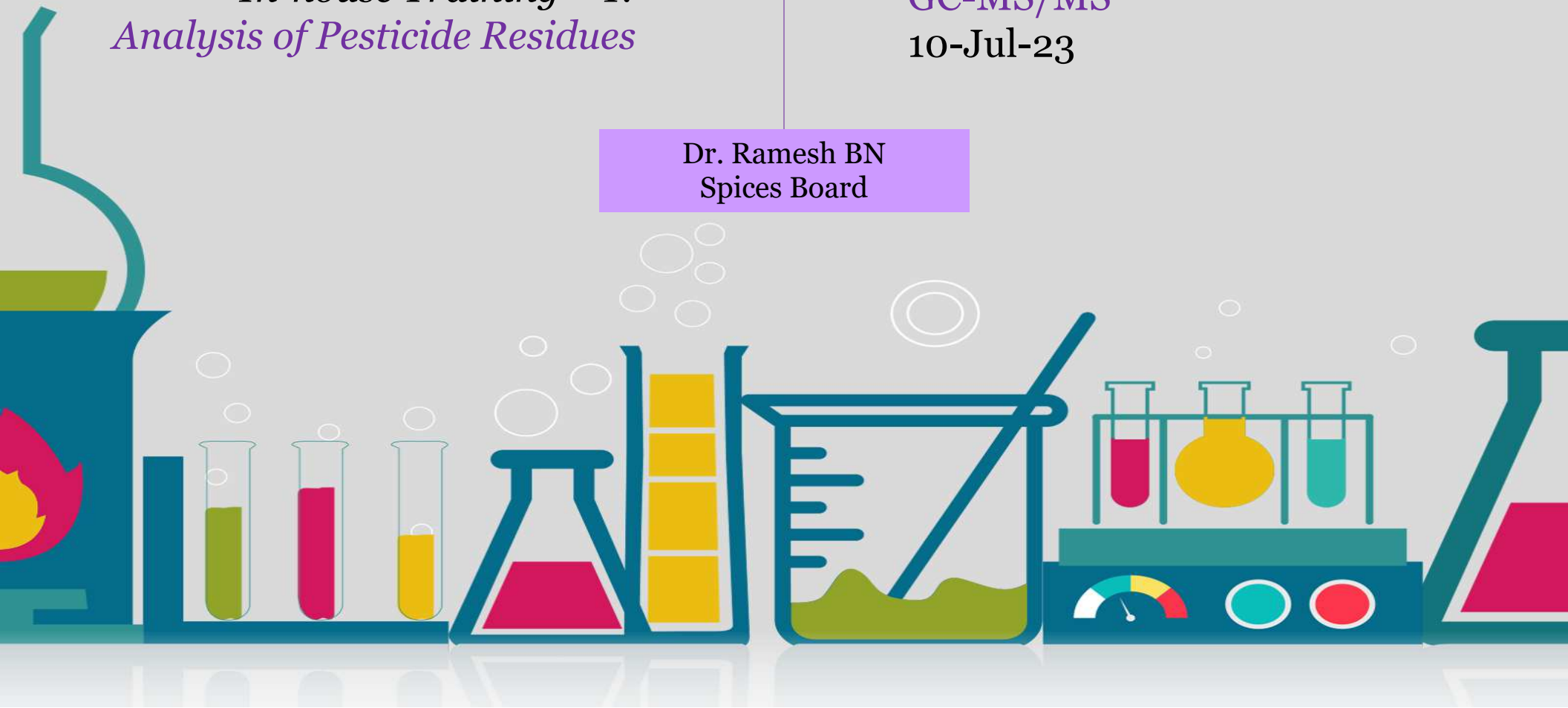
QEL, Spices Board

In-house Training – 1:
Analysis of Pesticide Residues

Lecture - 2
Introduction to instrumentation:
GC-MS/MS
10-Jul-23

Dr. Ramesh BN
Spices Board



Moving on

From last lecture…

What we have already covered:

- Basics of chromatography and HPLC
- General principles of mass spectrometry (MS) and hyphenated techniques
- General principles of LC-MS/MS
  - Quadrupole theory
  - How mass filtration happens in MS: scanning (RF-DC ramp) and selection (RF-DC fixed)

What we will cover today:

- Review of basics operation of GC
- General principles of GC-MS and GC-MS/MS
- How GC-MS differs from LC-MS
- Using GC-MS and GC-MS/MS for residue analysis

Gas Chromatography

A review…

⇒ Chromatographic technique for separation of mixtures of volatile compounds.

- ↳ Mobile Phase is a gas, He or N2.
- ↳ Mixtures are separated based on the difference in interaction of the components with the stationary phase.
- ↳ Identification of unknowns is based on matching TR of standards

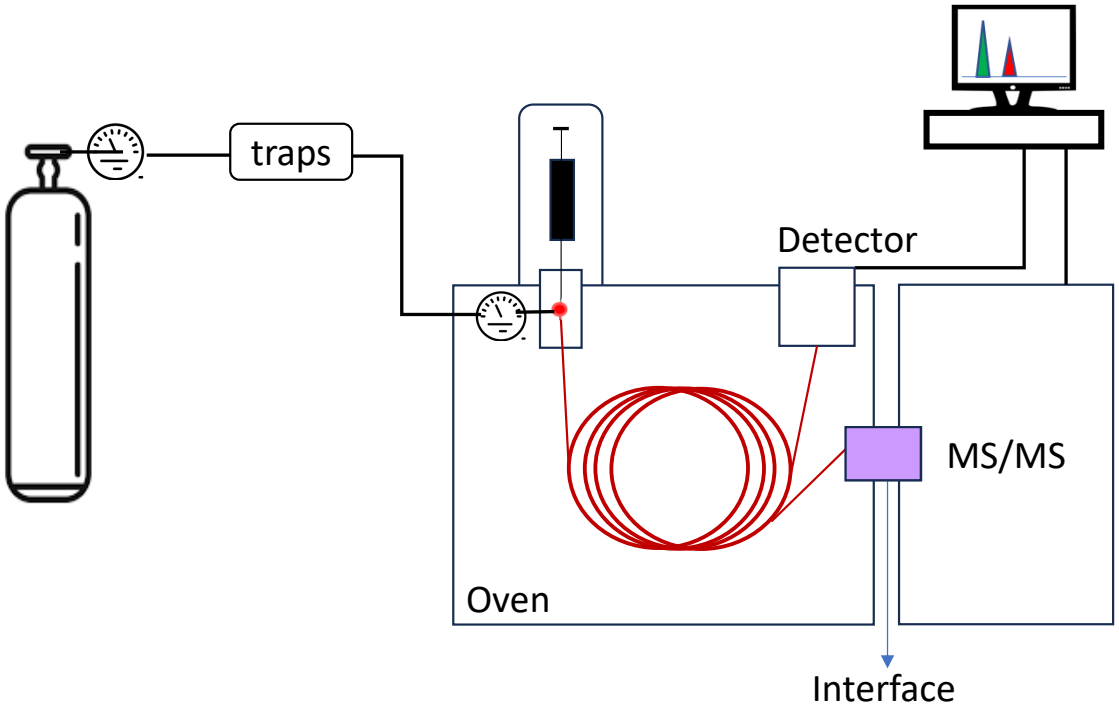
⇒ Using conventional detectors require relying on retention time matching for identification causes problems:

- ↳ False positives, false negatives

⇒ Mass spectrometry is used as the detector to avoid these problems

Gas Chromatography

parts

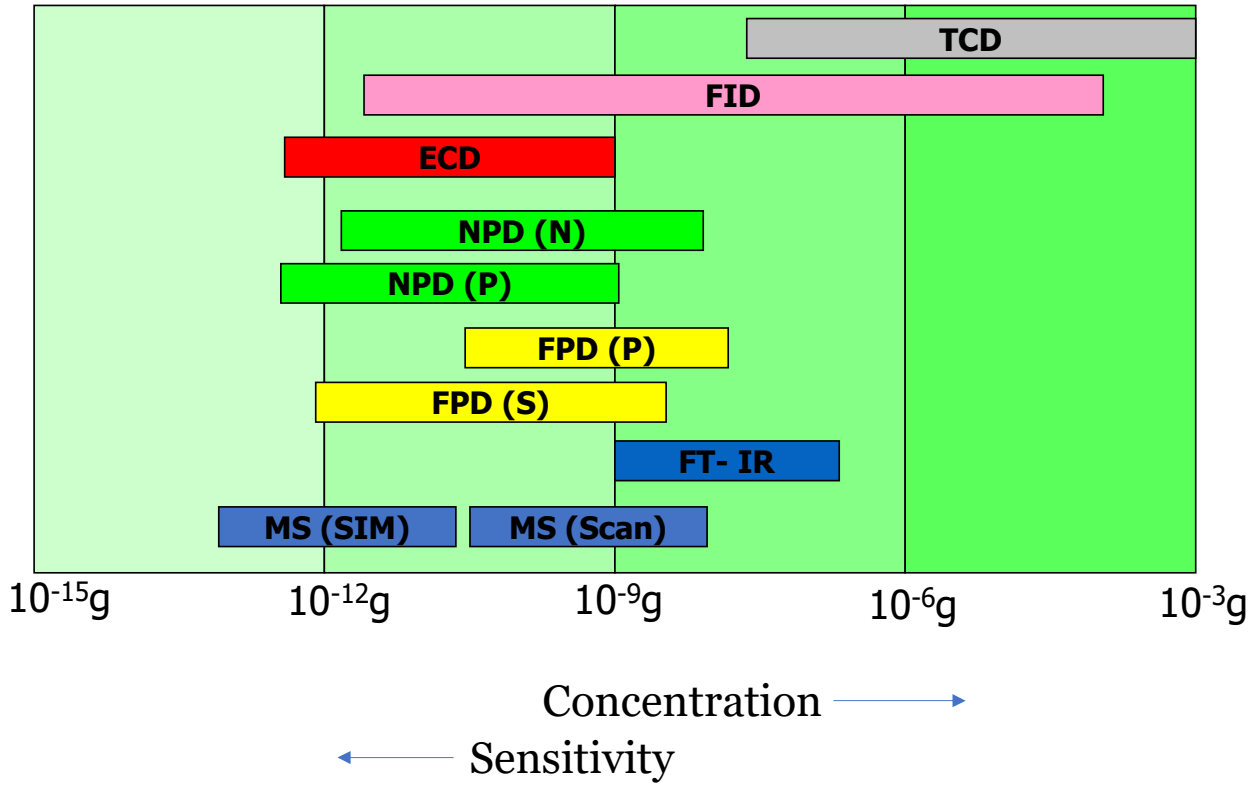


The components

- Helium gas is the carrier
- Moisture, oxygen and hydrocarbon traps
- The column is a capillary tube of fused silica, with the liquid stationary phase coated inside
- The electronic flow controller (EFC) manages the flow of carrier gas through the column
- The oven controls the temperature of the column
- The injector vaporizes the sample and mixes it with the carrier gas
- Detector detects the compounds separated in the column

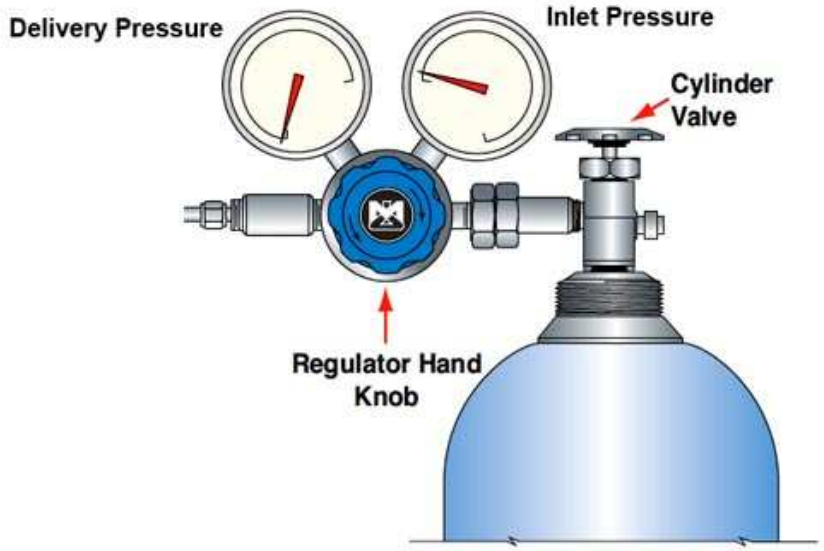
MS as a detector for GC

Sensitivity and range compared to conventional detectors



The carrier gas

Things to remember



⇒ The carrier gas cylinder should be replaced when the inner pressure falls below 200 psi

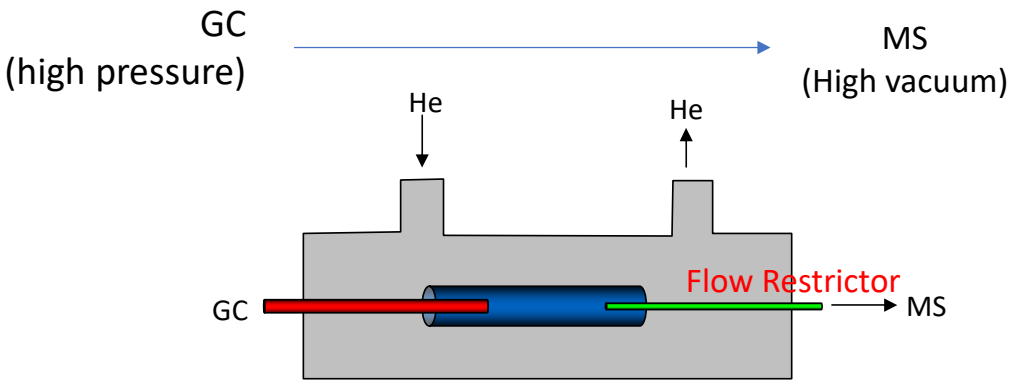
↳ Will affect MS performance if pressure is lower

GC-MS and –MS/MS

§ Components and operation

The interface

Hyphenation to GC



- Column outlet is placed directly opposite to the MS Inlet
- MS vacuum can handle around 1 ml/min carrier flow
  - A flow-restrictor is used
  - High GC flow – excess is vented out
  - Low GC flow – He from the reservoir is pulled in
- Best for capillary columns

How GC-MS/MS works

General principles

⇒ Very similar to LC-MS/MS, which we have already covered

- ↳ MS can 'see' only ions, so ionization is the first step in MS
- ↳ In GC-MS/MS,
  - ↳ ionization happens in vacuum (unlike LC-MS)
  - ↳ ionization is 'hard' (unlike LC-MS)
- ↳ Other operations are similar to LC-MS/MS, which has been covered already:
  - ↳ Quadrupole theory
  - ↳ Mass scanning / Mass filtration (RF/DC)
  - ↳ Collision induced dissociation
  - ↳ Detection by electron multiplier

The Vacuum system

In GC-MS

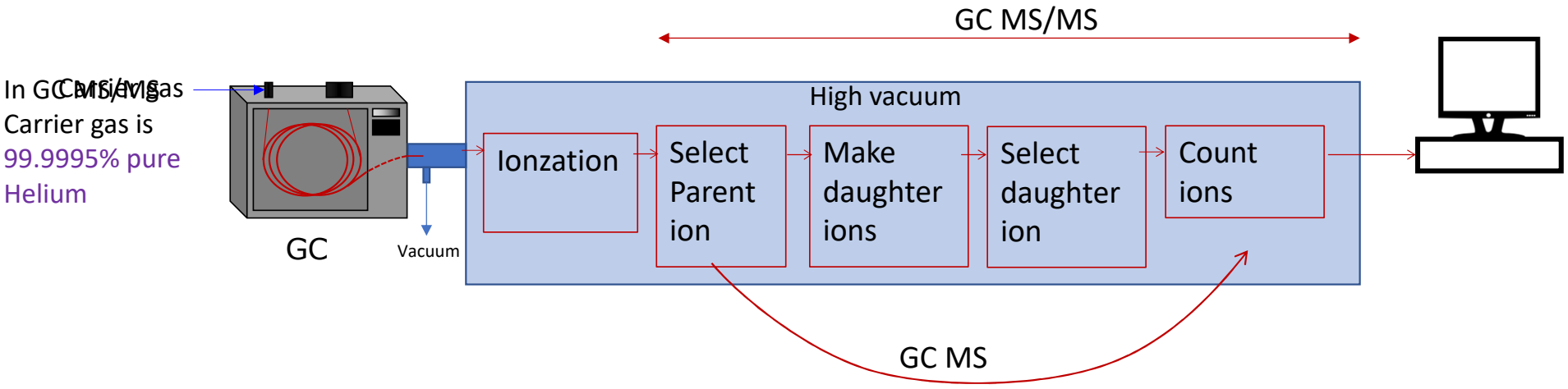
Like in LC-MS, in GC-MS also the vacuum is achieved in two stages:

- 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

GC-MS/MS

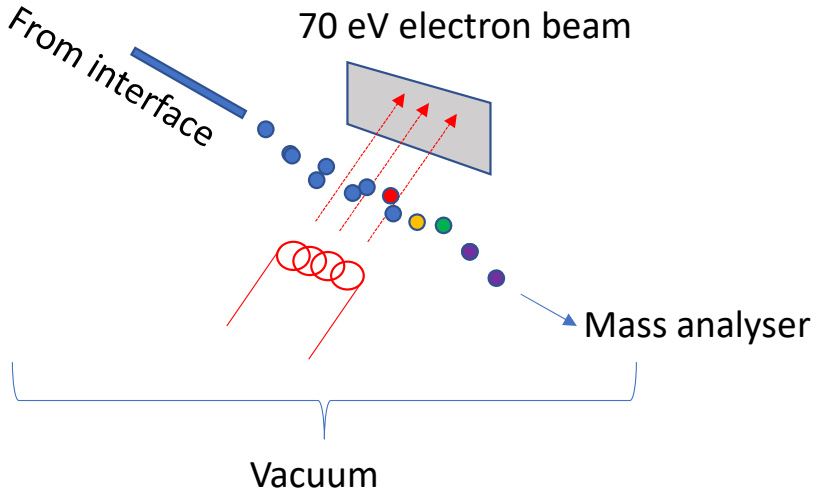
Hyphenation to GC



In GC MS/MS, ionization happens in vacuum
Ionization is hard: Molecular ion mostly doesn't survive
Standard spectrum library is possible in GC MS mode

Ionization

In GC-MS



Molecular Ion

$$M + \bar{e} \rightarrow M^{+\cdot} + 2\bar{e}$$

$$M^{+\cdot} \rightarrow I_1^+ + I_2^+ + m_1 + r^{\cdot} + \ldots$$

Mass spectrum
Which can be
library-matched

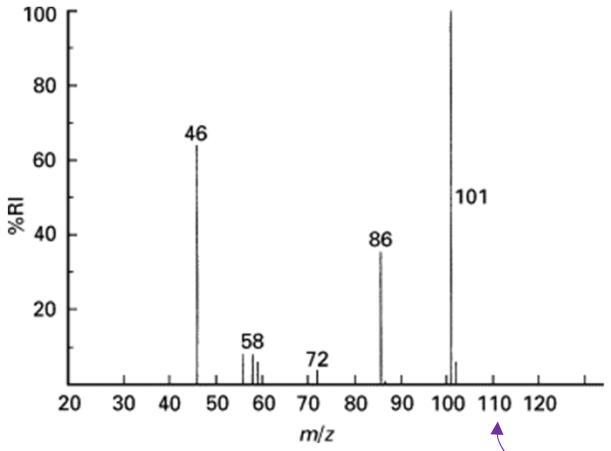
One or more of these ions
can become parent ions
for MS/MS analysis

GC-MS

Identification by library matching (1)

⇒ GC-MS ionization is hard
- ↳ The parent ion, $M^{+\cdot}$, most often does survive
- ↳ It breaks down into several smaller ions
- ↳ These smaller ions can be scanned (one by one, form lowest to highest), and detected
- ↳ A mass spectrum is produced

Base peak - ion with highest intensity (taken as 100%)



If molecular ion survives, it will show up as the ion with highest mass

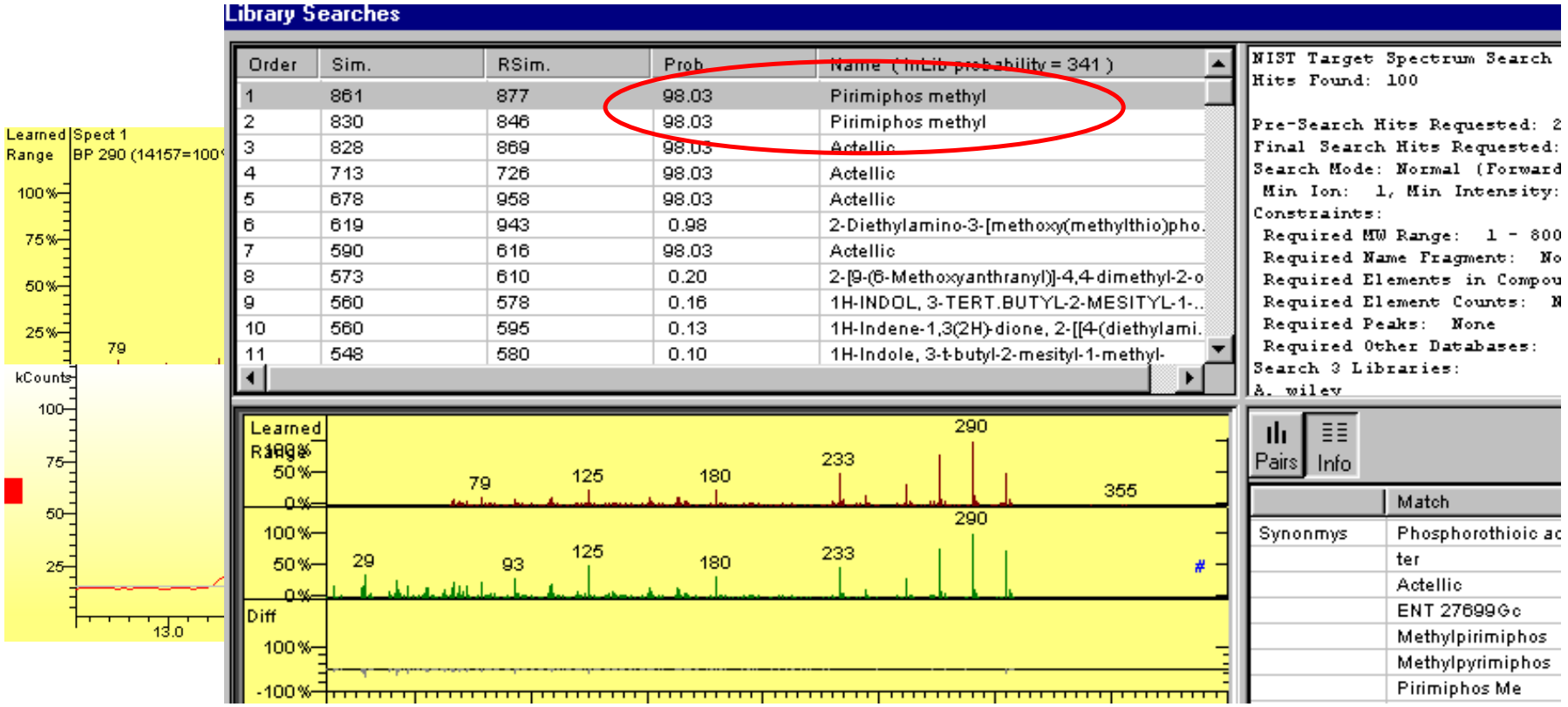
⇒ Conditions are standard (unless we choose to change them):
- ↳ Ionization is at 70 eV
- ↳ Vacuum is set at 10^{-5} torr
- ↳ Under standard conditions spectra produced are also standard
- ↳ Can be matched with standard libraries
  - ↳ NIST, Wily Pesticide Library etc.

NIST National Institute of Standards and Technology U.S. Department of Commerce

WILEY REGISTRY® 12th Edition/NIST 2020 Mass Spectral Library

GC-MS

Identification by library matching (2)



GC-MS is a very useful technique for identification (unlike LC-MS)

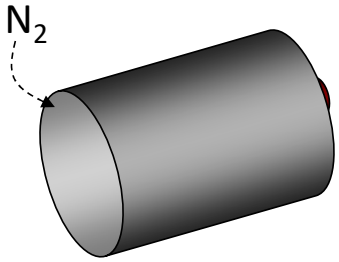
GC-MS/MS

Quantification by MRM mode(1)

⇒ The base peak (or any other intense ion) is selected as the parent ion from the mass spectrum

⇒ Subjected to collision induced dissociation

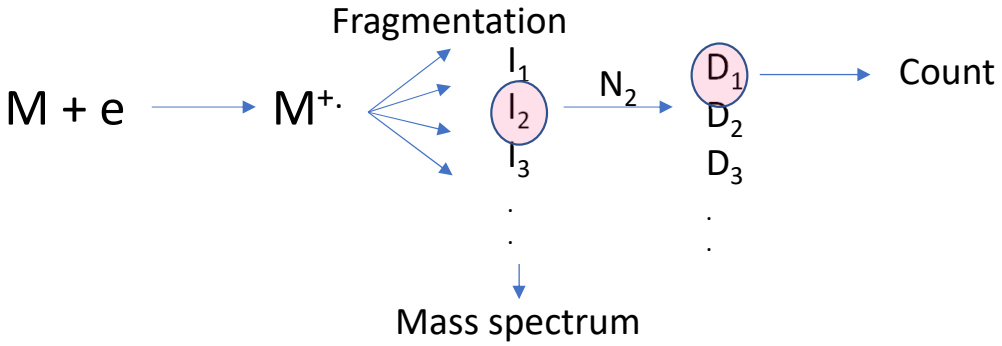
- ↳ In the second quadrupole, N_2 is used as collision gas
- ↳ The parent ion fragments into daughter ions
- ↳ A daughter ion is selected in the third quadrupole, followed by detection



⇒ Multiple reaction monitoring (MRM)

GC-MS/MS

Quantification by MRM mode



"Transition"

$I_2 \rightarrow D_1$

The MRM table

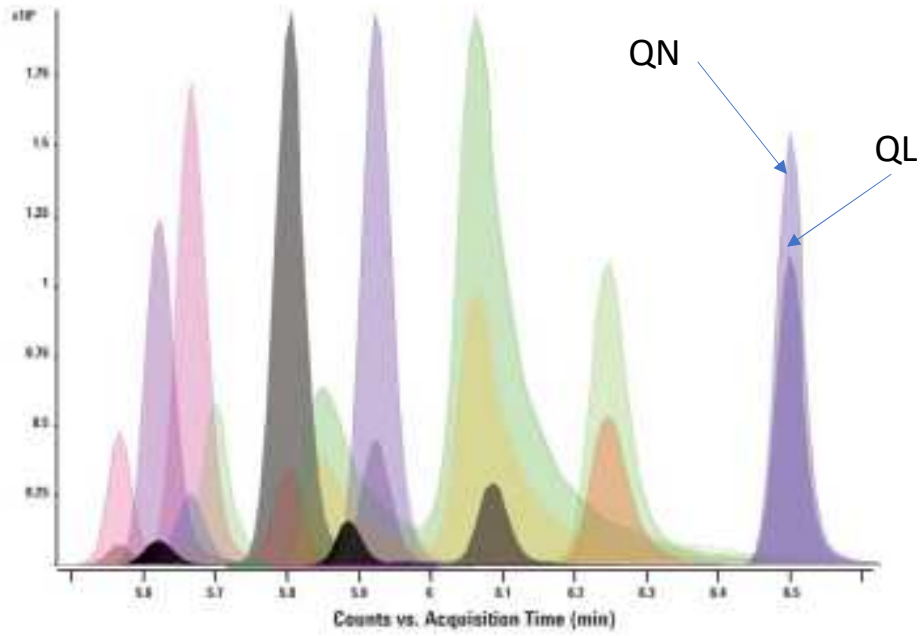
Name	Transition	Dwell time
Compound A	$P_A \rightarrow D_1$	100 ms
Compound A	$P_A \rightarrow D_2$	100 ms
Compound B	$P_B \rightarrow D_1$	100 ms
Compound B	$P_B \rightarrow D_2$	100 ms
….		

In GC-MS, best transitions can vary from matrix to matrix

GC-MS/MS Quantification

Quantifier and qualifier

- 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

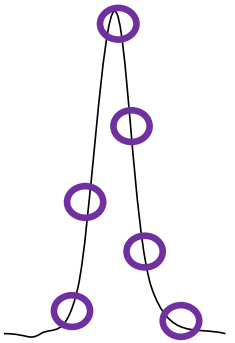


MRM time segmenting

Why?

Issue:

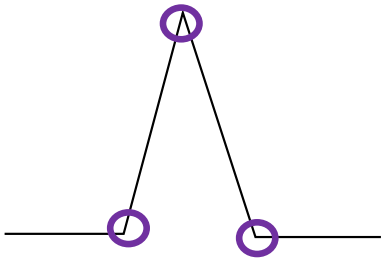
- GC peaks are typically sharp, usually only a few seconds wide
- When number of transitions in the MRM table is high, total cycle time will be high
- Number of data points per peak will be low
- Will affect peak shapes – a minimum of 5 points per peak needed



Number of data points high

Solution:

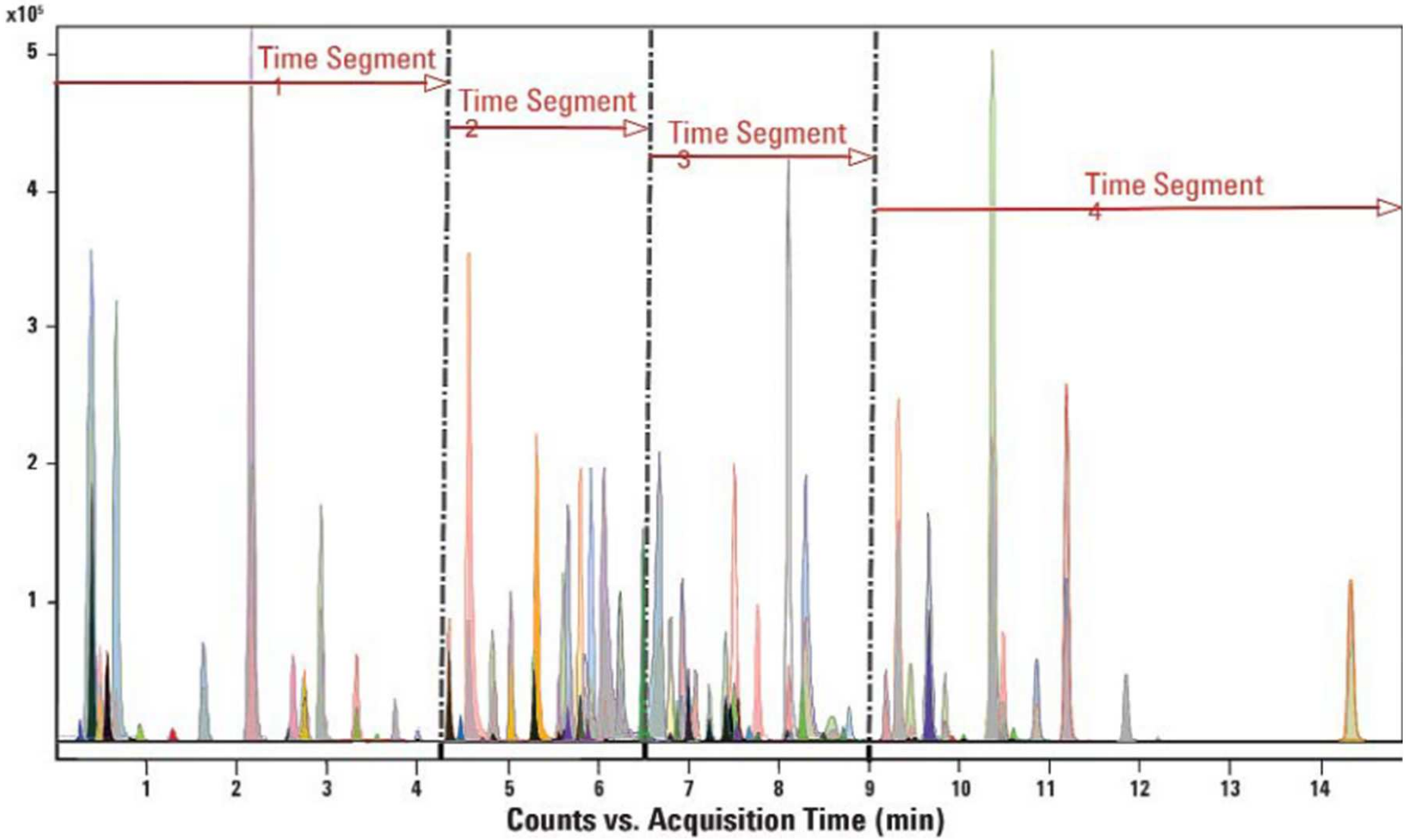
- Segment the total run-time into smaller periods
- Within each period, set an MRM cycle
- Also called 'dynamic' MRM



Number of data points low

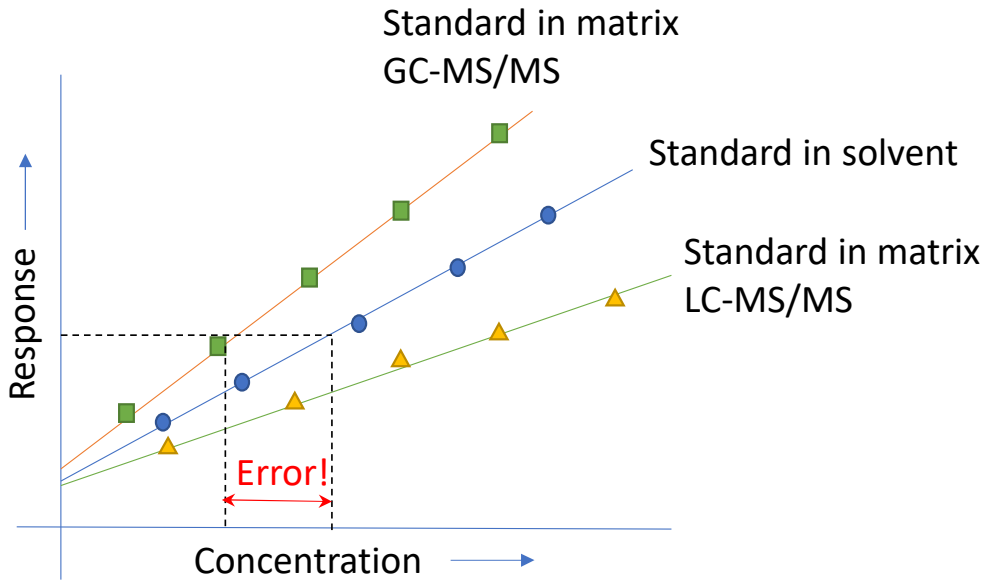
MRM time segmenting (2)

Also applicable in LC-MS/MS



Key difference between GC- and LC-MS/MS

Matrix effect



The calibration curve

Usually,

- Matrix suppression in LC-MS/MS
- Matrix enhancement in GC-MS/MS

We can't use solvent calibration curve for analytical work

Matrix-matched calibration curve required

Problem: availability of blank matrices

Practical aspects in GC-MS

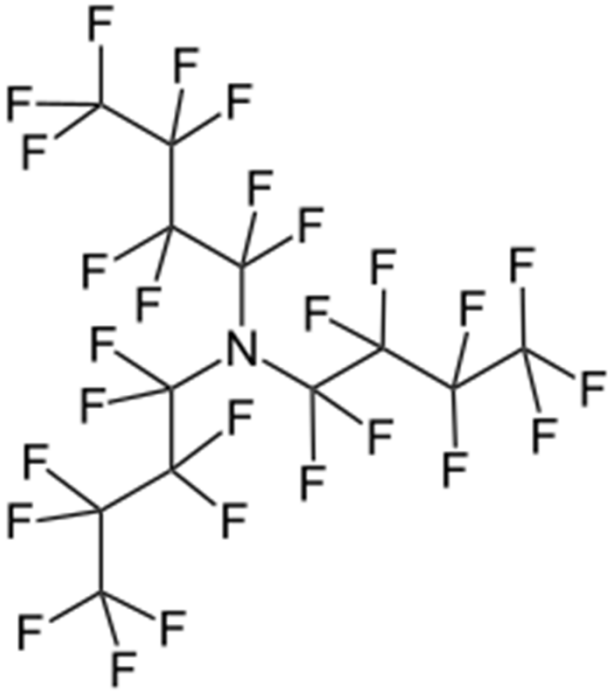
§ Some specific topics

Autotune in GC-MS

Startup checks

⇒ When MS shows errors or low sensitivity, or if there has been an unforeseen shutdown, we perform an autotune

- ↳ involves adjusting several mass spectrometer parameters through the infusion of a tune compound, commonly perfluorotributylamine (PFTBA)
- ↳ Usually the first step in troubleshooting



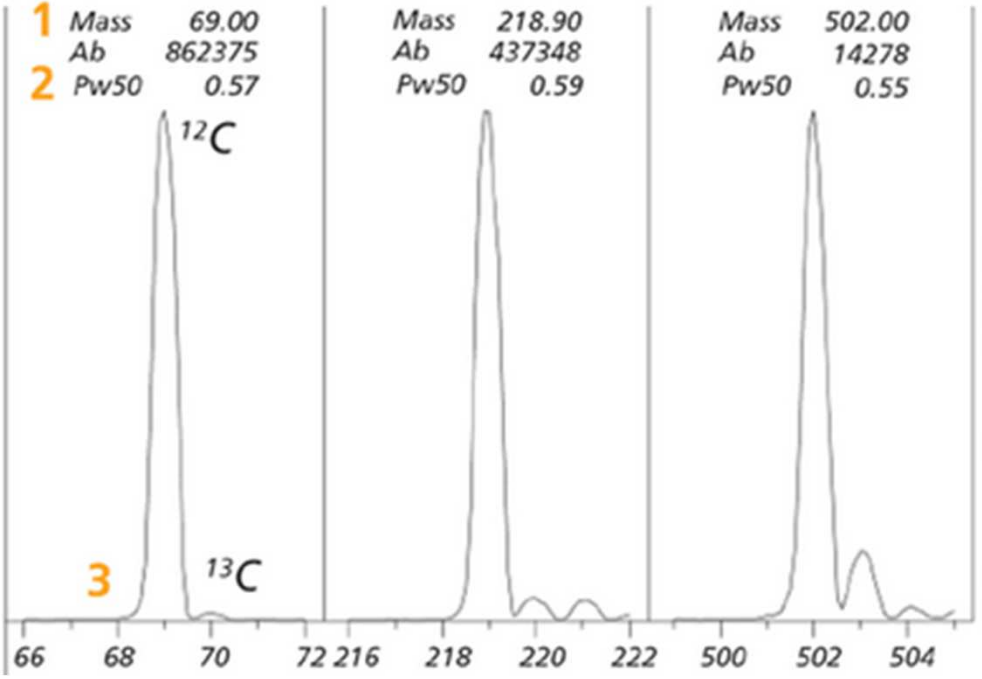
PFTBA

Autotune in GC-MS (2)

Startup checks

⇒ Autotune checks if:

- ↳ air/Water ratio is within limits (leaks and gas purity)
- ↳ background noise is low
- ↳ masses are correctly assigned (61, 219 and 502)
- ↳ the mass peak widths (PW50) are 0.55 ±0.1.
- ↳ the electron multiplier (EM) voltage is around 1500V. Higher values indicate saturation

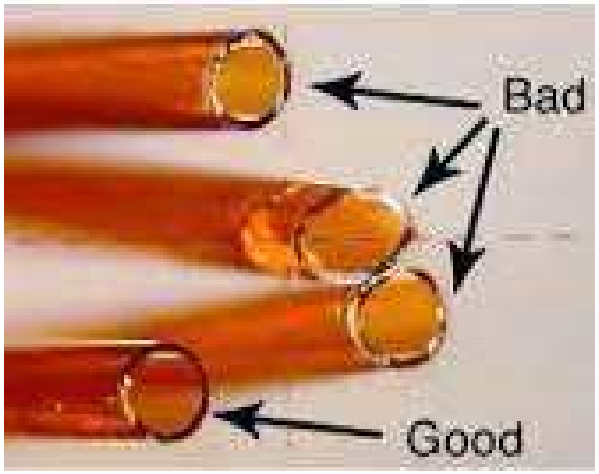


Column trimming

Retention time changes

⇒ After large number of injections, head of the capillary column might become contaminated:

- ↳ Head of the column might need trimming
- ↳ After many trimmings, RT shift might occur
- ↳ Might need to adjust the segmentation in the method
- ↳ RT-locking calculators are available

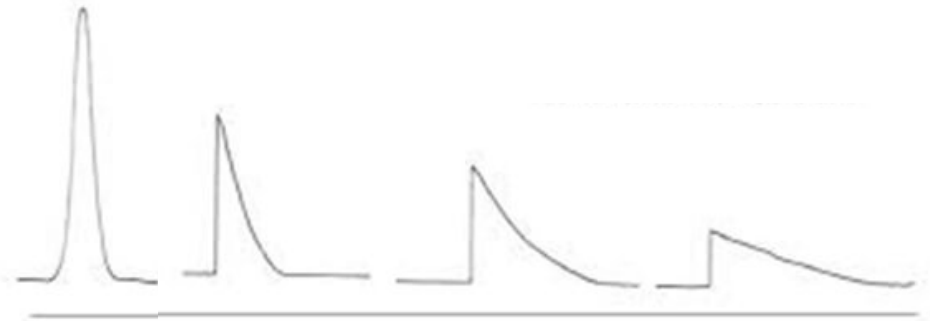


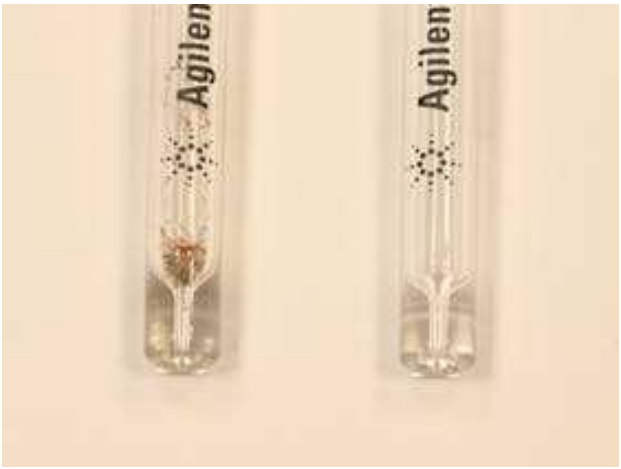
Liner changing

Contamination issues

⇒ After large number of injections, if there is sudden drop in linearity, or tailing of peaks, liner might be the cause:

- ↳ Liners can get contaminated
- ↳ With spices, liner change might be needed within around 100 injections
- ↳ Regeneration and repacking of liners possible





More to learn..

Next session 17th 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?