Introduction to Mass Spectrometry

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What Is Mass Spectrometry

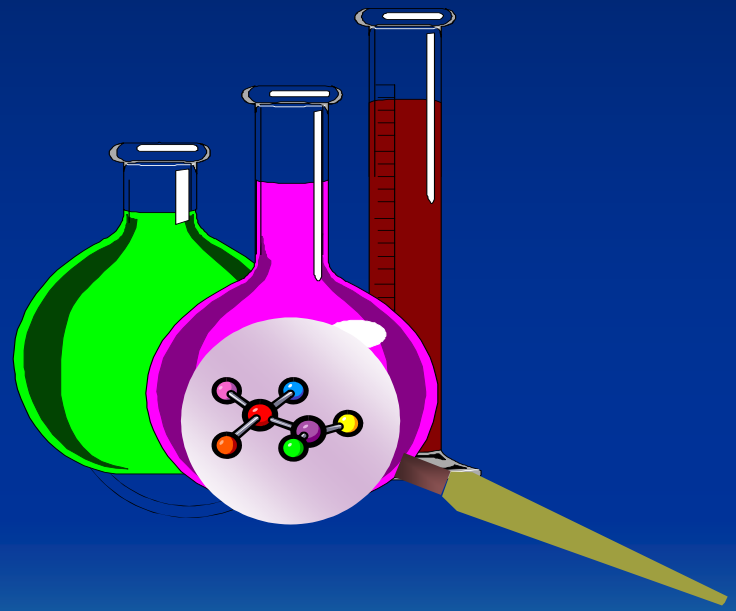
Mass Spectrometrist Definition:

Mass spectrometry is a powerful analytical technique that is used to identify unknown compounds, to quantify known materials, and to elucidate the structure and chemical properties of molecules.

Simple Definition:

Mass spectrometry is a technology that helps scientists:

1. ***identify molecules present in solids, liquids and gases.***
2. ***determine the quantity of each type of molecule.***
3. ***determine which atoms comprise a molecule and how they are arranged.***



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Where Can Mass Spectrometry Be Used?

Mass spectrometry provides valuable information to a wide range of professionals: physicists, chemists, biologists, physicians, astronomers, and more.

Mass spectrometry is used to...

- **Detect and identify the use of steroids in athletes**
- **Monitor the breath of patients by anesthesiologists during surgery**
- **Detect heart attack from a blood test**
- **Determine the composition of molecular species found in space**
- **Locate oil deposits by measuring petroleum precursors in rock**
- **Monitor fermentation processes for the biotechnology industry**
- **Detect dioxins in contaminated fish**
- **Determine how drugs are used by the body**
- **Determine gene damage from environmental causes**
- **Sequencing proteins, nucleic acid and oligosaccharides**
- **Determine the age and origins of specimens in geochemistry and archaeology**
- **... ...**

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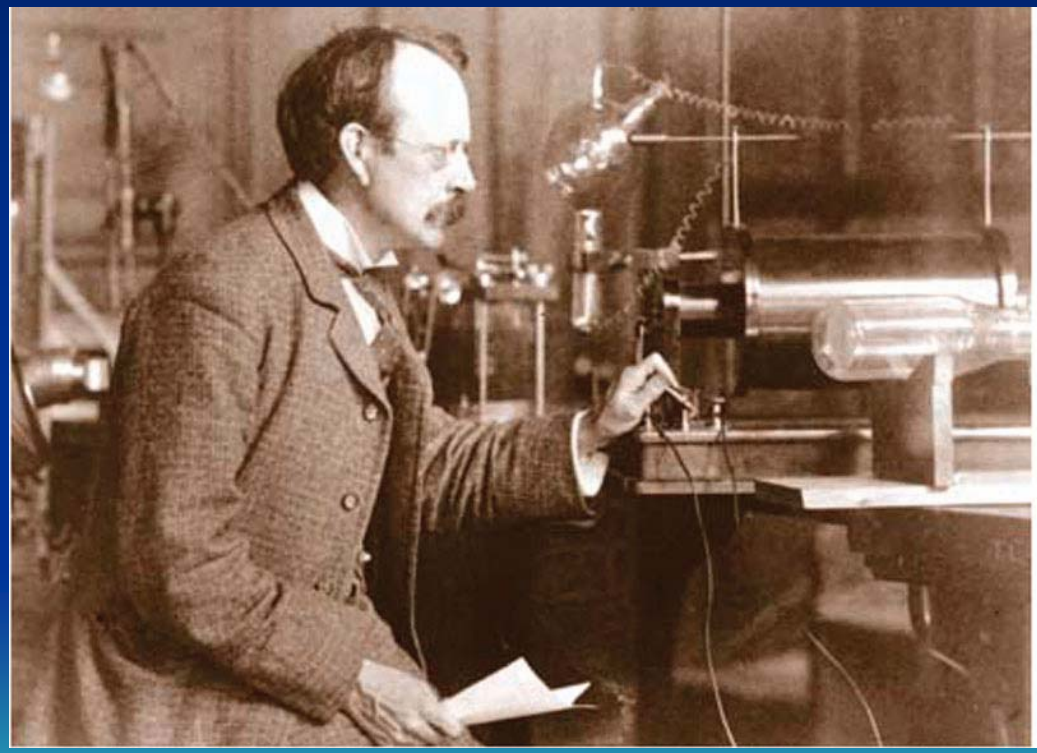
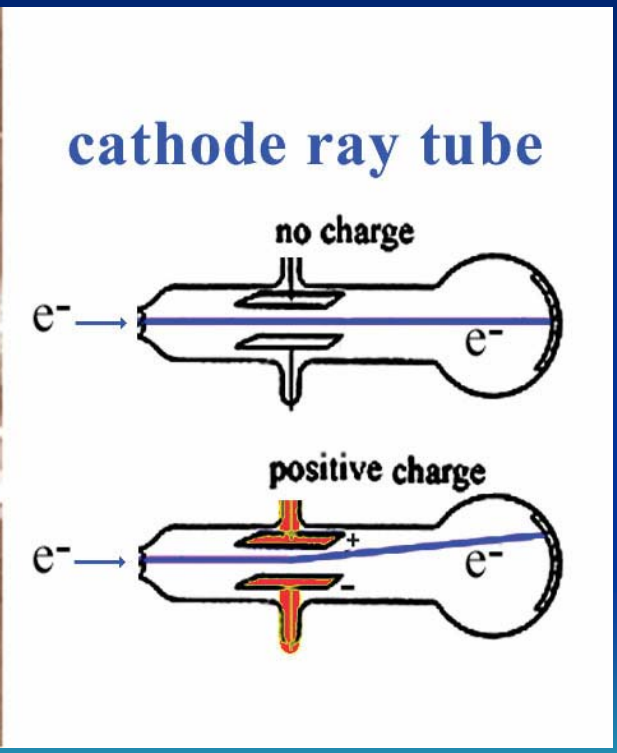
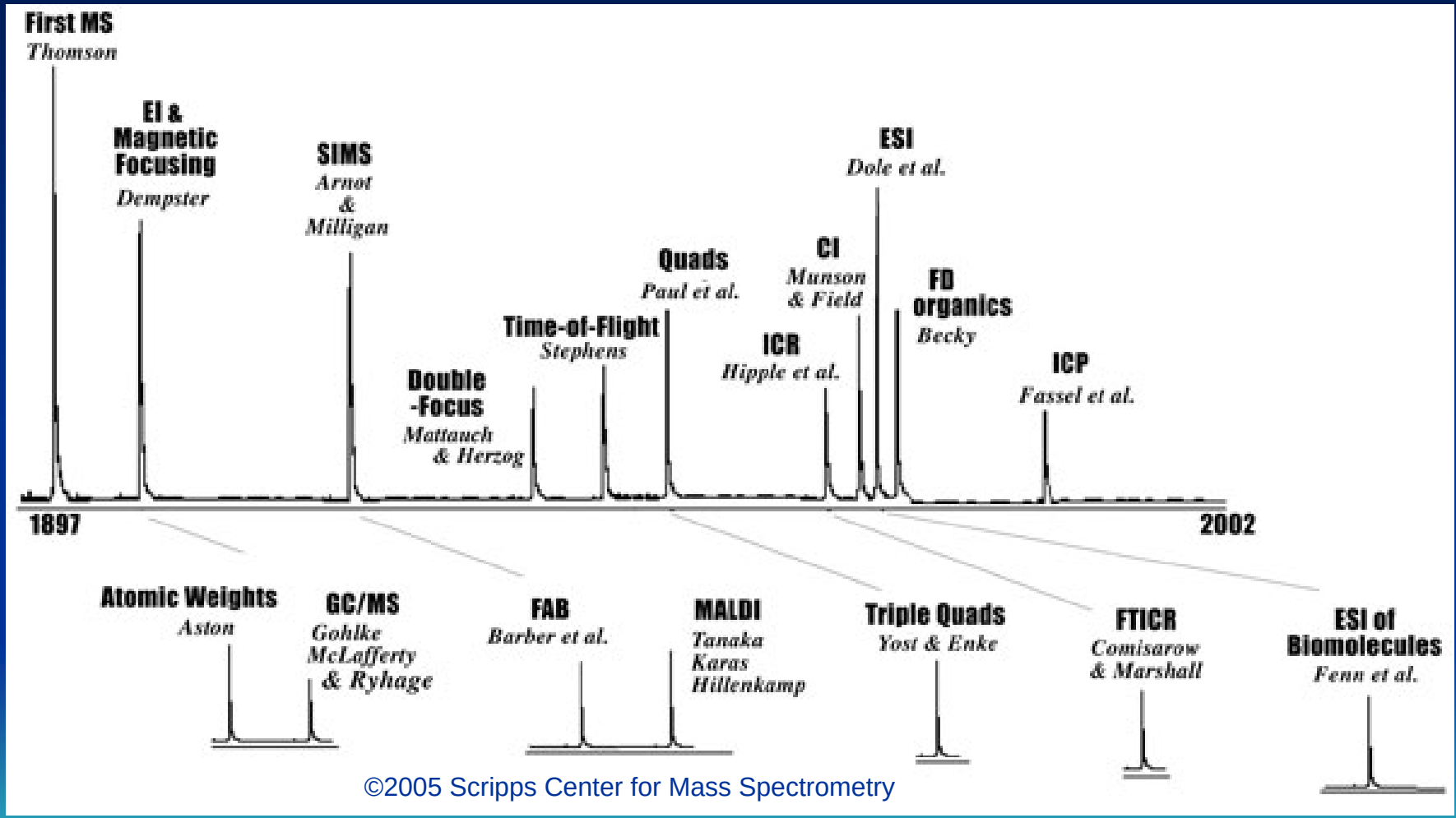
How Did Mass Spectrometry Originate?

The technique of mass spectrometry begins in J.J. Thomson's vacuum tube in 1889 – demonstrate the existence of electrons and "positive rays".





A History of Mass Spectrometry



Nobel Prize in Mass Spectrometry

- In 1899, J.J. Thompson built the first mass spectrometer awarded Physics Nobel Prize in 1906
- In 1919, Francis Anston observed isotopes using mass spectrometry, awarded Chemistry Nobel Prize in 1922
- In 1953, Wolfgang Paul invented ion quadrupole and ion trap mass spectrometers, awarded Physics Nobel Prize in 1989
- In 1988-9, John Fenn and Koich Tanaka developed ESI and MALDI for ionizing large molecules, awarded Chemistry Nobel Prize in 2002

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What Is A Mass Spectrometer

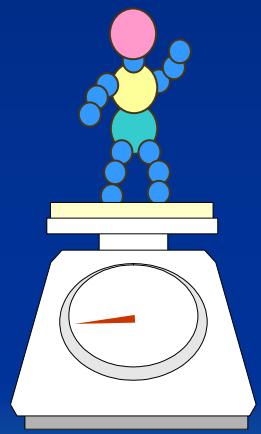
Mass Spectrometrist Definition:

A mass spectrometer is an instrument that measures the masses of individual molecules that have been converted to ions, i.e. molecules that have been electrically charged.

Simple Definition:

A machine used to weigh molecules.

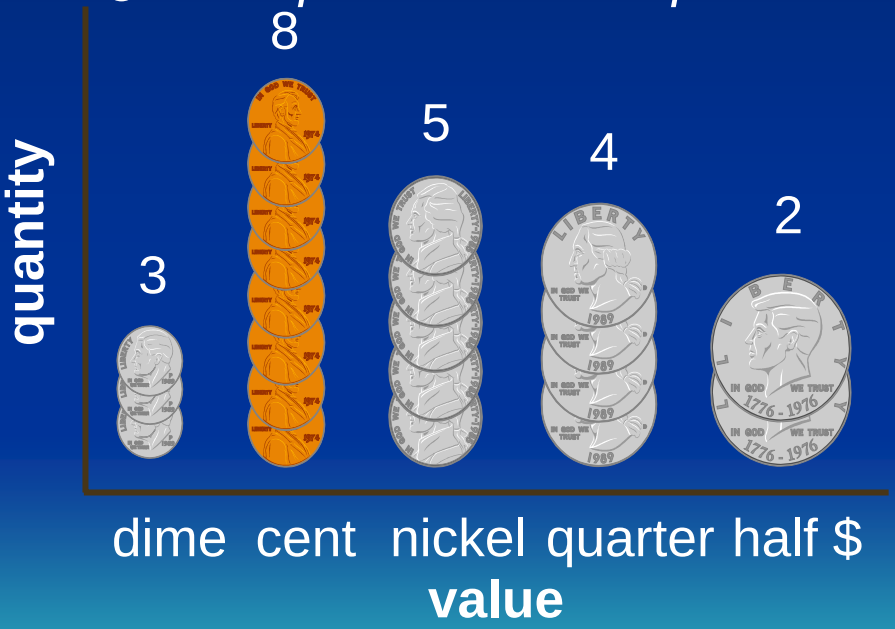
A molecular scale.



Concept of Mass Analysis

Sorting/counting coins analogy

- *Mixture of molecules* ⟷ *Pocket change (mixture of coins)*
- *Molecules of different weight, size* ⟷ *Penny, dime, nickel, quarter, half $*
- *Separation by "mass"* ⟷ *Sorting change by value or size*
- *spectrum* ⟷ *Concept of visual interpretation*



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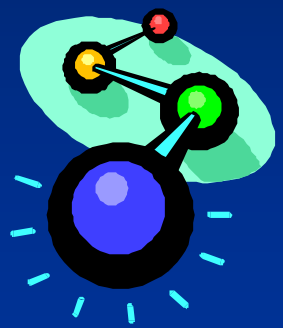
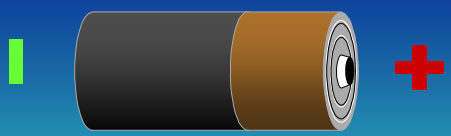
Concept of Ions and Charges

A mass spectrometer measures mass-to-charge ratio (*m/z*) instead of mass. Therefore, in order to be measured, molecules need to be charged (or ionized).

1. *An ion is an electrically charged molecule.*



2. *An ion can be positively (+) charged or negatively (-)charged. Consider the poles on a battery.*



3. *A mass spectrometer "weighs" molecules electronically by attracting and repelling ions. Consider magnets. Opposites attract. Like charges repel.*

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How Does A Modern Mass Spectrometer Look Like

An actual mass spectrometer ranges in size from about the size of a microwave oven to large research instruments that dominate entire rooms.

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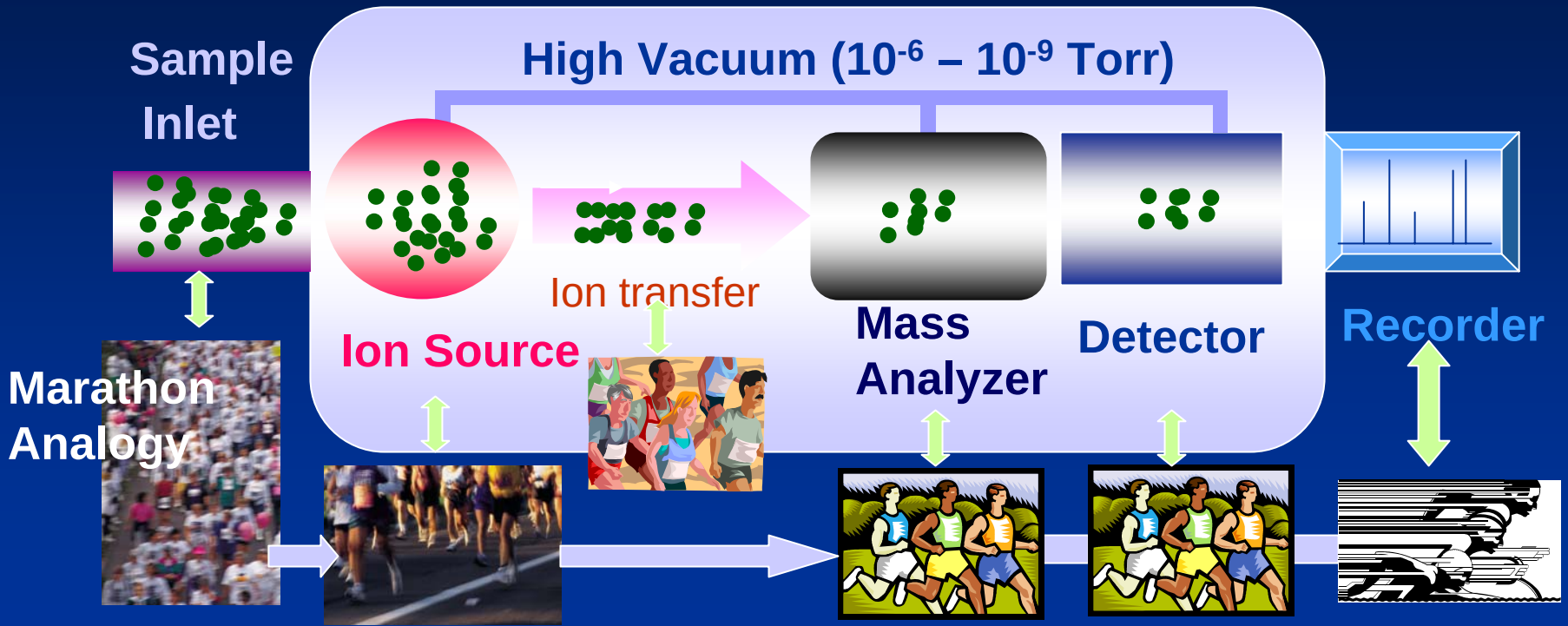
What's Inside A Mass Spectrometer

A mass spectrometer is composed of five essential parts:



1. Inlet: introducing samples from ambient room pressure into ion source
2. Ion source: converting sample molecules to ions
3. Mass analyzer: separating ions according to their mass
4. Detector: detecting ions and amplifying the signal
5. Recorder: receiving signal from detector, further amplifying, recording, creating mass spectrum

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How Is The Sample Introduced Into Mass Spectrometer?

An inlet system is needed to transfer the sample from the atmospheric pressure (760 Torr) into the source as mass spectrometers are operated in vacuum (~10^{-6} - 10^{-9} Torr).

Common Inlet Systems:

1. Chromatography inlets:
   - Gas chromatography (GC)
   - Liquid chromatography (LC)
   - Capillary electrophoresis (CE)

2. Syringe for direct infusion

3. Probe or plate

Agilent HPLC

Standard Infusion Syringe Pump from Harvard Apparatus

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What Are Ionization Techniques?

Ionization is a process of charging a molecule. Molecules must be charged in order to measure them using a mass spectrometer. "It makes a molecule fly in a mass spectrometer."

Common Ionization Technologies

Basic Type	Name and Acronym
Gas Phase (for volatile compounds)	Electron Ionization (EI)
	Chemical Ionization (CI)
Condensed Phase Desorption (for nonvolatile compounds)	Fast atom bombardment (FAB)
	Matrix-assisted desorption/ionization (MALDI)
	Atmospheric pressure chemical ionization (APCI)
	Electrospray ionization (ESI)

ESI and MALDI

ESI and MALDI are the two ionization techniques that make molecule elephant (i.e. Proteins) fly.

ESI (Electrospray):
formation of charged liquid droplets from which ions are desolvated or desorbed.

MALDI *(matrix assisted laser desorption ionization) impact of high energy photons on a sample imbedded in a solid organic matrix.*

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How Does Mass Analyzer Work?

The analyzer uses dispersion or filtering to sort ions according to their mass-to-charge ratios or a related property.

The most common analyzers:

1. Magnetic sectors**:**

   Magnetic field affect radius of curvature of ions -> *m/z*
2. Quadrupole mass filters

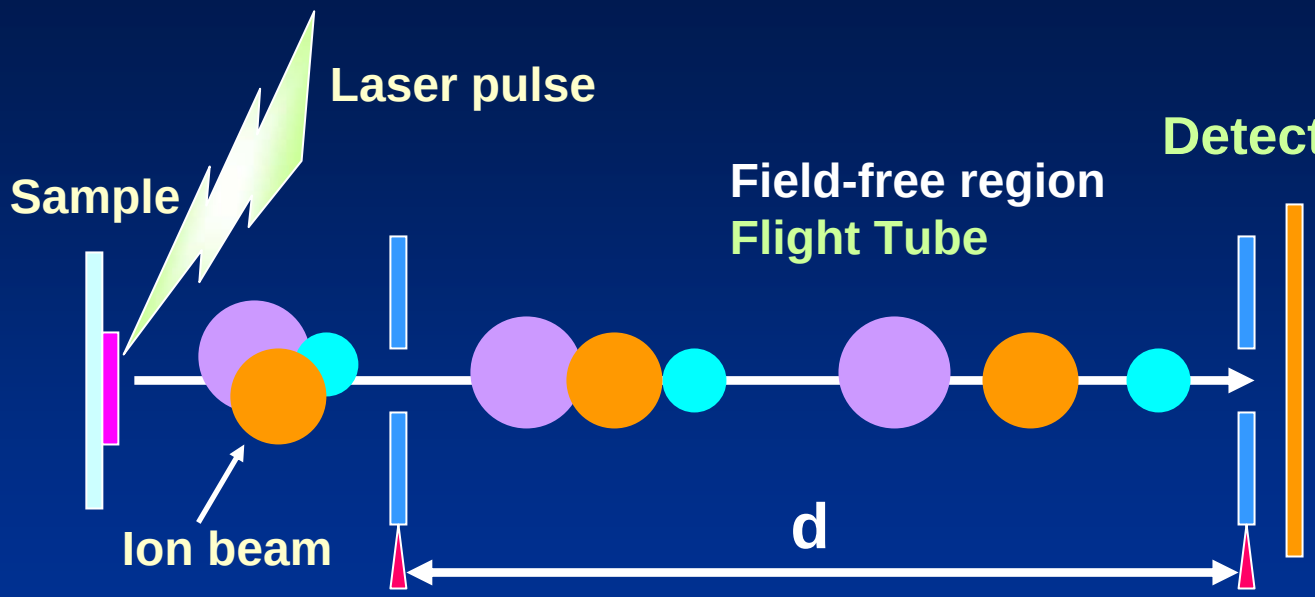
   Scan radio frequency field-> *m/z*
3. Quadrupole ion traps (QIT)

   Scan radio frequency field-> *m/z*
4. Fourier transform ion cyclotron resonance (FT ICR)

   Image current – ion cyclotron frequency -> *m/z*
5. Time-of-flight mass (TOF)

   Flight time - correlated directly with ion's *m/z*

How Does Time-Of-Flight Mass Analyzer Work?



Time-of-flight
$m/z = 2Vt^2/d^2$
m/z determined from ion's time of arrival
Smaller ions - higher V reach the detector earlier than larger ions

$m/z = 2Vt^2/d^2$

Rabbit runs faster than Sheep and faster than Elephant.

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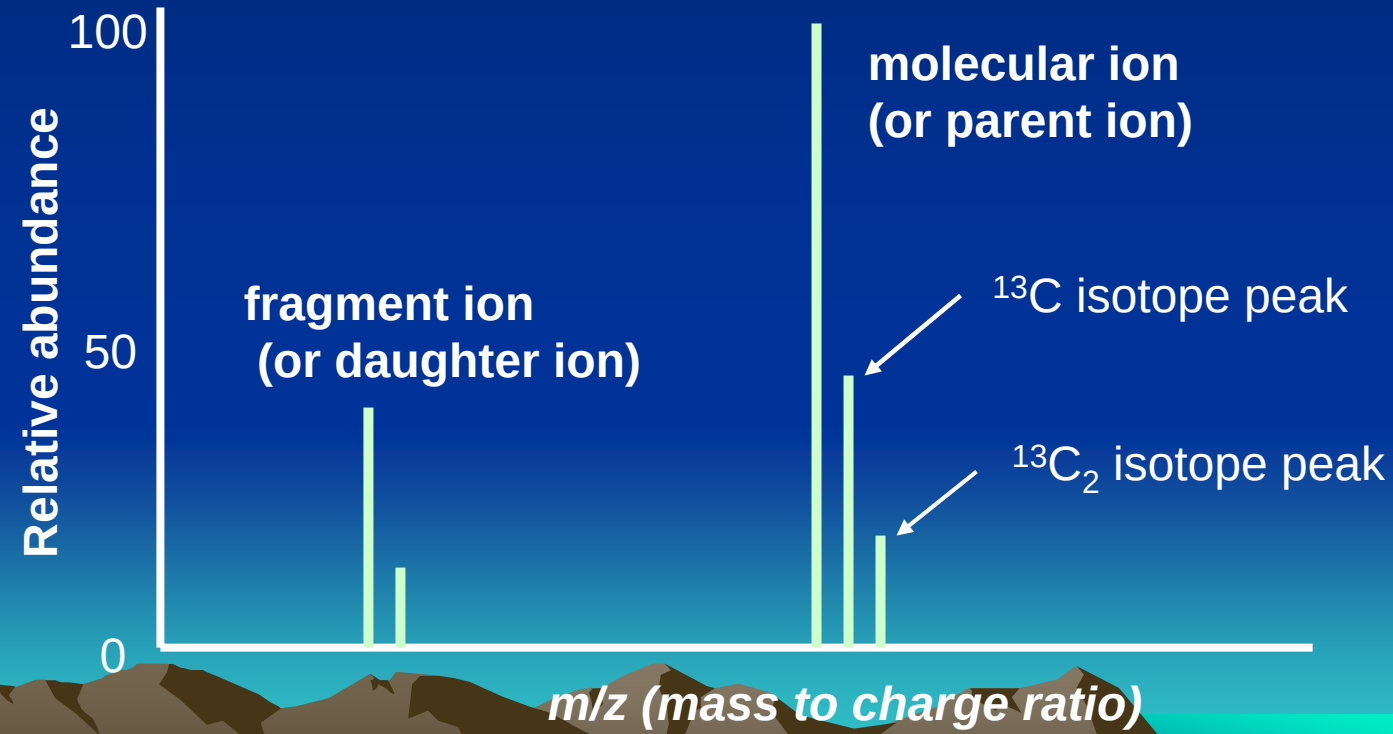
How Does A Mass Spectrum look like?
- A Bar Graph Format

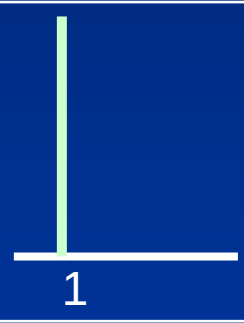
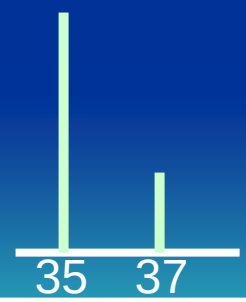
Molecular ion: An ion formed by the removal of one or more electrons to form a positive ion or the addition of one or more electrons to form a negative ion, also called parent ion or precursor ion.

Fragment ion: A product ion (or daughter ion) resulting from the dissociation of a precursor ion.



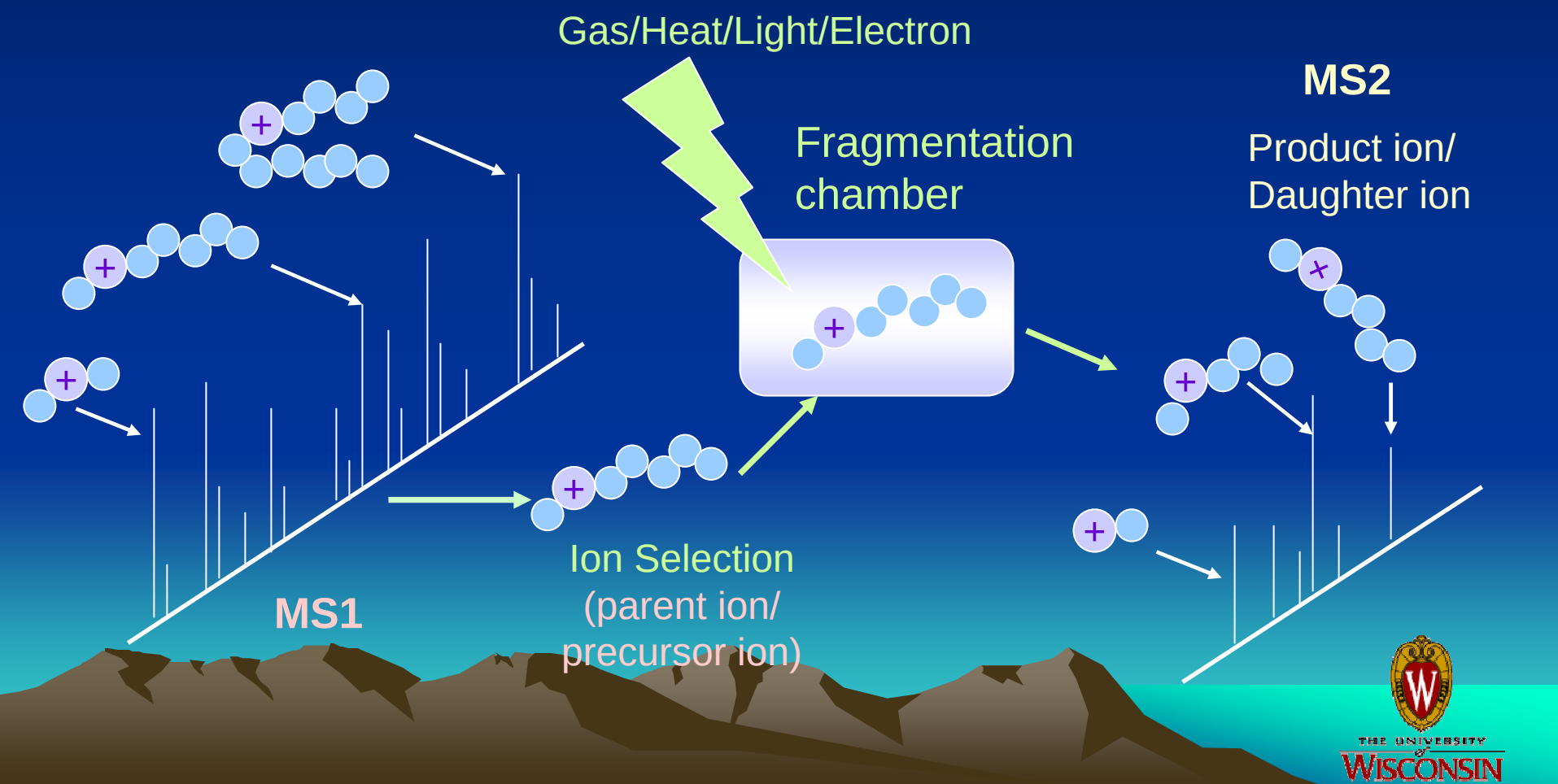
How Does Mass Spectra look like?
- Isotope Effect

Isotope: one of two or more forms of a single element; the atoms of each isotope have the same number of protons but different numbers of neutrons in the nuclei of an atom.

Element	M, %	M+1, %	M+2, %	Mass Spectrum
H	1, 100	2, 0.015		
Cl	35, 100		37, 32	

What Is Tandem Mass Spectrometry?

A sample is measured (weighed) in the first mass spectrometer, then a certain ion is selected and broken into pieces in the collision cell, and the fragments (product ion/daughter ion) are weighed in the second mass spectrometer.
Coupling two stages of mass analysis (MS/MS) to identify compounds in complex mixtures and in determining structures of unknown substances.



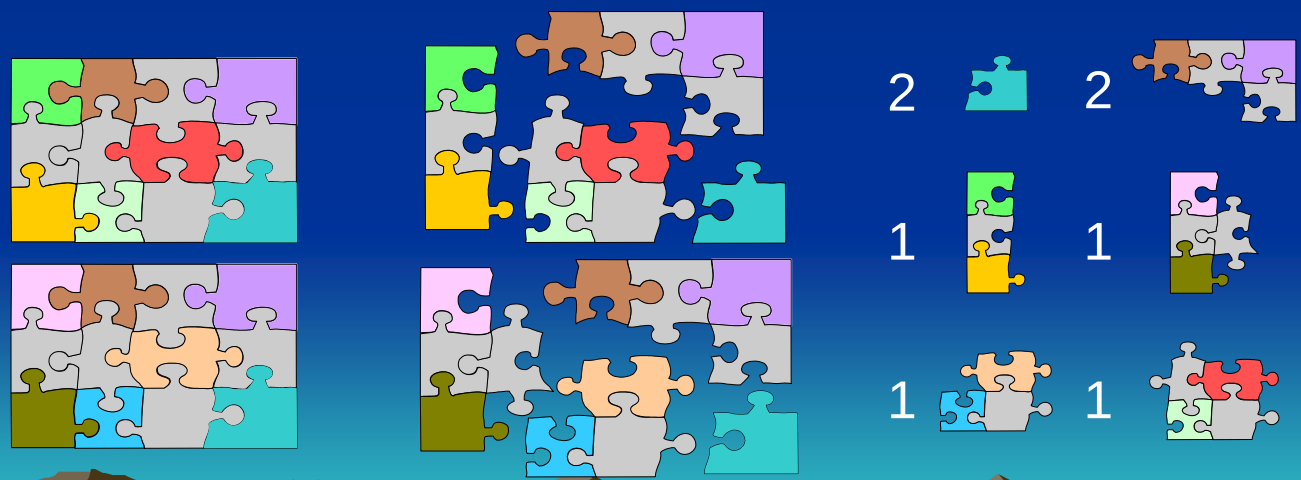
What Is Tandem Mass Spectrometry?

*Simple Definition: Two mass spectrometers joined by a chamber than breaks apart molecules. **

**This definition is appropriate for tandem-in-space but not for tandem-in-time.*

- Puzzle analogy

MS #1	Fragmentation Chamber	MS #2
Sorting molecules	*Breaking molecules*	*Sorting Pieces*



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What Is Tandem Mass Spectrometry? (Cont'd)

- Word Analogy

Molecules		Words
Comprised of atoms	⟷	Comprised of letters
Arrangement of atoms gives molecules function.	⟷	Arrangement of letters gives words meaning.
Special groups of atoms make functional groups.	⟷	Special groups of letters make syllables.
Common side chains = acids	⟷	Common endings = suffixes
$H_2NCH_2COOC_4H_9$ $H_2NCH(CH_2)COOC_4H_9$ $H_2NCH(C_6H_5)COOC_4H_9$	⟷	Talking Writing Playing

Detect all molecules containing a butyl formate functional group from an α-amino acid – Product ion scan. Use a neutrol loss scan function to detect only molecules that lose a butylformate function group weighing 102 Da. Neutral loss scan

List all words containing "ing" in the book of abstracts. Use a computer to search for the string "ing" and it displays all words containing "ing."

How to Use Mass Spectrometry for Quantification? - Isotope Dilution Mass Spectrometry

Simple Definition:

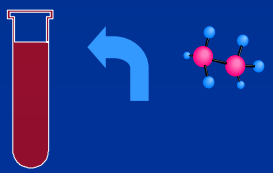
It is a method that measures how much compound X is present in a liquid, solid or gas. This method uses non-radioactive elements called stable isotopes to make a comparison of compound X with the standard that contains the stable isotope. Since the amount of stable isotope standard is known we can calculate how much compound X is present.

Jelly Bean Analogy

How much phenylalanine is present in Blood

How many Cherry Jelly Beans are in the jar?

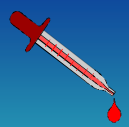
1. Add a marker or standard



10 picomoles d_5 Phenylalanine

10 Blueberry Jelly Beans

2. Obtain a sample.



10 uL (1 drop)

1 oz

How to Use Mass Spectrometry for Quantification?
- Isotope Dilution Mass Spectrometry (Cont'd)

- Jelly Bean Analogy

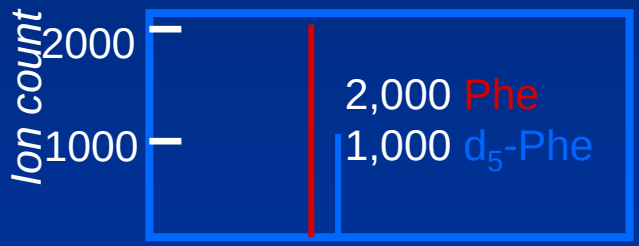
How many Cherry Jelly Beans are in the jar?

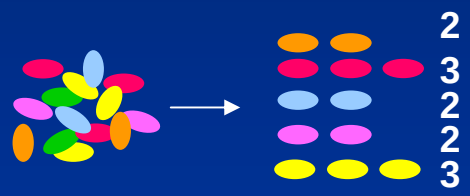
How much phenylalanine is present in Blood

Sort Phenylalanine by MS/MS Count how many.

3. Analysis

Sort jelly beans in the cup by flavor/color Count how many.





4. Calculations

a.) 2,000 Phe, 1,000 i.s.
b.) 2/1 ratio of Phe to I.S.
c.) 10 pmol i.s. added to 1 mL blood
d.) 10 x 2/1 = 20 pmol
e.) 20pmol Phe per mL of blood

a.) 3 Cherry Red, 2 Blueberry in 1 oz
b.) 3/2 ratio of Cherry to Blueberry
c.) 10 Blueberry added to 1 oz.
d.) 10 x 3/2 = 15
e.) 15 Cherry Red Jelly Beans in Jar

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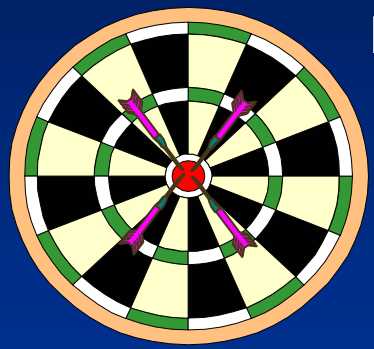
What Are Accuracy and Precision?

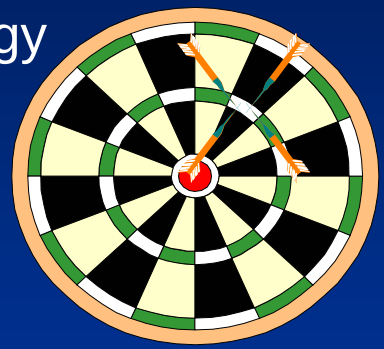
Mass Spectrometrists always say:

Mass Spectrometry is very accurate and precise.

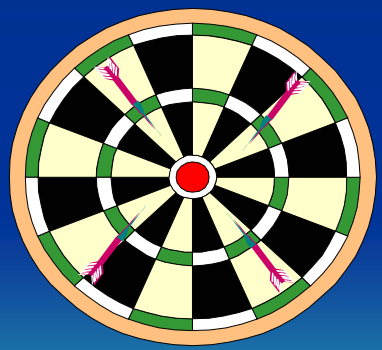
Dart Board Analogy



Accurate & Precise



Precise, Not Accurate



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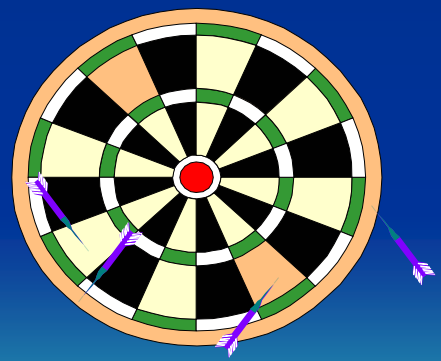

Not Accurate, Not Precise

References

1. Donald H. Chace , “Mass what?”, © Donald H. Chace, 2005 Pediatrix Medical Group.

2. Stu Borman, Hailey Russel, and Gary Siuzdak, “A Mass Spec Timeline”, SEPTEMBER 2003 TODAY’S CHEMIST AT WORK, ©2003 AMERICAN CHEMICAL SOCIETY

3. Chia M. Chiu and David C. Muddiman “What is mass spectrometry”, American Society for Mass Spectrometry: Education http://www.asms.org/whatisms/index.html

4. Gary Siuzdak, http://masspec.scripps.edu/MSHistory/ ©2005 Scripps Center for Mass Spectrometry

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- The author would like to thank Dr. Donald H. Chace for kindly granting the permission to use the materials in his poster, “Mass what?”, © Donald H. Chace, 2005 Pediatrix Medical Group.

- The author would like to thank Dr. Gary Siuzdak for kindly granting the permission to use the materials from his website http://masspec.scripps.edu/MSHistory/ ©2005 Scripps Center for Mass Spectrometry.

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