University of California
San Francisco

Basics of Mass Spectrometry in the Clinical Laboratory

Deborah French Ph.D., DABCC, FACB

Assistant Director of Chemistry

Director of Mass Spectrometry

UCSF Clinical Laboratories

San Francisco, CA

Mass Spectrometry in the Clinical Laboratory:
Best Practices and Current Applications

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Learning Objectives

After this presentation, you should be able to:

1. Explain the principles of mass spectrometry
2. Describe the different mass spectrometers available
3. Compare the data acquisition capabilities of the different instruments
4. Evaluate which mass spectrometer would best suit the applications required in your laboratory

Overview

- What is a mass spectrometer and what is mass spectrometry?
- Ionization techniques
- Mass analyzers
  - Single quadrupole, triple quadrupole, ion trap
    - SIM, SRM, ion ratios and product ion spectra for confirmation
  - High resolution mass analyzers and data acquisition
    - Nominal mass vs exact mass
- Comparison of mass analyzers
- Other considerations for implementation of mass spectrometry
- What's still needed?
- Conclusions

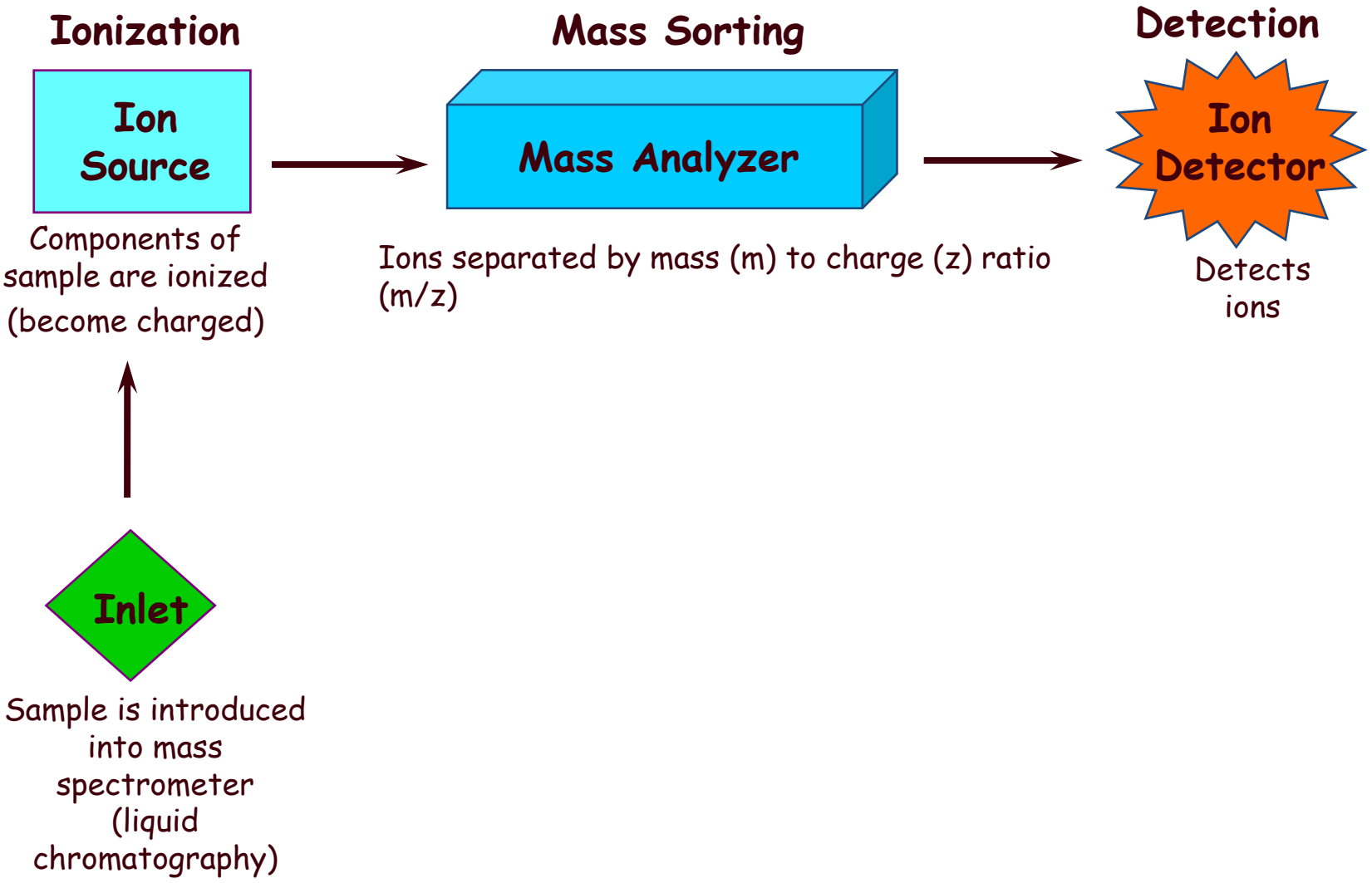
What is a mass spectrometer?

- an instrument that essentially weighs molecules

What is mass spectrometry?

- a technique that measures molecules in the gas phase
- charged species are generated and sorted based on the mass to charge ratio

What are the components of a mass spectrometry system?



Ionization

- have to convert flow of liquid from liquid chromatography system to gas before mass spectrometry analysis
- different forms of liquid to gas ionization
  - electrospray ionization (ESI)
  - atmospheric pressure chemical ionization (APCI)
  - atmospheric pressure photo ionization (APPI) (not commonly used in clinical laboratories)

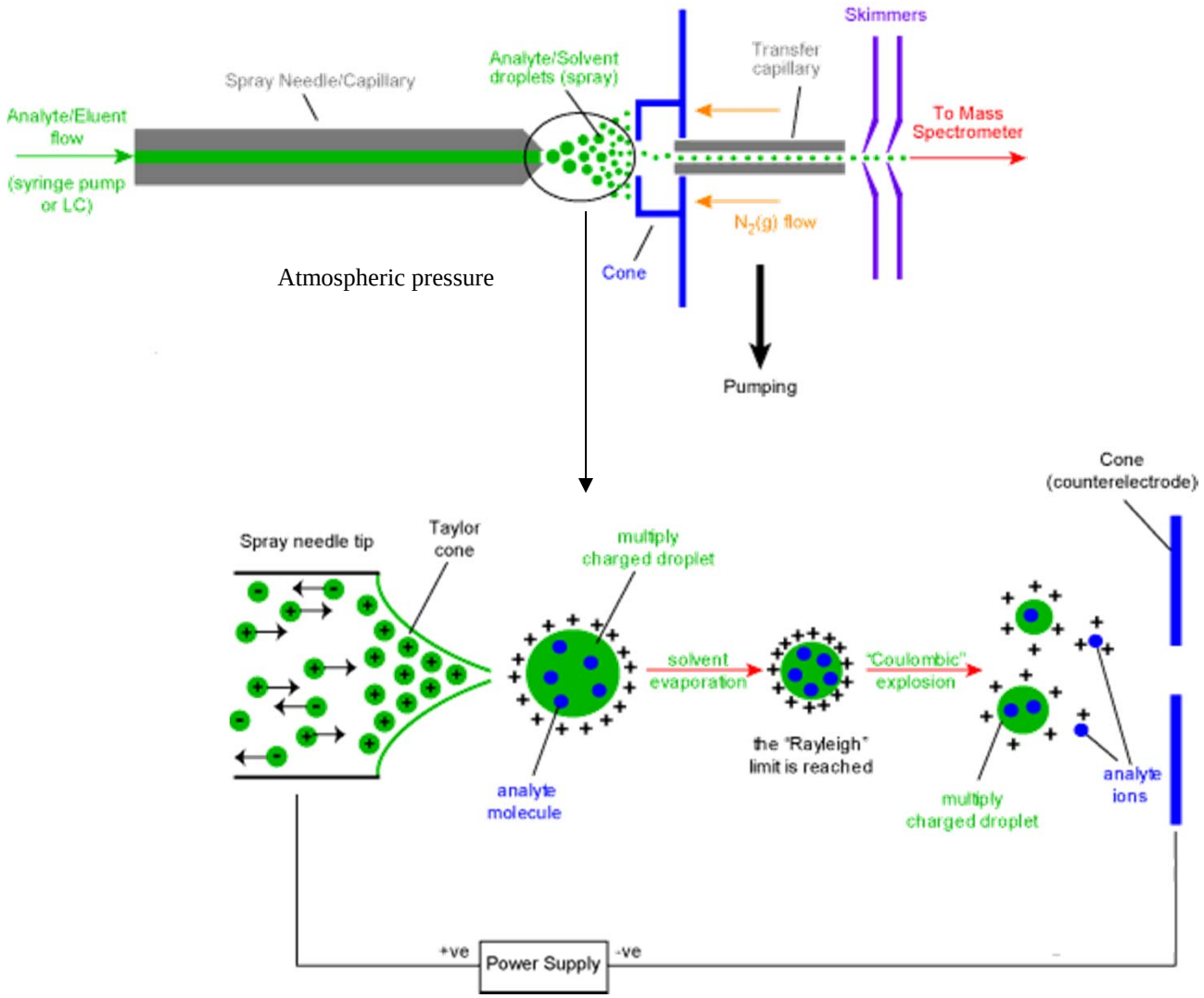
Convert flow of liquid from LC column to mist in order for ionization to occur

Flow from LC column

Converted to mist in ionization source

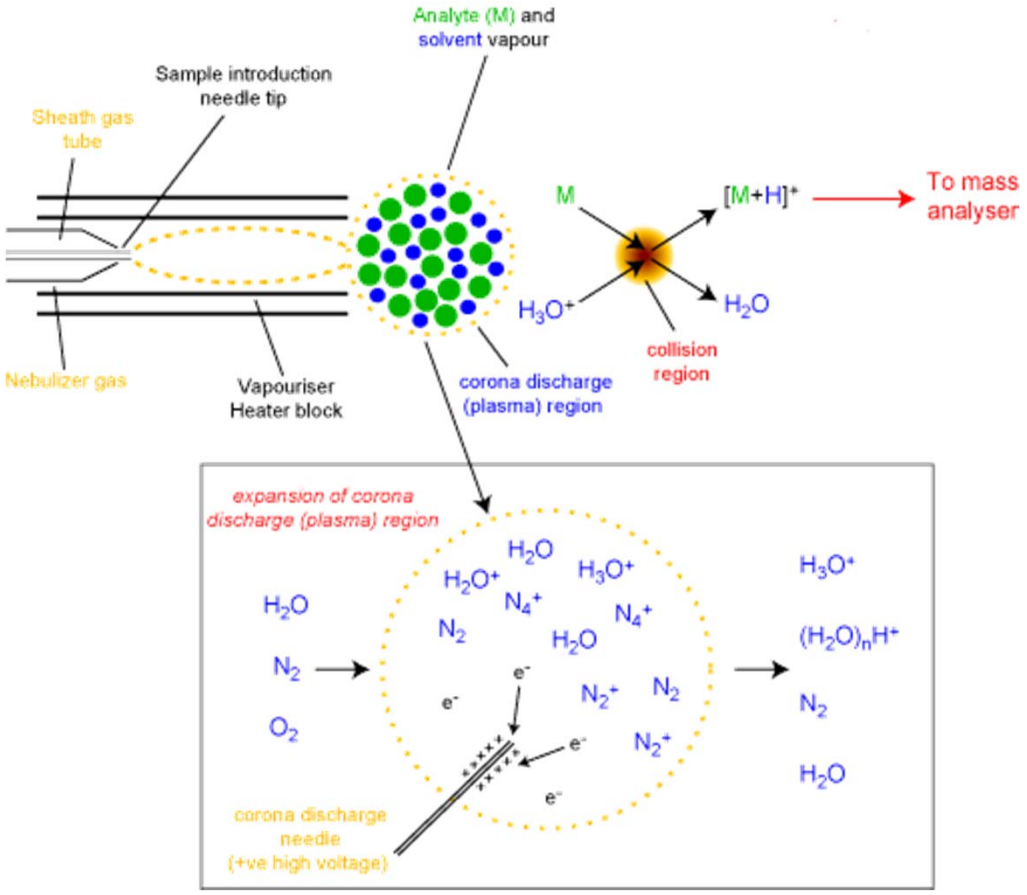
Pictures courtesy of Dr. Tom Annesley

Electrospray Ionization (ESI)



www.bris.ac.uk/nerclsmsf/techniques/hplcms.html

Atmospheric Pressure Chemical Ionization (APCI)



www.bris.ac.uk/nerclsmsf/techniques/hplcms.html

Ionization (cont)

- have to convert solid to gas before mass spectrometry analysis
  - matrix assisted laser desorption ionization (MALDI)

Picture courtesy of Dr. Tom Annesley

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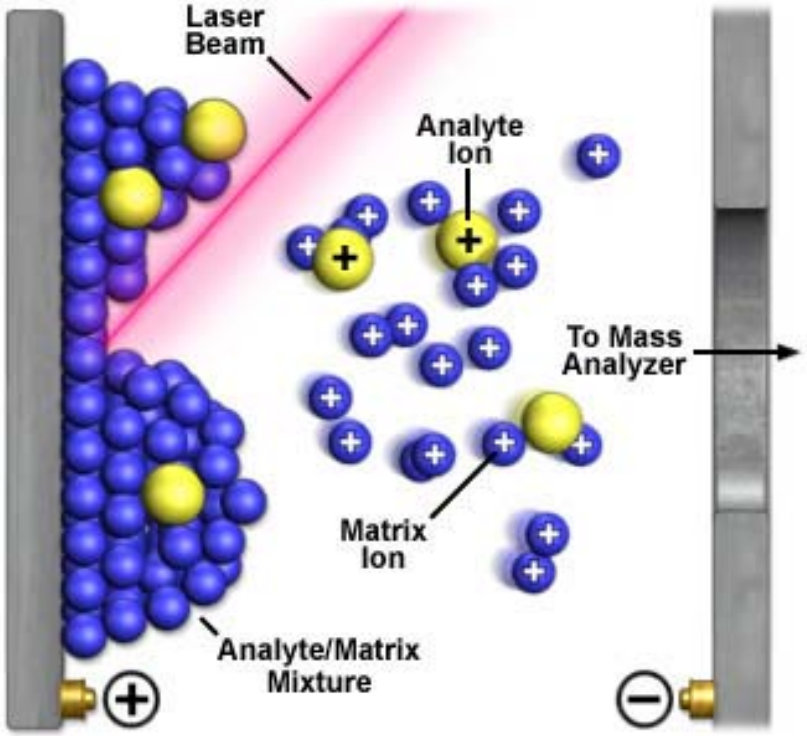

Picture courtesy of Dr. Tom Annesley

Overview

- What is a mass spectrometer and what is mass spectrometry?
- Ionization techniques
- Mass analyzers
  - Single quadrupole, triple quadrupole, ion trap
    - SIM, SRM, ion ratios and product ion spectra for confirmation
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- What's still needed?
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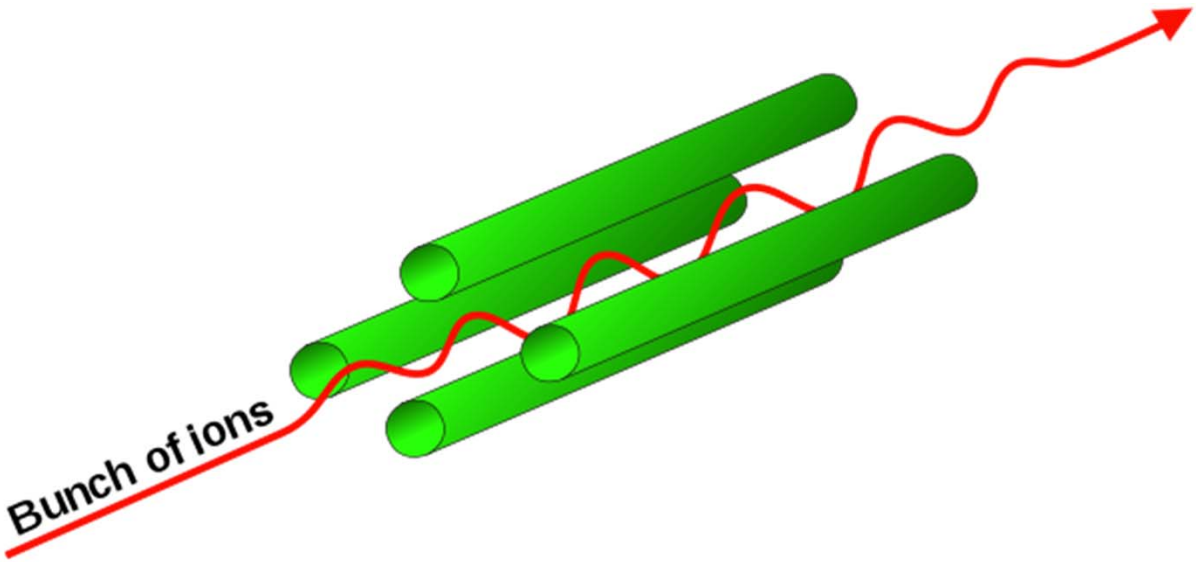
Mass Analyzers

Types of mass analyzers:

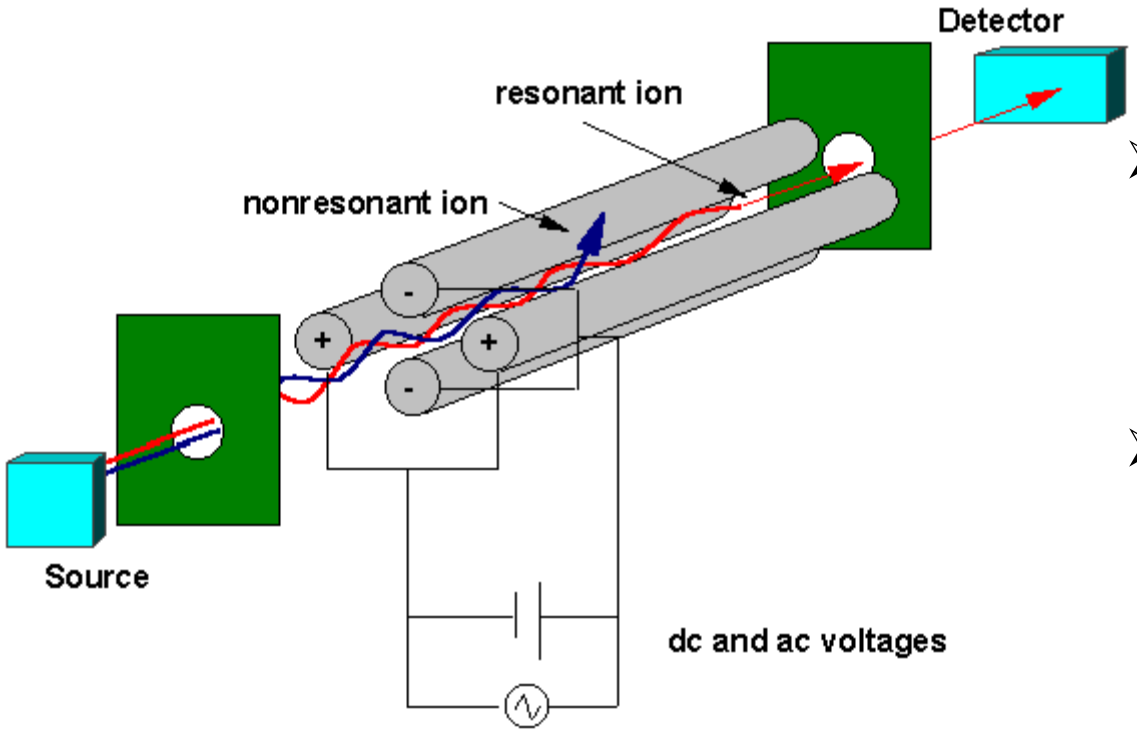
- single quadrupole
- triple quadrupole
- quadrupole ion trap
- time of flight
- fourier transform ion cyclotron resonance (FTICR)

What is a quadrupole?

- four metal rods set parallel to each other
- each opposing rod pair is connected electrically and a radio frequency (RF) voltage is applied between rod pairs
- direct current voltage is superimposed on RF voltage
- only ions with certain mass to charge ratio (m/z) will move through quadrupole at the specific voltages



What is a quadrupole?



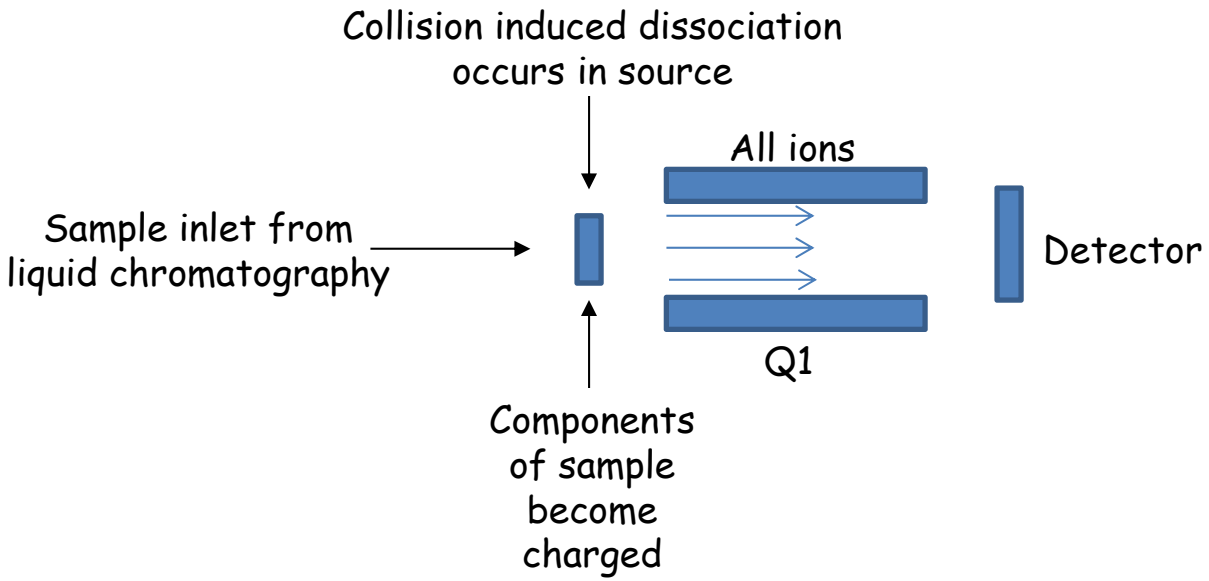
- allows one m/z to be monitored or to scan for a range of m/z by varying the voltages
- other ions will have unstable trajectories and will collide with the rods

http://www.chemicool.com/definition/quadrupole_mass_spectrometry.html

What is a mass to charge ratio or m/z?

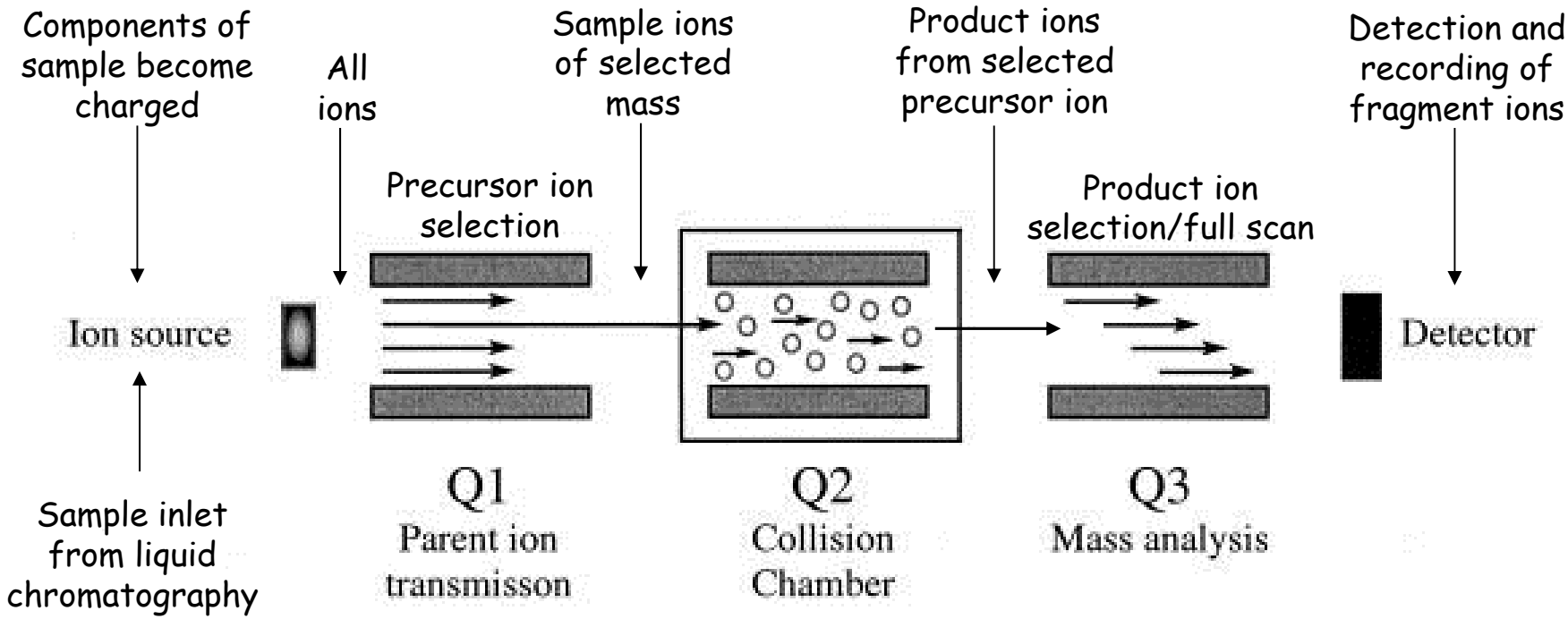
- the mass, m, of an analyte is the molecular weight
  - e.g. testosterone has a molecular weight of 288 g/mol
- when testosterone is ionized in positive mode, it becomes protonated (H^+) so it has one positive charge, or z
  - mass = 289 (i.e. m + 1 = 288 + 1)
    charge = +1
    mass to charge ratio (m/z) is $\frac{289}{1} = 289$

Single quadrupole MS



- only ions of desired mass to charge ratio reach detector when using optimized voltages for analyte of interest
- all analytes with that mass will be detected
- can also scan across a mass range by varying voltages
- not as specific as other instruments

Triple quadrupole MS



Q1. Ions of interest are selected (precursor/parent ions)
Q2. Fragmented into smaller product ions
Q3. Product ions separated by mass (m) to charge (z) ratio (m/z)

- also known as a tandem mass spectrometer (MS/MS)
- very selective so best for quantitative analysis
- poor scanning capabilities

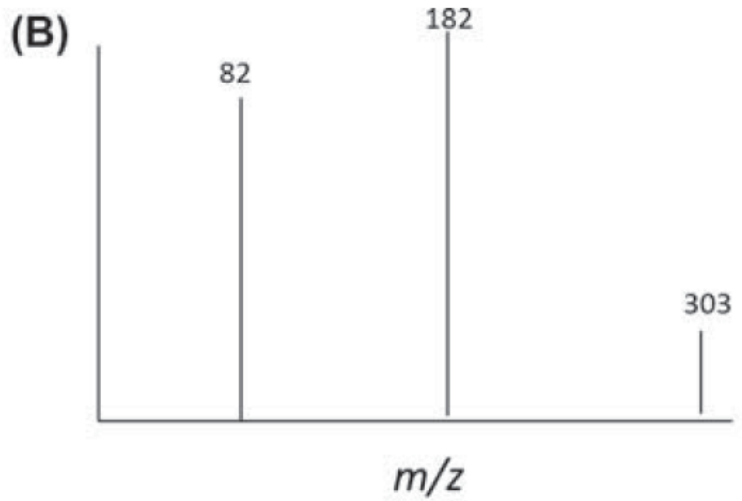
Quadrupole Ion trap MS

- quadrupole used to generate a field that functions to "trap" ions without destroying them
- ideal for qualitative analysis and elucidation of ion structure
- not as useful for quantitative analysis due to capacity limitations of the trap
- can be used to produce product ion spectra if used with MS/MS
    - an extra layer of selectivity

What are the commonly used different modes of operation using these instruments?

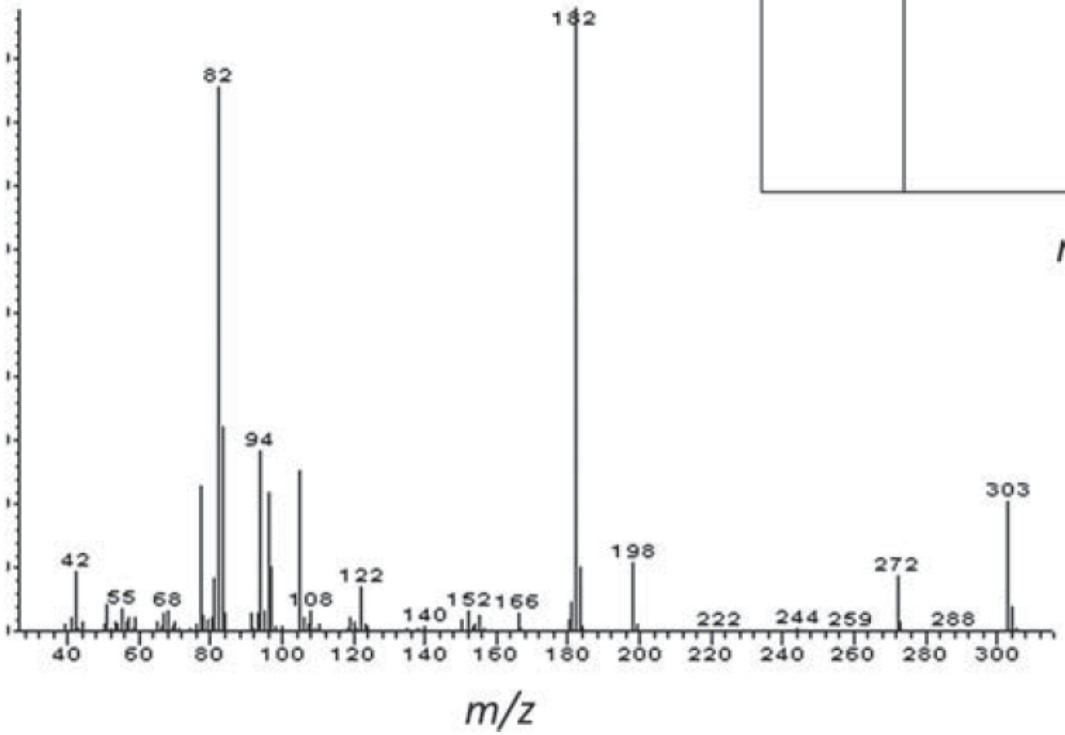
Selected Ion Monitoring (SIM)

Selected ion monitoring of cocaine



Ion ratios:
82/182= 0.88 ±20%
303/182= 0.23±20%

(A) Full mass spectrum of cocaine



Wu AHB, et al, 2012: 50(8):733-742, Clin Toxicol

Selected Ion Monitoring (SIM)

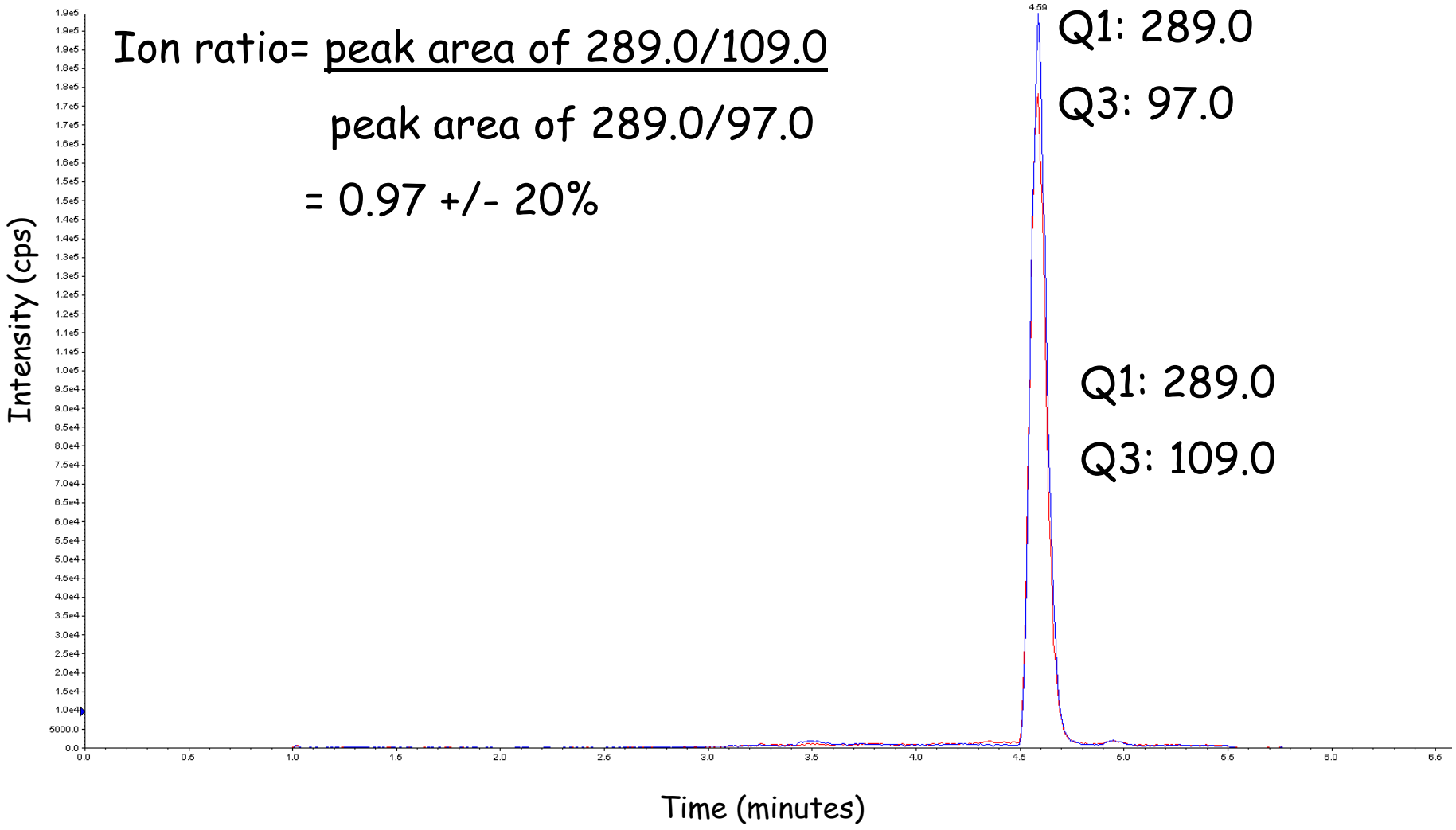
- typically employed in clinical laboratories using GC- or LC-MS
- targeted method
- monitoring fragmentation pattern of specific ions
- usually monitor 3 ions (may include molecular ion and fragment ions)
- use ratios between relative abundance of ions to ensure specificity
- ion ratios consistent across calibrators, controls and patient samples
- improves sensitivity, selectivity and precision of method

Selected Reaction Monitoring (SRM)

Ion ratio= peak area of 289.0/109.0

peak area of 289.0/97.0

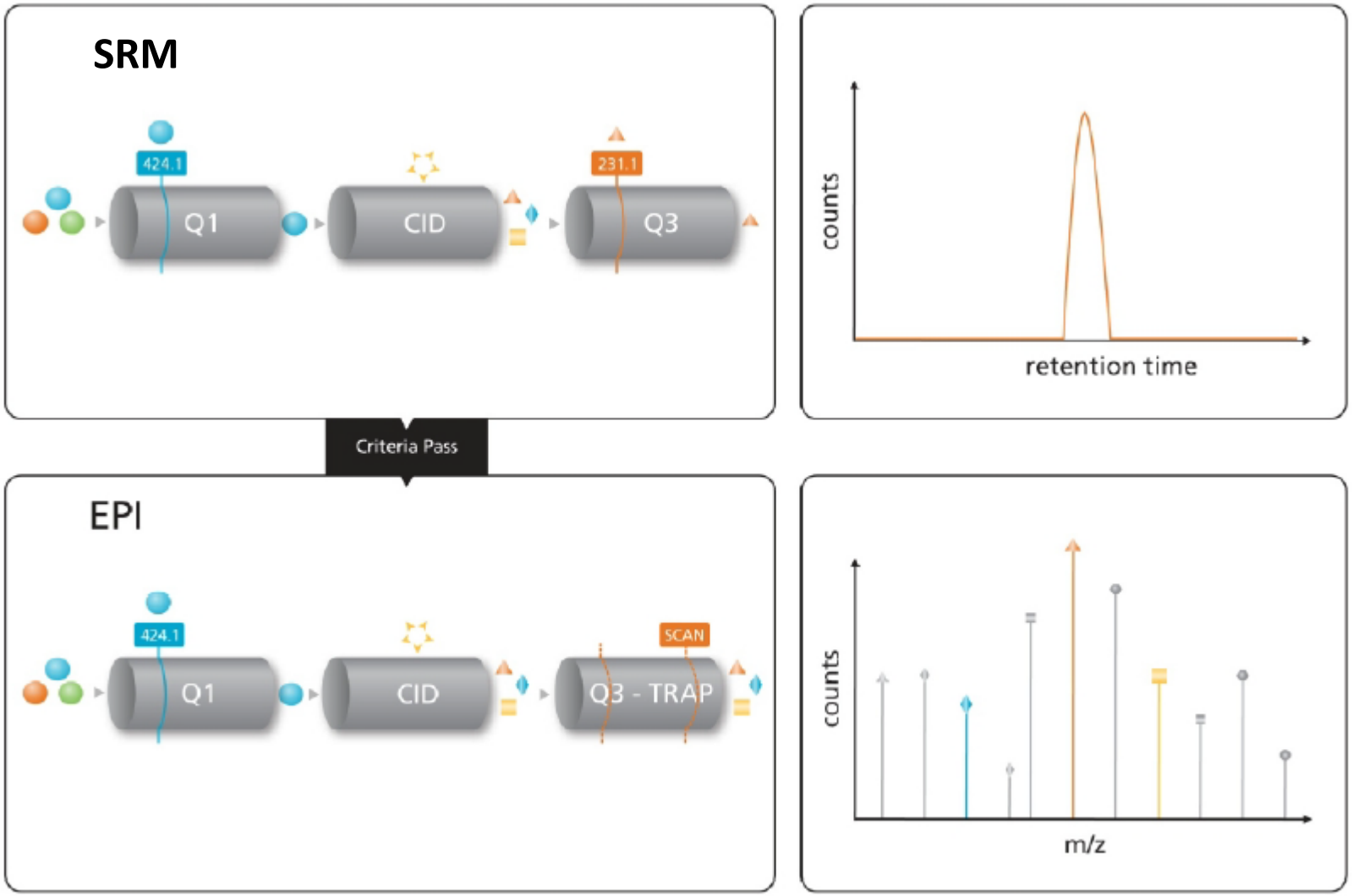
= 0.97 +/- 20%



Selected Reaction Monitoring (SRM)

- typically employed in clinical laboratories using LC-MS/MS
- targeted method
- monitoring of precursor/product ion pairs – transition
- usually monitor 2 transitions per analyte and internal standard
- use ratio between 2 transitions to help determine if there are interferences in the LC-MS/MS method – ion ratios
- ion ratios consistent across calibrators, controls and patient samples
- improves sensitivity, selectivity and precision of method

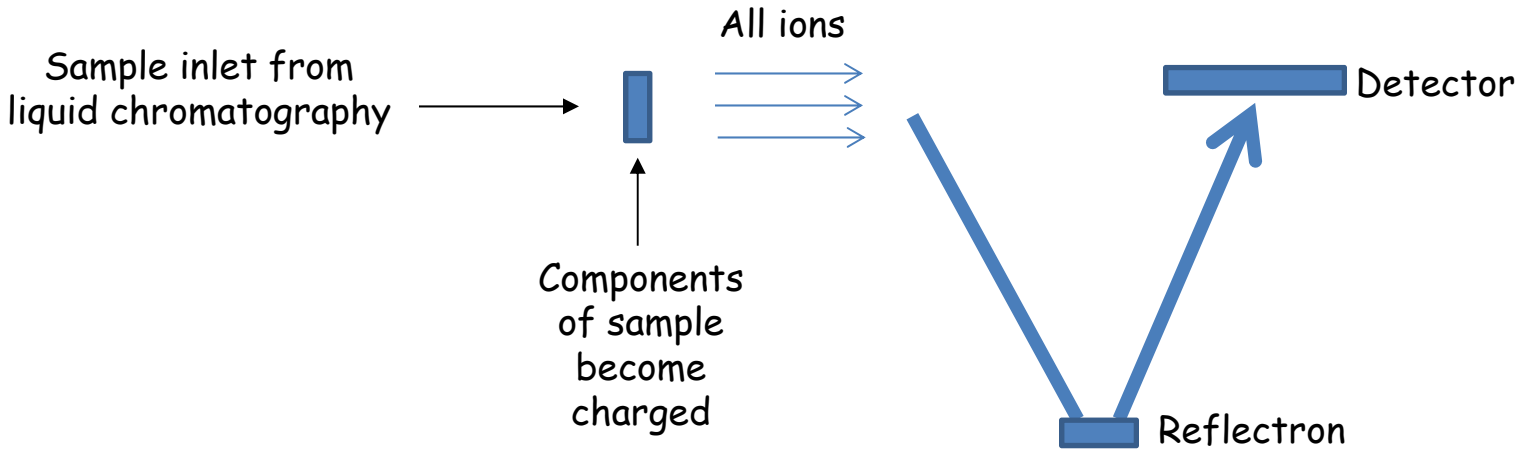
SRM and product ion spectra



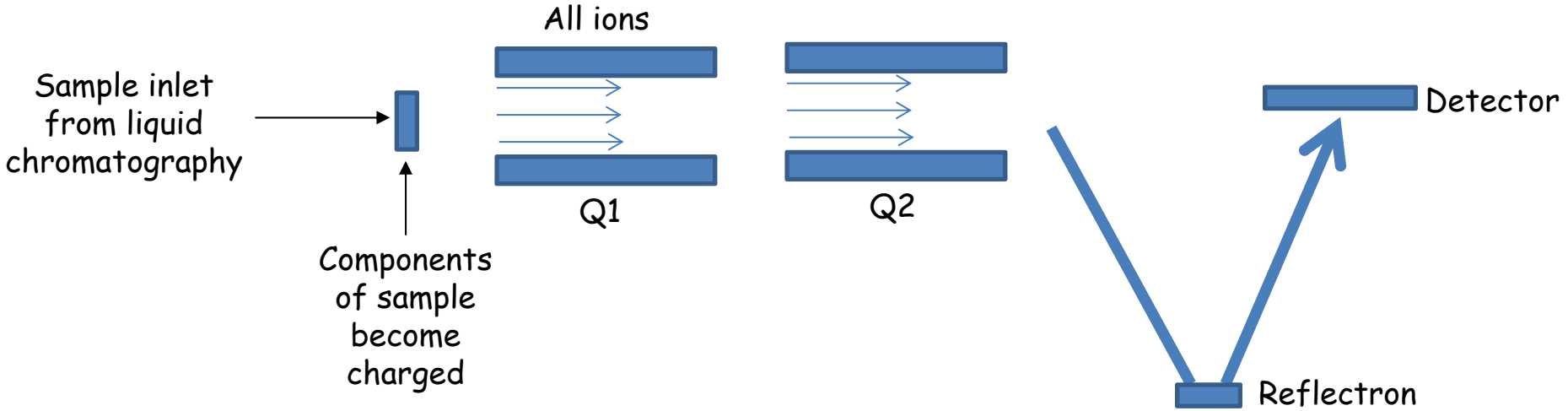
Courtesy of AB SCIEX

High resolution mass analyzers

Time of flight MS (TOF-MS)



Quadrupole time of flight MS (QTOF-MS)

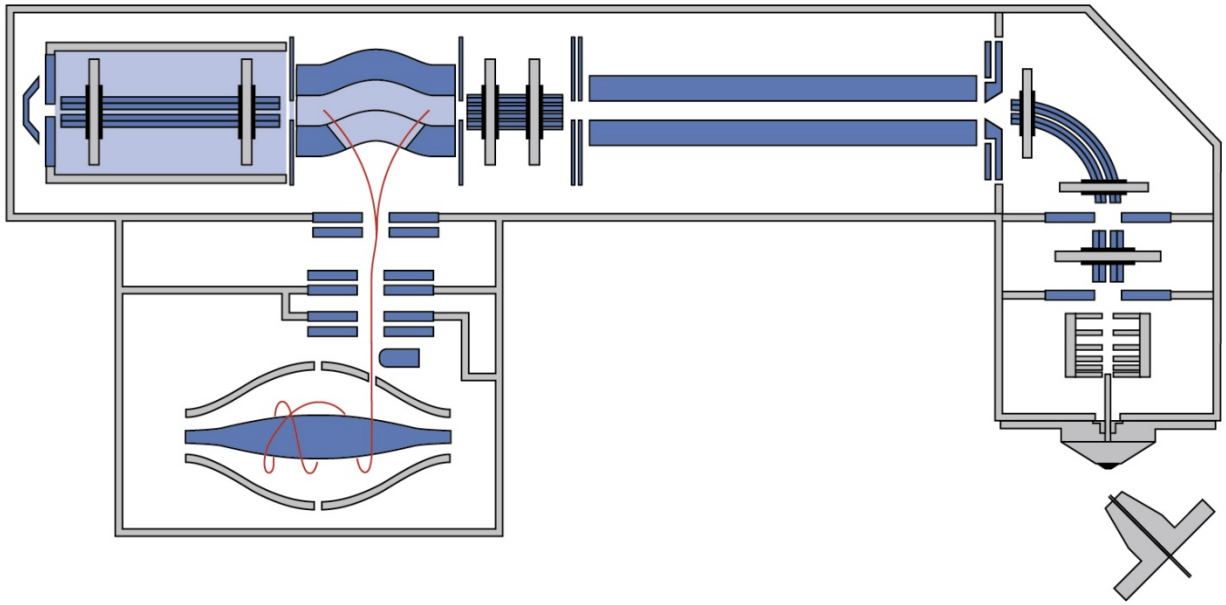


Time of flight MS (TOF-MS)

- based on time it takes for an ion to travel a specific path length when the same force is applied to all ions
- lighter ions arrive at detector earlier than heavy ions
- theoretically TOF-MS has no m/z range limit
- linear dynamic range limitations due to detector saturation
- useful for accurate mass determination
- not as useful for quantitative analysis unless using QTOF-MS

Fourier transform ion cyclotron resonance MS

- FTICR-MS (Orbitrap technology uses similar principles)



- ions trapped in a cell inside a strong magnetic field and move in circular orbits in a plane perpendicular to magnetic field
- RF electrical potential is applied to transmitter plates causing trapped ions to be excited into larger circular orbits
- frequency of motion of ion is inversely proportional to its mass

What defines a mass analyzer as "high resolution"?

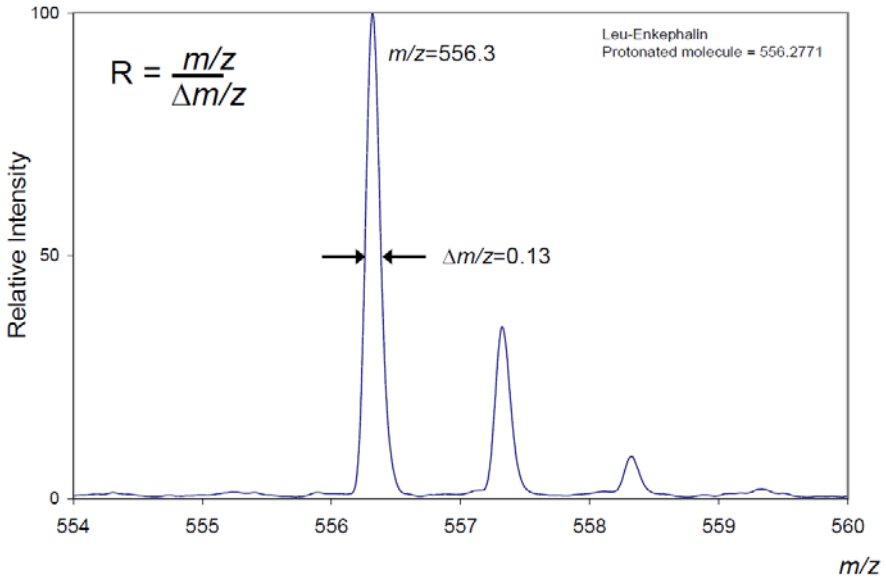
Mass Resolution

The ability to distinguish between ions differing slightly in m/z ratio

Can be calculated in two different ways:

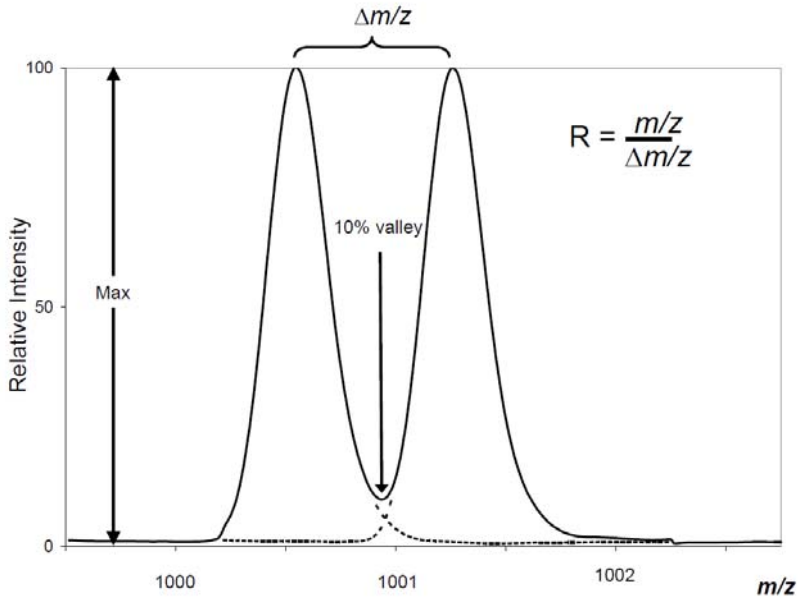
Δm/z is the full width of the peak at half its maximum height (FWHM).

Resolution = 556.3/0.13 = 4279



m/z of lowest mass peak is divided by the difference in m/z of the peaks (Δm/z).

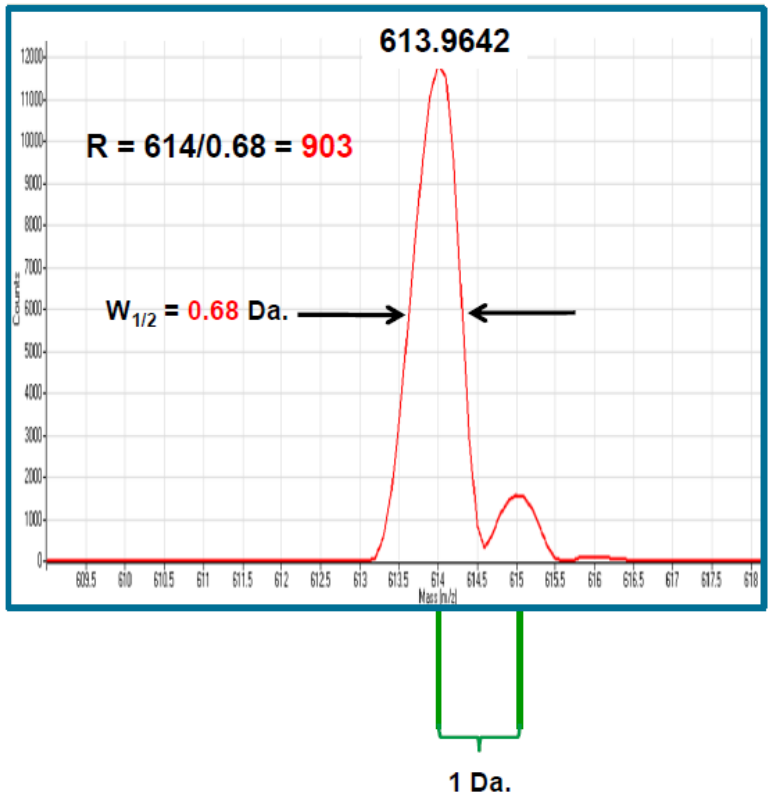
Resolution = 1000/1= 1000



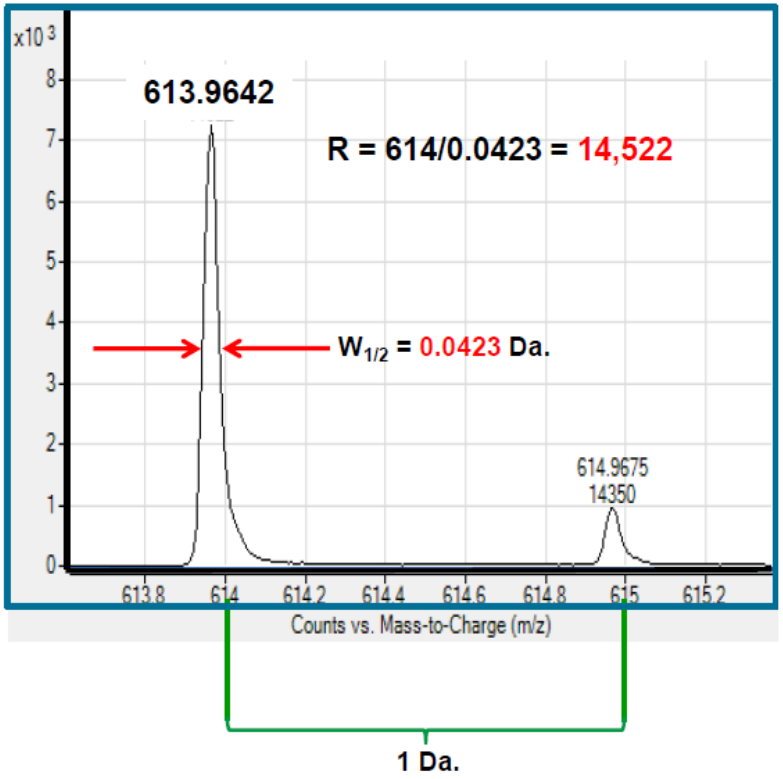
CLSI C50-A document

Mass Resolution

Single and Triple Quad, Ion Trap



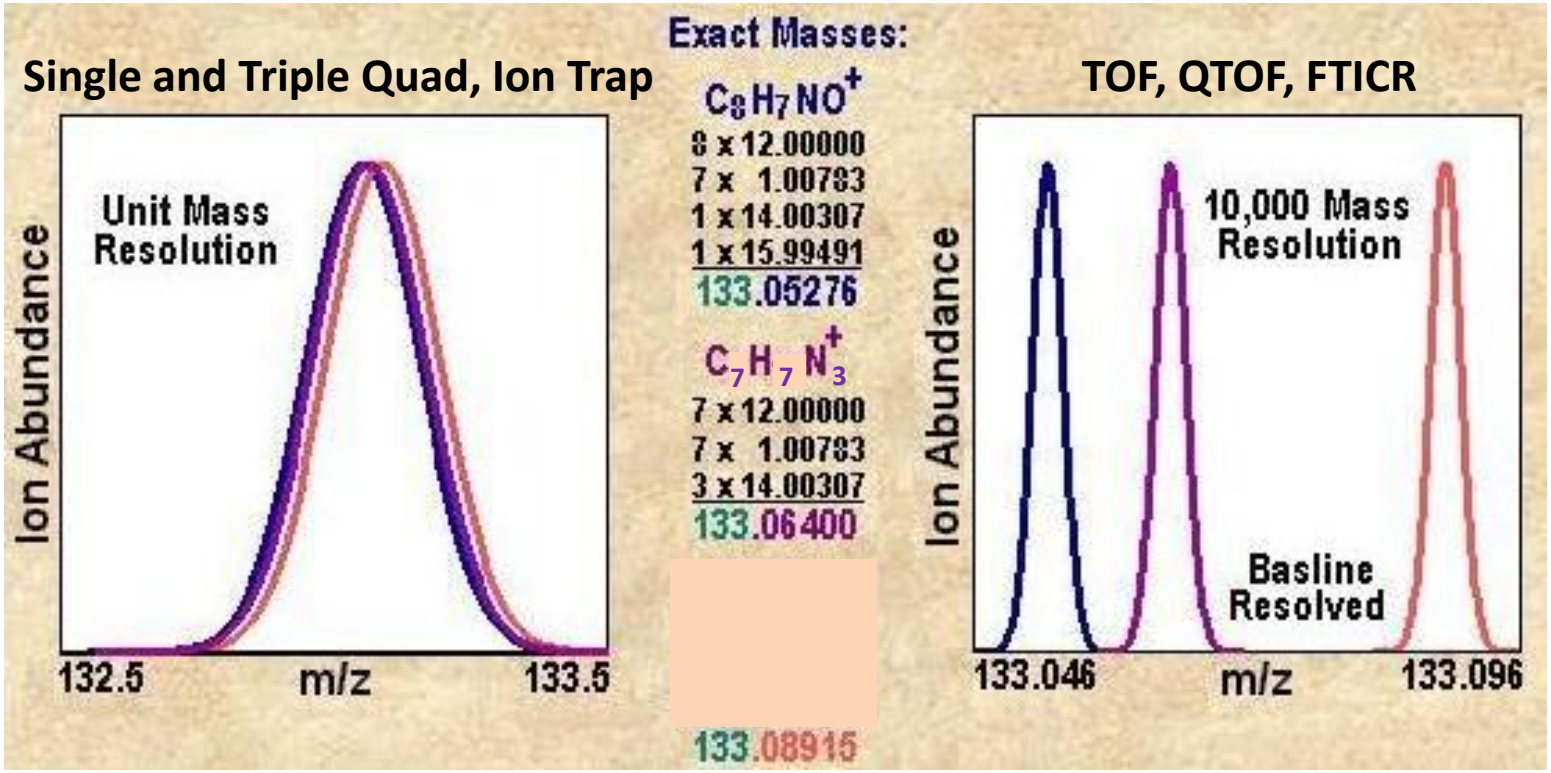
TOF, QTOF, FTICR



Courtesy of Agilent Technologies

Nominal Mass vs. Exact Mass

- nominal mass = mass of a molecule calculated using atomic masses of each atom taken as integers
- exact mass = calculated mass based on adding up the masses of each isotope in a molecule



http://www.epa.gov/esd/chemistry/ice/faq.htm#wiaem

What are the commonly used different modes of operation using these instruments?

TOF-MS

- full scan of all ions in sample
- extract chromatogram to obtain accurate mass
- database search to identify compound as well as matching LC retention time

QTOF-MS

- full scan of all ions in sample and set criteria to trigger MS/MS
- extract chromatogram to obtain accurate mass
- database/library search to identify compound based on fragmentation pattern, accurate mass, ion ratios, LC retention time

FTICR

- full scan and full scan fragmentation of all ions in sample
- extract chromatogram to obtain accurate mass
- database/library search to identify compound based on accurate mass, fragmentation pattern and LC retention time

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Which instrument do you need?

	LC-MS	LC-MS/MS	LC-TOF-MS	LC-QTOF-MS	FTICR
Specificity	++	+++	++	+++	+++
Sensitivity	++	+++	++	+++	+++
Resolution	Low	Low	High	High	Highest
Mass Accuracy	~0.1 units	~0.1 units	~0.01 units	~0.01 units	~0.0001 units
Ease of Use	++	+++	+++	++++	++++
Suited for which Applications?	Targeted Quant	Targeted Quant	Untargeted Qual	Targeted or untargeted Quant	Targeted or untargeted Quant
Cost	$$	$$$-$$$$	$$	$$$$	$$$$$

DO NOT FORGET THE COST OF A SERVICE CONTRACT – SIGNIFICANT $$$

Other considerations for implementing mass spectrometry

- electrical supply
- gas supply – nitrogen, argon
- exhaust
- UPS or back up power
- roughing pump and oil (and disposing of oil)

Mass spectrometry vendor should be able to give you a site guide documenting the requirements for the instrument

Optional - interface between mass spectrometer and laboratory information system

These can all add $$$ to the cost of implementation!

Additional factors to consider when choosing instrumentation

- what do you actually need for the applications you wish to implement?
    - take into account sensitivity, throughput, and robustness requirements for your lab
- what expertise do your technologists possess?
    - MS has to be tuned for every single analyte you want to measure – this is not a "plug and play" technology
- what is the cost – direct and indirect – of implementation?

Reminder

Electrospray ionization (ESI)

Atmospheric pressure chemical ionization (APCI)

Atmospheric pressure photoionization (APPI)

Matrix-assisted laser desorption ionization (MALDI)

Single quadrupole

Triple quadrupole

Ion trap

Time of flight (TOF)

Fourier transform ion cyclotron resonance (FTICR)

Electron multiplier

Image current measurement (in FTICR)

What is still needed in terms of MS in the clinical laboratory?

- automation of the whole process
- ready to use reagent kits
- more user friendly software
- autoverification of results
- easier and less expensive implementation of an interface between the MS and the laboratory information system
- service available 24/7
- reduction in cost ☺

Conclusions

- mass spectrometers essentially weigh molecules
- mass spectrometry is a technique that measures molecules in the gas phase
- mass spectrometers have 3 major parts:
  - ion source (for ionization)
  - mass analyzer
  - detector
- mass analyzers vary in specificity, sensitivity, cost and ease of use – should be chosen wisely in terms of desired applications
- technologists will require a significant amount of training
- don't forget the "extras" such as gas and electrical supply, exhaust, service contract etc as the cost is significant