Mass Spectrometry Fundamentals

Samples – Preparation – Separation – Mass Spec Analysis – Informatics – Reporting

Mass Spectrometry Fundamentals
Mass-to-Charge Measurement
Mass Analyzer Types
Scan Modes for Experiments

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MASS SPECTROMETRY FUNDAMENTALS MASS-TO-CHARGE MEASUREMENT AND INSTRUMENTATION

Updated April 2025

General Presentation on Basic Fundamentals of Mass Spectrometry

Overall Workflow Module Considerations

1. **Introduction to Mass Spectrometry Fundamentals**
2. **Components of a Mass Spectrometer**
3. **What is mass-to-charge?**
4. **What is Resolving Power?**
5. **Ionization Techniques (ESI, MALDI, etc.)**
6. **Types of Mass Analyzers (Q, QQQ, TOF, QTOF, IM, FTICR MS, and Orbi)**

MASS SPECTROMETRY FUNDAMENTALS

April 2025

Introduction

MASS SPECTROMETRY FUNDAMENTALS

Mass spectrometry is an important tool for identifying and quantifying specific molecules with high precision.

This sensitive technique detects, identifies, and quantifies molecules based on their **mass-to-charge (m/z) ratio**.

Applications of mass spectrometry range from food quality and safety; to carbon dating; to life sciences; and to clinical research - demonstrating a versatility across many industries.

COMPONENTS OF MASS SPEC

Ion Source

The Ion Source is responsible for “ionizing” the sample. Samples can be prepared in liquid, gas, or dried form. There are serval types of ionization methods, depending on the physiochemistry of the specific molecular class of interest.

Mass Analyzer

The **Mass Analyzer** separates ions based on their **mass-to-charge (m/z) ratio**, allowing for precise measurement of abundance as well. Coupled to ESI, it is a “concentration sensitive” technique. As well, different mass analyzer separate ions with different physics, and have different detector technologies for counting abundance – *Future slides in this slide deck*.

Ion Detector

The Ion Detector counts the number of ions (or transients), providing accurate “counts of ions” for each compound detected in a mass measurement. The ion detectors in mass spectrometers can vary, including electron multipliers, Faraday cups, arrays, etc. which influence sensitivity and detection limits for quantifying compounds.

“Ionization Modes”

Electrospray (ESI)
MALDI
DESI Type
APCI Type

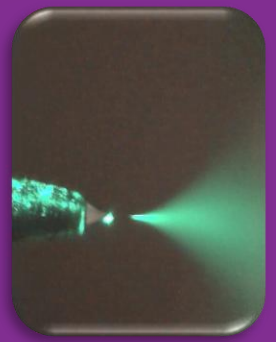
“Mass Analyzers”

Time-of-Flight (TOF)
Ion Mobility (IM)
SLIM, tims
Quadrupole Mass Analyzer (Q)
FTICR MS
Linear Ion Trap (LTQ)
Quadrupole Ion Trap (QIT)

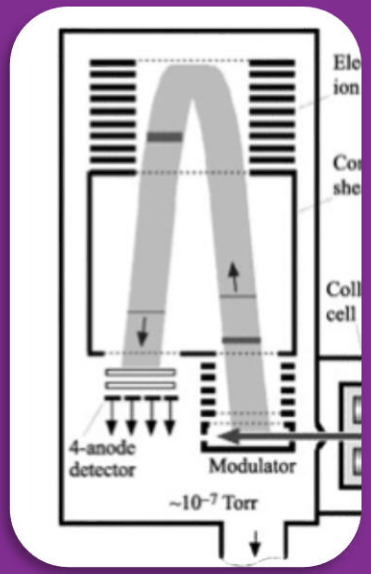
“Example Detectors”

ADC and TDC with ToF
FFT, etc. with ICR MS
Electron Multipliers (EM) with Q, LTQ, and QIT

Electrospray



Time-of-Flight



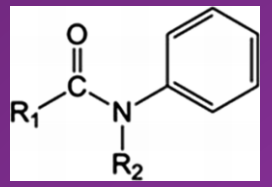
INTRODUCTION TO MASS SPECTROMETRY

Identifying Unknown Compounds

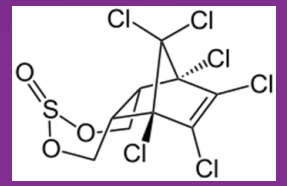
- Mass spectrometry is crucial for identifying unknown compounds, enabling researchers to analyze complex mixtures and determine the presence of specific substances.

Example – Pesticides in Sample?

Carbamates



Endosulfan



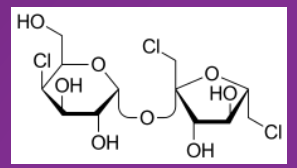
Present in Sample

?

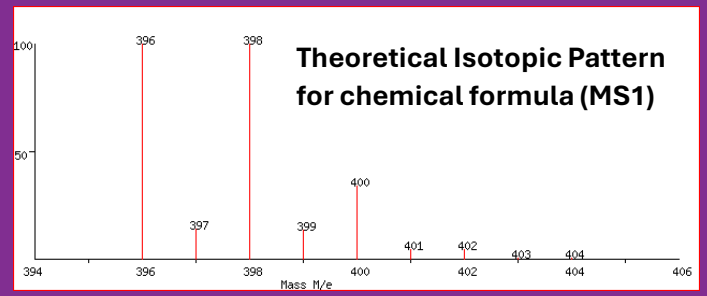
Isotopic Composition Analysis

- It plays a key role in determining the isotopic composition of elements in a molecule, providing insights into molecular structure and origin.

Example Sucralose



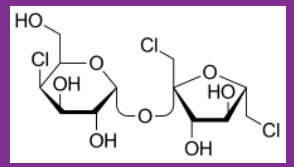
$C_{12}H_{19}Cl_3O_8$



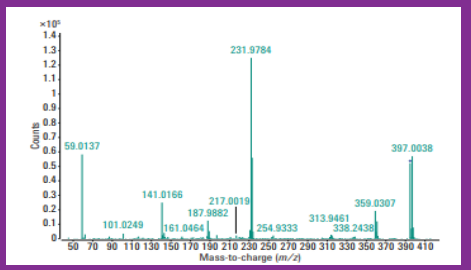
Fragmentation & Structure Determination

- Mass spectrometry helps in determining the structure of a compound based on fragmentation patterns, allowing for detailed structural analysis.

Example Sucralose



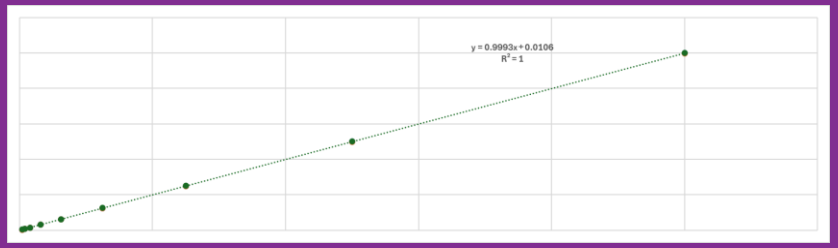
MSMS Fragment with Instrument



Quantifying Compound Amounts

- This technique is essential for quantifying the amount of a compound in a sample, making it invaluable in various scientific fields.

Example – Dilution Curve – Different Methods based on Need for quantification



MASS SPECTROMETRY WORKFLOW - GENERALIZE

- The ion source vaporizes and ionizes samples, creating charged particles.
- The mass analyzer separates ions based on their ***mass-to-charge (m/z) ratio***.
- The ion detector measures the abundance of ions, generating data for analysis.
- Samples can be prepared in liquid, gas, or dried form before analysis.
- The ion source ionizes the sample, allowing for detection and quantification.
- Variations in components allow for different mass spectrometer types and testing options.

- **Ionization Source**
  - Optimize temperature, voltage, and gas flow rates
  - Maximize ion production and minimize fragmentation
- **Mass Analyzer Parameters**
  - Adjust resolution, mass range, and scan speed
  - Achieve desired separation and detection of ions
- **Detector Settings**
  - Optimize detector gain and bias voltage
  - Achieve desired signal-to-noise ratio and sensitivity
- **Compound Optimization (for LC-MS/MS)**
  - Fine-tune precursor/product ions and collision energies
- **Data Acquisition Parameters**

MEASURING MASS-TO-CHARGE OF MOLECULES

- Mass spectrometry is an important tool for identifying and/or quantifying specific compounds or materials with high precision.
- Applications of mass spectrometry range from small molecules to much larger molecules like proteins.
- Applications of mass spectrometry range from food quality, environmental health, diagnostic testing, biomedical, and clinical research.
- This sensitive technique detects, identifies, and quantifies molecules based on the:

  mass-to-charge (m/z) ratio and relative response factor
- Thus – Must **"Ionize"** the Sample for LC MS Analysis
- ***Liquid(or Solid)-to-Gas Phase so that the instrument can measure the m/z ratio.***

Base Considerations for a Liquid Chromatography Mass Spectrometry (LC MS) Experiments

Sample Solubility

Choose a solvent that effectively dissolves your analyte.

Instrument Compatibility

Ensure the solvent is compatible with your ESI source and mass spectrometer.

Ionization Efficiency

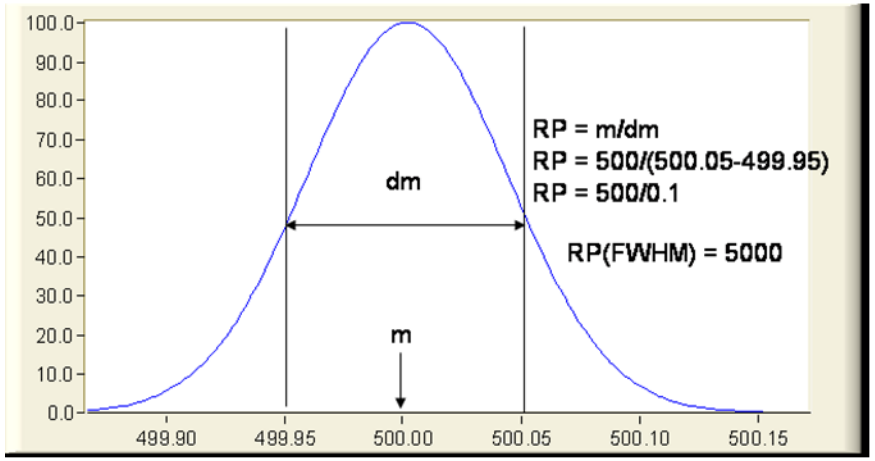
Some solvents may be better than others for specific analytes, impacting ionization efficiency.

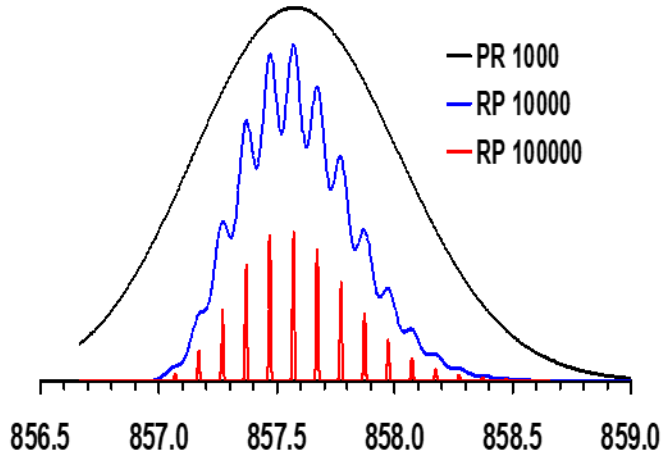
Compatibility with Other Techniques

If you are using ESI in conjunction with other techniques like LC, consider solvent compatibility with the chromatography method.

MASS SPECTROMETRY CONCEPTS – RESOLVING POWER

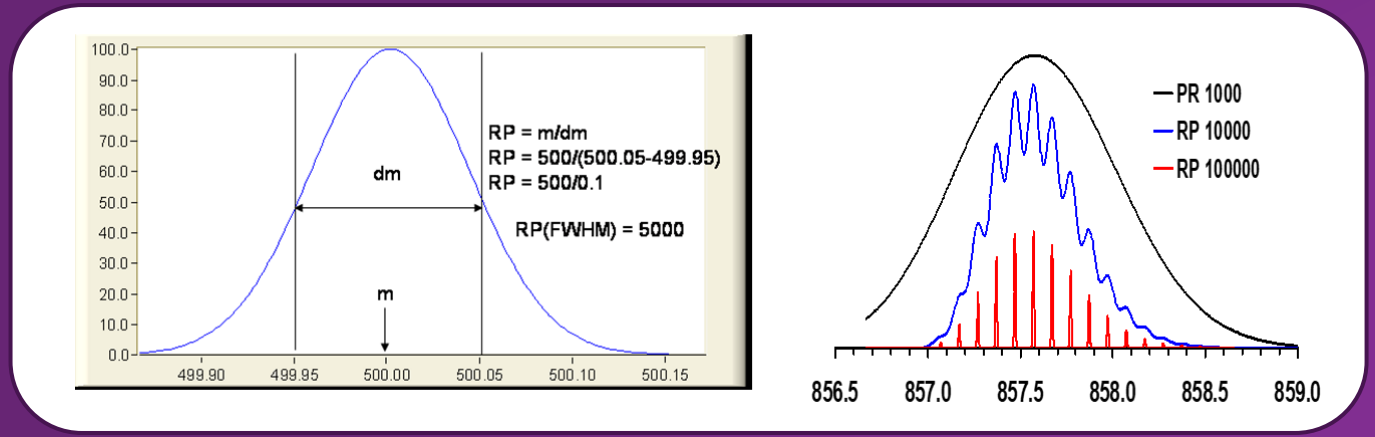
Mass spectrometers may feature diverse **mass analyzers** like *quadrupole mass filter (Q), time-of-flight (TOF), ion trap (IT), FT based, or ion mobility (IM)* each providing distinct resolution and accuracy for mass measurements. Hybrid instrumentation (combination of multiple analyzers) have improved the last 25 years.





Resolving Power Calculations with Mass Spectrometry Ubiquitin (10+ Charge State)
8560.62 (C_{378} H_{630} N_{105} O_{118} S_1)
[Note: in right figure – RP = Resolving Power]

MASS SPECTROMETRY CONCEPTS – RESOLVING POWER



Definition: Resolving power in mass spectrometry describes the instrument's ability to distinguish between ions with very similar mass-to-charge ratios (m/z). It quantifies how well two adjacent peaks in a mass spectrum can be separated.

Mathematical Expression: Resolving power (R) is often expressed as $R = m/\Delta m$, where 'm' is the nominal m/z of the ion and 'Δm' is the smallest difference in m/z between two peaks that can be distinguished (according to a specific definition, e.g., baseline separation or a certain percentage valley).

Importance: Higher resolving power is crucial for analyzing complex mixtures, identifying isobaric compounds (molecules with the same nominal mass but different exact masses), and accurately determining the mass of ions, which can aid in elemental composition determination.

Instrument Dependence: Different types of mass analyzers exhibit varying degrees of resolving power. For example, Fourier Transform-based instruments (FT-ICR, Orbitrap) typically offer much higher resolving power compared to quadrupole or time-of-flight (TOF) instruments (though high-resolution TOF instruments exist).

IONIZATION FOR MS ANALYSIS
ESI, MALDI, APCI, DESI

MASS SPECTROMETRY CONCEPTS – IONIZATION - ESI

ESI - One of the most widely used ionization methods for a wide variety of sample and molecule types

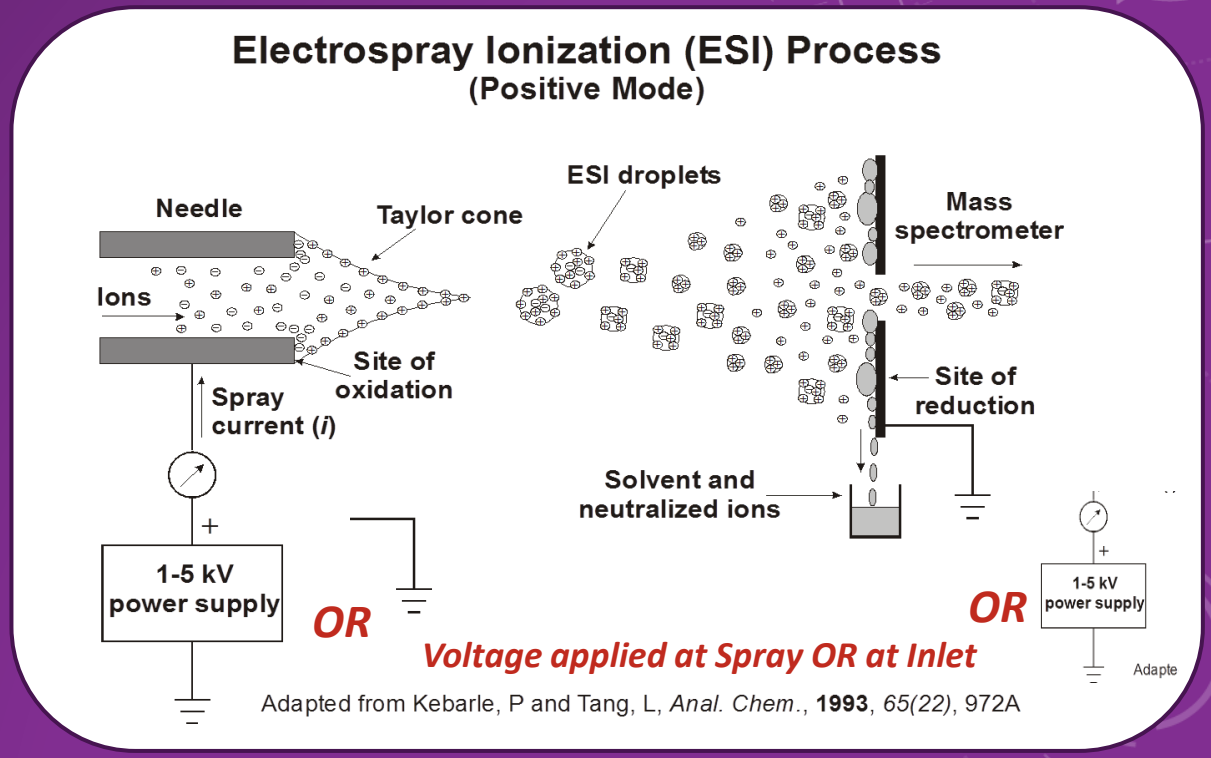
Different mass spectrometers utilize various ion sources such as electron impact, atmospheric chemical ionization (APCI), matrix assisted laser desorption ionization (MALDI) or **electrospray ionization (ESI)**, which affect the ionization efficiency and sample types.

Generally speaking, there are currently three "flow rates" associated with instrumentation, based on sample type and column inner diameters used for said sample type.

Standard Flow – 500 uL/min to 250 ul/min

Micro Flow – 50 to 1uL/min

Nano Flow – 500nl/min to 5 nL/min



MASS SPECTROMETRY CONCEPTS – IONIZATION - ESI

ESI - One of the most widely used ionization methods for a wide variety of sample and molecule types

- Sample Solubility

Choose a solvent that effectively dissolves your analyte.

- Instrument Compatibility

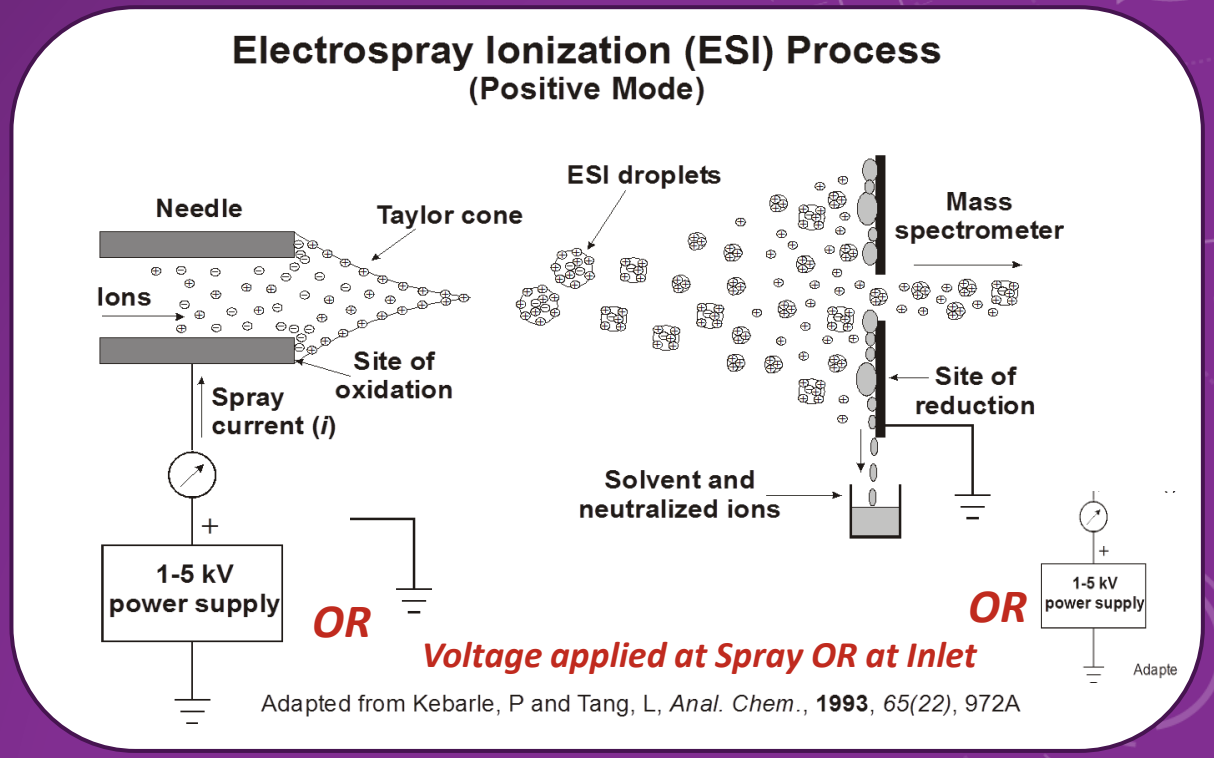
Ensure the solvent is compatible with your ESI source and mass spectrometer.

- Ionization Efficiency

Some solvents may be better than others for specific analytes, impacting ionization efficiency.

- Compatibility with Other Techniques

If you are using ESI in conjunction with other techniques like LC, consider solvent compatibility with the chromatography method.

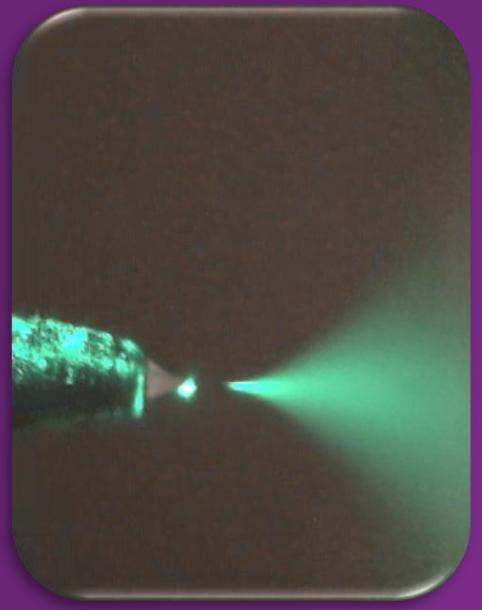


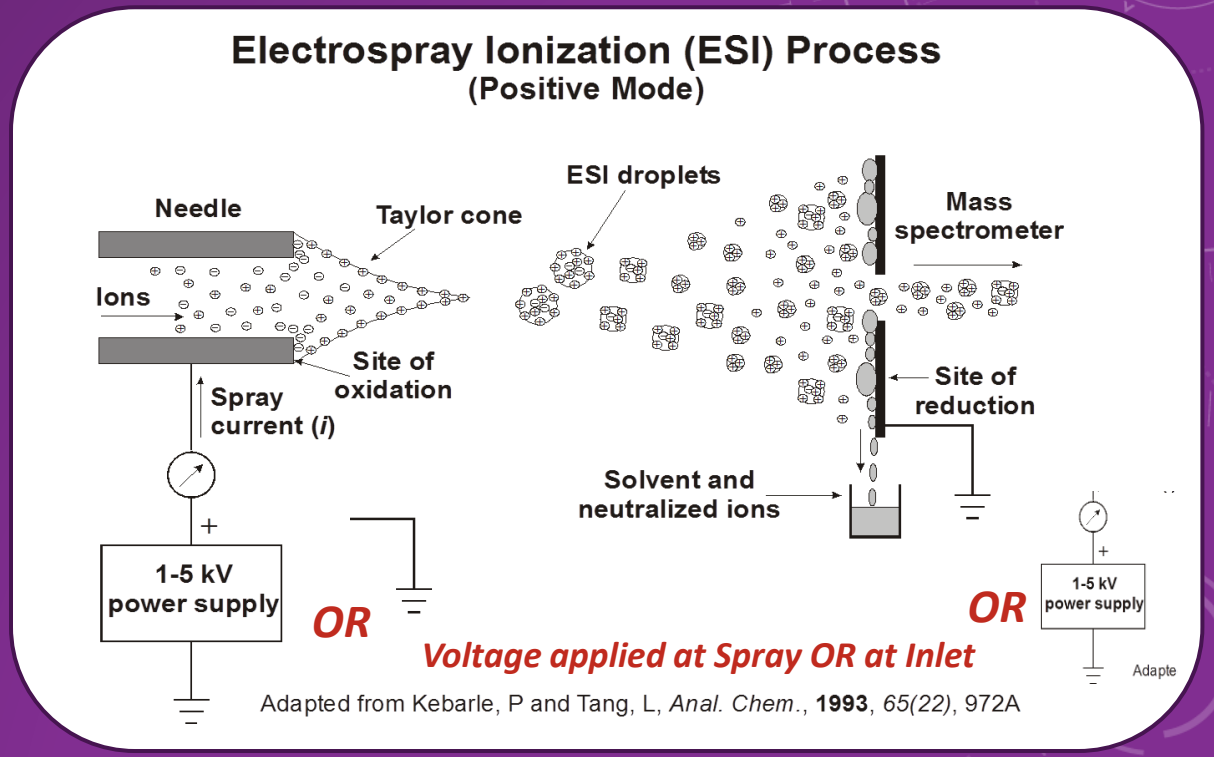
MASS SPECTROMETRY CONCEPTS – IONIZATION - ESI

ESI - One of the most widely used ionization methods for a wide variety of sample and molecule types

Concentration Sensitive Technique

Image - Electrospray Tailor Cone





Electrospray is a competition for charge during the ionization process. Complex Samples can create ion suppression (and sometimes enhancement). The ionization process is also very sensitive to salts in samples and the solvents used. Non-organic salts can suppress ionization of desired analytes. Modifiers such as formic acid, acetic acid, ammonium formate, etc. can enhance (or suppress) ionization. As well, the dynamic range of analytes may be affected is there are highly abundant molecules.

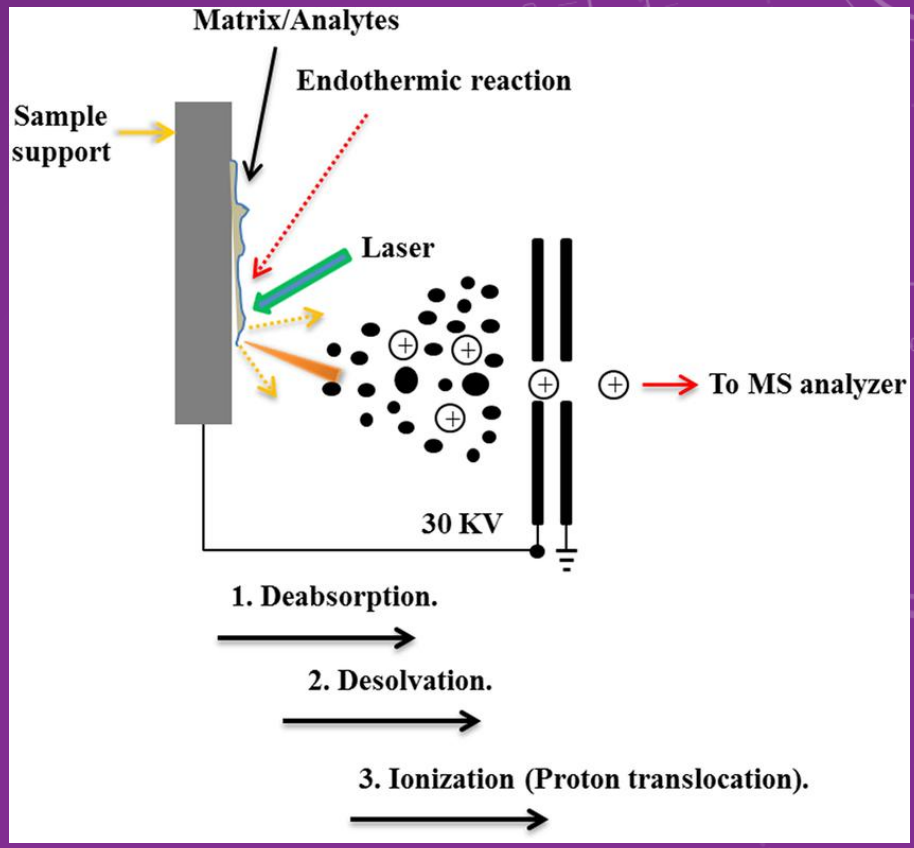
MASS SPECTROMETRY CONCEPTS – IONIZATION - MALDI

Matrix-Assisted Laser Desorption/Ionization (MALDI)

Matrix-Assisted Laser Desorption/Ionization (MALDI) is a soft ionization technique used in mass spectrometry, particularly well-suited for the analysis of large biomolecules like proteins, peptides, polymers, and oligonucleotides. It involves embedding the analyte in a matrix compound and then using a laser to induce desorption and ionization.

MALDI has found great interest and usage in imaging applications – displacing other similar detection needs, from other fields outside of normal MassSpec research.

MALDI



https://www.researchgate.net/publication/336750230_Advances_in_MS_Based_Strategies_for_Probing_Ligand-Target_Interactions_Focus_on_Soft_Ionization_Mass_Spectrometric_Techniques

MASS SPECTROMETRY CONCEPTS – IONIZATION - MALDI

How MALDI Works - Generally:

1. **Sample Preparation:** The analyte is dissolved in a suitable solvent and mixed with a solution of a matrix compound. The matrix is typically a small organic molecule that strongly absorbs light at a specific laser wavelength.
2. **Crystallization:** A small aliquot of the analyte-matrix mixture is deposited onto a MALDI target plate (usually metal). As the solvent evaporates, the analyte molecules become co-crystallized within the matrix. This isolates the analyte molecules from each other, preventing aggregation and fragmentation during the ionization process.
3. **Laser Irradiation:** The sample spot on the target plate is irradiated with a pulsed laser beam at a wavelength that is strongly absorbed by the matrix.
4. **Desorption and Ionization:** The matrix molecules rapidly absorb the laser energy, leading to rapid heating and sublimation (desorption) of the matrix along with the embedded analyte molecules into the gas phase. Simultaneously, ionization of the analyte occurs through various mechanisms, including:
    - **Proton transfer:** The matrix molecules, often acidic or basic, can transfer protons (H^+) to or abstract protons from the analyte molecules, creating singly or multiply charged ions (e.g., $[M+H]^+$, $[M-H]^-$).
    - **Adduct formation:** Analyte ions can also form adducts with alkali metal ions (e.g., Na^+, K^+) present as impurities in the matrix or sample.
5. **Ion Extraction and Analysis:** The generated gas-phase ions are then extracted from the ion source region by an electric field and directed into the mass analyzer (most commonly a Time-of-Flight (TOF) analyzer due to its suitability for high-mass ions).

MASS SPECTROMETRY CONCEPTS – IONIZATION - MALDI

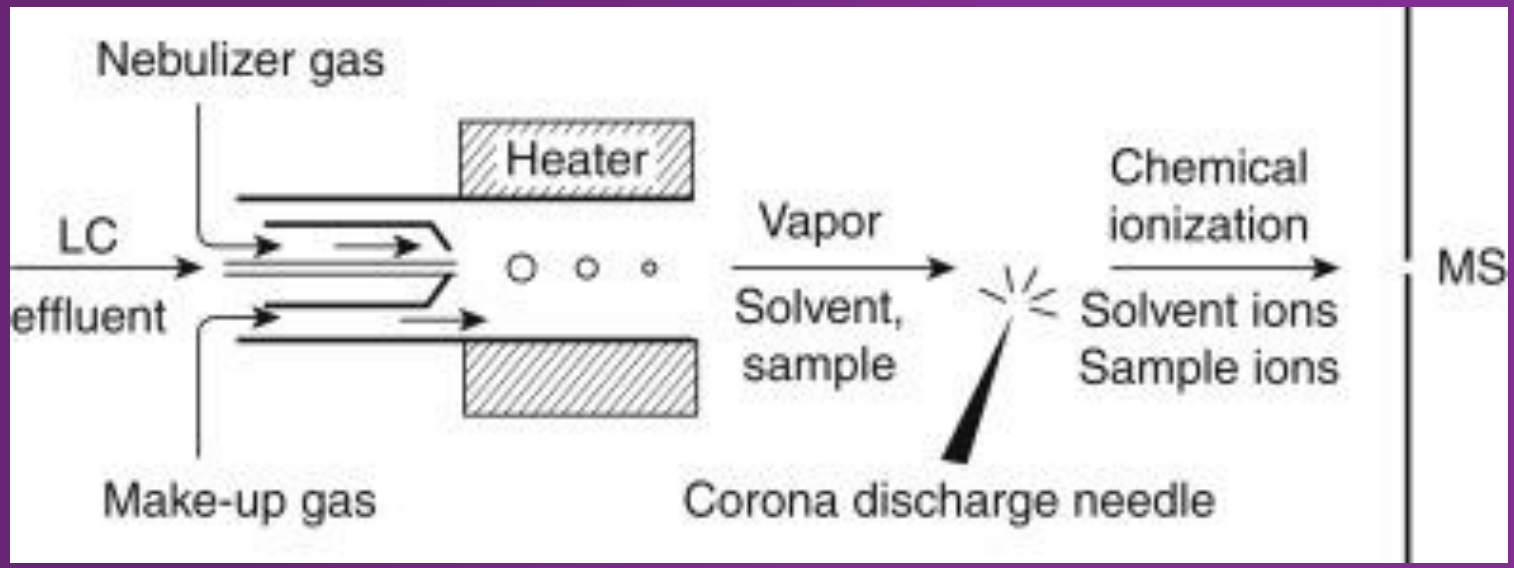
- **Soft Ionization:** MALDI is considered a "soft" ionization technique because it typically imparts low internal energy to the analyte molecules, minimizing fragmentation and allowing for the intact molecular ion to be observed. This is crucial for analyzing fragile biomolecules.
- **Matrix Dependency:** The choice of matrix is critical for successful MALDI analysis. The matrix must:
  - Strongly absorb the laser wavelength.
  - Co-crystallize well with the analyte.
  - Facilitate efficient desorption and ionization of the analyte.
  - Have a low mass to avoid interfering with the analysis of low-mass analytes.
- **High Mass Range:** MALDI is particularly effective for ionizing and analyzing large molecules with masses exceeding hundreds of thousands of Daltons, making it ideal for proteomics, polymer analysis, and other macromolecular studies.
- **Pulsed Technique:** MALDI is inherently a pulsed ionization method due to the use of a pulsed laser. This makes it well-suited for coupling with time-of-flight (TOF) mass analyzers, which also operate in a pulsed manner, allowing for efficient and accurate mass measurements.
- **Surface Technique:** MALDI involves analyzing the sample from a solid surface, which can be advantageous for certain types of samples and sample preparation methods.
- **Sensitivity:** MALDI can offer good sensitivity, particularly for higher molecular weight analytes.
- **Tolerance to Salts and Buffers:** Compared to some other ionization techniques like electrospray ionization (ESI), MALDI is generally more tolerant to the presence of salts and buffers in the sample, although careful sample preparation is still important.

MASS SPECTROMETRY CONCEPTS – IONIZATION - APCI

Atmospheric Pressure Chemical Ionization (APCI)

Atmospheric Pressure Chemical Ionization (APCI) is a soft ionization technique used in mass spectrometry that operates at atmospheric pressure. It is particularly well-suited for the analysis of relatively **non-polar** to moderately polar, thermally stable compounds with molecular weights typically up to around 1500 Da.

Sample is super heated, then chemical ionization with a corona discharge needle.



https://www.sciencedirect.com/topics/chemistry/atmospheric-pressure-chemical-ionization-mass-spectrometry

MASS SPECTROMETRY CONCEPTS – IONIZATION - APCI

How APCI Works:

1. **Sample Introduction:** The liquid sample is introduced into the APCI source, often via a heated nebulizer. The nebulizer creates a fine spray of the liquid eluent, which is then vaporized by a heated gas stream (e.g., nitrogen).
2. **Corona Discharge:** The vaporized sample passes through a region where a sharp needle or electrode maintained at a high voltage (corona discharge) creates a plasma. This plasma ionizes the surrounding gas molecules (primarily the nebulizing gas and solvent vapor) to form stable reagent ions. Common reagent ions include protonated solvent molecules (e.g., $[M+H]^+$ where M is methanol or water) or their clusters.
3. **Ion-Molecule Reactions:** The gas-phase reagent ions then collide and react with the neutral analyte molecules exiting the nebulizer. Ionization of the analyte typically occurs through proton transfer (H^+), adduct formation, or charge transfer, depending on the chemical properties of the analyte and the reagent ions.
    - **Positive Ion Mode:** Reagent ions like protonated solvent molecules ($[SH]^+$) can transfer a proton to the analyte (A) to form protonated analyte ions ($[A+H]^+$): $[SH]^+ + A \rightarrow [A+H]^+ + S$
    - **Negative Ion Mode:** Reagent ions can abstract a proton from the analyte or form adducts with it to create negative ions (e.g., $[A-H]^-$ or $[A+X]^-$).
4. **Ion Sampling:** The newly formed analyte ions are then sampled into the vacuum system of the mass analyzer through a small orifice or a series of skimmers.
5. **Mass Analysis and Detection:** Once inside the mass analyzer (e.g., quadrupole, time-of-flight, ion trap), the ions are separated based on their m/z ratio and detected, generating a mass spectrum.

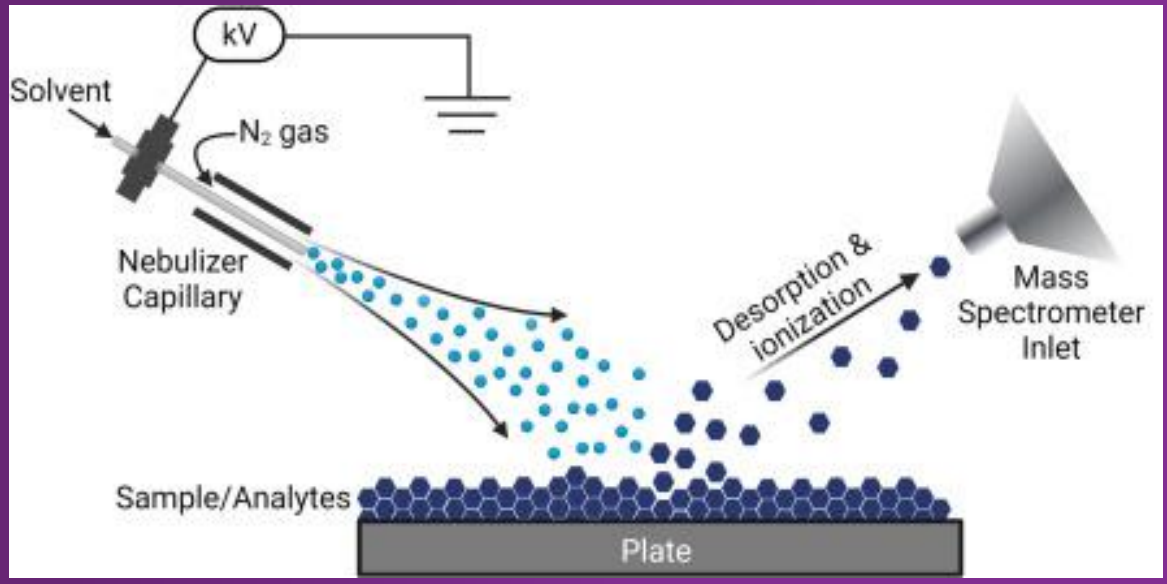
MASS SPECTROMETRY CONCEPTS – IONIZATION - APCI

- **Atmospheric Pressure Operation:** APCI occurs at atmospheric pressure, simplifying the interface with liquid chromatography (LC) systems, as the eluent does not need to be desolvated under vacuum before ionization.
- **Suitable for Thermally Stable Compounds:** The ionization process in APCI is relatively gentle, making it suitable for thermally labile compounds that might decompose under higher temperature ionization techniques like electron ionization (EI). However, the initial vaporization step still requires some thermal stability.
- **Polarity Range:** APCI is effective for a range of compounds with moderate polarity, generally less polar than those best suited for electrospray ionization (ESI). It works well for many pharmaceuticals, pesticides, and environmental contaminants.
- **Gas-Phase Ionization:** Ionization occurs in the gas phase through chemical reactions between reagent ions and the analyte, leading to relatively simple spectra, often dominated by the protonated or deprotonated molecular ion.
- **Solvent Dependent:** The choice of solvent in the liquid chromatography system significantly influences the reagent ions formed and thus the ionization efficiency of the analyte. Method development often involves optimizing the solvent system.
- **Corona Discharge:** The use of a corona discharge creates a robust and stable source of primary ions, making APCI a reliable ionization technique.
- **Interface with HPLC:** APCI is widely used as an interface with High-Performance Liquid Chromatography (HPLC) due to its atmospheric pressure operation and compatibility with typical LC flow rates and solvents.
- **Lower Sensitivity for Very High Molecular Weight Compounds:** APCI is generally less effective for very large biomolecules (above ~1500 Da) compared to techniques like ESI or MALDI

MASS SPECTROMETRY CONCEPTS – IONIZATION - DESI

Desorption Electrospray Ionization (DESI)

Desorption Electrospray Ionization (DESI) is an ambient ionization technique in mass spectrometry that allows for the direct analysis of samples under atmospheric conditions with minimal to no sample preparation. It achieves ionization by spraying a charged solvent stream onto a surface, which then desorbs and ionizes the analytes present.



https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/desorption-electrospray-ionization

MASS SPECTROMETRY CONCEPTS – IONIZATION - DESI

- **Ambient Ionization:** DESI operates at atmospheric pressure and room temperature, eliminating the need for vacuum conditions in the ionization source and allowing for the direct analysis of samples in their native environment.
- **Minimal Sample Preparation:** One of the key advantages of DESI is that it often requires little to no sample preparation. Samples can be analyzed directly as they are, whether they are solids, liquids, or even biological tissues.
- **Surface Analysis Technique:** DESI is primarily a surface analysis technique, providing information about the chemical composition of the outermost layers of a sample. It can be used for both localized spot analysis and imaging by rastering the solvent spray across the surface.
- **Soft Ionization:** Similar to ESI, DESI is a soft ionization technique, typically producing predominantly molecular ions (e.g., $[M+H]^+$, $[M-H]^-$) with minimal fragmentation, making it suitable for identifying intact molecules.

MASS SPECTROMETRY CONCEPTS – IONIZATION - DESI

How DESI Works:

1. **Charged Solvent Spray:** A high-velocity stream of electrically charged solvent droplets is directed at the sample surface at an angle. This spray is typically generated using electrospray ionization (ESI) principles, but directed towards a surface instead of directly into the mass spectrometer inlet.
2. **Surface Interaction:** The charged solvent droplets impact the sample surface, creating a thin film or extracting analytes into secondary microdroplets through a "splashing" or "droplet pick-up" mechanism.
3. **Analyte Desorption and Ionization:** As the secondary microdroplets are ejected from the surface, the solvent evaporates, and the analytes within them become ionized, similar to the processes in conventional ESI (e.g., protonation, deprotonation, adduct formation).
4. **Ion Transfer to Mass Spectrometer:** The gas-phase ions are then directed into the mass spectrometer inlet, which is positioned a short distance away from the sample surface. This transfer often occurs through air at atmospheric pressure.
5. **Mass Analysis and Detection:** Once inside the mass analyzer, the ions are separated based on their m/z ratio and detected, generating a mass spectrum of the surface components.

MASS SPECTROMETRY CONCEPTS – IONIZATION

Notes on Solvents and Ionization – Electrospray Ionization – with LC ESI MS

Compatible Solvents and Organic Salts for LC-ESI-MS

Liquid Chromatography (LC) coupled with Electrospray Ionization Mass Spectrometry (ESI-MS) is a powerful analytical technique. However, the compatibility of the mobile phase components, including solvents and salts, with both the LC system and the ESI source is crucial for optimal performance and data quality.

Compatible Solvents:

ESI is a "soft" ionization technique that relies on the formation of charged droplets from the liquid eluent, followed by solvent evaporation and Coulombic explosion to generate gas-phase ions. Therefore, **volatile solvents** are essential for efficient droplet formation and desolvation.

Preferred solvents for ESI MS.

- **Water (H_2O):** A fundamental component, especially in reversed-phase LC. High purity is crucial (LC-MS grade).
- **Methanol (MeOH):** A good organic modifier, often used in reversed-phase and hydrophilic interaction chromatography (HILIC). It enhances ionization for many compounds.
- **Acetonitrile (MeCN):** Another popular organic modifier, particularly in reversed-phase LC. It generally leads to better sensitivity in ESI compared to methanol for some analytes and can promote desolvation.
- **Isopropanol (IPA):** Sometimes used to improve the solubility of hydrophobic compounds or to modify ionization efficiency. Higher viscosity compared to MeCN or MeOH might require adjustments to LC flow rates.

MASS SPECTROMETRY CONCEPTS – IONIZATION

Notes on Solvents and Ionization – Electrospray Ionization – with LC ESI MS

Compatible Solvents and Organic Salts for LC-ESI-MS

Liquid Chromatography (LC) coupled with Electrospray Ionization Mass Spectrometry (ESI-MS) is a powerful analytical technique. However, the compatibility of the mobile phase components, including solvents and salts, with both the LC system and the ESI source is crucial for optimal performance and data quality.

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ESI is a "soft" ionization technique that relies on the formation of charged droplets from the liquid eluent, followed by solvent evaporation and Coulombic explosion to generate gas-phase ions. Therefore, **volatile solvents** are essential for efficient droplet formation and desolvation.

Use with caution – This List is not ideal - but can be used.

- **Ethanol (EtOH):** Can be used as an organic modifier, although less common than methanol or acetonitrile.
- **Acetone:** Can be used in some applications but might not be as universally compatible as methanol or acetonitrile.
- **Tetrahydrofuran (THF):** Can be used with caution and is often mixed with other compatible solvents. May not be suitable for all ESI sources or analytes.
- **Dichloromethane (DCM):** Can be used in mixtures with methanol or acetonitrile, especially for normal-phase LC applications coupled with ESI. However, it should be used with caution due to potential safety and environmental concerns.
- **Small amounts of other polar aprotic solvents:** Dimethylformamide (DMF) and Dimethylsulfoxide (DMSO) can be tolerated in small percentages if necessary for analyte solubility, but high concentrations can suppress ionization and contaminate the MS source.

MASS SPECTROMETRY CONCEPTS – IONIZATION

Notes on Organic Salts and Ionization – Electrospray Ionization – with LC ESI MS

Compatible Solvents and Organic Salts for LC-ESI-MS

Compatible Organic Salts and Buffers:

To achieve optimal chromatographic separation, buffers and salts are often added to the mobile phase to control pH and ionic strength. For ESI-MS, these additives must be **volatile** to prevent non-volatile residues from building up in the ESI source and causing signal suppression.

- **Ammonium Formate (NH_4COOH):** A widely used volatile buffer, effective in the low pH range (around 3-4). Provides good buffering capacity and compatibility with ESI. Typically used in concentrations of 5-20 mM.
- **Ammonium Acetate (CH_3COONH_4):** Another common volatile buffer, providing buffering in the near-neutral pH range (around 4-6). Good compatibility with ESI and often used in concentrations of 5-20 mM.
- **Ammonium Bicarbonate (NH_4HCO_3):** A volatile buffer used for slightly alkaline conditions (around pH 7-9). It decomposes to ammonia and carbon dioxide in the ESI source, making it compatible. Typically used in concentrations of 5-20 mM.
- **Formic Acid (HCOOH):** A volatile acid often used as a mobile phase modifier to improve ionization in positive ion mode. Typically used at concentrations of 0.1-0.5%. It also provides some pH control in the acidic range.
- **Acetic Acid (CH_3COOH):** Similar to formic acid, a volatile acid used to enhance positive ion mode ionization and provide acidic pH. Typically used at concentrations of 0.1-0.5%.
- ***Ammonium Hydroxide (NH_4OH):*** A volatile base used to enhance ionization in negative ion mode and provide alkaline pH. Typically used at concentrations of 0.01-0.1%.
- ***Triethylamine (TEA):*** A volatile amine that can be used as a modifier in reversed-phase LC, particularly for basic compounds. However, it can sometimes suppress ionization in ESI and should be used cautiously and at low concentrations (e.g., 0.01-0.1%).
- ***Other volatile organic amines:*** Diethylamine (DEA), triethanolamine (TEOA) can be used

MASS SPECTROMETRY CONCEPTS – IONIZATION

Notes on Organic Salts and Ionization – Electrospray Ionization – with LC ESI MS

Salts and Detergents Bad for LC and ESI:

Certain salts and detergents are detrimental to both the LC system and the ESI source. They can cause various problems, including:

- **Non-volatile residues:** These can accumulate in the ESI source, leading to signal suppression, contamination, and reduced sensitivity. They can also precipitate within the LC system, causing blockages and damage to the column and pump.
- **Ion suppression:** Some compounds can interfere with the ionization process in ESI, leading to a decrease or complete loss of signal for the analytes of interest.
- **Column damage:** Certain additives can react with or degrade the stationary phase of the LC column, leading to poor separation and reduced column lifetime.
- **System contamination:** Non-volatile compounds can persist in the LC system and MS source, contaminating subsequent analyses.

MASS SPECTROMETRY CONCEPTS – IONIZATION

Notes on Organic Salts and Ionization – Electrospray Ionization – with LC ESI MS

Salts and Detergents Bad for LC and ESI:

- **Inorganic Salts:**
  - **Sodium Chloride (NaCl), Potassium Chloride (KCl):** Common inorganic salts that are non-volatile and cause significant ion suppression in ESI, particularly by forming adducts with the analytes (e.g., $[M+Na]^+$, $[M+K]^+$).
  - **Phosphate Buffers (e.g., Sodium Phosphate, Potassium Phosphate):** Non-volatile and can precipitate in the ESI source, leading to severe contamination and signal suppression.
  - **Sulfate Salts (e.g., Sodium Sulfate):** Non-volatile and can cause similar problems as phosphate buffers.
  - **Citrate Buffers (e.g., Sodium Citrate):** Non-volatile and can contaminate the ESI source.
  - **Borate Buffers (e.g., Sodium Borate):** Non-volatile and should be avoided.
- **Detergents (Surfactants):**
  - **Sodium Dodecyl Sulfate (SDS):** A common anionic detergent that strongly suppresses ionization in ESI and can contaminate the MS source.
  - **Triton X-100, NP-40:** Non-ionic detergents that can also cause significant ion suppression and contaminate the system.
  - **Tween series (e.g., Tween 20, Tween 80):** Non-ionic detergents that interfere with ESI and are difficult to remove from the system.
  - **CHAPS:** A zwitterionic detergent that can be used in some specific cases at low concentrations, but it can still cause ion suppression and is generally best avoided if possible.

MASS SPECTROMETRY CONCEPTS – IONIZATION

Notes on Organic Salts and Ionization – Electrospray Ionization – with LC ESI MS

Salts and Detergents Bad for LC and ESI:

- **Other Problematic Additives:**
  - **Trifluoroacetic Acid (TFA):** While volatile and commonly used in reversed-phase LC for peptide and protein analysis, it can significantly suppress the signal of small molecules in positive ion mode ESI. Formic or acetic acid is often preferred for small molecule analysis with ESI.
  - **High concentrations of non-volatile acids or bases:** Mineral acids (e.g., sulfuric acid, hydrochloric acid) and strong non-volatile bases should be avoided.
  - **Ion-pairing reagents (e.g., perchloric acid, long-chain alkyl sulfonates):** These are often non-volatile or can strongly suppress ionization in ESI.
  - **Glycerol:** A non-volatile compound often used to stabilize proteins, it can contaminate the MS source and suppress ionization.
  - **Polyethylene Glycols (PEGs):** Common contaminants from plastics and some reagents, they can produce a series of adduct ions in the mass spectrum, interfering with analyte identification.
  - **EDTA:** A chelating agent that is non-volatile and can contaminate the MS source.

MASS SPECTROMETRY CONCEPTS – IONIZATION

Notes on Organic Salts and Ionization – Electrospray Ionization – with LC ESI MS

Best Practices:

- **Use LC-MS grade solvents and additives:** These are specifically purified to minimize contaminants that can interfere with LC and ESI-MS.
- **Filter samples and mobile phases:** This helps to remove particulate matter that can clog the LC system and ESI needle.
- **Avoid non-volatile salts and detergents whenever possible:** If they are necessary for sample preparation, implement a suitable clean-up step (e.g., solid-phase extraction, dialysis) before LC-ESI-MS analysis.
- **Consider the pH of the mobile phase:** The pH can significantly affect the ionization efficiency of the analytes in ESI. Choose a volatile buffer system that provides the desired pH for chromatography and ionization.
- **Keep additive concentrations low:** Even volatile additives can cause ion suppression at high concentrations. Optimize the concentration for both chromatography and MS sensitivity.

MASS SPECTROMETER OPTICS
TRANSFERRING AND MOVING IONS AROUND ION GUIDES FOR ION BEAMS

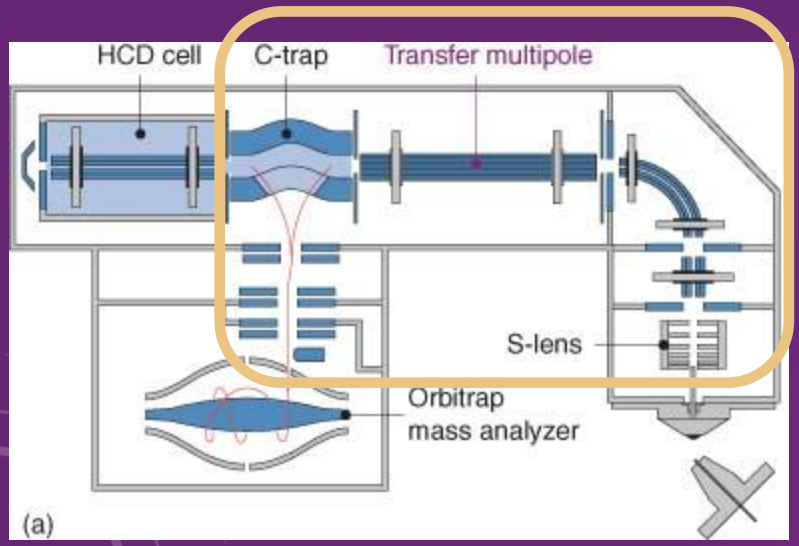
MASS SPECTROMETERS AND OPTICS - BASICS

Mass spectrometry instruments rely on carefully controlled electric and magnetic fields to manipulate and direct ions from the ion source, through the mass analyzer, and to the detector. These fields, and the physical components that generate and shape them, are often referred to as **ion optics**. Efficient ion transfer and collection are crucial for maximizing sensitivity and resolution. Here are some common ion optic elements used in mass spectrometers *(please contact for more information):*

Fundamentals and Advances of Orbitrap Mass Spectrometry

Elizabeth S. Hecht, Michaela Scigelova, Shannon Eliuk, Alexander Makarov

https://onlinelibrary.wiley.com/doi/10.1002/9780470027318.a9309.pub2



Some Optics in the *Classic* Thermo QE

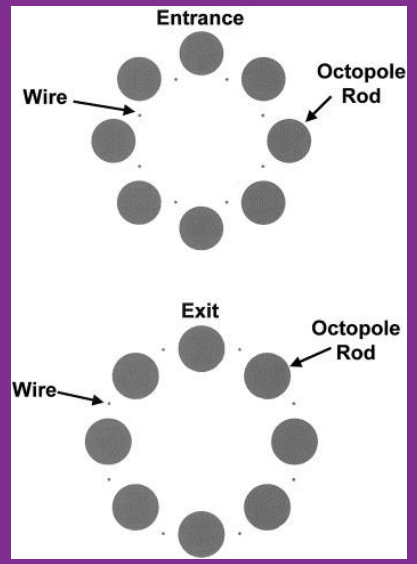
***C-Trap*: for concentration of ion packet**

***Transfer Multipole*: based on the Example Octupole Optics (to the right)→**

***S-lens*: help with ion Beam focus**

Example - Octopole Optics

For transferring of ions (or beam) to mass analyzer



https://www.sciencedirect.com/science/article/pii/S1044030502006220

"RF octopole ion guide used to bridge a differential pumping stage of an ESI interface in a Finnigan LCQ instrument. (a) Head-on view to show the octopole alignment and (b) side view." *see link below*

https://ms-textbook.com/for-instructors/

QUADRUPOLE MASS FILTERS (Q) AND TRIPLE QUADRUPOLE MASS SPEC (QQQ)

QUADRUPOLE MASS FILTERS

Quadrupole Mass Filter

A **quadrupole mass filter (Q)** is a type of mass analyzer used in mass spectrometry to selectively transmit ions based on their mass-to-charge ratio (m/z). It consists of four parallel, cylindrical or hyperbolic rods arranged symmetrically around a central axis.

Components:

- **Four Rod Electrodes:** Precisely aligned and equally spaced. Opposite rods are electrically connected in pairs.
- **Voltage Supply:** Applies a combination of a direct current (DC) voltage (U) and a radio frequency (RF) alternating current (AC) voltage ($V\cos(\omega t)$) to the rod pairs. One pair receives $+(U + V\cos(\omega t))$, and the opposite pair receives $-(U + V\cos(\omega t))$.
- **Ion Inlet:** Introduces ions into the space between the rods, typically along the z-axis.
- **Ion Outlet & Detector:** Allows selected ions to exit the filter and be detected.
- **Vacuum System:** The entire assembly operates under vacuum to minimize collisions between ions and other gas molecules.

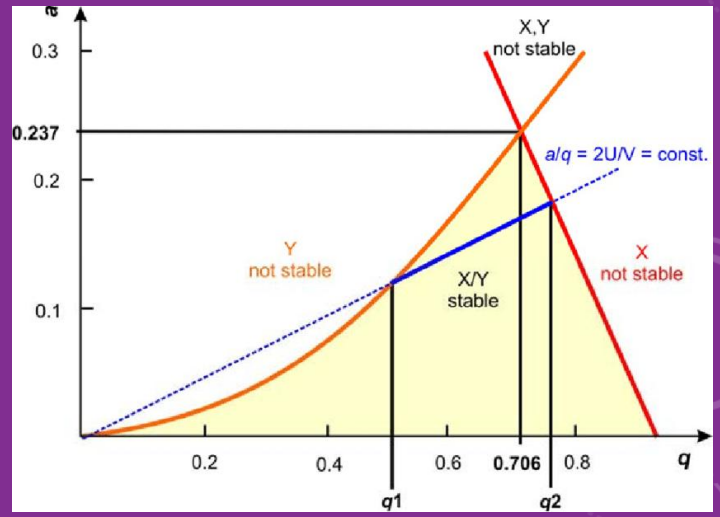
QUADRUPOLE MASS FILTERS

Principle of Operation:

- Ions entering the quadrupole region are subjected to the electric fields created by the applied DC and AC voltages.
- These fields cause the ions to oscillate in the x and y directions (perpendicular to the ion path along the z-axis).
- For a given set of DC and AC voltages and frequency, only ions with a specific m/z ratio will have stable trajectories through the quadrupole filter.
- Ions with unstable trajectories will experience oscillations of increasing amplitude, eventually colliding with the rods and being neutralized.
- By varying the DC and AC voltages (while maintaining a fixed ratio for a specific resolution), the quadrupole can selectively transmit ions of different m/z values, allowing for a mass spectrum to be scanned.

Quadrupole Mass Filter

Stability diagram of the quadrupole mass filter. Only ions on the straight line (blue) and in the yellow area reach the detector



https://www.researchgate.net/publication/33428451_Partial_and_Total_Oxidation_of_Methane_in_Monolithic_Catalysts_at_Short_Contact_Times

QUADRUPOLE MASS FILTERS

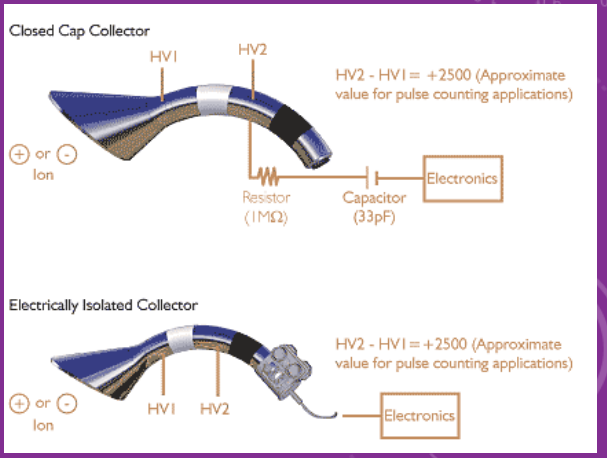
In essence, the quadrupole mass filter acts as a mass-selective gate. By carefully controlling the electric fields, it allows only ions within a narrow m/z window to pass through at any given time. Scanning these voltages allows for the sequential transmission and detection of ions across a mass range, resulting in a mass spectrum.

Steps to Generate a Mass Spectrum:

1. **Ion Introduction:** Ions from the ion source are injected into the quadrupole region.
2. **Voltage Application:** DC (U) and RF (Vcos(ωt)) voltages are applied to the quadrupole rods.
3. **Ion Filtering:** For a specific U/V ratio and RF frequency (ω), only ions with a particular m/z have stable trajectories and pass through the filter. All other ions collide with the rods.
4. **Voltage Scanning:** The magnitudes of the DC and AC voltages are scanned (typically while keeping their ratio constant to maintain a desired resolution) over a range.
5. **Ion Detection:** The ions that successfully traverse the quadrupole at each voltage setting are detected, and their abundance is measured.
6. **Spectrum Generation:** The detected ion abundance is plotted against the corresponding m/z value (which is related to the applied voltages at the time of detection) to generate the mass spectrum.

Quadrupole Mass Filter

Electron Multipliers - Ion Detection

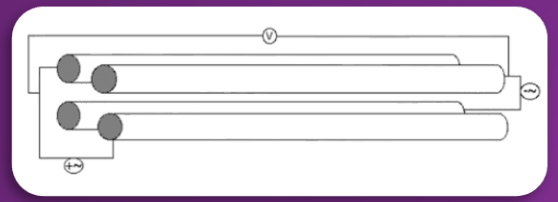


Think of it as – Counting the number of ions at each mass-to-charge (m/z) measured.

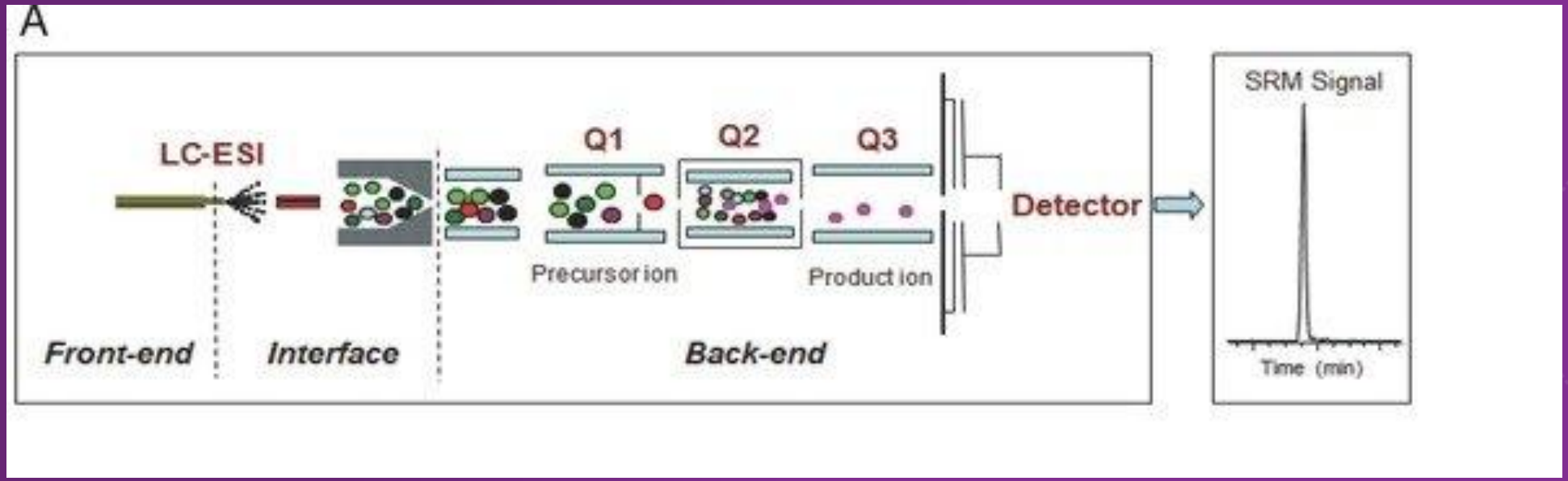
TRIPLE QUADRUPOLE MASS SPECTROMETER

Quadrupole Mass Filter 1 (Q1)

Quadrupole Mass Filter – Collision Cell Q2

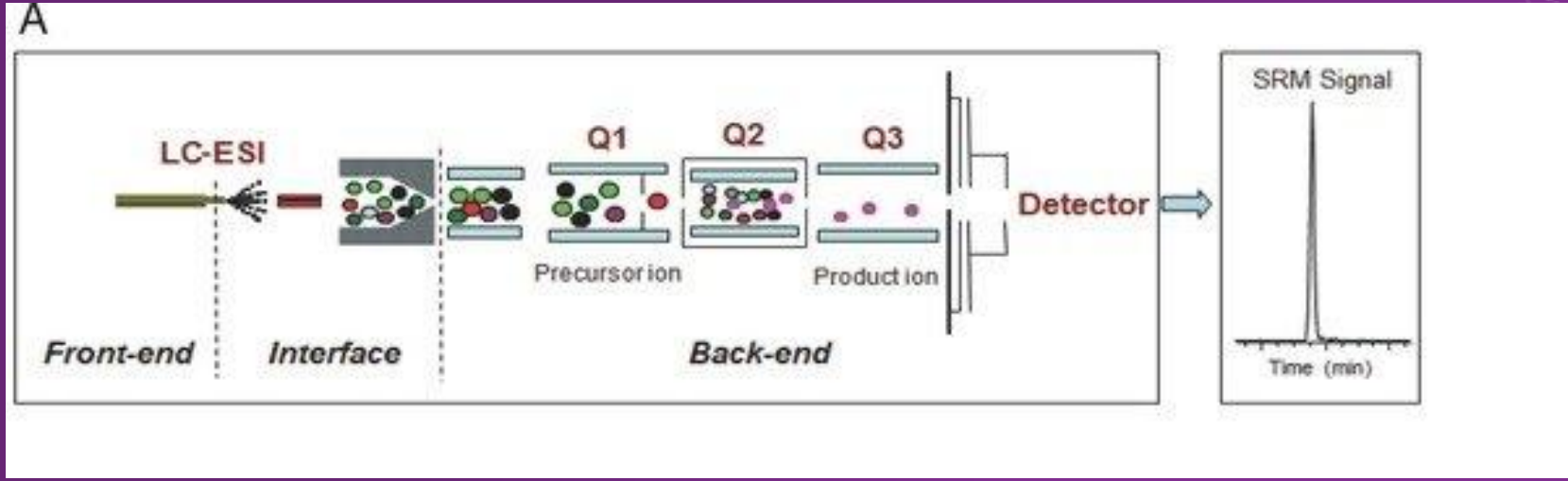


Quadrupole Mass Filter 3 (Q3)



https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/pmic.201100436

TRIPLE QUADRUPOLE MASS SPECTROMETER



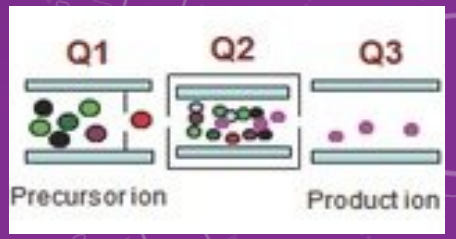
https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/pmic.201100436

A triple quadrupole mass spectrometer (QqQ) is a tandem mass spectrometer consisting of **three quadrupole mass analyzers arranged in series**. It is often abbreviated as **Q1-q2-Q3**, where:

- **Q1:** The first quadrupole mass filter, used for selecting a precursor ion of a specific m/z.
- **q2:** The second quadrupole, operated in **RF-only mode** (no DC voltage). This acts as a **collision cell**, where the selected precursor ions are fragmented by colliding with an inert gas (e.g., argon, nitrogen). It does not select for mass.
- **Q3:** The third quadrupole mass filter, used for analyzing the m/z of the fragment ions (product ions) generated in q2.

TRIPLE QUADRUPOLE MASS SPECTROMETER

QqQ MS

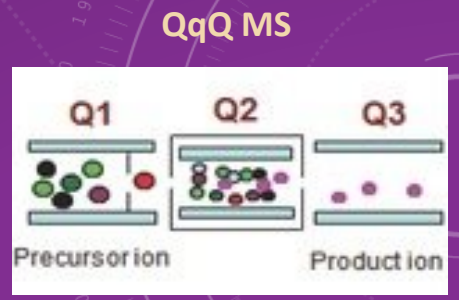


The triple quadrupole configuration allows for highly selective and sensitive analysis, making it a powerful tool for quantitative analysis and structural elucidation.

How a Triple Quadrupole Works:

1. **Ionization:** Sample molecules are first ionized in an ion source (e.g., ESI, APCI).
2. **Precursor Ion Selection (Q1):** The first quadrupole (Q1) is set to selectively pass ions with a specific m/z value, known as the precursor ion. All other ions are filtered out by their unstable trajectories.
3. **Collision-Induced Dissociation (CID) (q2):** The selected precursor ions enter the second quadrupole (q2), the collision cell. Here, they collide with an inert gas, gaining internal energy and subsequently fragmenting into smaller product ions.
4. **Product Ion Analysis (Q3):** The resulting fragment ions are then passed into the third quadrupole (Q3). Q3 can be operated in different ways (as described in the scan types below) to analyze these product ions.
5. **Detection:** Ions that pass through Q3 are detected, and their abundance is measured.

TRIPLE QUADRUPOLE MASS SPECTROMETER



Different Scan Types in Triple Quadrupole Mass Spectrometry:

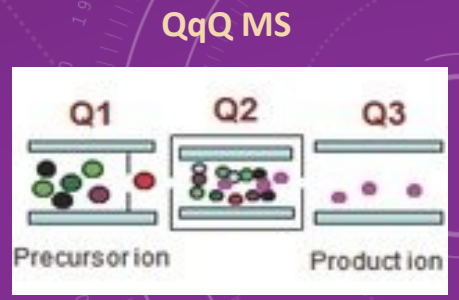
The unique arrangement of the three quadrupoles enables several distinct scan modes, each providing different types of information:

1. **Product Ion Scan:**

- **Q1:** Fixed to select a specific precursor ion m/z.
- **q2:** Collision cell for fragmentation.
- **Q3:** Scans across a range of m/z values to detect all the fragment ions produced from the selected precursor.

Information Obtained: Provides structural information about the precursor ion by identifying its fragmentation pathways. This is useful for method development (identifying optimal transitions for SRM/MRM) and structural elucidation.

TRIPLE QUADRUPOLE MASS SPECTROMETER



Different Scan Types in Triple Quadrupole Mass Spectrometry:

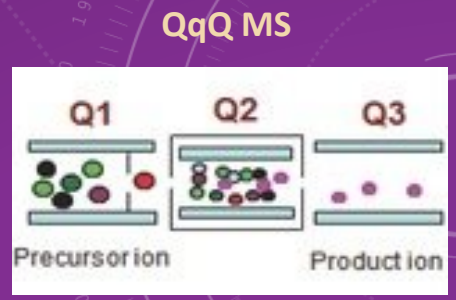
The unique arrangement of the three quadrupoles enables several distinct scan modes, each providing different types of information:

2. **Precursor Ion Scan**

- **Q1:** Scans across a range of m/z values
- **q2:** Collision cell for fragmentation (set to a specific collision energy).
- **Q3:** Fixed to select a specific product ion m/z.

Information Obtained: Identifies all precursor ions in a sample that fragment to yield the selected product ion. This can be useful for identifying compounds with a common structural motif or functional group that leads to the characteristic loss of the selected product ion.

TRIPLE QUADRUPOLE MASS SPECTROMETER



Different Scan Types in Triple Quadrupole Mass Spectrometry:

The unique arrangement of the three quadrupoles enables several distinct scan modes, each providing different types of information:

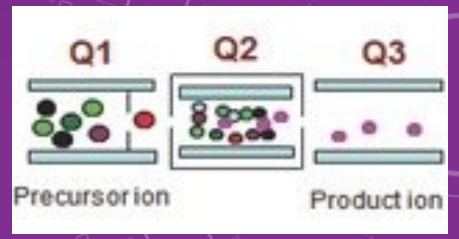
3. **Neutral Loss Scan:**

- **Q1:** Scans across a range of m/z values.
- **q2:** Collision cell for fragmentation (set to a specific collision energy).
- **Q3:** Scans across the same m/z range as Q1, but with a constant mass offset corresponding to the mass of a specific neutral fragment loss (e.g., H_2O, NH_3, CO_2).

Information Obtained: Identifies all precursor ions in a sample that lose a specific neutral fragment during fragmentation. This is useful for selectively identifying compounds with a common labile moiety.

TRIPLE QUADRUPOLE MASS SPECTROMETER

QqQ MS



Different Scan Types in Triple Quadrupole Mass Spectrometry:

The unique arrangement of the three quadrupoles enables several distinct scan modes, each providing different types of information:

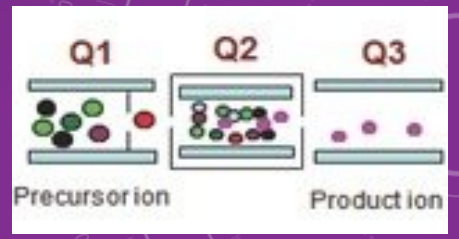
4. **Selected Reaction Monitoring (SRM) / Multiple Reaction Monitoring (MRM):**

- **Q1:** Fixed to select a specific precursor ion m/z.
- **q2:** Collision cell for fragmentation (optimized collision energy for a specific transition).
- **Q3:** Fixed to select a specific product ion m/z that is a characteristic fragment of the chosen precursor.

Information Obtained: Provides highly selective and sensitive quantitative analysis of target analytes. By monitoring a specific precursor-to-product ion transition, background noise is significantly reduced. MRM involves monitoring multiple such transitions simultaneously for one or more analytes, increasing throughput and confidence in identification and quantification. This is the most common mode for quantitative work in complex matrices.

TRIPLE QUADRUPOLE MASS SPECTROMETER

QqQ MS



Quick Review – QqQ MS These different scan types make the triple quadrupole mass spectrometer a versatile instrument for a wide range of applications, from targeted quantitative analysis to structural investigations and compound screening.

Summary – MRM Scan Mode

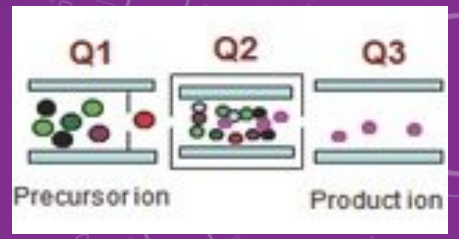
- **Purpose of MRM**
  - Quantitative analysis of specific target compounds
- **Mechanism of MRM**
  - Selectively monitors specific precursor-product ion transitions
  - Provides high sensitivity and specificity
- **Some Applications of MRM Assays**
  - Drug analysis
  - Food safety
  - Environmental monitoring

Summary – Product Ion Scan

- **Purpose of Product Ion Scan**
  - Identifying fragment ions from a selected precursor ion
- **Mechanism of Product Ion Scan**
  - Q1 selects a specific precursor ion
  - Q2 fragments the selected precursor ion
  - Q3 scans to detect the resulting product ions
- **Applications of Product Ion Scan**
  - Structural elucidation
  - Identifying metabolites

TRIPLE QUADRUPOLE MASS SPECTROMETER

QqQ MS



Quick Review – QqQ MS These different scan types make the triple quadrupole mass spectrometer a versatile instrument for a wide range of applications, from targeted quantitative analysis to structural investigations and compound screening.

Summary – SRM Scan Mode

- Purpose of SRM
  - Optimizes MRM scans for targeted analysis of multiple compounds
- Mechanism of SRM
  - Schedules MRM scans for specific compounds at different times
  - Maximizes sensitivity and throughput
- Applications of SRM
  - High-throughput analysis
  - Targeted analysis of complex samples

Summary – NL Scan Mode

- Purpose of Neutral Loss Scan
  - Detect compounds losing a specific neutral fragment during fragmentation
- Mechanism of Neutral Loss Scan
  - Q1 and Q3 scan with a constant mass difference
  - Detect precursor-product ion pairs
- Applications of Neutral Loss Scan
  - Identifying specific modifications
  - Detecting specific reactions

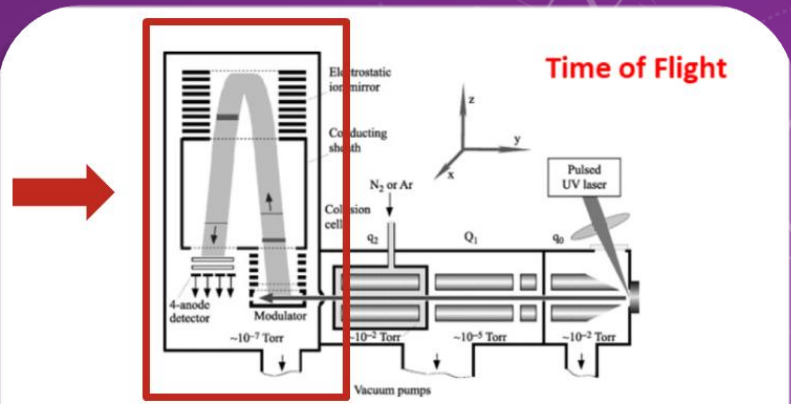
TIME-OF-FLIGHT (TOF) AND QUADRUPOLE TIME-OF-FLIGHT (Q-TOF) MS MORE “COUNTING IONS” FOR MEASUREMENT

TIME-OF-FLIGHT MASS ANALYZERS

Time-of-Flight (TOF) Mass Spectrometry

Time-of-Flight (TOF) mass spectrometry separates ions based on the time it takes them to travel a fixed distance through a field-free region under vacuum after being accelerated by an electric field. Ions with the same kinetic energy will have velocities inversely proportional to the square root of their mass-to-charge ratio (m/z). Lighter ions will reach the detector faster than heavier ions with the same charge. Here's a step-by-step description of how it works and how it converts to a mass spectrum.

Q-ToF MS



Hybrid Quadrupole/Time-of-Flight Mass Spectrometers for Analysis of Biomolecules Werner Ens; Kenneth G. Standing, Methods in Enzymology, Volume 402, 2005, Pages 49–78 Biological Mass Spectrometry

Ion Generation and Acceleration:

- Ions are generated from an ion source (e.g., MALDI, ESI) and introduced into the acceleration region of the TOF analyzer.
- A pulsed electric field is applied to accelerate these ions. All ions entering this region at the same time and with the same charge will ideally gain the same kinetic energy (KE).
- The kinetic energy gained by an ion with charge *z* accelerated through a potential difference *V* is given by: **KE = zV**.
- Since kinetic energy is also defined as **KE = ½mv²**, where *m* is the mass and *v* is the velocity, ions with different m/z ratios will acquire different velocities. Lighter ions (lower m/z) will have higher velocities, and heavier ions (higher m/z) will have lower velocities.

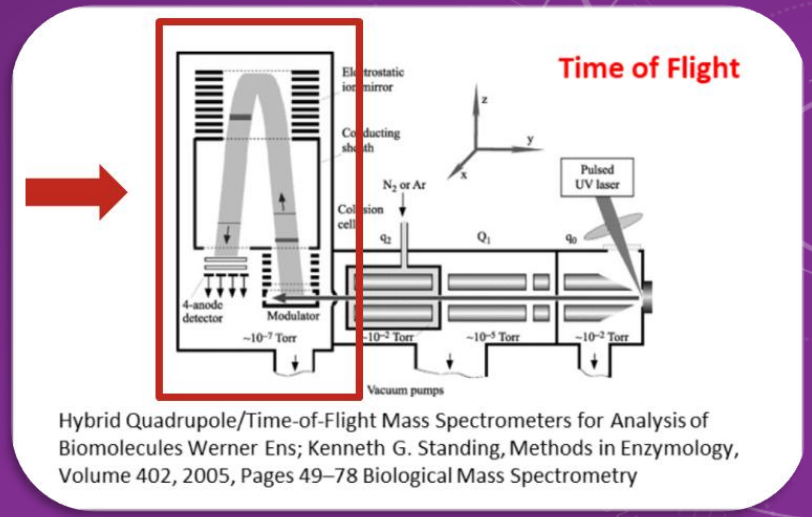
Flight Through the Field-Free Region:

- After acceleration, the ions enter a field-free region (drift tube) of a known length (*L*).
- In this region, there is no electric field acting on the ions, and they travel at a constant velocity determined by their kinetic energy gained during acceleration.
- The time (*t*) it takes for an ion to travel through the drift tube is directly proportional to the length of the tube and inversely proportional to its velocity: **t = L/v**.
- Since velocity is related to m/z, ions with different m/z ratios will take different times to reach the detector. Lighter ions will have shorter flight times, and heavier ions will have longer flight times.

TIME-OF-FLIGHT MASS ANALYZERS

TOF-MS offers the advantage of high acquisition speed, as all ions entering the analyzer are detected sequentially based on their flight time. It is often coupled with pulsed ionization techniques like MALDI and is widely used for analyzing a broad range of molecules, including large biomolecules. Factors like initial kinetic energy spread and spatial distribution of ions at the start of the flight path can affect resolution, and techniques like reflectrons (ion mirrors) are often employed to improve the focusing of ions and enhance mass resolution.

Q-ToF MS



Hybrid Quadrupole/Time-of-Flight Mass Spectrometers for Analysis of Biomolecules Werner Ens; Kenneth G. Standing, Methods in Enzymology, Volume 402, 2005, Pages 49–78 Biological Mass Spectrometry

Ion Detection:

- At the end of the field-free region, ions strike a detector (e.g., electron multiplier, microchannel plate).
- The detector records the arrival time of each ion.
- Ions with the same m/z will ideally arrive at the detector at approximately the same time, forming a "packet" of ions.

Note a Q is added for the Q-ToF:

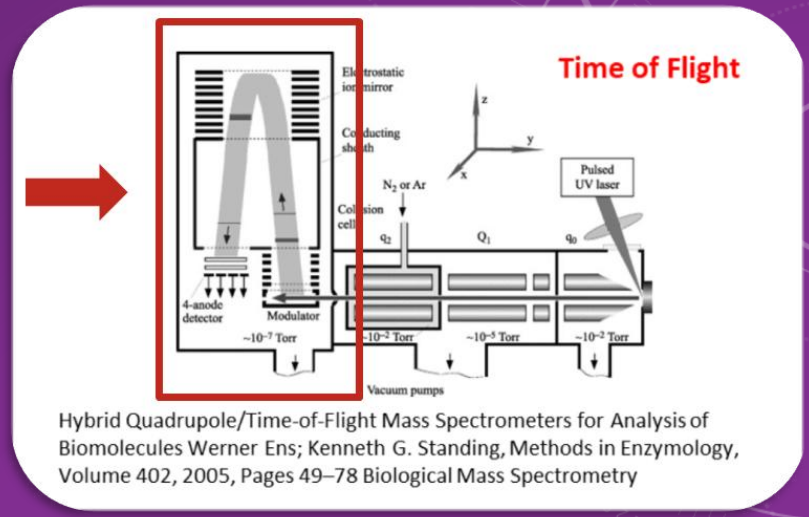
- Enable Fragmentation to march to libraries or for qualitative analysis.
- Enbables both Data Dependent Acquisition (DDA) and Data Independent Acquisition (DIA)

Time-to-m/z Conversion:

- The detector generates a signal (current or voltage pulse) at each ion arrival time.
- The recorded arrival times are then converted to m/z values using the relationship derived from the kinetic energy equation and the flight time equation:
  - $KE = zV = \frac{1}{2}mv^2$
  - $v = \sqrt{(2zV/m)}$
  - $t = L/v = L / \sqrt{(2zV/m)} = L * \sqrt{(m/(2zV))}$
- From this, the m/z ratio can be expressed as: $\mathbf{m/z = k * t^2}$, where *k* is a constant that depends on the acceleration voltage (*V*) and the length of the flight tube (*L*). This constant is typically determined through calibration using ions of known m/z values.

TIME-OF-FLIGHT MASS ANALYZERS

Q-ToF MS



Hybrid Quadrupole/Time-of-Flight Mass Spectrometers for Analysis of Biomolecules Werner Ens; Kenneth G. Standing, Methods in Enzymology, Volume 402, 2005, Pages 49–78 Biological Mass Spectrometry

Mass Spectrum Generation:

- The intensity of the signal at each arrival time (which has been converted to an m/z value) is proportional to the abundance of ions with that specific m/z.
- A mass spectrum is then generated by plotting the ion abundance (intensity of the detector signal) as a function of the calculated m/z values.
- The resulting spectrum shows peaks at different m/z values, with the height or area of each peak representing the relative abundance of the corresponding ions.

Generalized, TOF-MS achieves mass analysis by:

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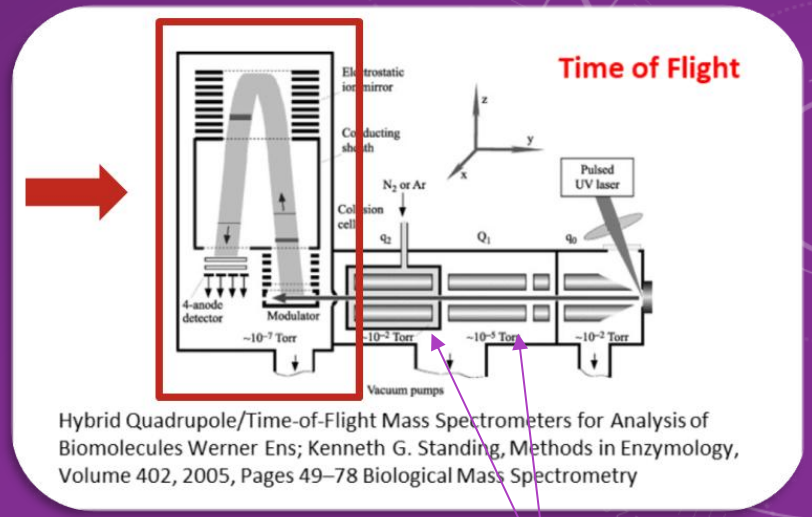
2. **Packets of ions to** "drift" through a field-free region, where their velocity is inversely proportional to the square root of their *m/z*.
3. **Detecting** the arrival time of ions at the end of the flight path.
4. **Converting** the measured flight times to *m/z* values using a known relationship based on the instrument parameters.
5. **Generating** a mass spectrum by plotting ion abundance against the calculated *m/z* values.

TIME-OF-FLIGHT MASS ANALYZERS

Main Scan Modes for Q-TOF Instrument

A Quadrupole Time-of-Flight (QTOF) instrument combines a **quadrupole mass filter (Q)** with a **Time-of-Flight (TOF)** mass analyzer. This hybrid design allows for precursor ion selection (in the quadrupole) followed by accurate mass measurement of both the **precursor and fragment ions (in the TOF)**. The scan modes on a **QTOF** instrument leverage these capabilities to provide different types of information. Here are some common scan modes:

Q-ToF MS



Hybrid Quadrupole/Time-of-Flight Mass Spectrometers for Analysis of Biomolecules Werner Ens; Kenneth G. Standing, Methods in Enzymology, Volume 402, 2005, Pages 49–78 Biological Mass Spectrometry

Quadrupole Mass Filter (Q) and Collision Cell for "CID Fragmentation"

1. **Full Scan MS (Q1 Scan):**
   - The quadrupole (Q1) is set to a wide mass range, allowing all ions entering it to pass through to the collision cell (q2 - often a hexapole or octopole) and then to the TOF analyzer.
   - The TOF analyzer measures the accurate mass of all ions present in the sample.
   - This mode is used for qualitative analysis, identifying all components in a sample and obtaining their accurate masses.

TIME-OF-FLIGHT MASS ANALYZERS

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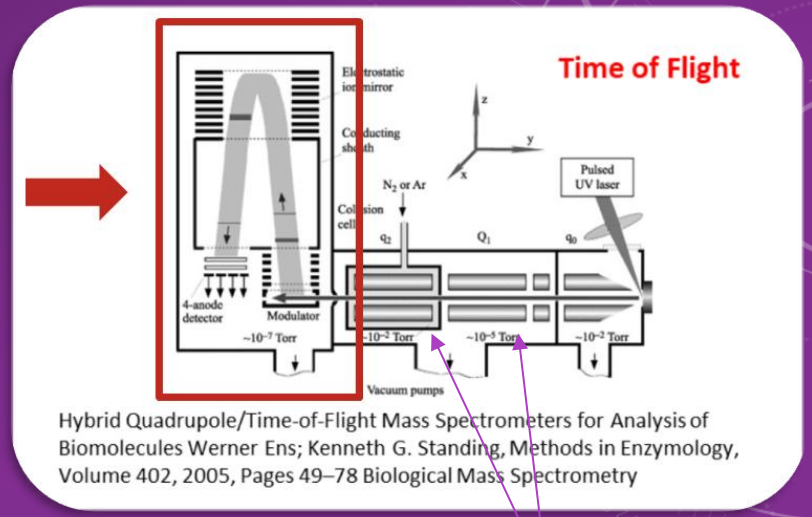

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Quadrupole Mass Filter (Q) and Collision Cell for "CID Fragmentation"

2. Product Ion Scan

- The quadrupole (Q1) is set to select a specific precursor ion m/z.
- This selected precursor ion is then fragmented in the collision cell (q2) by collision-induced dissociation (CID).
- The TOF analyzer then detects a *m/z* range to with the fragment ions produced from the selected precursor, providing accurate mass measurements for these MSMS fragments.
- This mode is used for structural elucidation and confirmation of compound

TIME-OF-FLIGHT MASS ANALYZERS

Main Scan Modes for Q-TOF Instrument

A Quadrupole Time-of-Flight (QTOF) instrument combines a **quadrupole mass filter (Q)** with a **Time-of-Flight (TOF)** mass analyzer. This hybrid design allows for precursor ion selection (in the quadrupole) followed by accurate mass measurement of both the **precursor and fragment ions (in the TOF)**. The scan modes on a **QTOF** instrument leverage these capabilities to provide different types of information. Here are some common scan modes:

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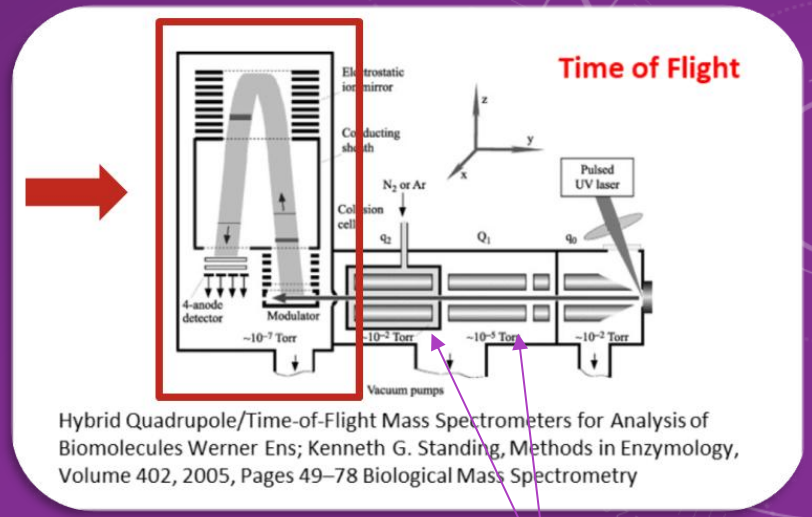

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Quadrupole Mass Filter (Q) and Collision Cell for "CID Fragmentation"

3. **Data-Dependent Acquisition (DDA) / Information-Dependent Acquisition (IDA):**
   - The instrument performs a Full Scan MS (Q1 scan) to identify the most abundant precursor ions.
   - Based on predefined criteria (e.g., intensity thresholds, charge states), the instrument automatically switches to a **Product Ion Scan (MS/MS)** for selected precursor ions.
   - This allows for automated acquisition of fragmentation data for the most abundant compounds in a sample – for more complex samples.

TIME-OF-FLIGHT MASS ANALYZERS

Main Scan Modes for Q-TOF Instrument

3. Data Independent Acquisition (DIA)- Newest Applications

- **Comprehensive Precursor Fragmentation:** In DIA, a wide or stepped series of precursor m/z windows are sequentially selected (or all are allowed to pass simultaneously in some approaches) into the collision cell, where all ions within each window are fragmented. This results in the acquisition of MS/MS spectra for virtually all detectable precursors across the chosen mass range, without relying on pre-selection based on abundance.
- **Unbiased Data Acquisition:** Unlike data-dependent acquisition (DDA), DIA acquires fragmentation data in a systematic and pre-defined manner, independent of the real-time abundance of ions. This leads to a more complete and reproducible dataset, enabling retrospective analysis, the ability to re-mine data for new analytes, and more reliable quantitative comparisons across samples.
- **Complex Data Processing:** DIA datasets are typically more complex than DDA data due to the co-fragmentation of multiple precursors within each isolation window. Specialized bioinformatics tools and algorithms are required to deconvolute these multiplexed spectra, identify and quantify target analytes, and link precursor ions to their corresponding fragment ions for accurate analysis.

Example for DIA - SWATH Acquisition (Sequential Window Acquisition of All Theoretical Fragment Ion Spectra) – Earliest Commercialization of DIA:

- This is a data-independent acquisition (DIA) method where Q1 sequentially selects a series of overlapping mass windows across the entire mass range.
- Within each window, all ions are fragmented in q2, and the TOF analyzer (Q3) records a high-resolution, accurate mass MS/MS spectrum.
- This comprehensive data acquisition strategy allows for retrospective analysis and quantification of all detectable analytes and their fragments without prior selection, providing a complete digital archive of the experiment.

TIME-OF-FLIGHT MASS ANALYZERS – QUICK REVIEW

Mass Spectrum Generation:

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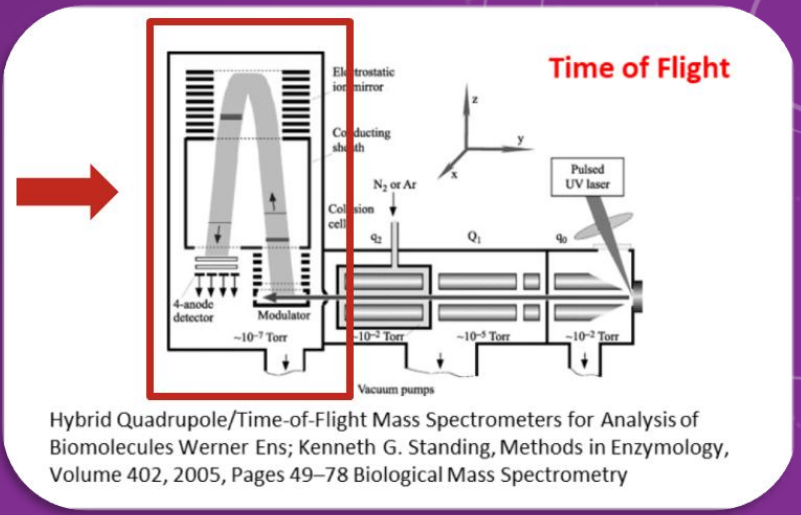

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5. **Generating** a mass spectrum by plotting ion abundance against the calculated *m/z* values.

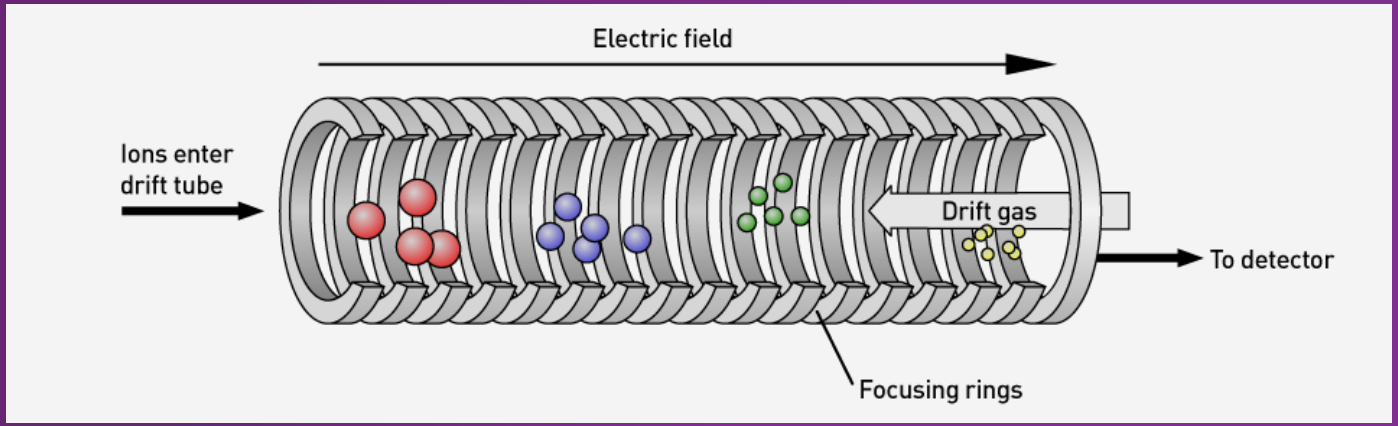
ION MOBILITY BASED SEPARATIONS AND MASS SPECTROMETRY

ION MOBILITY - BASICS

Basics of Ion Mobility Spectrometry (IMS)

Ion Mobility Spectrometry (IMS) is an analytical technique that separates gas-phase ions based on their mobility through a buffer gas under the influence of an electric field. The fundamental principle is that ions with different sizes, shapes, and charge states will experience varying degrees of collisions with the buffer gas molecules as they drift through the electric field, resulting in different drift velocities. This difference in velocity allows for the separation of ions over time or space.

Basic Ion Mobility Spectrometry



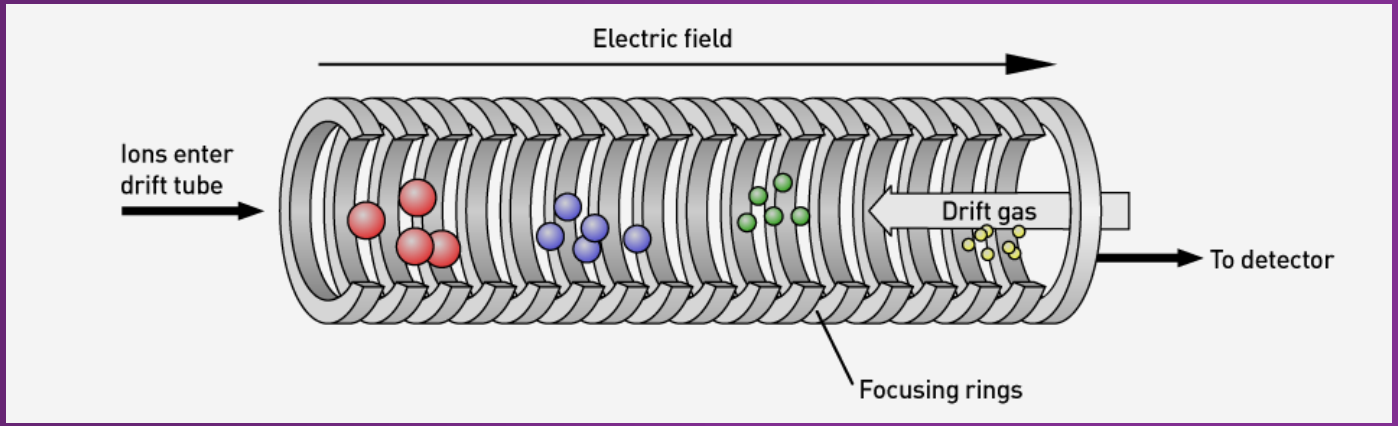
https://www.analyticon.eu/en/ion-mobility-spectrometry.html

ION MOBILITY - BASICS

Basics of Ion Mobility Spectrometry (IMS)

These different IMS techniques offer unique advantages in terms of resolution, sensitivity, speed, and the types of information they provide, making them valuable tools in various analytical applications, often coupled with mass spectrometry for enhanced separation and identification capabilities.

Basic Ion Mobility Spectrometry



https://www.analyticon.eu/en/ion-mobility-spectrometry.html

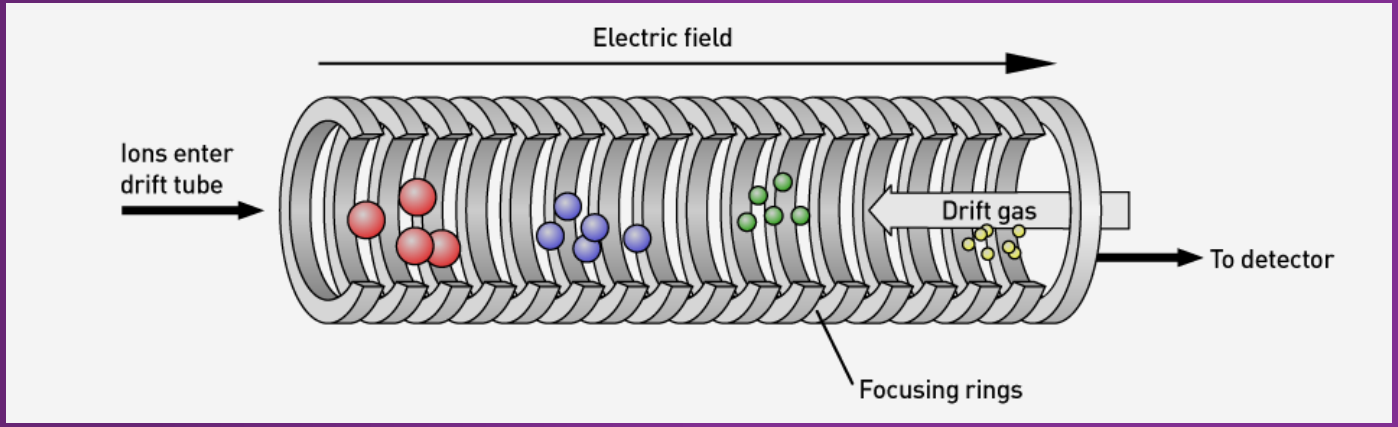
ION MOBILITY – SUB-GROUPS

Different Types of Ion Mobility Spectrometry:

Drift-Time Ion Mobility Spectrometry (DTIMS):

- **Technology:** Ions are introduced as a pulse into a tube filled with a buffer gas (e.g., nitrogen, helium) under a constant, uniform electric field. The time it takes for ions to travel the length of the tube and reach a detector is measured. Smaller, more compact ions with higher charge states experience fewer collisions and thus have higher mobility and shorter drift times.
- **Examples:** Traditional IMS instruments used in security screening for explosives and drugs often employ DTIMS. High-resolution DTIMS instruments coupled with mass spectrometry are used for detailed structural analysis of biomolecules, allowing for the measurement of collision cross-sections (CCS).

Drift-Time Ion Mobility Spectrometry (DTIMS)



https://www.analyticon.eu/en/ion-mobility-spectrometry.html

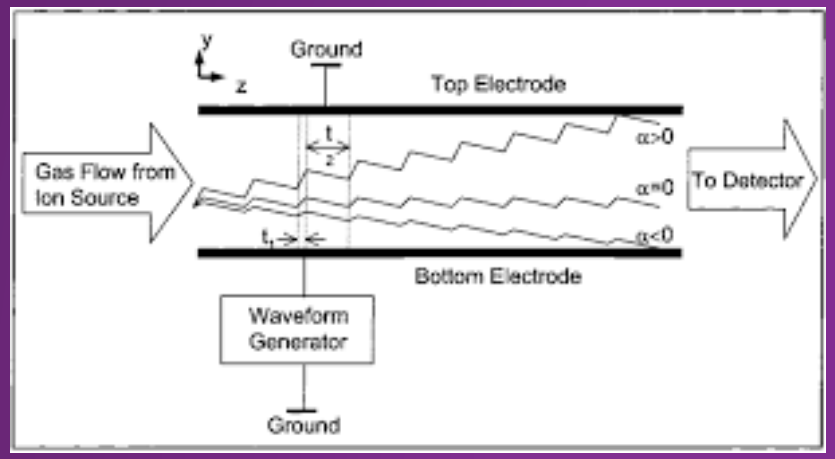
ION MOBILITY – SUB-GROUPS

Different Types of Ion Mobility Spectrometry:

Differential Mobility Spectrometry (DMS) / Field Asymmetric Ion Mobility Spectrometry (FAIMS):

- **Technology:** Ions are separated based on the difference in their mobility at low and high electric field strengths. An asymmetric alternating high-voltage waveform is applied perpendicular to the direction of ion flow, combined with a DC compensation voltage. Only ions with a specific mobility profile will traverse the device without colliding with the electrodes.
- **Examples:** FAIMS is used in various applications, including the analysis of volatile organic compounds (VOCs), biomarker discovery in breath analysis, and as a pre-filter in mass spectrometry to reduce chemical noise and separate isobaric ions. Portable devices for rapid detection of hazardous substances often utilize DMS/FAIMS.

Differential Mobility Spectrometry (DMS) and Field Asymmetric Ion Mobility Spectrometry (FAIMS) - *Basics*

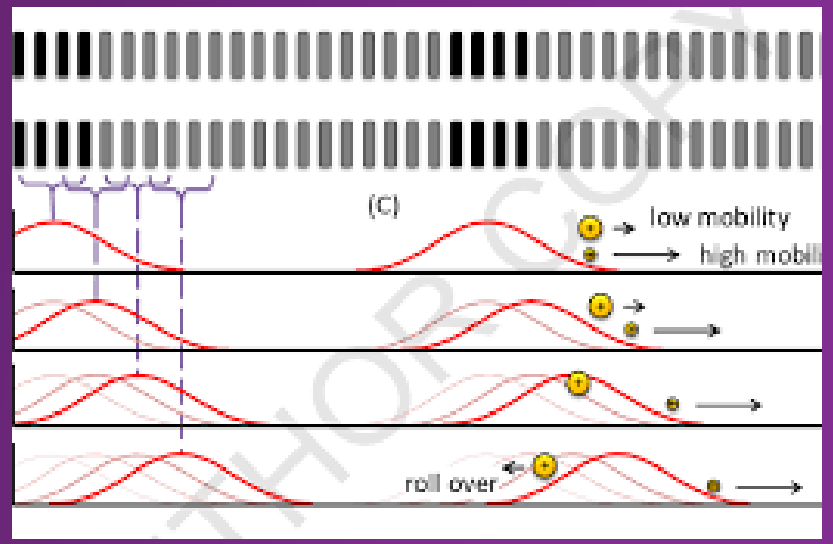


ION MOBILITY – SUB-GROUPS

Different Types of Ion Mobility Spectrometry:

Traveling-Wave Ion Mobility Spectrometry (TWIMS):

- **Technology:** Ions are propelled through a buffer gas by a series of traveling potential waves generated by applying RF and DC voltages to a stack of ring electrodes. Ions with different mobilities interact differently with the traveling wave, leading to their separation based on their drift time through the cell.
- **Examples:** TWIMS is commercially integrated into some mass spectrometers and is used for separating isomers, conformers, and analyzing the size and shape of proteins and other biomolecules. It allows for CCS determination through calibration.



The Influence of Drift Gas Composition on the Separation Mechanism in Traveling Wave Ion Mobility Spectrometry: Insight from Electrodynamic Simulations
June 2003 International Journal for Ion Mobility Spectrometry 16(2):85-94

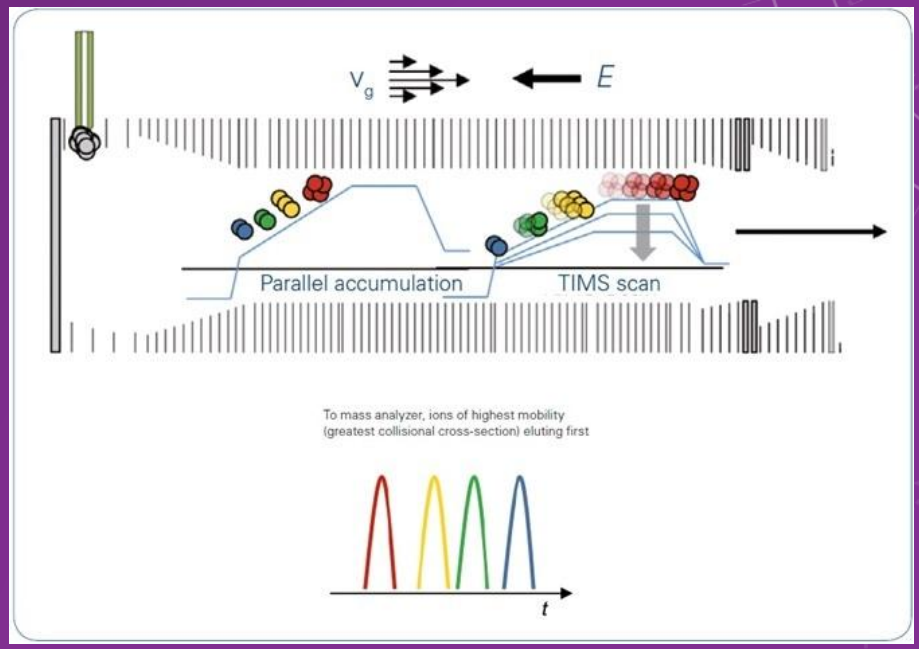
ION MOBILITY – SUB-GROUPS

Different Types of Ion Mobility Spectrometry:

Trapped Ion Mobility Spectrometry (TIMS):

- **Technology:** Ions are trapped in a spatially varying electric field created within an RF ion guide, counteracted by a flow of buffer gas. Ions are then released based on their mobility by gradually decreasing the electric field. Higher mobility ions are eluted at lower electric field strengths. Molecules exit from larger CCS to smaller CCS.
- **Examples:** TIMS coupled with high-resolution mass spectrometry offers high separation efficiency in a compact design and is used for complex mixture analysis in proteomics and metabolomics, enabling the separation of isobaric and isomeric species and CCS measurements.

Parallel Accumulation Serial Fragmentation (PASEF) with *tims and Bruker timsTOF*



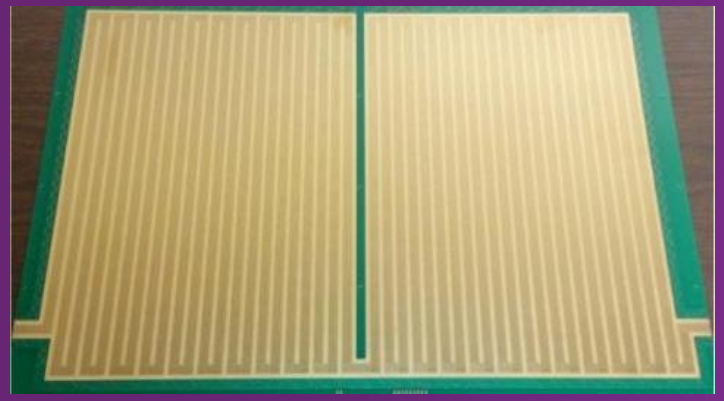
https://www.news-medical.net/whitepaper/20190315/PASEF-Redefining-New-Standards-for-Proteomics-Research.aspx

ION MOBILITY – SUB-GROUPS

Different Types of Ion Mobility Spectrometry:

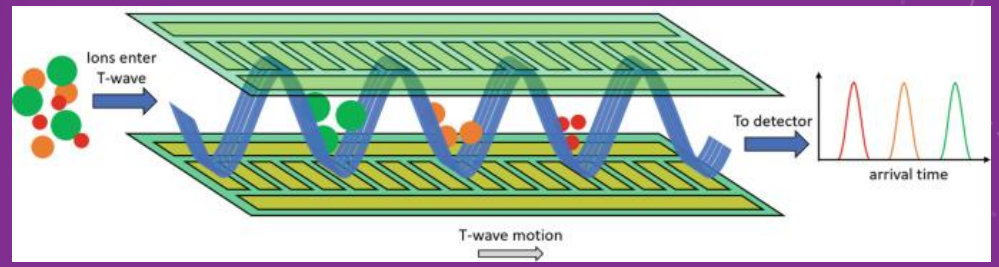
Structures for Lossless Ion Manipulations (SLIM):

- **Technology**: SLIM utilizes a serpentine path defined by arrays of microelectrodes on closely spaced parallel surfaces. Ions are propelled along this extended path by traveling wave (TW) electric fields, while RF and DC fields provide lossless radial confinement. The extended path lengths (potentially exceeding tens of meters with multiple passes) enable ultra-high resolution separations based on ion mobility.
- **Examples:** SLIM is currently used in advanced research settings, often coupled with high-resolution mass spectrometry, for extremely high-resolution separation of complex mixtures, including isomers, conformers, and even isotopologues. It shows promise in proteomics, metabolomics, and other omics fields where resolving closely related species is critical.



https://www.pnnl.gov/mass-spectrometry-based-measurement-technologies

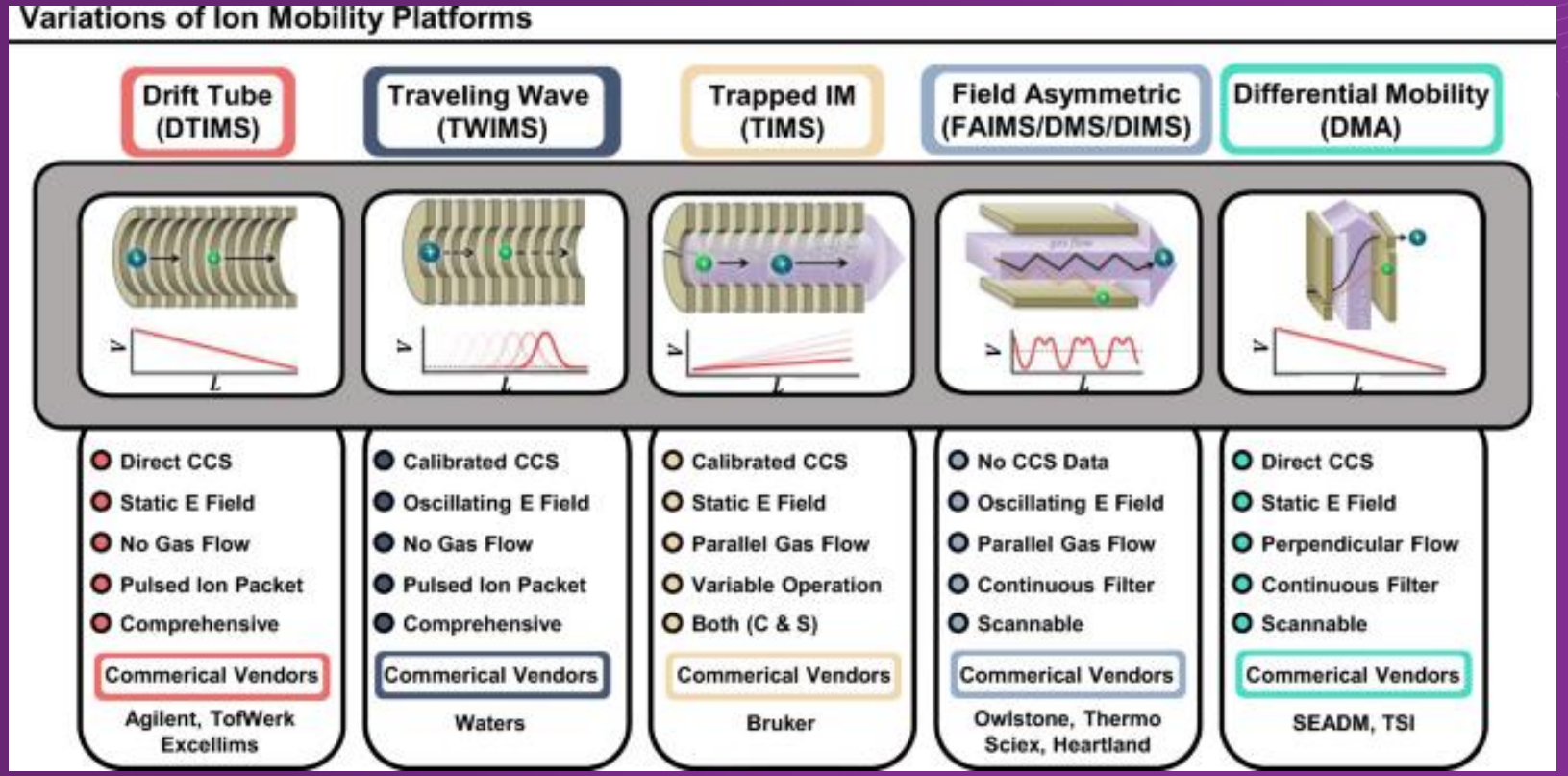
Implementation of Ion Mobility Spectrometry-Based Separations in Structures for Lossless Ion Manipulations (SLIM) - 2022



https://link.springer.com/protocol/10.1007/978-1-0716-1811-0_23

ION MOBILITY – SUB-GROUPS

Different Types of Ion Mobility Spectrometry:



https://link.springer.com/article/10.1007/s13361-019-02288-2

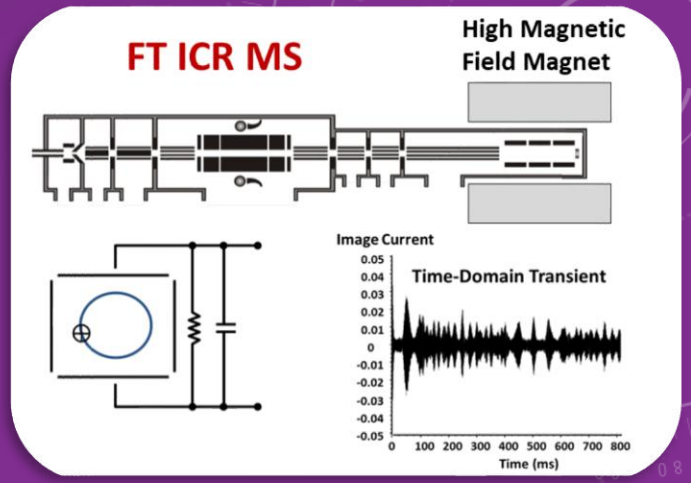
Ion Mobility Spectrometry: Fundamental Concepts, Instrumentation, Applications, and the Road Ahead – 2019

RESONANCE BASED MASS SPEC

- FOURIER TRANSFORM ION CYCLOTRON RESONANCE (FT ICR MS)
- ORBITRAP DETECTION MS
- “TRAPPING” MASS SPECTROMETRY

FOURIER TRANSFORM ION CYCLOTRON RESONANCE

FT-ICR MS



Ion Injection and Trapping:

- Ions generated from an ion source are introduced into the ICR cell, which is located within a powerful superconducting magnet.
- The magnetic field (B) interacts with the ions to move in “circular paths” perpendicular to the field lines. This circular motion is called **cyclotron motion**.
- Simultaneously, an electrostatic potential applied to trapping plates at either end of the ICR cell confines the ions axially, preventing them from escaping along the magnetic field lines.

Cyclotron Motion and Excitation:

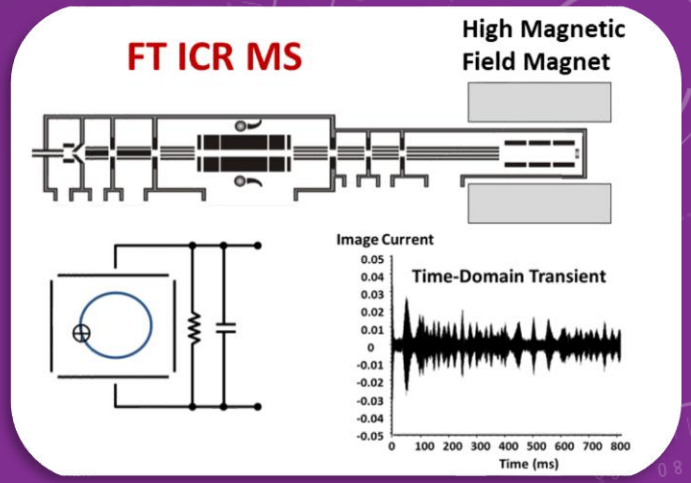
- The frequency of the cyclotron motion (ω_c) is directly proportional to the ion's charge (z) and the magnetic field strength (B), and inversely proportional to its mass (m): **ω_c = zB/m**. This fundamental relationship is the basis of mass analysis in FT-ICR-MS.
- To detect the ions, a radio frequency (RF) pulse is applied to excitation plates within the ICR cell.
- This RF pulse is tuned to the cyclotron frequencies of the ions of interest, causing them increase the radius of their cyclotron orbits. Importantly, ions with the same *m/z* will be excited coherently, forming a packet of ions orbiting in phase.

Image Current Detection:

- As the coherent packets of ions orbit near detection plates within the ICR cell, they induce a time-dependent current on these plates. This is the **image current**.
- Each packet of ions with a specific *m/z* will generate a sinusoidal signal at its characteristic cyclotron frequency.
- The total detected signal is a complex waveform, which is a superposition of all the different cyclotron frequencies corresponding to the different m/z ions present in the ICR cell. This signal decays over time as the coherent ion packets lose phase coherence due to ion-ion interactions and collisions (this decay is related to relaxation processes).

FOURIER TRANSFORM ION CYCLOTRON RESONANCE

FT-ICR MS



Fourier Transform Analysis:

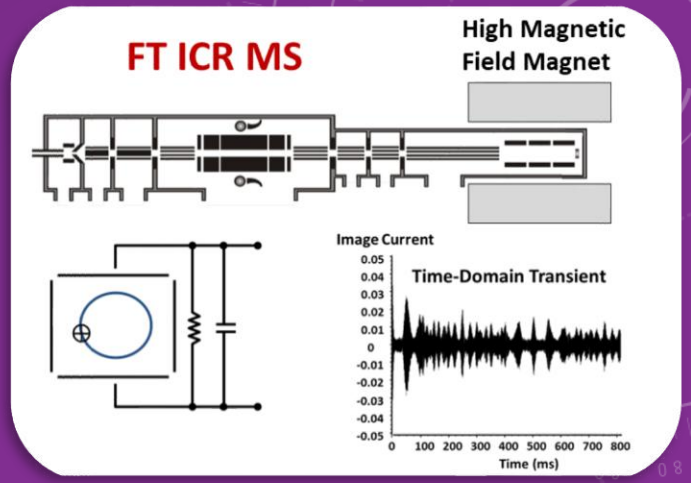
- The detected time-domain signal (the image current as a function of time) is then subjected to a **Fourier Transform (FT)**.
- The Fourier Transform decomposes the complex time-domain signal into its individual frequency components.
- Each frequency component corresponds to the cyclotron frequency of a specific m/z ion within the ICR cell.
- The intensity of each frequency component in the FT spectrum is proportional to the abundance of ions with that particular m/z.

Conversion to Mass Spectrum:

- The output of the Fourier Transform is a frequency-domain spectrum, where the x-axis represents the cyclotron frequency and the y-axis represents the intensity of that frequency.
- Since the cyclotron frequency (ω_c) is directly related to the m/z ratio ($m/z = zB/\omega_c$), and the charge state (z) and magnetic field strength (B) are known, the frequency axis can be converted into an m/z axis.
- The intensities of the frequency peaks are then plotted against their corresponding m/z values to generate the final **mass spectrum**.
- The mass spectrum is a graphical representation where the x-axis shows the m/z values of the detected ions, and the y-axis shows their relative abundance.

FOURIER TRANSFORM ION CYCLOTRON RESONANCE

FT-ICR MS



Ultra-High Resolving Power in FT-ICR MS: Description and Impact

Ultra-High Resolving Power FT-ICR MS stands out for its exceptionally high resolving power, often reaching values of **1,000,000 and beyond (m/Δm)**, and in some specialized research settings even exceeding **10,000,000 or even 200,000,000**. This is significantly higher than other mass analyzers like quadrupole, ion trap, or even typical high-resolution TOF and Orbitrap instruments.

However, this does sacrifice speed, as the length of the measurement increases.

What This Does for Measurement: This ultra-high resolving power has profound implications for mass spectrometry measurements:

- **Separation of Isobaric Ions:** It enables the baseline separation of ions with very small mass differences, including isobaric ions that have the same nominal mass but different exact masses due to variations in elemental composition (e.g., $C_6H_{12}O_6$ vs. $C_5H_{10}O_5N_2$). Differences as small as a few milli-Daltons (mDa) can be resolved.
- **Accurate Mass Determination:** The high resolution allows for more precise determination of the ion's cyclotron frequency, which directly translates to highly accurate mass measurements, often in the **sub-ppm (parts per million)** range. This accuracy is crucial for confirming the elemental composition of identified molecules.
- **Resolution of Isotopic Fine Structure:** In some cases, the resolving power is sufficient to resolve the isotopic fine structure of molecular ions, revealing the relative abundance of different isotopes (e.g., ^{12}C vs. ^{13}C, ^{16}O vs. ^{17}O). This isotopic pattern can provide additional confidence in elemental composition assignments.

FOURIER TRANSFORM ION CYCLOTRON RESONANCE

Current Applications Leveraging Ultra-High Resolving Power:

Intact Protein Analysis (Top-Down Proteomics): FT-ICR MS is a powerful tool for top-down proteomics, where intact proteins are analyzed without prior digestion. The high resolution is essential for resolving different proteoforms (protein isoforms and those with post-translational modifications - PTMs) that often have subtle mass differences. It allows for accurate mass determination of intact proteins and their fragments generated by techniques like ECD or ETD, facilitating the identification and characterization of PTMs, sequence variants, and protein structure.

Lipid Analysis (Lipidomics): The complexity of lipidomes, with numerous isobaric and isomeric lipid species, necessitates the high resolving power of FT-ICR MS. It allows for the separation and identification of lipids with the same nominal mass but differing in fatty acyl chain length, degree of unsaturation, and head group composition. Accurate mass measurements aid in determining the precise elemental composition of lipids, crucial for comprehensive lipid profiling and biomarker discovery.

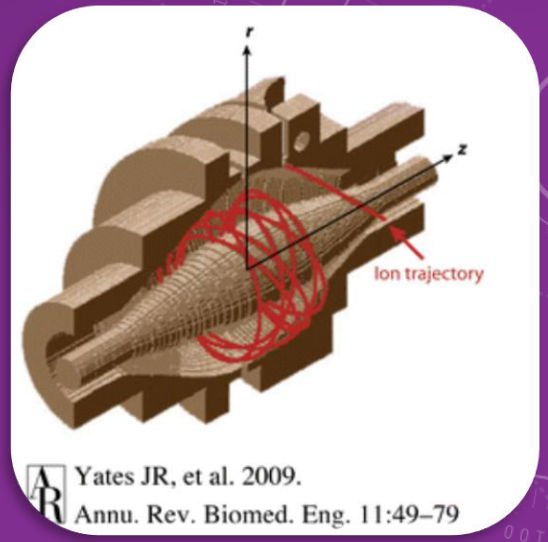
Petroleum Analysis (Petroleomics): Petroleum is an extremely complex mixture containing thousands of hydrocarbons and heteroatom-containing compounds with very similar masses. FT-ICR MS is a cornerstone of petroleomics, enabling the resolution and identification of these individual components based on their unique elemental compositions. This detailed molecular-level characterization is vital for understanding petroleum properties, refining processes, and environmental impact.

“ORBITRAP” MASS ANALYZER

The Orbitrap is a high-resolution, accurate-mass analyzer used in mass spectrometry. It works by trapping ions in an electrostatic field and measuring the frequency of their axial oscillation to determine their mass-to-charge ratio (m/z). Here's a step-by-step description of how it works and how it converts to a mass spectrum:

- The Orbitrap consists of a central spindle-like electrode surrounded by a barrel-like outer electrode.
- A static DC voltage applied to the central electrode creates a complex electrostatic field within the Orbitrap.
- As ions enter the Orbitrap, they are electrostatically attracted towards the central electrode.
- Simultaneously, their tangential velocity causes them to orbit around the central electrode, similar to planets orbiting a star.
- The specific shape of the electrodes and the applied voltage also induce a harmonic axial oscillation (back and forth movement) of the ions along the central electrode. This axial frequency is key to mass analysis.

OrbiTrap



Orbital and Axial Motion

- Each ion within the Orbitrap follows a unique trajectory that is a combination of its orbital motion around the central electrode and its harmonic oscillation along the z-axis (axial direction).
- The **axial oscillation frequency** of an ion is inversely proportional to the square root of its m/z ratio. This means ions with different m/z values will oscillate at different frequencies.
- The radial (orbital) motion ensures the ions remain trapped within the Orbitrap due to a balance between electrostatic attraction and centrifugal force.

“ORBITRAP” MASS ANALYZER

The Orbitrap is a high-resolution, accurate-mass analyzer used in mass spectrometry. It works by trapping ions in an electrostatic field and measuring the frequency of their axial oscillation to determine their mass-to-charge ratio (m/z). Here's a step-by-step description of how it works and how it converts to a mass spectrum:

OrbiTrap

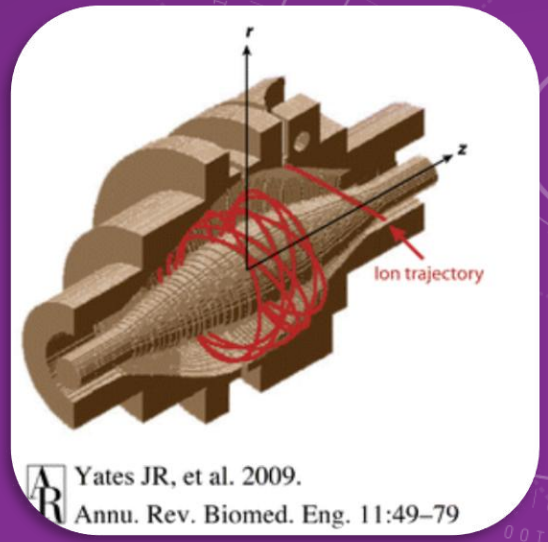


Image Current Detection:

- The outer barrel electrode of the Orbitrap is typically split into two halves.
- As the packets of ions with the same m/z oscillate axially between these two halves, they induce a time-dependent current on the outer electrodes. This is called the **image current**.
- Each group of ions with a specific m/z will generate a sinusoidal signal at their characteristic axial frequency.
- The total signal detected is a complex waveform that is a superposition of all the different frequencies corresponding to the different m/z ions present in the Orbitrap.

Fourier Transform Analysis:

- The detected time-domain signal (the image current as a function of time) is then subjected to a mathematical process called the **Fourier Transform (FT)**.
- The Fourier Transform decomposes the complex time-domain signal into its individual frequency components.
- Each frequency component corresponds to a specific m/z value of the ions oscillating within the Orbitrap.
- The intensity of each frequency component in the FT spectrum is proportional to the abundance of ions with that particular m/z.

“ORBITRAP” MASS ANALYZER

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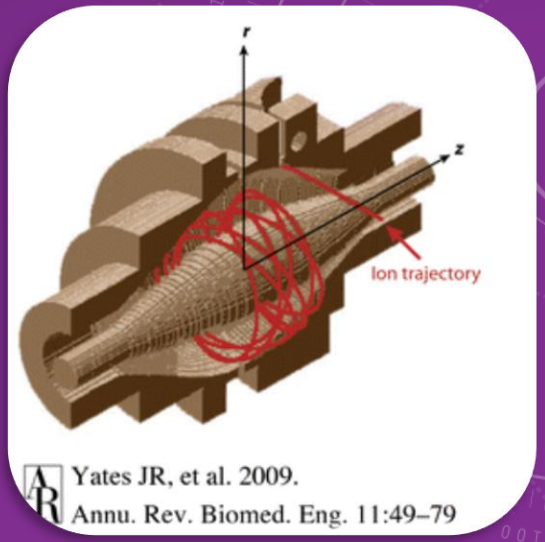
Conversion to Mass Spectrum:

- The output of the Fourier Transform is a frequency-domain spectrum, where the x-axis represents the frequency of axial oscillation and the y-axis represents the intensity of that frequency.
- Since the axial oscillation frequency is directly related to the m/z ratio ($\omega = \sqrt{k/(m/z)}$, where ω is the angular frequency and k is a constant related to the Orbitrap geometry and voltage), the frequency axis can be converted into an m/z axis.
- The intensities of the frequency peaks are then plotted against their corresponding m/z values to generate the final **mass spectrum**.
- The mass spectrum is a graphical representation where the x-axis shows the m/z values of the detected ions, and the y-axis shows their relative abundance.

OrbiTrap

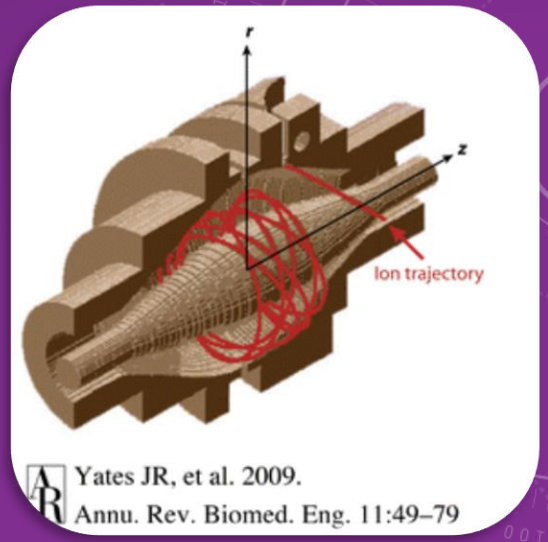


In summary, the Orbitrap achieves mass analysis by:

1. **Trapping** ions in a specific electrostatic field, causing them to orbit and oscillate axially.
2. **Measuring** the characteristic axial oscillation frequencies of the ions through the induced image current on the outer electrodes.
3. **Using Fourier Transform** to convert the time-domain signal into a frequency-domain spectrum, where each frequency corresponds to a specific m/z.
4. **Converting** the frequency spectrum into a mass spectrum by relating the frequencies to their corresponding m/z values and plotting their abundances.

“ORBITRAP” MASS ANALYZER – *HISTORY AND LEGACY*

OrbiTrap



Early Integrations & Hybrid Instruments:

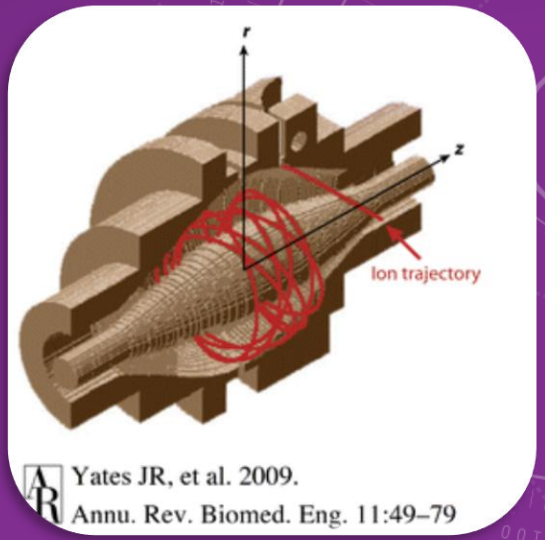
- **LTQ Orbitrap (Classic):** The first commercially successful instrument to integrate the Orbitrap mass analyzer with a linear ion trap (LTQ). Released in the mid-2000s, it provided a significant leap in resolution and mass accuracy compared to traditional ion traps.
- **LTQ Orbitrap XL:** An advancement of the original LTQ Orbitrap, offering improved sensitivity and scan speeds.
- **LTQ Orbitrap Velos:** Introduced a dual-pressure ion trap and further enhanced sensitivity and speed, becoming a workhorse for proteomics.
- **Orbitrap Elite:** Combined a linear ion trap with a high-field Orbitrap, pushing the boundaries of resolution and offering versatile fragmentation techniques.

The "Q Exactive" Series (Quadrupole-Orbitrap Hybrids):

- **Q Exactive:** A benchtop quadrupole-Orbitrap (Q-Orbitrap) system that offered high resolution, accurate mass, and good quantitative capabilities, becoming popular in various fields.
- **Q Exactive Plus:** An evolution of the Q Exactive with improved performance and additional features.
- **Q Exactive HF (High Field):** Featured a more compact, high-field Orbitrap, leading to even higher resolution and faster scan speeds.
- **Q Exactive HF-X:** Further enhanced performance with improved sensitivity and robustness.
- **Q Exactive UHMR (Ultra-High Mass Range):** Specialized for analyzing very large molecules and non-covalent complexes.
- **Q Exactive GC Orbitrap:** Integrated a gas chromatograph with a Q-Orbitrap mass spectrometer, combining the separation power of GC with high-resolution accurate mass detection.

“ORBITRAP” MASS ANALYZER – *HISTORY AND LEGACY*

OrbiTrap



The "Orbitrap Fusion" Series (Tribrid Instruments):

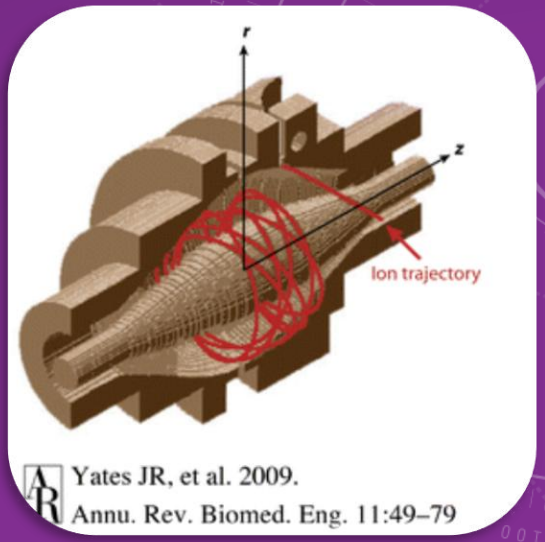
- **Orbitrap Fusion:** A tribrid instrument combining a quadrupole mass filter, a linear ion trap, and an Orbitrap mass analyzer, offering exceptional versatility in terms of ion selection, fragmentation, and detection options.
- **Orbitrap Fusion Lumos:** An advancement of the Fusion platform with increased sensitivity, faster scan speeds, and new fragmentation techniques like UVPD.
- **Orbitrap Tribrid Nova:** Further enhanced sensitivity and speed for demanding applications.
- **Orbitrap Eclipse Tribrid:** The latest generation of the Tribrid platform, offering advanced ion management, real-time search capabilities, and enhanced performance for complex analyses.

The "Orbitrap Exploris" Series (High-Performance Benchtop Instruments):

- **Orbitrap Exploris 120:** A high-performance benchtop Orbitrap system focused on delivering robust and reliable high-resolution accurate mass data.
- **Orbitrap Exploris 240:** Offers higher resolution and sensitivity compared to the 120 model, expanding its application range.
- **Orbitrap Exploris 480:** Provides even higher performance for advanced research and high-throughput analyses.
- **Orbitrap Exploris MX:** A more compact and accessible high-resolution accurate mass system.

“ORBITRAP” MASS ANALYZER – *HISTORY AND LEGACY*

OrbiTrap



The "Orbitrap Ascend" Series (Mid-Range Tribrid Instruments):

- **Orbitrap Ascend:** A mid-range tribrid system offering a balance of performance and accessibility.
- **Orbitrap Ascend MD:** A version tailored for clinical research applications.

The "Orbitrap IQ-X" Series (Advanced Tribrid Instruments):

- **Orbitrap IQ-X Tribrid:** A high-end tribrid system designed for ultimate performance and versatility in complex analyses.

Key Trends and Observations:

- **Hybrid Architectures:** The trend has been towards hybrid instruments combining the Orbitrap with other mass analyzers (quadrupoles, ion traps) to provide a wider range of capabilities for precursor selection, fragmentation, and analysis.
- **Increased Performance:** Over the years, Orbitrap-based instruments have consistently improved in terms of resolution, mass accuracy, sensitivity, and scan speed.
- **Benchtop Form Factors:** While early Orbitrap instruments were often larger, newer generations, like the Q Exactive and Orbitrap Exploris series, have brought high-resolution accurate mass capabilities to more compact benchtop systems.
- **Application-Specific Instruments:** Thermo Fisher has also developed specialized Orbitrap-based instruments tailored for specific applications like GC-MS and ultra-high mass range analysis.

“ORBITRAP” MASS ANALYZER

OrbiTrap

Modern “Hybrid” – Orbitrap Scan Types

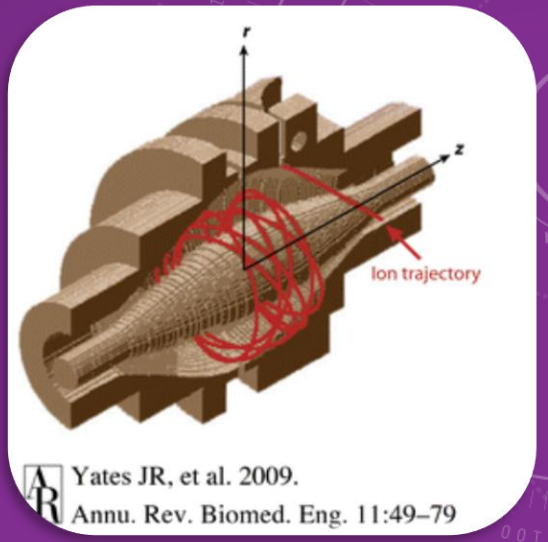
Modern "Hybrid" Orbitrap mass spectrometers, such as the **Thermo Scientific Q Exactive, Orbitrap Fusion, and Orbitrap Exploris series**, offer a versatile range of scan types that leverage the unique combination of a quadrupole mass filter (Q) with the high-resolution, accurate-mass (HRAM) Orbitrap analyzer. These instruments often also include an ion trap (IT) for additional fragmentation and analysis capabilities (Tribrid systems). Here's a description of some common scan types and experiments:

1. Full Scan MS (OrbiTrap Scan):

- **Description:** The quadrupole (Q) acts as a wide mass filter or allows all ions to pass into the Orbitrap. The Orbi then acquires a high-resolution, accurate-mass spectrum across a broad m/z range.
- **Experiment/Use:** This is the fundamental scan for qualitative analysis, identifying all ions present in a sample and determining their accurate masses for potential elemental composition determination. It's also used for quantitative analysis by extracting ion chromatograms of specific m/z values.

“ORBITRAP” MASS ANALYZER

OrbiTrap



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2. Targeted Selected Ion Monitoring (t-SIM) / Selected Ion Monitoring (SIM) (Q-Orbi):

- **Description:** The quadrupole (Q) is used to selectively pass ions within a narrow m/z window of interest into the Orbitrap for high-resolution, accurate-mass detection.
- **Experiment/Use:** This mode enhances sensitivity for target analytes by reducing the complexity of ions entering the Orbi, allowing for longer acquisition times for the specific ions of interest. It's primarily used for quantitative analysis of known compounds where high sensitivity and mass accuracy are required.

“ORBITRAP” MASS ANALYZER

OrbiTrap

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3. Parallel Reaction Monitoring (PRM) / Targeted-MS^2 (t-MS^2) (Q-HCD-Orbi):

- **Description:** The quadrupole (Q) selects a specific precursor ion, which is then fragmented in the Higher-energy Collisional Dissociation (HCD) cell. The resulting product ions are then analyzed at high resolution and accurate mass in the Orbitrap.
- **Experiment/Use:** This is a targeted quantitative MS/MS method. The high resolution of the Orbi for product ions provides increased selectivity compared to traditional SRM/MRM on triple quadrupoles, especially in complex matrices. It's used for sensitive and specific quantification of target analytes with structural confirmation.

“ORBITRAP” MASS ANALYZER

OrbiTrap

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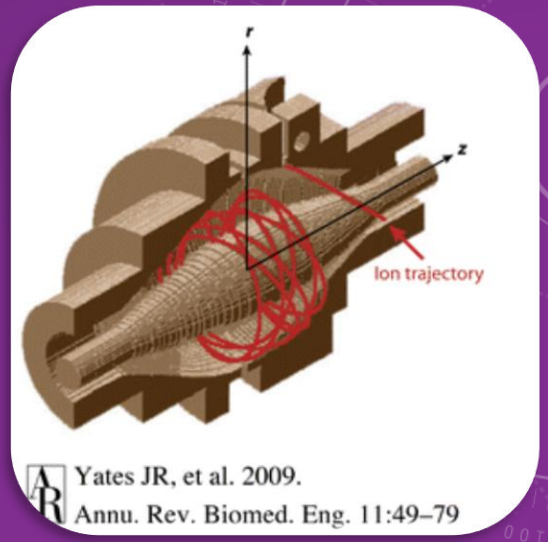
4. Data-Dependent Acquisition (DDA) / Information-Dependent Acquisition (IDA) (Q-HCD-Orbi, Q-CID/ETD-Orbi, etc.):

- **Description:** The instrument performs a Full Scan MS to identify the most abundant ions. Based on predefined criteria (e.g., intensity thresholds), the instrument automatically switches to an MS/MS scan (e.g., PRM-like using HCD or other fragmentation methods like CID or ETD if an ion trap is present) on the selected precursor ions.
- **Experiment/Use:** This is a common mode for qualitative analysis and protein/peptide identification in proteomics and metabolomics. It allows for automated acquisition of fragmentation data for the most abundant compounds to gain structural information.

“ORBITRAP” MASS ANALYZER

OrbiTrap



Modern “Hybrid” – Orbitrap Scan Types

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5. Data-Independent Acquisition (DIA) (Q-HCD-Orbi with wide windows or SWATH-Like):

- **Description:** The quadrupole (Q) sequentially selects a series of wider m/z windows (or in some cases, all ions are allowed to enter). All ions within each window are fragmented in the HCD cell, and the resulting complex MS/MS spectra are acquired at high resolution and accurate mass in the Orbitrap. *Techniques like SWATH (Sequential Window Acquisition of All THeoretical Fragment Ion Spectra) fall under this category – with SCIEX QTOF Instruments*.
- **Experiment/Use:** DIA provides comprehensive fragmentation data for all detectable analytes, enabling more reliable quantitative analysis and retrospective data mining. It's particularly valuable for complex samples in proteomics and metabolomics where unbiased quantification is crucial.
- .

“ORBITRAP” MASS ANALYZER

OrbiTrap

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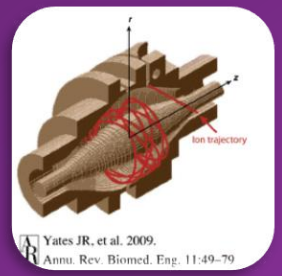
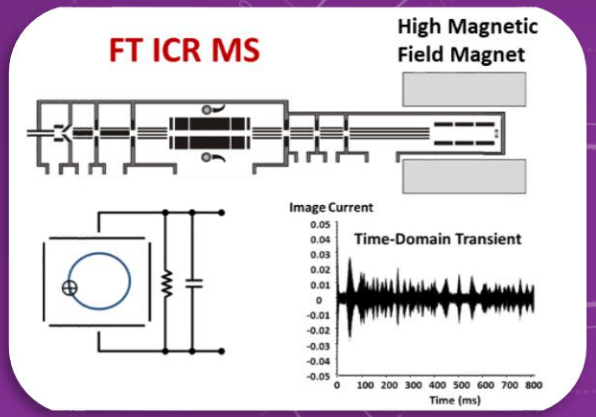
6. MS^n Experiments (Utilizing the Ion Trap - e.g., Q-CID-IT-CID-Orbi):

- **Description:** Tribrid instruments can leverage the ion trap (IT) for multiple stages of fragmentation (MS^n). A precursor ion is selected by the quadrupole (Q), fragmented in the IT (e.g., by CID), a specific fragment ion is then isolated in the IT and further fragmented, with the final fragment ions often analyzed in the high-resolution Orbitrap.
- **Experiment/Use:** This provides detailed structural information, especially for complex molecules like glycans or natural products, by stepwise fragmentation and high-resolution accurate mass analysis of the resulting fragments.

“FT” BASED DETECTION – CONVERSATION OF MEASURED DATA TO M/Z SPECTRUM

Summary for FT Mass Analyzers Data Conversion from Measurement





Ion Detection & Signal Acquisition: The oscillating ions induce a time-domain signal (transient). This signal is recorded as a series of data points over a specific acquisition time.

Zero-Filling: Before performing the Fourier Transform, additional data points with a value of zero are appended to the end of the acquired time-domain signal. This increases the total number of data points.

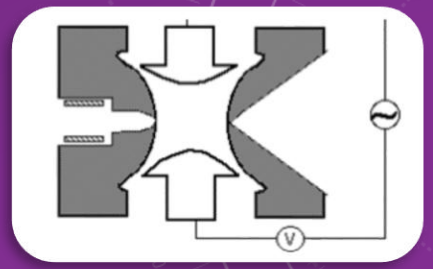
Fourier Transform: The zero-filled time-domain signal is then subjected to a Fourier Transform, converting it into a frequency-domain spectrum. The increased number of data points from zero-filling results in a frequency spectrum with more data points

Frequency to m/z Conversion: The frequencies in the spectrum are converted to mass-to-charge ratio (m/z) values based on the fundamental relationship of the FT MS analyzer. The zero-filling does not change this relationship but provides more discrete points along the m/z axis.

Spectrum Generation: The intensity of each frequency (now representing a specific m/z) is plotted against the m/z value. Due to zero-filling, the resulting mass spectrum will have more data points, leading to a smoother appearance of the peaks, effectively interpolating between the original frequency points.

QUADRUPOLE ION TRAP MASS SPECTROMETRY (QIT)

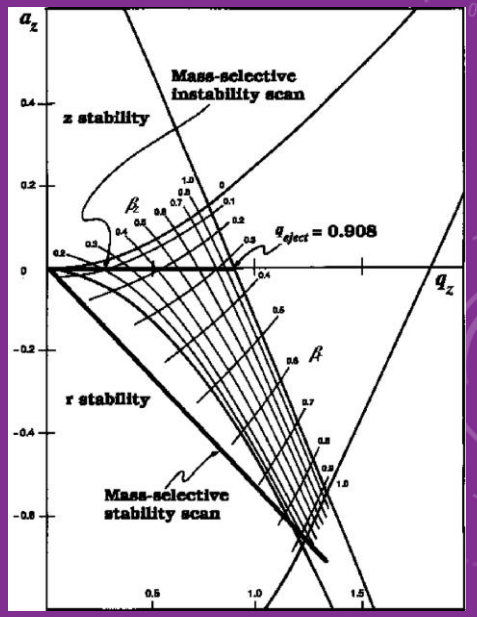
QIT



Ion Traps (QIT): Confining Ions with Electric Fields

Ion traps are devices that use **electric fields**, *(and sometimes magnetic fields)*, to **confine and manipulate charged particles (ions) in a defined space**. They are a fundamental component of many mass spectrometers, allowing for the isolation, storage, and analysis of ions based on their mass-to-charge ratio (m/z).

Typical stability diagram for a quadrupole ion trap



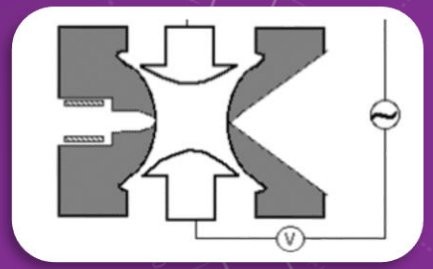
https://soar.wichita.edu/items/1ea09553-f4fe-4259-b26e-db1f96f03529

1. The Basic Principle: Electric Fields and Ion Motion

- Ions, being charged particles, experience a force when placed in an electric field (F=qE, where q is the charge and E is the electric field).
- By carefully shaping and controlling electric fields in three dimensions, it's possible to create a potential well that traps ions. Imagine a valley where if you place a ball (the ion), it will tend to stay within the confines of the valley due to gravity (the electric field).
- **Earnshaw's Theorem** states that a static electric field alone cannot create a stable three-dimensional trap for charged particles. This is why ion traps typically employ **dynamic (time-varying) electric fields**, often in the radio frequency (RF) range.

QUADRUPOLE ION TRAP MASS SPECTROMETRY (QIT)

Paul Trap - QIT



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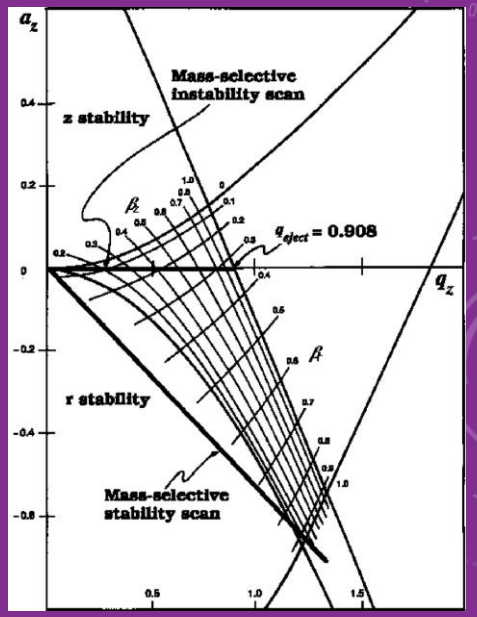
2a. Common Ion Trap Geometries and Operation:

- ***Quadrupole Ion Trap (QIT) / Paul Trap (3D Ion Trap):***
  - Consists of three electrodes with hyperbolic or near-hyperbolic surfaces: a ring electrode and two end-cap electrodes.
  - An **oscillating RF voltage** is applied to the ring electrode, while the end-caps are typically held at a DC voltage or are grounded.
  - The combination of the static and oscillating electric fields creates a complex, time-varying potential that confines ions within the trap.
  - Ions with a stable trajectory within this field are trapped. The stability of an ion's trajectory depends on its m/z ratio and the applied RF and DC voltages.

Typical stability diagram for a quadrupole ion trap



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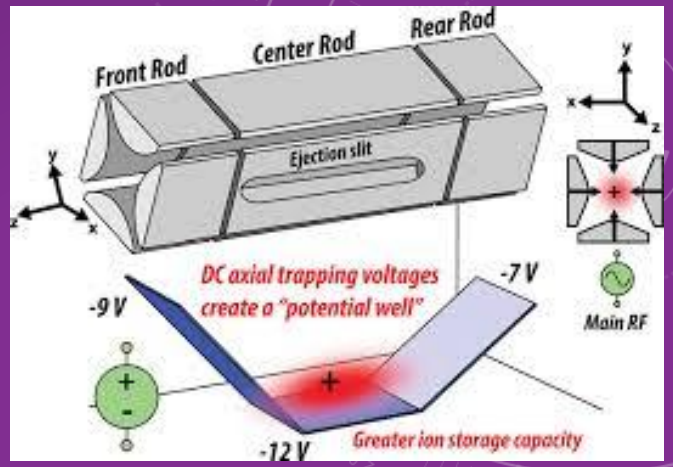
2b. Common Ion Trap Geometries and Operation:

- ***Linear Ion Trap (LIT):***
  - Uses a set of parallel rod electrodes (typically four).
  - An RF voltage is applied to one pair of opposing rods, and the other pair is either grounded or has an opposite RF voltage.
  - **Static DC voltages** are applied to segmented end electrodes to confine ions along the axial (longitudinal) direction of the trap.
  - Similar to the 3D trap, ions with stable trajectories in the combined RF and DC fields are trapped.

Linear Ion Trap - QIT



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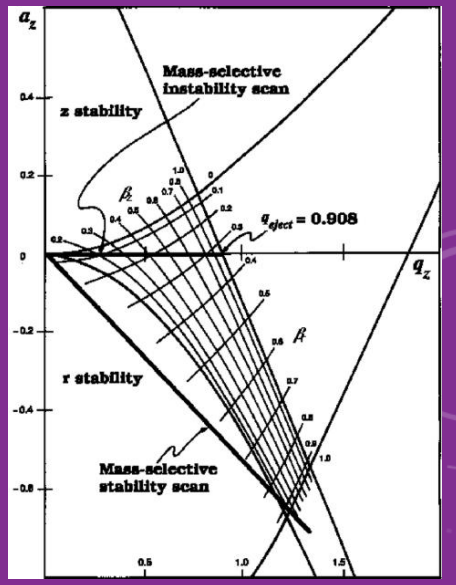

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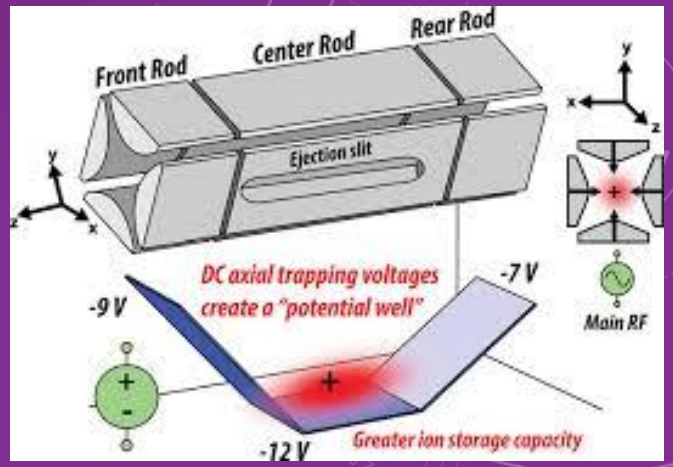
3. Ion Motion within the Trap:

- Within the trapping field, ions undergo complex oscillatory motion at frequencies that are related to their m/z ratio and the applied electric field parameters. These are often referred to as **secular frequencies**.

- The amplitude and stability of these oscillations are crucial for trapping. Ions with unstable trajectories will collide with the electrodes and be lost.

Linear Ion Trap - QIT



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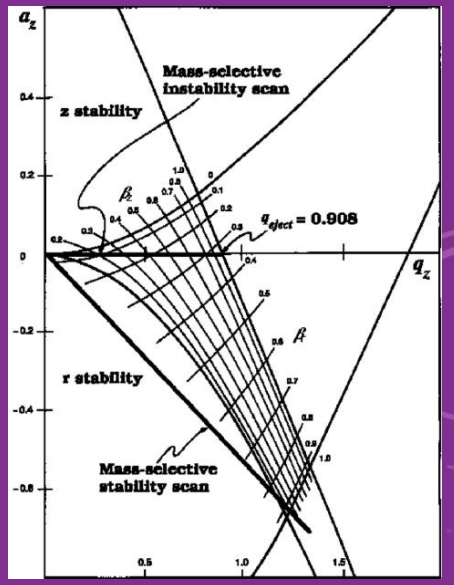

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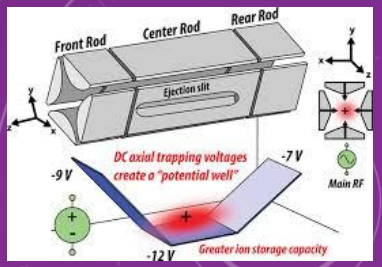


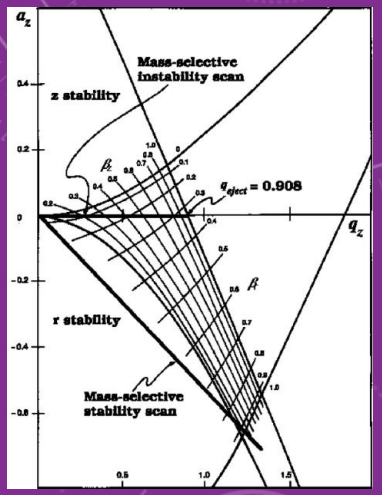
4. Ion Ejection Based on Electric Fields:

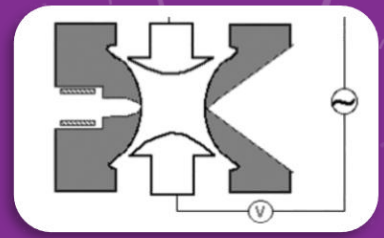
To analyze the trapped ions in a mass spectrometer, they need to be selectively ejected from the trap and directed towards a detector. This ejection process is primarily controlled by manipulating the electric fields applied to the trap electrodes. Here are common methods:

A. **Ramping the RF Voltage (Mass-Selective Instability):**
- In a QIT, by gradually increasing the amplitude of the RF voltage applied to the ring electrode, the stability of ions within the trap is altered.
- As the RF voltage increases, ions with progressively higher m/z values become unstable and are sequentially ejected from the trap.
- The ejected ions are typically focused through an exit aperture in one of the end-cap electrodes and directed to a detector.
- This sequential ejection creates a mass spectrum as ions of different m/z values reach the detector at different times.

B. **Applying Supplemental AC or RF Voltages (Resonance Ejection):**
- A small AC or RF voltage at a specific frequency can be applied to the end-cap or ring electrode.
- If this frequency matches the secular frequency of ions with a particular m/z ratio, those ions will absorb energy and their oscillations will become amplified.
- The increased amplitude of oscillation leads to instability, and the ions are ejected from the trap.
- By scanning the frequency of the supplemental voltage, ions of different m/z values can be selectively ejected. This method can offer better mass resolution in some cases.

C. Applying DC Voltage Pulses (Axial Ejection in LITs):
- In LITs, ions are confined axially by DC potentials on the end segments.
- To eject ions, these DC potentials can be rapidly changed or pulsed.
- By carefully timing and shaping these DC pulses, ions can be pushed out of the trap along the axial direction towards the detector.
- This ejection can be made mass-selective by combining it with RF manipulation to isolate specific m/z ranges within the trap before ejection.





MASS SPEC – ANALYZER TYPE - GENERALIZATIONS

Instrument	Resolving Power	Mass Accuracy	Scan Speed	Typical Applications
Quadrupole (QqQ)	Low to Medium (up to ~4,000)	Medium (0.1 - 1 Da)	Fast	Quantitative analysis, MRM, SIM
Time-of-Flight (Q-TOF)	Medium to High (up to ~100,000)	High (1 - 10 ppm)	Fast	Proteomics, Metabolomics, Imaging, etc.
Orbitrap (Q+Orbi)	High to Very High (up to >1,000,000)	High to Very High (<1 ppm)	Medium to Slow	High-resolution mass spectrometry, Proteomics
FT ICR MS	High to Very High (up to >10,000,000)	High to Very High (<1 ppm)	Medium to Slow	Highest-resolution mass spectrometry, Proteomics
Ion Trap	Low to Medium	Medium (0.1 - 1 Da)	Slow	Qualitative analysis, MS^n fragmentation

THAT’S IT FOR NOW – MASS SPECTROMETRY BASICS AND REVIEW

Updated - April 2025

MASS SPECTROMETRY FUNDAMENTALS MASS-TO-CHARGE MEASUREMENT AND INSTRUMENTATION

Updated April 2025

General Presentation on Basic Fundamentals of Mass Spectrometry

Overall Workflow - Module Considerations

1. **Introduction to Mass Spectrometry Fundamentals**
2. **Components of a Mass Spectrometer**
3. **What is mass-to-charge?**
4. **What is Resolving Power?**
5. **Ionization Techniques (ESI, MALDI, etc.)**
6. **Types of Mass Analyzers (Q, QQQ, TOF, QTOF, IM, FTICR MS, and Orbi)**