

MS Goals and Applications

- Several variations on a theme, three common steps

- **Form gas-phase ions**

- choice of ionization method depends on sample identity and information required

- **Separate ions on basis of m/z**

- “Mass Analyzer”
- analogous to monochromator, changing conditions of analyzer results in different ions being transmitted

- **Detect ions**

- want (need) high sensitivity
 - “Resolution”

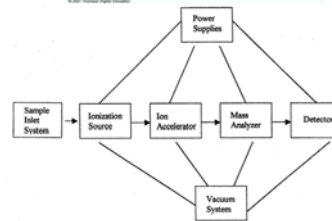
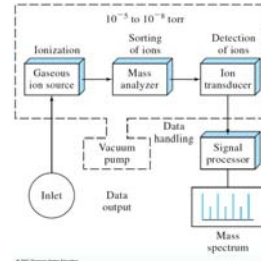


Figure 9.4 Block diagram of a mass spectrometer.

MS Goals and Applications

- All MS experiments are conducted under vacuum, why?
 - Mean free path (λ):

$$\lambda = \frac{RT}{\sqrt{2}\pi d^2 N_A P} \approx \frac{5 \text{ cm}}{\text{mtorr}}$$

- Ion Optics: Electric and magnetic fields induce ion motion
 - Electric fields most common: Apply voltage, ions move
 - Magnetic fields are common in mass analyzers. “Bend” ions paths (Remember the right hand rule?)

MS Figures of Merit: Resolving Power and Resolution

- Relate to ability to distinguish between m/z
 - Defined at a particular m/z
- Resolving Power, Resolution...
 - Variety of definitions
 - Δm at a given m

$$\text{Resolving Power} = \frac{m}{\Delta m}$$

$$\text{Resolution} = \frac{m}{\Delta m}$$

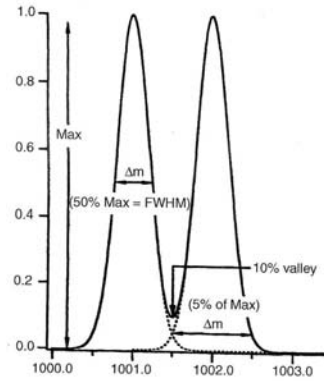


Figure 9.3 Illustration of the peaks used to calculate

MS Components: Mass Analyzers

• Magnetic Sector Mass Analyzers

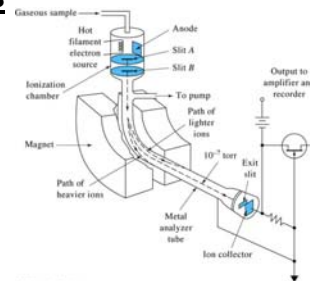
- Accelerate ions by applying voltage (V)
- velocity depends on mass and charge (m/z)

$$KE = zeV = \frac{1}{2}mv^2$$

- Electromagnet introduces a magnetic field (variable)
- The path on an ion through the sector is driven by magnetic force and centripetal force
 - For an ion to pass through, These must be equal

$$F_M = Bzev = \frac{mv^2}{r} = F_c \quad \longrightarrow \quad \frac{m}{z} = \frac{B^2 r^2 e}{2V}$$

- For a given geometry (r), variation in B or V will allow different ions to pass
- “Scanning” B or V generates a mass spectrum



MS Components: Mass Analyzers

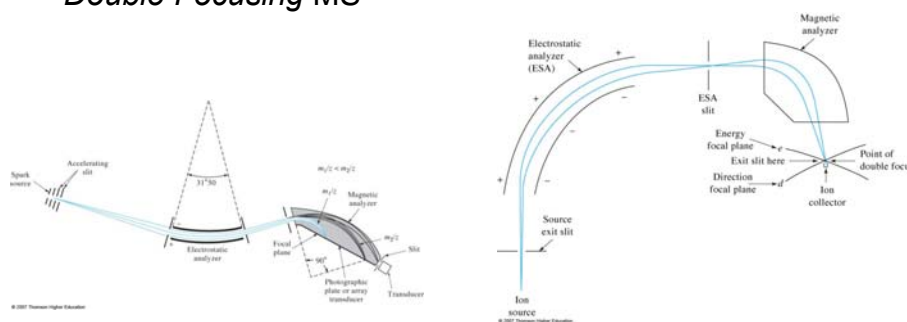
- In practice, ions leaving the source have a small spread of kinetic energies (bandwidth?)

$$R = \frac{m}{\Delta m} \leq 2000 \text{ for mag. sector alone}$$

- Result is a spread in paths through magnetic field
 - leads to broadened bands and decreased resolution
- Problem is minimized using *Double Focusing MS*
 - Two sectors:
 - Electrostatic sector focuses on the basis of translation energy: “Energy Analyzer”
 - Magnetic sector focuses on the basis of momentum: “Momentum Analyzer”
 - Results in better M/Z discrimination and higher resolution (up to 100,000!).
 - Often more \$\$

MS Components: Mass Analyzers

- *Double Focusing MS*



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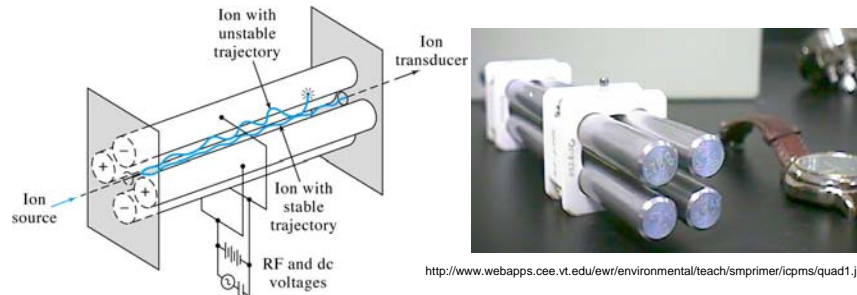
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<http://www.ms-textbook.com/1st/downloads>

MS Components: Mass Analyzers

• Quadrupole Mass Filter



- Opposing AC voltage applied between pairs of rods
- $U_{dc} + V \cos \omega t$ and $-(U_{dc} + V \cos \omega t)$
- Because of positive potential superimposed on AC, quad acts as high-pass mass filter in plane with positive DC offset
- Because of negative potential, quad acts as a low-pass mass filter in plane with negative DC offset.

MS Components: Mass Analyzers

- By changing AC and DC potentials, different m/z will have "stable" trajectories
 - acts like a "notch" filter!
 - Tunable up to $m/z \sim 4000$ with unit mass resolution
- Many benefits over Double Focusing
 - Smaller, Less Expensive
 - More Rugged
 - Possible to "scan" spectra in <0.1 sec
- Can't get the high resolution like double focusing!

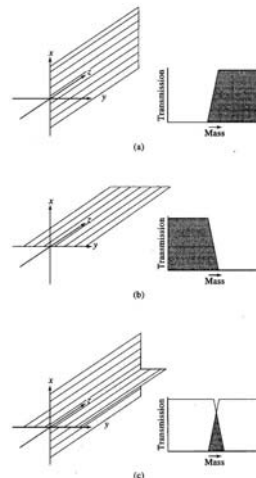


Figure 11-6 Quadrupole acts as (a) a high-pass mass filter in the xz plane, (b) a low-pass mass filter in the yz plane, and (c) a narrow-band filter when high-pass and low-pass filters are both in operation. (Reprinted with permission from F. E. Miller and M. B. Denton, *Anal. Chem.*, 1986, 63, 619. Copyright 1986 by American Chemical Society.)

MS Components: Mass Analyzers

• Ion Traps

- Ions are “stored” and selectively cycled out

• Quadrupole Ion Trap (QIT)

- Similar concept to quadrupole
- RF and DC electric fields
- Only certain m/z are “stable”

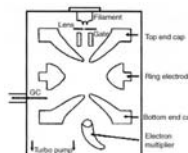


Figure 9.24 Cross-section of a quadrupole ion trap mass spectrometer. This schematic shows a gas phase sample introduced from a GC and ionized inside the trap by electrons from the filament. (From Niessens and van der Greef, used with permission.)

http://cdn.arstechnica.net/wp-content/uploads/2014/02/bollen_pen_open.preview.jpg

• FT-Ion Cyclotron Resonance (FT-ICR)

- Magnetic field traps ions
- RF pulse is added to augment motion
- Current at receiver relates to m/z
- <http://www.magnet.fsu.edu/education/tutorials/magnetacademy/fticr/>

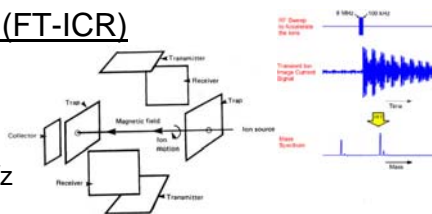


Figure 9.25 “Exploded” view of an ICR ion trap. The ICR has been the primary mass analyzer used in FTMS, both alone and in newer “hybrid” FTMS instruments.

MS Components: Mass Analyzers

• Time of Flight Mass Analyzer:

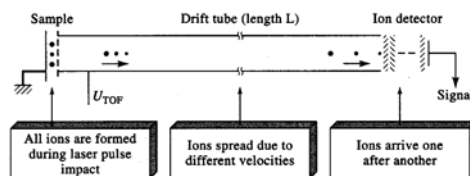


Figure 11-8 Principle of a time-of-flight mass spectrometer. A bunch of ions



• “Pulse” of ions are accelerated into analyzer

- Very small range of kinetic energies (ideally all have same KE)
- Since masses vary, velocity must also vary

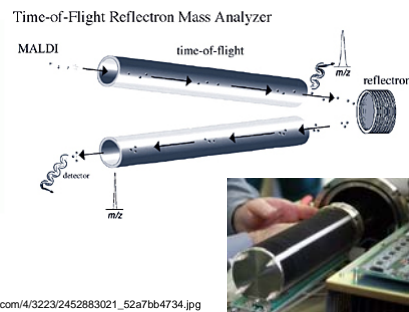
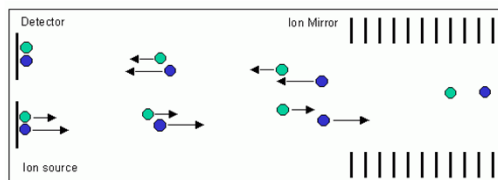
• Ions enter a field-free region, the *drift tube*, where they are separated on the basis of their velocities

- Lighter ions (smaller m/z) arrive at the detector first, heavier ions (larger m/z) arrive later

http://www.chem.agilent.com/en-US/products-services/Instruments-Systems/Mass-Spectrometry/6545-Q-TOF-LC-MS/PublishingImages/6545_226x191.png

MS Components: Mass Analyzers

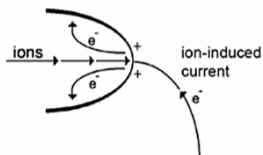
- Potential for very fast analysis (sub millisecond)
- Simple instrumentation
- Resolution depends on applied voltage (kinetic energy) and flight time
 - use internal standards to calibrate
 - Resolution is enhanced by use of **reflectron**
 - Like a concave “ion mirror”



https://c2.staticflickr.com/4/3223/2452883021_52a7bb4734.jpg

MS Components: Detectors

- Two common types of detectors:
 - Faraday Cup
 - Electron Multiplier
- Faraday Cup
 - Ions are accelerated toward a grounded “collector electrode”
 - As ions strike the surface, electrons flow to neutralize charge, producing a small current that can be externally amplified.
 - Size of this current is related to # of ions in
 - No internal gain → less sensitive



<http://www.ccrtechnology.de/Designbilder/Faraday.png>

MS Components: Detectors

- **Electron Multiplier**
 - Analogous to PMT
 - Durable, applicable to most analyzers
 - Ions strike surface of dynode
 - Generate electrons
 - $>1 e^-/\text{ion}$
 - Ejected electrons are accelerated to other dynodes
 - $>1 e^- \text{ out}/e^- \text{ in}$
 - Current is related to number of ions in times large gain (10^7 or so)

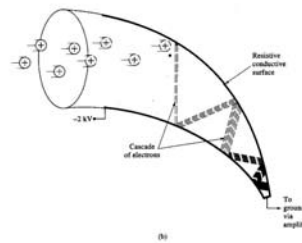
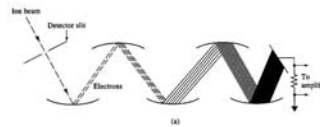
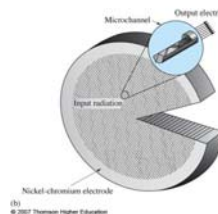
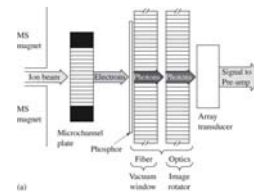


Figure 11-2 (a) Discrete dynode electron multiplier. Dynodes are kept at successively higher potentials via a multistage voltage divider. (b) Continuous dynode electron multiplier. (Adapted from J. T. Mason, Introduction to Mass Spectrometry, p. 247, New York: Raven Press, 1985, 1998 permission.)



MS Components: Detectors

- Single channel vs. array detectors
 - “Single” m/z vs “whole spectrum at a time”
 - Often a tradeoff between sensitivity and speed
- Microchannel Plate
 - Converts ions to electrons
 - Gains approaching electron multipliers
 - $\sim 10^4$ for single, more if “stacked”
 - Electrons can be detected in two dimensions.
 - One approach: convert electrons to photons and use optical detection (i.e. camera!)

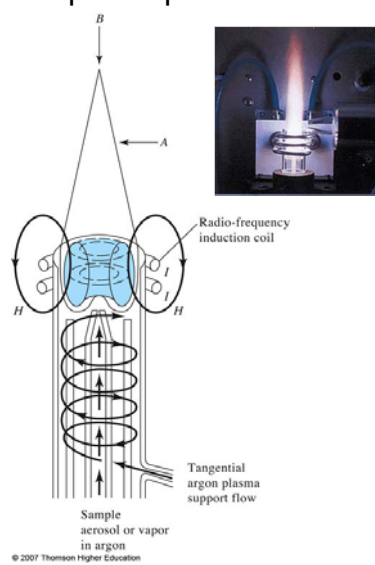


MS Components: Sources

- Ion sources are the component with the greatest number of variations
- Choice of source depends on identity of analyte
 - solid/liquid
 - organic./inorganic
 - reactive/nonreactive
- Common requirements of sources
 - produce ions!
 - Ideally small spread in kinetic energies
 - Produce ions uniformly, without mass discrimination
 - Accelerate ions into analyzer
 - Series of ion optics

MS Components: Atomic Sources

- **Inductively Coupled Plasma:** Atmospheric pressure discharge
- Relatively high argon flow rate (Liters per minute)
- After ignition, coupling of ionic charge with RF magnetic field “forces” ions to move
 - Heating results, plasma is sustained

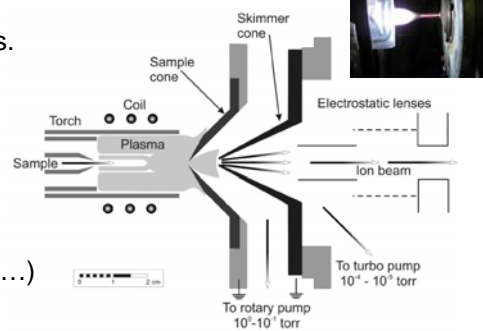


MS Components: Atomic Sources

- **The ICP as an ionization source:**
 - High temperature in the source results in the formation of ions
 - best for atomic mass spec.
 - Challenges:
 - How do we get from atmospheric pressure in the ICP to vacuum in the MS without filling the MS with argon?
 - How do we keep the high temperature of the ICP from melting/ionizing components of the MS instrument?

MS Components: Atomic Sources

- Pressure is reduced by inserting a cooled cone (sampler) into the plasma. This allows only a small fraction of the plasma material to pass.
 - mechanical pump maintains lower pressure of ~1 torr
- A small fraction of this material passes through a second cone (the skimmer) into the high vacuum chamber
 - ion optics accelerate the ions into the mass analyzer
- Typically used with quadrupoles.
 - Unit mass resolution up to ~1000-2000
 - Large LDR
- Isobaric Interference
- Polyatomic ions
- Matrix effects (refractory oxides...)



http://iramis.cea.fr/images/astimg/886_1.jpg

MS Components: Hard vs Soft Sources

- Parent or Molecular Ion Formation
 - needed to establish molecular weight
- Hard (energetic) sources leads to excited-state ions and fragmentation
 - Good for structural information
- Soft sources cause little fragmentation
 - Good for molecular weight determination

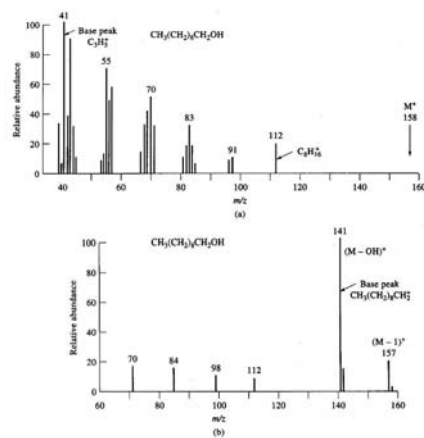


Figure 20-2 Mass spectrum of 1-decanol from (a) a hard source and (b) a soft source.

Molecular Ionization Sources

- More exist than are on this list!
- Need to transfer energy to analyte and ultimately produce ions.
 - Mechanism determines the extent of fragmentation

TABLE 20-1 Ion Sources for Molecular Mass Spectrometry

Basic Type	Name and Acronym	Ionizing Agent
Gas phase	Electron impact (EI)	Energetic electrons
	Chemical ionization (CI)	Reagent gaseous ions
Desorption	Field ionization (FI)	High-potential electrode
	Field desorption (FD)	High-potential electrode
	Electrospray ionization (ESI)	High electrical field
	Matrix-assisted desorption-ionization (MALDI)	Laser beam
	Plasma desorption (PD)	Fission fragments from ^{252}Cf
	Fast atom bombardment (FAB)	Energetic atomic beam
	Secondary-ion mass spectrometry (SIMS)	Energetic beam of ions
	Thermospray ionization (TS)	High temperature

MS Components: Molecular Sources

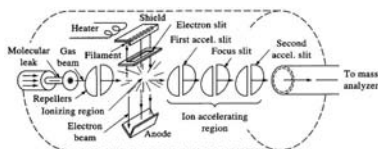


Figure 20-3 An electron-impact ion source. (From R. M. Silverstein, G. C. Bassler, and T. C. Merrill, *Spectroscopic Identification of Organic Compounds*, 5th ed., p. 4, New York: Wiley, 1991. Reprinted by permission of John Wiley & Sons, Inc.)

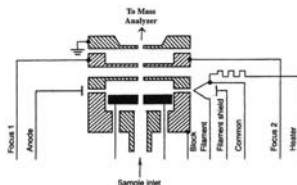
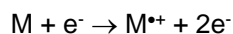


Figure 9.6 Cross-section of an EI source. The filament and anode define the electron beam. The ion slit formed in the gap above the two repellers (the solid color blocks). A positive charge on the repellers together with a negative potential on the focus electrodes, cause positive ions to be accelerated upward in the diagram. (Modified from Ewing, used with permission.)



- **Electron Ionization (EI or Electron Impact):**

- Sample is vaporized by heating and “leaked” into source
- Electrons are formed at a hot filament and accelerated across the path of the sample gas
- As electrons “impact” gas molecules, ionization may occur (electrostatic repulsion). Forms “molecular ion”



MS Components: Molecular Sources

- **EI cont'd**

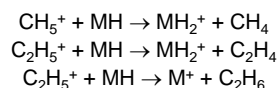
- High energy of electrons results in excited state ions
 - energy may be lost through collisions or reactions
 - Results in fragmentation of molecular ion to form daughter ions
 - Reactions may be unimolecular (fragmentation, rearrangement) or bimolecular
 - “Hard” ionization source
 - Fragmentation pattern is characteristic of molecule → Structure Identification

- **Chemical Ionization (CI):**

- Excess of small, gaseous molecule is added to ionization chamber
- Odds of collision of e^- produce by filament with the additive \gg than with analyte
- Result is production of ionized additive species
- These less-energetic ions serve to ionize analyte

MS Components: Molecular Sources

- CI Example: methane
 - Forms CH_4^+ , CH_3^+ , CH_2^+ by ionization
 - These ions react to form primarily CH_5^+ , and C_2H_5^+
 - Analyte (MH) is ionized by proton transfer or hydride transfer



- Result is a spectrum dominated by $(\text{M}+1)^+$ or $(\text{M}-1)^+$ peaks and little fragmentation
- Soft Ionization Source!
- **Field Ionization**
 - Gas flows past "emitter" subject to large electric field
 - electron tunneling causes ionization
 - Little fragmentation

MS Components: Molecular Sources for Nongaseous Samples

- Applicable to large molecules, nonvolatile species

- **Electrospray Ionization (ESI):**

- Atmospheric pressure method
- Sample is pumped through a needle that is held at high voltage compared to cylindrical electrode
- Produces fine spray of charged droplets
- As solvent evaporates, charge density increases \rightarrow ionization
- Often produces multiply charged ions: good for large molecules!
 - Making elephants fly!

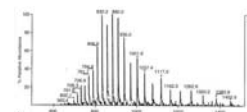
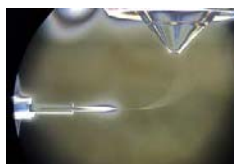
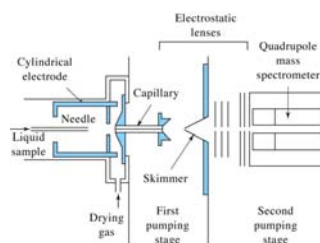


Figure 9.8 ESI mass spectrum (a) and deconvoluted mass spectrum (b) of carbonic anhydride. (Reprinted with permission from Franzen.)

MS Components: Molecular Sources for Nongaseous Samples

• Matrix-Assisted Laser Desorption/Ionization (MALDI)

- Sample is placed in a matrix containing a good optical absorber (chromophore), solvent is removed
- Sample is irradiated with a pulsed laser. Absorption by matrix aids in sublimation/ionization of analyte (HOW?)
- Essentially no fragmentation! Good for big molecules (biopolymers, etc.)

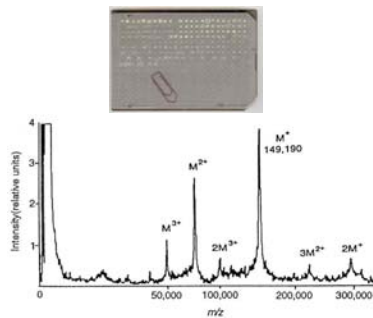
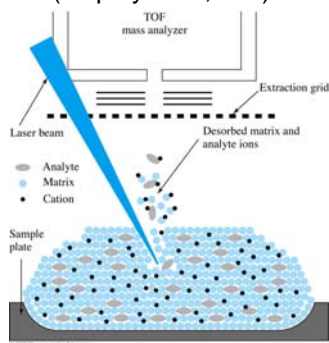


Figure 9.11 The MALDI mass spectrum of a mouse monoclonal antibody. The matrix used was nicotinic acid; the laser radiation used was 266 nm. (Reprinted from Karas and Bahr, with permission from Elsevier.)

MS Components: Molecular Sources for Nongaseous Samples

• Fast Atom Bombardment (FAB):

- Molecule dispersed in a glycerol matrix, bombarded by a beam of atoms from an *atom gun* (energetic)
- Energy transfer results in production of positive and negative ions, matrix helps to aid ejection

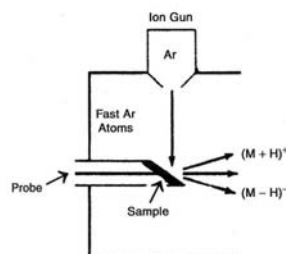
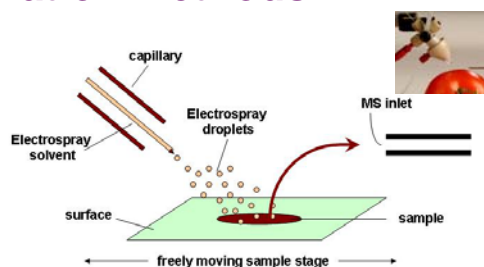


Figure 9.12 Schematic FAB ionization source. The sample, dissolved in solvent, is spread in a thin film on the end of a metal probe and bombarded by fast-moving argon atoms. Both positive and negative ions are produced.

“New” Ionization Methods

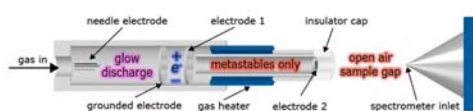
- DESI – Desorption Electro spray Ionization

- Minimal sample prep
- Imaging capabilities



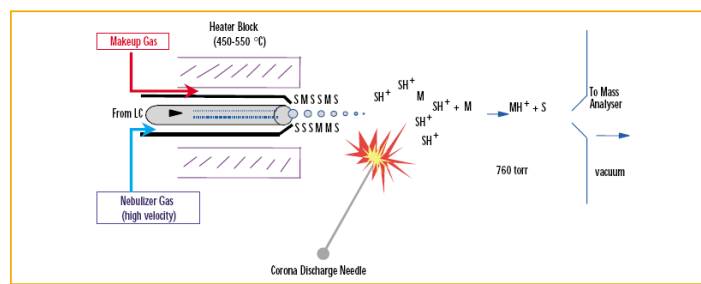
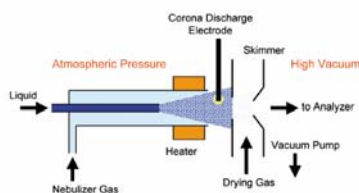
- DART – Direct Analysis in Real Time

- Interaction between metastables and analyte
- $M^* + A \rightarrow A^{*+} + M + e^-$
- No sample prep!



“New” Ionization Methods

- APCI - Atmospheric Pressure Chemical Ionization
- Typically coupled with HPLC



Hyphenated MS Techniques

- **Tandem MS (MS-MS):**

- Multiple MS (often quads) coupled together.
 - Each serves a different purpose
- Soft ionization source produces parent ions that are filtered by the first MS
- Field-free region is filled with inert gas to allow collisions and fragmentation, producing “daughter ions”
- Daughter ions are analyzed
- Since each MS can be scanned, several applications are possible: separations,

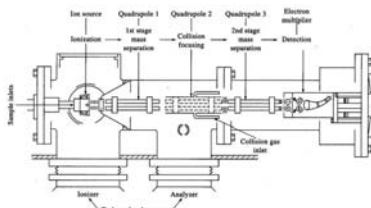


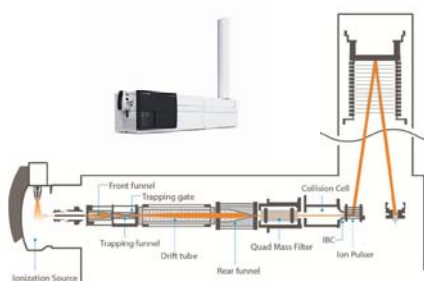
Figure 20-24 Schematic of a tandem quadrupole MS/MS instrument. Courtesy of PerkinElmer.

Hyphenated MS Techniques

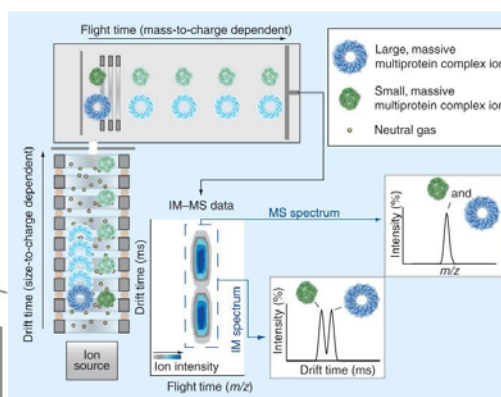
- **Ion Mobility Spectrometry – Mass Spectrometry**

- Separation in two dimensions
 1. Size to charge
 2. Mass to charge

- Application particularly for large molecules



<http://www.chem.agilent.com/en-US/products-services/Instruments-Systems/Mass-Spectrometry/6560-Ion-Mobility-TOF-1-C-MS/Pages/default.aspx>



Expert Rev Proteomics. 2012 ; 9(1): 47–58. doi:10.1586/ep.11.75

Hyphenated MS Techniques

• GC-MS

- Need to deal with the presence of carrier gas and the pressure difference b/w GC and MS
 - Capillary GC is usually no problem
 - Packed Column GC can be a problem
 - use “jet separator” to remove carrier gas
- Typically combined with quads, but also ion-trap detectors: fast scans for rapid separations
- Detection modes: Total ion chromatogram, Selected ion chromatogram or Mass spectra
 - Possible 3-D data containing separation and identification!

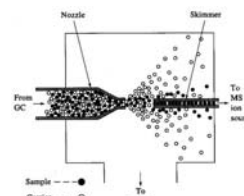


Figure 27-14 Schematic of a jet separator. (Courtesy of DuPont de Nemours and Co., Wilmington, DE.)

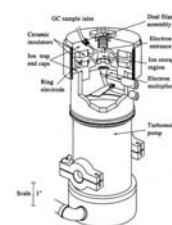
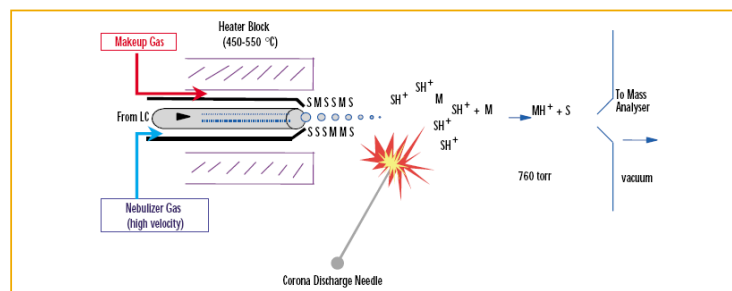


Figure 27-15 Schematic of the ion trap detector. Adapted with permission from U. C. Saffell Jr., P. A. Adley, and D. C. Hoelzel, *Mass Spectrometry*, 1982, 11(1), 11. Copyright 1982 by International Science Communications, Inc.

Hyphenated MS Techniques

• LC-MS

- HUGE difference b/w LC and MS conditions
- Interface is critical
 - Many variations (thermospray, electrospray), nothing is ideal (yet)
 - Most common are ESI and Atmospheric Pressure Chemical Ionization (APCI)



Hyphenated MS Techniques

- **CE-MS**
 - CE is probably best suited for coupling to MS
 - low volume flow rates
 - ESI is most common
 - “End” of the capillary is metalized
 - Allows application of potential for both separation and ionization
 - $E(\text{injection}) > E(\text{ionization}) > \text{ground}$

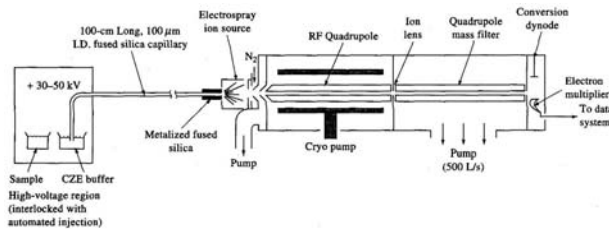


Figure 30-7 An instrument for capillary electrophoresis/mass spectrometry. The voltage between the buffer solution on the left and the metalized silica capillary is 30 to 50 kV. The flow of nitrogen is 3 to 5 kV. The flow of nitrogen at -70°C for desolvation is 3 to 6 L/min. (From R. D. Smith, J. A. Oliveira, N. T. Nguyen, and H. R. Udseth, *Anal. Chem.*, 1988, 60, 437. With permission.)

Strategies for Quantitation (not exclusive to MS)

- Key challenges involve two considerations
 - Instrument limitations
 - Sample limitations
- Ideally, choose the simplest method that provides required level of accuracy and precision
 - Basic calibration curve
- Internal Standards
 - Deal with precision issues by measuring a relative signal of Int. Standard and Analyte
 - Internal Standard and Analyte are different species!

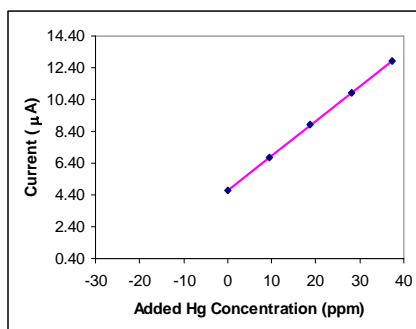
Strategies for Quantitation (not exclusive to MS)

- Standard Additions
 - Often components present in an analyte sample (other than the analyte itself) also contribute to an analytical signal, causing **matrix effects**.
 - It is difficult to know exactly what is present in a sample **matrix**, so it is difficult to prepare standards.
 - Possible to minimize these effects by employing **standard additions**
 - Add a known amount of standard to the sample solution itself.
 - Perform the analysis.
 - The resulting signal is the sum of the signal for the sample and the standard.
 - By varying the concentration of the standard in the solution, it is possible to extract a value for the response of the unknown itself.

Strategies for Quantitation (not exclusive to MS)

- Graphical Approach to Standard Add's:

Hg added (ppm)	Current (μA)
0	4.66
9.36	6.76
18.72	8.83
28.08	10.86
37.44	12.8



- Unknown concentration is derived by extrapolating line to x-intercept.

Strategies for Quantitation (not exclusive to MS)

- Isotope dilution - more MS exclusive
 - Artificially change isotope ratios of a sample by spiking with isotope-enriched standard
 - Standard has same identity as analyte, but different, and known, isotopic abundance.
 - Analyte has natural abundance (typically)
 - Measured isotope ratio from MS reflects combination of analyte and spike signal
 - Signal at m/z for isotope A = $f(C_{\text{unk}}F_A + C_{\text{spike}}F_{A,\text{spike}})$
 - Signal at m/z for isotope B = $f(C_{\text{unk}}F_B + C_{\text{spike}}F_{B,\text{spike}})$
 - C = total concentration of all isotopes of element
 - F_X = Fractional abundance of isotope X
 - Since we know F_X , $F_{X,\text{spike}}$, and C_{spike} , a little algebra gets us to C_{unk}