Food Value Chain Development Project

ASEAN - JICA

OUPUT 2 AJFVC

MATERIALS FOR PESTICIDE RESIDUE ANALYSIS TRAINING HELD IN THE PHILIPPINES FOR CAMBODIAN PARTICIPANTS IN JANUARY 2025

- An Introduction to Gas Chromatography Mass Spectrometry
- GC-MS Importance of Preventive Maintenance And Day-To-Day Basic Troubleshooting
- Analytical Quality Control and Method Validation Procedures For Pesticide Residues Analysis In Food and Feed Sante 11312/2021 V2
- Method Validation Parameters and Criteria (Quantitative)
- Proficiency Testing on Pesticide Residue Analysis
- Estimation of Measurement Uncertainty
- Pesticides: Principle and Classification
- Guidelines on Good Laboratory Practice in Pesticide Residue Analysis
- Liquid Chromatography/ Mass Spectrometry Fundamentals
- Pesticide Residue Analysis Using Modular QuEChERS - BS EN 15662:2018

An Introduction to Gas Chromatography Mass Spectrometry

Pesticide Analytical Laboratory Section
Plant Product Safety Services Division
Bureau of Plant Industry
Quezon City, metro manila, philippines

PRESENTED BY
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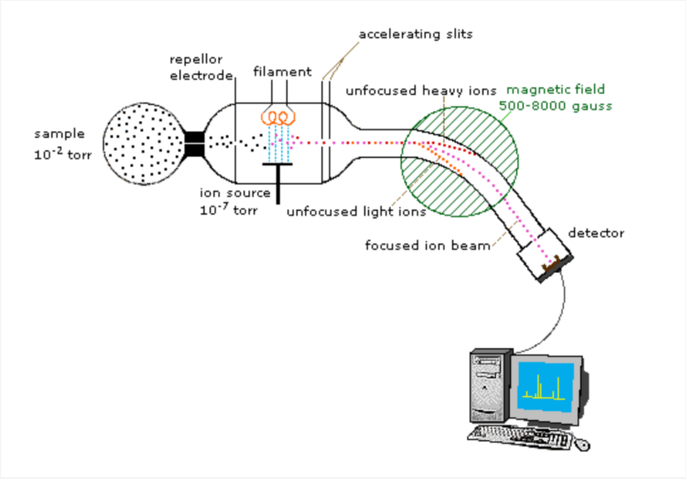
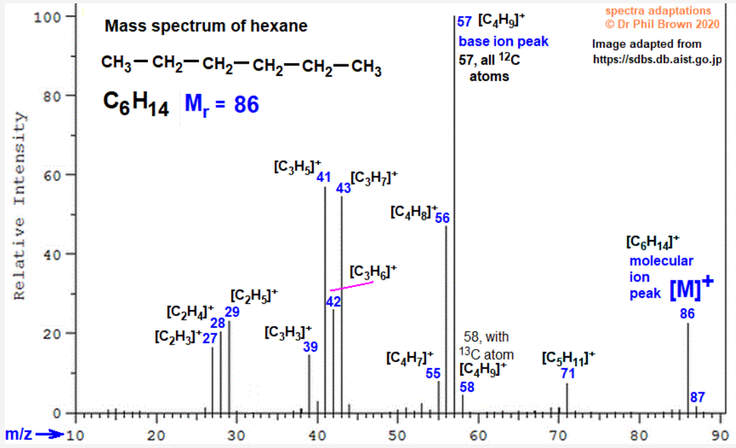
PESTICIDE RESIDUE UNIT

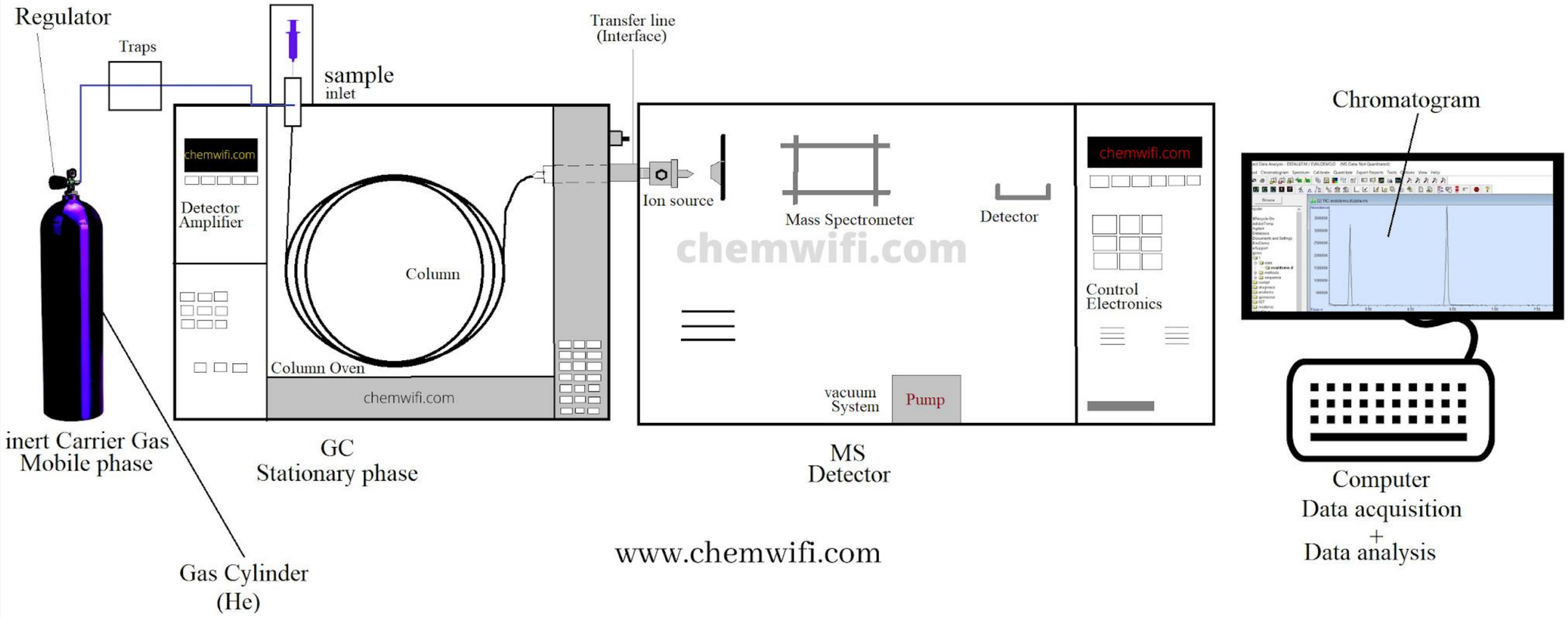




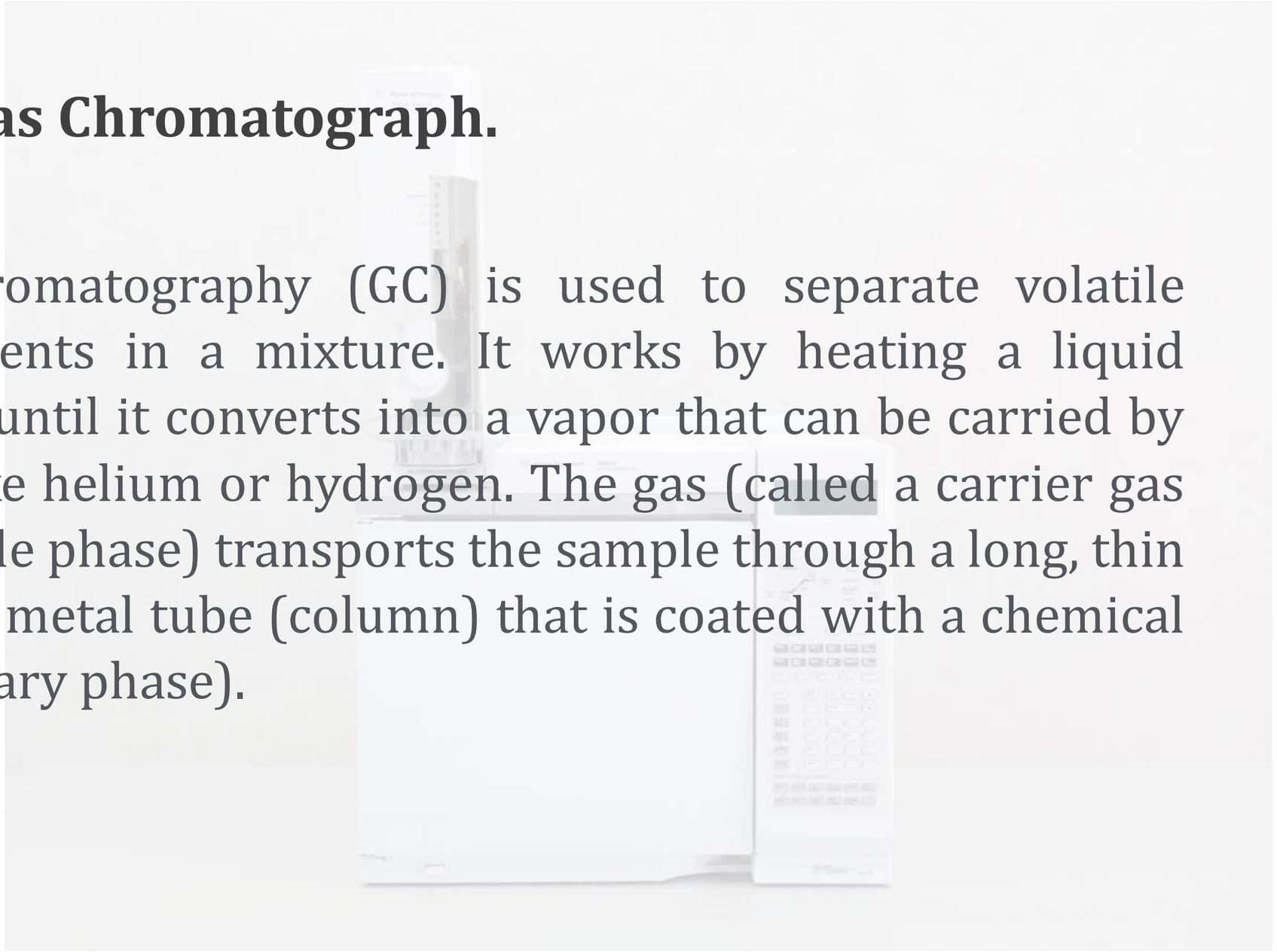
What is Gas Chromatography / Mass Spectrometry?

Gas chromatography/mass spectrometry (GC/MS) combines two analytical tools to identify and measure the concentration of chemicals found in foods, consumer products, pharmaceuticals, fuels, the environment, and more.

Fundamental of GC-MS
(Diagram)



The Gas Chromatograph.

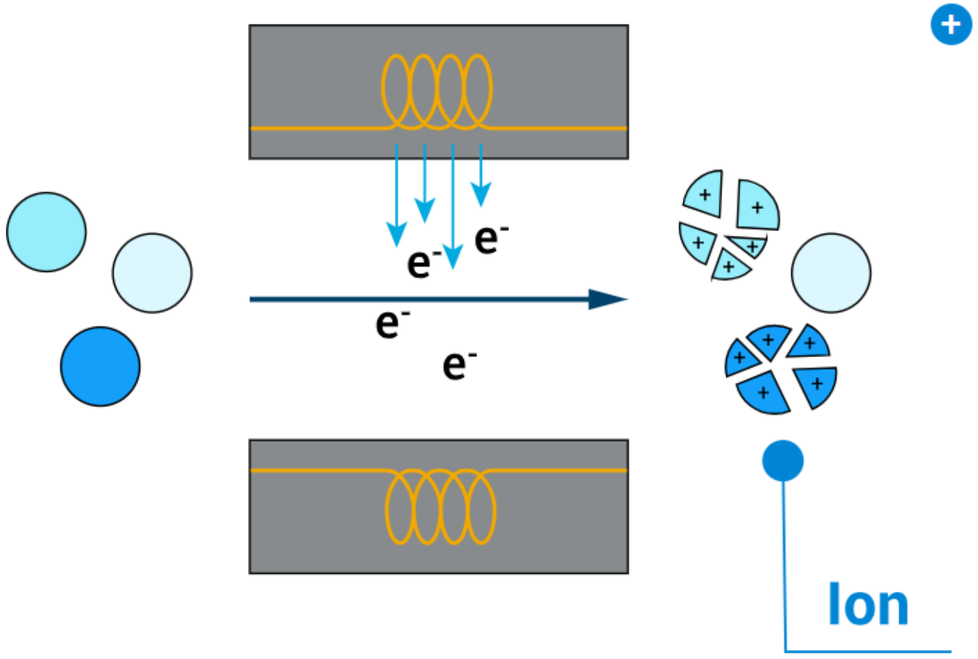


Gas chromatography (GC) is used to separate volatile components in a mixture. It works by heating a liquid sample until it converts into a vapor that can be carried by a gas like helium or hydrogen. The gas (called a carrier gas or mobile phase) transports the sample through a long, thin glass or metal tube (column) that is coated with a chemical (stationary phase).

The Mass Spectrometer

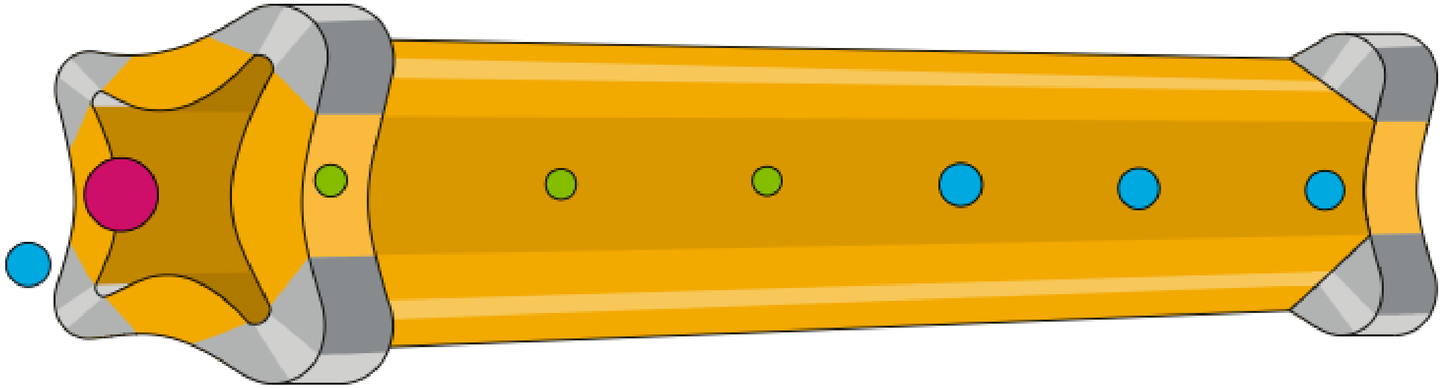
A mass spectrometer (MS) is a detector that identifies and measures the vaporized compounds separated in the GC. While GC provides retention time and peak intensity information, mass spectrometry adds a third dimension: mass information. Mass information can be used to identity, quantify, and determine the structural and chemical properties of molecules.

The first component the chemicals encounter in the mass spectrometer is called the **ion source**, where neutral molecules that elute from the GC column are ionized. A common ion source is an electron ionization (EI) source that usually contains a metal filament, similar to the filament in a light bulb. When an electrical charge is applied to the filament, it emits a stream of electrons at the incoming compounds, breaking them into fragments, and many of them with a positive charge. The pattern of resulting fragments acts as a highly specific “fingerprint” that can be used to identify the chemical.



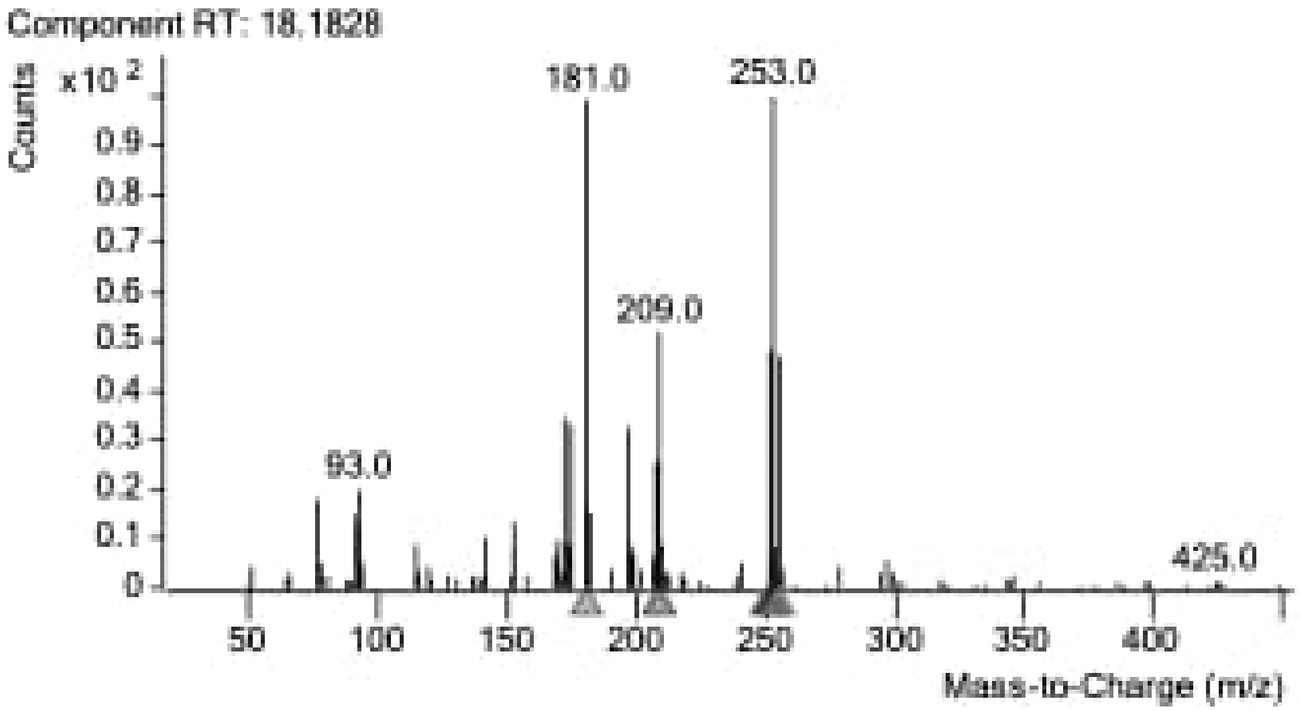
The Mass Spectrometer

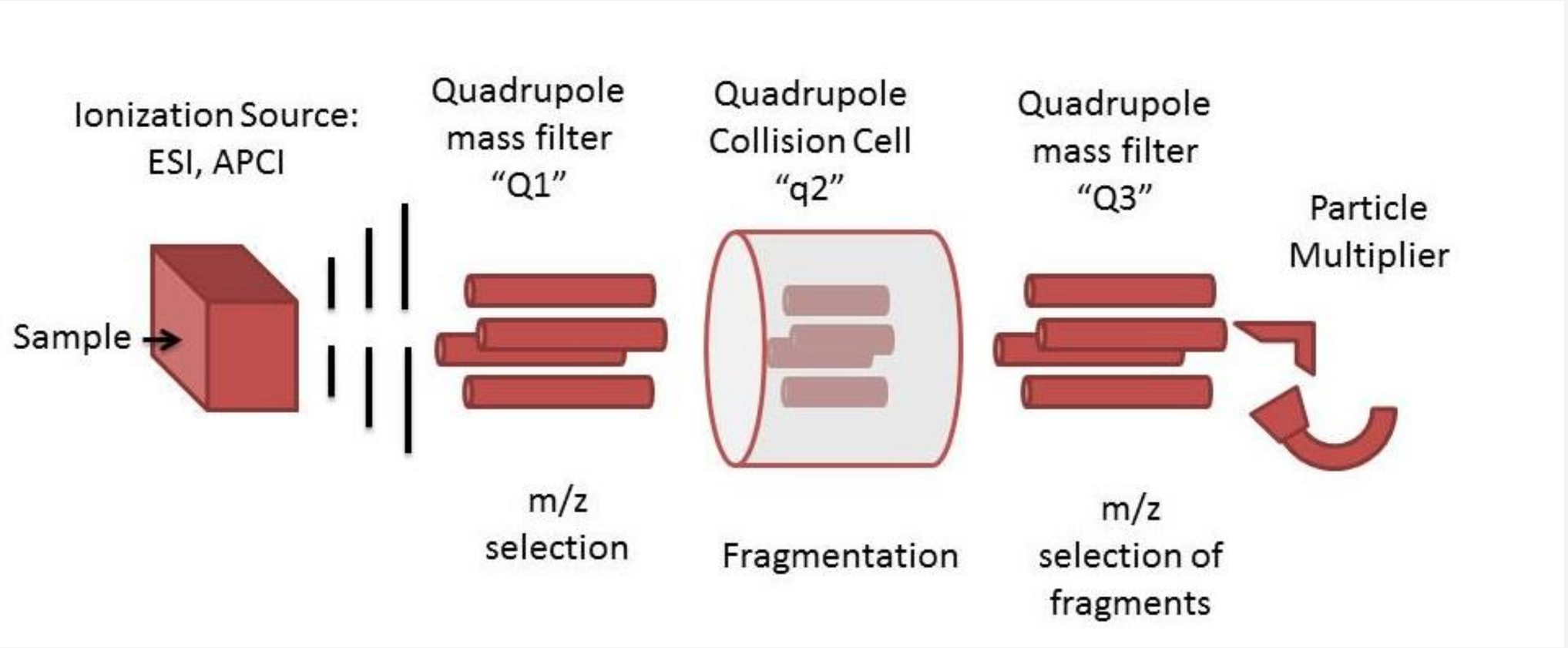
Within the ion source, a series of electrodes known as lenses direct the charged molecules away from the source and into a **quadrupole** mass analyzer (or mass filter). A quadrupole consists of four rods to which a direct current voltage and radio frequency are applied. Various combinations of these forces ensure that only fragments of a specific mass (called a mass-to-charge ratio or m/z) will travel down the electric field of the quadrupole toward the detector at a given time.



The Mass Spectrometer

The mass spectrometer rapidly cycles through different voltages, measuring many m/z ratios. The ions that pass through the instrument are measured by a **detector** called an electron multiplier, which provides a signal intensity for each ion species present. The recorded data at each point in time of an experiment is called a mass spectrum. The pattern of this mass spectrum can be used for identification purposes, much like a fingerprint. The response recorded for the different ion species can be calibrated for quantitative purposes.





A MS/MS system, **consists of two mass analyzers connected in series with a collision or fragmentation cell in between**. Ions are separated in the first mass analyzer (MS1), enter the collision cell and undergo fragmentation, resulting in generation of ions called product ions which are separated in the second mass analyzer (MS2) and detected.

Definitions concerning instruments, mass and *m/z* and ions

What are ions? Ions are atoms, molecules or fragments of molecules that carry one or more positive or negative electrical charges.

What is mass to charge (m/z) ratio? of an ion is the number obtained by dividing the mass of the ion (m) by the number of electrical charges (z) acquired by the sample during the ionization process. The m/z of an ion is dimensionless number:- m and z are always written in italics.

What is *m* in *m/z*? The scales of atomic masses are based upon an agreed standard by IUPAC. Today carbon 12C is taken to have an atomic mass of 12.000000000 Da.

The atomics masses of the other elements and their isotopes are measured relative to this.

12C =12.00000000

1H = 1.007825035

14N =14.003074002

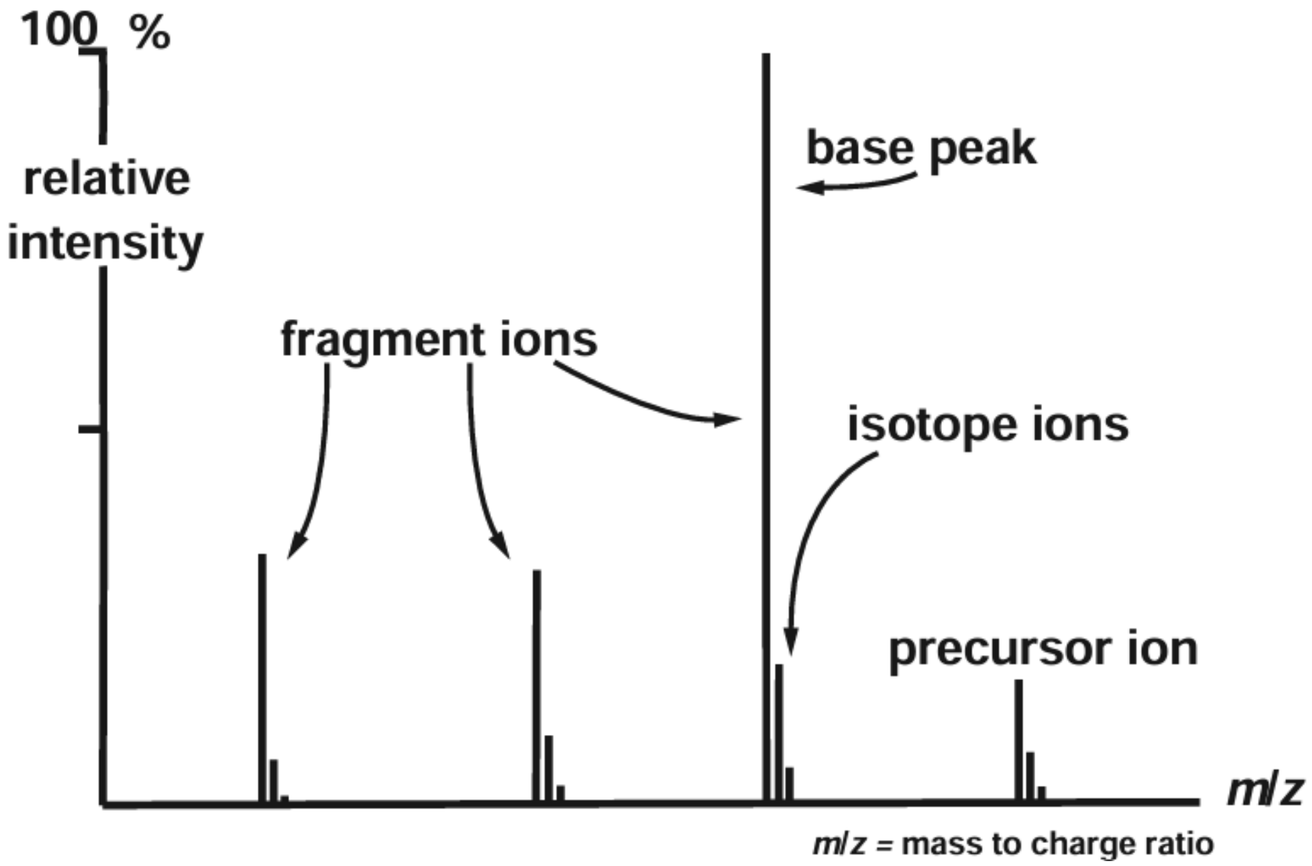
16O =15.99491463

Definitions concerning instruments, mass and *m/z* and ions

What is *z* in *m/z*? The electrical charge (positive of negative) present on an ion is represented by z. In most cases there is only one charge on an ion; thus, the measured m/z value is equivalent to the mass of the ion (z=1)

What is ionic mass? The ionic mass of an ion takes into account the mass of an electron (0.000548Da =0.548 mDa) that is removed or added during the formation of the ion.

What is a mass spectrum?



• Energy is added to molecules during ionization. The distribution of the energy may result in the breaking of chemical bonds and, consequently, in fragment ion formation. The fragmentation may be so extensive that no precursor ion is observed.

• The form of the molecular/precursor ion depends on the mode of ionization and can include for EI [M]+. and CI [M+H]+. [M+NH4]+., for ESI [M]+, [M + H]+ and other adduct ions, e.g., [M + Na]+.

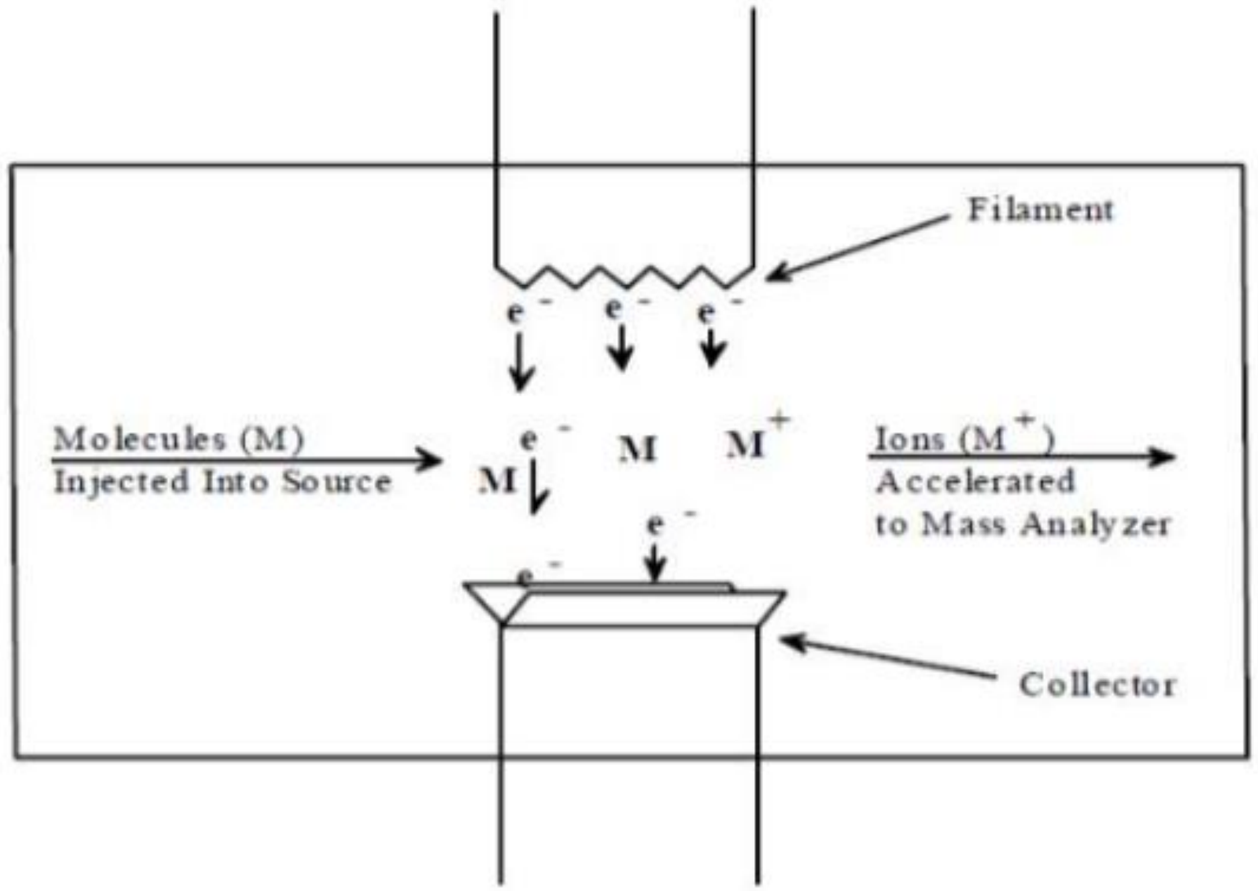
• The **base peak** represents the most stable ion resulting from the ionization process and is, therefore, the most intense (abundant) peak in the spectrum. The intensities of all other ions are usually normalized with respect to the base peak.

• Ions, normally of lesser intensity and to the right of each precursor/fragment ion, generally represent isotopic species. Typically, but not always, isotope ions reflect the presence of carbon-13 (13C).

Types of Ionization

Ionization Method	Typical Analytes	Sample Introduction	Mass Range	Method Highlights
Chemical ionization (CI)	Relatively small, volatile	GC or liquid/solid probe	Up to 1000 Daltons	Soft method, molecular ion peak $[M+H]^+$
Electron Impact Ionization (EI)	Relatively small, volatile	GC or liquid/solid probe	Up to 1000 Daltons	Hard method, versatile, provides structure info
Elecrtospray Ionization (ESI)	Peptides, proteins, nonvolatile	Liquid chromatography	Up to 20000 Daltons	Soft method, ions often multiply charge
Fast Atom Bombardment (FAB)	Carbohydrates, organometallics, peptides, nonvolatile	Sample mix in viscous liquid	Up to 6000 Daltons	Harder than ESI or MALDI
Matrix Assisted Laser Desorption Ionization (MALDI)	Peptides, proteins, nucleotides	Sample mix in solid matrix	Up to 500 Daltons	Soft method, very high mass

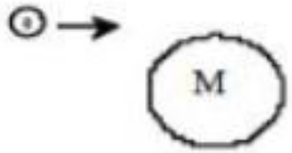
Electron Impact (EI)



1.EI is the most widely used ionization mode in GC/MS analysis. Almost all commercial GCMS instruments equip this mode as standard ionization.
2.EI causes much fragmentation of a molecule whose spectral pattern is useful for identifying sample compound.
3.Library search is available. EI mass spectra obtained by 70eV electron bombardment can be used for identification by comparing with spectra registered in the mass spectral library.
4.An open type of ion source is used. Vacuum pressure inside the source, mainly determined by carrier gas, is about less than 10-2 Pa.

Electron Ionization Process

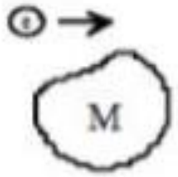
A)



A) Ionizing electron approaches the electron cloud of a molecule

$M+e^- \rightarrow M^+ + e^- + e^-$

B)



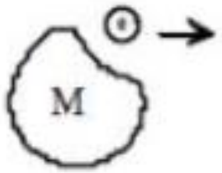
B)Electron cloud distorted by ionizing electron

C)

C) Electron cloud further distorted by ionizing electron

Electron Ionization Process

D)

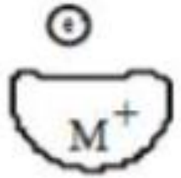


D) Ionizing electron passes by the molecule

E)

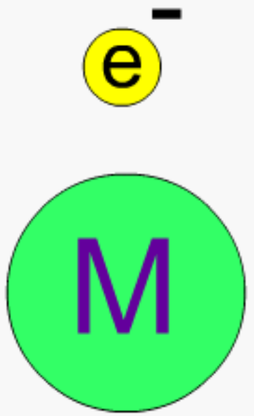
E) Electron cloud of molecule ejecting an electron

F)

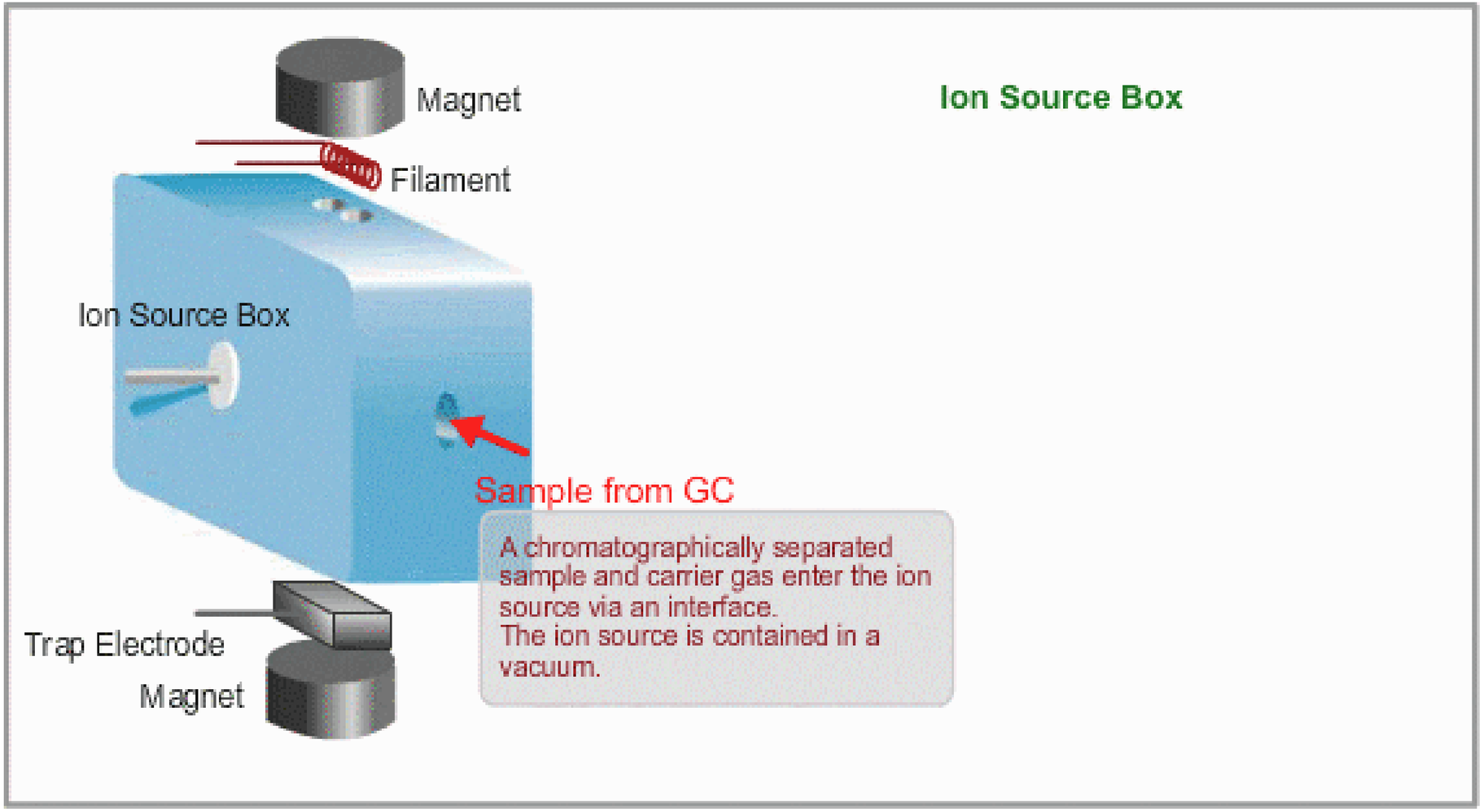


F) Molecular ion and ejected electron.

$M+e^- \rightarrow M^{+*}+e^-+e^- \rightarrow m^+ + R + e^- + e^-$

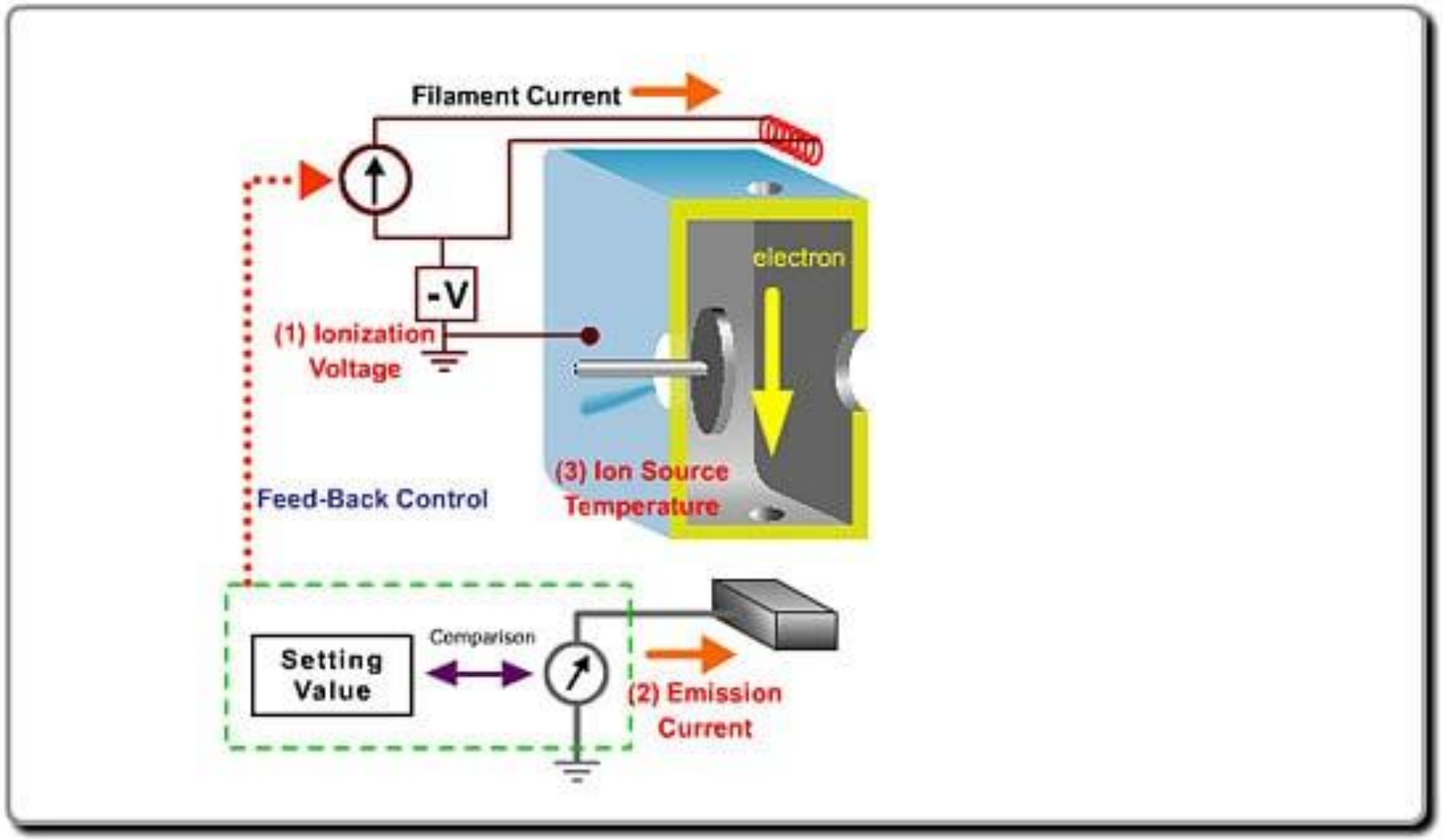


The Ion Source

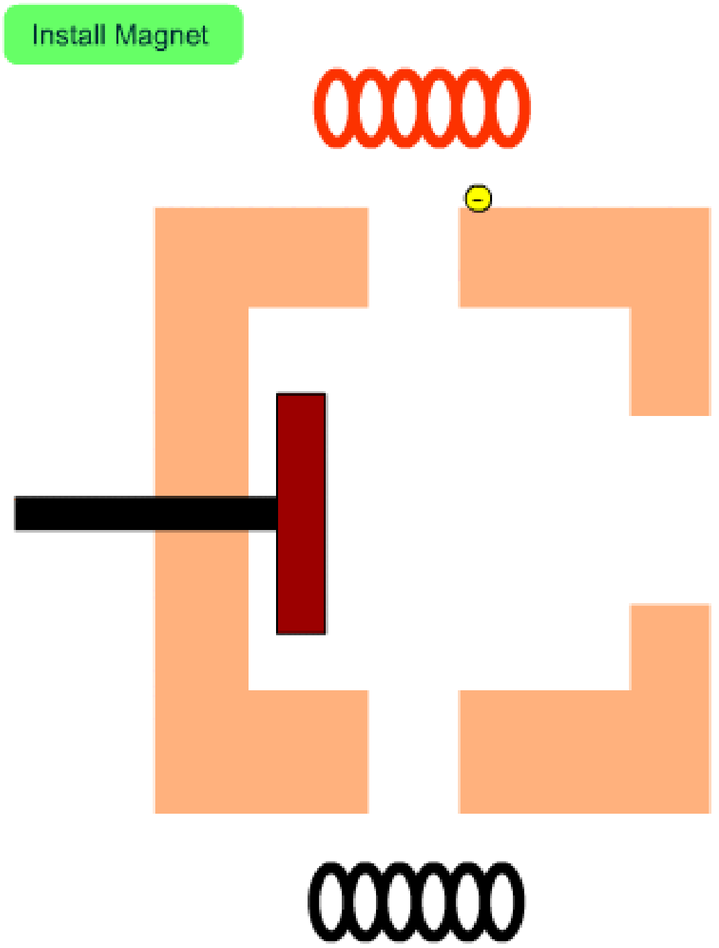


How to Control Ionization

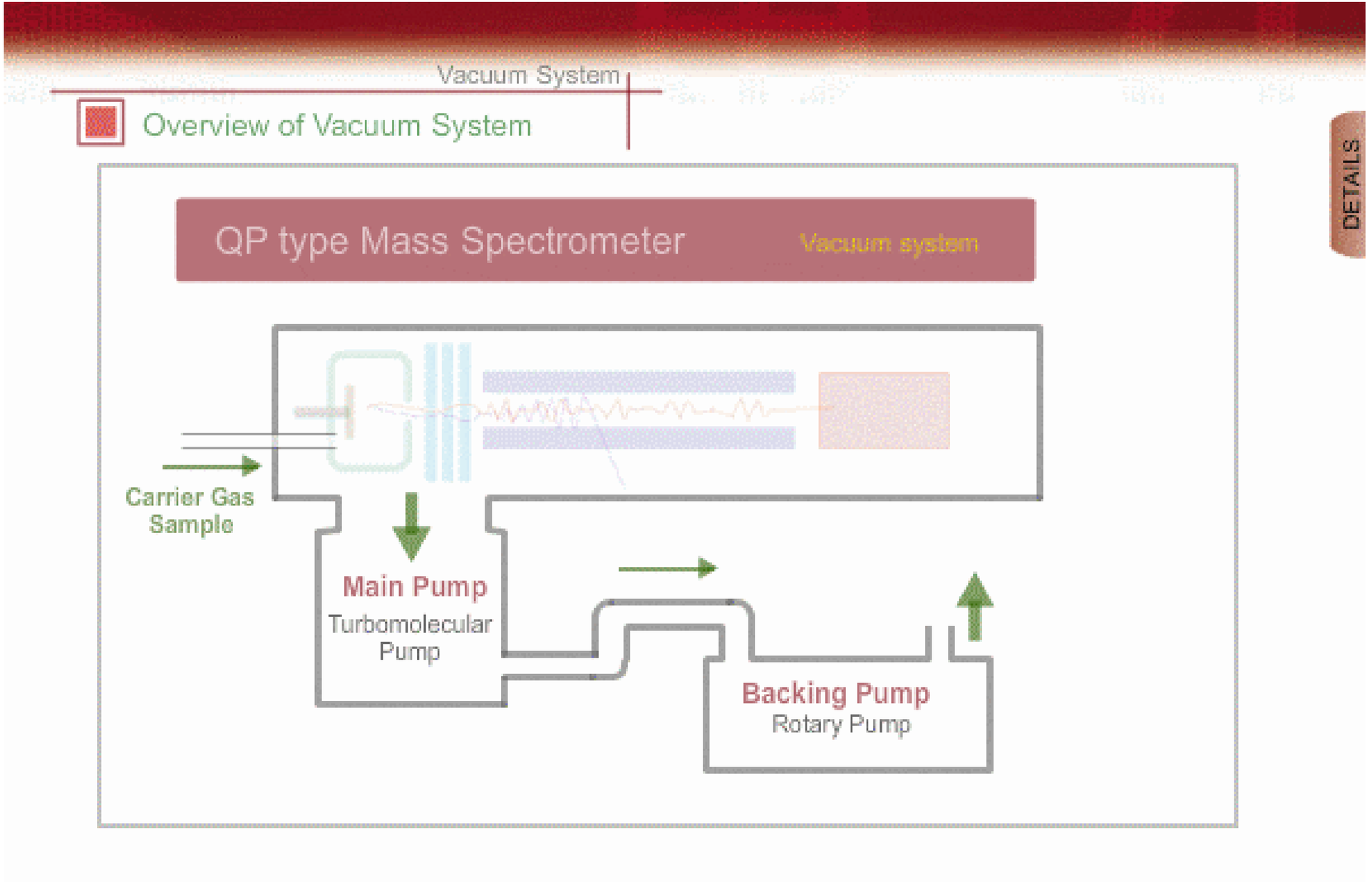
To Control Ionization



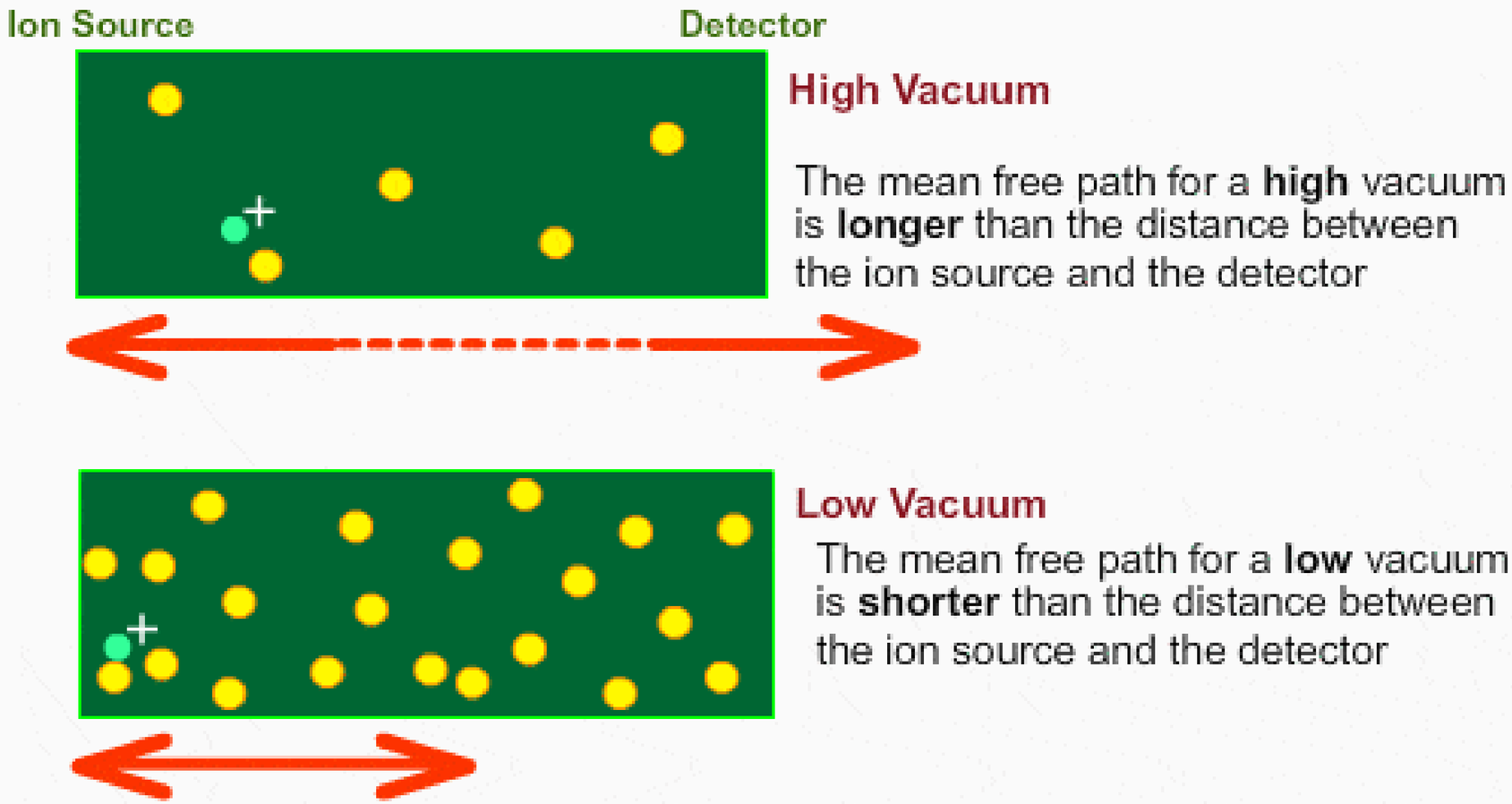
The Role of Magnet



The Vacuum System



Why in Vacuum System?



SCAN vs SIM Mode

SCAN MODE

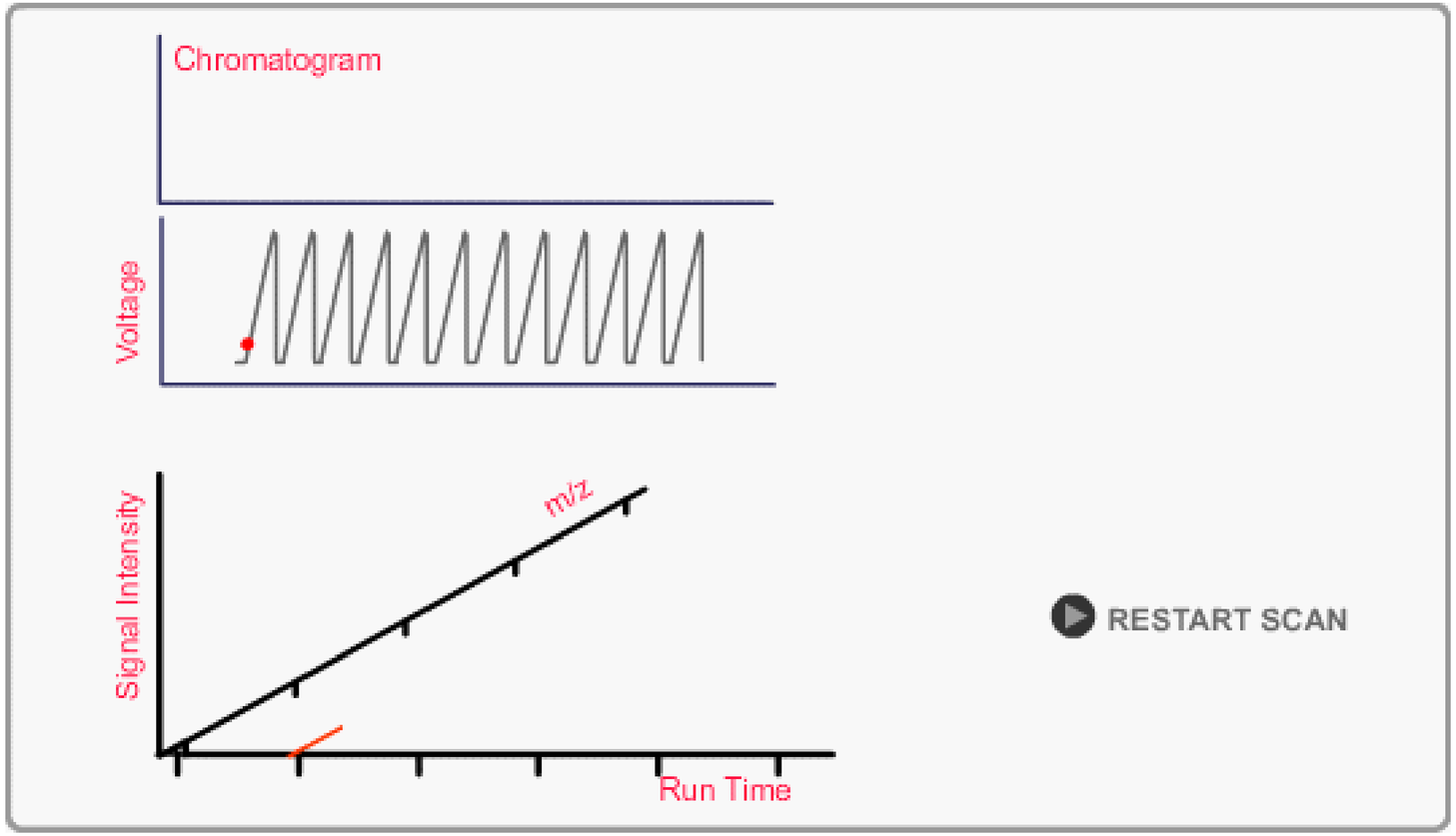
Used for the identification of chemical components using a mass spectrum, quantitative analysis and determination of some parameters for SIM analysis

SIM MODE

Lower detection limits can be obtained with SIM mode than the scan mode in quantitative analysis because of the sensitivity is 10 to 100 times better

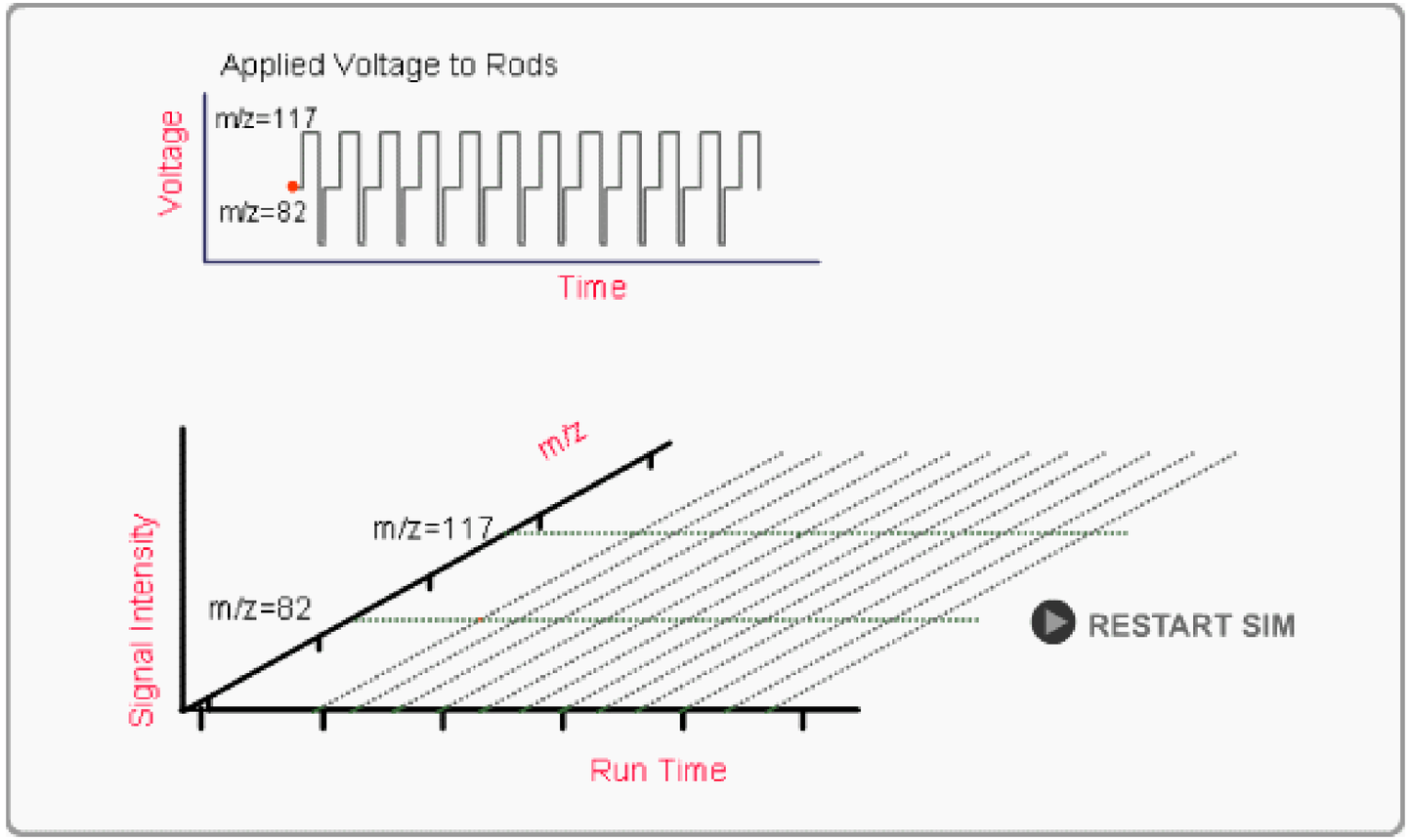
SCAN Mode

Mass spectral data are acquired in sequence at specified intervals, for example 0.5 sec, by changing the voltage applied to rods. All of the measured spectra are stored in a computer to be processed.



SIM Mode

In SIM mode, the mass spectrometer is set to measure only the specified mass, the sensitivity of SIM is tens to hundreds times higher than a SCAN.



GC-MS

IMPORTANCE OF PREVENTIVE MAINTENANCE AND DAY-TO-DAY BASIC TROUBLESHOOTING

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I. GAZA
PESTICIDE RESIDUE UNIT

Today's Discussion

Outline of Topics

01 Why is routine maintenance of your GC essential?

02 Establishing a maintenance schedule

03 Troubleshooting common day-to-day problems

04 Tune report

WHY IS ROUTINE MAINTENANCE OF YOUR GC ESSENTIAL?

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January, 2025

ASEAN JICA Food Value Chain Development Project

ENSURES ACCURACY AND RELIABILITY OF RESULTS

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EXTENDS INTRUMENT LIFESPAN

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EXTENDS INTRUMENT LIFESPAN

MINIMIZES INTRUMENT DOWNTIME

ENSURES SENSITIVITY AND PRECISION

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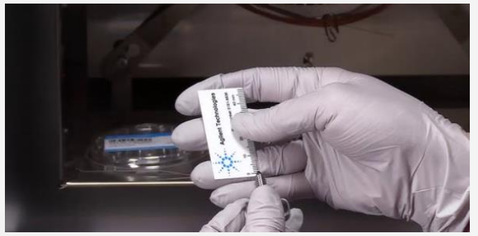
SUPPORTS REGULATORY COMPLIANCE

ENSURES SENSITIVITY AND PRECISION



Investing time in routine maintenance enhances system efficiency, reliability, and accuracy, making it an indispensable part of gas chromatography operations.

SUPPORTS REGULATORY COMPLIANCE

ESTABLISH A MAINTENANCE SCHEDULE

- A scheduled maintenance plan acts as a reminder to check and service the GC regularly, ensuring nothing is overlooked.
- It also helps with planning your workflow

01 BEFORE USE

02 AFTER MULTIPLE INJECTIONS

03 MONTHLY

04 EVERY 3 MONTHS

05 EVERY 6 MONTHS

06 ANNUALY

01 BEFORE USE

- ✓ Check logbook for previous log
- ✓ Check gas tank supply, pressure, & flow
- ✓ Check baseline
- ✓ Tune instrument
- ✓ Trial run your LOQ, check if GC needs cleaning

- Retention time shifts
- Unstable baseline
- Inlet pressure shutdown
- Column damage

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02 AFTER MULTIPLE INJECTIONS

> After 100 injections/as needed

- ✓ Trim column, inlet side
- ✓ Replace inlet liner
- ✓ Replace inlet ferrule, & septa

- Poor peak separation
- Poor peak shape
- Poor reproducibility
- Decreased sensitivity
- Retention time shifts

- Poor peak shape
- Sample carryover
- Poor reproducibility
- Column & detector contamination

- Leak
- Sample carryover
- Poor reproducibility

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03 MONTHLY

- ✓ Switch the ion source filaments from #1 to # 2

- No emission current
- Low or no signal

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- ✓ Check the syringe for visible dirt
- ✓ Clean the syringe plunger if it does not slide smoothly
- ✓ Replace syringe if clogged, bent, or abnormal wear on the septa

- Inaccurate volume injection
- Poor reproducibility
- Peak tailing
- Split peaks

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05 EVERY 6 MONTHS

- ✓ Clean the ion source
- ✓ Replace filament if sagging or uncoiled
- ✓ Replace foreline pump oil discolored
- ✓ Replace gold plated inlet seal if dirty
- ✓ Refill calibration vial if needed

- Decreased sensitivity
- Poor peak shape
- Unstable and noisy baseline
- Ghost peaks
- Failed tuning (Mass calibration)
- Poor reproducibility

- High foreline pressure
- Pump failure
- Instrument shutdown

- Leak
- Retention time shift
- Contamination

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- Poor reproducibility

- High foreline pressure
- Pump failure
- Instrument shutdown

- Leak
- Retention time shift
- Contamination

06 EVERY 12 MONTHS

- ✓ Replace GC column
- ✓ Replace gas clean filters (75% discolored)
- ✓ Preventive maintenance by third party service provider

- Decreased sensitivity
- Poor peak shape
- Ghost peaks
- Poor reproducibility
- Column bleed

01 BEFORE USE

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03 MONTHLY

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Planned maintenance downtime

Unexpected maintenance downtime

04 EVERY 3 MONTHS

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- ✓ Replace syringe if clogged, bent, or abnormal wear on the septa

- Inaccurate volume injection
- Poor reproducibility
- Peak tailing
- Split peaks

ONTHS

- Decreased sensitivity
- Poor peak shape
- Unstable and noisy baseline
- Ghost peaks
- Failed tuning (Mass calibration)
- Poor reproducibility

ging or uncoiled
oil discolored
let seal if dirty
needed

- High foreline pressure
- Pump failure
- Instrument shutdown

- Leak
- Retention time shift
- Contamination

EVERY 12 MONTHS

- ✓ Replace GC column
- ✓ Replace gas clean filters (75% discolored)
- ✓ Preventive maintenance by third party service provider

- Decreased sensitivity
- Poor peak shape
- Ghost peaks
- Poor reproducibility
- Column bleed

KEEP RECORDS AND LOG ALL ACTIVITIES

DATE	ACTIVITY/MAINTENANCE	# OF INJECTIONS	REMARKS	ANALYST
December 10,2024	- Lower GC heated areas to ambient temperature. - Turn off inlet pressure - Replace inlet septa, inlet liner - Trim GC column, inlet side ~ 6in - Replace inlet ferrule, reconnect column - Purge system,(Purge flow to 200ml/min for 10 mins.) - Reload MR1_2025.m & stabilize instrument - Retighten column nut and retainer nut - Tune instrument		Gas saver: OFF Status OK, ready for injection	I. Gaza
December 11,2024	- Load MR1_2025.m - Quick tune instrument - Check retainer nut, retighten - Re-stabilize instrument, 20 minutes - Quick tune instrument - Inject samples MPR-24-2710 to 2730, Market monitoring samples	46	- Nitrogen at 44%, Oxygen at 11%, possible leak - Status OK, ready for injection	I. Gaza M. Alava
December 13,2024	- Retrieve MPR-24-2710 to 2730		r^2= 0.998 MDL = 0.005ppm	M. Alava

COMMON DAY-TO-DAY PROBLEMS

AIR LEAKS

Symptoms

- higher than normal foreline pressure
- higher than normal background noise
- 4:1 oxygen-to-nitrogen ratio in tune
- Peak characteristics of air:
  - m/z 18: Water (H_2O)
  - m/z 28: Nitrogen (N_2)
  - m/z 32: Oxygen (O_2)
  - m/z 44: Carbon Dioxide (CO_2)
  - m/z 16: Atomic Oxygen (O)

Cause:

- Commonly due to loose seals or nuts
- Broken inlet liner or column

What to do:

- Tighten retainer nut
- Tighten inlet and MSD column nut,use self-tightening nuts if available
- Check inlet liner, septum and ferrule
- Check GC column, check for small cracks
- Purge system at 200ml/min for 10 mins.

COMMON DAY-TO-DAY PROBLEMS

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- Check GC column, check for small cracks
- Purge system at 200ml/min for 10 mins.

POOR PEAK SYMMETRY, GHOST PEAKS etc.

Symptoms

- Peak broadening
- Peak fronting/tailing
- Peak splitting
- Ghost peaks
- Inconsistent response
- Decrease in sensitivity
- Sample carryover
- RT shift
- Poor repeatability
- No peak

Cause:

- Contaminant build up in the front end of column, Inlet contamination, dirty Gold seal, dirty syringe, worn out septa/ferrule

What to do:

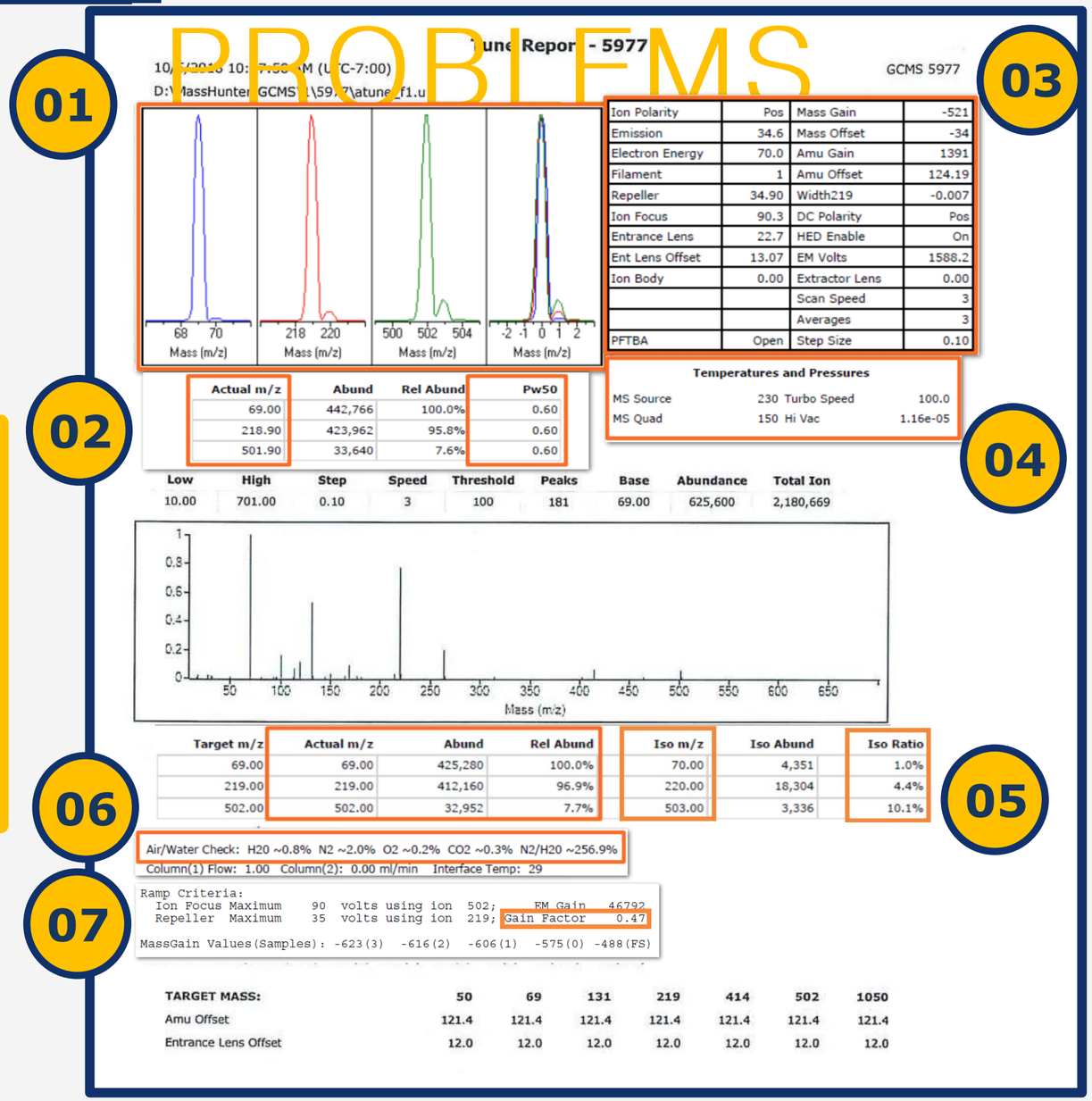
- Check method
- Check syringe
- Trim column at inlet side, replace inlet liner, ferrule, and septum
- Bakeout column
- Bakeout MSD

COMMO DAY-TO-DAY PROBLEMS

OUT OF BOUNDS TUNE

- May be due to air leaks
- MS needs more time to stabilize
- Calibrant level is low
- Dirty ion source
- EM horn needs replacing
- Pump oil needs replacing

1. **Mass peak profiles of PFTBA perfluorotributylamine (m/z 69, 219 and 502)**
2. **Actual m/z and PW50 of (m/z 69, 219 and 502)**
3. **MS parameter**
4. **Temperatures and pressures during the tune**
5. **Spectrum scan result**
6. **Air/water checklist**
7. **Autotune report gain factor**



Ion Polarity	Pos	Mass Gain	-521
Emission	34.6	Mass Offset	-34
Electron Energy	70.0	Amu Gain	1391
Filament	1	Amu Offset	124.19
Repeller	34.90	Width219	-0.007
Ion Focus	90.3	DC Polarity	Pos
Entrance Lens	22.7	HED Enable	On
Ent Lens Offset	13.07	EM Volts	1588.2
Ion Body	0.00	Extractor Lens	0.00
		Scan Speed	3
		Averages	3
PFTBA	Open	Step Size	0.10

Actual m/z	Abund	Rel Abund	Pw50
69.00	442,766	100.0%	0.60
218.90	423,962	95.8%	0.60
501.90	33,640	7.6%	0.60

Low	High	Step	Speed	Threshold	Peaks	Base	Abundance	Total Ion
10.00	701.00	0.10	3	100	181	69.00	625,600	2,180,669

Target m/z	Actual m/z	Abund	Rel Abund	Iso m/z	Iso Abund	Iso Ratio
69.00	69.00	425,280	100.0%	70.00	4,351	1.0%
219.00	219.00	412,160	96.9%	220.00	18,304	4.4%
502.00	502.00	32,952	7.7%	503.00	3,336	10.1%

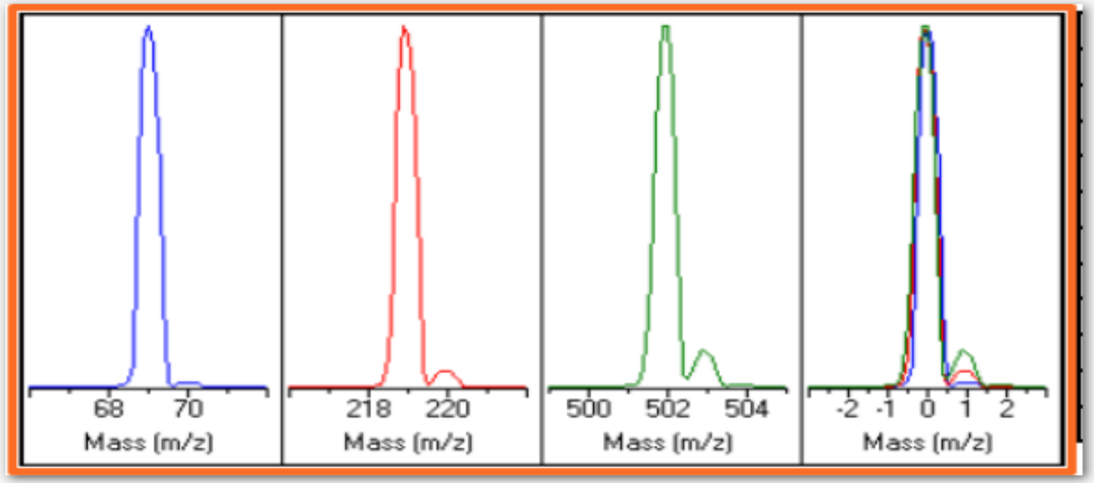
TARGET MASS:	50	69	131	219	414	502	1050
Amu Offset	121.4	121.4	121.4	121.4	121.4	121.4	121.4
Entrance Lens Offset	12.0	12.0	12.0	12.0	12.0	12.0	12.0

CHECKING THE AUTOTUNE REPORT

1. Mass peak profiles of PFTBA

- Shows you the signal for the three main ions from PFTBA that the instrument tunes on.

✓The mass peak profiles should be smooth, symmetrical and even in width, the mass peak profiles should not have visible noise, splits, or precursors visible.



2. Actual m/z and PW50 of (m/z 69, 219 and 502)

- This section presents the measured mass-to-charge ratios (m/z) and the peak widths at half maximum (PW50) for the tuning ions.

✓Actual m/z should be within +/- 0.2 m/z of the actual mass at m/z 69.0, 219.0, and 502.0.
✓The PW50 of all masses should be 0.60 +/- 0.05 m/z if the target width is set to 0.60.

Actual m/z	Abund	Rel Abund	Pw50
69.00	442,766	100.0%	0.60
218.90	423,962	95.8%	0.60
501.90	33,640	7.6%	0.60

CHECKING THE AUTOTUNE REPORT

3. MS parameter

- Tells you which settings the instrument is using to obtain the data presented.

✓A clean ion source with a new EM horn typically has a gain voltage of 1400–1600 V. *Clean ion source or replace EM horn if EM volts is repeatedly at 2800-3000V*

4. Temperature and pressure

- The temperatures and pressures actual values of the Mass Spectrometer during the tune

Ion Polarity	Pos	Mass Gain	-521
Emission	34.6	Mass Offset	-34
Electron Energy	70.0	Amu Gain	1391
Filament	1	Amu Offset	124.19
Repeller	34.90	Width219	-0.007
Ion Focus	90.3	DC Polarity	Pos
Entrance Lens	22.7	HED Enable	On
Ent Lens Offset	13.07	EM Volts	1588.2
Ion Body	0.00	Extractor Lens	0.00
		Scan Speed	3
		Averages	3
PFTBA	Open	Step Size	0.10

Temperatures and Pressures

MS Source	230	Turbo Speed	100.0
MS Quad	150	Hi Vac	1.16e-05

CHECKING THE AUTOTUNE REPORT

5. Mass spectrum result

- displays the spectrum scan data

Target m/z	Actual m/z	Abund	Rel Abund	Iso m/z	Iso Abund	Iso Ratio
69.00	69.00	425,280	100.0%	70.00	4,351	1.0%
219.00	219.00	412,160	96.9%	220.00	18,304	4.4%
502.00	502.00	32,952	7.7%	503.00	3,336	10.1%

✓Measured Mass Assignment (Actual m/z) should be within +/- 0.1 m/z of 69.0, 219.0, and 502.0.

✓Relative Mass Abundance (Rel Abund) should be >40% for m/z 219 and >2% for m/z 502.

✓Isotope Measured Mass (Iso m/z) should be within +/- 0.1 m/z of 70.0, 220.0, and 503.0.

✓Isotope Abundance (Iso Abund) should all be > 1000 counts.

✓Isotope Ratio (Iso Ratio) should be close to the theoretical values (m/z 70 at 1.08%, m/z 220 at 4.32% and m/z 503 at 10.09%).

CHECKING THE AUTOTUNE REPORT

6. Air/water checklist

- displays the spectrum scan data

Target m/z	Actual m/z	Abund	Rel Abund	Iso m/z	Iso Abund	Iso Ratio
69.00	69.00	425,280	100.0%	70.00	4,351	1.0%
219.00	219.00	412,160	96.9%	220.00	18,304	4.4%
502.00	502.00	32,952	7.7%	503.00	3,336	10.1%

✓H2O: < 20%, N2: < 5%, O2:< 1.5%
for systems under vacuum and at default operating temperature for at least 2hrs.
For >24 hrs, water <5%.

7. Gain factor result of current autotune

- displays the gain factor of current tune, gain factor is the calculation of the current sensitivity of the system.
- Typically a clean ion source and new Filaments has a gain factor of 0.3 to 0,5.

```
Ramp Criteria:
  Ion Focus Maximum     90  volts using ion  502;      EM Gain    46792
  Repeller  Maximum     35  volts using ion  219;  Gain Factor     0.47

MassGain Values(Samples): -623(3)  -616(2)  -606(1)  -575(0)  -488(FS)
```

✓Clean ion source if gain factor reaches 2 to 3 times of the present gain factor.

ANALYTICAL QUALITY CONTROL AND METHOD VALIDATION PROCEDURES FOR PESTICIDE RESIDUES ANALYSIS IN FOOD AND FEED SANTE 11312/2021 v2

Pesticide Analytical Laboratory Section
Plant Product Safety Services Division
Bureau of Plant Industry
Quezon City, metro manila, philippines

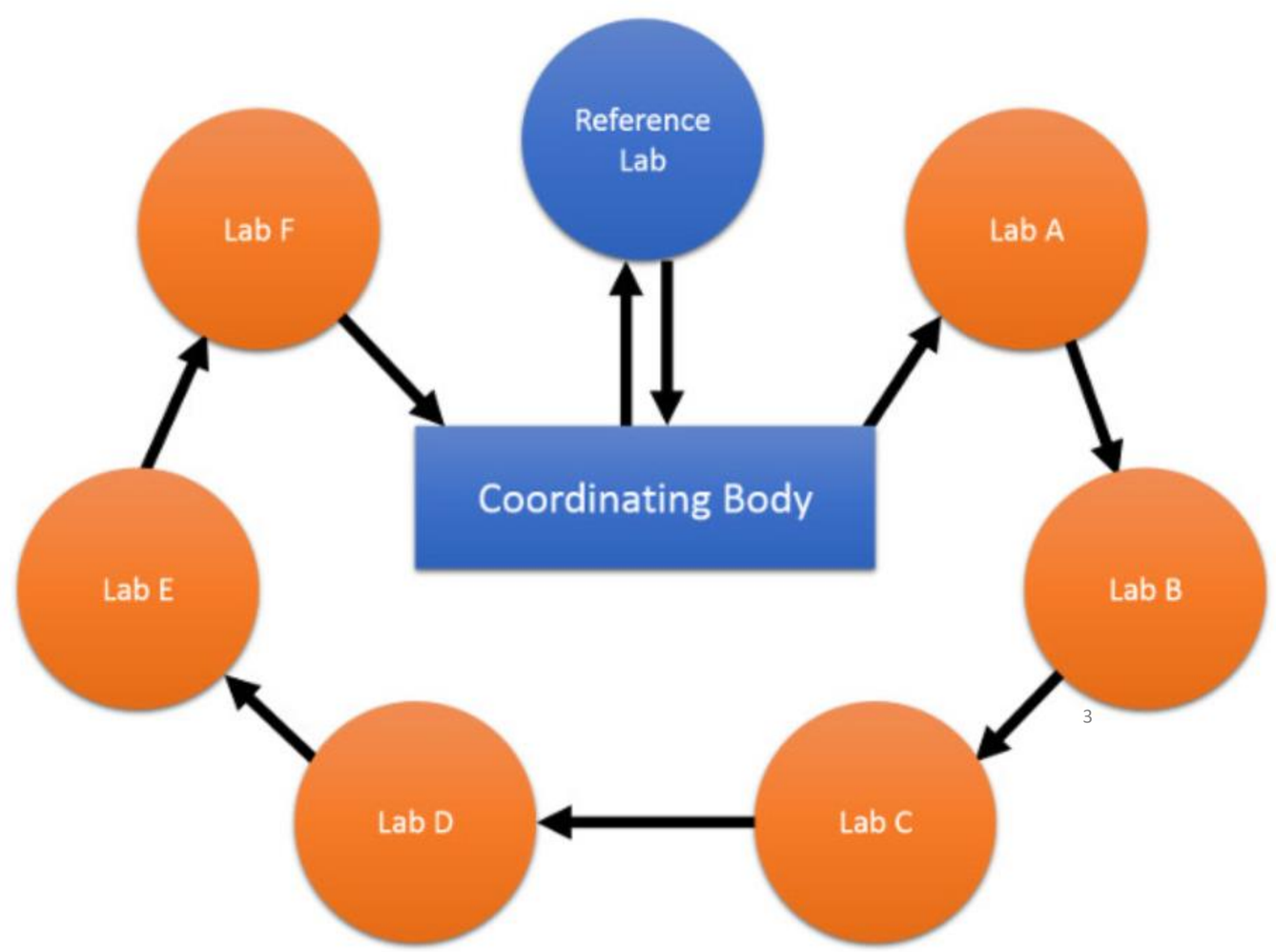
PRESENTED BY
JULIO SALVADOR C. VALEZA
PESTICIDE RESIDUE UNIT

METHOD VALIDATION

"A process that is used to demonstrate the suitability of an analytical method for an intended purpose"

"Documented program which provides a high degree of assurance that an analytical method will consistently determine the presence, absence or quality of one or more attributes with predetermined acceptance criteria"

2



- A coordinating body sends a test item to a reference laboratory for testing. Then, the coordinating body sends the item to each participating laboratory for subsequent testing.
- Each participant laboratory will independently test the item, submit their results to the coordinating body, and forward the item to the next participating laboratory.
- After each participating laboratory has completed testing, the artifact is returned to the coordinating body.
- The coordinating body will evaluate the all the test results and issue a performance report to each participating laboratory.
- This is typically referred to as Round Robin Testing, and is one of the most common proficiency testing schemes used by PT providers.

Why Validate

01 Regulatory Guidelines Requirement

02 Customer expectation

03 Suitable in specific needs

ISO 17025:2017 ACCREDITED LABORATORY

When to Validate?

- Before initial use in routine testing
- When transferred to another laboratory
- Whenever the conditions or method parameters for which the method has been validated change and the change is outside the original scope of the method
- When an established method is used in a different laboratory, with different analysts and different equipment

What to Validate?

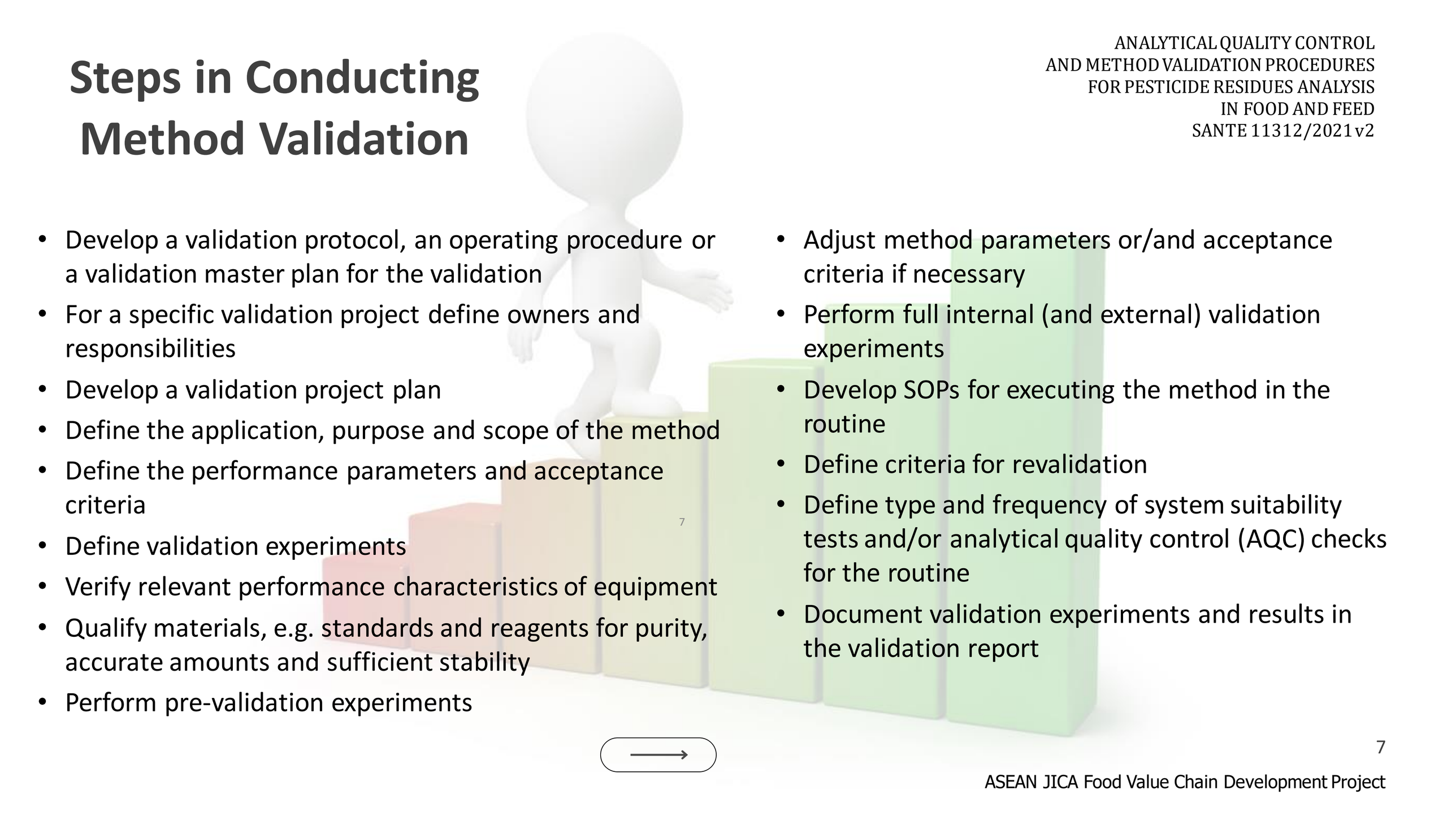
- Laboratory developed method
- Non standard methods

Points to Consider in Method Validation

The scope of the method and its validation criteria should be defined early in the process. These include the following questions:

- What analytes should be detected
- What are the sample matrices
- Are there any specific legislative or regulatory requirements
- Should it be qualitative or quantitative
- What are the required detection and quantitation limits
- What precision and accuracy is expected
- Which type of equipment to be used

Steps in Conducting Method Validation



- Develop a validation protocol, an operating procedure or a validation master plan for the validation
- For a specific validation project define owners and responsibilities
- Develop a validation project plan
- Define the application, purpose and scope of the method
- Define the performance parameters and acceptance criteria
- Define validation experiments
- Verify relevant performance characteristics of equipment
- Qualify materials, e.g. standards and reagents for purity, accurate amounts and sufficient stability
- Perform pre-validation experiments

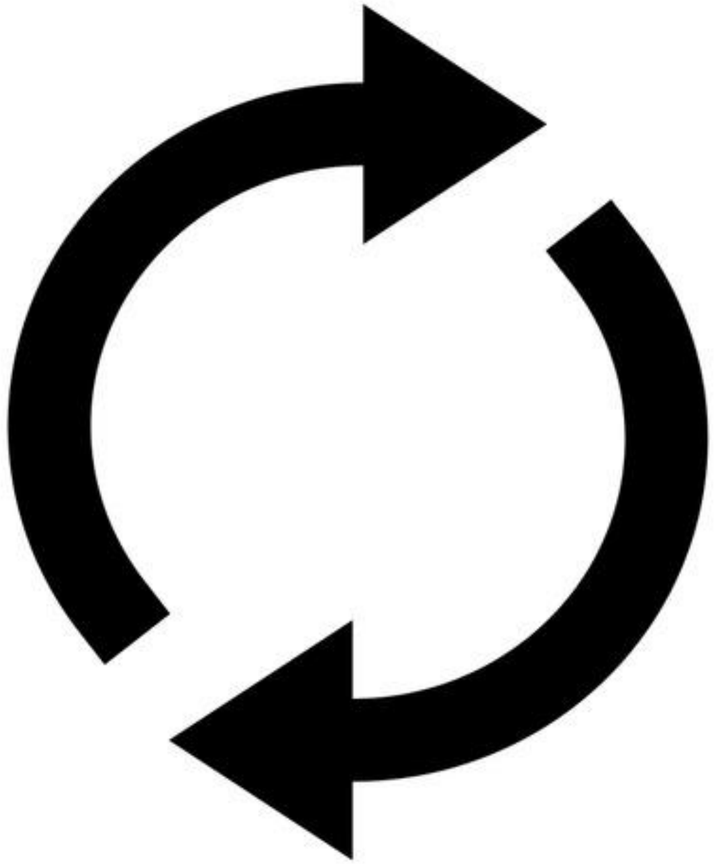
7

- Adjust method parameters or/and acceptance criteria if necessary
- Perform full internal (and external) validation experiments
- Develop SOPs for executing the method in the routine
- Define criteria for revalidation
- Define type and frequency of system suitability tests and/or analytical quality control (AQC) checks for the routine
- Document validation experiments and results in the validation report

Revalidation

- Revalidation is necessary whenever a method is changed, and the new parameter lies outside the operating range.
- Revalidation is also required if the scope of the method has been changed or extended

8

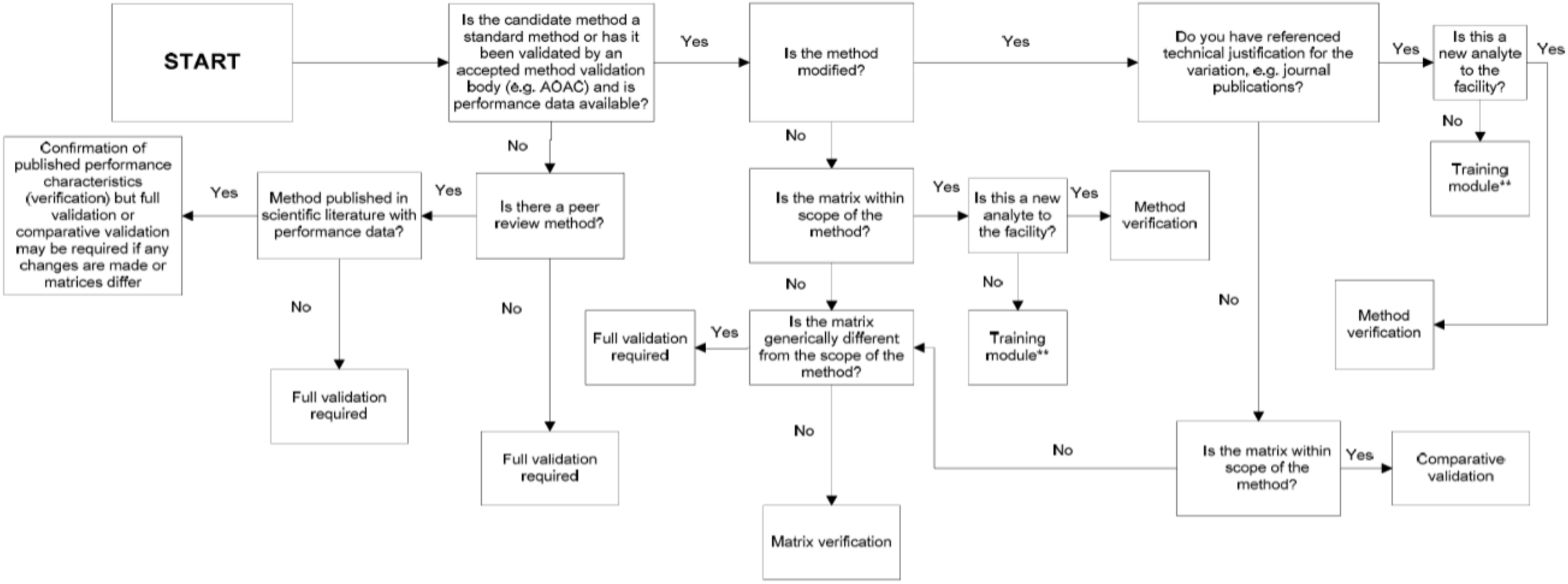


Method Validation vs Verification

- ***Validation*** is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled (ISO/IEC 17025:2017)

- ***Verification*** is the provision of objective evidence that a given item fulfils specified requirements or where the specified requirements are adequate for an intended use (ISO/IEC Guide 99:2007)

Method Validation and Verification Decision Tree



Method Validation Parameters and Criteria (Quantitative)

1. Sensitivity / Linearity

The sensitivity (or inclusivity) of a method is the rate of change of the measured response with change in the concentration (or amount) of analyte (or microorganism). For instrumental systems, sensitivity is represented by the slope (b) of the calibration curve (y = a + bx) and can be determined by a classical least squares procedure (for linear fits), or experimentally, using samples containing various concentrations of the analyte.

Criterion:

Deviation of back-calculated concentration from true concentration ≤± 20 %

$$\frac{Cmeasured - Ctrue}{Ctrue} \times 100$$

2. Recovery

Recovery of an analyte through an analytical method, is referred to as **'apparent recovery'**. The proportion of analyte determined remaining at the final point of the analytical method following its addition (usually to a blank sample) prior extraction. Usually expressed as a percentage. Routine recovery refers to the determination(s) performed with the analysis of each batch of samples.

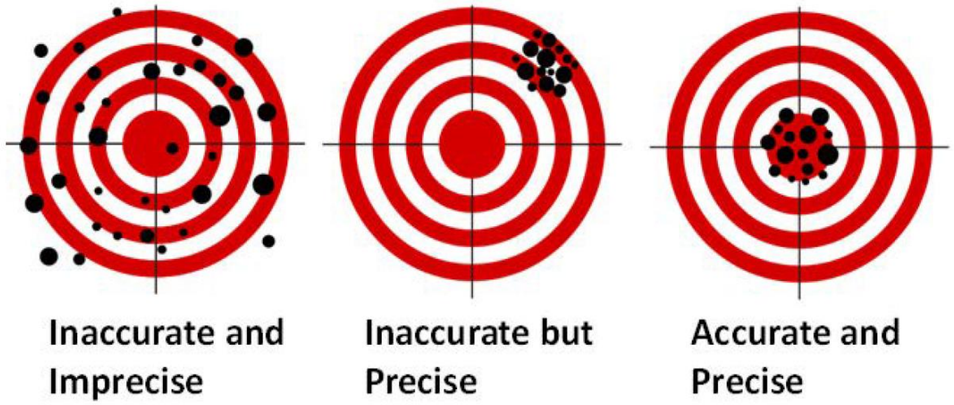
Criterion: 70-120 %

Recovery of an analyte after extraction and clean up steps. The proportion of analyte (yield) remaining at the point of the final determination following its addition (usually to a blank analytical test portion) prior to extraction. Usually expressed as a percentage. Also referred to as 'extraction recovery', 'absolute recovery'.

How? Average recovery for each spike level tested

3. Precision

The closeness of agreement between independent analytical results obtained by applying the experimental procedure under stipulated conditions. The smaller the random part of the experimental errors which affect the results the more precise the procedure. A measure of precision (or imprecision) is the standard deviation



Criterion: ≤ 20 %

How? Repeatability RSDr for each spike level tested

3a. Repeatability

Repeatability is the precision estimate obtained when measurement results are produced in one facility and tests are performed on identical test items during a short interval of time by one operator using the same equipment under conditions that are as constant as possible (e.g. incubation time and temperature). It can be expressed as standard deviation (*s*), variance (*s*2), probability distribution function, etc for a suitable number of measurements made under repeatability conditions.

Criterion: ≤ 20 %

Instrumental repeatability may be determined by the injection of the standard solutions that are used to prepare the working calibration curve as well as an incurred or fortified sample at each of the spike levels 7 times. These injections should be done in random order to minimize bias. Calculate mean, standard deviation and percent relative standard deviation.

How? Repeatability RSDr for each spike level tested

3b. Reproducibility

Reproducibility is the precision estimate obtained when a series of measurements are made under **more variable conditions**, i.e. **the same method on identical test items used by different operators with different equipment in different facilities at different times.** It can be expressed as standard deviation (s), variance, probability distribution factor, etc. of suitable number of determinations on identical specimens analyzed over several days with at least two different calibration standards.

Criterion: ≤ 20 %

How? Repeatability RSDr for each spike level tested

4. Limit of Quantitation / Lowest Validated Level

The lowest concentration or mass of the analyte that has been validated with acceptable accuracy by applying the complete analytical method and identification criteria.

Refers to the smallest analyte concentration or mass, which can be quantitatively analyzed with a reasonable reliability by a given procedure.

How? Lowest spike level meeting the identification and method performance criteria for recovery and precision

Criterion: ≤MRL , 70-120 %

5. Limit of Detection (LOD)

means the validated lowest residue concentration which can be quantified and reported by routine monitoring with validated control methods; in this respect it can be regarded as the LOQ

- ***Method Detection Limit (MDL)***

MDL is a term that should be applied to extraction and analysis methods developed for the analysis of specific analytes within a matrix. The MDL can be defined as the smallest amount or concentration of an analyte that can be reliably detected or differentiated from the background for a particular matrix (by a specific method).

- ***Instrument Detection Limit (IDL)***

the smallest amount of an analyte that can be reliably detected or differentiated from the background on an instrument (i.e. instrumental noise). As the sensitivity increases, the IDL decreases, and as the instrumental noise decreases, so does the IDL.

Approach in Determining LOD

- **LOD based on visual evaluation**

Visual evaluation may be used for non-instrumental methods but may also be used with instrumental methods. It is also useful for establishing the LOD for qualitative measurements.

- The detection limit is determined by the analysis of sample blanks samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. Sample blanks are spiked with an analyte at a range of concentration levels. At each concentration level, it will be necessary to measure approximately 7 independent replicates (as mentioned previously, in reality this number is often surpassed). Measurement of the replicates at various levels should be randomised. A response curve of percentage positive (or negative) results versus concentration should be constructed from the data, from which it should be possible to determine, by inspection, the threshold concentration at which the test becomes unreliable.

Approach in Determining LOD

- **LOD based on the standard deviation of the blank**

The detection limit may be determined by the analysis of a large number of blanks ($n \geq 20$ is recommended). Where independent sample blanks are measured once each ($n \geq 10$ is recommended) and independent sample blanks fortified at lowest acceptable concentration are measured once each ($n \geq 10$ is recommended). The LOD is expressed as mean sample blank value plus three standard deviations ($+ 3s$).

- **LOD based on the range in which the calibration equation applies**

Using the estimate of LOD as the blank plus three standard deviations of the blank, the instrument response to a blank is taken as the intercept of the calibration (a), and the standard deviation of the instrument response is taken as the standard error of the calibration ($s_{y/x}$). Therefore from the calibration equation if $y_{LOD} = a + 3\, s_{y/x} = a + bx_{LOD}$, then $x_{LOD} = 3\, s_{y/x}/b$.

6. Specificity

The ability of the detector (supported by the selectivity of the extraction, clean-up, derivatization or separation, if necessary) to provide signals that effectively identify the analyte. GC-MS with EI is a fairly non-selective determination system capable of high specificity. High resolution mass MS and MSMS can be both highly selective and highly specific.

How? Response in reagent blank and blank control samples

Criterion: ≤ 30% of Reporting Limit

7. Matrix Effect

An influence of one or more co extracted compounds from the sample on the measurement of the analyte concentration or mass. It may be observed as increased or decreased detector response compared with that produced by solvent solutions of the analyte. The presence or absence of such effects may be demonstrated by the difference of response from standard in matrix extract and standard in solvent

How? Difference of response from standard in matrix extract and standard in solvent

Criterion: in case of more than 20 % signal suppression or enhancement, matrix effects need to be addressed in calibration

$$\%ME = \left(\frac{Rstd\ in\ matrix\ extract}{Rstd\ in\ solvent} - 1\right) X\ 100$$

8. Ruggedness / Robustness

The ruggedness (a measure of robustness) of a method is the degree to which results are unaffected by minor changes from the experimental conditions described in the method, for example, small changes in temperature, pH, reagent concentration, flow rates, extraction times, composition of mobile phase. Ruggedness testing provides an indication of the methods reliability during normal usage.

How? Average recovery for each spike level tested

Criterion: 70-120 %

Table 4. *Validation parameters and criteria*

Parameter	What/how	Criterion	Cross reference to AQC document
Sensitivity/linearity	Linearity check from five levels	Deviation of back-calculated concentration from true concentration ≤± 20 %	C14-C19
Matrix effect	Difference of response from standard in matrix extract and standard in solvent	*	C21-C29 Glossary
LOQ	Lowest spike level meeting the identification and method performance criteria for recovery and precision	≤ MRL	G6[10]
Specificity	Response in reagent blank and blank control samples	≤ 30 % of RL	C41
Recovery	Average recovery for each spike level tested	70-120 %	G3,G6
Precision (RSD_r)	Repeatability RSD_r for each spike level tested	≤ 20 %	G3, G6
Precision (RSD_{wR})	Within-laboratory reproducibility, derived from on-going method validation / verification	≤ 20 %	G3, G6
Robustness	Average recovery and RSD_{wR}, derived from on-going method validation / verification	See above	G6, C39-C44
Ion ratio	Check compliance with identification requirements for MS techniques	Table 3	Section D
Retention time		±0.1 min.	D2

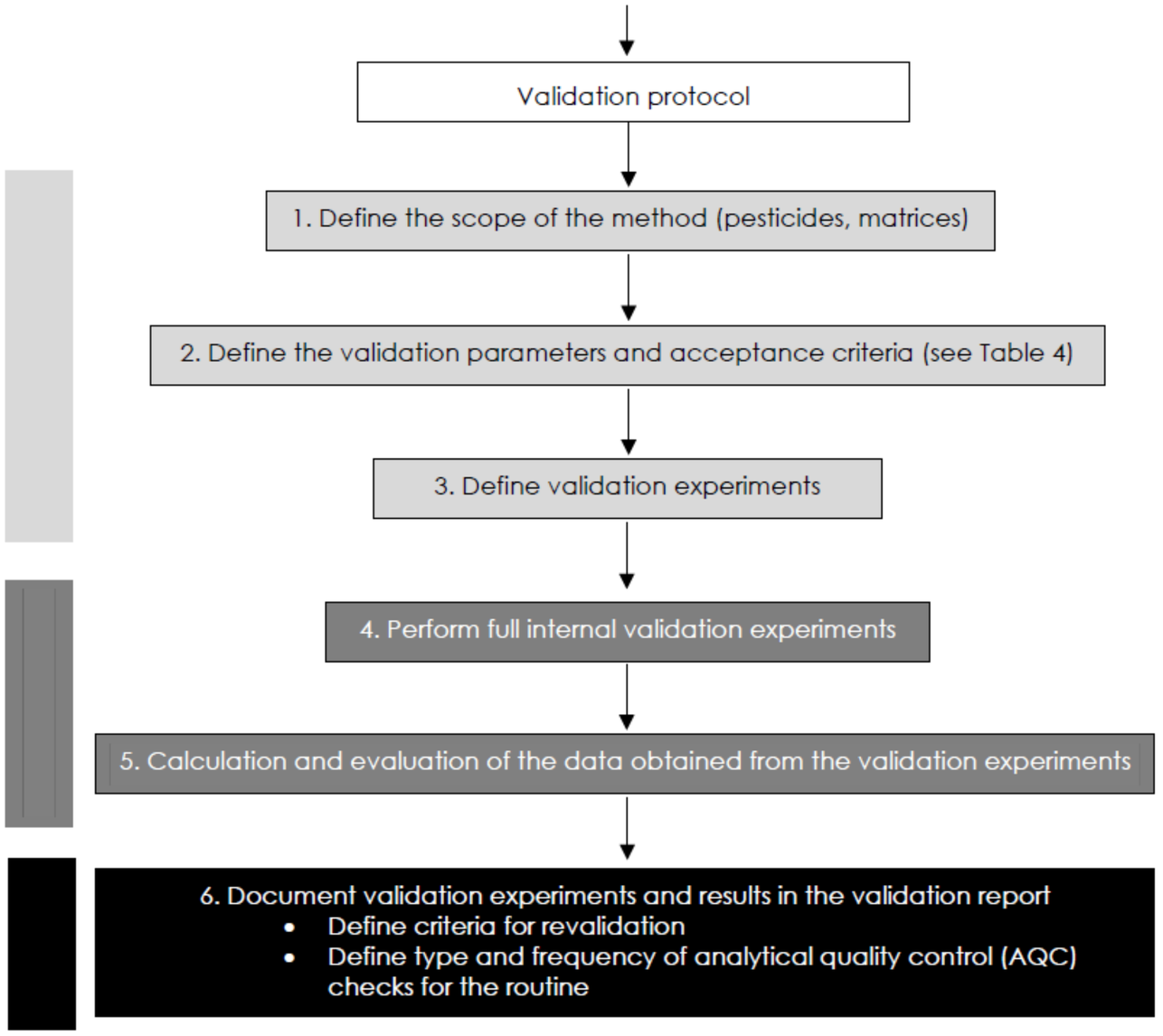
*in case of more than 20 % signal suppression or enhancement, matrix effects need to be addressed in calibration (C21-C29).

Method Validation Parameters and Criteria (Quanlitative)

- ***Screening Method***
  - If the method is intended to be used as a qualitative method, there are no requirements with regard to recovery of the analytes.
  - the possible presence of false detects should be checked using non-spiked / blank samples. – determination of selectivity

- The validation of a screening method based on an SDL can be focused on detectability.
  - at least 20 samples spiked at the estimated SDL.
  - The SDL of the qualitative screening method is the lowest level at which an analyte has been detected
  - The samples selected should represent multiple commodities from the same commodity group, with a minimum of two samples for each individual commodity included and will be representative for the intended scope of the laboratory.
  - Additional validation data can be collected from on-going AQC-data and method performance verification during routine analysis.

INITIAL VALIDATION PLAN FOR QUANTITATIVE METHODS



Method Validation Procedure: outline and example approach

Validation needs to be performed
• for all analytes within the scope of the method
• for at least 1 commodity from each of the commodity groups (as far as they are within the claimed scope of the method or as far as applicable to samples analyzed in the laboratory)

Experimental:
A typical example of the experimental set up of a validation is:
Sample set (sub-samples from 1 homogenized sample):
• Reagent blank
• 1 blank (non-spiked) sample
• 5 spiked samples at target LOQ
• 5 spiked samples at 2-10x target LOQ

Instrumental sample sequence:
• Conditioning blanks in GC
• Calibration standards
• Reagent blank
• Blank sample
• 5 spiked samples at target LOQ
• 5 spiked samples at 2-10 x target LOQ
• Calibration standards

Method Validation Procedure: outline and example approach

Spiking of commodities is a critical point in validation procedures. In general, the spiking procedure should reflect as much as possible the techniques used during routine application of the method. If for example, samples are milled cryogenically and extracted in frozen condition spiking must be done on frozen analytical test portions of blank material and extracted immediately. Where samples are milled at room temperature and extracted on average after 20 min, spiking should be done on blank analytical test portions at room temperature. In general, spiking of samples will not simulate incurred residues even if the spiked sample is left standing for a certain time. To study the relative extractability of incurred residues agriculturally treated samples should be taken.

Data evaluation:

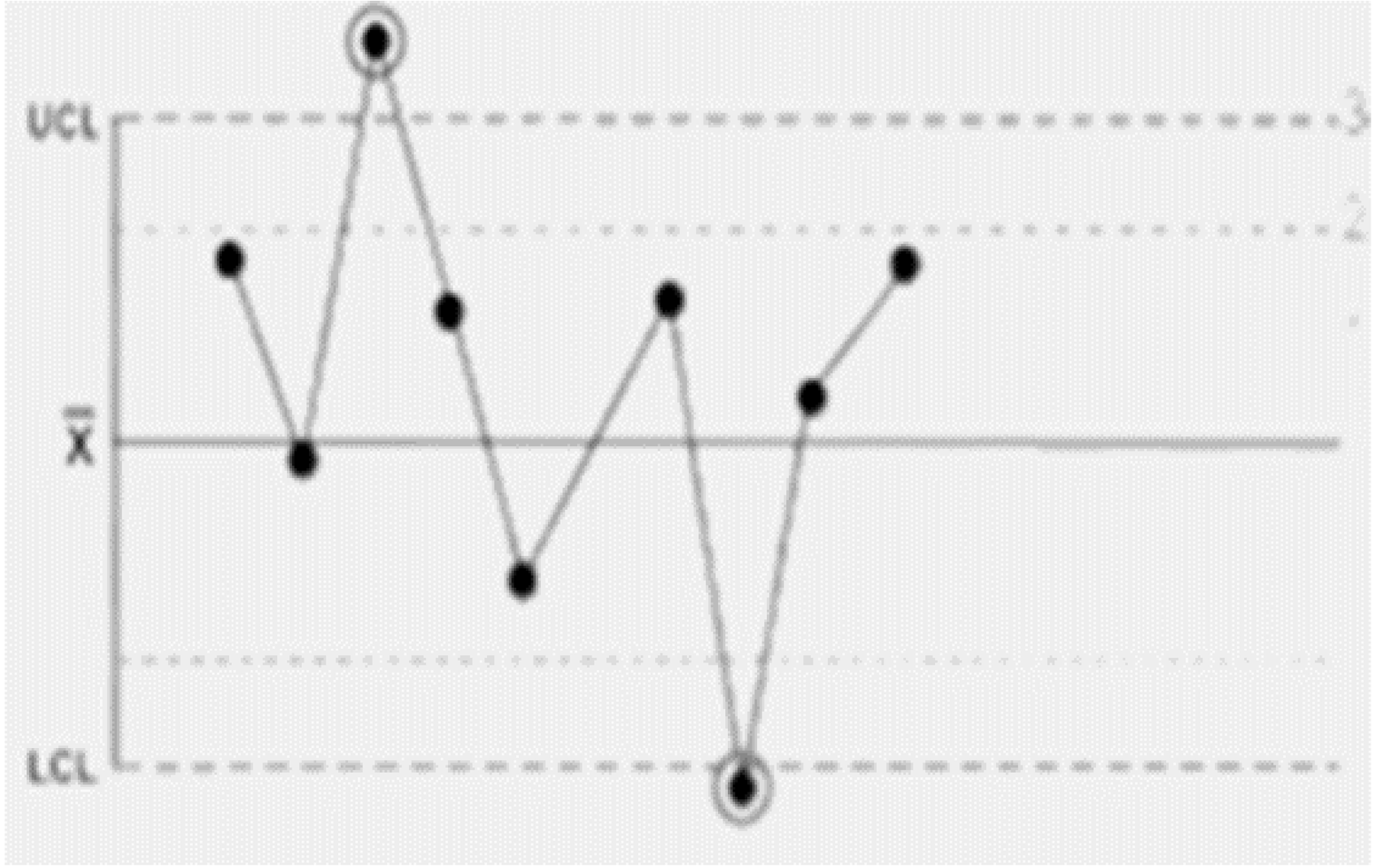
Inject the sample sequence, calibrate and quantify as is described in this AQC document.

Evaluate the parameters from Table 4 and verify them against the criteria.

Routine Recovery Checks

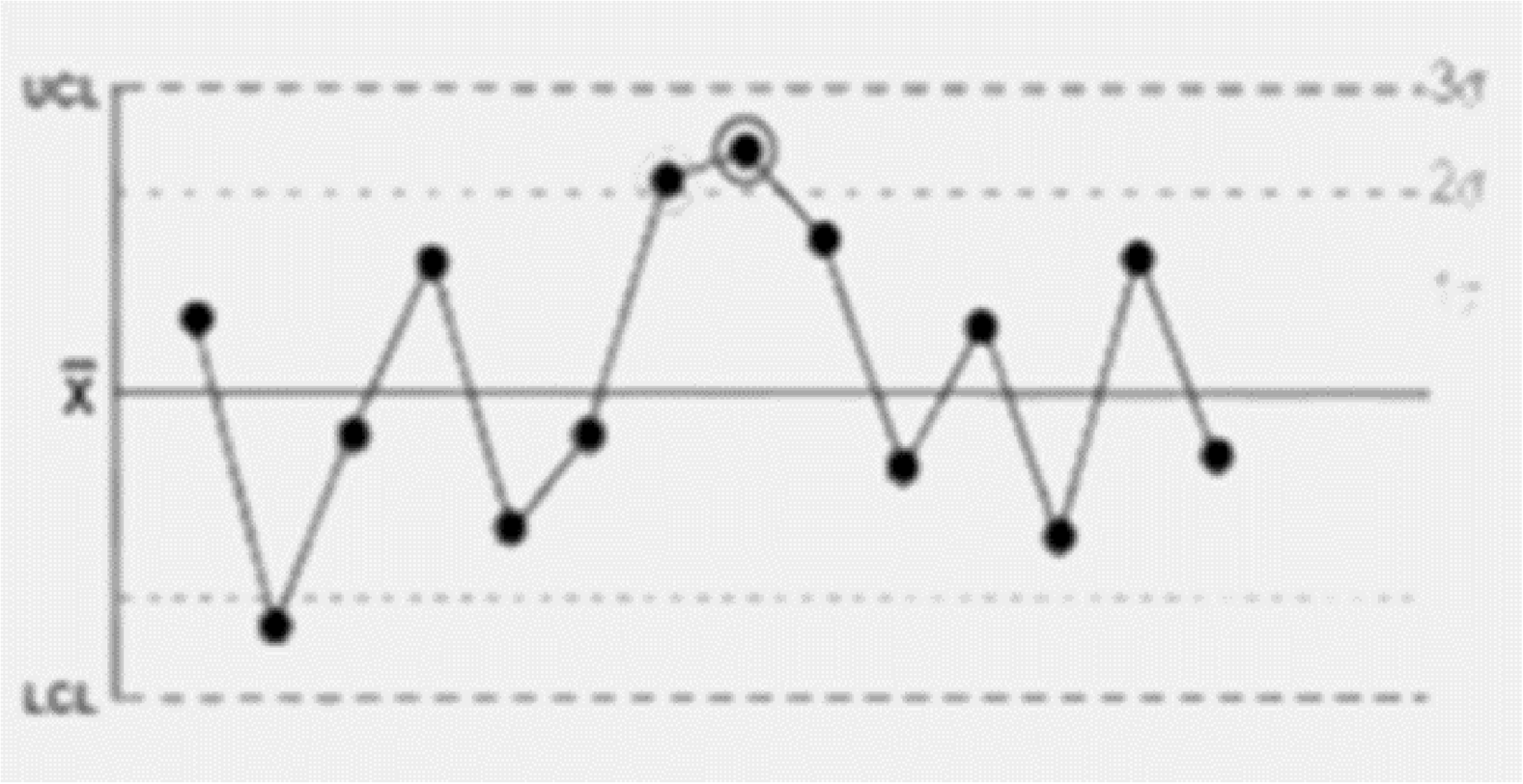
	Analytes for recovery check (minimum)	All other analytes
Number of analytes	At least 10 % of the scope per detection system covering all critical aspects of the method	Within a rolling programme to include all other analytes as well as representative commodities from different commodity groups
Minimum frequency *of* recovery checks	Every batch	At least every 12 months, preferably every 6 months
Level	Reporting Limit	Reporting Limit

Control Charts Guidelines



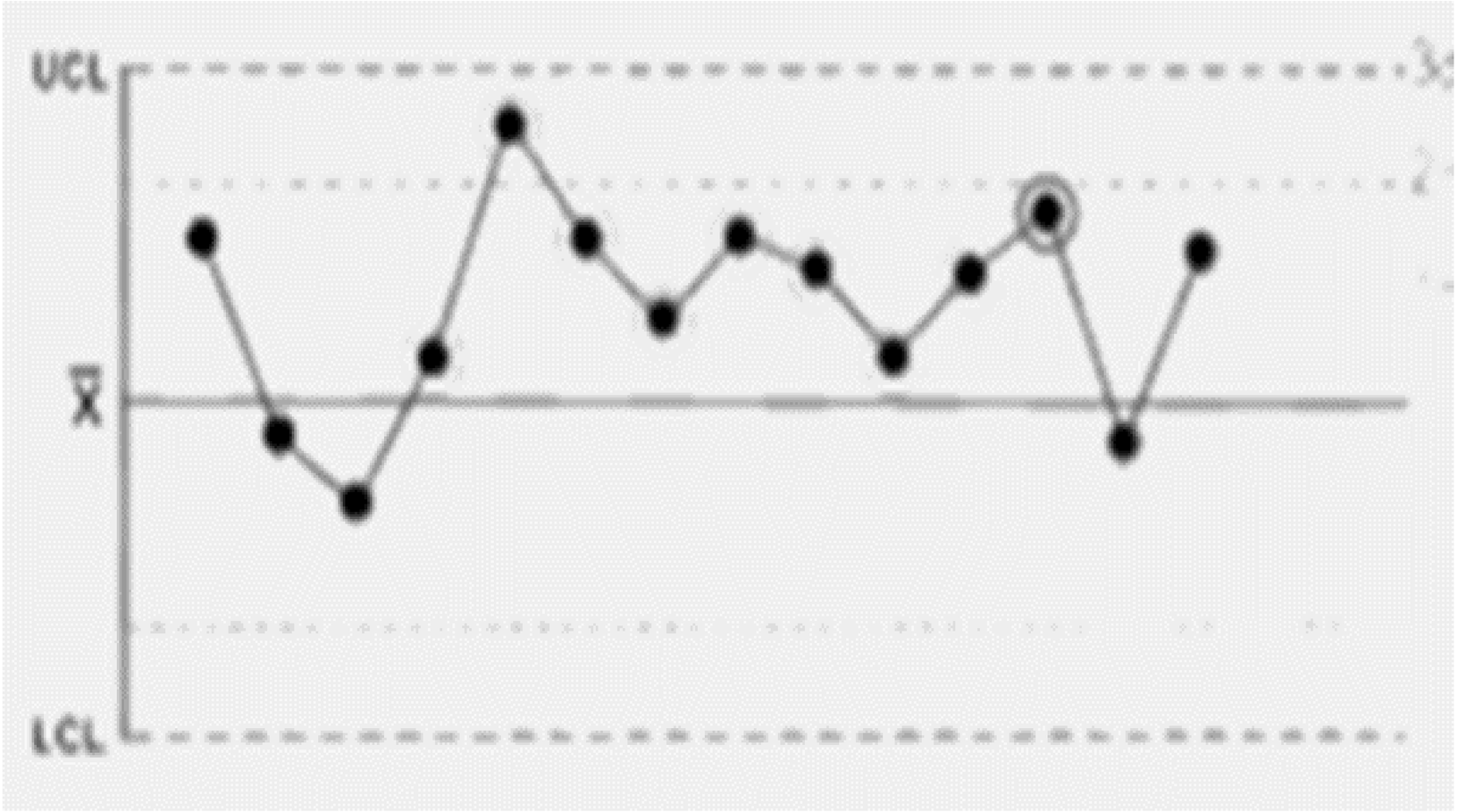
1. One value or more fall outside the upper and lower action limit

Control Charts Guidelines



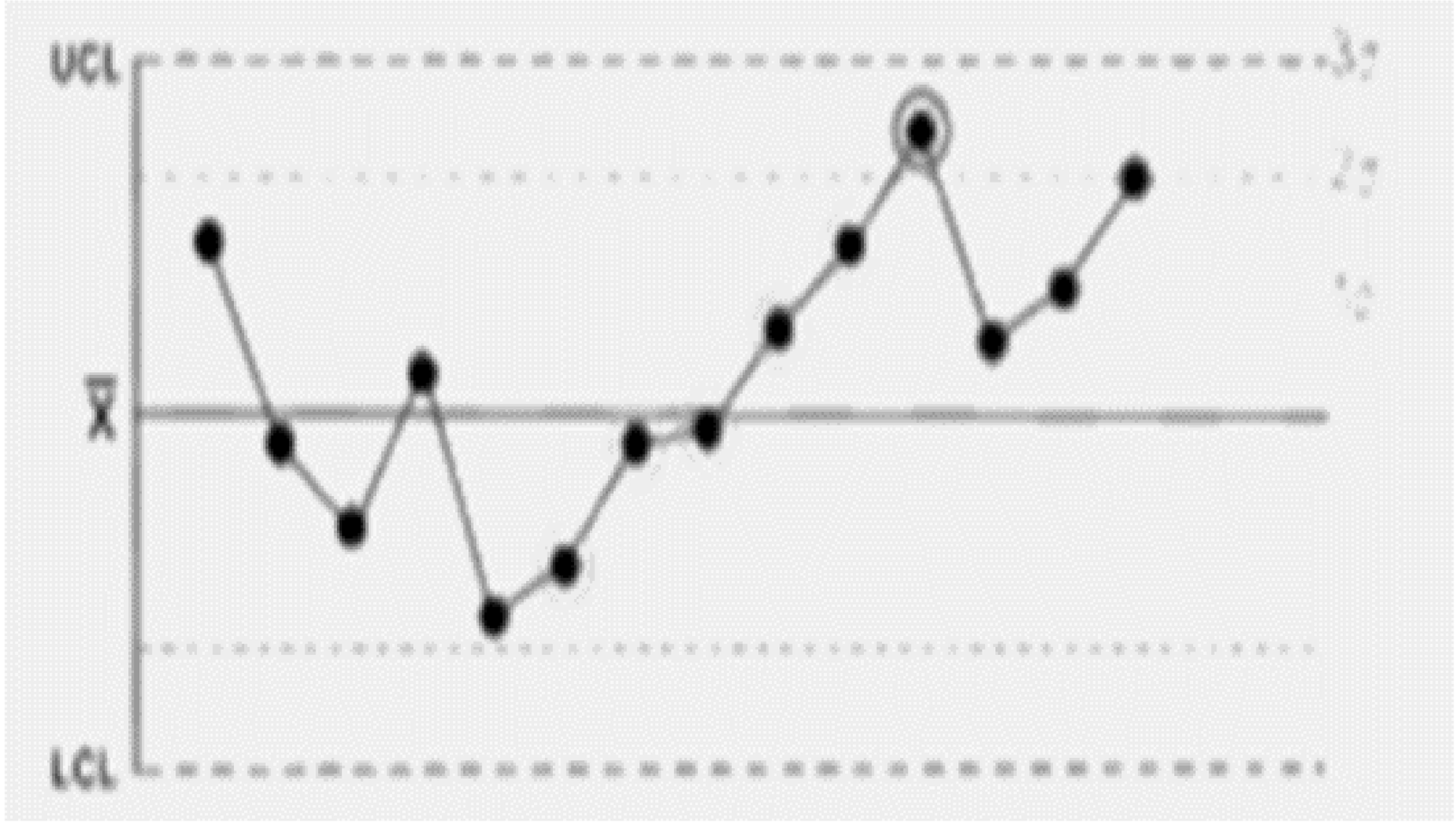
2. Two out of three consecutive values fall outside lower or upper warning limit on the same side

Control Charts Guidelines



3. A series of seven or eight consecutive values fall all above or all below the mean

Control Charts Guidelines



4. Seven points in a row are continually decreasing or increasing

PROFICIENCY TESTING on PESTICIDE RESIDUE ANALYSIS

1

Pesticide Analytical Laboratory Section
Plant Product Safety Services Division
Bureau of Plant Industry
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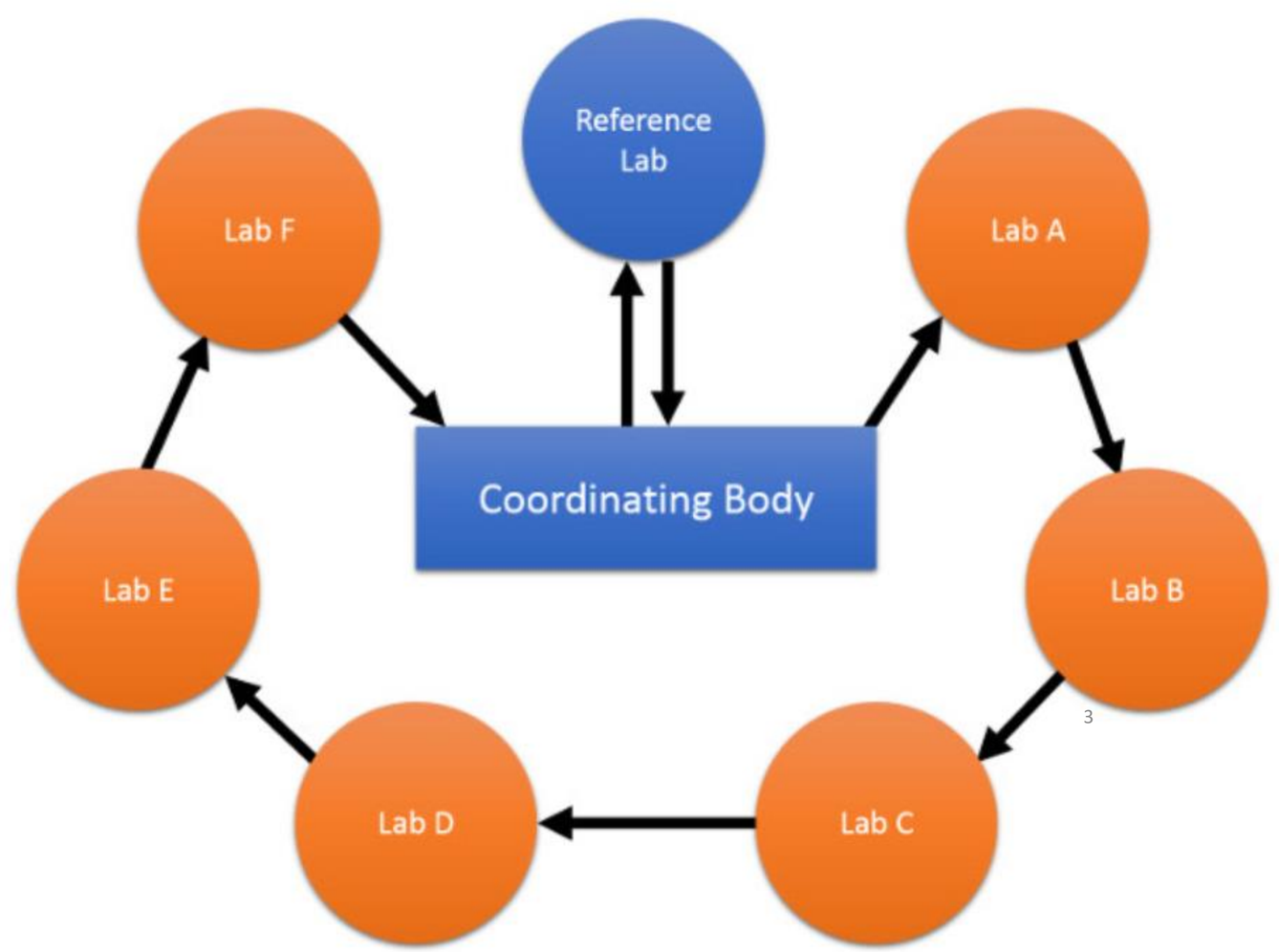
What is Proficiency Testing

According to ISO/IEC 17043:2010, proficiency testing (PT) is the evaluation of participant performance against pre-established criteria by means of interlaboratory comparisons.

a proficiency test is a method used to demonstrate competency and validate a laboratory's measurement process by comparing your results to the results of a reference laboratory and other participant laboratories.



- A coordinating body sends a test item to a reference laboratory for testing. Then, the coordinating body sends the item to each participating laboratory for subsequent testing.
- Each participant laboratory will independently test the item, submit their results to the coordinating body, and forward the item to the next participating laboratory.
- After each participating laboratory has completed testing, the artifact is returned to the coordinating body.
- The coordinating body will evaluate the all the test results and issue a performance report to each participating laboratory.
- This is typically referred to as Round Robin Testing, and is one of the most common proficiency testing schemes used by PT providers.

Why Proficiency Testing Important

01 A measurement of method

02 Technical training of personnel

03 Traceability of standards

04 Estimates of measurement uncertainty

Proficiency Testing

- According to ISO Guide 43, Proficiency Testing is a formal exercise **managed by a coordinating body** which includes a **standard or reference laboratory**. The results are issued in a formal report that clearly provides the En and Z score.

Interlaboratory Comparison

- According to ISO/IEC 17043:2010, inter-laboratory comparison (ILC) is the organization, performance, and evaluation of measurements or tests on the same or similar items by two or more laboratories or inspection bodies in accordance with predetermined conditions.
- ISO Guide 43 describes an inter-laboratory comparison as an exercise that is performed by agreement between two or more participating laboratories where the results are issued in a formal report.
- An inter-laboratory comparison does not require the use of a reference laboratory or a coordinating body. Therefore, participant laboratories are only comparing performance amongst the group of participating members.

How to Evaluate Proficiency Testing Results?

- Proficiency testing results are commonly evaluated using two methods described in ISO/IEC 17043;

1. Normalized Error
2. Z-Score.

Normalized error is a statistical evaluation used to compare proficiency testing results between the participant and the reference laboratory where the uncertainty in the measurement result is included.

- *When the value of |En| ≤ 1 (i.e. between -1 and +1), the results are considered satisfactory.*
- *When the value of |En| > 1 (i.e. greater than +1 or less than -1), the results are considered unsatisfactory.*

$$E_n = \frac{x_{Lab} - x_{Ref}}{\sqrt{U_{Lab}^2 + U_{Ref}^2}}$$

Where,
x_{Lab} = measurement result of participating lab
x_{Ref} = measurement result of reference lab
U_{Lab} = Expanded Uncertainty (i.e. 95%) of participating lab
U_{Ref} = Expanded Uncertainty (i.e. 95%) of reference lab

How to Evaluate Proficiency Testing Results?

- Proficiency testing results are commonly evaluated using two methods described in ISO/IEC 17043;

1. Normalized Error
2. Z-Score.

Z-score is a statistical measurement of a score's relationship (i.e. how many **standard deviations** above or below the population mean) to the mean in a set of scores.
It is a statistical evaluation used to review the results of all participants and identify outliers and exclude their data from proficiency testing results.

When determining whether a participant's results are satisfactory, unsatisfactory, or questionable, the following rules are used;
- *When the value of Z <=2, the results are considered satisfactory.*
- *When the value of Z >=3, the results are considered unsatisfactory.*
- *When the value of Z >=2 and Z <=3, the results are considered questionable.*

$$z_i = \frac{(y_i - \bar{y})}{\sigma}$$

Where,
y_i = measurement result of participating lab
y_{bar} = the population mean (i.e. average)
σ = the standard deviation of the population

ISO/IEC 17025 Requirement for Proficiency Testing

Ensuring the Validity of Results

In section 7.7.2, the ISO/IEC 17025 standard states that laboratories shall monitor their performance by comparing their results with other laboratories.

The two methods that are recommended are:

a. Proficiency Testing or
b. Interlaboratory Comparisons

See the excerpt below:

*"**7.7 Ensuring the validity of results***
***7.7.2** The laboratory shall monitor its performance by comparison with results of other laboratories, where available and appropriate. This monitoring shall be planned and reviewed and shall include, but not be limited to, either or both of the following:*

***a)** participation in proficiency testing;*

NOTE – ISO/IEC 17043 contains additional information on proficiency tests and proficiency testing providers. Proficiency testing providers that meet the requirements of ISO/IEC 17043 are considered to be competent.

***b)** participation in interlaboratory comparisons other than proficiency testing."*

ISO/IEC 17025 Requirement for Proficiency Testing

Externally Provided Products and Services

In section 6.6.1, the ISO/IEC 17025 standard states that laboratories must use only suitable externally provided services when it affect laboratory activities. If you read the note below the section, you will see that proficiency testing services should be included in externally provided services.

- If you maintain an Approved Supplier List (like many other labs), then you may want to add your proficiency testing providers to it.

See the excerpt below:

*"**6.6 Externally provided products and services***

***6.6.1** The laboratory shall ensure that only suitable externally provided products and services that affect laboratory activities are used, when such products and services:*

***a)** are intended for incorporation into the laboratory's own activities;*

***b)** are provided, in part or in full, directly to the customer by the laboratory, as received from the external provider;*

***c)** are used to support the operation of the laboratory.*

NOTE Products can include, for example, measurement standards and equipment, auxiliary equipment, consumable materials and reference materials. Services can include, for example, calibration services, sampling services, testing services, facility and equipment maintenance services, proficiency testing services and assessment and auditing services."

ISO/IEC 17025 Requirement for Proficiency Testing

Improvement

In section 8.6.1, the ISO/IEC 17025 standard states that laboratories must identify and select opportunities for improvement and implement any necessary actions. If you read the note just below the section, you will see that the standard recommends using proficiency testing results to find opportunities for improvement.

See the excerpt below:

*"**8.6 Improvement (Option A)***

***8.6.1** The laboratory shall identify and select opportunities for improvement and implement any necessary actions.*

NOTE Opportunities for improvement can be identified through the review of the operational procedures, the use of the policies, overall objectives, audit results, corrective actions, management review, suggestions from personnel, risk assessment, analysis of data, and proficiency testing results."

As you can see, the ISO/IEC 17025:2017 standard requires you to participate in a proficiency testing or interlaboratory comparison program (where available and appropriate).

Additionally, the standard recommends that you;

- consider proficiency testing providers as service providers and
- use proficiency testing results to find opportunities for improvement.

Estimation of Measurement Uncertainty

**ANALYTICAL QUALITY CONTROL
AND METHOD VALIDATION PROCEDURES FOR PESTICIDE RESIDUES ANALYSIS
IN FOOD AND FEED
SANTE 11312/2021 v2**

Pesticide Analytical Laboratory Section
Plant Product Safety Services Division
Bureau of Plant Industry
Quezon City, metro manila, philippines

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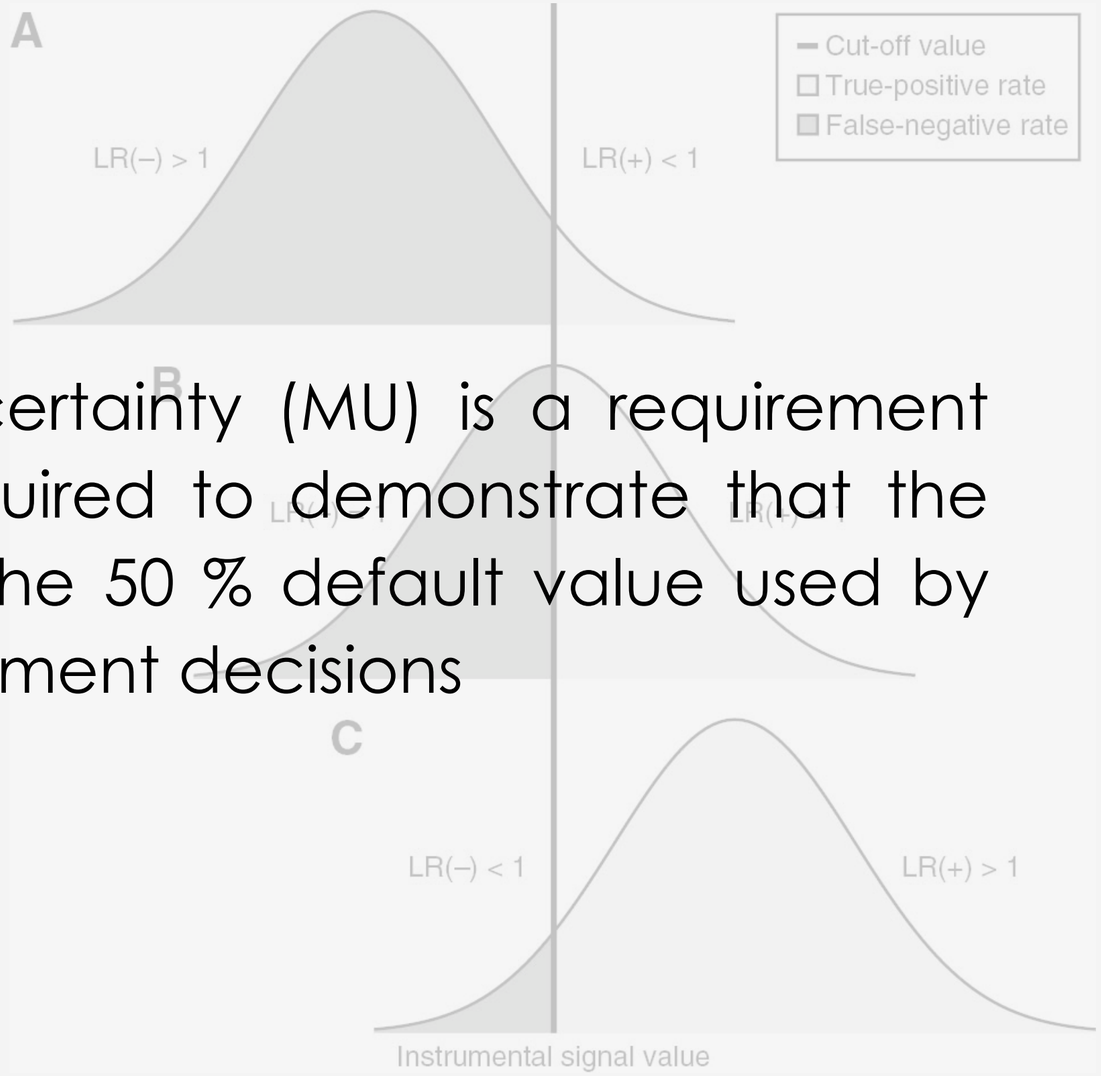
MEASUREMENT UNCERTAINTY



Establishment of the measurement uncertainty (MU) is a requirement under ISO/IEC 17025 (E5). It is also required to demonstrate that the laboratory's own MU is not exceeding the 50 % default value used by regulatory authorities in cases of enforcement decisions

$$U' = k \times u' \qquad \text{Eq. 1}$$

Documents Recommended

01 Eurachem

02 Nordtest

03 Eurolab

04 Codex CAC/GL 59-2006

Approach 1. Estimating MU based on intra-laboratory validation/QC data.

$$u' = \sqrt{u'(bias)^2 + u'(precision)^2}$$

Eq. 2

with u' = measurement uncertaintly

u'(bias) = uncertainty component for the bias

u'(precision) = uncertainty component for the precision

first estimate of u'(bias) and u'(precision) is usually obtained at the initial validation stage for each pesticide/representative matrix/level combination. However, a much ***more realistic estimation is calculated for each pesticide from a number (usually, ≥10) of long-term QC tests (spiked samples) for each pesticide for one or more matrices of the same commodity group.***

Estimation of the u'(bias) component **without correction for recovery**

- bias is the difference between the measured value and the true value. In absence of CRM or PT assigned values, the true value is the spiked concentration, and the bias is the difference between the spiked and the measured concentration. The bias is given by:

$$r\ bias = \frac{measured\ concentration - spiked\ concentration}{spiked\ concentration} \times 100\%$$

Eq. 3

Estimation of the u'(bias) component **without correction for recovery**

u'(bias) can be calculated using the following equation:

$$u'(bias) = \sqrt{RMS'(bias)^2 + u'(Cref)^2}$$

Eq. 4

with RMS'(bias) = root mean square of the bias = $\sqrt{\frac{\sum bias_i^2}{N}} = \sqrt{mean_{bias}^2 + SD.P_{bias}^2}$

with $\text{mean}_{\text{bias}}$ = the mean of the bias

$\text{SD.P}_{\text{bias}}$ = the population standard deviation of the bias (stdev.p in Excel)

$u'(C_{ref})$ = uncertainty of the spiked concentration.

Estimation of the u'(bias) component **without correction for recovery**

When certified analytical standards and calibrated/verified volumetric material/balances are used to prepare the spiked samples, it can be assumed that the uncertainty associated with the spiking level is negligible. Equation 4 then simplifies to:

$$u'(bias) = \sqrt{mean_{bias}^2 + SD.P_{bias}^2}$$ Eq. 5

Estimation of the u'(bias) component **with correction for recovery**

In case the analysis result is mathematically corrected for recovery using a recovery factor, then the u'(bias) can be calculated using the following equation:

$$u'(bias) = \sqrt{\left(\frac{RSD_{wR}}{\sqrt{N}}\right)^2 + u'(Cref)^2} \qquad \text{Eq. 6}$$

with RSD_{wR} = within-laboratory reproducibility of the recovery

N = number of recovery tests

Estimation of the u'(bias) component **with correction for recovery**

When certified analytical standards and calibrated/verified volumetric material/balances are used to prepare the spiked samples, it can be assumed that the uncertainty associated with the spiking level is negligible. Equation 6 then simplifies to:

$$u'(bias) = \frac{RSD_{wR}}{\sqrt{N}} \qquad \text{Eq. 7}$$

Estimation of the u'(precision) component

The RSD_{wR} is preferably derived from spiked samples from ≥10 sample batches over a longer period of time (on-going validation). When multiple matrices from a commodity group are analysed and one RSD_{wR} value is used for that group, the RSD_{wR} should be based on spiked samples of different matrices reflecting the scope of analysis in order to obtain a realistic estimate for the commodity group.

$$u'(\text{precision}) = RSD_{wR} \qquad \text{Eq. 8}$$

Estimation of the combined measurement uncertainty

The combined measurement uncertainty is estimated by equation 2, and using equation 5 and 8 is:

$$u' = \sqrt{mean_{bias}^2 + SD.P_{bias}^2 + RSD_{rw}^2} \qquad \text{Eq. 9}$$

When analysis results are mathematically corrected for recovery using a recovery factor, the combined measurement uncertainty is estimated by equation 2, using equation 7 and 8:

$$u' = \sqrt{\left(\frac{RSD_{wR}}{\sqrt{N}}\right)^2 + RSD_{wR}^2} \qquad \text{Eq. 10}$$

***Table C1**. Example A, pesticide X (low bias, good within-lab reproducibility)*

Date	QC samples spiked at 0.05 mg/kg	Measured (mg/kg)	Rel. relative bias (%) [equation 3]
10/Jan	Apple	0.051	2
26/Jan	Pear	0.045	-10
04/Feb	Lettuce	0.050	0
08/Feb	cauliflower	0.056	12
22/Feb	Cherries	0.052	4
28/Feb	Onion	0.046	-8
05/Mar	French beans	0.048	-4
06/Mar	Carrots	0.045	-10
22/Mar	Leek	0.037	-26
	N	9	
	mean	0.0478	-4.44
	SD.P bias (stdev.p) (%)		10.232
	standard dev. measured (mg/kg) (stdev.s)	0.00543	
	RSD_{wR} (%)	11.357	
	u'(bias) (%) [equation 5]		11.1555
	u'(precision) = RSD_{wR} (%) [equation 8]	11.357	
	u' combined (%) [equation 2 and 9]	15.920	
	U' (expanded MU) (%) [equation 1]	31.839	

The estimated expanded measurement uncertainty is 32 %. For pesticide X, the laboratory has demonstrated that the expanded MU is not exceeding the 50 % default value (E12). The regulatory authorities can use the 50 % default value for enforcement decisions.

***Table C2**. Example B, pesticide Y (high bias, good within-lab reproducibility)*

Date	QC sample spiked at 0.05 mg/kg	Measured (mg/kg)	Rel. bias (%)
10/Jan	Apple	0.038	-24
26/Jan	Pear	0.034	-32
04/Feb	Lettuce	0.037	-26
08/Feb	cauliflower	0.042	-16
22/Feb	Cherries	0.039	-22
28/Feb	Onion	0.034	-32
05/Mar	French beans	0.036	-28
06/Mar	Carrots	0.034	-32
22/Mar	Leek	0.028	-44
	N	9	
	Mean	0.0358	-28.4
	SD.P bias (stdev.p) (%)		7.470
	standard dev. measured (mg/kg) (stdev.s)	0.00396	
	RSD_{wR} (%)	11.073	
	u'(r bias) (%) [equation 5]		29.4090
	u'(precision) = RSD_{wR} (%) [equation 8]	11.073	
	u' combined (%) [equation 2 and 9]	31.424	
	U' (expanded MU) (%) [equation 1]	62.849	

For pesticide Y, the laboratory has demonstrated that the expanded MU is exceeding the 50 % default value (E12) when results are not corrected for recovery. If, at the end of the analytical program, the results were corrected for the average recovery achieved over the 3 month period, then the u'(bias) need only to reflect the uncertainty associated with the mean recovery and equation 7 applies.[21] The average recovery in example B is [100 %- bias%]=71.6 %. The RSD_{wR} of this recovery is the same as the RS_{wR} of the measured concentrations (11.073 %). With that, the u'(bias) according to equation 7 is 3.691%, resulting in a combined u' of 11.672 % and an expanded MU of 23 %.

Pesticides:
Principle and Classification

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Jefcor Laboratories, Inc.

JEFCOR LABORATORIES, INC.
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Rationale:

Pesticides play a significant role in food production.

They protect or increase yields and may increase the number of times each year a crop can be grown on the same land, which is particularly important in countries that face food shortages.

Pesticides are intrinsically toxic and are deliberately spread in the environment. Their production, distribution and use requires strict regulation and control.

WHO, in collaboration with FAO, is responsible for assessing the risks to humans from pesticides, whether through direct exposure or residues in food, and for recommending adequate protection measures.

Pesticide (FAO, 2024)

Any substance intended for preventing, destroying, attracting, repelling, or controlling any pest including unwanted species of plants or animals during the production, storage, transport, distribution, and processing of food, agricultural commodities.

Includes substances on animal feeds or which may be administered to animals for the control of ectoparasites.

Pesticides (Cont'd.)

Includes substances intended for:

plant-growth regulator, defoliant, desiccant, fruit thinning agent, or sprouting inhibitor and substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport.

The term normally excludes fertilizers, plant and animal nutrients, food additives and animal drugs.

Pesticide Development

From Test Tube to Registration

PhP 5-11 Billion

5-12 YEARS

		Number of Compounds:
SYNTHESIS		**40,000-60,000**
INITIAL SCREENING		**150**
TOXICOLOGY/ ENVIRONMENT	 	**10**
LABORATORY/ FIELD TRIALS		**2**
REGISTRATION/ MARKETING		**1**

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Pesticide Composition

Active ingredient

+

Inert / Auxillary Materials

- *Most important component*
  - *Biologically active*
  - *Poison*
  - *destroys or controls the target pest*
- all materials in the pesticide formulation other than active ingredient
  - *solvents, emulsifiers, carriers, binders, wetting agents, etc.*

Name Classification of Pesticide

Chemical Name	- Name given by the International Union of Pure and Applied Chemists (IUPAC) standards as based on the structure of the compound - Example: Diazinon IUPAC name is: O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate
Common Name	- Adapted name accepted by international organizations - Example: Diazinon
Trade Name or Brand Name	- Name given by the manufacturer of the pesticide formulation - Example: Diazinon is marketed as Diazol , Basudin

CLASSIFICATION OF PESTICIDES

Pesticides can be classified based on:

- Chemical composition
- Mode of Entry
- Target Pests or Organism
- Toxicity and Hazard
- Use / Usage
- Source/ Origin

Classification of Pesticides based on PEST Controlled

Pesticides are generally formulated for a **specific type of pest**

PESTICIDE TYPE	PEST/S CONTROLLED
Acaricide/Miticide	Mites, ticks and spiders
Avicide	Birds
Bactericide	Bacteria
Fungicide	Fungi
Insecticide	Insects
Molluscicide	Mollusks such as slugs and snails
Nematicide	Nematodes
Herbicide	Weeds
Rodenticide	Rodents such as rats, mice

Classification of Pesticides based on USE/ USAGE

1. **General Use Pesticide**

Products in this category may be handled by the general public in conformity with label directions.

2. **Restricted Use Pesticide**

Handled only by trained and certified applicators; present a greater degree of human and environmental danger; require more careful and precise application in conformity with label directions.

3. **Banned Pesticide**

Cannot be used under any circumstances

Classification of Pesticides based on MODE OF ENTRY

- **Stomach**

  poison generally enters the pest through the mouth by ingestion and absorption in the digestive tract

- **Contact**

  poison penetrates the insect body as a result of contact of the legs and other parts of the body on treated surfaces

- **Fumigant**

  poison is volatile and enters the body through the respiratory system of the insect

- **Systemic**

  poison is taken into the plant thorugh the roots of leaves and transported via the vascular system to the different parts of the plant. Sucking, boring and mining insects inquire the poison through feeding.

Classification of Pesticides based on TOXICITY and HAZARD

- **Toxicity**
  - **Innate property** of a substance to produce harm/injury.
    - *Acute – effect after a single exposure*
    - *Chronic – effect after repeated and long exposure*

- **Hazard**
  - **Probability** or likelihood of an adverse effect.

TOXICITY AND HAZARDS (FAO, 2022)

i) GHS – Acute toxicity

	Hazard category					
	Category 1	Category 2	Category 3	Category 4	Category 5	Not classified i.e. toxicity lower than Cat 5
Pictogram/ Symbol					*No pictogram*	*No pictogram*
Signal Word	Danger	Danger	Danger	Warning	Warning	*No signal word*
Hazard Statement						
Oral	Fatal if swallowed	Fatal if swallowed	Toxic if swallowed	Harmful if swallowed	May be harmful if swallowed	
Dermal	Fatal in contact with skin	Fatal in contact with skin	Toxic in contact with skin	Harmful in contact with skin	May be harmful in contact with skin	
Inhalation	Fatal if inhaled	Fatal if inhaled	Toxic if inhaled	Harmful if inhaled	May be harmful if inhaled	
Colour band	PMS red 199 C	PMS red 199 C	PMS Yellow C	PMS Blue 293 C	PMS Blue 293 C	PMS Cool Grey 7C

Guidance on good labelling practice for pesticides (Second revision)

https://www.who.int/publications/i/item/9789240005662

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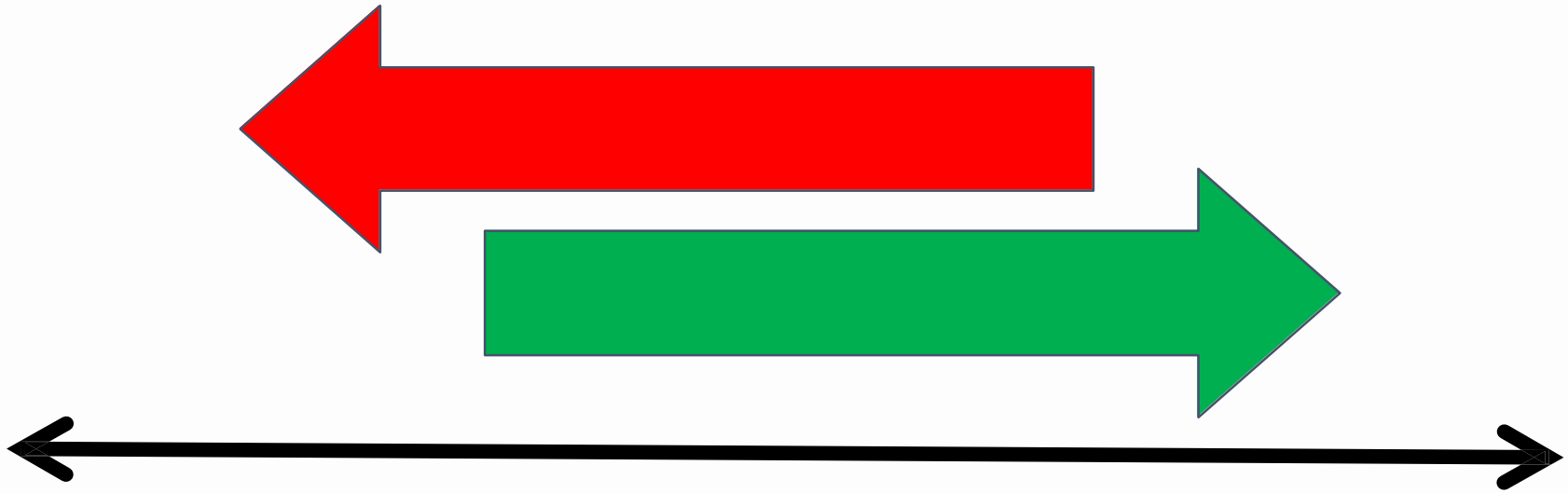
Classification of Pesticides based on TOXICITY and HAZARD



LD50 values (mg/kg)

Pesticides with **LOW** LD50 values *are more toxic*

than Pesticides with **HIGH** LD50 values

Classification of Pesticides based on HAZARD

Class		LD_{50} for the rat (mg/kg body weight)	
		Oral	Dermal
Ia	Extremely hazardous	< 5	< 50
Ib	Highly hazardous	5–50	50–200
II	Moderately hazardous	50–2000	200–2000
III	Slightly hazardous	Over 2000	Over 2000
U	Unlikely to present acute hazard	5000 or higher	

Source: The WHO Recommended Classification of Pesticides by Hazard,2019

Sample Computation:

- **Given:**

  Body weight: 100 kgs

  Product: MsK 25EC

  Oral LD_{50}: >5000 mg/kg BW

How much of MsK should be ingested by the farmer to get 50% chance of dying (relative to the product toxicity)?

$$\frac{5{,}000\ \text{mg}}{1\ \text{kg}} \times \frac{X\ \text{grams}}{1{,}000\ \text{mg}} = \boxed{5\ \text{g / kg LD50}}$$

$$\frac{5\ \text{g}}{1\ \text{kg}} \times 100\ \text{kg} = \boxed{500\ \text{g}}$$

Lethal dose (for a 100kg person)

Lethal dose for MsK is 500 g or ml for a 100 kg person (LD_{50} of > 5,000 mg/kg bw).

HAZARDS due to Pesticides

Activity	Type of Hazard
Manufacturing and Formulation	Inhalation, Dermal, Oral,
Transport	Spillage (Inhalation, Dermal)
Storage	Inhalation, Dermal, Oral (children)
Application	Inhalation, Oral, Dermal
General Public	Residues

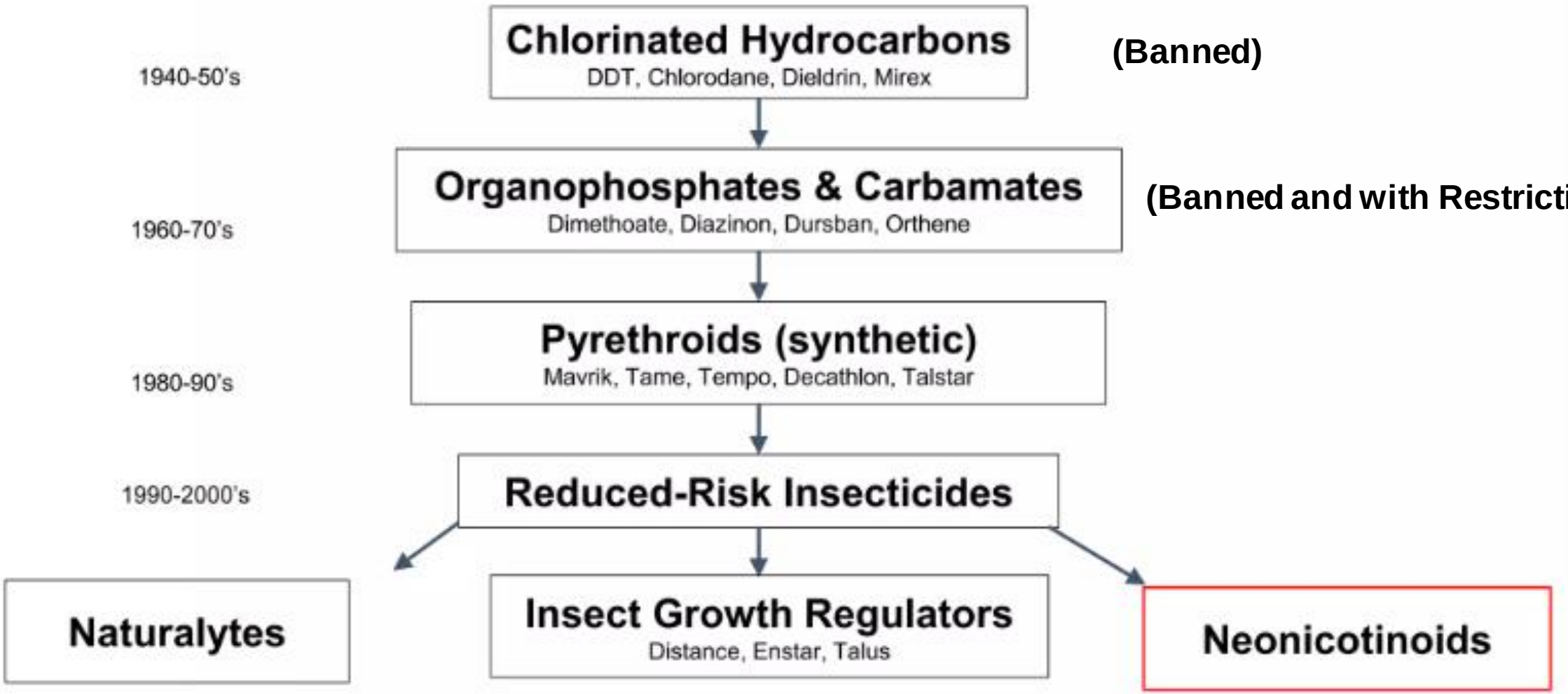
Classification of Pesticides based on Formulation Type

- **Bait** – mixture of active ingredient and food that attracts pests in the form of meal, pellets
- **Soluble Powder (SP)** – dry powder which dissolves in water to spray solution
- **Emulsion Concentrate (EC)** – contains active ingredient, petroleum solvent and emulsifiers. Pesticide is suspended in spray which is milky colored.
- **Fumigant** – volatile liquids or solids packaged to release a toxic gas
- **Granule (G or GR)** – dry inert materials (clay, walnut shell, corn cob) combined with active ingredient

Classification of Pesticides based on Chemical Composition

- Organochlorines
- Organophosphates
- Carbamates
- Pyrethroids

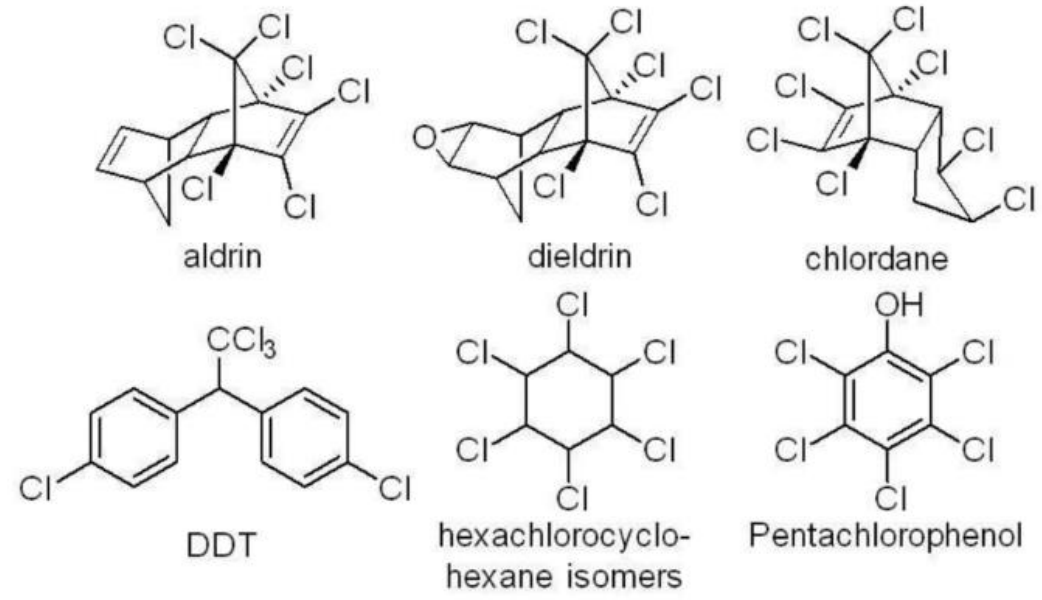
History - Evolution of Insecticides



1. Organochlorines (chlorinated hydrocarbon insecticides)

Insecticides that generally have a wide spectrum of insecticidal activity

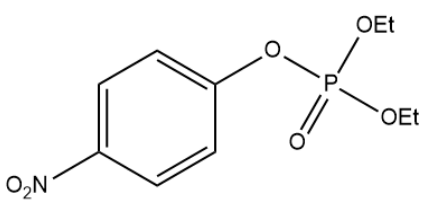
a. Diphenyl aliphatics: DOT, dicofol, methoxychlor
b. Benzene derivatves: BHC/HCH, lindane, Pentachlorophenol (PCP)
c. Cyclodienes: chlordane, aldrin, endrin, endosulfan, heptachlor
d. Polychloroterpenes; toxaphene



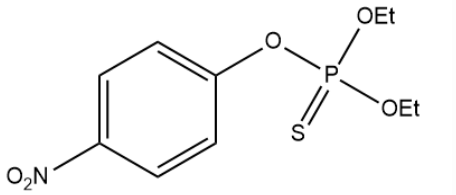
- Very persistent in the environment, does not degrade easily
- Bioaccumulative
- Most if not all are **banned globally**

2. Organophosphates

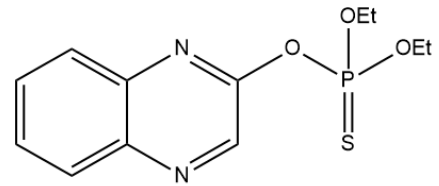
- Organophosphorus (OP) compounds are organic derivatives of phosphorus that have largely been used as pesticides and nerve agents, several of which are **highly toxic.**
- OPs interfere with the **acetylcholinesterase** enzyme, disrupting nerve impulses and killing or disabling the insect.



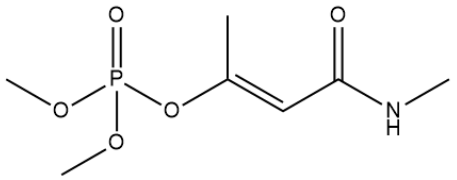
Paraoxon



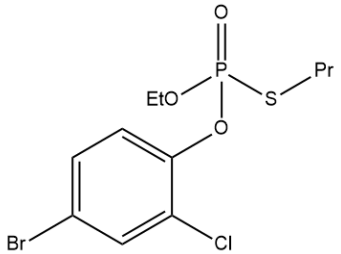
Parathion



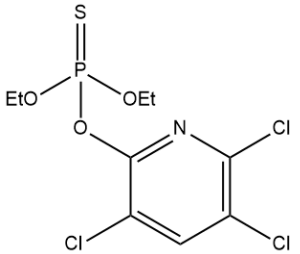
Quinalphos



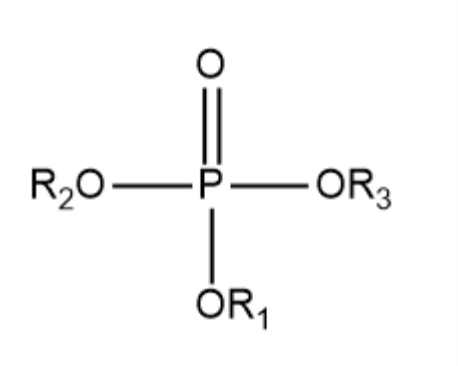
Monocrotophos



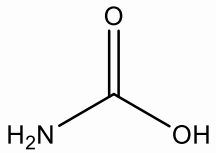
Profenofos



Chlorpyrifos



3. Carbamates (CMs)

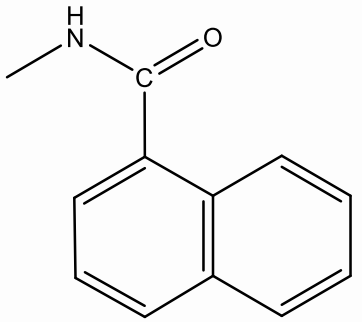


Carbamic acid

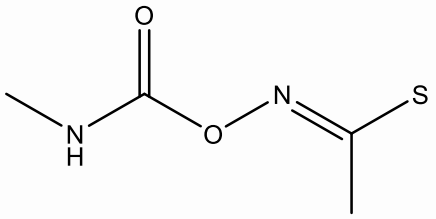
Derivatives of carbamic acid

Acetylcholinesterase inhibitor, with improved degradability

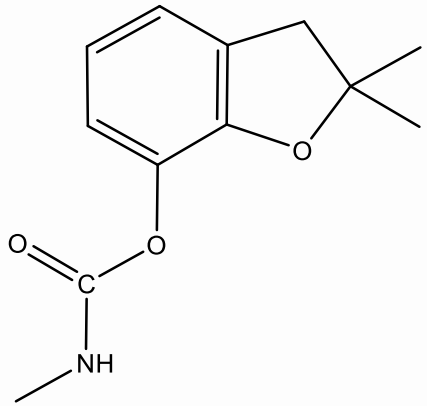
Carbaryl
Methomyl
Carbofuran
Carbosulfan
Oxamyl



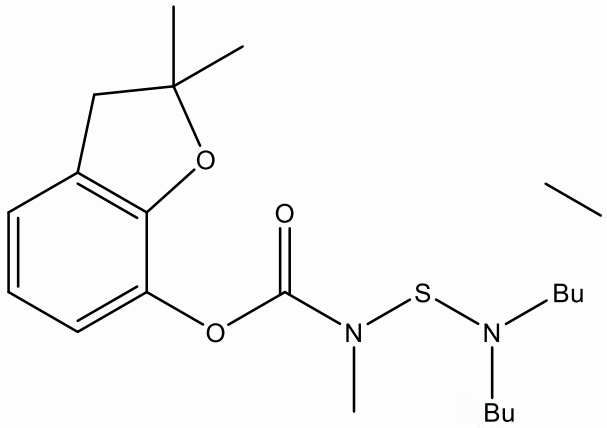
Carbaryl



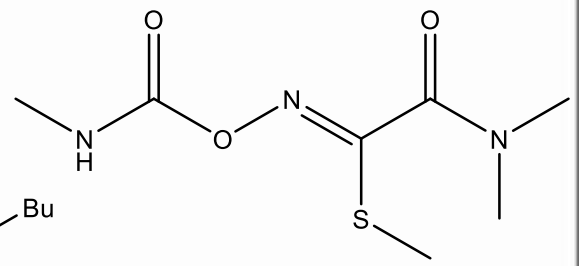
Methomyl



Carbofuran



Carbosulfan



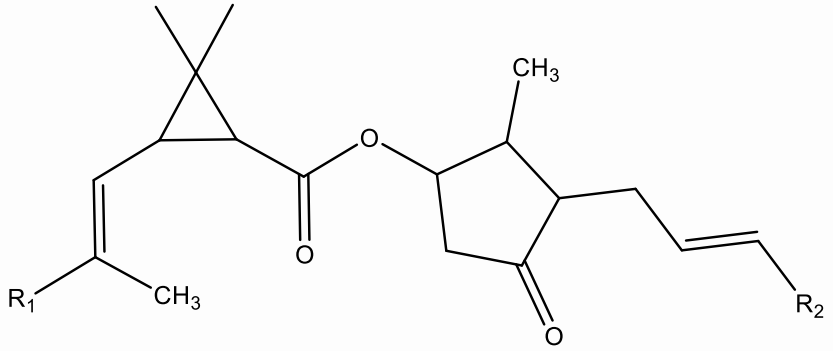
Oxamyl

4. Pyrethroids (-thrins)

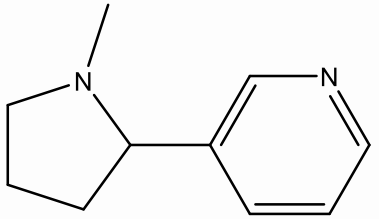
- an organic compounds analogous to the natural pyrethrins from Chrysanthemum.

- **Type I pyrethroids** are derivatives of pyrethrin.
  - ✓ Do not have a cyano group
- **Type II pyrethroids** have cyano group.

Type I Pyrethroids	Type I Pyrethroids
Allethrin	Cyfluthrin
Bifenthrin	Cyhalothrin
Permethrin	Cypermethrin
Phenothrin	Deltamethrin
Resmethrin	Fenvalerate
Tefluthrin	Fenpropathrin
Tetramethrin	Flucythrinate
	Flumethrin
	Fluvalinate

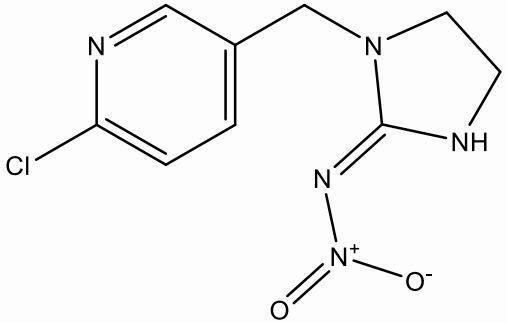


Neonicotinoids

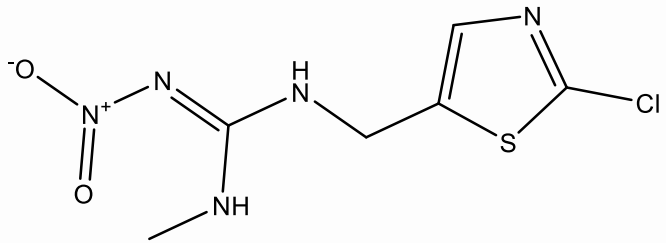


Nicotine

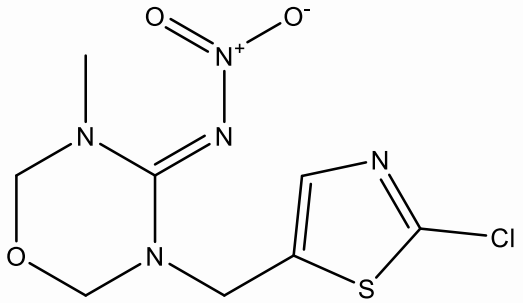
- Nicotine analog– antagonist to postsynaptic nicotine acetylcholine receptor (nAChR)
- Selectively toxic to insects



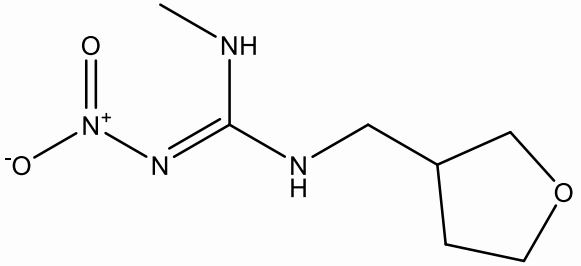
Imidacloprid



Clothianidin



Thiamethoxam



Dinotefuran

Organonitrogen Pesticides

- Pesticides that contain nitrogen in its chemical structure.
- Characterized by low detection limits in the range of low nanograms per liter with the use of mass spectrometer and nitrogen-specific detectors.

Biopesticides

- ✓ types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals.

- ✓ include naturally occurring substances that control pests (biochemical pesticides), microorganisms that control pests (microbial pesticides), and pesticidal substances produced by plants containing added genetic material (plant-incorporated protectants) or PIPs

Classes of Biopesticides

- **Biochemical pesticides** are naturally occurring substances that control pests by non-toxic mechanisms; include substances that interfere with mating, such as insect sex pheromones, as well as various scented plant extracts that attract insect pests to traps.

- **Microbial pesticides** consist of a microorganism (e.g., a bacterium, fungus, virus or protozoan) as the active ingredient; the most widely used microbial pesticides are subspecies and strains of Bacillus thuringiensis, or Bt.

- **Plant-Incorporated-Protectants (PIPs)** are pesticidal substances that plants produce from genetic material that has been added to the plant. For example, scientists can take the gene for the Bt pesticidal protein and introduce the gene into the plant's own genetic material. Then the plant, instead of the Bt bacterium, manufactures the substance that destroys the pest.

ANALYSIS OF PESTICIDES

Pesticide Analysis

- Chromatographic techniques are used to detect pesticides, and the type of chromatography used depends on the properties of pesticides such as its:
  - ✓ **Volatility**
  - ✓ **Solubility**

Pesticide Analysis

- **Solubility** is a measure of the ability of a pesticide to dissolve in a solvent, which is usually water.
  - ✓ pesticides that are highly soluble in water dissolve easily and are more likely to move with water in surface runoff or to move through the soil in water than less-soluble pesticides.

- **Volatility** is the tendency of a pesticide to turn into a gas or vapor
  - ✓ some pesticides are more volatile than others.
  - ✓ pesticide volatilization increases as temperatures and wind increase
  - ✓ volatility is also more likely under conditions of low relative humidity

Pesticide Analysis

- **Gas Chromatography (GC)** - used to analyze volatile pesticides, which can be vaporized and separated using a GC column.
  - ✓ Capillary gas chromatography is the most common technique used in pesticide analysis

- **Liquid chromatography (LC) -** used to analyze nonvolatile and semi-volatile pesticides, which are dissolved in a liquid solvent and separated using an LC column.
  - ✓ High-performance liquid chromatography (HPLC) and ultrahigh-performance liquid chromatography (UHPLC) are examples of LC.

Safety Data Sheet (SDS)

- A **safety data sheet (SDS)** is a document that contains information about the hazards of a chemical and how to use it safely.
  - are used to help reduce or eliminate the risks of using, storing, and handling hazardous chemicals.
  - should be arranged into 16 sections and has to provide certain information as prescribed under the REACH regulations
    - ✓ *Sections 1 through 8 contain general information about the chemical, identification, hazards, composition, safe handling practices, and emergency control measures (e.g., fire fighting).*
    - ✓ *Sections 9 through 11 and 16 contain other technical and scientific information, such as physical and chemical properties, stability and reactivity information, toxicological information, exposure control information, and other information including the date of preparation or last revision.*

- Employers must ensure that the SDSs are readily accessible to employees for all hazardous chemicals in their workplace.

Safety Data Sheet (SDS)

- ✓ Section 1: Identification
- ✓ Section 2: Hazard(s) Identification
- ✓ Section 3: Composition/Information on Ingredient
- ✓ Section 4: First-Aid Measures
- ✓ Section 5: Fire-Fighting Measures
- ✓ Section 6: Accidental Release Measures
- ✓ Section 7: Handling and Storage
- ✓ Section 8: Exposure Controls/Personal Protection
- ✓ Section 9: Physical and Chemical Properties
- ✓ Section 10: Stability and Reactivity
- ✓ Section 11: Toxicological Information
- ✓ Section 12: Ecological Information
- ✓ Section 13: Disposal Considerations
- ✓ Section 14: Transport Information
- ✓ Section 15: Regulatory Information
- ✓ Section 16: Other Information

Guidelines on Good Laboratory Practice in Pesticide Residue Analysis

Edna C. Mijares, RCh., MSc.
Jefcor Laboratories, Inc.

JEFCOR LABORATORIES, INC.
Quality Service is Our Business.

Normative References:

- CAC/GL 40-1993. Guidelines on Good Laboratory Practice in Pesticide Residue Analysis

- Maximum Residue Limits. *Retrieved from: https://croplife.org/crop-protection/regulatory/product-management/chemical-safety/maximum-residue-limits/*

- Codex Alimentarius. *Retrieved form https://www.fao.org/fao-who-codexalimentarius/en/*

OUTLINE

- General flowchart of pesticide residue analysis and corresponding CODEX guidelines per step
- CAC/GL 40-1993 (Rev.2003, Amend. 2010)

- **Guidelines** are intended to assist in ensuring the reliability of analytical results in checking compliance with maximum residue limits of foods moving in international trade.

- **Reliable analytical results** are essential to protect the health of consumers and to facilitate international trade.

Codex Guidelines for Pesticide residues

Reference	Title	Committee	Modified
CXG-33-1999	Recommended Methods Of sampling for the Determination Of Pesticide Residues for compliance with MRLs	CCPR	1999
CXG 40-1993	Guidelines on Good Laboratory Practice in Pesticide Residue Analysis	CCPR	2010
CXG 49-2003	Harmonized IUPAC Guidelines for Single-Laboratory Validation of Methods of Analysis	CCMAS	2003
CXG 59-2006	Guidelines on Estimation of Uncertainty of Results	CCPR	2011
CXG 84-2012	Principles and Guidance on the selection of Representative Commodities for the Extrapolation of Maximum Residue Limits for Pesticides to Commodity Groups	CCPR	2010
CXG 90-2017	Guidelines on Performance Criteria for Methods of Analysis for the Determination of Pesticide Residues in Food and Feed	CCPR	2017

Source: https://www.fao.org/fao-who-codexalimentarius/thematic-areas/pesticides/en/#c452840

Pesticide Residue Analysis

CAC/GL 40-1993. Guidelines on Good Laboratory Practice in Pesticide Residue Analysis

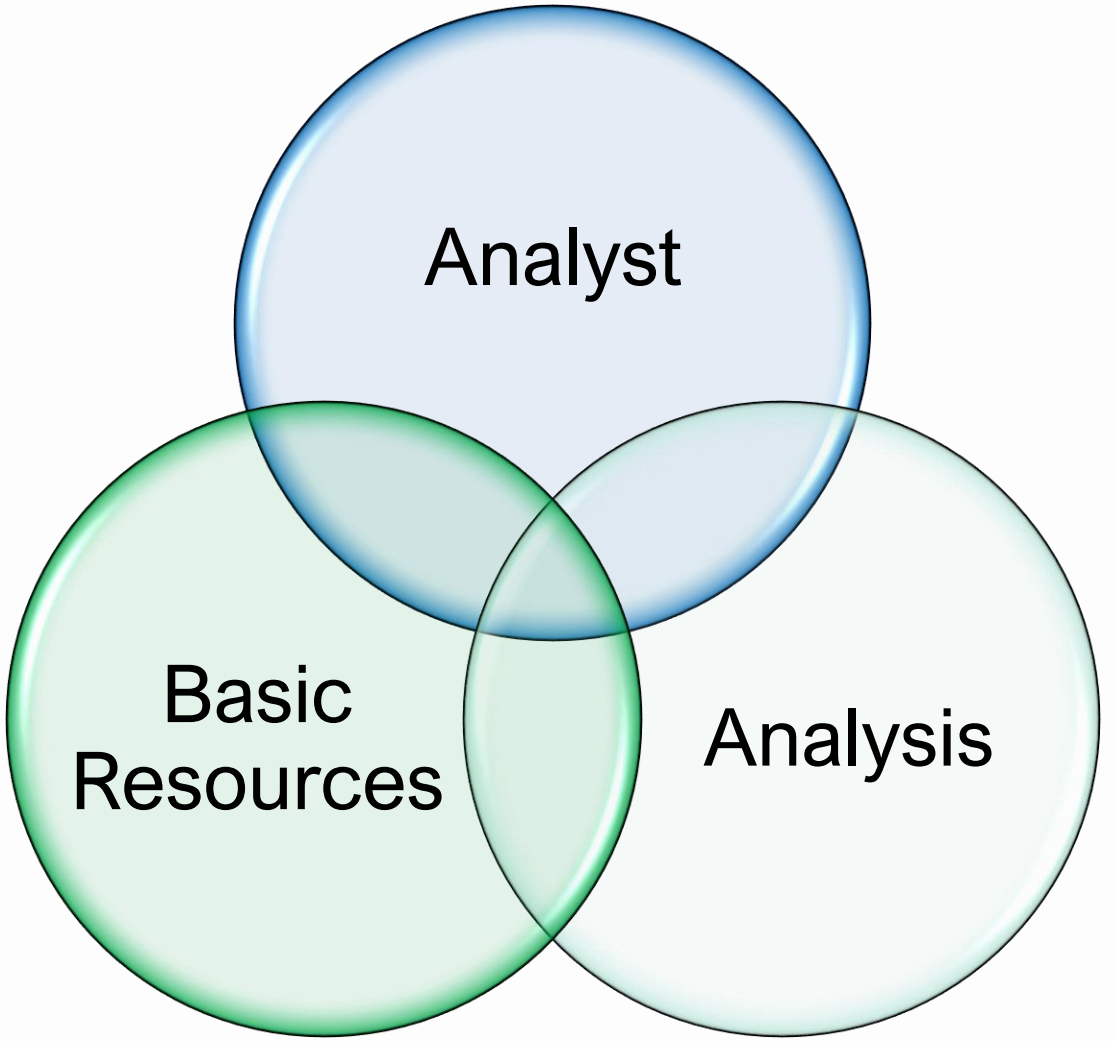
Sampling	• CAC/GL 33-1999. ***Recommended Methods of Sampling for the Determination of Pesticide Residues for Compliance with MRLS***
Sample Preparation	• CAC/GL 41-1993. Portion of Commodities to which MRL Apply and which is Analyzed
Sample Analysis	• CAC/GL 90-2017. ***Guidelines on Performance Criteria for Methods of Analysis for Determination of Pesticide Residues in Food and Feed*** • ***EU:SANTE 11312/2021***
Data Evaluation	• CAC/GL 56-2005. Guidelines on the Use of Mass Spectrometry (MS) for Identification, Confirmation and Quantitative Determination of Residues
Reporting	• CAC/GL 59-2006. Guidelines on Estimation of Measurement Uncertainty of Results

Pesticide analysis laboratory:

- An essential part of a national food control system
- Verify the safety and quality of food (both domestically produced, imported and/or exported) to be able to protect the consumers.

- provides support for food law enforcement, by providing scientific information needed for policy and decision making process related to food safety and quality.

Good Analytical Practice in Pesticide Residue Analysis



Codex CAC/GL 40-1993 (Rev.2003, Amend. 2010)

Codex CAC/GL 40-1993 (Rev. 2003. Amendment 2010 "Guidelines on Good Laboratory Practice In Pesticide Residue Analysis"

- **Analyst**
- **Basic Resources (Laboratory)**
- **Analysis**
  - ✓Validation of methods
  - ✓Performance Verification
  - ✓Confirmatory tests
  - ✓Mass spectrometry
  - ✓Lowest calibrated level (LCL)
  - ✓Expression of results

Codex CAC/GL 40-1993 (Rev. 2003. Amendment 2010 "Guidelines on Good Laboratory Practice In Pesticide Residue Analysis"

- **Parameters to be addressed in**
  - ✓**Method Validation**
  - ✓**Extension of validated method**
  - ✓**Adaptation of validated method by another laboratory**
  - ✓**Performance Verification**

- **Representation commodities/ samples for validation of analytical procedures**

A. Analyst

- ***Adequate number*** of Analysts with:
  - ✓ appropriate professional qualification
  - ✓ training and experience in operation of laboratory instruments
  - ✓ appropriate laboratory skills

A. Analyst

“Must” have experience and competent in pesticide residue analysis:

- ✓ Demonstrate that they can use the method within the expected performance parameters during method validation
- ✓ Have an understanding of the principles of pesticide residue analysis
- ✓ Have an understanding on the requirements of Analytical Quality Assurance systems
- ✓ Understand the purpose of each stage in the method
- ✓ Have ***training*** in the evaluation and interpretation of analytical results

B. Basic Resources

➢ Laboratory

- ✓ laboratory and facilities must be designed to allow tasks to be allocated to well defined areas where maximum safety and minimum chance of contamination of samples prevail.
- ✓ there must be active separation of activities in the laboratory
- ✓ must be equipped with all laboratory safety and waste management provisions

B. Basic Resources

Equipment and Supplies

- ✓laboratory will require adequate, reliable supplies of electricity and water

- ✓there must be ***adequate*** supplies and ***suitable quality*** of reagents, solvents, glassware and chromatographic materials

- ✓equipment used must be ***fit for purpose***

- ✓regular ***calibration and maintenance*** of all measuring equipment must be done and records must be kept up-to-date on file

B. Basic Resources

Equipment and Supplies

- ✓ Pesticide reference standards must be of known and acceptably high purity
- ✓ All analytical standards, stock solutions and reagents must be properly labelled
  - ✓ identity
  - ✓ analyst identification
  - ✓ preparation date
  - ✓ solvent used
  - ✓ storage conditions

C. Analysis

- Avoidance of Contamination
- Reception and Storage of Samples
- Standard Operating Procedures (SOPs)
- Validation of Methods
- Performance Verification
- Confirmatory Tests
- Mass Spectrometry
- Derivatization
- The Concept of Lowest Calibrated Level
- Expression of Results

Avoidance of Contamination

- Contamination and interferences are the critical areas in pesticide residue analysis
  - can give rise to false positive or false negative result or loss of sensitivity

- Possible sources of contamination:
  - Sampling
  - Sample transport and storage
  - Analyses
  - Chemical reagents
  - Glassware, syringe and chromatographic columns

Reception and Storage of samples

- The laboratory shall have a procedure for the transportation, receipt, handling, protection, storage, retention, and disposal or return of test item.
  - ✓ Precautions shall be taken to avoid deterioration, contamination, loss or damage to the item.

- The laboratory shall have a system for identification of test item and shall be retained while the item is under the responsibility of the laboratory.

- Samples must be stored at (1-5°C) away from direct sunlight if it cannot be analyzed immediately

- Samples received deep frozen must be kept at ≤ -16°C until analysis

- Storage of samples for longer period of time require storage at approximately -20°C

Standard Operating Procedures (SOPs)

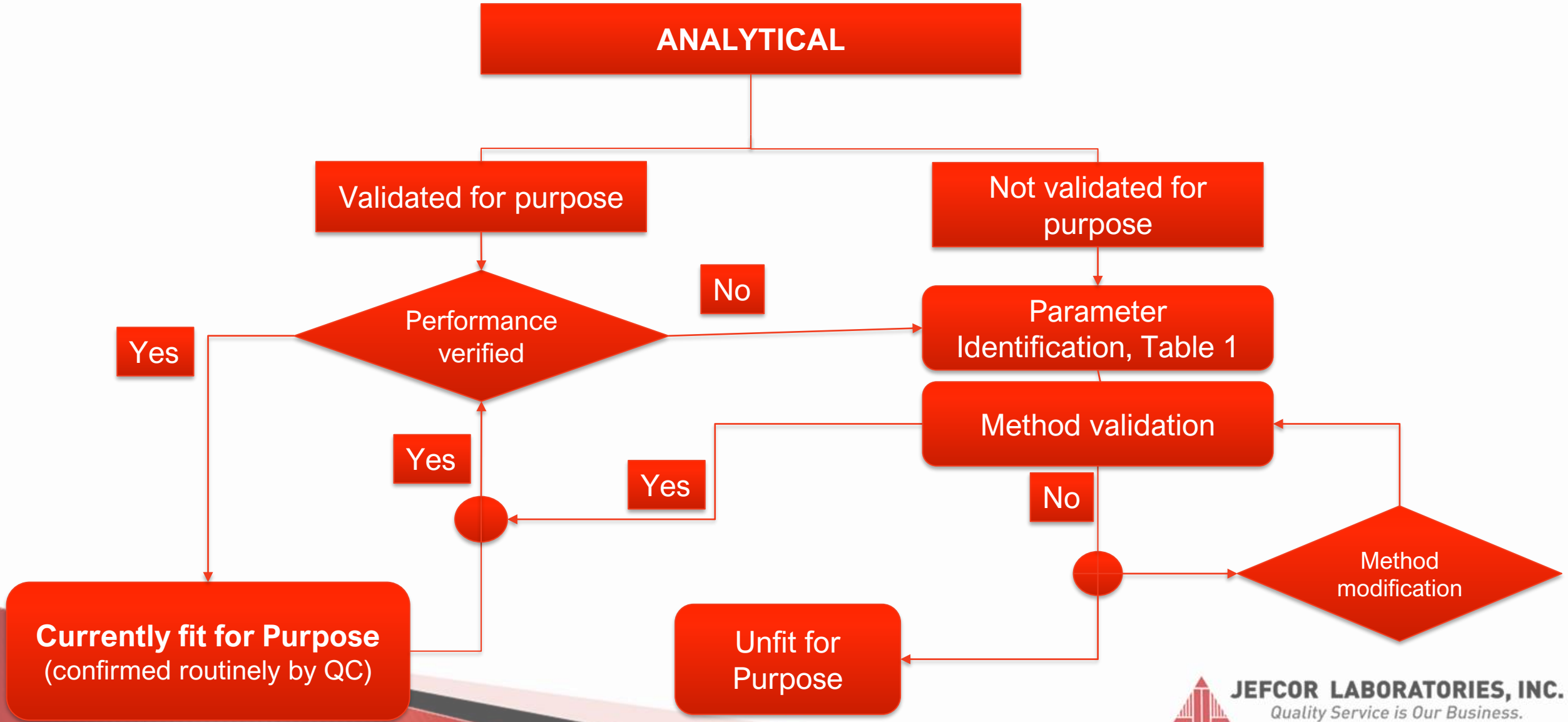
- **SOPs** should be used for all operations:
  - full working instruction & information on applicability, expected performance, internal QC requirements and calculation of results
  - information on any hazards arising from the method, from standards or form reagents

Validation of Methods

- **Analytical method –** is the series of procedures from receipt of sample to the production of final result.

- **Validation** is the process of verifying that a method is fit for intended purpose

METHOD VALIDATION WORKFLOW



Parameters to be assessed for method validation of new methods

- Specificity
- Analytical range
- Calibration range
- LOD and LOQ
- Analyte stability in sample extracts
- Extraction efficiency
- Homogeneity of analytical samples

Table 1 of Codex CAC/GL 40-1993 (Rev.2003, Amend. 2010)

Within Laboratory Method Validation
Criteria for Analysis of Pesticide Residues

Concentration	Repeatability		Reproducibility		Trueness
	CV_A%	CV_L%	CV_A%	CV_L%	Range of mean % recovery
≤1 µg/kg	35	36	53	54	50-120
> 1 µg/kg ≤ 0.01 mg/kg	30	32	45	46	60-120
> 0.01 mg/kg ≤ 0.1 mg/kg	20	22	32	34	70-120
> 0.1 mg/kg ≤ 1 mg/kg	15	18	23	25	70-110
> 1 mg/kg	10	14	16	19	70-110

Table 3 of CAC/GL 40-1993.

Representative commodities/samples for validation of analytical procedure for pesticide residues

Commodity Group	Common Properties	Commodity Class	Representative
I.	High water and chlorophyll content	Leafy vegetables, Brassica leafy vegetables, legume vegetables	spinach or lettuce broccoli, cabbage, kale
II,	High water, low to no chlorophyll content	Pome Fruits Stone Fruits Berries Small Fruits Fruiting Vegetables Root Vegetables	apple, pear, peach. cherry Strawberry, grape, tomato. bell pepper, melon Mushroom, potato, carrot, parsley
III,	High acid content	Citrus Fruits	Orange, lemon
IV.	High sugar content		Raisins, dates
V.	High oil or fat	Oil seeds Nuts	avocado, sunflower seed walnut, pecan nuts, pistachios
VI.	Dry Materials	Cereals Cereal Products	wheat. rice or maize grains wheat bran, wheat flour
	Commodities requiring individual test		garlic, hops, tea, spices, cranberry

Representative analyte *may* be used to assess the performance of a method

- The representative analyte(s) selected should:
  - ✓possess sufficiently wide range of physico-chemical properties
  - ✓regularly detected analyte or for which critical decisions will be made based on the results

- All analytes included in the initial validation process should be those which will have to be tested regularly and determined simultaneously

- The concentration of the analyte used to characterize a method should cover the high and low acceptable limits of all analytes in all commodities

Table 4 of Codex CAC/GL 40-1993 (Rev.2003, Amend. 2010)

Performance Verification

- **Purpose:**

  - monitor the performance of the method under actual condition;
  - consider the effect of analytical variations ;
  - demonstrate that the performance characteristic of the method are similar to the established method validation, showing that method is under “statistical control”;
  - data obtained during method validation may be updated with data collected from performance verification during the regular use of the method

Parameters for Within Laboratory (single laboratory) performance of optimized method

- Analyte stability in extracts and standard solutions
- Calibration function ; Matrix effect
- Analytical range
- Specificity and selectivity of analyte detection
- Selectivity of separation
- Homogeneity of analyte in analytical sample
- Analyte stability during sample processing
- Extraction efficiency
- Analyte stability during sample storage

Table 2 of Codex CAC/GL 40-1993 (Rev.2003, Amend. 2010)

Parameters for extension of the validated method

- Analyte stability during sample storage, processing, and in extracts and standard solutions
- Calibration function, matrix effect
- Accuracy
- Specificity and selectivity of analyte detection
- Selectivity of separation
- Extraction efficiency

Table 2 of Codex CAC/GL 40-1993 (Rev.2003, Amend. 2010)

Parameters for adaptation of the validated method in another laboratory

- Purity and suitability of chemicals, reagents and sorbents
- Analyte stability in extracts and standard solutions
- Calibration function; matrix effect
- Analytical range; accuracy and precision; limit of detection; limit of quantitation
- Specificity and selectivity of analyte detection
- Analyte “homogeneity”
- Analyte stability in extracts and standard solutions

Table 2 of Codex CAC/GL 40-1993 (Rev.2003, Amend. 2010)

Performance Verification

- **Use of Control Charts**
  - tool for demonstrating the performance of a method and the reproducibility of the selected parameters
  - for a large number of the same type of sample for the analysis of the same active ingredient, the control chart is based on the mean recovery and its standard deviation obtained during the regular use of the method

Requirements for performance verification (*methods used regularly*)

- Suitability of chemicals, adsorbents and reagents
- Calibration and analytical range
- Accuracy and precision
- Selectivity of separation; Specificity of detection and performance of detectors
- Analyte homogeneity in processed sample
- Extraction efficiency
- Duration of analysis

Table 4 of Codex CAC/GL 40-1993 (Rev.2003, Amend. 2010)

Confirmatory Tests

- **Use of Control Charts**
  - tool for demonstrating the performance of a method and the reproducibility of the selected parameters
  - for a large number of the same type of sample for the analysis of the same active ingredient, the control chart is based on the mean recovery and its standard deviation obtained during the regular use of the method
  - control charts cannot be applied for multiresidue analysis

Mass Spectrometry

- confirmatory technique for residue analysis
- can also be used for residue screening purposes
- the most definitive confirmation of the presence of a residue is the acquisition of its “complete” electron-impact ionization mass spectrum
- the relative abundance of ions in the spectrum and the absence of interfering ions are important consideration in confirming identity

The Concept of Lowest Calibrated Level (LCL)

- **For MRLs or other Accepted Limits (ALs) compliance**
  - the residue methods must be sufficiently sensitive to reliably determine the residues present in a sample at or around MRL or AL
  - it is not necessary to use methods to determine residues at levels two or more orders of magnitude lower, due to very high cost

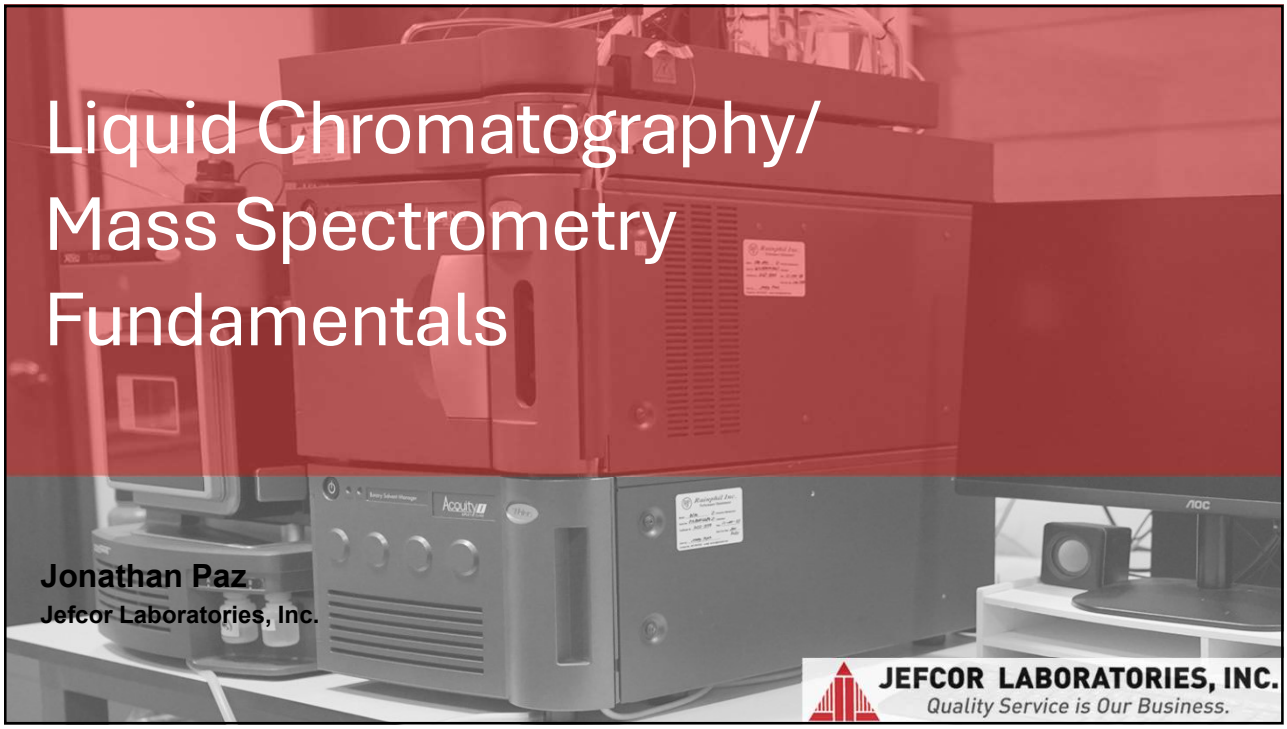
- When the MRL is set at the limit of determination of the analytical method, the LCL will also be at this level

Setting the Lowest Calibrated Level

MRL (mg/kg)	LCL (mg/kg)
5 or greater	0.5
0.5 up to 5	0.5
0.05 up to 0.5	0.02 increasing to 0.1 for MRLs
Less than 0.05	0.5 x MRL

Expression of Results

- only confirmed data should be reported, expressed as defined by MRL
- null values should be reported as being less than the lowest calibrated level
- results are not corrected for recovery, and they may only be corrected if the recovery is significantly different from 100%



1

Normative References

- Waters LC/MS Booklet
- Agilent University - Liquid Chromatography/Mass Spectrometry Fundamentals
- E. Michael Thurman, Imma Ferrer, Amadeo Fernández-Alba, Chapter 8 LC-MS. I: Basic principles and technical aspects of LC-MS for pesticide analysis,
- Comprehensive Analytical Chemistry,
- Codex GL56 "Guidelines on the Use of Mass Spectrometry (MS) for Identification, Confirmation and Quantitative Determination of Residues

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2

Recap: Chromatography

- Physical separation technique.

  The components of a mixture are distributed between a stationary phase and the mobile phase.

- Separation is based on the affinity of the components for each phase,

  Increased affinity = longer retention on the stationary phase = separation.

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3

Recap

- Samples prepared in a suitable solvent, are pumped through a chromatography column and separated by interactions within the stationary phase.
- After separation by retention time, compounds may continue to an orthogonal detector for detection and quantification.

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What is Mass Spectrometry?

An analytical technique where the components of a sample are separated by their **mass (m) and electrical charge (Z)**.

It's used for both quantitative and qualitative chemical analysis of sample compounds.

unknown compounds by determining molecular weight and elucidating the structural properties of the molecular components.

quantifying known compounds by total mass determination

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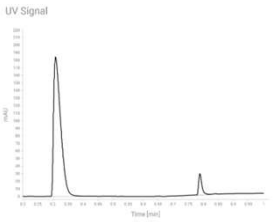
Why add a MS system to HPLC

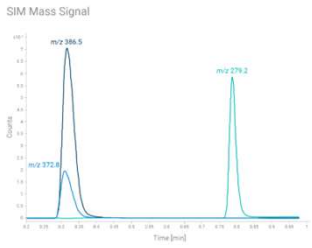
A wide variety of detector types can be integrated into an LC system.

Photometric (UV & DA) detectors are typically used when the analyte has significant chromophores

Mass spectrometry provides unique, valuable, and **orthogonal** information.

Allows quantitating compounds that are not amenable to UV detection or incomplete chromatographic separation (co-elution)





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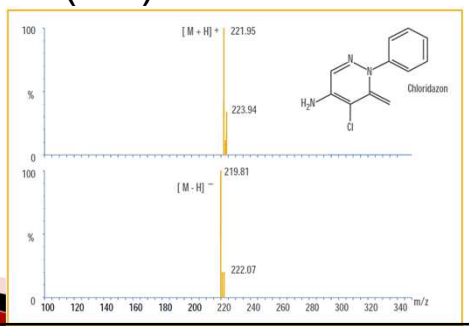
Mass Spectrometry Instrumentation

Mass spectrometers operate by converting the analyte molecules to a charged (ionized) state, with subsequent analysis of the ions and any fragment ions that are produced during the ionization process, on the basis of their mass to charge ratio (m/z).



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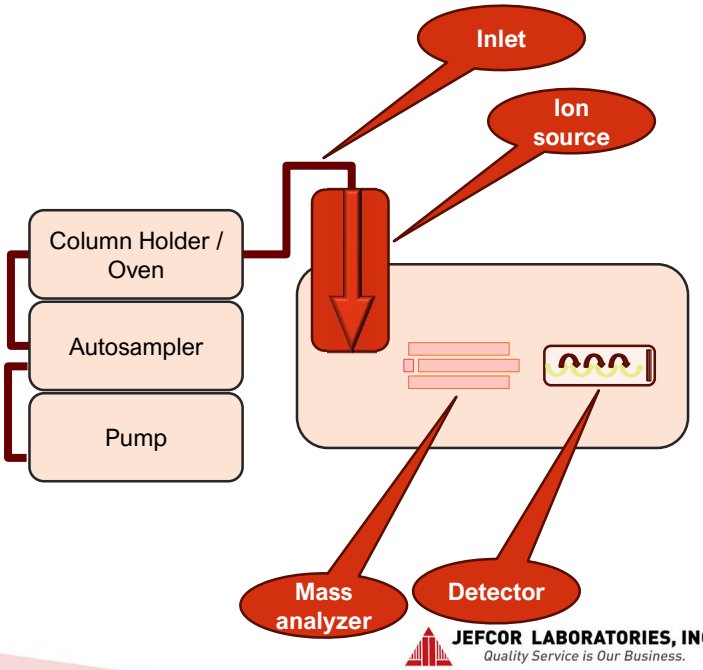
Components of a LC-MS System

Inlet system: introduces a small amount of sample into the ion source with minimal loss of vacuum.

Ion source: It is the heart of the mass spectrometer. It is where the sample is ionized

Mass analyzer: It is responsible for taking the ionized masses and sorting them according to their mass-to-charge ratio (m/z).

Detector: converts the sorted ionized masses to quantifiable data.



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Sample introduction to the MS

Mass spectrometry (MS) is used to determine the mass of **gas phase ions**

First step: create a spray. At very low flow rate.

The difference in potential is sufficient to create the spray.

LC–MS. I: Basic principles and technical aspects of LC–MS for pesticide analysis

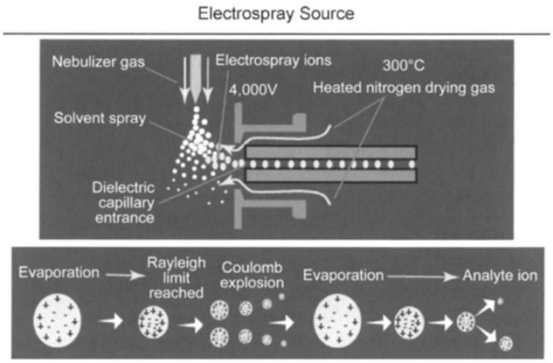
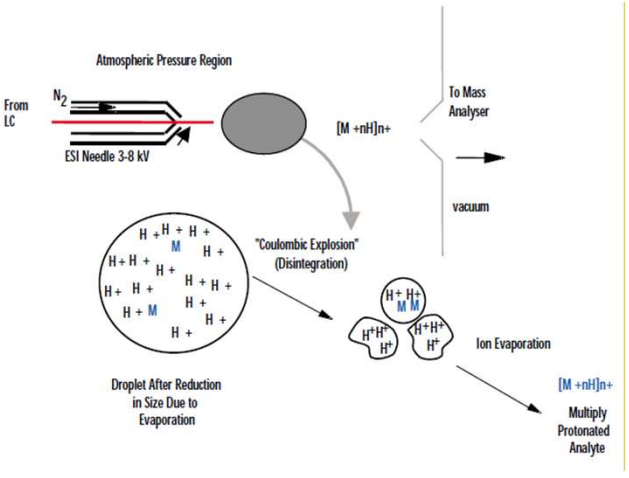


Fig. 8.2. An ESI source.

9

the HPLC is connected to the electrospray probe, which consists of a metallic capillary surrounded with a nitrogen flow.

A voltage is applied between the probe tip and the sampling cone. In most instruments, the voltage is applied on the capillary, while the sampling cone is held at low voltage.



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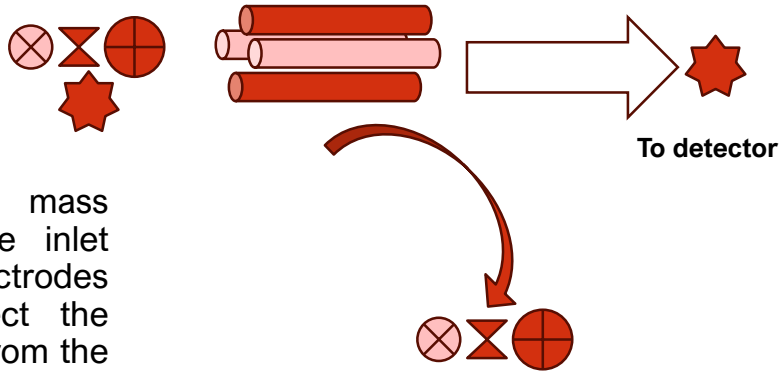
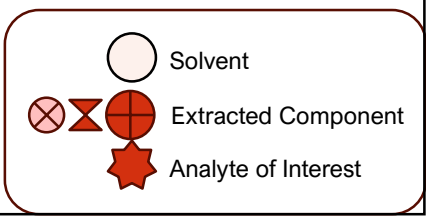
Mass analyzer:
Triple Quadrupole System



After entering the mass spectrometer through the inlet capillary, a series of electrodes known as "lenses" direct the charged molecules away from the source toward the quadrupole mass analyzer

"filters and selects masses that enter the detector"



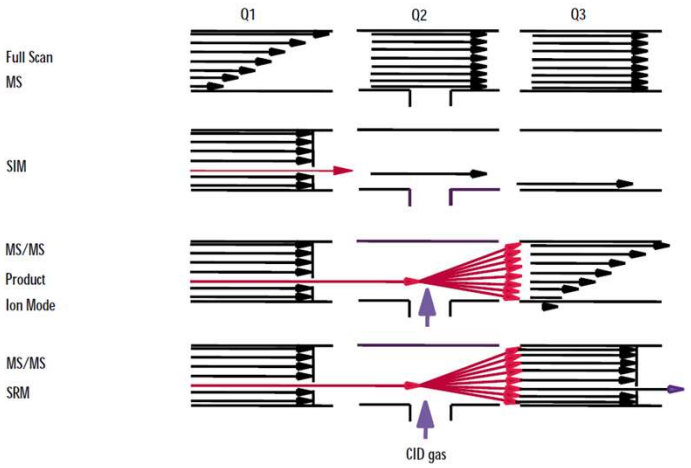
https://www.waters.com/waters/gpl.htm

11

MS/MS WITH TRIPLE QUADRUPOLES

A triple quad instrument can be used in various ways,

1. **Full scan** MS: all mass fragments enter the detector
2. **Selected Ion** Monitoring (SIM): one or more fragments are allowed to enter the detector
3. MS/MS **Product Ion** Mode (PIC): selected parent ion is fragmented and all product ions are detected
4. **Selected Reaction** Monitoring (SRM): one parent ion and one or more product ions are detected



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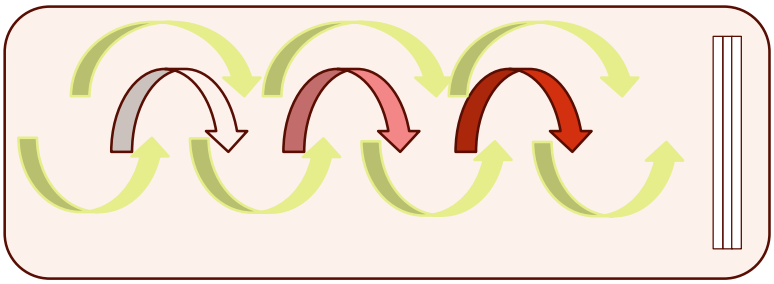
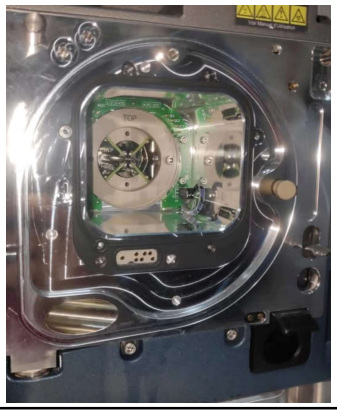
Mass Detectors

To produce the mass spectrum, the detector records the signal intensity from ions arriving **at each given time.**

The pattern of this mass spectrum can be used for identification, much like a fingerprint. In addition, the response recorded for the different ion species can be calibrated for quantitative purposes.





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the advantages of LC/MS?

- LC/MS provides an additional dimension of selectivity
- Analytes are separated by liquid chromatography (HPLC or UHPLC), creates individual peaks for each analyte separated by retention time.
- For each time point, a mass spectrum is also created and can be integrated to identify the m/z of components eluting at that time point.
- With mass information, analytes can be identified without the need for a reference, and co-eluting peaks can be resolved by mass

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Application for regulation: CAC GL 56

Identification of analytes should be based on chromatographic peaks observed in the extracted ion chromatograms of two or more selective ions.

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The following identification criteria should be met:

- Analyte retention time
- Ion ratio reference values
- signal to noise ratios for measured peaks must be greater than 3
- ion transitions chosen for identification purposes should make chemical/structural sense
- reagent and matrix blank samples are free of contamination, and/or interferences, a response >20% of the LOQ. For matrix blank samples, 30% of LOQ may be acceptable.

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Table 3. *Identification requirements for different MS techniques*[2]

MS detector/Characteristics		Acquisition	Requirements for identification	
Resolution	Typical systems (examples)		minimum number of ions	additionally
Unit mass resolution	Single MS Quadrupole, ion trap, TOF	Full scan, limited m/z range, SIM	3 ions	S/N ≥ 3[d] Analyte peaks from both product ions in the extracted ion chromatograms must fully overlap.
	MS/MS Triple quadrupole, ion trap, Q-trap, Q-TOF, Q-Orbitrap	Selected or multiple reaction monitoring (SRM, MRM), mass resolution for precursor-ion isolation equal to or better than unit mass resolution	2 product ions	Ion ratio from sample extracts should be within **±30 % (relative)** of average of calibration standards from same sequence
Accurate mass measurement	High resolution MS: (Q-)TOF (Q-)Orbitrap	Full scan, limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof	2 ions with mass accuracy ≤ 5 ppm[a, b, c]	S/N ≥ 3[d] Analyte peaks from precursor and/or product ion(s) in the extracted ion chromatograms must fully overlap. Ion ratio: see D12

[a] preferably including the molecular ion, (de)protonated molecule or adduct ion
[b] including at least one fragment ion
[c] <1 mDa for m/z <200
[d] in case noise is absent, a signal should be present in at least five subsequent scans

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Gathered mass data must make chemical sense

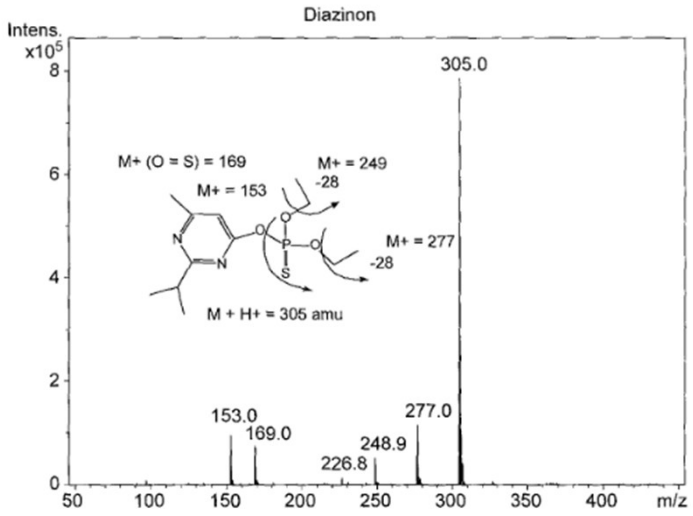

Fig. 8.5. Mass spectrum of diazinon.

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LCMS TROUBLESHOOTING

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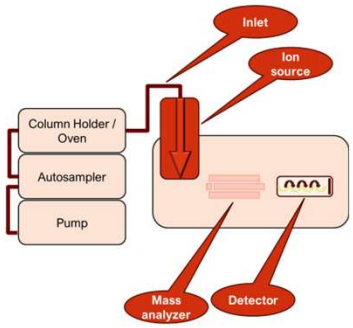
Common Problems Encountered in LC-MS

- Interface Problem
- LC Problem
- MS Problem



Two approaches to Troubleshooting:
1)Preventive maintenance.
2)Troubleshooting:
a) Isolate area where problem arise.
b) Identify problem.
c) Solve it.

Duty of analyst:
Able to identify problems &
Conduct preliminary troubleshooting measures.

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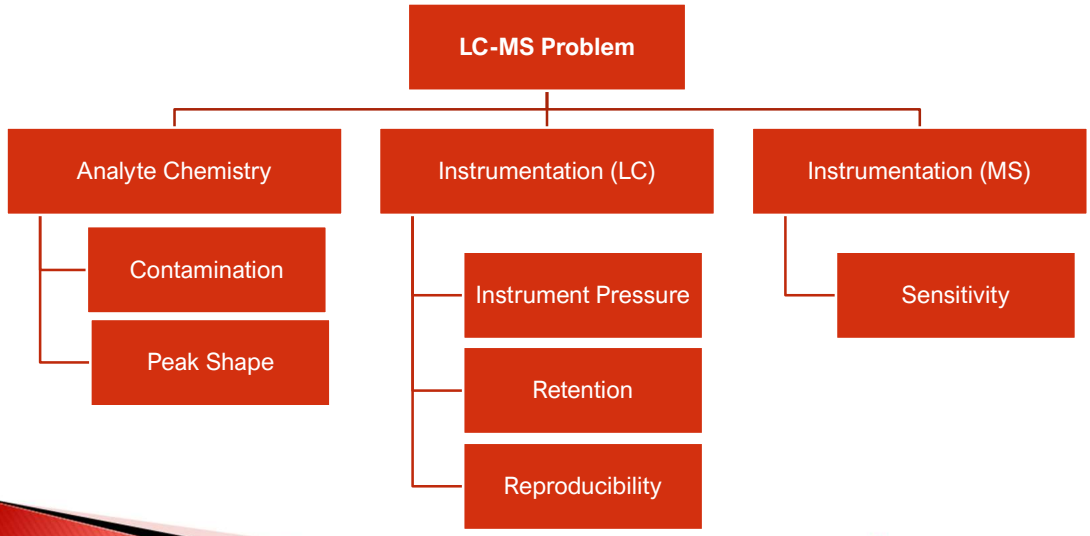
Strategies to Troubleshooting



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LC Pressure Issues

Observation: High Pressure increase

Potential Problems:

1. Plugged Inlet Frit
2. Purge Valve Frit
3. Column contamination
4. Plugged Capillary or Needle
5. Seat Plugged
6. Plugged Column Packing

Low Pressure

- Typically a connection or LC pump/seal issue; unless the column
- has been improperly used and disassembled or lost all its packing.

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Preventing Column Pressure Problems

1. Filter mobile phase:
   - filter non-HPLC grade solvents
   - filter buffer solutions
2. Filter all samples and standards
3. In-line filters
   - Install an in-line filter between auto-sampler and column
4. Perform sample clean-up on dirty samples.
5. Appropriate column flushing — flush buffers from entire system with water/organic mobile phase
6. Replace buffers every 24-48 hours, never add to the bottle, to avoid microbial growth

23

Reproducibility problems: Effect of mobile phase pH

Arises from changes in pH of the mobile phase.

Develop mobile phase that not sensitive to pH changes.

Use proper buffer for mobile phase

Negative effect of silanol group on column, resulting in active sites for retention shifts

24

Reproducibility problems: Solvents used

Improper injection solvent selection causes

- Poor retention.
- Inaccurate peak.
- Precipitation in parts of MS.

Remedies :

- Choose injection solvent compatible with the mobile phase.

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Sound Sample Preparation

Sample cleanup in LC-MS is essential.

Required to remove unwanted compounds, to increase method sensitivity and specificity.

Step 1

1)Solid phase extraction

2)Liquid-liquid extraction

3)protein precipitation

Step 2

Separate analyte from unwanted compound by LC

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More Matrix Removal = Less Matrix Entering System = Time and Cost Savings!

Less matrix build-up
- Less interferences
- Improved S/N
- Better reproducibility

Better chromatography
- Less time spent on data
- analysis/manual integration
- Less time spent on re-runs/recalibrations

Less maintenance
— Less instrument down-time
— Saves $$ on consumables/services

Less troubleshooting
— "Is it my column or my MS"?
— Less instrument down-time

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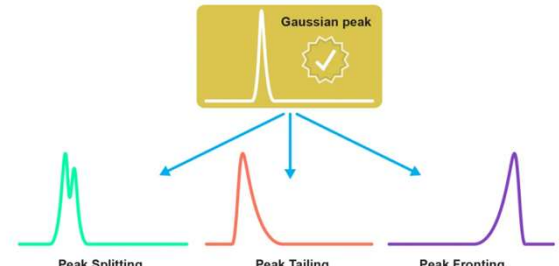
Peak Shape Changes: Splitting, Fronting and Tailing

Peak splitting often observed when injecting a large volume of sample in a solvent that is stronger than the mobile phase

Tip
- When injecting a sample in strong solvent, limit the size of the injection
- Inject the sample in a solvent that is no stronger than the starting conditions for the method



https://www.acdlabs.com/blog/an-introduction-to-peak-tailing-fronting-and-splitting-in-chromatography/

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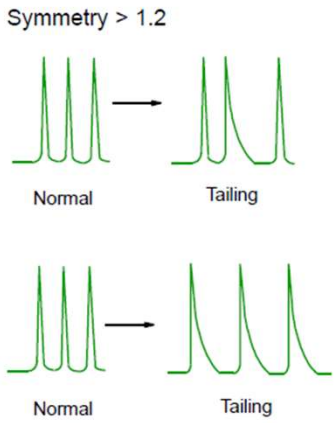
Peak Shapes: Tailing



Causes

Some Peaks Tail:

- Secondary interactions
- Small peak eluting on tail of larger peak

All Peaks Tail:

- Extra-column effects i.e. poor connections, too much volume
- Build up of contamination on column inlet (partially plugged frit)
- Bad column

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Retention Shifts

All Peaks Shift to Lower Retention (acids, bases, neutrals)

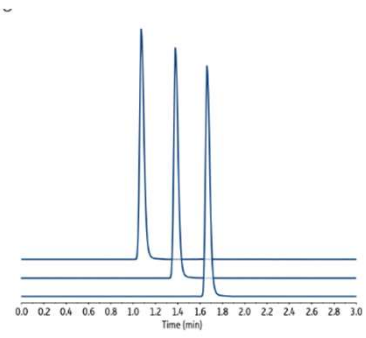
- a) Loss of Bonded-Phase
- b) Mobile Phase Unstable (less likely)
- c) Solvent Delivery System (flow rate)

All Peaks Shift to Greater Retention

- a) Loss of Organic Solvent in Aqueous / Organic Mix
- b) Column Change (less likely)
- c) Solvent Delivery System (flow rate)

Ionic Peaks Shift Retention

- a) Loss of Volatile MP Component (ionic strength, pH shift)
- b) Column Change (bonded phase or contamination)



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Ghost Peaks: Potential sources of contamination

- **Solvent bottles**: they are not flushed or specific for MS applications
- **Solvents:** use branded MS grade solvents from reliable suppliers
- Bottle head assembly & solvent inlet filters: ***don't touch with hands,***
- **Degasser:** flush all solvent channels with aqueous and organic solvents, switch channels for troubleshooting
- Packing materials for all parts

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Thank You!

Maraming salamat!

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ASEAN JICA Food Value Chain Development Project

01/2025

PESTICIDE RESIDUE ANALYSIS USING MODULAR

QuEChERS - BS EN 15662:2018

"Foods of plant origin - Multimethod for the determination of pesticide residues using GC- and LC-based analysis following acetonitrile extraction/partitioning and clean-up by dispersive SPE - Modular QuEChERS-method"

Pesticide Analytical Laboratory Section

Plant Product Safety Services Division

Bureau of Plant Industry

Quezon City, metro manila, philippines

I.Gaza

PESTICIDE RESIDUE UNIT

What is QuEChERS?

What is QuEChERS?

2003

Sample Preparation Technique

Pesticide Residue Analysis

Quick Minimal steps and time

Easy User-friendly process

Cheap Low solvent and resource usage

Effective High recovery for diverse pesticides

Rugged Reliable across various conditions

Safe Reduces exposure to hazardous chemicals

Sodium acetate buffer
AOAC standard compliance
Focus on AOAC-related foods
Harmonized in AOAC

Original QuEChERS

Anastassiades et al. in 2003

EXTRACTION

ACETONITRILE

CEN Method

European Committee for Standardization

Sodium citrate buffers
EU regulatory compliance
Emphasis on European foods
Better for pH-sensitive
Harmonized in EU

CLEAN-UP

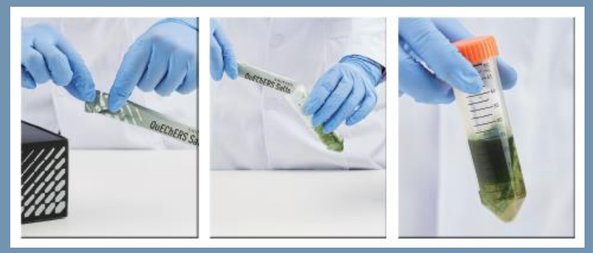
dSPE

AOAC Method

Association of Official Analytical Chemists

No buffer
Flexible compliance
General food
Limited for pH-sensitive
Not standardized

SAMPLE PREP



EXTRACTION

CLEAN-UP

DETECTION

BS EN 15662:2018

Acetonitrile extraction/partitioning and clean-up by dispersive SPE - Modular QuEChERS-method

BS EN 15662:2018

Acetonitrile extraction/partitioning and clean-up by dispersive SPE - Modular QuEChERS-method

BS EN 15662:2018 is a European standard for determining pesticide residues in food and feed, detailing a modified QuEChERS method for multi-residue analysis of various matrices of plant origin.

Widely used in analytical laboratories for regulatory compliance and quality assurance.

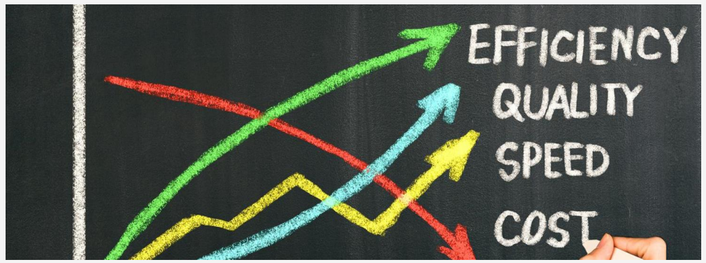
BS EN 15662:2018

Acetonitrile extraction/partitioning and clean-up by dispersive SPE - Modular QuEChERS-method

- Fruits and vegetables (e.g., apples, tomatoes, leafy greens)
- Cereals and cereal-based products
- Processed foods of plant origin (e.g., juices, jams)

- Wide range of pesticide residues, including organochlorines, organophosphates, carbamates, and pyrethroids
- Applicable to both non-polar and polar compounds
- Targets multiresidue analysis with GC-MS and LC-MS detection

IMPORTANCE



ENSURES FOOD SAFETY

Consumer Protection and Harmonized Limits

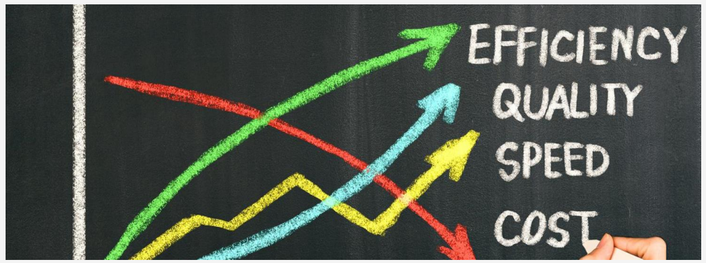
- Identifies and quantifies pesticide residues in food to ensure they are within permissible limits.
- Supports compliance with Maximum Residue Limits (MRLs) set by regulatory agencies like the EU and WHO.

METHOD STANDARDIZATION and STRAIGHTFORWARD APPROACH

Standardized Methodology and Quality Assurance

- Provides a consistent and reliable approach for pesticide residue analysis, accepted globally.
- Simple and straightforward method approach.

IMPORTANCE



COMPLANCE WITH LEGAL REQUIREMENTS AND PROMOTION OF GLOBAL TRADE

-Ensures food producers and exporters meet strict pesticide regulations, avoiding trade barriers.

ANALYTICAL EFFICIENCY

-Multi-Residue Analysis: Capable of detecting a wide range of pesticides in a single analysis, saving time and resources.

-Versatility: Modular design allows adjustments for different food matrices and varying pesticides.

- Cost-Effective: Reduces the need for expensive equipment or complex methods while delivering reliable results.

BS EN 15662:2018 MODULES in FOCUS

Extraction Modules (E1-E9)

These modules outline the procedures for extracting residues from various food matrices.

E1 – General purpose module for high-water content plant material (Fruits and Vegetables, Juices)
E2 – pH-sensitive matrices requiring buffer salt to stabilize analytes (Lemon, Limes)
E3 – Plant material of intermediate water content, and where pH control is unnecessary (Bananas,Dates)
E4 – Plant material with low water content (Dried fruits)
E5 – Plant material with very low water content (Grains, Honey)
E6 – Plant material with intermediate water content and high oil content (Garlic, Onion)
E7 – Plant material with very low water content and high matrix load, also used for freeze-dried products(Spices, Coffee)
E8 – Extraction for determining acidic pesticides, Plant material and edibles with neutral or acidic pH and high water content
E9 – Extraction for determining acidic pesticides, Plant material and edibles with low water content

BS EN 15662:2018 MODULES in FOCUS

Clean-up Modules (C0-C5)

C0 – NO CLEAN-UP, Plant materials with low matrix load, determination of base sensitive and acidic pesticides
C1 – FREEZING-OUT, clean-up used to reduce fat in extracts of high fat/oil content by freezing out and precipitation of fats overnight
C2 – dSPE with PSA, clean-up used for determining neutral and alkaline pesticides, this is the standard cleanup for commodities not specified
C3 – dSPE WITH a.) 50 mg PSA /mL extract, or b.) 75 mg PSA /mL extract, clean-up for plant material with low water content
C4 – dSPE WITH PSA and C18, simultaneous clean-up of raw extracts and removal of co-extracted fat
C5 – dSPE WITH PSA and GCB, clean-up used for determining neutral and alkaline pesticides in highly pigmented extracts.

BS EN 15662:2018 MODULES in FOCUS

Stabilization Modules (S0-S1)

S0 – No Stabilization, use if acid-labile pesticides are to be determined
S1 – Stabilization with formic acid, use if acid-stable pesticides are to be determined

For every 1 mL of extract, 10 µL of 5% formic acid in acetonitrile is added.

This ensures the chemical stability of sensitive analytes.

METHOD (E1-C2)

1. SAMPLE PREPARATION, EXTRACTION, PARTITIONING (E1)

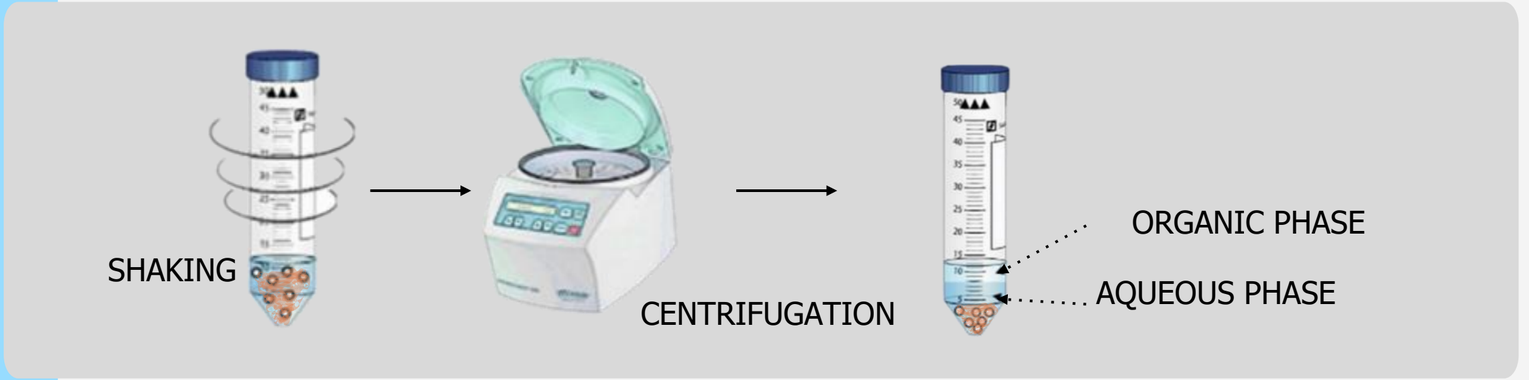
- *Weigh 10g (±0.1g) of homogenized sample into a 50 mL centrifuge tube.*

\+ *Add 10 mL of Acetonitrile to the sample*

- *Shake sample using a vortex,*
  1-3mins for room temperature sample
  or 15 mins for frozen samples

\+ *Add prepared buffer-salt mixture (EN salt)* to the suspension and immediately shake vigorously (second extraction)*

- *Centrifuge for 5 mins at 4500rpm.*



*4g of anhydrous magnesium sulfate, 1g sodium chloride, 1g of trisodium citrate dihydrate and 0.5 g of disodium hydrogen citrate sesquihydrate – Agilent Part Number:5982-5650,

METHOD (E1-C2)

AMBIENT TEMPERATURE

1 TO 3 MINUTES SHAKING

FROZEN STATE

15 MINUTES SHAKING

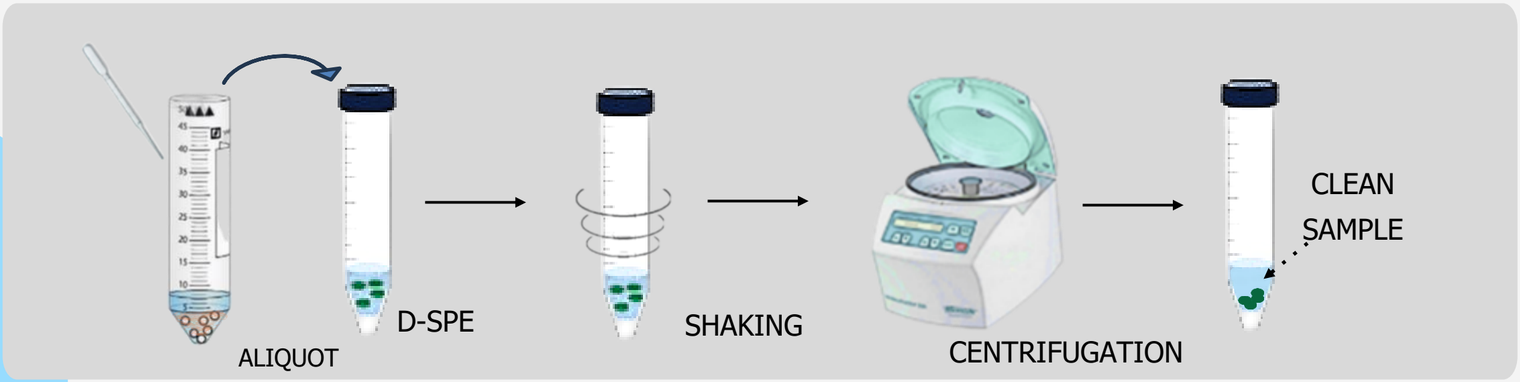
METHOD (E1-C2)

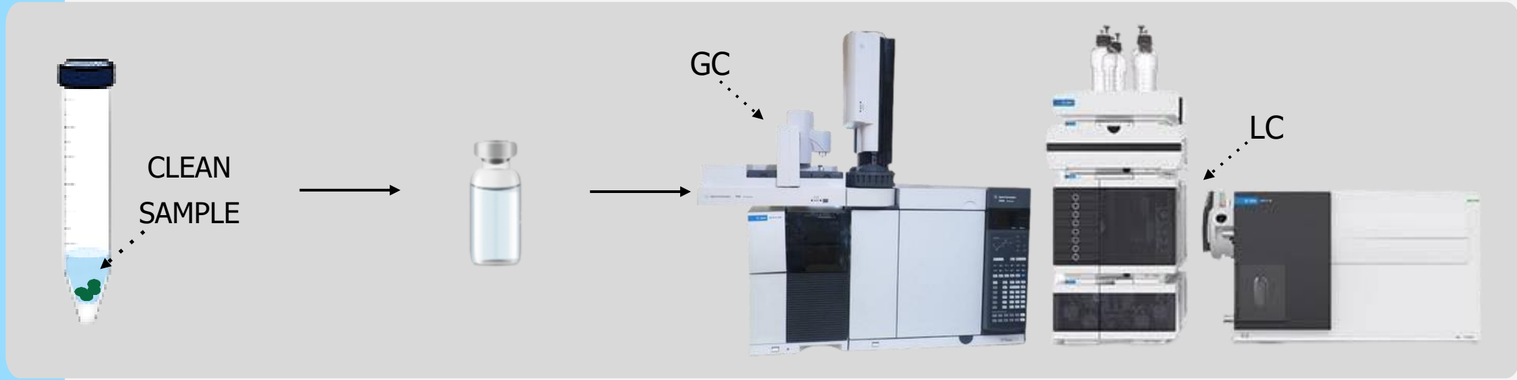
2. SAMPLE CLEAN-UP (C2)

- *Aliquot 6 mL of Acetonitrile extract, transfer to dSPE tube with amine sorbent*
  (150 mg PSA, 900 mg of magnesium sulfate)
- *Shake sample vigorously for 30 s using a vortex.*
- *Centrifuge for 5 mins at 4500rpm.*

Acidify using module S1 if necessary

- Inject to GC or LC





CLEAN-UP

ADSORBENTS USED IN STANDARD

Primary Seconday Amine (PSA)
- Fatty acids
- Organic acids
- Sugars
- Polar pigments

(e.g., anthocyanins)

C18 (Octadecyl Silica)
- Non-polar interferences
- Lipids
- Waxes

Magnesium Sulfate ($MgSO_4$)
- Removes residual water by dehydration

Graphitized Carbon Black (GCB)
- Pigments

(e.g., chlorophyll, carotenoids)
- Sterols

*May adsorb some planar analytes like certain pesticides (e.g., thiabendazole, chlorothalonil).

EXTRACTION - WITHOUT HYDROLYSIS

MODULE	DESCRIPTION	PREFFERED APPLICATION	EXAMPLES
E1	A test portion of 10 g without any addition of water is extracted with 10ml acetonitrile	Plant material and edibles with high water content (≥80 %)	Fruit, vegetables, juices
E2 (E2a or E2b)	10 g test portion is extracted by 10 ml acetonitrile with addition of E2a) 0.6 ml or E2b) 0.2 ml sodium hydroxide solution	Plant material and edibles with high water content (≥80 %) and high acid content	Lemons, lime, red currant raspberry, blackberry
E3 (E2a or E2b)	E3a) 2.5 ml or E3b) 4.5 ml of water is added to a test portion of 10 g and then extracted with 10ml acetonitrile	Plant material and edibles with intermediate water content (> 40 % and < 80 %)	Bananas, root and tuber vegetables (potatoes, yam, parsnip), fresh dates, chestnuts
E4	Test sample is homogenized with water and a test portion of 13.5 g of the homogenate is extracted with 10ml acetonitrile.	Plant material and edibles with low water content (15 % to 40 %)	Dried fruit und similar commodities
E5	A test portion of 5 g is completed with 10 ml of water and then extracted with acetonitrile, 15mins shaking time or soaking time	Plant material and edibles with very low water content (<15 %) and honey	Cereal grain, Creal grain products, Honey
E6	A test portion of 5 g is completed with 6 ml of water and then extracted with acetonitrile.	Plant material and edibles with intermediate water content (>40 % to 80 %) and high matrix load or high oil content (>5 %)	Garlic, avocados
E7	A test portion of 2 g is completed with 10 ml of water and then extracted with acetonitrile, 15mins shaking time or soaking time	Plant material and edibles with very low water content (<15 %) and high matrix load as well as freeze-dried products	Spices, coffee, tobacco, tea, lentils, freeze-dried fruit

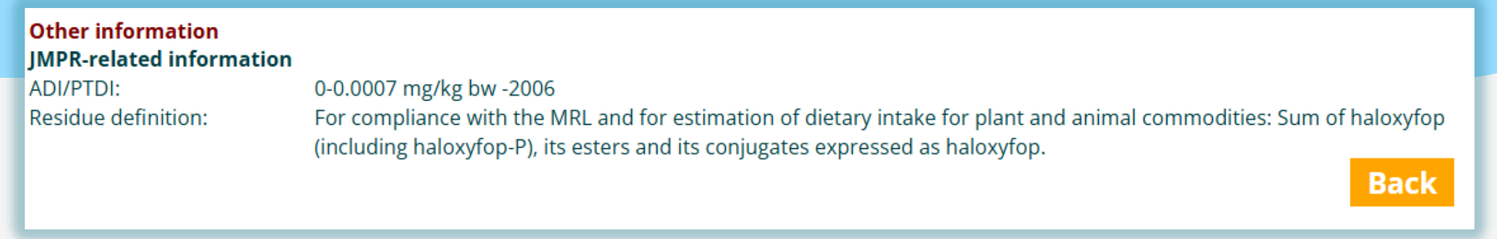
RESIDUE DEFINITON

A pesticide residue is any specified substance in food, agricultural commodities, or animal feed resulting from the use of a pesticide. In general, residue definition includes all or a combination of the following, parent compound, derivatives of the pesticide, such as conversion products, metabolites, reaction products, and other related impurities.

For each pesticide used on food or feed commodities, regulatory authorities need to choose which residue(s) will be used for dietary risk assessment and setting MRLs. Residue definitions are set by JMPR (Joint Meeting on Pesticide Residues) and other major registrars.

a. For example is, "**CARBOFURAN**", a carbamate insecticide
Parent compound: Carbofuran | Metabolites included: 3-hydroxycarbofuran
The residue definition for it's MRL setting is the **sum of carbofuran and 3-hydroxycarbofuran, expressed as Carbofuran**

b. "HALOXYFOP", an herbicide, In the context for compliance with MRLs set by the CODEX Alimentarius,
The residue definition is the **sum of Haloxyfop (including haloxyfop-P), its esters and its conjugates expressed as Haloxyfop**
(Reference: Codex Alimentarius pesticide database)



Other information

JMPR-related information

ADI/PTDI:	0-0.0007 mg/kg bw -2006
Residue definition:	For compliance with the MRL and for estimation of dietary intake for plant and animal commodities: Sum of haloxyfop (including haloxyfop-P), its esters and its conjugates expressed as haloxyfop.

Back

EXTRACTION - WITH HYDROLYSIS

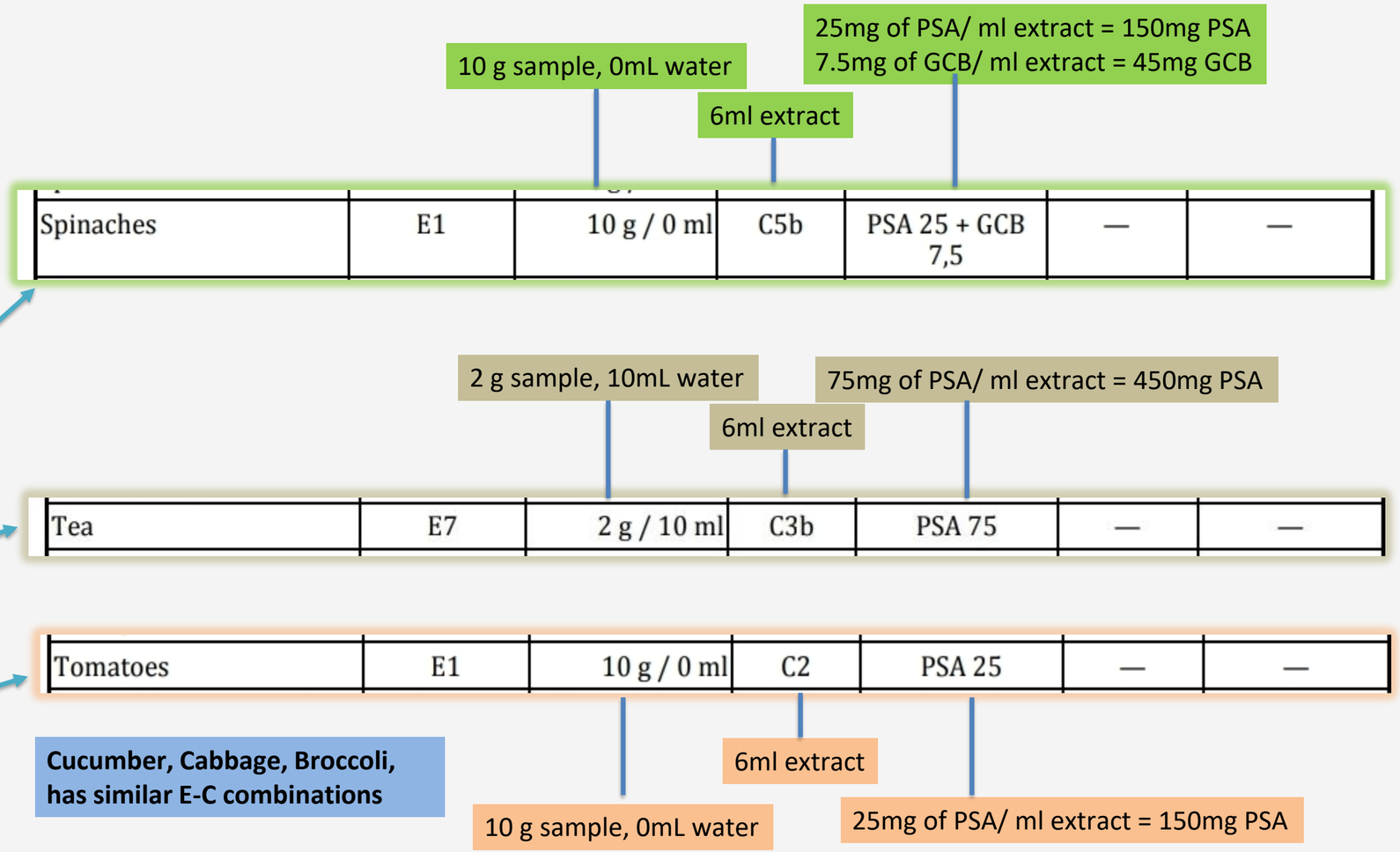
MODULE	DESCRIPTION	PREFFERED APPLICATION	EXAMPLES
E8	Hydrolysis of esters and conjugates of acidic pesticides in the slurry of 10 g sample in acetonitrile followed by extraction with acetonitrile (2ml or 1ml of 5M Sodium Hydroxide, 1.4ml or 1ml of 2.5M sulfuric acid)	Plant material and edibles with neutral or acidic pH and high water content (≥80 %)	Fruit and vegetables, Juices
E9	Hydrolysis of esters and conjugates of acidic pesticides in the slurry of 2 g to 5 g sample in acetonitrile followed by extraction with acetonitrile (1ml of 5M Sodium Hydroxide)	Plant material and edibles with low water content	Cereal grain, cereal grain prodcuts, garlic, spices, coffee, tobacco, tea, lentils, freeze-dried fruit

CLEAN-UP MODULES

MODULE	DESCRIPTION	PREFFERED APPLICATION	EXAMPLES
C0	No clean-up	Base-sensitive and acidic pesticides (pKa < 5) that interact with the aminosorbent (PSA) used in modules C2 to C5, analysis of extracts with low matrix-load	Cucumber, apples, sufficiently diluted rawextracts
C1	- 8mL from E2,E5, or E6, stored in freezer overnight - 6ml cold extract to C2, C3, or C5	Removal of co-extracted fat by freezing (can be used in combination with further clean-up steps, e.g. C2, C3, C5)	Oranges, lemons, cereal grain
C2	- 6mL from E1-E4 or E6 - Dispersive SPE with 150mg PSA/900mg Magnesium Sulfate *25mg PSA /mL extract, 150mg Magnesium Sulfate/mL extract	Clean-up of raw-extracts prior to the determination of basic and neutral pesticides	Standard-procedure for any commodity not shown separately
C3	- 6mL from E5, E7 - Dispersive SPE with a larger amount of amino sorbent **C3a** (50mg PSA/mL Extract) = 300mg PSA/900mg Magnesium Sulfate **C3b** (75mg PSA/mL extract) = 450mg PSA/900mg Magnesium Sulfate	Clean-up of raw-extracts of foods of plant origin with high matrix-load prior to the determination of basic and neutral pesticides	Raw-extracts from modules E5 (e.g. cereal grain and products thereof) and E7 (e.g. coffee, tea, dried herbs, spices)
C4	- 6mL from E2,E5,E6 Dispersive SPE with 150mgPSA/150mg C18/900mg Magnesium Sulfate	Simultaneous clean-up of raw extracts and removal of coextracted fat	Citrus fruit, cereal grain and products thereof, avocados, olives
C5	- 6mL from E1 or E7 - Dispersive SPE with a mixture of amino-sorbent and graphitized carbon black **C5a** 2.5mg GCB/mL Extract = 15mg GCB/900mg Magnesium Sulfate **C5b** 7.5mg GCB/mL Extract = 45mg GCB/900mg Magnesium Sulfate	Clean-up of intensely coloured raw-extracts prior to the determination of basic and neutral pesticides	Iceberg lettuce, head lettuce, rocket salad

Examples of modular combinations

Commodity	Extraction (E)	Description (E)[a]	Clean-up (C)	Description (C)[b]	Clean-up (C altern.)	Description (C altern.)[b]
Rocket	E1	10 g / 0 ml	C5b	PSA 25 + GCB 7,5	—	—
Romaine lettuce	E1	10 g / 0 ml	C5a	PSA 25 + GCB 2,5	—	—
Rosemary (68 % water)	E6	5 g / 6 ml	C2	PSA 25	—	—
Rosemary, fresh	E3a	10 g / 2,5 ml	C2	PSA 25	—	—
Sage, fresh	E6	5 g / 6 ml	C2	PSA 25	—	—
Sage, fresh	E3a	10 g / 2,5 ml	C5b	PSA 25 + GCB 7,5	—	—
Salsifies	E1	10 g / 0 ml	C2	PSA 25	—	—
Savoy cabbage	E1	10 g / 0 ml	C2	PSA 25	—	—
Shallots	E1	10 g / 0 ml	C2	PSA 25	—	—
Shallots, freeze-dried	E7	2 g / 10 ml	C2	PSA 25	—	—
Spearmint	E1	10 g / 0 ml	C5b	PSA 25 + GCB 7,5	—	—
Spices	E7	2 g / 10 ml	C2	PSA 25	—	—
Spinaches	E1	10 g / 0 ml	C5b	PSA 25 + GCB 7,5	—	—
Strawberries	E1	10 g / 0 ml	C2	PSA 25	—	—
Strawberries, freeze-dried	E7	2 g / 10 ml	C2	PSA 25	C3a	PSA 50
Sweet potatoes	E3a	10 g / 2,5 ml	C2	PSA 25	—	—
Tamarind (31 % water)	E4	500 g / 850 ml	C2	PSA 25	—	—
Taro/eddoe	E3a	10 g / 2,5 ml	C2	PSA 25	—	—
Tea	E7	2 g / 10 ml	C3b	PSA 75	—	—
Thyme, dried	E7	2 g / 10 ml	C2	PSA 25	C5b	PSA 25 + GCB 7,5
Thyme, fresh	E3a	10 g / 2,5 ml	C5b	PSA 25 + GCB 7,5	—	—
Thyme, fresh (65 % water)	E6	5 g / 6 ml	C2	PSA 25	—	—
Tomatoes	E1	10 g / 0 ml	C2	PSA 25	—	—
Tomatoes, dried (14,5 % water)	E4	500 g / 850 ml	C2	PSA 25	—	—
Vegetables	E1	10 g / 0 ml	C2	PSA 25	—	—
Vicia faba (with pods)	E3a	10 g / 2,5 ml	C2	PSA 25	—	—
Wheat sprouts (47 % water)	E3b	10 g / 4,5 ml	C2	PSA 25	—	—
Wine	E1	10 g / 0 ml	C2	PSA 25	—	—
Yams	E3a	10 g / 2,5 ml	C2	PSA 25	—	—



HANDS-ON ACTIVITY
Analysis of Rice using using modular QuEChERS and laboratory developed method

Pesticide Analytical Laboratory Section
Plant Product Safety Services Division
Bureau of Plant Industry
Quezon City, metro manila, philippines

Michael Alava
Ench Gaza
Jay Valeza
PESTICIDE RESIDUE UNIT

ACTIVITIES

Sample Processing
- Grinding of rice
- weighing of test portion

Standard preparation
MATRIX-MATCH, FOR LABORATORY DEVELOPED METHOD
- 5ppm
- 1ppm
- 0.10ppm
- 0.05ppm
- 0.02ppm
- 0.01ppm
- 0.005ppm

Reagent preparation
- 3:1 Acetonitrile: Toluene

Analysis
- Rice analysis using Modular QuEChERS method for GC

Analysis
- Rice analysis using Modular QuEChERS method for LC

Analysis
- Rice analysis using laboratory developed method for GC

Data processing
- Retrieve and computation of results

Modular QuEChERS

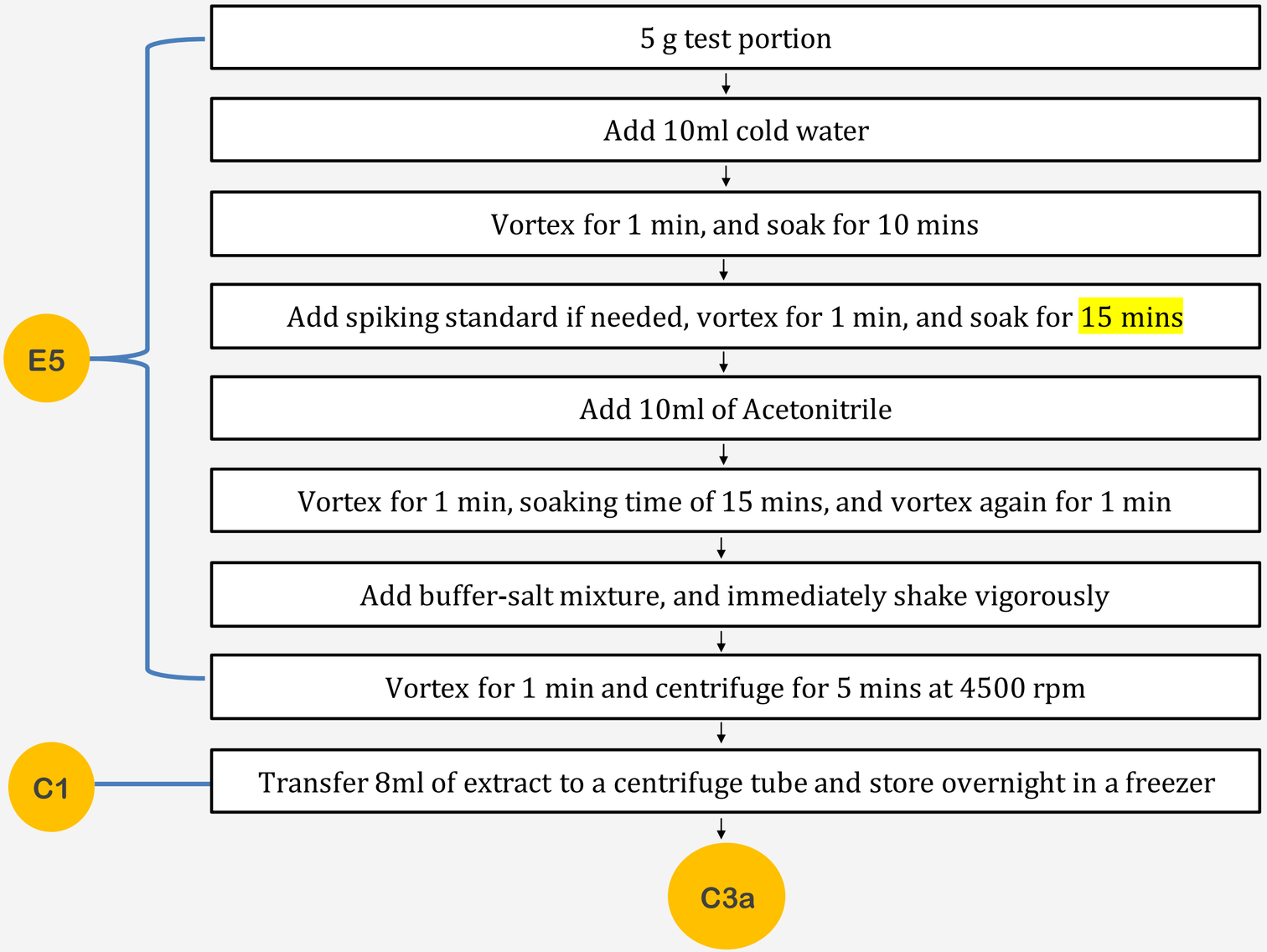
E5-C1-C3a

Chemicals
Reagents
SPE
Apparatus

- QuEChERS Extraction Kit, EN 15662 Method
- QuEChERS Dispersive Kit, General Fruits and Vegetables, 15 mL EN
- Acetonitrile, HPLC Grade
- Acetonitrile, LC-MS Grade
- Acetone, Pesticide Grade
- Water, LC-MS Grade
- Distilled Water

- Grinder
- Top loading balance
- Vortex Mixer
- Centrifuge
- Rotary Evaporator
- Pipettor
- Fumehood
- GC-MS

E5-C1-C3a Extraction + First Clean-up



E5-C1-C3a
Extraction
+
First Clean-up

GC INJECTION
Control: 1
Recovery: 3

Transfer 6ml of cold raw extract into a dSPE tube containing 150mg PSA/900mg Magnesium sulfate

↓

Vortex for 1 min and centrifuge for 5 mins at 4500 rpm

↓

Transfer 3.6 ml of clean extract to a round bottom flask

↓

Solvent Change

Dry, and reconstitute with 1.8 ml of Acetone

↓

Inject to GC

FOR LC Injection, omit solvent change to Acetone

LC INJECTION
Control: 1
Recovery: 3

Modified QuEChERS

(Laboratory developed method)

Chemicals
Reagents
SPE
Apparatus

- QuEChERS Extraction Kit, EN 15662 Method
- 500 mg GC-e/500 mg PSA SPE
- Acetonitrile, HPLC Grade
- Acetone, Pesticide Grade
- Water, LC-MS Grade
- Toluene, AR Grade
- Distilled Water

- Grinder
- Top loading balance
- Vortex Mixer
- Centrifuge
- Rotary Evaporator
- Pipettor
- Fumehood
- GC-MS

Modified QuEChERS
(Laboratory developed method)

GC INJECTION
Control: 1
Recovery: 3

SPE Conditioning
500 mg GC-e/500 mg PSA
10ml 3:1 Acetonitrile : Toluene

Extraction

- 10 g test portion
- Add 10ml cold water
- Vortex for 1 min, and soak for 10 mins
- Add spiking standard if needed, vortex for 1 min, and soak for 10 mins
- Add 10ml of Acetonitrile
- Vortex for 1 min, soaking time of 15 mins, and vortex again for 1 min
- Add buffer-salt mixture, and immediately shake vigorously
- Vortex for 1 min and centrifuge for 5 mins at 4500 rpm

Clean-up

- Elute 5 ml of extract into 500 mg GC-e/500 mg PSA SPE tube
- Re-elute SPE with 20 ml of 3:1 Acetonitrile : Toluene
- Dry, and reconstitute with 5 ml of Acetone
- Inject to GC