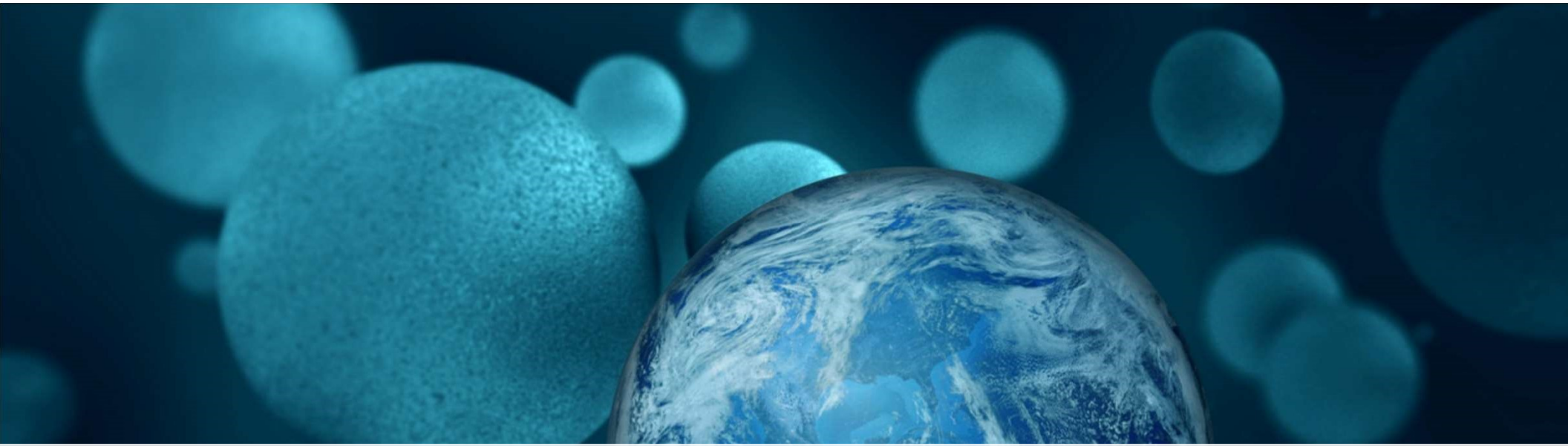

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- **Basics of liquid chromatography and tandem mass spectrometry**
- **Pesticides analysis in cereals, fruits and vegetables, fatty matrices using LC-MS/MS**

Ramiz M.R.Azad
Application Development Scientist
Thermo Fisher Scientific,
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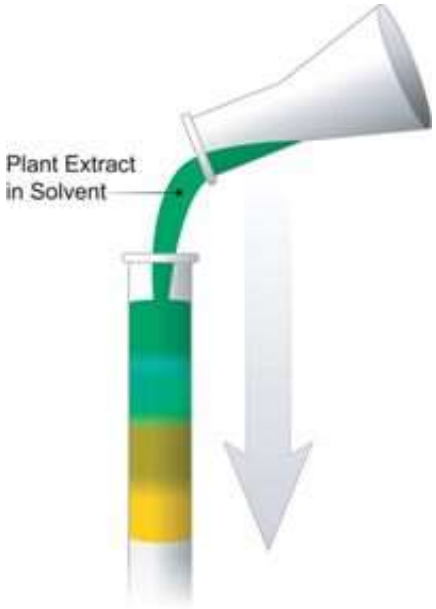
Outline

- Basics of Liquid Chromatography
- Components of HPLC/UHPLC
- Basics of Mass Spectrometry
- Working principle of LCMSMS
- API-Ionization techniques
- Quadrupole mass analyzer
- Maintenance and how to improve your method performance
- Different food safety applications of LCMSMS

Brief History

Liquid chromatography (LC) was defined in the early 1900s by the work of the Russian botanist, Mikhail S. Tswett. His pioneering studies focused on separating compounds [leaf pigments], extracted from plants using a solvent, in a column packed with particles



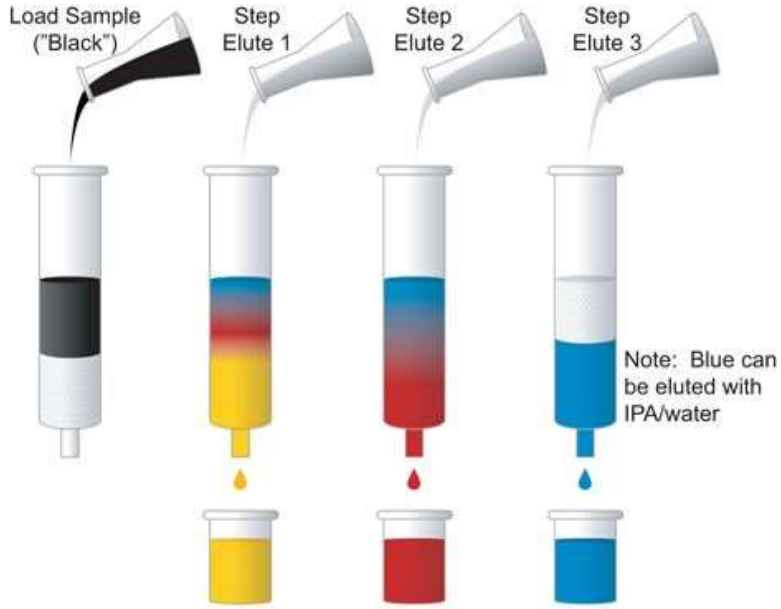
Tswett filled an open glass column with particles. Two specific materials that he found useful were powdered chalk [**calcium carbonate**] and alumina. He poured his sample [solvent extract of homogenized plant leaves] into the column and allowed it to pass into the particle bed. This was followed by pure solvent. As the sample passed down through the column by gravity, different colored bands could be seen separating because some components were moving faster than others

HPLC Basics

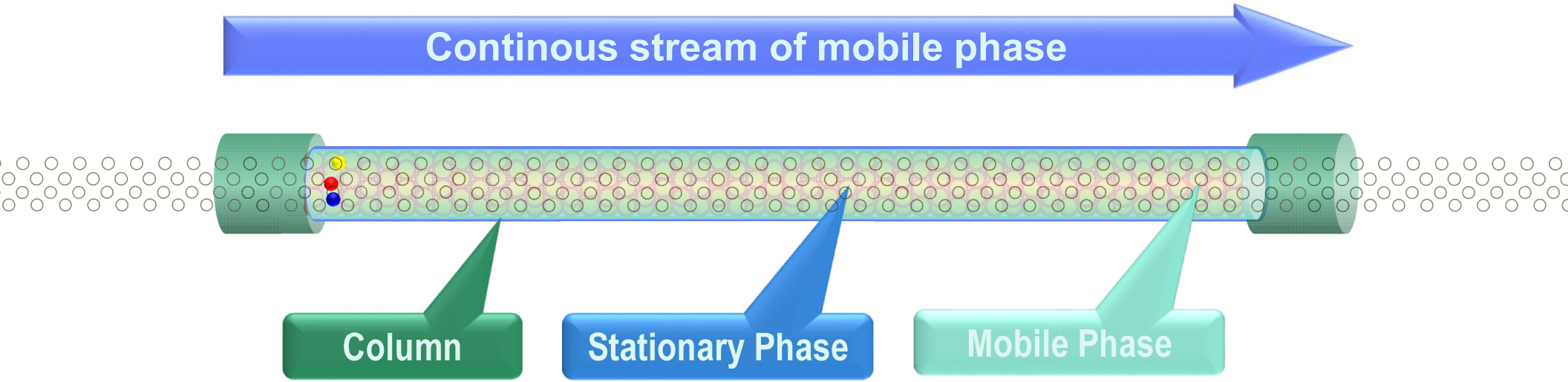
High performance liquid chromatography or commonly known as HPLC is an analytical technique used to separate, identify or quantify each component in a mixture. The mixture is separated using the basic principle of column chromatography and then identified and quantified by spectroscopy. A computer analyzes the data show the output in display

HPLC Theory

HPLC works following the basic principle of thin layer chromatography or column chromatography, where it has a stationary phase (solid like silica gel) and a mobile phase (liquid or gas). The mobile phase flows through the stationary phase and carries the components of the mixture with it. Different components travel at different rates. Thus the components separated and found in different region in chromatography to separate, identify and quantify.



the chromatographic process



- The stationary phase retains analytes due to various interactions.
- When different chemical components pass through the column at different rates they become separated in single zones.

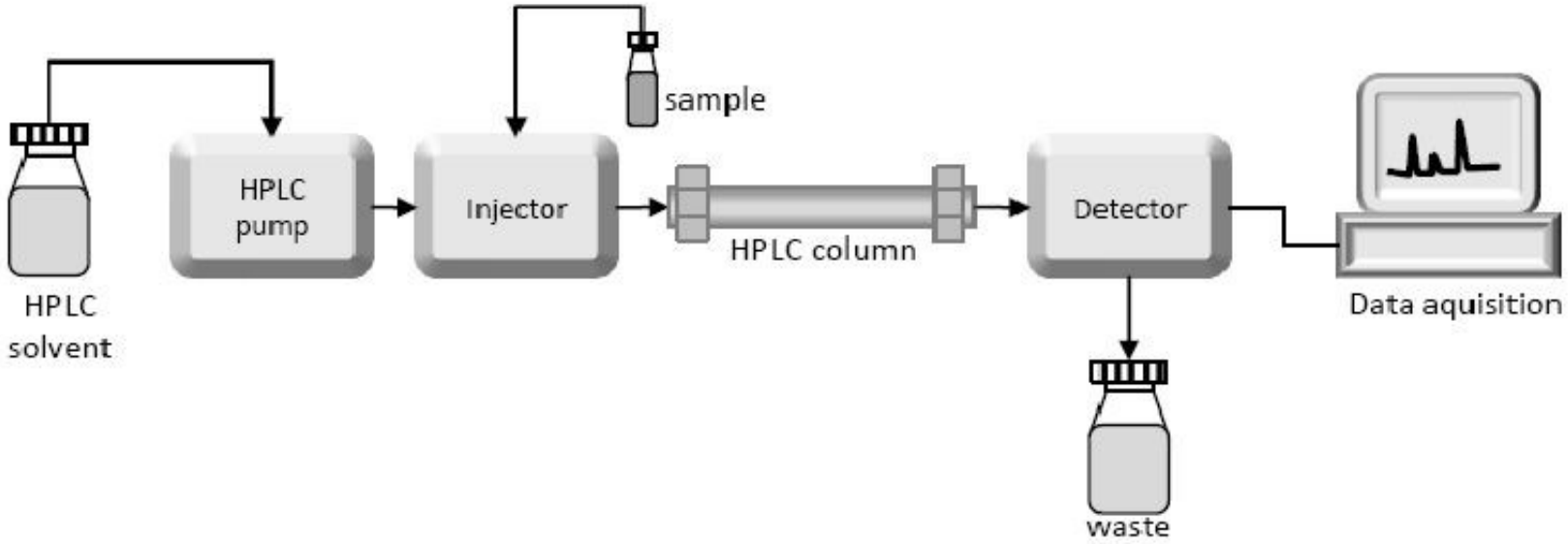
Introduction – Size of Silica Particles

- The smaller the particles, the better the separation performance
- But: Smaller particles generate higher back pressure

	Typical Particle Size	**Typical Back Pressure**	**Typical Column Diameter**
Preparative HPLC	100 – 10 µm	10 - 100 bar	21 mm
Conventional HPLC	5 – 3 µm	100 – 300 bar	4.6 mm
UHPLC	≤ 3 µm	≥ 600 bar	2.1 mm

- HPLC: High Performance Liquid Chromatography
- UHPLC: Ultra High Performance Liquid Chromatography

components of HPLC



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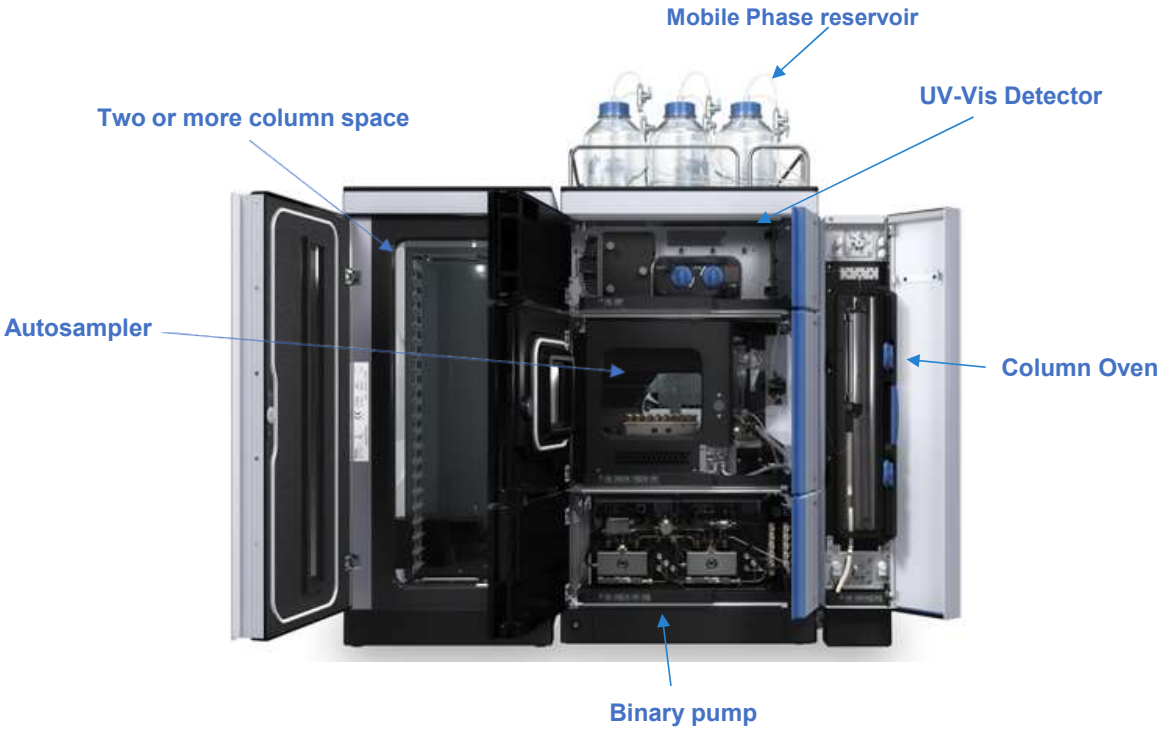
Vanquish flex UHPLC



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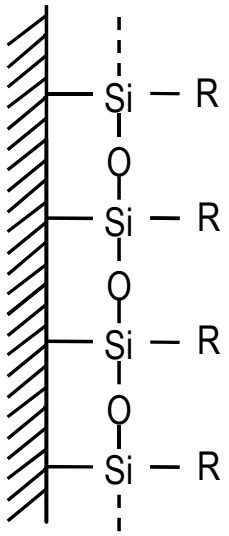
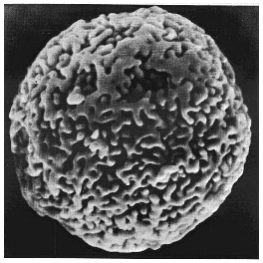
Column Chemistry – HPLC-Modes

- **Normal Phase** (NP): The stationary phase is polar and the mobile phase is non-polar.
  - ➢Niche mode in modern (prep) LC (e.g. separation of enantiomers)
- **Reversed Phase** (RP): The stationary phase is made of chemically modified silica (normally with non-polar surface) and the mobile phase is polar.
  - ➢Most common HPLC mode
  - ➢Good for separation of broad polarity range
- Denotation is due to history: Early HPLC experiments worked with pure silica and polar solvents.

General rule: ”Similia similibus attrahuntur“ (“like dissolves like”).

Column Chemistry– Normal Phase Chromatography





- Common **NP stationary phases:**

R= OH	Silica
$R=(CH_2)_3NH_2$	Amino
$R=(CH_2)_3CN$	Cyano
$R=(CH_2)_2OCH_2CH(OH)CH_2OH$ Diol	

- Common **NP mobile phases:**

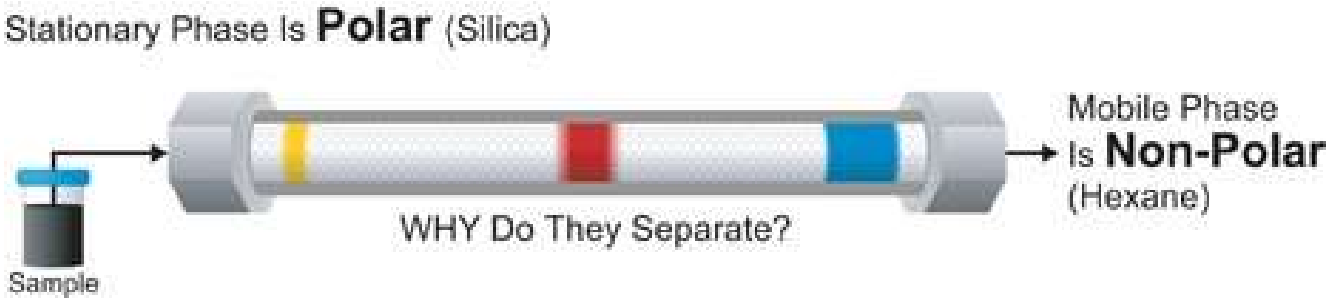
  Heptane < Hexane < Toluene < Chloroform < Ethyl acetate < Isopropanol < Acetonitrile < Methanol

  (eluotropic series for SiO_2: decreasing retention)

- **Difficulties:** High influence of water traces (in ppm range) or other polar additives on retention behavior

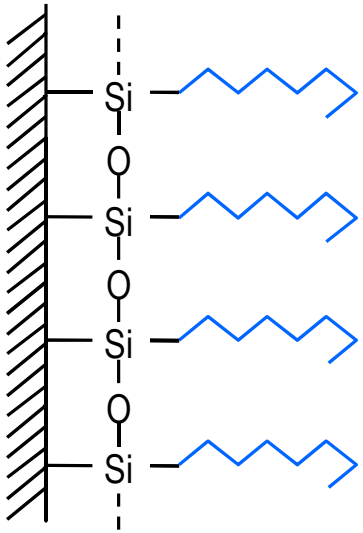
Column Chemistry- Normal Phase Chromatography

- The least polar component elutes first.
- Increasing the polarity of the mobile phase decreases the elution time.
- Check sample solubility in mobile phase!
- Check the NP compatibility of the HPLC system (e.g. piston seals)!

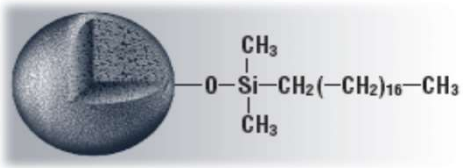


Column Chemistry– Reversed Phase Chromatography

Example: C8



Silica- C8



- Common **RP stationary phases**:

  Normal silica treated with **R**Me_2SiCl

  R=$(CH_2)_{17}CH_3$: C18

  R=$(CH_2)_7CH_3$: C8

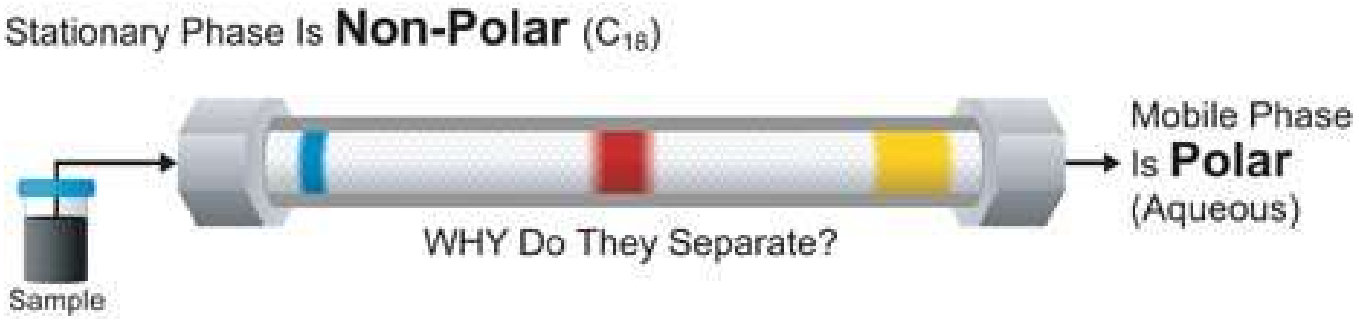
- Common **RP mobile phases**:

  Water < Methanol < Acetonitrile

  (elutropic series for RP: decreasing retention)

Column Chemistry– Reversed Phase Chromatography

- Most common HPLC mode: 80% RP separation
- Good for separation of solutes with broad polar range.
- The most polar component elutes first.
- Increasing the polarity of the mobile phase increases the elution time.



HPLC Versions

1. Isocratic HPLC is carried out with only one eluent composition within a single run. Therefore, the interaction between mobile and stationary phases is constant during the entire time span of the separation. Especially similar substances can be separated well with this method. This type of implementation is particularly popular especially in routine applications and should be as far as possible strived for all separation tasks. Another advantage of this method is that you only need a single pump for pumping the eluents.

2. In **Gradient HPLC** the composition of the eluent is changed continuously over time. A constant change in interaction between eluent and stationary phase is achieved this way. The sample substances are displaced quicker from the exchange sites on the stationary phase. This renders the separability of components with very different polarity in acceptable time possible. Since eluent composition at the beginning of this separation procedure differs from that in the end, make sure the system is in equilibrium before starting the separation (= equilibration). The mixing of the eluent can be carried out differently depending on the technically circumstances of each device.

3. Low-Pressure Gradients- eluent mixed through valve and pumped by single pump

4. High-Pressure Gradients- each eluent is pumped by a separate pump and the mixing is carried out on the pressure side of the system

Detectors

1 The **UV detector** is currently the most frequently used detector in the HPLC. UV-light with a specific wavelength from a deuterium or quartz lamp in the detector passes through the flow cell

2 The **diode array detector (DAD)** or also referred to as photodiode array detector (PDA), measures, just as the UV-detector, light absorption by the mobile phase in the ultraviolet and visual wavelength range

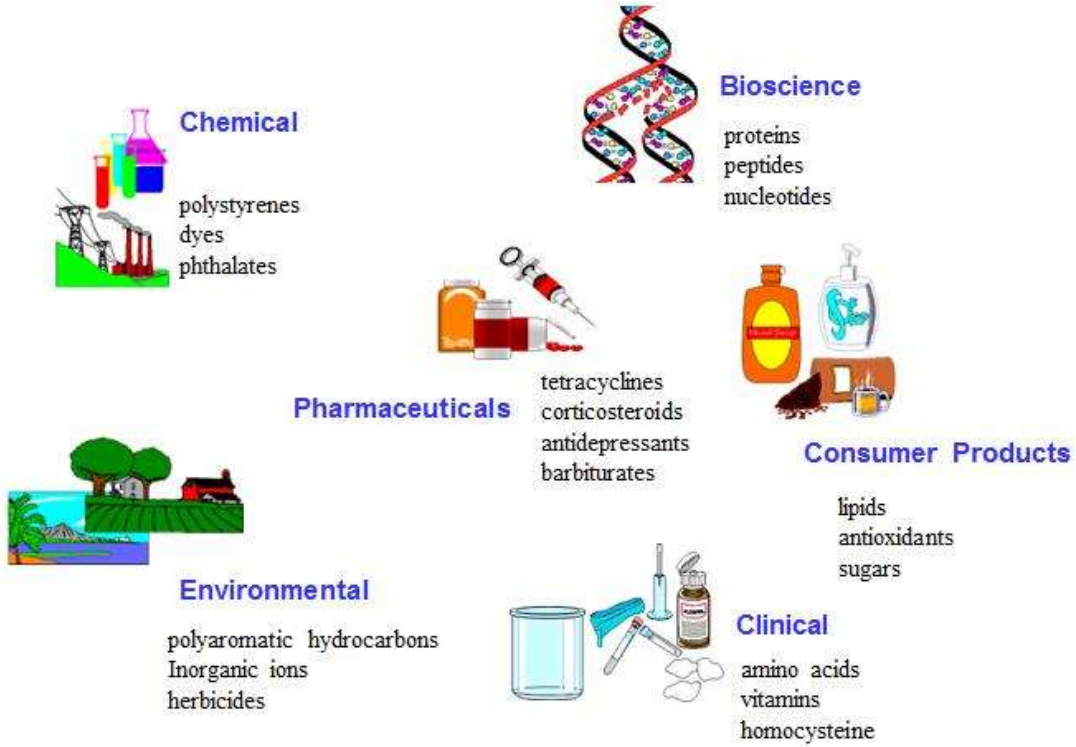
3 The **Refractive Index detector (RI)** is less sensitive than the UV-detector, but it can detect substances that show no UV absorbance. It measures the difference of the refractive index between pure eluent and eluent mixed with the sample

4 The **Fluorescence Detector (FLD)** is up to 1000 times more sensitive than the UV detector. It uses the property of fluorescent substances to detect those

5 The **Mass Spectrometer**

Applications of HPLC

- Pharmaceutical
- Forensic
- Clinical
- Environmental
- Food
- Consumer products
- Etc.….



A Brief History of Mass Spectrometry

•1897 – Modern mass spectrometry (MS) is credited to the cathode-ray-tube experiments of J.J. Thomson of Manchester, England.

•1953 – Wolfgang Paul's invention of the quadrupole and quadrupole ion trap earned him the Nobel Prize in physics.

•1968 – Malcolm Dole developed contemporary electrospray ionization (ESI) but with little fanfare. Creating an aerosol in a vacuum resulted in a vapor that was considered too difficult to be practical. Liquid can represent a volume increase of 100 to 1000 times its condensed phase (1 mL/min of water at standard conditions would develop 1 L/min of vapor).

•1974 – Atmospheric pressure chemical ionization (APCI) was developed by Horning based largely on gas chromatography (GC), but APCI was not widely adopted.

•1983 – Vestal and Blakely's work with heating a liquid stream became known as thermospray. It became a harbinger of today's commercially applicable instruments.

•1984 – Fenn's work with ESI was published leading to his Nobel Prize-winning work published in 1988.

What is MS and LCMS??

MS

Mass spectrometry (MS) ionizes atoms or molecules to facilitate their separation and detection in accordance with their molecular masses and charges (mass to charge ratio). MS is used in various applications, e.g., biochemical and Food analysis

LCMS

The combined technique between MS and HPLC is commonly known as LC-MS. Combining the two analytical methods reduces experimental error and improves accuracy. This technique is very useful in applications that involve a huge number of compounds, such as environmental effluents.

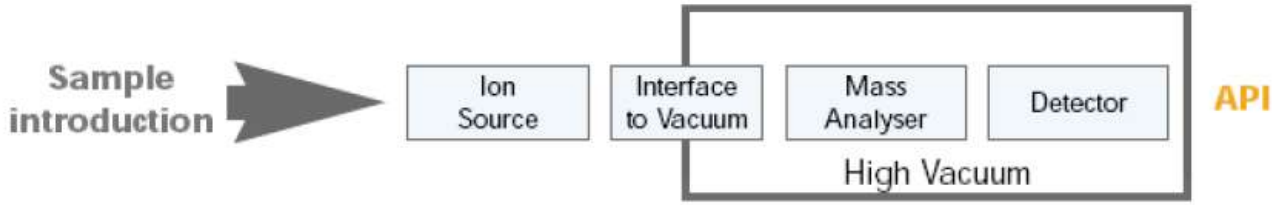
Mass Spectrometry – Simplified……

Generate	→	Ion Production
Move	→	Ion Optics
Select	→	Quadrupole
Detect	→	Electron Multiplier

The lifetime of an ion from the point of formation
to detection is approximately 50 to 100 microseconds

How LC-MS Works

1. **Sample Introduction**: sample is converted into a gaseous phase (except with gaseous samples or samples that are thermally unstable) and is introduced through the inlet to the ionization chamber
2. **Ionization**: gaseous sample is ionized to generate cations/anions
3. **Separation**: ions separate according to their mass/charge ratio by a mass analyzer
4. **Detection**: a detector is used to determine the species and quantity of each ion



Ion generation (API)

Atmospheric Pressure Ionization

Source Types

1. Electrospray (ESI) – Solution phase process.
2. Atmospheric Pressure Chemical Ionization (APCI) – Gas phase process.
3. Atmospheric Pressure Photo-Ionization (APPI) – Gas phase process.

Source Purpose

1. Desolvate sample LC flow for introduction into mass spectrometer.
2. Baffle the first vacuum region of the mass spectrometer from the atmospheric pressure region in the source.
3. Ionize the analyte or allows the transport of ions from solution into the gas phase.
4. Pump away neutrals and opposite charged ions, which would otherwise interfere with the analysis of ions of desired polarity.

Chemistry considerations

ESI:

Ions formed by solution chemistry

Good for thermally labile analytes

Good for polar / semi-polar analytes

Good for high MW molecules (proteins / peptides)

APCI / APPI:

Ions formed by gas phase chemistry

Good for volatile / thermally stable analytes

Good for non-polar / semi-polar analytes

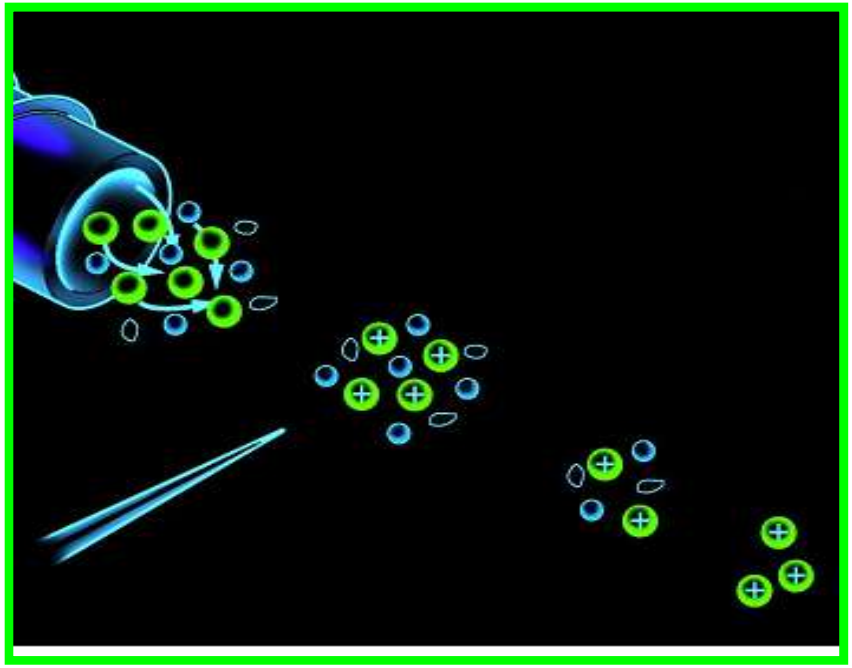
Good for small molecules (i.e. steroids)

Good for ions containing a chromophore (APPI)

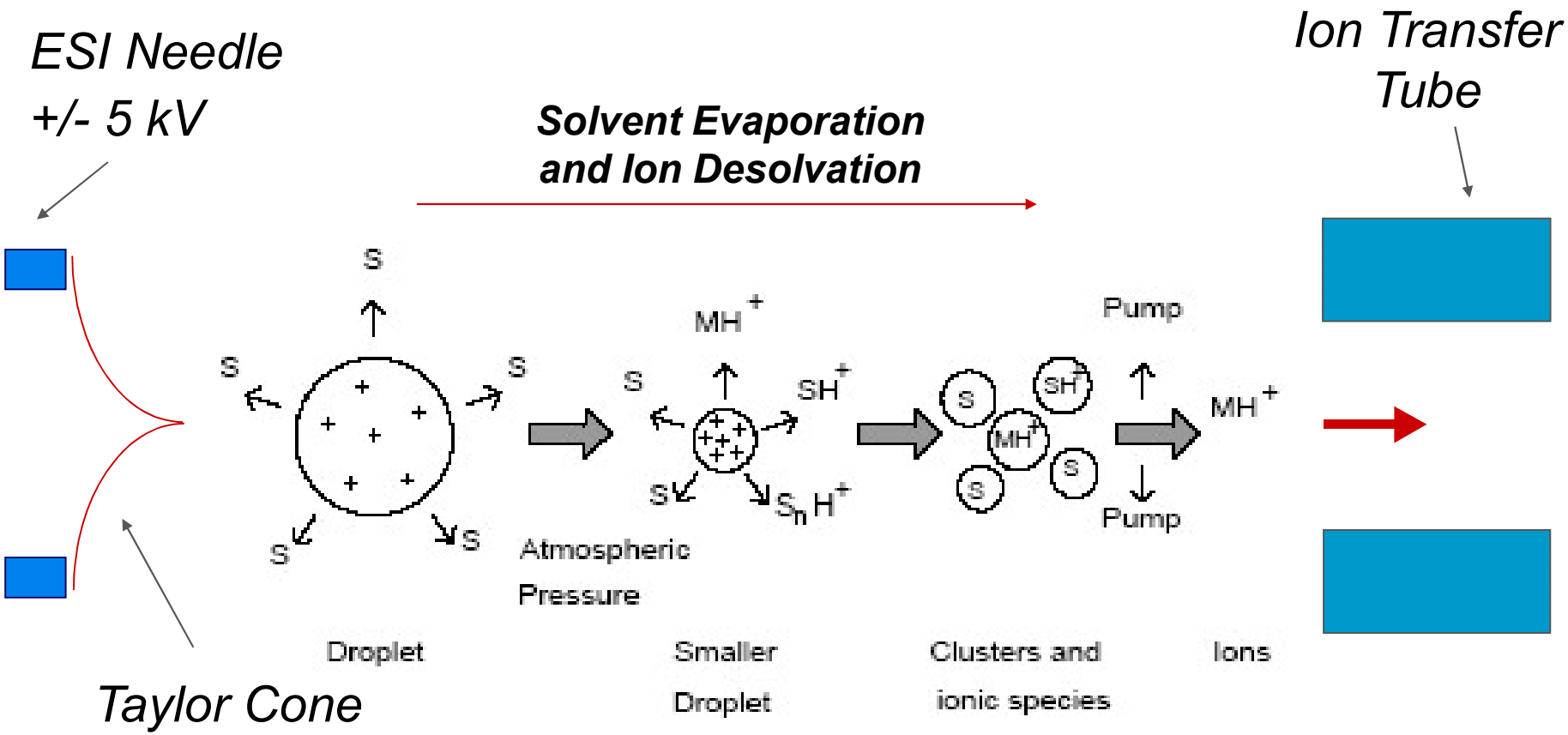
Ion generation - Two main ionization methods :

Electro**S**pray **Ionization** (ESI)

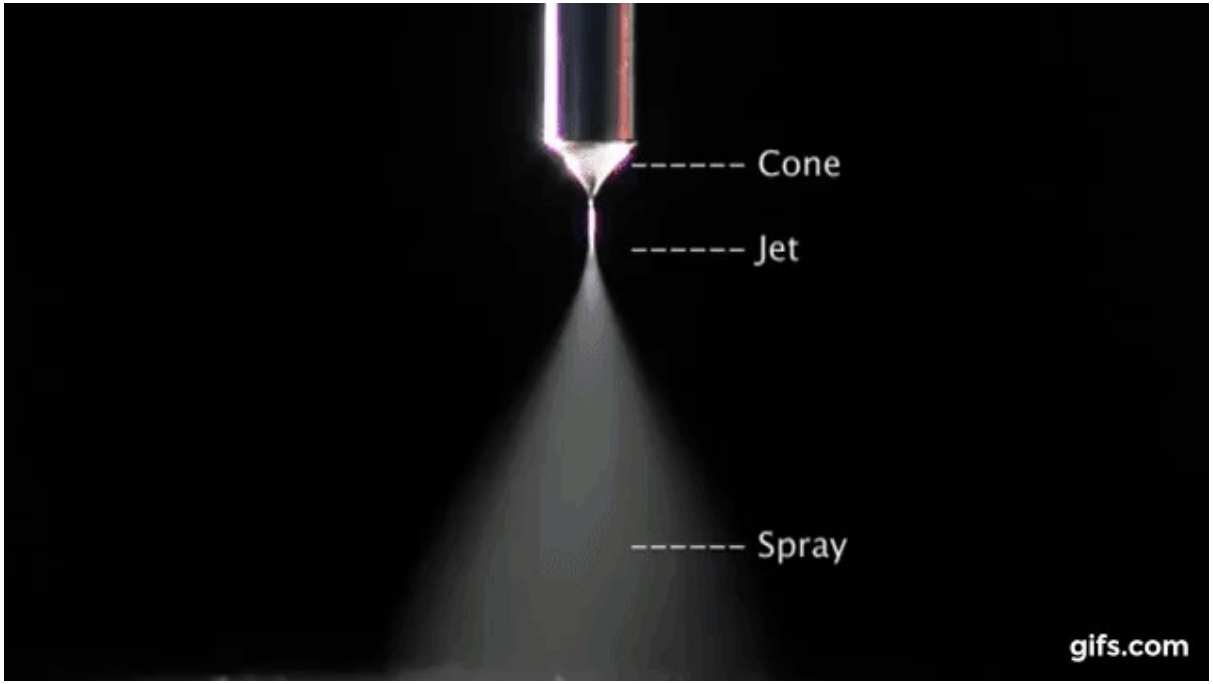
Atmospheric **P**ressure **C**hemical **Ionization** (APCI)



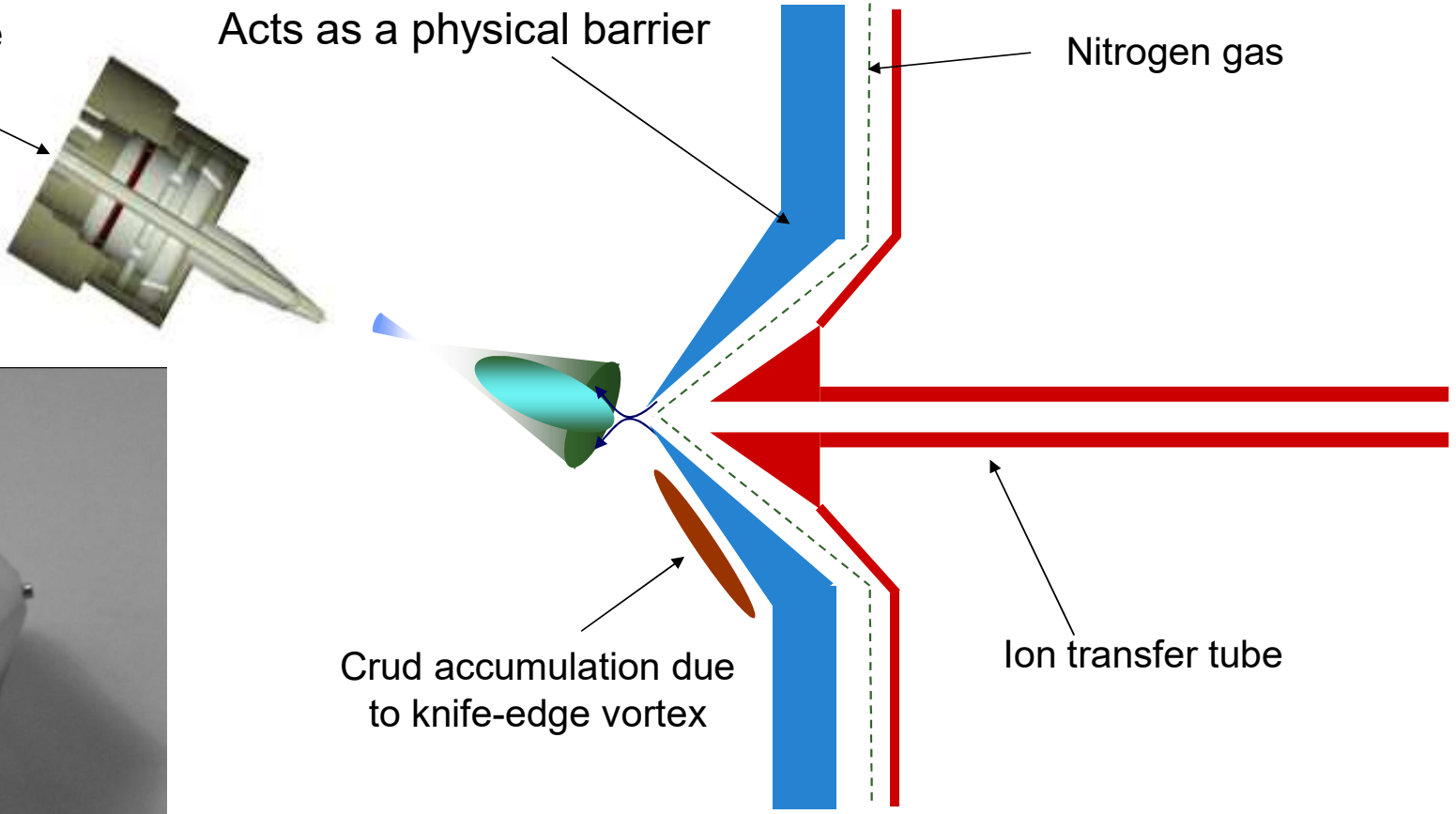
Electrospray - Basic Principle



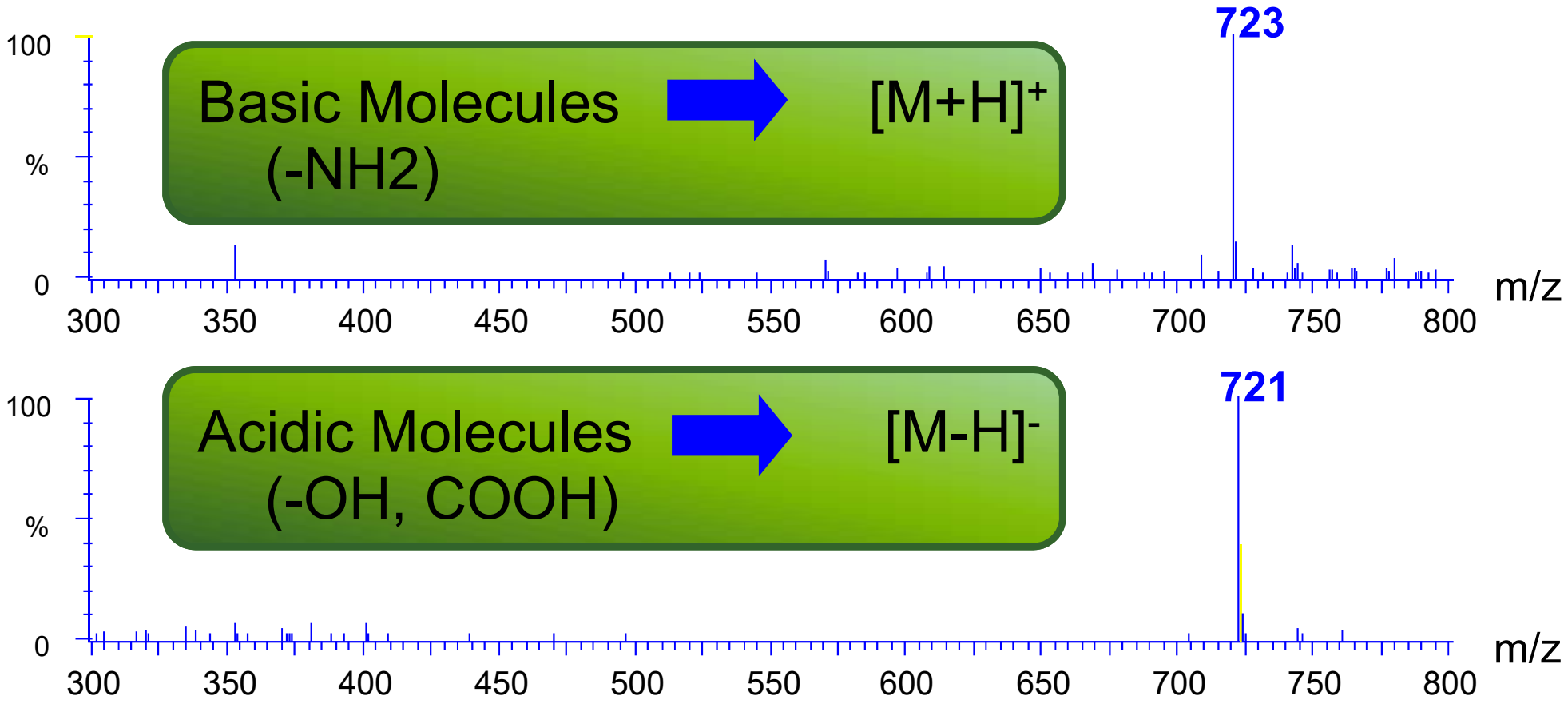
Nebulizaton



The Ion Sweep Gas Function



Positive or Negative Ionization ?



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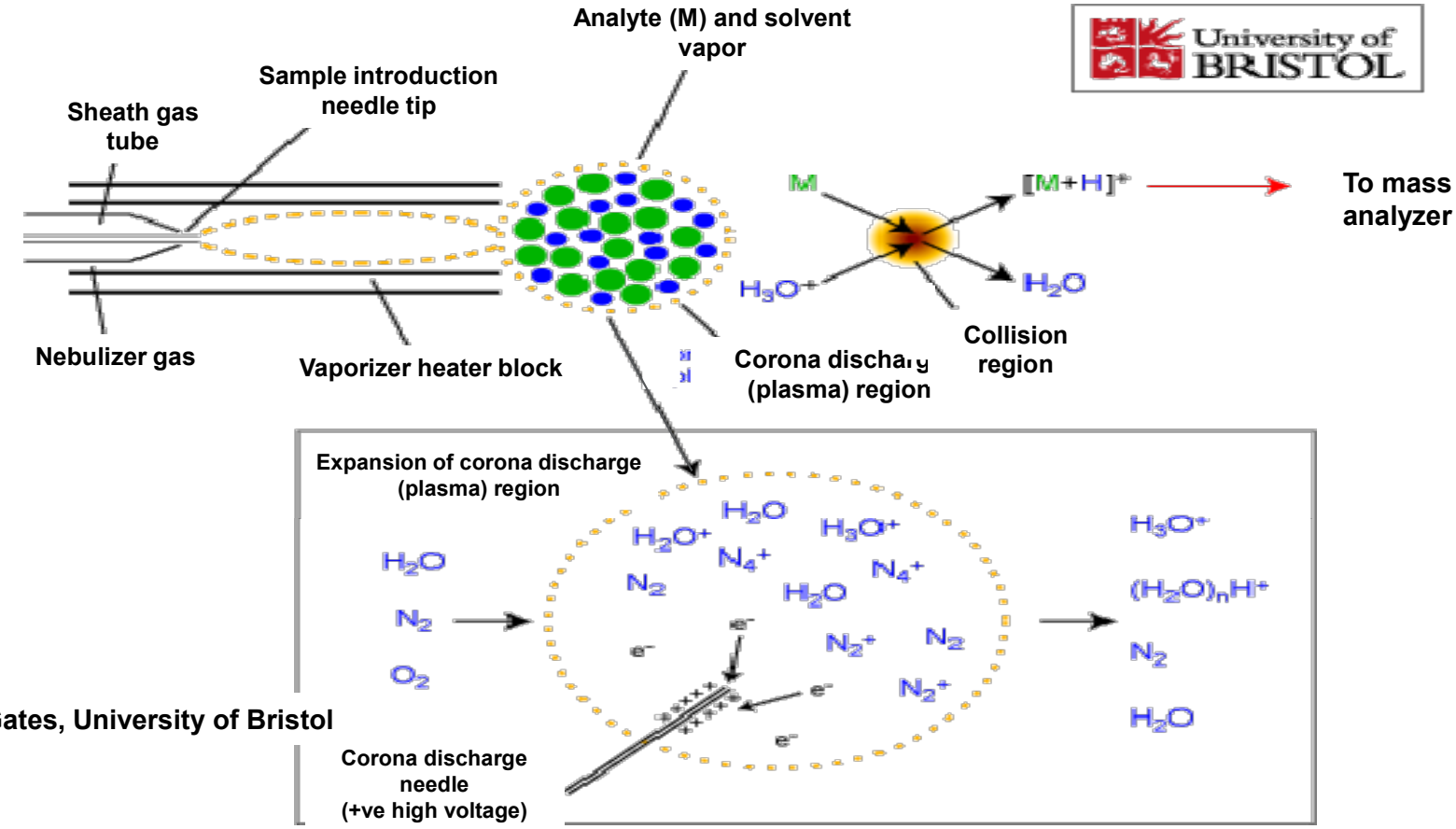
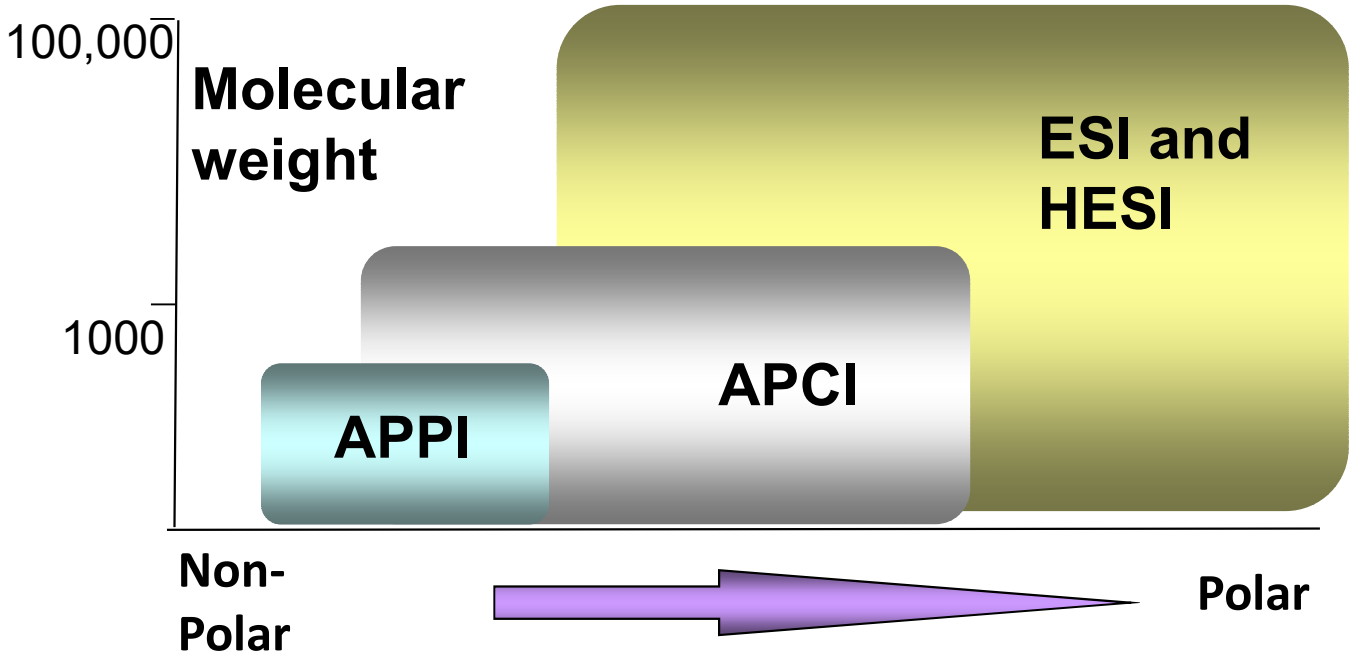
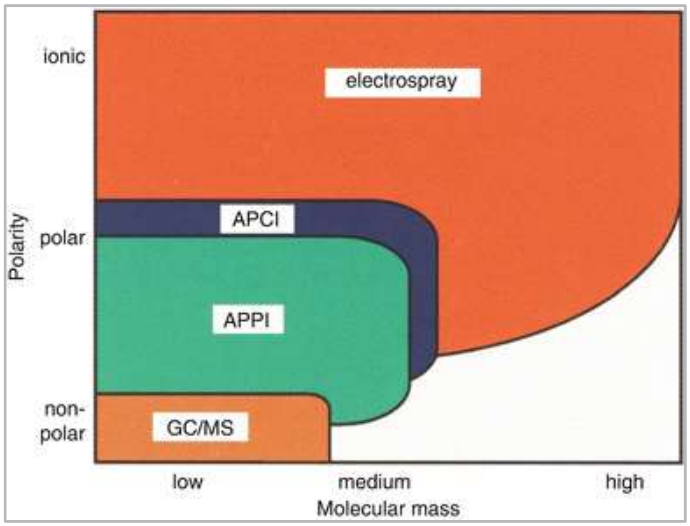
Atmospheric Pressure Chemical Ionization (APCI)



Which Ionization Mode to Use ?





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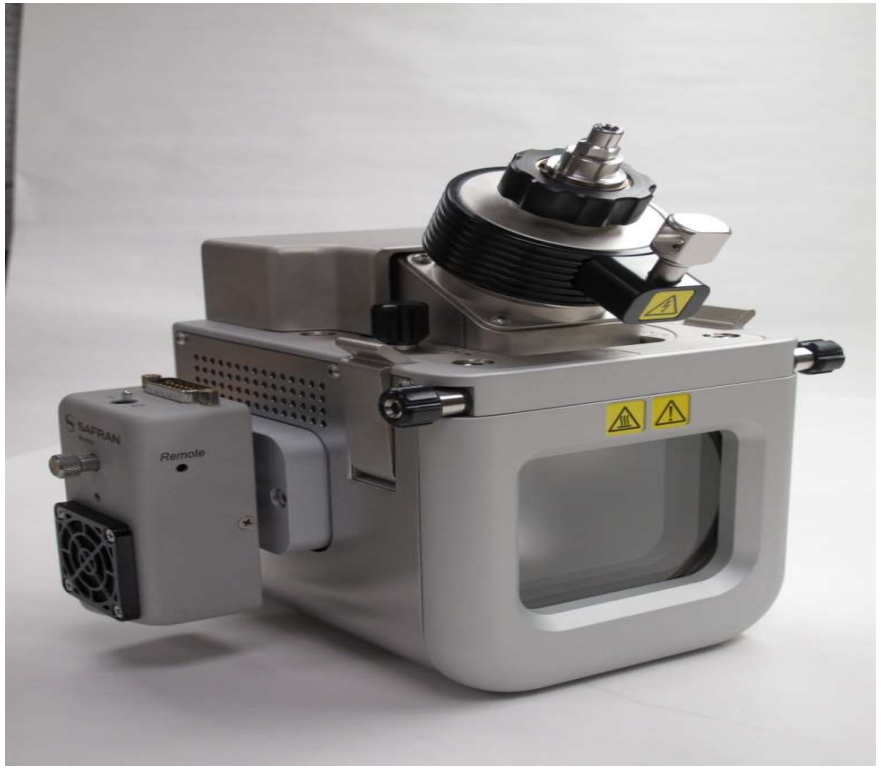
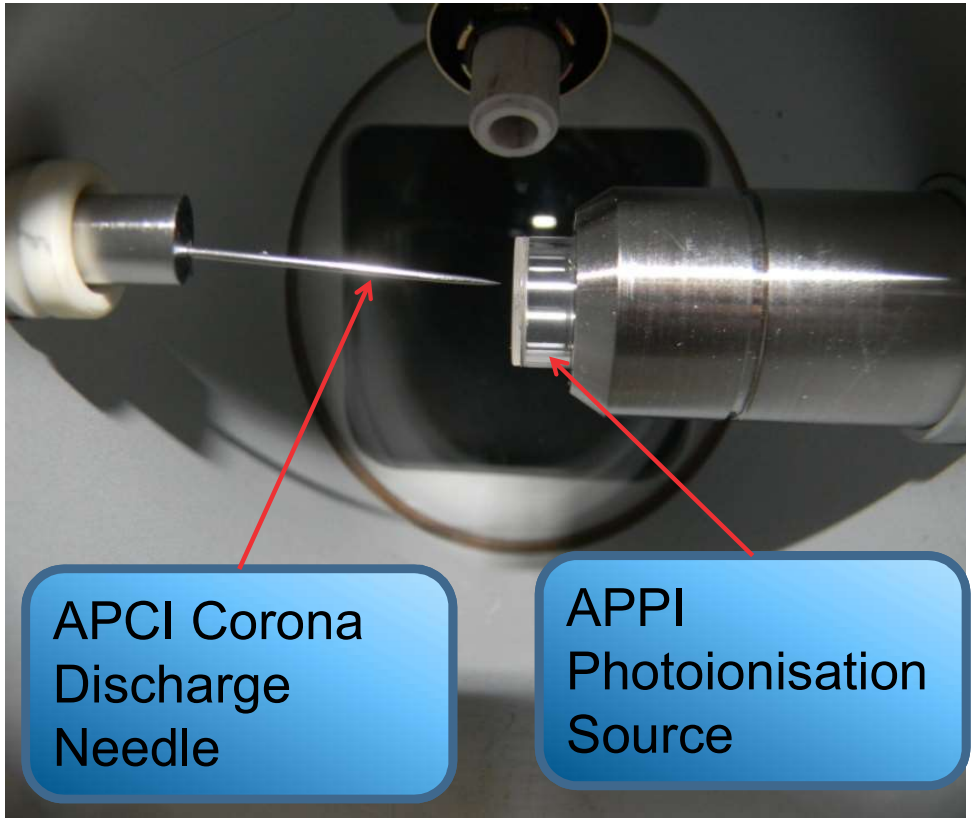
APPI Photoionisation Source





ESI: Mobile phase considerations

- ☑ Acetic Acid (0.1 - 1%)
- ☑ Formic Acid (0.1 - 1%)

Proton Donors

- ☑ Ammonium Hydroxide
- ☑ Ammonia Solutions

Proton Acceptors

- ☑ Trichloroacetic Acid (< 0.1% v/v)
- ☑ Trifluoroacetic Acid (< 0.1% v/v)

Chromatographic Separation

- ☑ Triethylamine (< 0.1% v/v)
- ☑ Trimethylamine (< 0.1% v/v)

Negative Ion Formation

- ☑ Ammonium Acetate (5 - 50 mM)
- ☑ Ammonium Formate (5 - 50mM)

Buffers

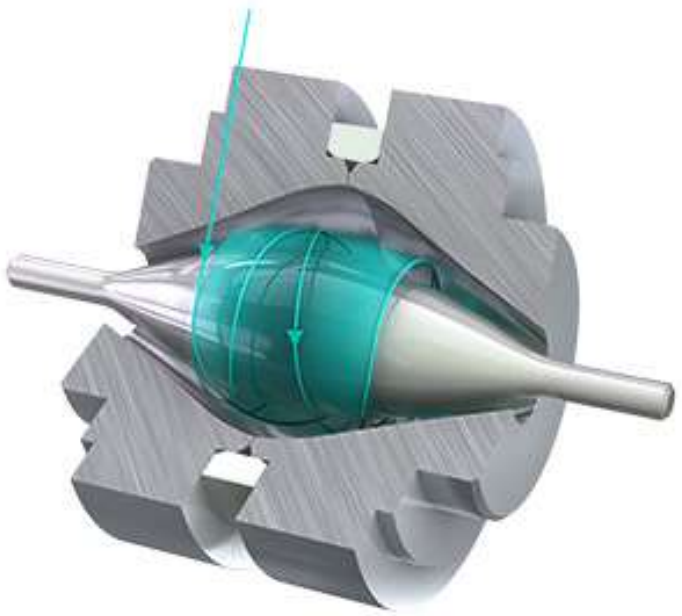
ESI: Mobile phase considerations

LC additives to avoid:

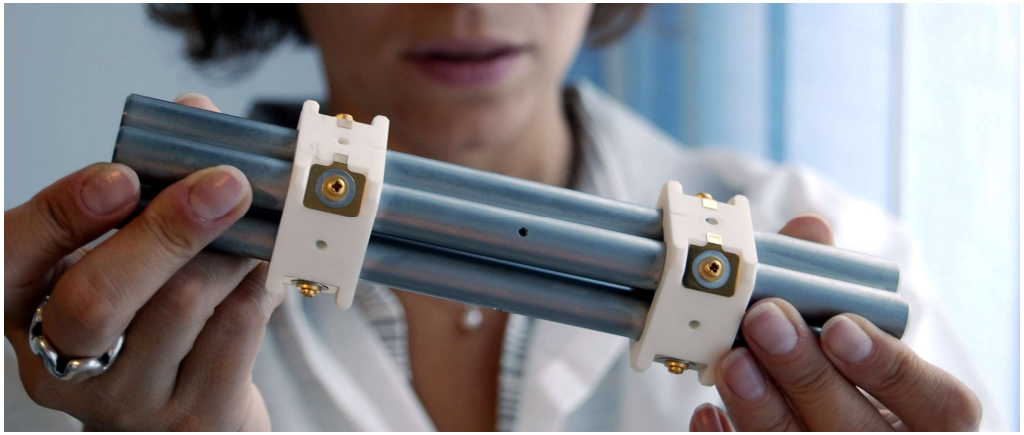
- **Inorganic acids** such as HCl, H_2SO_4, H_3PO_4 or **alkali metal bases** such as NaOH may cause source corrosion.
- **Detergents**, **surfactants** and other **surface active agents** (SDS, PEG, Triton X 100) can suppress ionization.
- **Trifluoroacetic acid** (TFA) at C>0.1 % v/v causes ion suppression in both negative and positive ion mode.
- **Involatile buffers** such as phosphate, citrate, borate buffers suppress Ionization.
- **Tetrahydrofuran** (THF) reacts with the PEEK in the ESI probe.

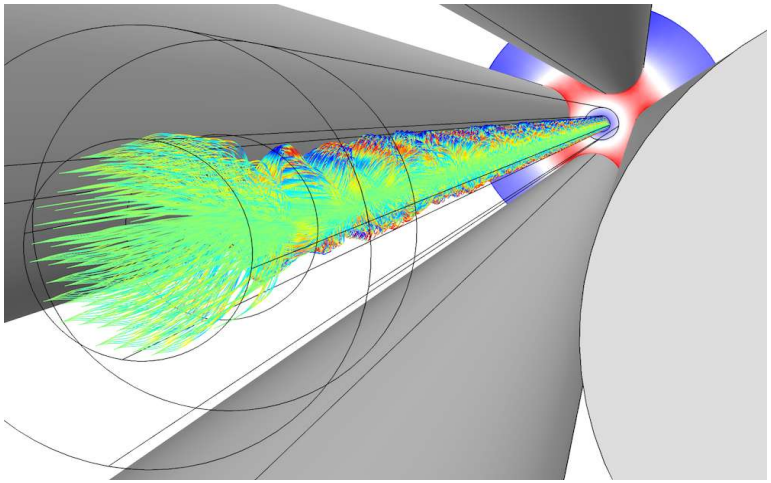
Mass Analyzer

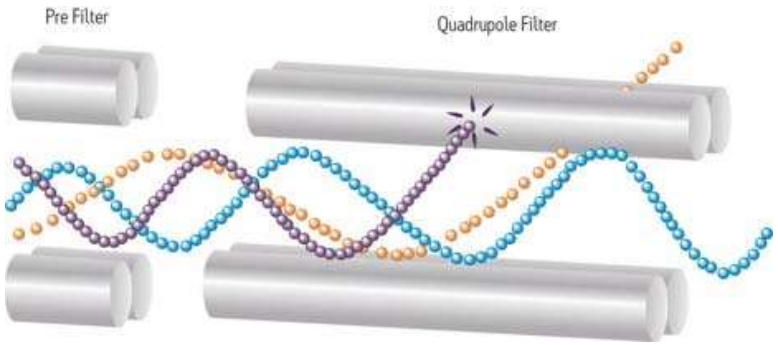
- **Quadrupole Analyzers**
- **Time-of-flight Analyzers**
- **Ion Trap Analyzers**
- **Orbitrap**
- **Hybrid Analyzers**

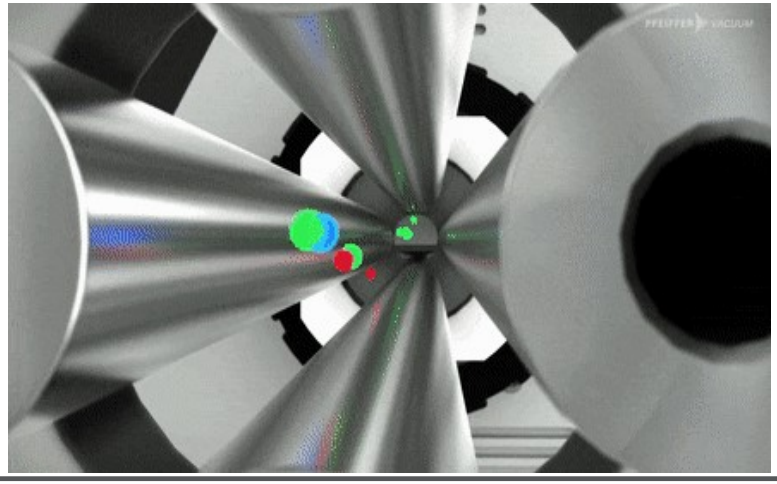


Quadrupole





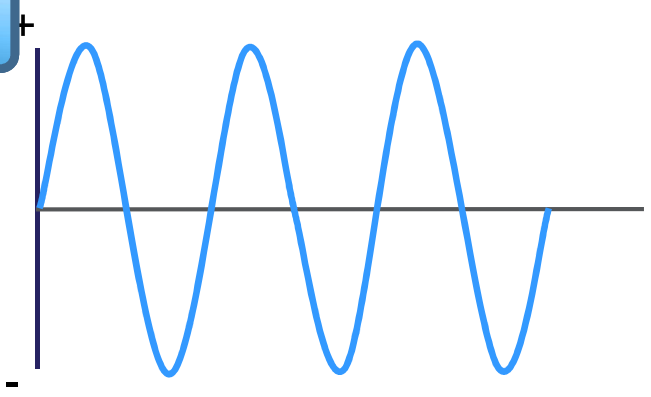


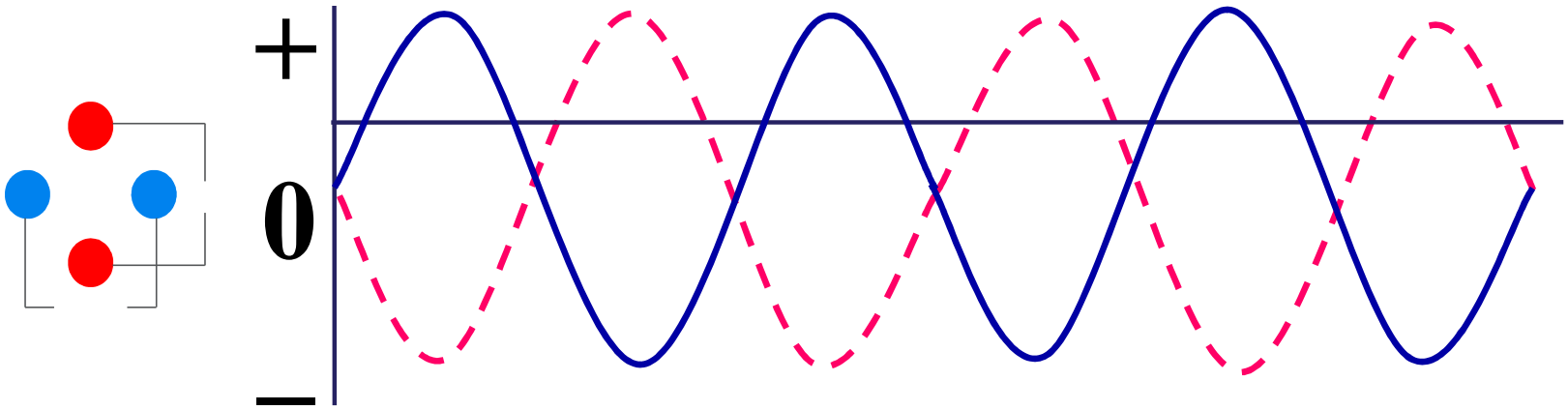


Quadrupole

Radio-Frequency Alternating Voltages

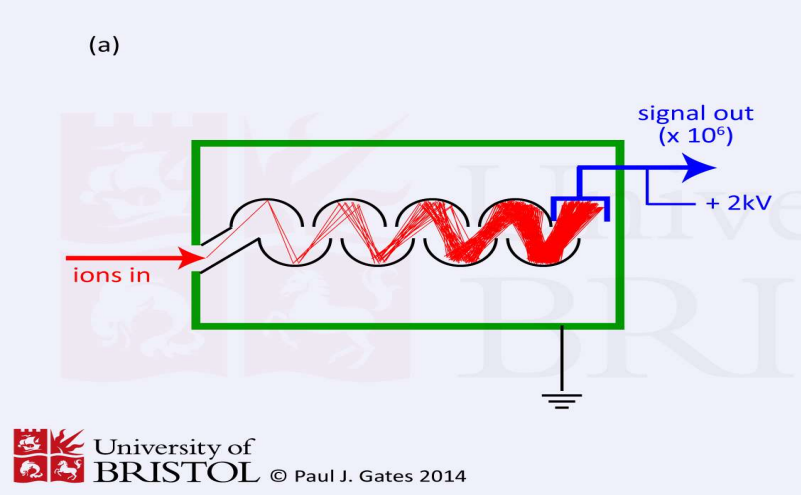
RF Has Two Components:
- Frequency (cycles/second)
- Power (amplitude of wave)





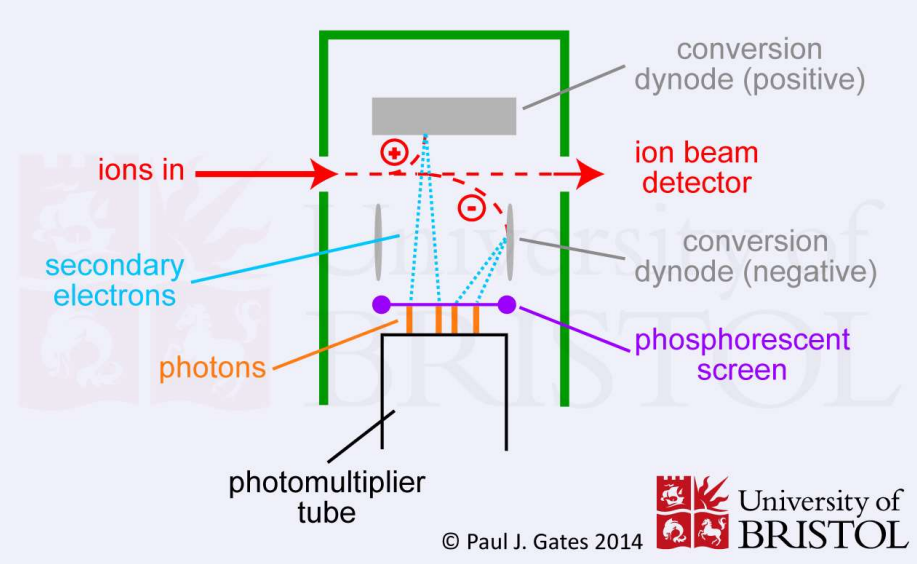
detectors

EMT



Electron multipliers are probably the most common means of detecting ions, especially when positive and negative ions need to be detected on the same instrument. Electron multiplier has series of dynodes maintained at increasing potentials resulting in a series of amplifications

PMT

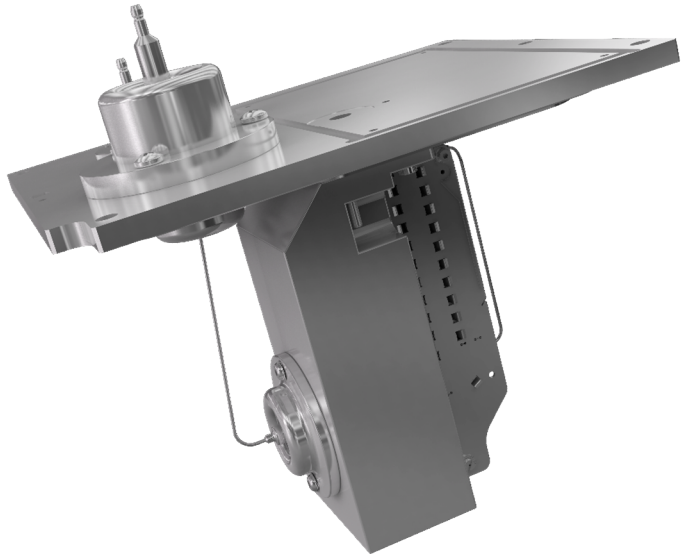


In a photomultiplier (or scintillation counter) the ions initially strike a dynode which results in electron emission. These electrons then strike a phosphorous screen which in turn releases a burst of photons. The photons then pass into the multiplier where amplification occurs in a cascade fashion - much like with the electron multiplier.

Detector

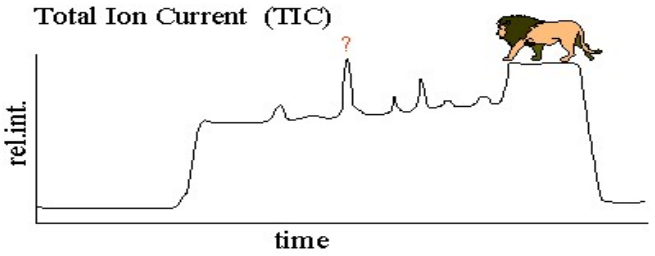
Benefits: Increased electron multiplier lifetime. Increased Uptime!

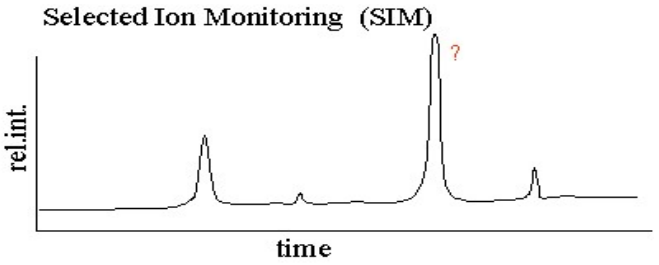
- Increased number of dynodes (21) for extended lifetime.
- Improved electron multiplier calibration routine.
- Excellent linearity and dynamic range across the mass range.
- Reduced number of service visits leading to more uptime.

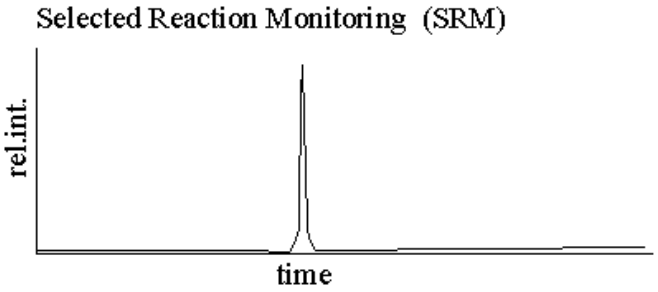


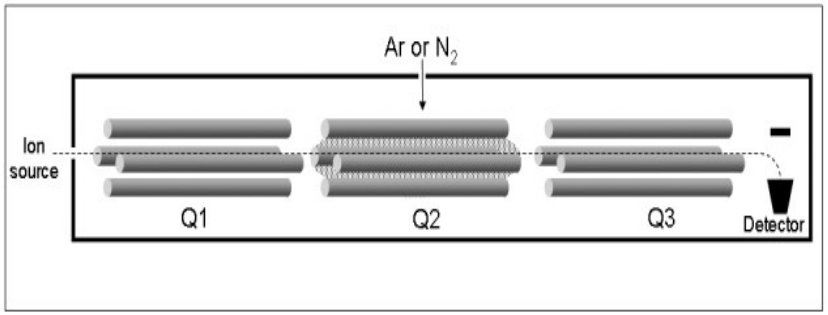
What is triple quadrupole MS?

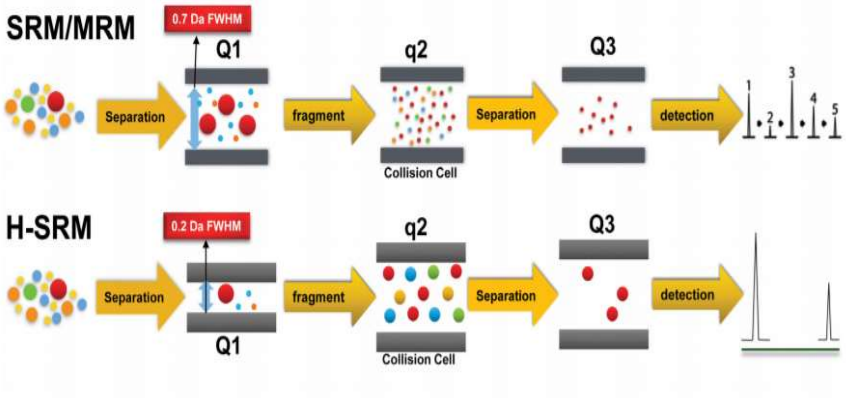
- **Full Scan**
- **SIM**
- **SRM/MRM**



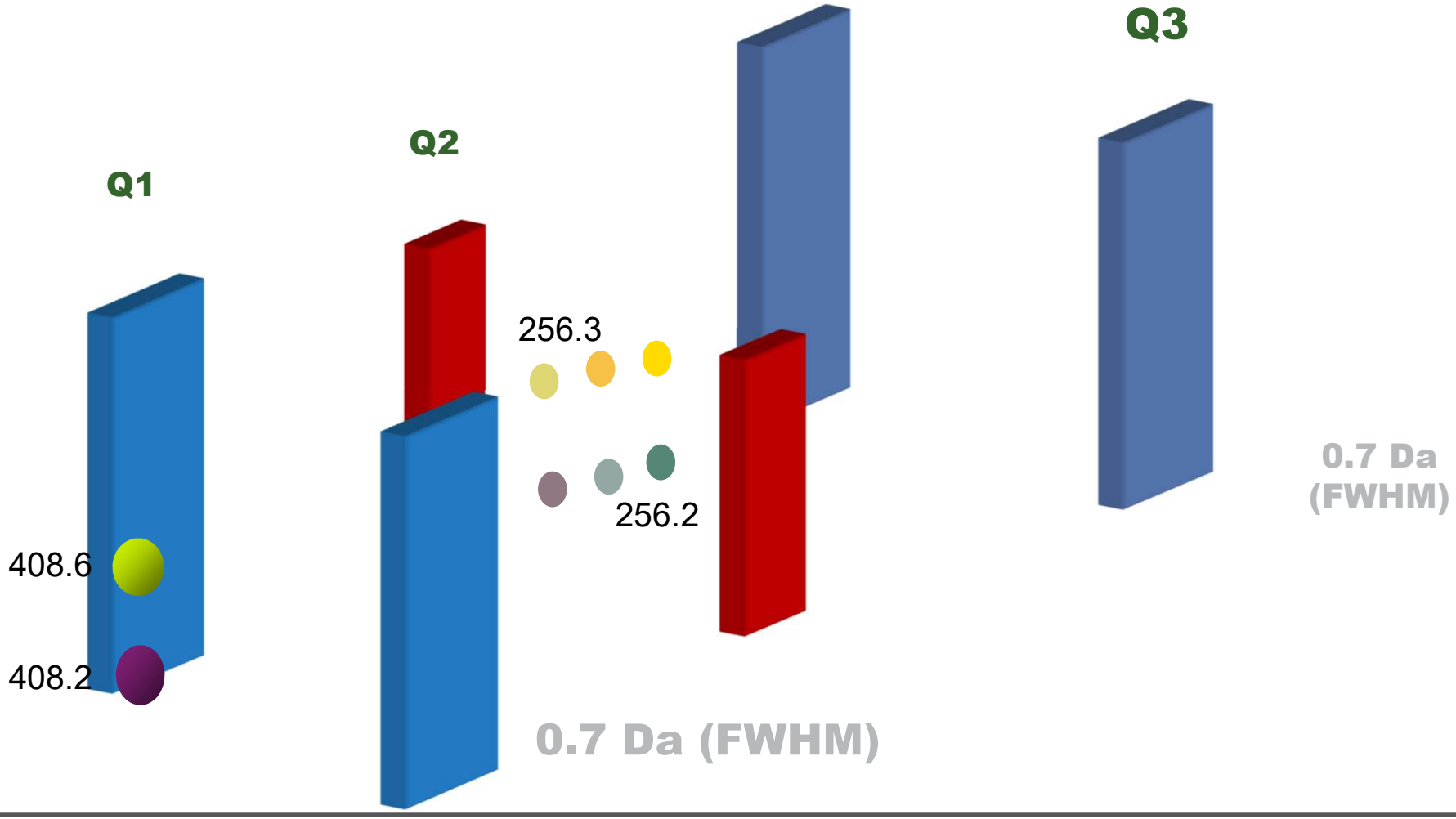








SRM - Selected Reaction Monitoring at Unit Resolution



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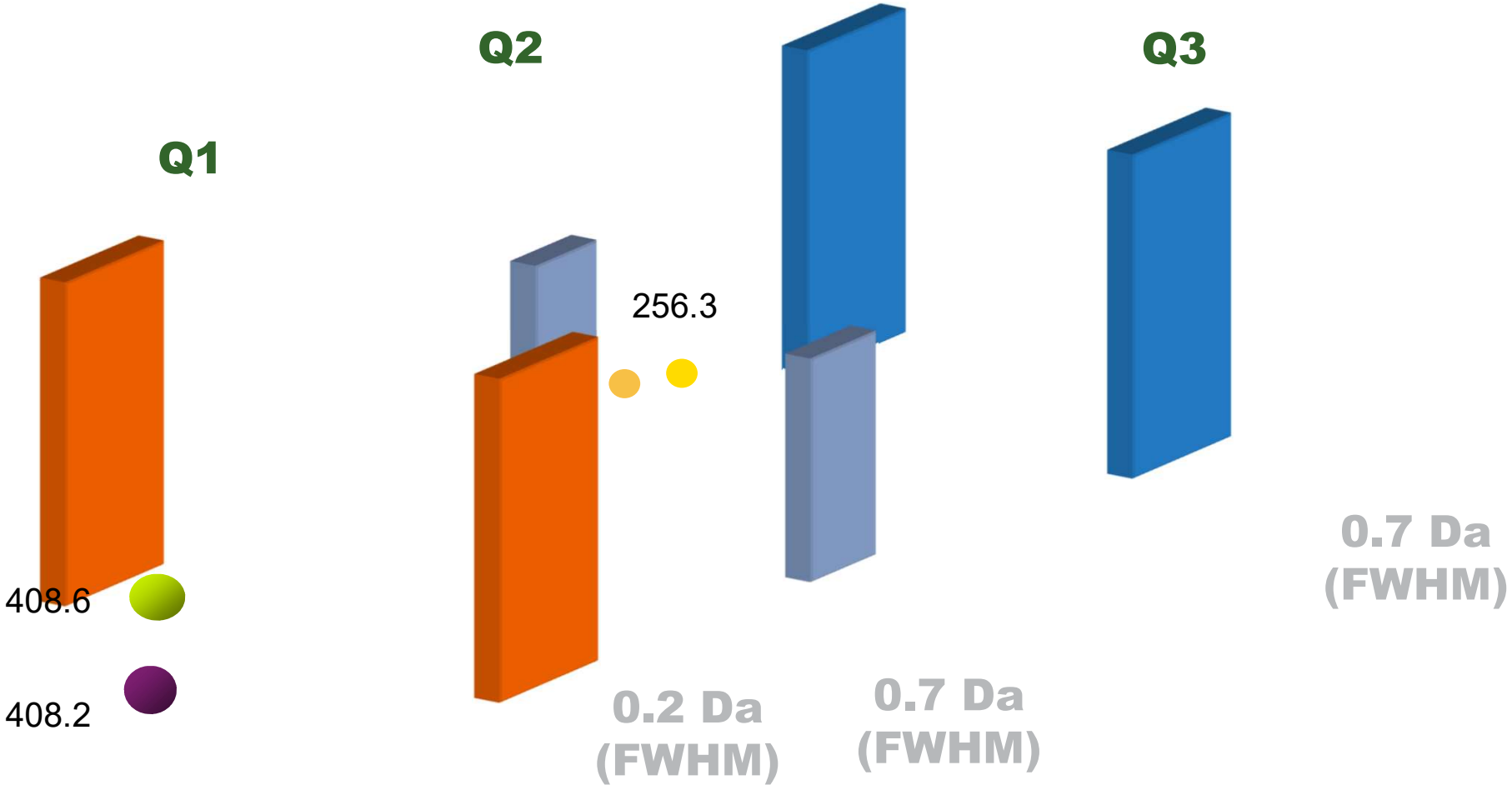
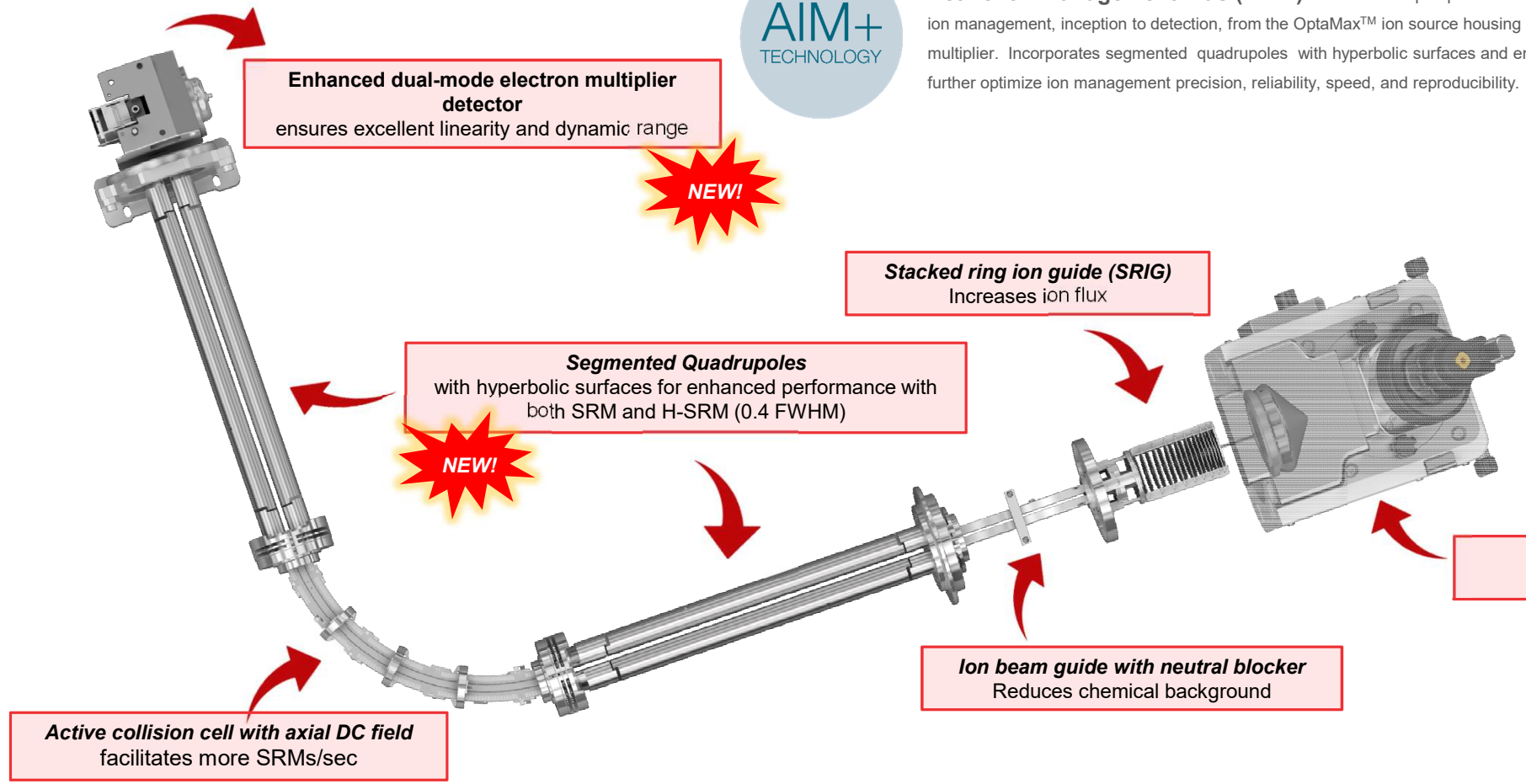
H-SRM - Selected Reaction Monitoring at High Resolution



TSQ Quantis: Unprecedented Robustness, Day After Day

AIM+ TECHNOLOGY

Active Ion Management Plus (*AIM+*) - The next step in precision design delivers the ultimate in ion management, inception to detection, from the OptaMax™ ion source housing to the enhanced electron multiplier. Incorporates segmented quadrupoles with hyperbolic surfaces and enhanced RF Electronics to further optimize ion management precision, reliability, speed, and reproducibility.



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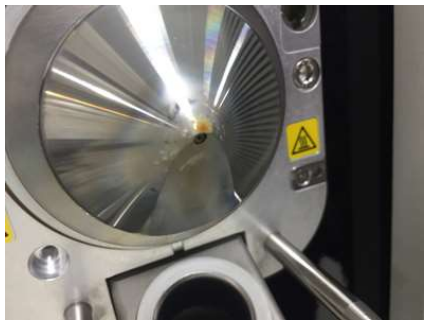
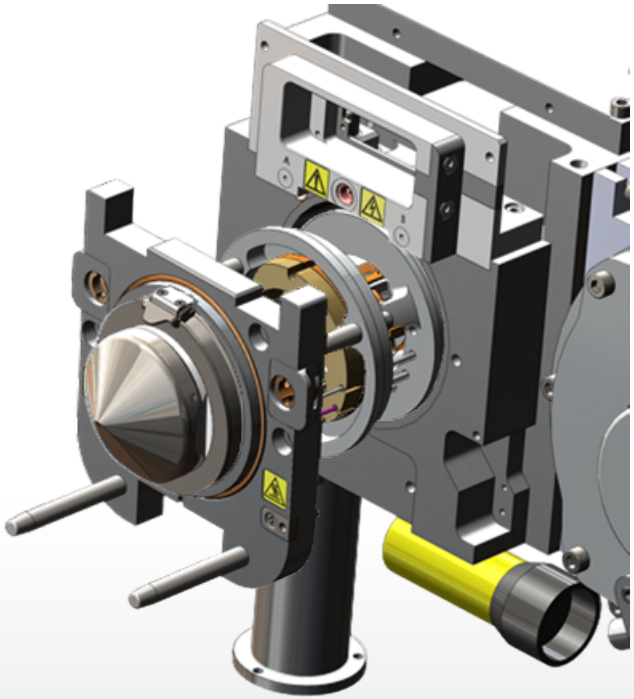
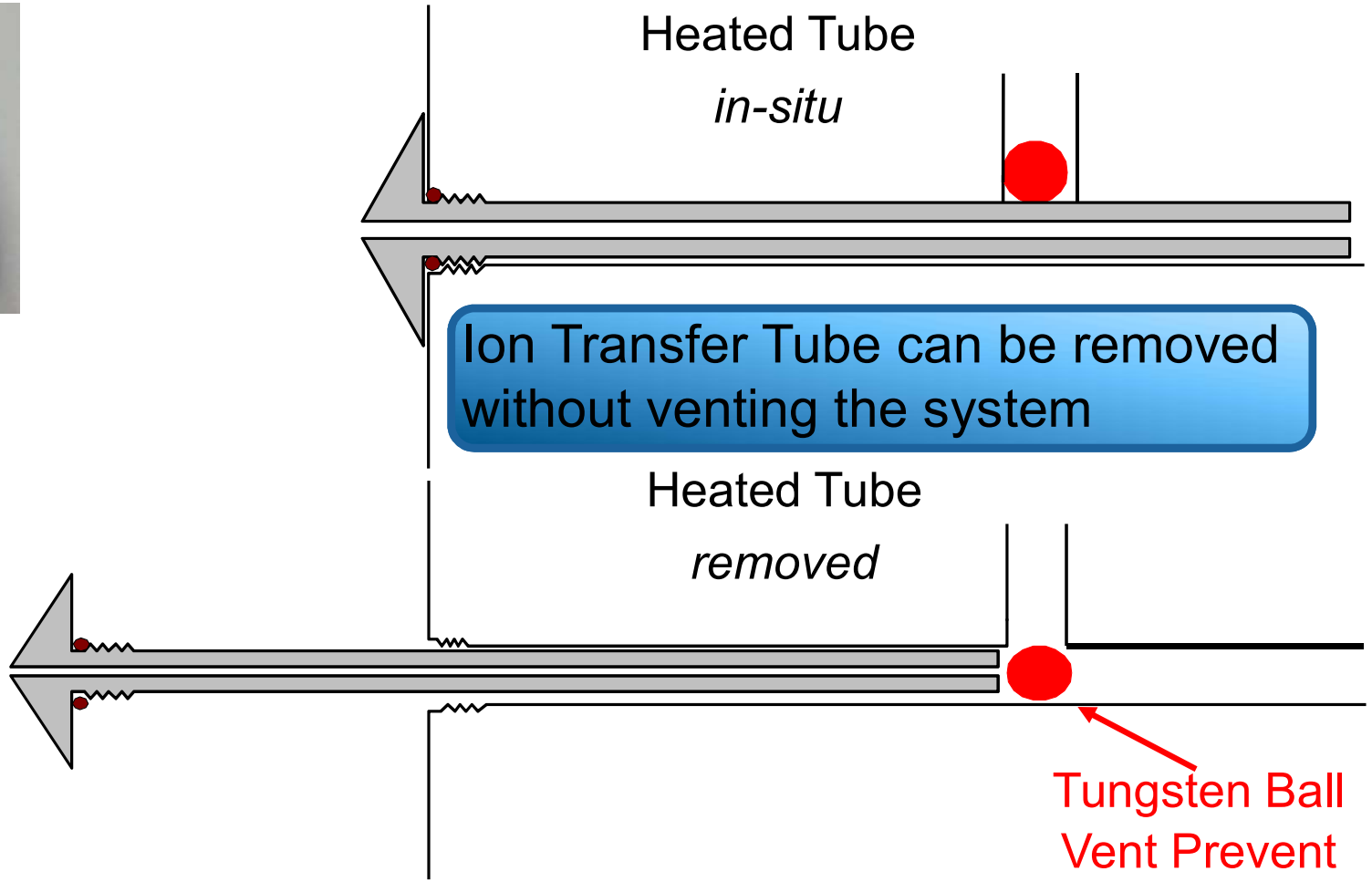
TSQ series MS—Easy to Clean

No tools required for removal of ion optics





Heated Ion Transfer Tube



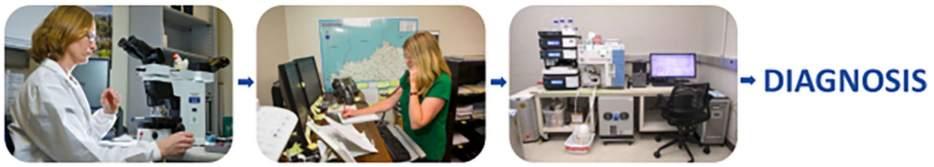
tips to improve method performance in LCMSMS

Sr. No.	Issue	Tip
1	Wide or split chromatographic peak shape	diluent, column, isomer, pump mixer, column overload
2	Poor recovery of compounds	acidity, reconstitution, temp effect
3	No response of compound	tuning, RF lens, standard, column chemistry
4	Pressure fluctuation	priming/purging, check mobile phase, purge valve
5	Poor precision	injection volume, temp (AS, column), number of data points, sensitivity
6	Separation of epimers (tetracyclines)	column, mixer, mobile phase composition
7	Source issue (temp, gas, water droplet)	fixing, MS on mode
8	Carryover	needle wash, time of wash, when to wash, column cleaning
9	RT fluctuation gradient	proper gradient, temp, column performance
10	Sensitivity	tuning, source cleaning, injection volume
11	Communication	software, PC, machine restart, plugs
12	Not finding mass during tuning	check adduct, add buffer, multiple charge

any other…… please let us know

Applications

- **Multiresidue Pesticides analysis in variety of Food commodities**
- **Multiresidue Pesticides analysis in variety of Beverages**
- **Multiresidue Pesticides analysis in variety of Confectioneries**
- **Aflatoxins analysis**
- **Antibiotics & Vet Drug analysis**
- **Biochemical Screening for Genetic Disorders**
- **Therapeutic Drug Monitoring and Toxicology**
- **Vitamins and Related Metabolites**
- **Steroid Hormones**
- **Pharmaceutical**
- **Clinical**
- **Forensic**
- **Dope testing**



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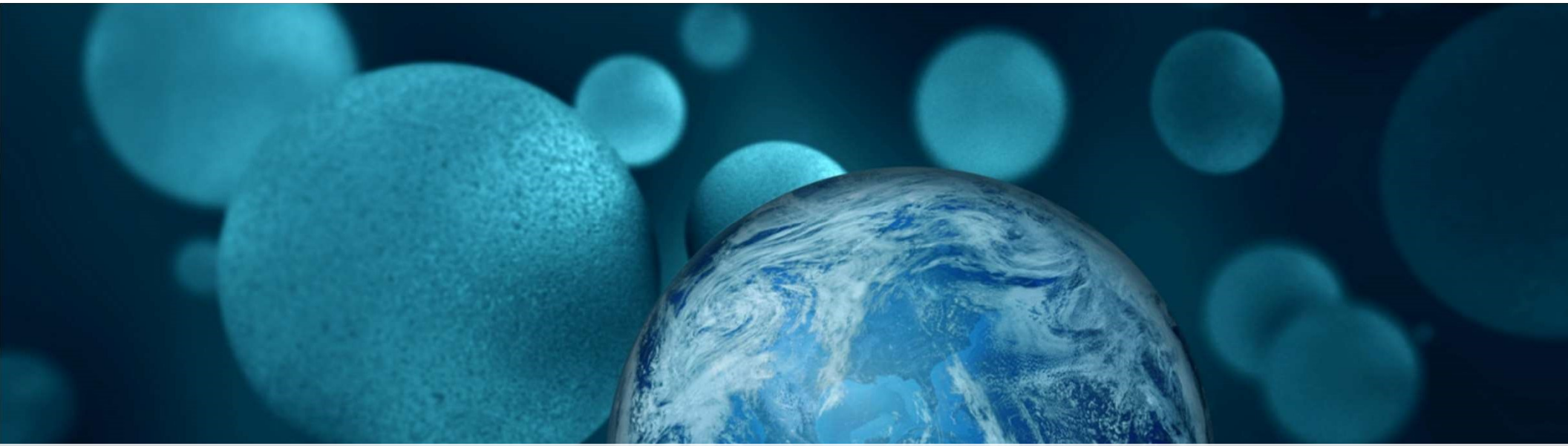


ThermoFisher SCIENTIFIC

Food Safety applications for trace level contaminant analysis using LC-MS/MS in variety of food samples

Ramiz M.R.Azad
Application Development Scientist
Thermo Fisher Scientific,
Customer Solution Center, India

What is Food Safety- key facts

- **Food safety** refers to the proper handling, cooking, and preservation of food in order to protect people from foodborne illnesses
- **Unsafe food** containing harmful **bacteria, viruses, parasites or chemical** substances, causes more than 20(ranging from diarrhea to cancers
- **An estimated 600 million** – **almost 1 in 10 people in the world** – fall ill after eating contaminated food and 420 000 die every year.
- **Children under 5 years** of age carry 40% of the foodborne disease burden, with **125 000 deaths** every year
- **Food contamination** happens when food are corrupted with another substance. It can happen In the process of production, transportation, packaging, storage, sales and cooking process. The contamination can be physical, chemical and biological
- Physical contamination-When the foreign object comes into the food e.g. hair, glass or metal, pests, jewelry, dirt and fingernails
- Chemical contamination- pesticides, herbicides, veterinary drugs, mycotoxins food additives, adulterants, environmental sources (water, air or soil pollution),
- Biological contamination-food that has been contaminated by substances produced by living creatures, such as humans, rodents, pests or microorganisms e.g. bacteria, virus

source: https://www.who.int/news-room/fact-sheets/detail/food-safety

What is our role- Chemical contamination

- Commercial testing labs
- Government labs
- Regulatory bodies
- Validation/compliance

Manufactures- Low to high end machines
Technical/Application Support

Analysts
Researchers
Scientists

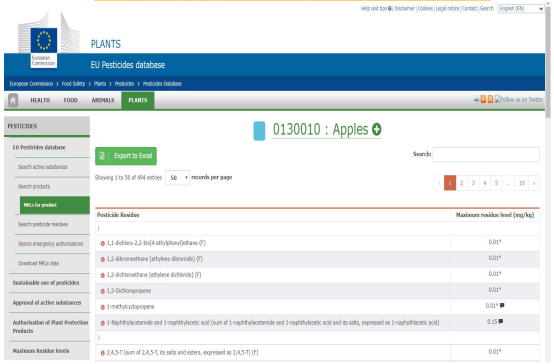
testing

wet chemistry/Classical technique
e.g. kjeldahl

analytical	**advanced analytical**	
HPLC	LCMSMS	Pesticides
UHPLC	GCMSMS	Vet Drugs
UV-Vis	ICP-MS	Mycotoxins
FTIR	Orbitrap	Heavy
AAS	Fusion/Tribrid	metals PAH,
ICP	Ion trap/TOF	PCB

Regulation…

- **Regulations by jurisdiction and agency**
- Codex
- European regulation
- FSSAI
- CFDA so on……



https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN

TABLE

Sl. No.	Name of the Insecticide	Food	Maximum Residue Limit (MRL) in mg/kg
(1)	(2)	(3)	(4)
1.	2,4-Dichlorophenoxy Acetic Acid	Sugarcane	0.05
		Food grains	Maize-0.05, Wheat-2 and Rice-0.1and other food grains- 0.01
		Milled food grains	0.01
		Potato	0.2
		Milk and Milk products	0.05
		Meat and Poultry	0.2
		Eggs	0.05 (shell free basis)
		Fruits	2
2.	Acephate (expressed as mixture of Methamidophos and acephate).	Rice	1
		Safflower seed	2
		Cottonseed	2
		Milk and Milk products	0.02
		Meat and Meat products	0.05
3.	Acetamiprid	Chilli	2
		Dried Chilli	20
		Rice	0.01
		Okra	0.1
		Cabbage	0.7
		Milk and Milk products	0.02
		Meat and Meat products	0.05
		Cotton seed Oil	0.1

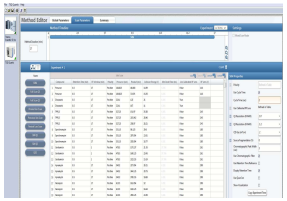
Resources required to start analysis

- TSQ Quantis with Vanquish Flex UHPLC
- Supporting equipment's (centrifuge, vortex ,etc.)
- Consumables
- LC Column Chemistry
  - Hypersil Gold C18
  - Accucore C18
- Pesticide Kit Restek (200)
- Vet drug standard (56)
- Solvents
- QuEChERS Buffers and Salts

Workflow

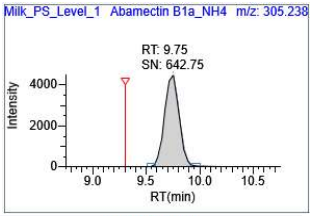
1. Instrument Method- LC and MS

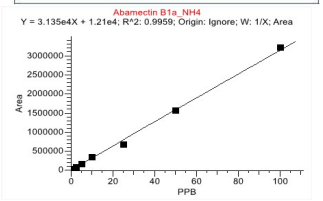
No	Time	Flow [ml/min]	%B	Curve
1	0.000	Run		
2	0.100	0.300	5.0	5
3	1.000	0.300	5.0	5
4	9.000	0.300	100.0	5
5	13.000	0.300	100.0	5
6	13.500	0.300	5.0	5
7	17.000	0.300	5.0	5
8	New Row			
9	17.000	Stop Run		



2. Method development

- Chromatography
- Sensitivity
- Linearity





3. Sample Preparation

- e.g. QuEChERS, VDX, any other

Validation and Data review

- Guideline SANTE/12682/2019 and EC657

- Matrix effect
- Linearity
- Sensitivity
- Specificity
- Recovery (3 levels)
- Precision (n=6)
- Ion ratio
- Robustness
- RT ±0.1min

European Commission

LCMSMS method- Multiresidue Pesticides

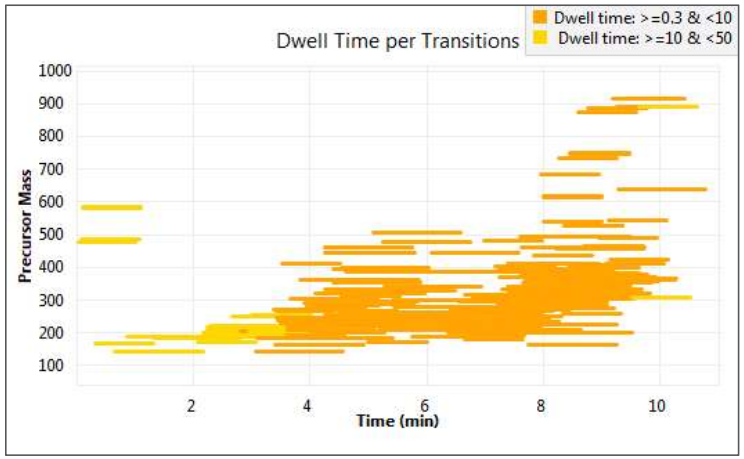
UHPLC Instrumentation	Vanquish UHPLC Flex (binary pump F Thermo Scientific™ (P/N VF-P10-A)
Column	Accucore aQ column, 100 mm x 2.1 mm, 2.6 µm (Thermo Scientific™, P/N 17326-102130)
Sample Compartment temp	10°C (Still air) (Split Sampler FT Thermo Scientific™, P/N VF-A10-A)
Column oven temp	25°C (Column Compartment ,Thermo Scientific™, P/N VH-C10-A)
Injection volume	10 µL
Needle wash	80% Methanol and 20% Water
Mobile Phase	A: 5mM ammonium formate + 0.1% formic acid in Water B: 5mM ammonium formate + 0.1% formic acid in Methanol
Set inline filter	35µl, VF-P1 (10µl mixer kit) (Thermo Scientific, P/N 6044.3870)
Total run time	15.0 min

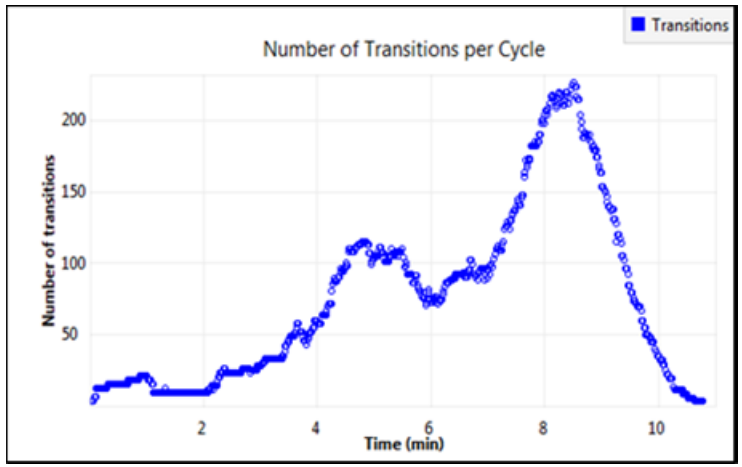
LC Gradient Program

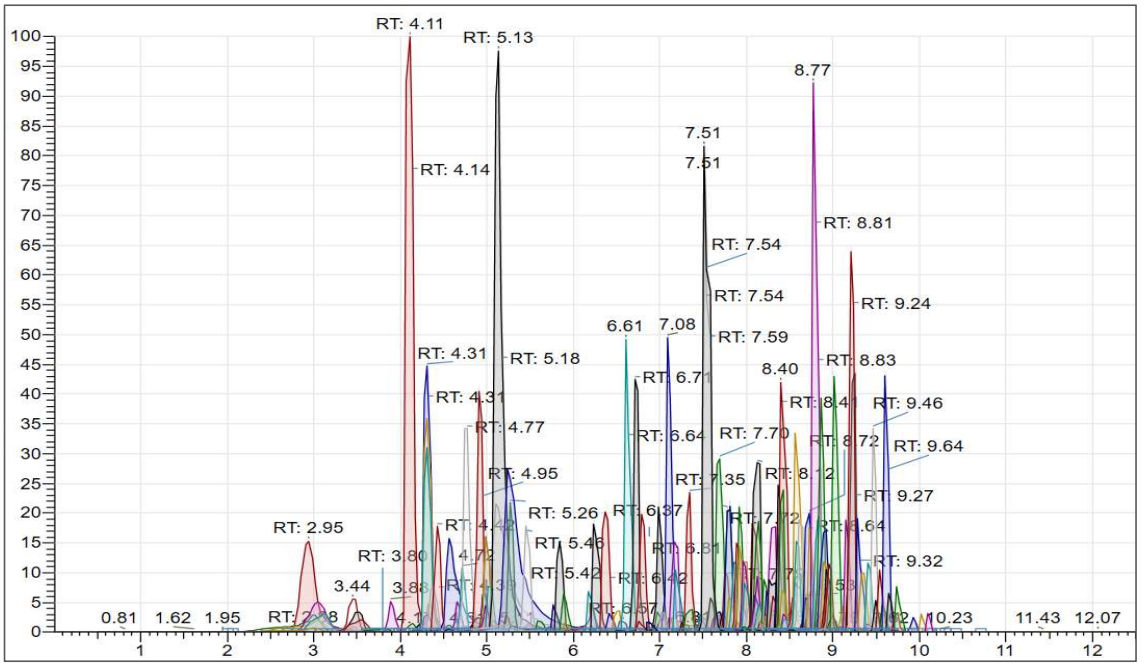
Time (min)	Flow rate (ml/min)	%B	Curve
0.000	0.300	0	5
0.500	0.300	0	5
7.000	0.300	70	5
9.000	0.300	100	5
12.000	0.300	100	5
12.100	0.300	0	5
15.000	0.300	0	5

Mass Spectrometer Instrumentation	TSQ Quantis Triple Quadrupole Mass Spectrometer (Thermo Scientific™)
Method type	Acquisition-Timed (SRM mode)
Ion Source Type	H-ESI
Spray Voltage	Static Positive- 3700V Negative- 2500V
Sheath Gas	30 Arb
Aux Gas	6 Arb
Sweep Gas	1 Arb
Ion Transfer tube temp	325 °C
Vaporizer temp	350 °C

Dwell time







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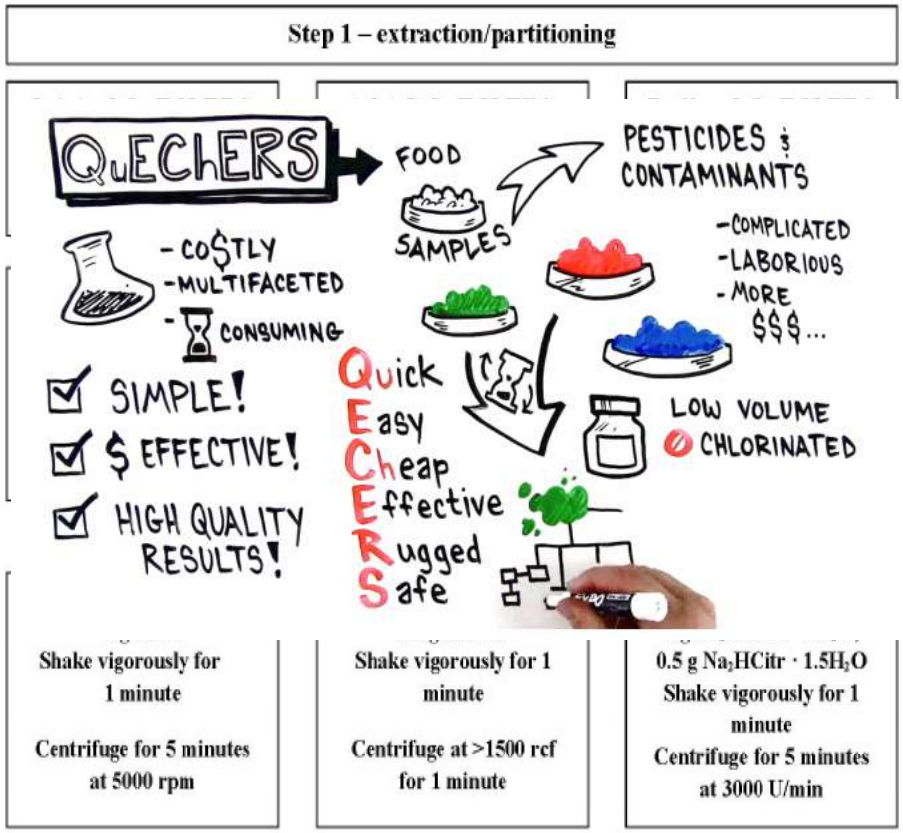
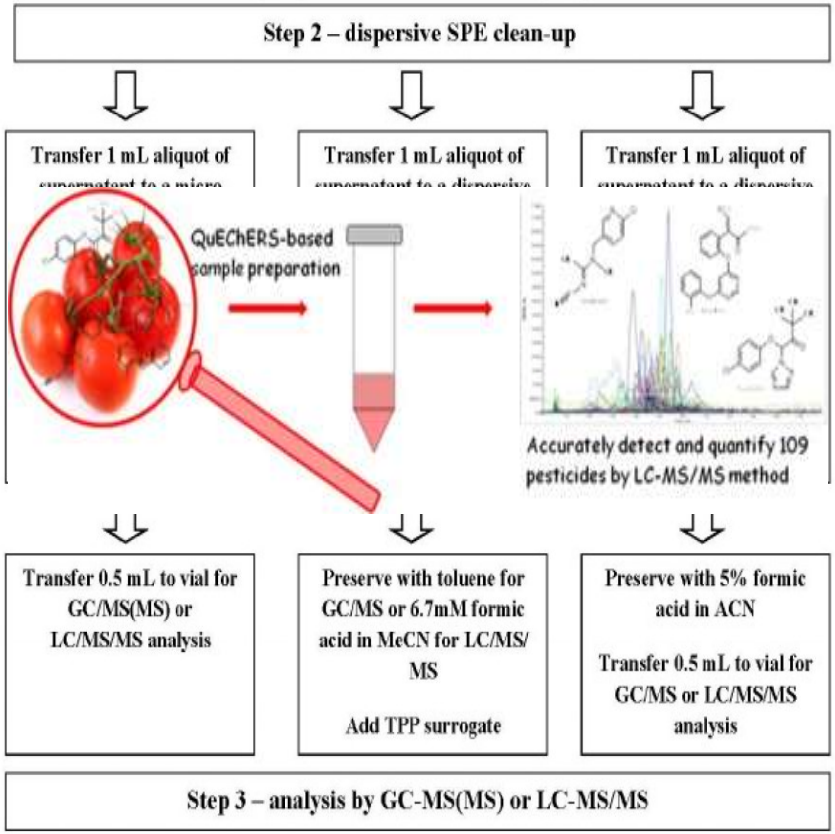
Sample Preparation Approach- Pesticides

Extraction



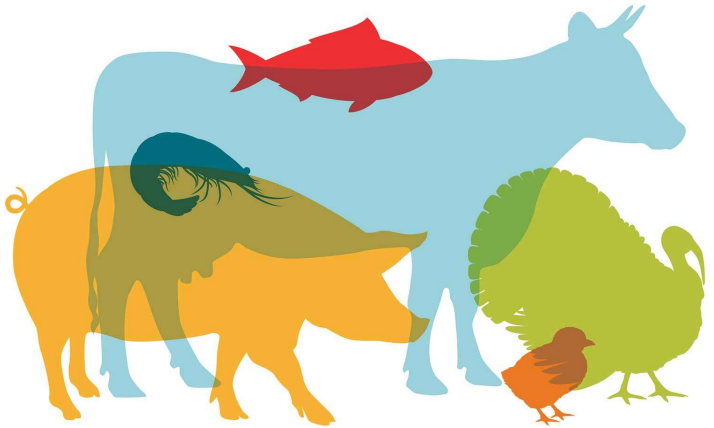
Clean up



Sample Preparation Approach- Veterinary Drugs

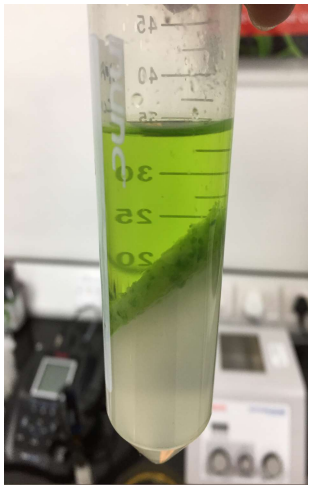
Veterinary drug explorer (VDX)

- Weigh 4 g milk sample in 50ml falcon tube-(Spiking will be done at this step)
- Add 10ml Acidified acetonitrile (1% Acetic Acid), Vortex for 1 min, 2500 rpm
- Add ammonium oxalate/EDTA solution (1mL)
- Add 3g sodium sulfate, shake well and vortex for 5 min at 2500rpm
- Centrifuge at 4500 rpm for 5 minutes
- Decant 8ml of supernatant in 15mL falcon tube
- Add 500mg C_{18} material into the tube
- Vortex and centrifuge at 4500 rpm for 10minutes
- Evaporate 5mL of supernatant to dryness using Nitrogen evaporator
- Reconstitute in 1ml with water:methanol:acetonitrile (70:15:15)
- Inject into LC-MS/MS
- **A- Matrix match standard**
- **B-Matrix extracted spike/Procedural standard**

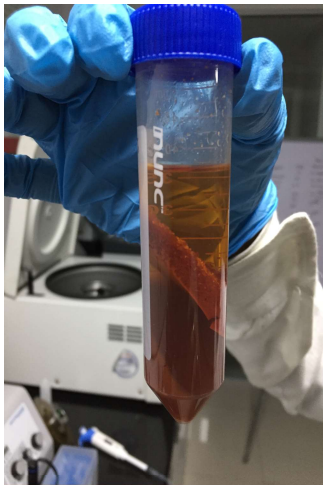


How to decide sample quantity and clean up material??


tomato
15g + 15ml acidified acetonitrile
clean up
150mg MgSO4
50mg PSA

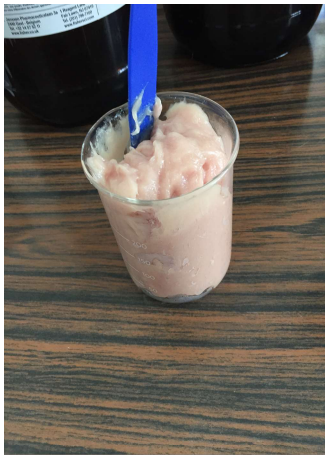
cucumber
15g + 15ml acidified acetonitrile
clean up
150mg MgSO4
50mg PSA

chilli
2g + 15ml acidified water (soak 10 min) + 15ml acetonitrile
clean up
150mg MgSO4
50mg PSA
50mg C18
10mg GCB

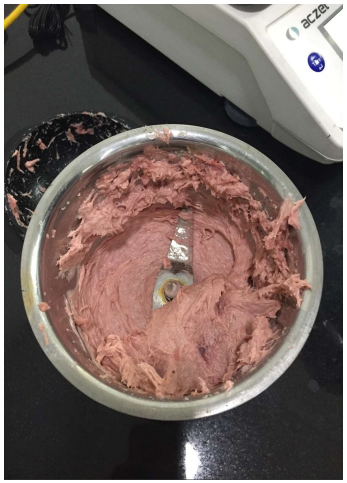
How to decide sample quantity and clean up material??





meat products (lamb, chicken, fish)
5g + 10ml acidified water + 15ml acetonitrile
clean up
150mg $MgSO_4$
50mg PSA
50mg C18
freezing step (optional)



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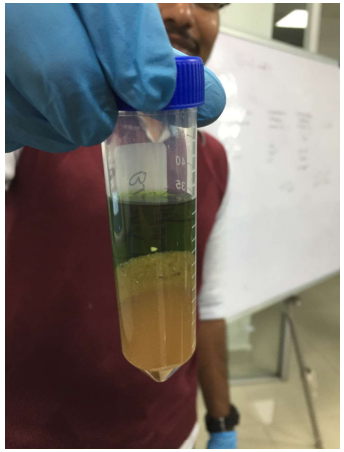
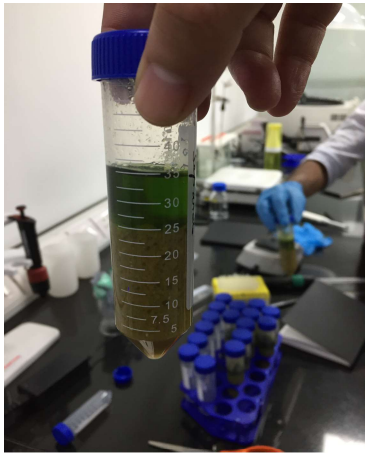
Okra, spinach cereals sample preparation




Okra
Homogenize sample with water (50:50)
15g + 15ml acidified acetonitrile
clean up
150mg MgSO4
50mg PSA
10mg GCB

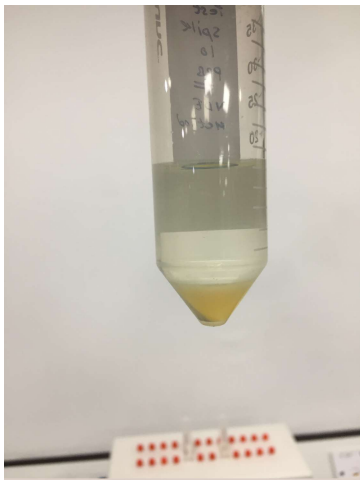
Spinach
15g + 15ml acidified acetonitrile
clean up
150mg MgSO4
50mg PSA
10mg GCB

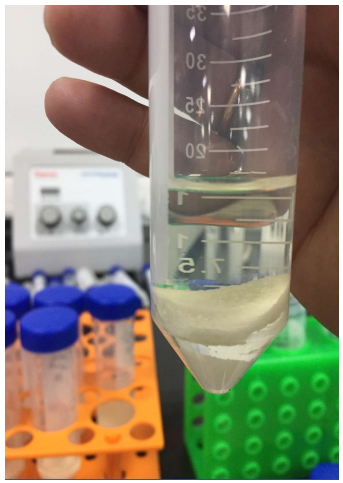
wheat/rice
5g + 15ml acidified water (soak 10 min)+ 15ml acetonitrile
clean up
150mg MgSO4
50mg PSA

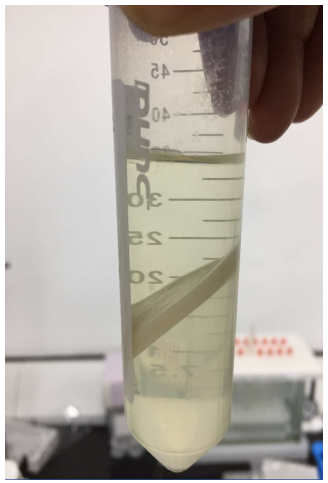
reconstitution in case of CAP, antibiotics

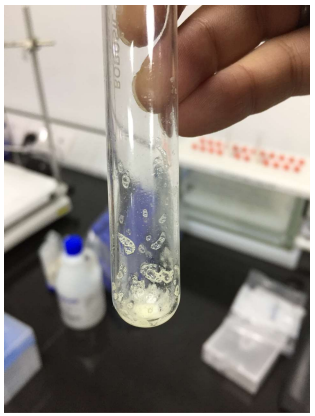
Without sodium sulphate, partial evaporation, time 90 min

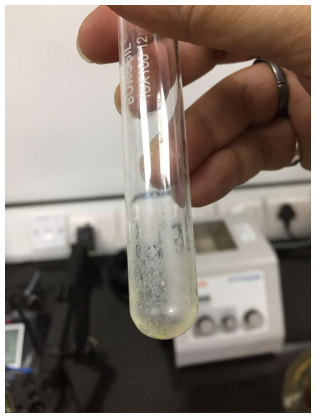
With sodium sulphate, complete evaporation, time 30 min











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Validation guideline SANTE/12682/2019

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	Comparison of response from solvent standards and matrix-matched standards	*	C21-C29
LOQ	Lowest spike level meeting the identification and method performance criteria for recovery and precision	≤ MRL	G6[8]
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

MS detector/Characteristics		Acquisition	Requirements for identification	
Resolution	Typical systems (examples)		minimum number of ions	other
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. Ion ratio from sample extracts should be within **±30 % (relative)** of average of calibration standards from same sequence
	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	
Accurate mass measurement	High resolution MS: (Q-)TOF (Q-)Orbitrap FT-ICR-MS sector MS	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

https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2019-12682.pdf

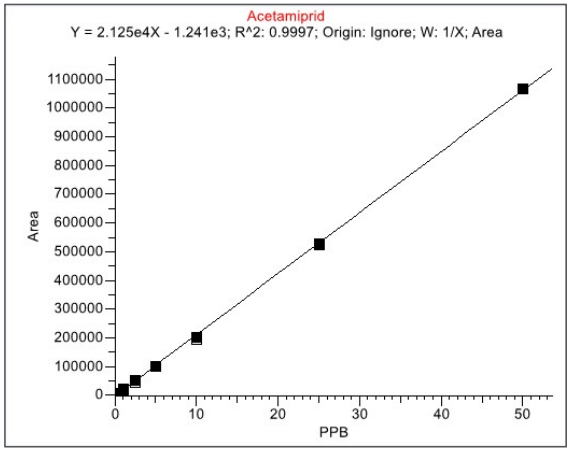
A typical example of the experimental set up of a validation is:

Sample set (sub-samples from 1 homogenized sample):

- Solvent Blank
- Solvent linearity (minimum 5 levels)
- Solvent Blank
- 1blank (non-spiked)sample
- Matrix match linearity/Procedural standard/MES
- 1blank (non-spiked)sample
- 5 spiked samples at target LOQ
- 5 spiked samples at target LOQx2
- 5 spiked samples at target LOQx5
- 1blank (non-spiked)sample
- Bracketing standard/linearity
- Ruggedness injections- 100

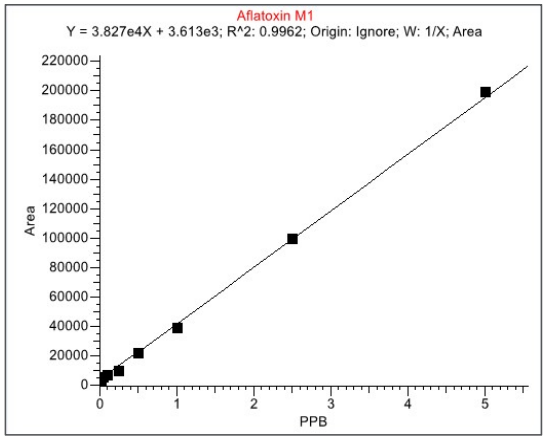
1. Sensitivity/linearity

The lowest calibration level (LCL) must be equal to, or lower than, the calibration level corresponding to the reporting limit (RL). The RL must not be lower than the LOQ.



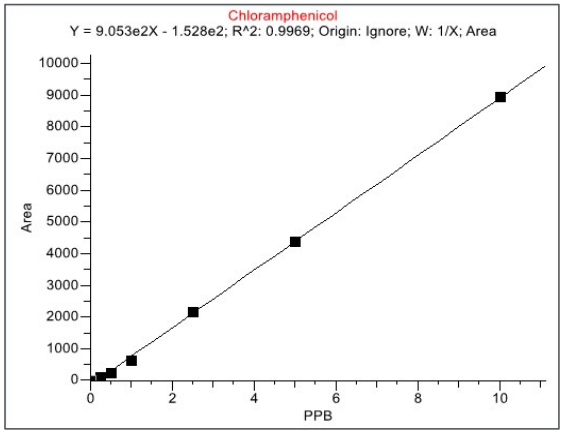
Linearity range- 0.0005-0.05mg/kg
LOQ – 0.005mg/kg

Matrix- Apple, Grape, Chilli, Spinach, Cabbage, Wheat, Rice, Juice, tomato, cucumber, Fish, Chicken, Lamb



Linearity range- 0.01-5ug/kg
LOQ – 0.025ug/kg

Matrix- Milk

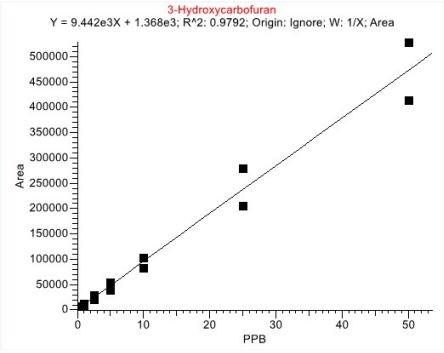


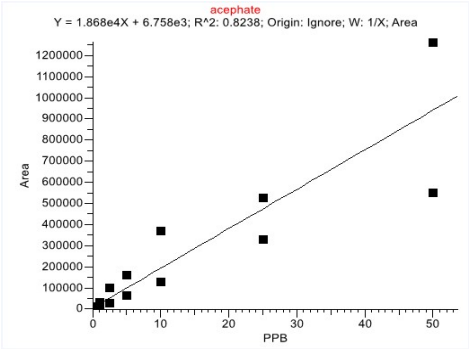
Linearity range- 0.05-10ug/kg
LOQ – 0.1ug/kg

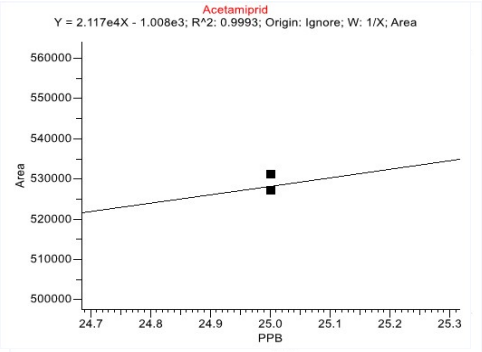
Matrix- Milk, Honey

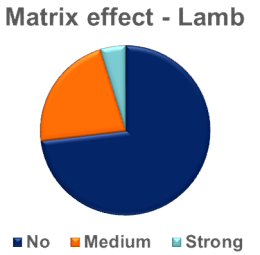
2. Matrix effect

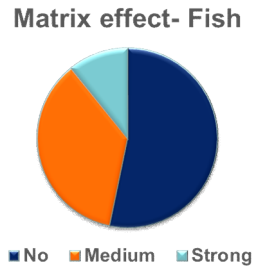
Matrix effects or matrix interferences from natural constituents of samples are frequent. The interference may be peculiar to the determination system used, variable in occurrence and intensity, and may be subtle in nature. If the interference takes the form of a response overlapping that of the analyte, a different clean-up or determination system may be required. Matrix effects in terms of suppression or enhancement of the detection system response.

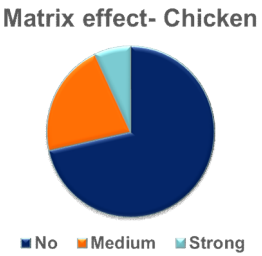










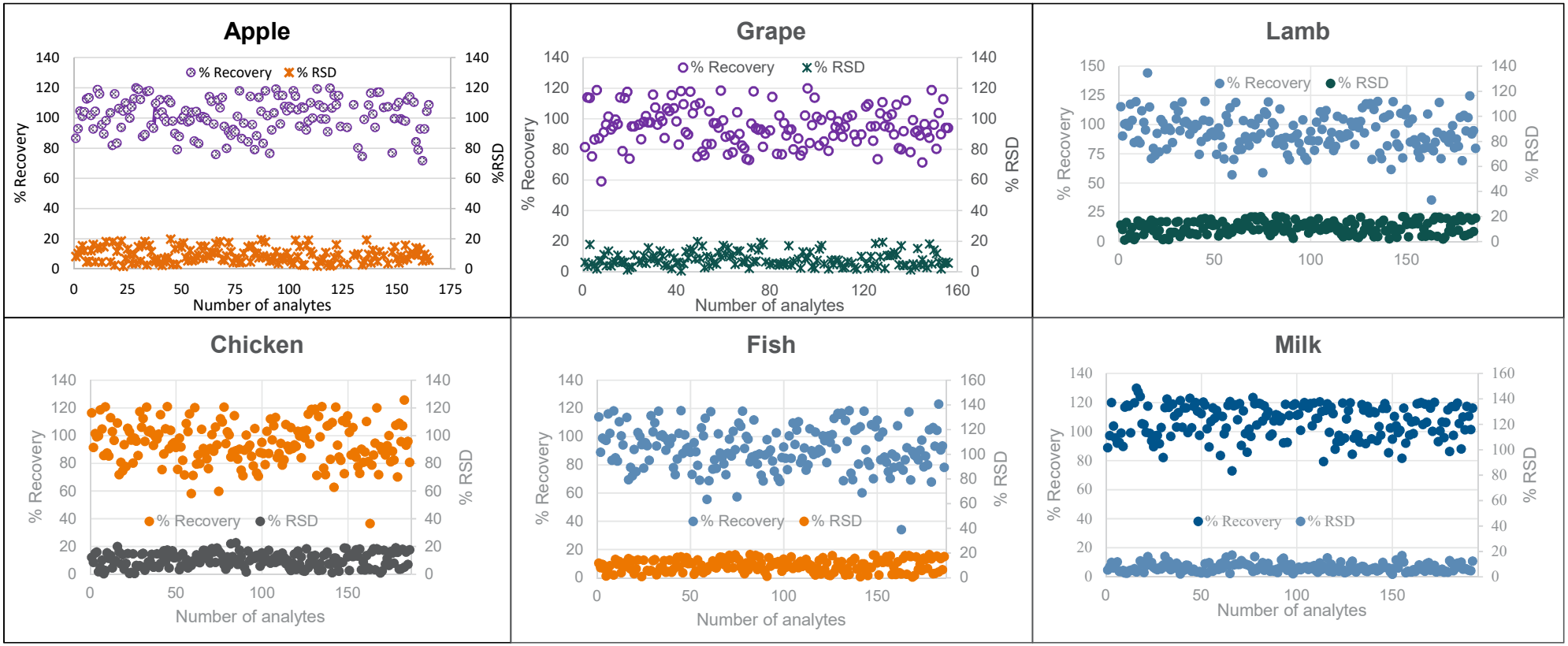


Formula- ((matrix response/solvent response)-1)*100

Sr. No	Compound	SS 10 PPB	EN_Tomato_10 PPB	Matrix Effect	Remark
1	3-Hydroxycarbofuran	2131986	2023293	-5.10	Pass
2	Acetamiprid	122385	111381	-8.99	Pass
5	Aldicarb sulfone	15796	13652	-13.57	Pass
6	Aldicarb sulfoxide	569	2425	0.00	Pass
7	Ametryn	210141	204289	-2.78	Pass
8	Aminocarb	1121225	1084677	-3.26	Pass
10	Azoxystrobin	207411	183968	-11.30	Pass

3. Limit of quantification (LOQ), Recovery

Limit of quantitation (quantification). The lowest concentration or mass of the analyte that has been validated with acceptable accuracy by applying the complete analytical method. ≤MRL



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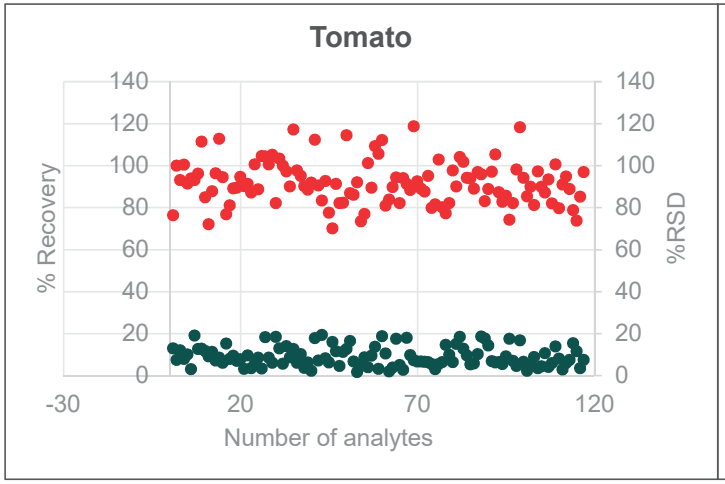
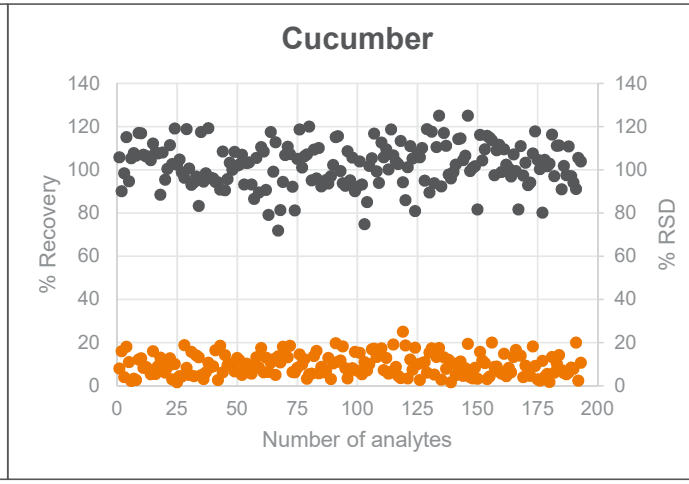
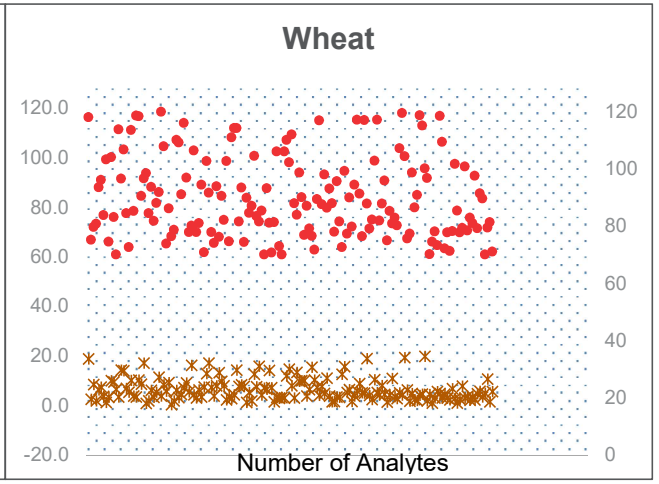
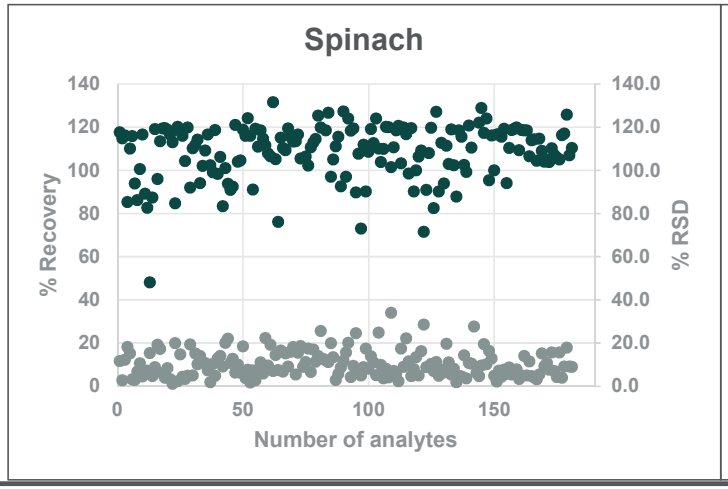
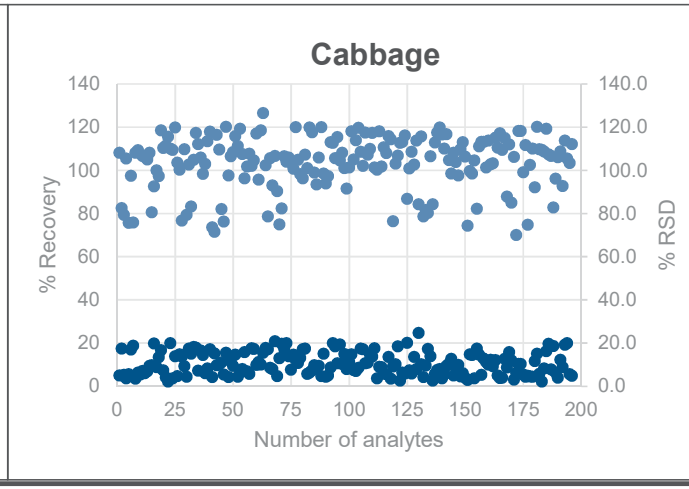
Few more matrix…

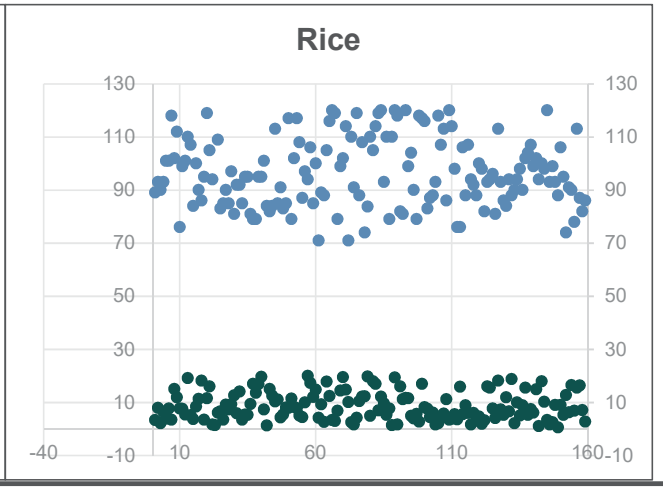












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4. Robustness

Average recovery and $RSD_{wR,}$ derived from on-going method validation / verification

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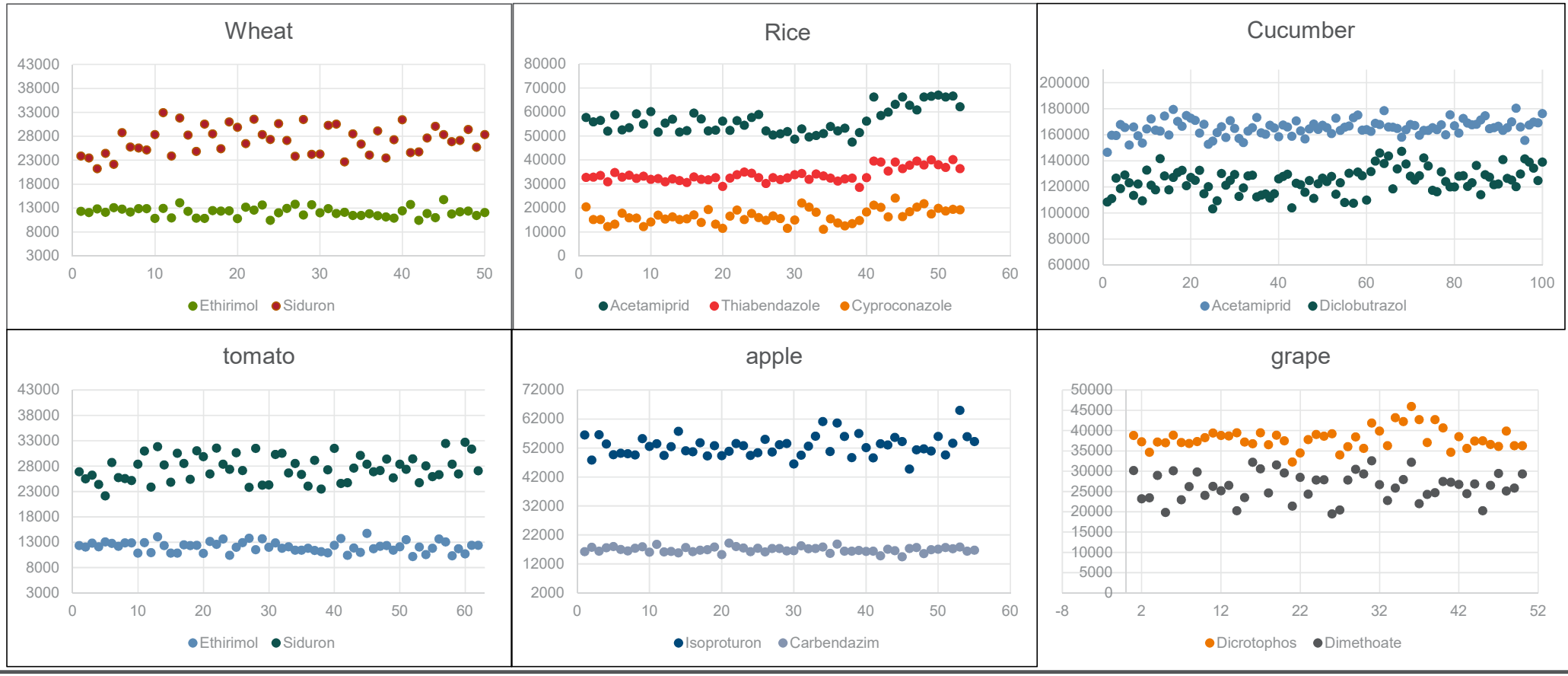
4. Robustness

Average recovery and $RSD_{wR,}$ derived from on-going method validation / verification



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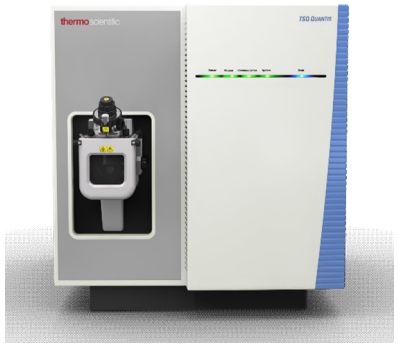
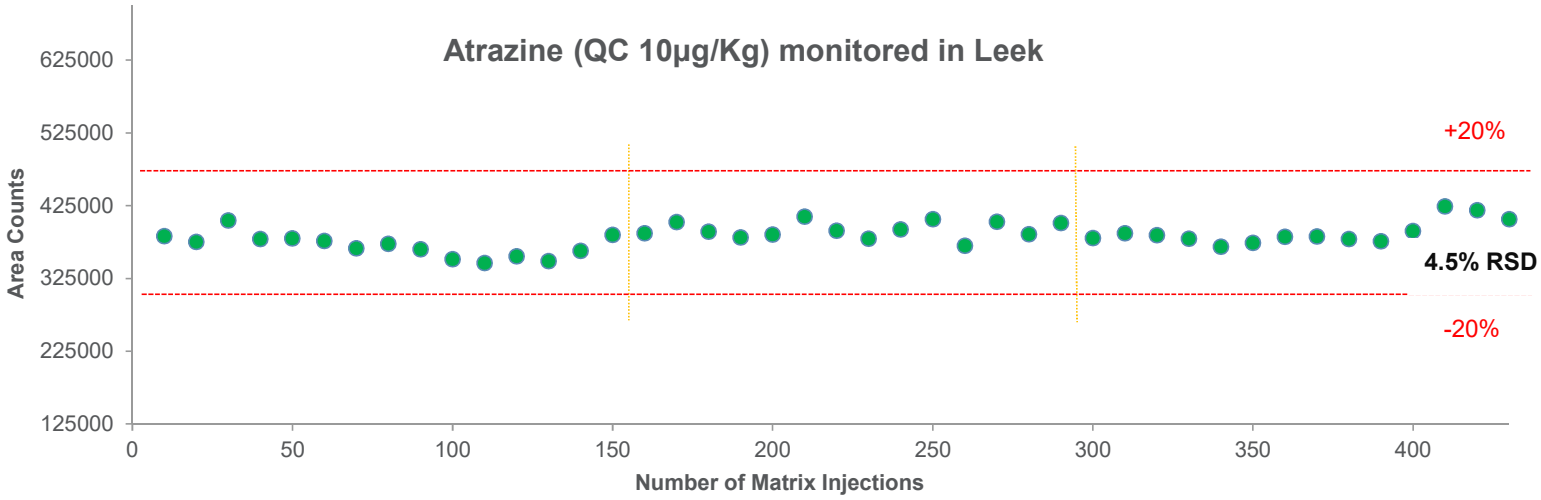
TSQ Quantis MS: Demonstration of Robustness – Food Safety



Atrazine QC monitored in leek for more than 400 injections with 4.5% RSD . Red lines represent ± 20% response at 10 µg/Kg. Yellow lines show the time the system was placed in standby mode for 12h to demonstrate consistent performance after standby period



FOOD SAFETY

Application Note 64971

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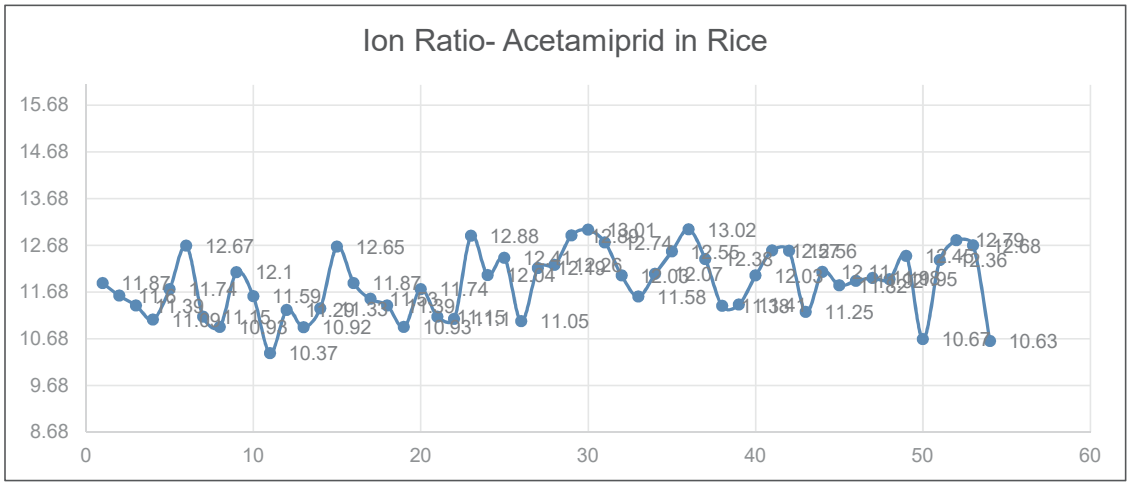
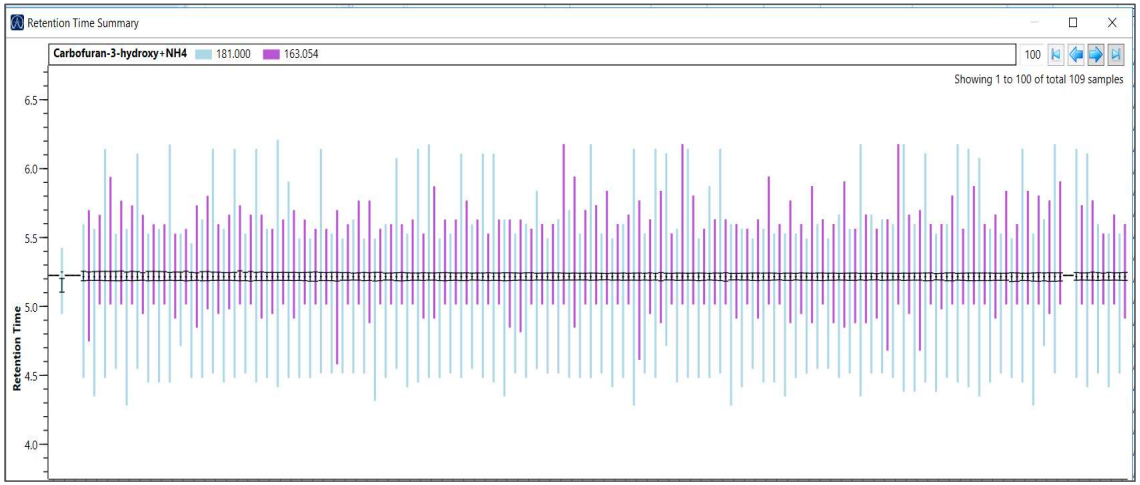
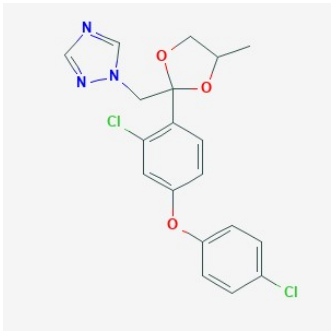
5. Ion ratio, Retention time and Specificity







Response in reagent blank and blank control samples ≤30% of LOQ

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1. Pesticide residues in fresh fruits using LC-MS/MS

Food category- Fruits

Highlights

- LCMSMS- TSQ Quantis
- Linearity-0.0005 to 0.050mg/kg
- LOQ-0.005mg/kg
- Validation as per SANTE/12682/2019 guidelines
- Sample Preparation AOAC QuEChERS 2007.01
- No cleanup, acetonitrile extract dilute-and-shoot method
- Sub-ppb level sensitivity (e.g. 0.001 mg/kg)
- Low cost, simple, sensitive and rugged method
- Number of molecule-160 (multiresidue method)
- Compliance - EU and FSSAI MRLs

Ref: https://www.revbase.com/TagTeam/link.asp?el=79324BC

Sample extraction and cleanup:

- Weigh 15 g homogenized sample into a 50 mL extraction tube.
- For the recovery experiment, spike the samples before the addition of the extraction solvent.
- Add 15 mL of acetonitrile (containing 1% acetic acid).
- Shake vigorously and vortex for 1 minute on a vortex mixer at 2500 rpm.
- Add 6 g anhydrous $MgSO_4$ and 1.5 g sodium acetate to the tube and again mix vigorously for 1 minute on a vortex mixer at 2500 rpm.
- Centrifuge at 5000 rpm for 5 min.
- Dilute the supernatant with water (1:4 ratio, v:v).
- Transfer the extract into an LC vial for instrumental analysis.
- Inject 5 µL of extract into the LC-MS/MS.

Part number- AN73021

Authors
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Customer Solution Center, Ghaziabad,
Thermo Fisher Scientific, India

Keywords
TraceFinder, pesticide residues, grape, apple, QuEChERS, LC-MS/MS, TSQ Quantis

Application benefits

- No cleanup, acetonitrile extract dilute-and-shoot method
- Sub-ppb level sensitivity (e.g. 0.001 mg/kg)
- Low cost, simple, sensitive and rugged method
- Compliance with the EU and FSSAI MRLs

Goal

The objective was to provide an analytical solution for the trace level quantitation of 160 pesticides (parent, isomers and metabolites) in table grapes and in apple using liquid chromatography-tandem mass spectrometry. The optimized method was validated in accordance with the EU SANTE guidelines and further evaluated for compliance with the Food Safety and Standards Authority of India (FSSAI) as well as European Union (EU) MRLs.

Introduction

In India, the commercial cultivation of grapes and apples requires frequent applications of pesticides throughout the growing season to control a variety of pests and diseases. Consequently, the occurrence of pesticide residues is a primary concern for the stakeholders of both crops. The minimization of

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2. Pesticide residues in red chili powder using LC-(HESI)-MS/MS

Food category- Spices

Highlights

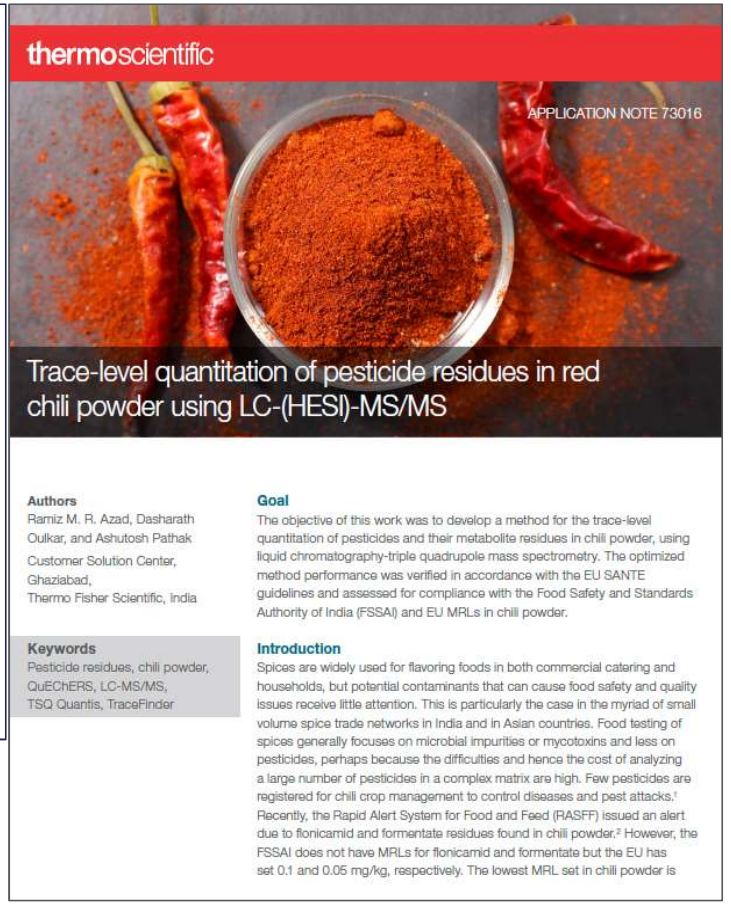
- LCMSMS- TSQ Quantis
- Sample Preparation method- AOAC QuEChERS 2007.01
- Dilute and shoot method- C18 treated acetonitrile extract
- Linearity-0.0001 to 0.050mg/kg
- LOQ 0.005mg/kg
- Validation as per SANTE/12682/2019 guidelines
- Number of molecule-127 (multiresidue method)
- Low cost, simple, sensitive and rugged method
- Compliance - EU and FSSAI MRLs

Ref: https://www.revbase.com/TagTeam/link.asp?el=379D4BE0

Sample extraction:

- Weigh 2 g chilli powder into a 50 mL extraction tube.
- For recovery experiment, spike samples before the addition of water and extraction solvent.
- Add 15 mL of HPLC grade water (containing 1% acetic acid) and leave the sample for 10 min soaking.
- Add 15 mL acetonitrile to the above tube.
- Mix vigorously for 1 minute on a vortex mixer at 2500 rpm.
- Add 6 g anhydrous $MgSO_4$ and 1.5 g sodium acetate to the tube and again mix vigorously for 1 minute on a vortex mixer at 2500 rpm.
- Centrifuge at 5000 rpm for 5 min at ambient conditions.
- Take an aliquot (1 mL) of the acetonitrile supernatant layer.
- Add 50 mg PSA + 7 mg GCB + 150 mg $MgSO_4$.
- Centrifuge at 5000 rpm for 5 min at ambient conditions.
- Take 0.25 mL supernatant and dilute with 0.75 mL of water.
- Inject 5 µL into the LC-MS/MS.

Part number- AN73016



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3. Pesticide residues in rice using LC-(HESI)-MS/MS

Food category- Cereals

Highlights

- LCMSMS- TSQ Quantis
- Sample Preparation method- AOAC QuEChERS 2007.01
- No cleanup, acetonitrile extract dilute-and-shoot method
- Linearity-0.0005 to 0.100mg/kg
- LOQ- 0.005mg/kg
- Number of molecule-145 (multiresidue method)
- Validation as per SANTE/12682/2019 guidelines
- Low cost, simple, sensitive and rugged method
- Compliance - EU and FSSAI MRLs

Ref:https://www.revbase.com/TagTeam/link.asp?el=1D793BAC

Sample extraction:

- Weigh 5 g sample into a 50 mL extraction tube.
- Spike recovery samples before addition of extraction solvent.
- Add 15 mL of HPLC grade water (containing 1% acetic acid) and leave the sample for 10 min soaking.
- Add 15 mL acetonitrile to the tube.
- Shake vigorously for 1 min on a vortex mixer at 2500 rpm.
- Add 6 g anhydrous $MgSO_4$ and 1.5 g sodium acetate to the tube and again mix vigorously for 1 min on a vortex mixer at 2500 rpm.
- Centrifuge with 5000 rpm for 5 min at ambient conditions.
- Filter the extract through a syringe filter and dilute with water (50:50).
- Inject into the LC-MS/MS.

Part number- AN72982

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4. Pesticide residues in rice using LC-(HESI)-MS/MS

Food category- Cereals

Highlights

- LCMSMS- TSQ Quantis
- Sample Preparation method- AOAC QuEChERS 2007.01
- No cleanup, acetonitrile extract dilute-and-shoot method,
- Number of molecule-160 (multiresidue method)
- Linearity-0.0005 to 0.050mg/kg
- LOQ- 0.005mg/kg
- Validation as per SANTE/12682/2019 guidelines
- Low cost, simple, sensitive and rugged method
- Compliance - EU and FSSAI MRLs

Ref: : https://www.revbase.com/TagTeam/link.asp?el=56DF4625

Sample extraction

- Weigh 5 g homogenized sample into a 50 mL extraction tube (Note sample spiking at this step).
- Add 15 mL of HPLC grade water (containing 1% acetic acid) and leave the sample to soak for 10 min.
- Add 15 mL acetonitrile.
- Shake vigorously for 1 minute on a vortex mixer at 2500 rpm.
- Add 6 g anhydrous $MgSO_4$ and 1.5 g sodium acetate to the tube and again mix vigorously for 1 minute on a vortex mixer at 2500 rpm.
- Centrifuge with 5000 rpm for 5 min at ambient conditions.
- Filter the acetonitrile supernatant through a 0.2 µm PTFE membrane filter and dilute with HPLC grade water (1:1) before analyzing by LC-MS/MS.

Part number- AN72961

Authors
Ramiz M.R. Azad and Dasharath Oulkar
Customer Solution Center, Ghaziabad,
Thermo Fisher Scientific, India

Keywords
TraceFinder, pesticide residues, rice, QuEChERS, LC-MS/MS, TSQ Quantis

Goal
The objective of this work was to develop a screening solution followed by quantitation of 160 pesticide residues in rice matrix using liquid chromatography-triple quadrupole mass spectrometry. The optimized method was validated in accordance with the SANTE guidelines and in compliance with the requirements of FSSAI and the European Commission (EC) MRLs.

Introduction
The Central Insecticide Board and Registration Committee (CIBRC) has few chemicals registered for rice.[1] The European Commission (EC) and FSSAI have set the maximum residue levels (MRLs) for many pesticides in rice at 0.01 mg/kg, but the MRLs for fipronil and fipronil sulfone are set at 0.005 mg/kg.[2,3] The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method has been adopted for pesticide residue extraction in most food samples.[4] Additionally, the instrument method plays an important role in delivering accurate and precise results to meet the regulatory requirements.

The aim of this work was the optimization and method validation of a multi-residue method for pesticides in rice by using LC-MS/MS with a Thermo Scientific™ TSQ Quantis™ triple quadrupole mass spectrometer. Sample

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5. Pesticide residues in high water content vegetables using LC-(HESI)-MS/MS

Food category- high water content vegetable

Highlights

- LCMSMS- TSQ Quantis
- Sample Preparation method- AOAC QuEChERS 2007.01
- Matrix match calibration
- Number of molecule-184 (multiresidue method)
- Linearity-0.0005 to 0.1mg/kg
- LOQ- 0.005mg/kg
- Validation as per SANTE/12682/2019 guidelines
- Compliance - EU and FSSAI MRLs

Ref: : https://www.revbase.com/TagTeam/link.asp?el=2AC9E65A

Matrix (µL)	Std stock (µg/mL)	Std volume (µL)	Water (µL)	Final concentration (ng/mL)
250	0.1	5	745	0.5
250	0.1	10	740	1.0
250	0.1	25	725	2.5
250	0.1	50	700	5.0
250	0.1	100	650	10.0
250	1.0	25	725	25.0
250	1.0	50	700	50.0
250	1.0	100	650	100.0

Sample extraction and clean-up

- Homogenized sample (15 g) was weighed into a 50 mL extraction tube.
- For the recovery experiment, the samples were spiked before the addition of an extraction solvent.
- Acetonitrile (containing 1% acetic acid) (15 mL) was added to the tube.
- The tube was shaken vigorously for 1 minute on a vortex mixer at 2500 rpm.
- QuEChERS salts (6 g $MgSO_4$ and 1.5 g sodium acetate) were added to the tube and it was again mixed vigorously for 1 minute on a vortex mixer at 2500 rpm.
- The tube was centrifuged at 5000 rpm for 5 min at room temperature.
- The supernatant (1 mL) was transferred into an Eppendorf tube and 50 mg primary secondary amine (PSA) with 150 mg $MgSO_4$ were added.
- The samples were shaken vigorously and vortexed for 1 min on a vortex mixer at 2500 rpm.
- The samples were centrifuged at 7500 rpm for 5 min.
- The supernatant (0.250 mL) was diluted with 0.75 mL water (1:4 ratio, v/v).
- The diluted extract was transferred into an LC vial for instrumental analysis.

Part number- AN65606

thermo scientific

APPLICATION NOTE 65606

Trace level quantitation of pesticide residues in high water content vegetables using LC-(HESI)-MS/MS

Authors: Ramiz M.R. Azad, and Dasharath Oulkar, Customer Solution Center, Ghaziabad, Thermo Fisher Scientific, India

Keywords: QuEChERS, LC-MS/MS, pesticide residues, cucumber, tomato, TraceFinder, TSQ Quantis

Goal

A simple and sensitive method for trace level quantification of pesticides in high water content vegetables by using LC-MS/MS. The optimized method was validated as per SANTE guidelines and evaluated for the Food Safety Standards Authority of India (FSSAI) as well as European Commission (EC) maximum residue levels (MRLs) compliance in tomato and cucumber.

Introduction

Tomatoes and cucumbers are used in many dishes including salads. To ensure the quality of the products, agrochemicals are used to kill pests, including insects, rodents, fungi, and unwanted plants or weeds[1]. These pesticides must be used safely and disposed of properly due to their toxic effects on humans.

The EC and FSSAI set the MRLs for these chemicals, and their metabolites in tomato and cucumber[2, 3]. The QuEChERS (quick, easy, cheap, effective, rugged, and safe) method is well known for its applicability in simultaneous analysis of many pesticides in a variety of fruits and vegetable samples[4].

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6. Single residue method for dithianon in apple and apple juice using LC-MS/MS

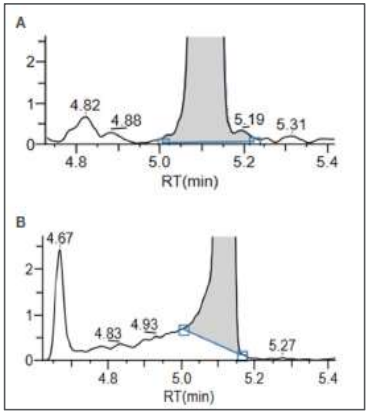
Food category- Fruits

Highlights

- LCMSMS- TSQ Quantis
- Sample Preparation method- AOAC QuEChERS 2007.01
- Number of molecule- 1 (single residue method)
- Linearity-0.0005 to 0.025mg/kg
- LOQ- 0.001mg/kg
- Validation as per SANTE/12682/2019 guidelines
- No competitor reported AN
- Compliance - EU and FSSAI MRLs

Ref: https://www.revbase.com/TagTeam/link.asp?el=76FE53D8

- Weigh 15 g homogenized sample into a 50 mL QuEChERS extraction tube *(Note: for recovery/QC, spiked before extraction).*
- Add 15 mL acetonitrile (containing 1% acetic acid).
- Vortex for 1 minute using a vortex mixer at 2500 rpm (approximately).
- Add QuEChERS 2007.01 salts (6 g $MgSO_4$, 1.5 g sodium acetate) to the tube and vortex for 1 min using a vortex mixer at 2500 rpm (approximately).
- Centrifuge at 4000 rpm for 5 min at ambient temperature.
- Filter 1 mL acetonitrile extract through a PTFE membrane filter into the LC vial.
- Inject 5 µL to LC-MS/MS for analysis.



Part number- AN72953

Authors
Ramiz M.R. Azad, Dasharath Oulkar
Customer Solution Center, Ghaziabad, Thermo Fisher Scientific, India

Keywords
UHPLC, TraceFinder, pesticide residues, apple, apple juice, QuEChERS, LC-MS/MS

Goal
The objective of this work is to develop and deliver a complete solution that can be implemented for routine analysis of dithianon in apple and apple juice by using LC-MS/MS.

Introduction
Apple is the primary fruit crop grown in temperate geographical zones in the northern part of India (Jammu and Kashmir, and Himachal Pradesh). A substantial share of apple production is from these two states only. To meet the current market demand, a large yield of high quality apples is required. Therefore, many chemicals are used to control the diseases and attacks from insect pests. For example, the fungal infections such as apple scab (*Venturia inaequalis*), powdery mildew (Podosphaera leucotricha), and apple canker (*Nectria galligena*) are prevented in their early development phase by use of fungicides.

Dithianon is a broad-spectrum fungicide for the reduction of scab (*Venturia spp.*) on apples and is a registered product for foliar spray applications in grapes, pome fruits, stone fruit, berries, spinach, lettuce, brassica crops, solanaceous crops, and rice.[1] Frequent applications of dithianon can result in residues on the apples, which poses a danger to consumer health. Therefore, the European (EC) and the Food Safety and Standards Authority of India (FSSAI) have set maximum residue limits (MRLs) for dithianon in apple at 3.0 and 0.1 mg/kg, respectively.[2,3]

7. Pesticide residues in leafy vegetables using LC-(H-ESI)-MS/MS

Food category- Leafy vegetables

Highlights

- LCMSMS- TSQ Quantis
- Sample Preparation method- AOAC QuEChERS 2007.01
- Matrix match calibration
- Number of molecule- 181 (multiresidue method)
- Linearity-0.0005 to 0.025mg/kg
- LOQ- 0.001mg/kg
- Validation as per SANTE/12682/2019 guidelines
- Compliance - EU and FSSAI MRLs

- Weigh 15 g homogenized sample into a 50 mL extraction tube
- Add internal standard Triphenyl phosphate (TPP)
- For the recovery experiment, the samples were spiked before the addition of an extraction solvent.
- Add 15 mL of Acetonitrile (containing 1% acetic acid).
- Shake vigorously and vortex for 1 minute on a vortex mixer at 2500 rpm.
- Add salts, i.e., 6 g $MgSO_4$ and 1.5 g Na-acetate to the tube.
- Mix vigorously for 1 minute on a vortex mixer at 2500 rpm.
- Centrifuge with 5000 rpm for 5 min at room temperature.
- Take 1ml of supernatant in the 2 mL Eppendorf tube.
- Add 50 mg PSA with 150 mg $MgSO_4$ and 5 mg GCB
- Shake vigorously and vortex for 1 minute on a vortex mixer at 2500 rpm.
- Centrifuge at 10000 rpm for 5 min.
- Dilute supernatant (0.250 mL) with 0.75 mL water (1:4 ratio, v/v).

AN in designing

Trace level quantitation of pesticide residues in leafy vegetables using LC-(H-ESI)-MS/MS

Ramiz M.R. Azad, Dasharath Oulkar

Customer Solution Center, Ghaziabad, Thermo Fisher Scientific, India

Key Words

TraceFinder, pesticide residues, leafy vegetables, spinach, cabbage QuEChERS, LC-MS/MS, TSQ Quantis, Polarity Switching

Goal

The goal of this project is to demonstrate the performance and versatility of the TSQ Quantis for trace level quantification of multi-residue pesticides in leafy vegetables. The optimized method was validated as per SANTE guideline and evaluated for the Food Safety and Standards Authority of India (FSSAI) as well as European Commission (EC) MRLs compliance in spinach and cabbage.

Introduction

Nowadays, the consumption of green leafy vegetables has been increasing, especially as a counterbalance of the growing number of degenerative diseases. Several bioactive compounds i.e., vitamins, minerals, antioxidants, as well as pigments (chlorophylls and carotenoids) are available in vegetables[1]. Spinach & cabbage is one of the most commonly planted vegetables worldwide. High chlorophyll content makes

8. Quantitation of Chloramphenicol in Honey using LC-(H-ESI)-MS/MS

Food category- Honey

Highlights

- LCMSMS- TSQ Quantis
- Sample Preparation method- In house
- Matrix match calibration
- Number of molecule- 1 (single residue method)
- Linearity-0.005 to 1.00 ug/kg
- LOQ- 0.1ug/kg
- Validation as per SANTE/12682/2019 guidelines
- Compliance - EU and FSSAI MRLs

Sample Extraction

- Weigh 1g sample into a 50-mL extraction tube
- For the recovery experiment, the samples spiked before the addition of the extraction solvent.
- Add 5 mL of HPLC grade water.
- Add 10 mL acetonitrile to the above tube.
- Shake vigorously for 1 minute on a vortex mixer at 2500 rpm.
- Add 1 g anhydrous sodium acetate to the tube and again mix vigorously for 1 minute on a vortex mixer at 2500 rpm.
- Centrifuge with 5000 rpm for 5 min at ambient conditions.
- Evaporate 5 mL supernatant to dryness under N2 gas.
- Reconstitute to 1mL using water: acetonitrile (9:1) and inject to LC-MS/MS.

AN in designing

Quantitation of Chloramphenicol residue in Honey using LC-(H-ESI)-MS/MS

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Key Words

TraceFinder, Chloramphenicol, antibiotic, Honey, LC-MS/MS, TSQ Quantis.

Goal

The objective of this work is to demonstrate the capability of LC-MS/MS (TSQ Quantis) for a trace level quantitation of chloramphenicol residues in honey by using liquid chromatography tandem mass spectrometry. The optimized method was evaluated as per EC 657/2002 guideline and assessed for the compliance of the FSSAI as well as the European Commission (EC) MRLs requirement in honey.

Introduction

Chloramphenicol (CAP) has been used as a broad-spectrum antibiotic in animal and human medicine because of its high bacteriostatic potency. Nowadays, its use has been greatly reduced due to severe side effects. In the EU, chloramphenicol has been banned for use in food-producing animals since 1994. In practical terms, this was achieved by including the ban in Annex IV (i.e., list of pharmacologically active substances for which maximum levels cannot be established) of the European Commission Regulation (EC) 2377/90. The (EU) Regulation 37/2010 includes this prohibition and hence replaces the annex mentioned above to (EEC) 2377/90. The ban is based on suspicion of CAP causing aplastic anemia in humans, as well as on the indication of possible reproductive toxicity. Repeatedly, residues of antibiotics are found in honey, even though these have been banned in the EU. The EU has set Minimum Required Performance Limits (MRPL) of 0.3 ppb. This does not mean that concentrations below these limits are permitted, but it should be zero tolerance. The MRPL is an instruction to the analyst that the method of analysis must be able to achieve this level or below.

The aim of this work was the optimization and method validation of a chloramphenicol analysis in the honey by using LC-MS/MS (Thermo Scientific™ TSQ Quantis). The data acquisition and processing carried out by using Thermo Scientific™ TraceFinder™ software. The optimized method was validated according to the EC 657/2002 guideline[1]. This method was applied to the real samples to demonstrate the application of streamlined workflow in compliance with the EU and FSSAI MRLs requirements.

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9. Pesticides in chicken, lamb, and fish using LC-MS/MS

Food category- Meat

Highlights

- LCMSMS- TSQ Quantis
- Sample Preparation method- In house
- Matrix match calibration
- Number of molecule- 186 (multi residue method)
- Linearity-0.0005 to 0.1 mg/kg
- LOQ- 0.005mg/kg
- Validation as per SANTE/12682/2019 guidelines
- Compliance - EU and FSSAI MRLs

Extraction and Clean-up

- Weigh 5 g homogenized sample into a 50 mL extraction tube
- Add internal standard Triphenyl phosphate (TPP)
- For the recovery experiment, the samples were spiked before the addition of an extraction solvent.
- Add 10ml of water and vortex for 1 minute on a vortex mixer at 2500 rpm
- Add 15mL of Acetonitrile (containing 1% acetic acid).
- Shake vigorously and vortex for 1 minute on a vortex mixer at 2500 rpm.
- Add salts, i.e., 6 g $MgSO_4$ and 1.5 g Na-acetate to the tube.
- Mix vigorously for 1 minute on a vortex mixer at 2500 rpm.
- Centrifuge with 5000 rpm for 5 min at room temperature.
- Take 1ml of supernatant in the 2 mL Eppendorf tube.
- Add 150mg MgSO4, 50mg PSA and 50mg C18 to the tube
- Shake vigorously and vortex for 1 minute on a vortex mixer at 2500 rpm.
- Centrifuge at 10000 rpm for 5 min.
- Dilute supernatant (0.500 mL) with 0.5 mL water (1:1 ratio, v/v).
- Transfer the diluted extract into a LC vial for instrumental analysis.

AN in review

A multi-residue method for detection and quantification of pesticides in chicken, lamb, and fish using LC-MS/MS

Ramiz M.R. Azad, Dasharath Oulkar

Customer Solution Center, Ghaziabad, Thermo Fisher Scientific, India

Key Words

TraceFinder, pesticide residues, fish,chicken,lamb,QuEChERS, LC-MS/MS, TSQQuantis, Polarity Switching

Goal

The goal of this project is to demonstrate the performance and versatility of the TSQ Quantis for trace level quantification of multi-residue pesticides in Lamb, Chicken and Fish. The optimized method will be validated as per SANTE guideline and evaluated for the Food Safety and Standards Authority of India (FSSAI) as well as European Commission (EC) MRLs compliance in Lamb, Chicken and Fish.

Introduction

Meat is an important food that can be contaminated by pesticides. This may also include analytes that exhibit high acute toxicity in humans or animals. Tolerances limits for meat, poultry and some egg products are monitored and enforced by the U.S. Department of Agriculture (USDA). Pest control in intensive

10. A generic approach for simultaneous detection and quantification of pesticides, veterinary drugs and aflatoxin M1 in milk by using LC-MS/MS

Food category- Milk

Highlights

- LCMSMS- TSQ Quantis
- Sample Preparation method- Vet Drug Explorer
- Matrix extracted spiked/procedural standard calibration
- Number of molecule- 246 (multi residue method)
- Linearity-variable according to different MRLs
- LOQ- variable according to different MRLs
- Validation as per SANTE/12682/2019 and EC 657 guidelines
- Compliance - EU and FSSAI MRLs

AN in review

A generic approach for simultaneous detection and quantification of pesticides, veterinary drugs and aflatoxin M1 in milk by using LC-MS/MS

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Key Words

TraceFinder, pesticide residues, veterinary drugss, aflatoxin, milk, LC-MS/MS, TSQ Quantis, polarity switching.

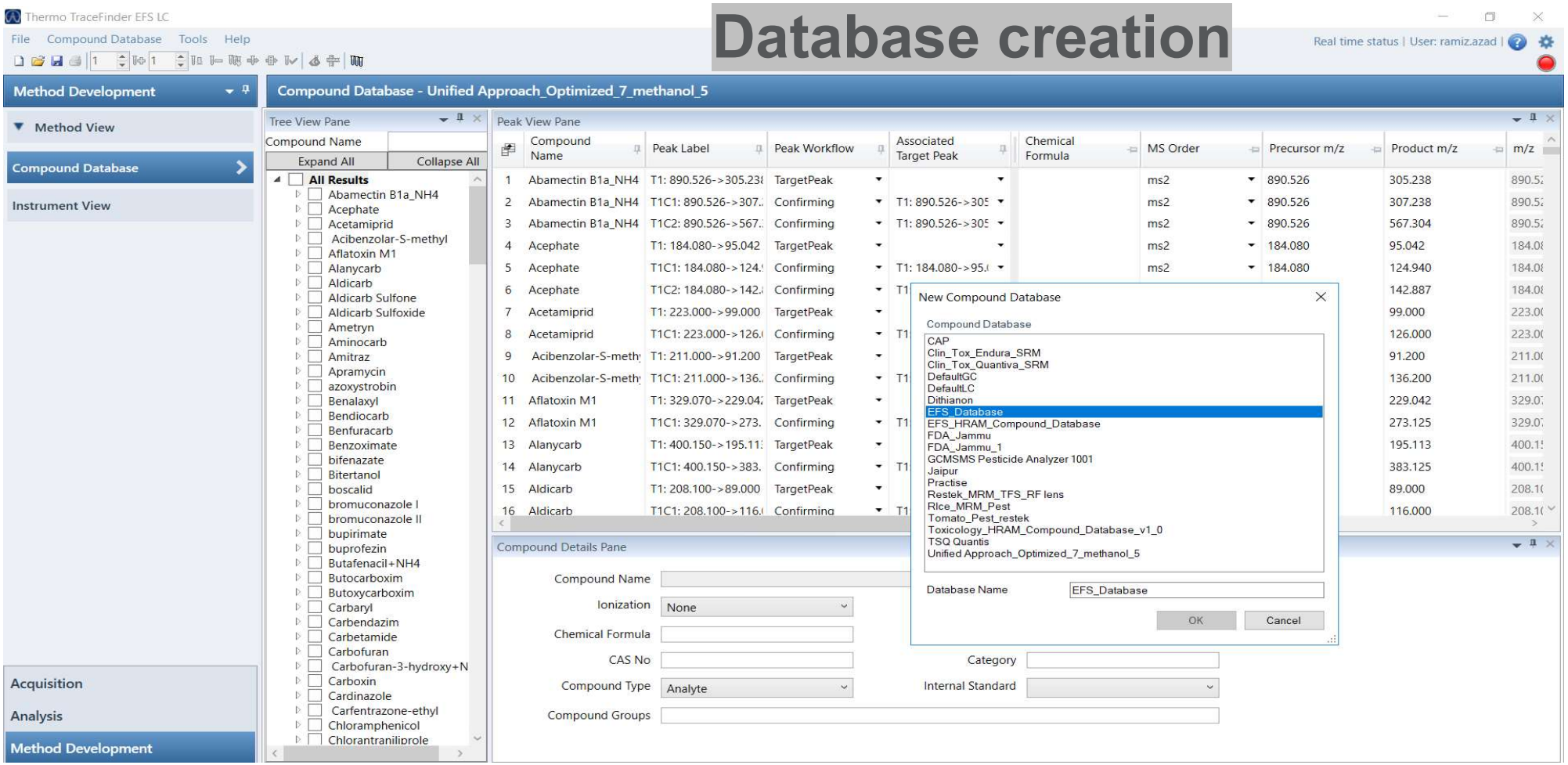
Goal

This study aimed to detect and quantify simultaneously multiclass of pesticides, veterinary drugss, and mycotoxins in milk using liquid chromatography tandem mass spectrometry. The optimized method performance will be verified following SANTE 11813/2018 and EC/657/2002 guidelines.

Introduction

Veterinary drugss are drugs used to treat bacterial infections in animals and control the growth of harmful bacteria. These drugs have been given to farm animals like cows, pigs, and poultry to treat infections or prevent an illness from spreading. Low doses of veterinary drugss are also added to animal feed to promote

Trace Finder Software



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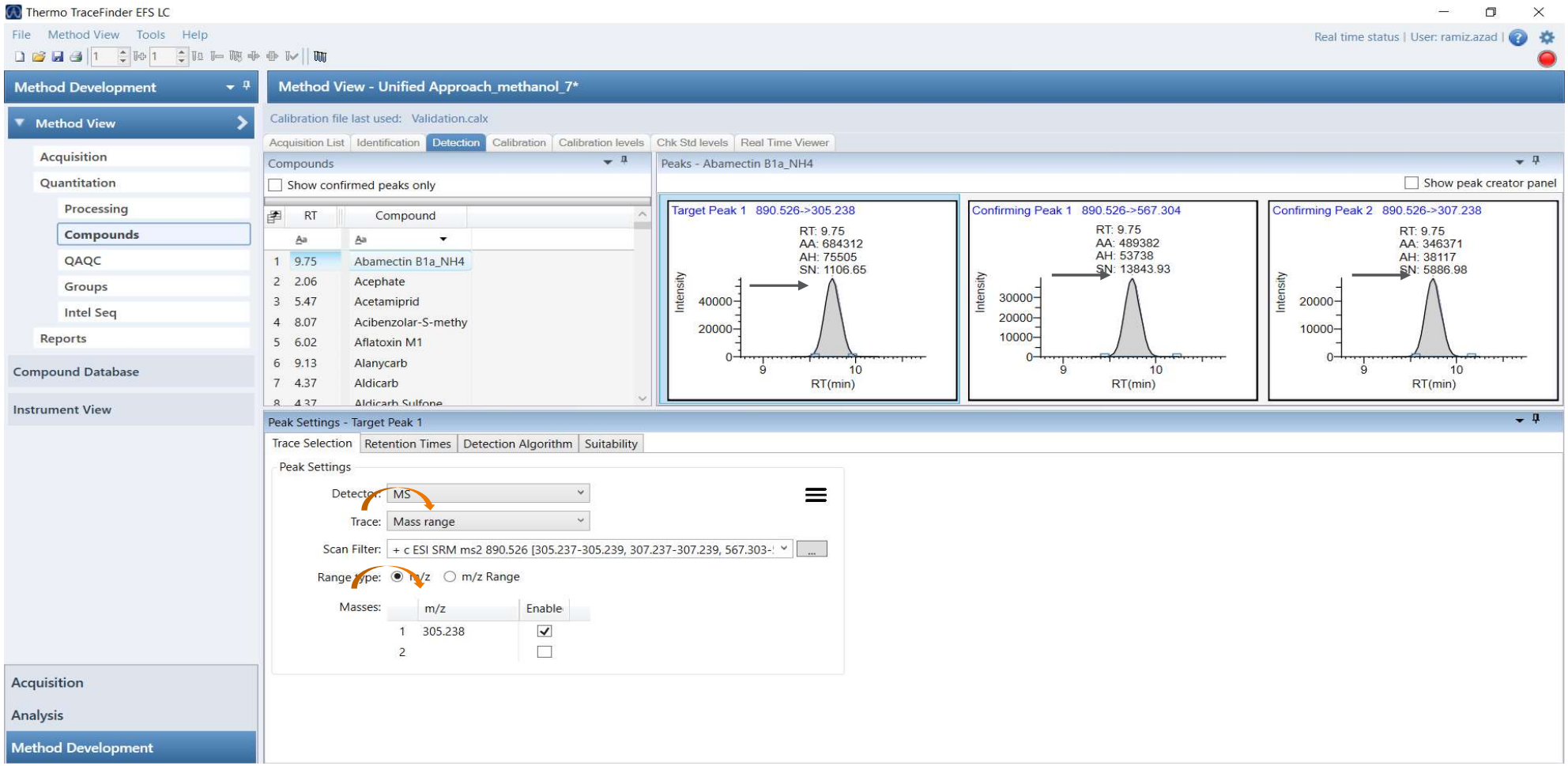
Trace Finder Software



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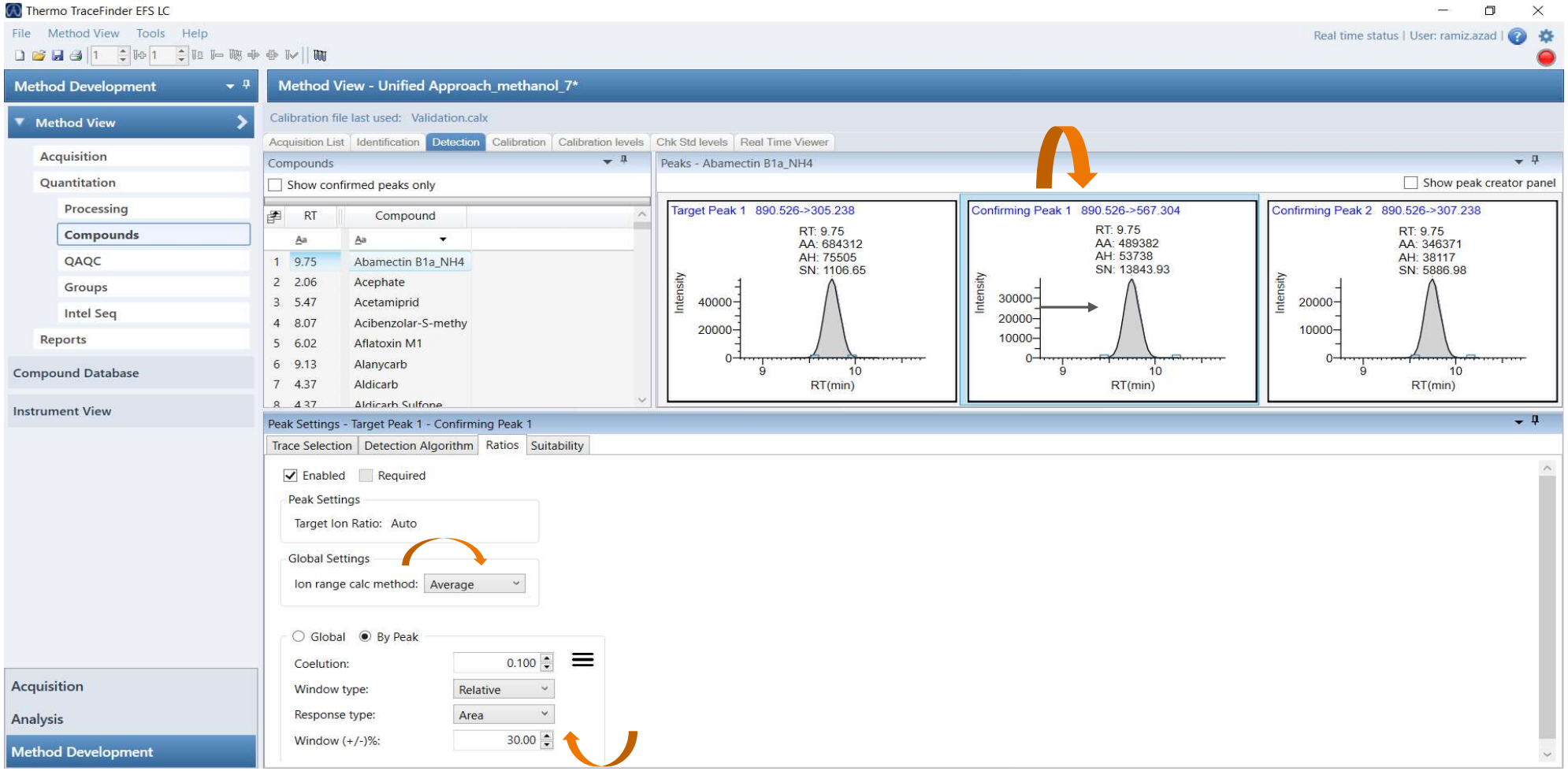
Trace Finder Software



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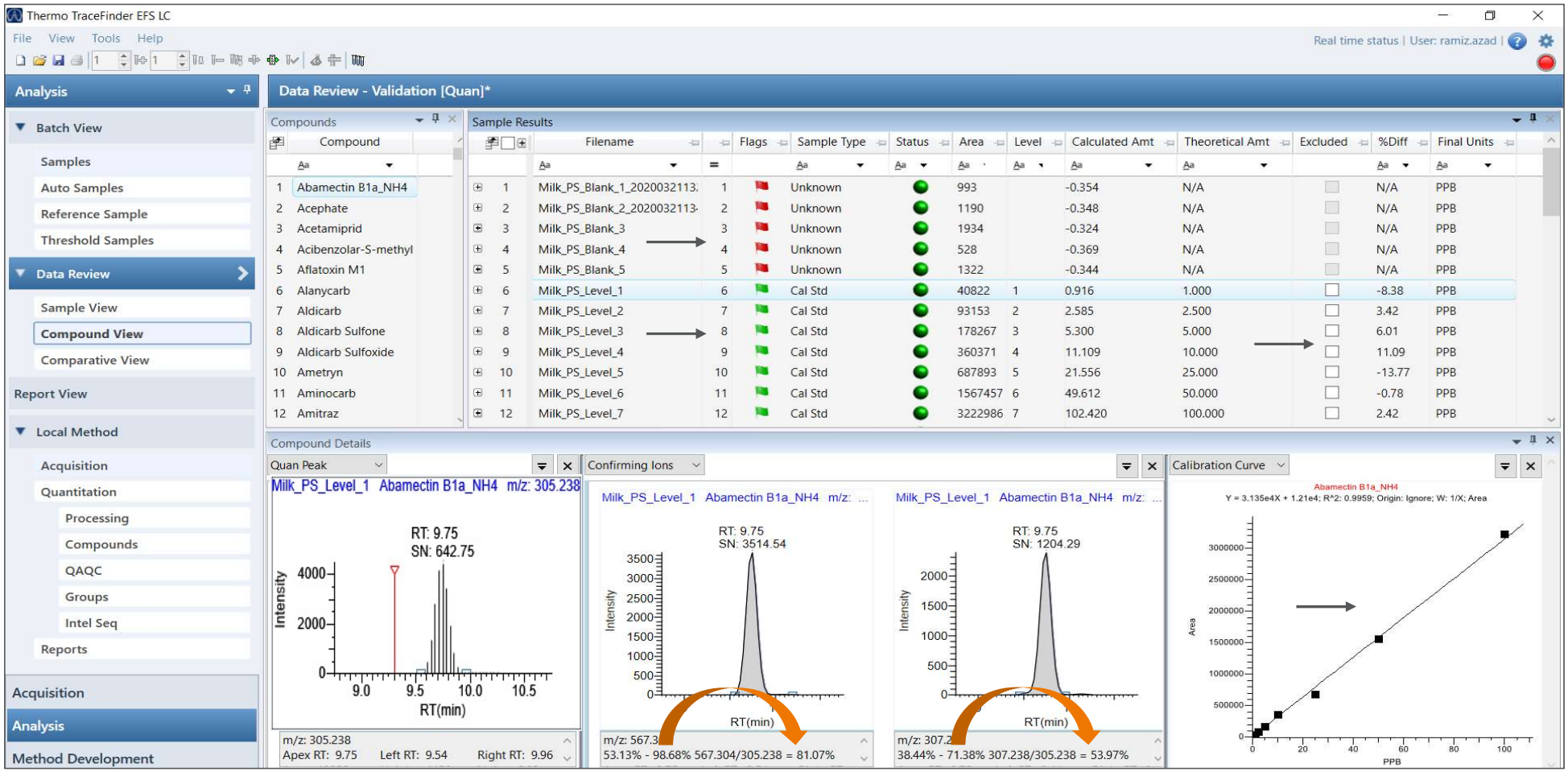
Trace Finder Software



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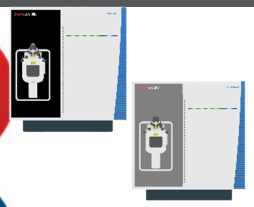
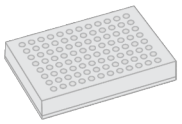
- **Service and Application Support –** Insurance you need at every moment





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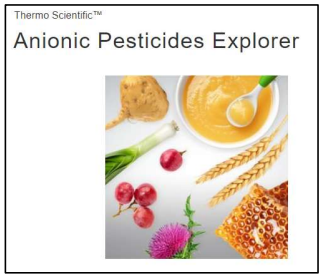
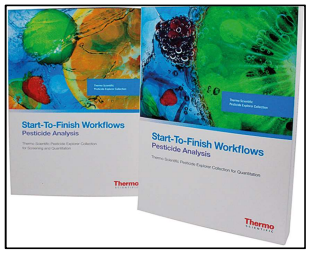
- **Partnership**
  - A collaboration that you can bank on





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