

# Introduction to Walk-Up Mass Spectrometry

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## Topics Covered

- Introduction to MS and the MSF
- Molecular Weight and Isotope Distributions
- Accuracy and Resolution
- Sources for Walk-Up MS
- Mass Analyzers for Walk-Up MS
- Upcoming Application Seminars

## Mass Spectrometry Facility

- Located in A411
- Staffed from 9:30-5:30, M-F except holidays
- Staff includes:
  - Jonathan A. Karty, Ph.D. (Jon), facility manager
  - Angela M. Hansen (Angie), Sr. Mass Spectrometrist
  - Undergraduate technicians for 2008-2009
    - Derek Zipkin
    - LaDasa Jones
- Instruments for walk-up use
  - Agilent 6890/5973 GC-MS
  - Bruker Biflex III MALDI-TOF
  - 2 Waters LCT Classic ESI-TOF
  - 1 Agilent ESI-Quadrupole (coming soon?!?)

## Why Mass Spectrometry

- **Information is composition-specific**
  - Very selective analytical technique
  - Most other spectroscopies can describe functionality present, but not absolute formula
- **MS is VERY sensitive**
  - MSF personnel dilute NMR samples 1:500
  - Picomole sensitivity is common in the MSF
- **Mass spectrometers have become MUCH easier to use in the last 15 years**

## Three Questions

- **Did I make my compound?**
  - Molecular weight is an intrinsic property of a substance
  - Molecular weight can therefore confirm identity
- **Did I make anything else?**
  - Mass spectrometry is readily coupled to chromatographic techniques
  - Not all compounds ionize easily (cf. UV-VIS)
- **How much of it did I make?**
  - Response in the mass spectrometer is proportional to analyte concentration ( $R = \alpha[M]$ )
    - Each compound has a unique response factor,  $\alpha$

## Common MS Applications

- Quick product identification (TLC plate)
- Confirmation of elemental composition
  - Much more precise than EA
- Selective detector for GC/HPLC
  - MS provides molecular weight information about each chromatographic peak
- Reaction monitoring
  - Crude reaction mixture MS
  - Stable isotope labeling
  - Stability studies

## Mass Spectrometer Components

- Inlet
  - Get samples into the instrument
- Source
  - Ionize the molecules in a useful way
- Mass Analyzer
  - Separates the ions by mass to charge ( $m/z$ ) ratio
- Detector
  - Converts ions into electronic signal or photons
- Data system
  - Photographic plates to computer clusters

## Important Concepts to Remember

- Mass spectrometers analyze gas-phase ions, not neutral molecules
  - Neutrals don't respond to electric and magnetic fields
  - If your molecule cannot ionize, MS cannot help
- MS is not a "magic bullet" technique
  - MS can describe atomic composition of an ion
  - Connectivity of the atoms is much more challenging
- Although MS requires a vacuum, it cannot be performed in a vacuum of information
  - Deriving useful information from MS data often requires some foreknowledge of the system under investigation

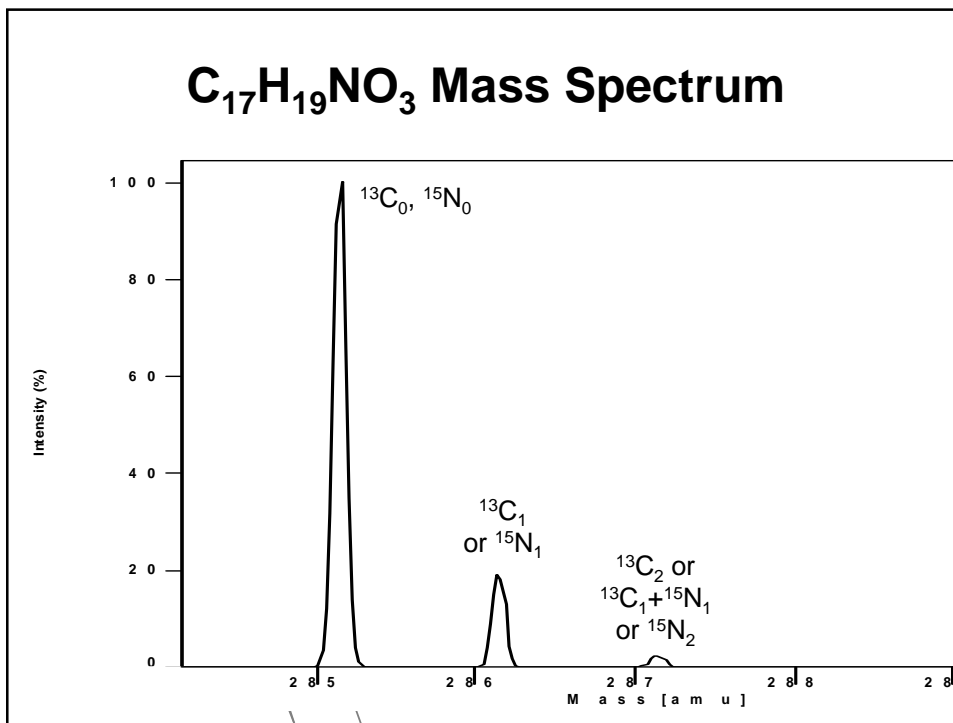
## Molecular Weight Calculations

- The molecular weight of a compound is computed by summing the masses of all atoms that comprise the compound.
  - Morphine:  $C_{17}H_{19}NO_3 = 12.011(17) + 1.008(19) + 14.007 + 15.999(3) = 285.34 \text{ Da}$
- Yet this is not the mass we observe
  - 285.136 is observed by EI-MS
- Molecular weight is calculated assuming a natural distribution of isotopes

## Monoisotopic vs. Average Masses

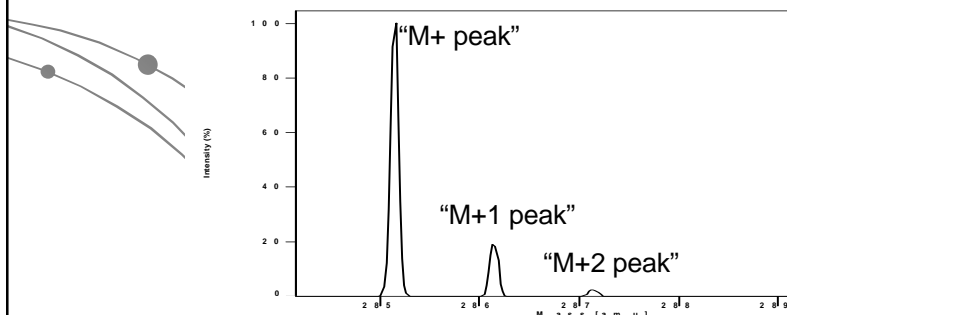
- **Most elements have a variety of isotopes**
  - C  $\rightarrow$   $^{12}\text{C}$  is 98.9% abundant,  $^{13}\text{C}$  is 1.1% abundant
    - For  $\text{C}_{20}$ , 80% chance  $^{13}\text{C}_0$ , 18% chance  $^{13}\text{C}_1$ , 2% chance  $^{13}\text{C}_2$
  - Sn has 7 naturally occurring isotopes @ >5% ab.
  - F, P, Na, Al, Co, I, Au have only 1 natural isotope
- **Mass spectrometers can often resolve these isotopic distributions**
- **Monoisotopic masses must be considered**
  - Monoisotopic masses for multi-isotope species are computed using most intense isotopes of all elements ( $^{12}\text{C}$ ,  $^1\text{H}$ ,  $^{35}\text{Cl}$ ,  $^{32}\text{S}$ ,  $^{79}\text{Br}$ ,  $^{58}\text{Ni}$ )
  - For morphine, monoisotopic mass = 285.1365
    - $12.0000(17) + 1.0078(19) + 14.0031 + 15.9949(3)$

## $C_{17}H_{19}NO_3$ Mass Spectrum



## Isotopic Envelopes

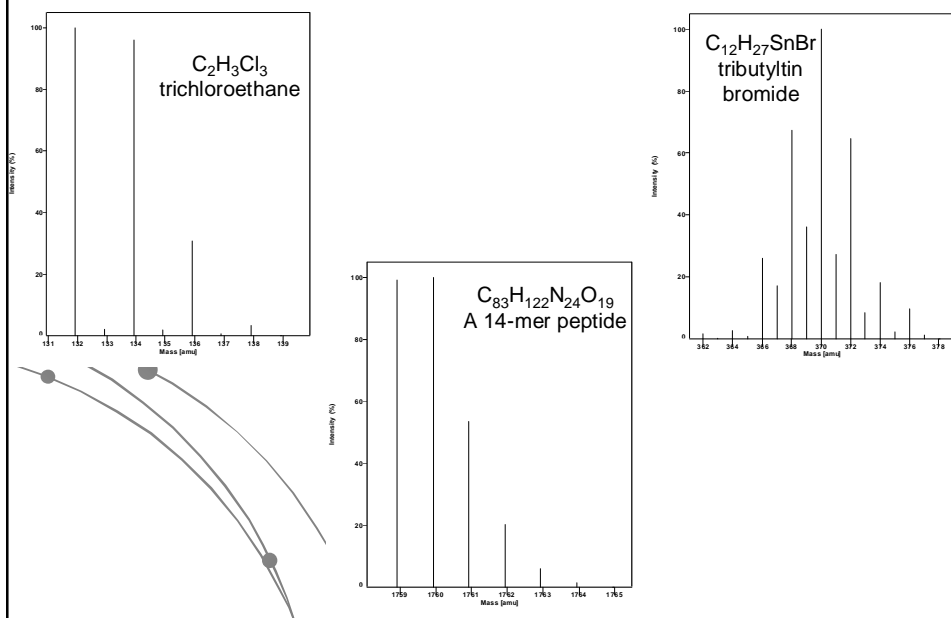
- Mass spectrometers measure ion populations
  - Any single ion only has 1 isotopic composition
  - $10^2 - 10^6$  or more ions in a reliable peak
- The observed mass spectrum represents the sum of all those different compositions



## Isotopic Envelopes 2

- Isotope envelopes can be used to preclude some elements from ionic compositions
  - Lack of intense M+2 peak precludes Cl or Br
  - Many metals have unique isotopic signatures
- M+1/M+ ratio can be used to count carbons
  - $[(M+1)/M+]/0.011 \approx \# \text{ carbon atoms}$
  - For morphine:  $(0.1901/1)/0.011 = 17.28 \rightarrow 17$
- Isotope table can be found on NIST website
  - Link from MSF "Useful Information" page

## A few isotope patterns



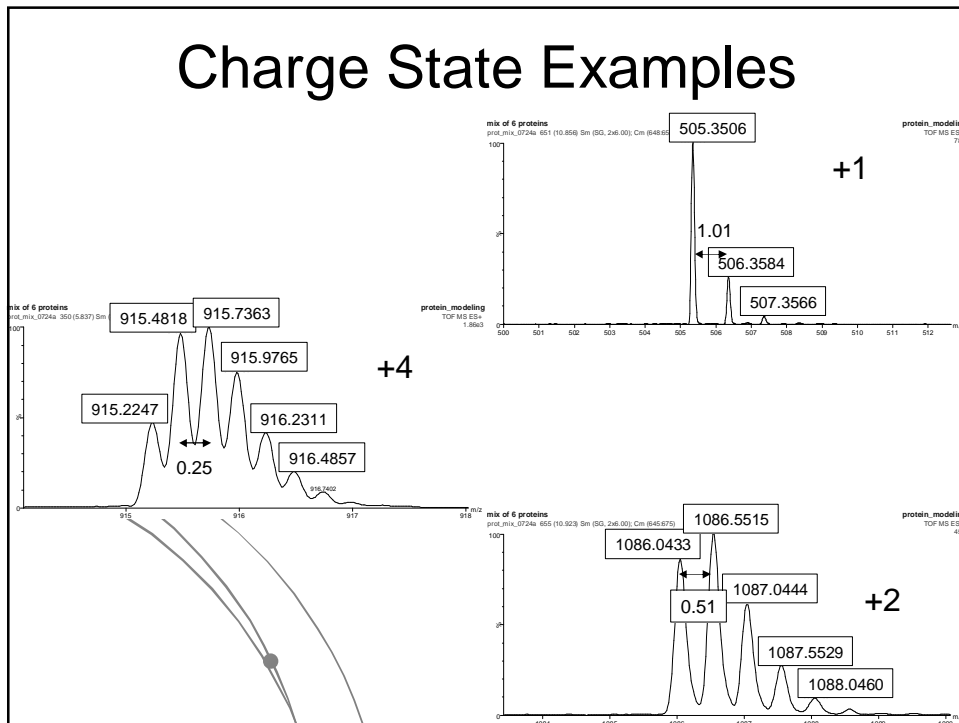
## A little more on molecular ions

- Be aware of ionization mechanism
  - EI, LDI, and CI generate radical cations
    - $M^+$  is an odd electron ion
    - Nitrogen rule is normal
      - Even parent ion mass implies even # of N atoms
      - $M^+$  for morphine by EI is 285.136, odd # N (1)
  - ESI, MALDI, and CI generate cation adducts
    - $M+H$  and  $M+Na$  are even electron ions
    - Nitrogen rule is inverted for odd mass cations
      - Even parent ion mass implies odd # of N atoms
      - $M+Na$  for morphine by ESI is 308.126, odd # N (1)
  - Metal atoms and pre-existing ions or radicals can alter observations

## Charge State Determination

- Mass spectrometrists use 2 units of mass
  - Dalton  $\rightarrow 1 \text{ Da} = 1 \text{ amu}$  (1/12 of a  $^{12}\text{C}$  atom)
  - Thompson  $\rightarrow 1 \text{ Th} = 1 \text{ Da}/z$  ( $z$  is electron charge)
- Thompson is more correct when referring to data from a mass spectrum
  - For a +1 ion,  $m/z$  in Th  $\approx$  mass in Da
- High molecular weight ions generated by ESI and MALDI often carry more than one charge
  - Determined by measuring spacing between adjacent isotopes (e.g.  $^{13}\text{C}_1$  and  $^{13}\text{C}_2$ ) (charge =  $1/\text{spacing}$ )
  - 0.33 Th between isotopes, +3 charge

# Charge State Examples



# Mass Accuracy

- **Mass accuracy reported as a relative value**
  - ppm = parts per million (1 ppm = 0.0001%)
    - 5 ppm @ m/z 300 =  $300 * (5/10^6) = \pm 0.0015$  Th
    - 5 ppm @ m/z 3,000 =  $3,000 * (5/10^6) = \pm 0.015$  Th
- **High resolving power facilitates precise mass measurements**
- **Mass accuracies for MSF instruments**
  - LCT: <50 ppm (ext. calib.), <5 ppm (int. calib.)
  - Biflex MALDI-TOF: depends on mass range
    - Under 3,000 Da w/ internal calibration: 60 ppm
    - Over 3,000 Da w/ internal calibration: 200 ppm
  - Quadrupole (GC-MS):  $\pm 0.2$  Th (absolute)

# What is Resolution?

- Resolution is the ability to separate ions of nearly equal mass/charge

- e.g.  $C_6H_5Cl$  and  $C_6H_5OF$  @ 112 m/z

- $C_6H_5Cl = 112.00798$  amu (all  $^{12}C$ ,  $^{35}Cl$ ,  $^1H$ )
- $C_6H_5OF = 112.03244$  amu (all  $^{12}C$ ,  $^{16}O$ ,  $^1H$ ,  $^{19}F$ )
- Resolving power >4700 required to resolve these two

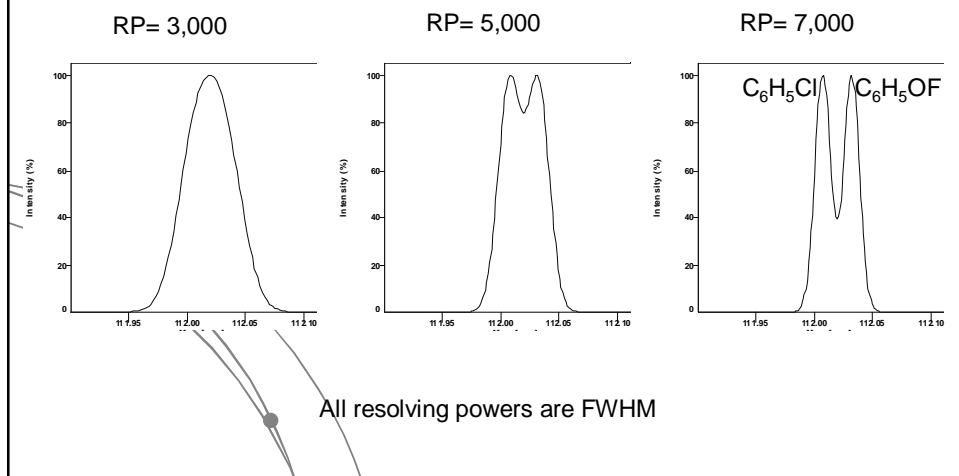
- Two definitions

- Resolution =  $\Delta m/m$  ( $0.024/112.03 = 0.00022$  or  $2.2 \cdot 10^{-4}$ )
- Resolving power =  $m/\Delta m$  ( $112.03/0.024 = 4668$ )

- Walk-up instrument capabilities

- Biflex is capable of 10,000 resolving power
- LCT is capable of 5,000 resolving power
- All peaks in GC-MS are about 0.6 Th wide

# Resolving Power Example



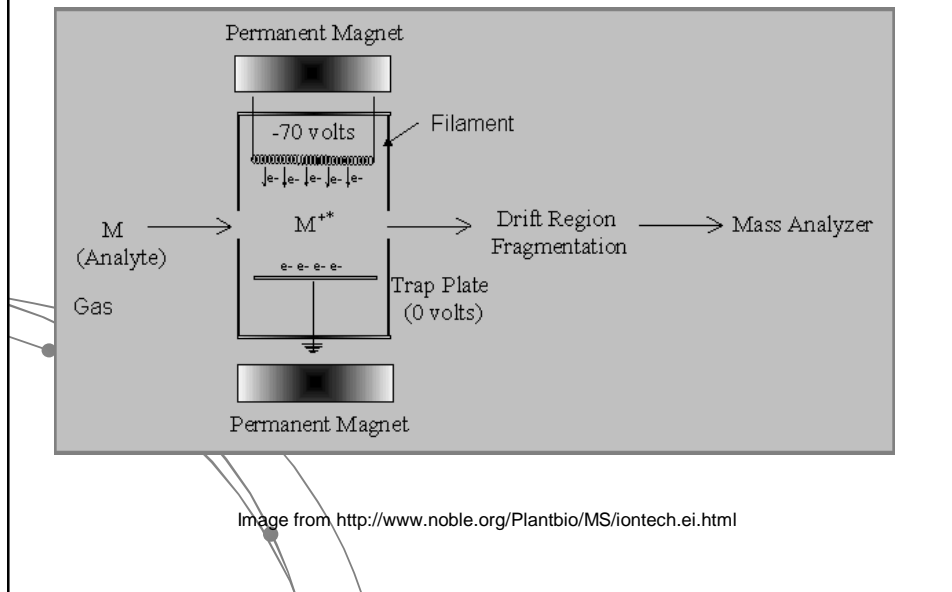
## Some useful software tools

- The “exact mass” feature in ChemDraw will give you a monoisotopic mass
- IsisDraw exact mass is not correct for large (>2,000 Da) compounds
- IsoPro (freeware) can be used to predict isotopic envelopes
  - See MS Links page for URL
- MassLynx “Isotope Model” can be used to predict isotope patterns
- BioLynx module of MassLynx can be used to predict oligopeptide, oligosaccharide, and oligonucleotide masses

## Electron Ionization (EI)

- Gas phase molecules are irradiated by beam of electrons
- Interaction between molecule and beam results in electron ejection
  - $M + e^- \rightarrow M^{+\bullet} + 2e^-$
- Radical species dominate
- EI is a very energetic process
  - Molecules often fragment right after ionization

## EI Diagram



## EI Advantages

- Simplest source design of all
- Very high yield (up to 0.1% ionization)
- Simple, robust ionization mechanism
  - Even noble gases are ionized by EI
- Fragmentation patterns can be used to identify species
  - NIST '08 library has over 220,000 spectra
  - Interpretation allows functionalities to be deduced in novel compounds

## EI Disadvantages

- Fragmentation often makes intact molecular ion difficult to observe
- Analytes must be in the gas phase
  - Not applicable to most salts
  - Labile compounds not amenable to EI
- Databases are very limited
  - NIST'08 has 192,000 unique compounds
  - Interpreting EI spectra *de novo* is an art
- EI only generates positive ions

## EI Mass Spectrum

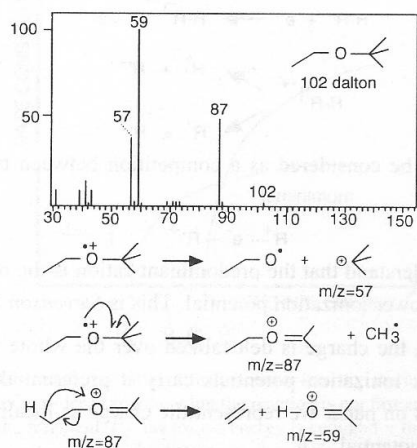


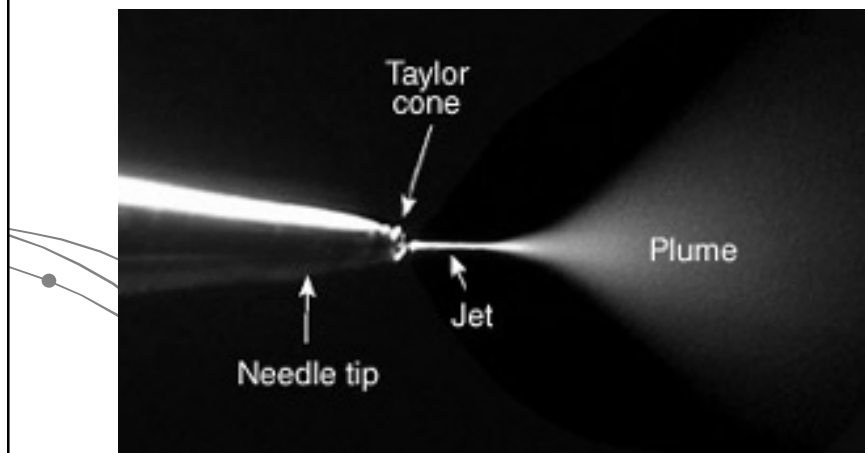
Figure 6.5: Fragmentation of *t*-butyl ethyl ether.

Figure from *Mass Spectrometry Principles and Applications*  
E. De Hoffmann, J. Charette, V. Strooband, eds., ©1996

## Electrospray Ionization (ESI)

- Dilute solution of analyte (<1 mg/L) infused through a fine needle in a high electric field
- Very small, highly charged droplets are created
- Solvent evaporates, droplets split and/or ions evaporate to lower charge/area ratio
- Warm nebulizing gas accelerates drying
- Free ions are directed into the vacuum chamber
- Ion source voltage depends on solvent
  - Usually  $\pm 2500 - \pm 4500$  V
    - +HV makes positive ions, -HV makes negative ions

## ESI Picture



## Characteristics of ESI Ions

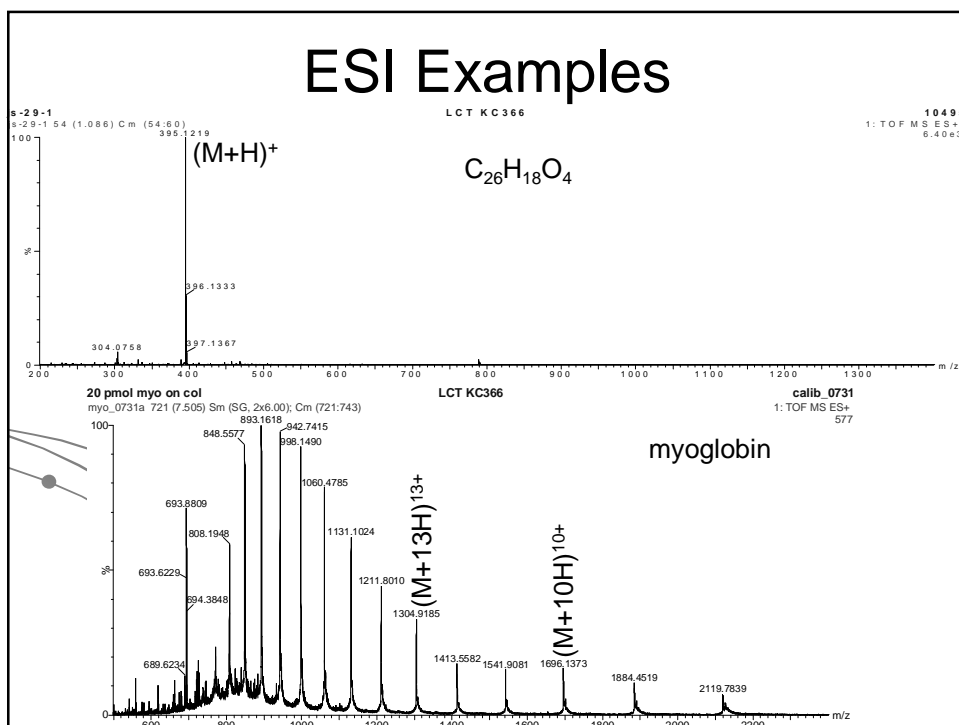
- ESI is a thermal process (1 atm in source)
  - Little fragmentation due to ionization (cf EI)
- Solution-phase ions are preserved in MS
  - e.g. organometallic salts
- ESI ions are generated by ion transfer
  - $(M+H)^+$ ,  $(M+Na)^+$ , or  $(M-H)^-$ , rarely  $M^{+•}$  or  $M^{•-}$
- ESI often generates multiply charged ions
  - $(M+2H)^{2+}$  or  $(M+10H)^{10+}$
  - *Most ions are 500-1500 m/z*
- ESI spectrum x-axis must be mass/charge (m/z or Th, not amu or Da)

## Advantages of ESI

- Gentlest ionization process
  - Greatest chance of observing molecular ion
  - Very labile analytes can be ionized
- Molecule need not be volatile
  - Proteins/peptides easily analyzed by ESI
  - Salts can be analyzed by ESI
- Easily coupled with HPLC
- Both positive and negative ions can be generated by the same source

## ESI Disadvantages

- Analyte must have an acidic or basic site
  - Hydrocarbons and steroids not readily ionized by ESI
- Analyte must be soluble in polar, volatile solvent
- ESI is less efficient than other sources
  - Most ions don't make it into the vacuum system
- ESI is very sensitive to contaminants
  - Solvent clusters can dominate spectra
- Distribution of multiple charge states can make spectra of mixtures hard to interpret
  - e.g. polymer mass spectra



## Matrix-Assisted Laser Desorption/Ionization (MALDI)

- Analyte is mixed with UV-absorbing matrix
  - ~10,000:1 matrix:analyte ratio
  - Analyte does not need to absorb laser
- A drop of this liquid is dried on a target
  - Analyte incorporated into matrix crystals
- Spot is irradiated by a laser pulse
  - Irradiated region sublimates, taking analyte with it
  - Matrix is often promoted to the excited state
  - Charges exchange between matrix and analyte in the plume (very fast <100 nsec)
- Ions are accelerated toward the detector

## MALDI Diagram

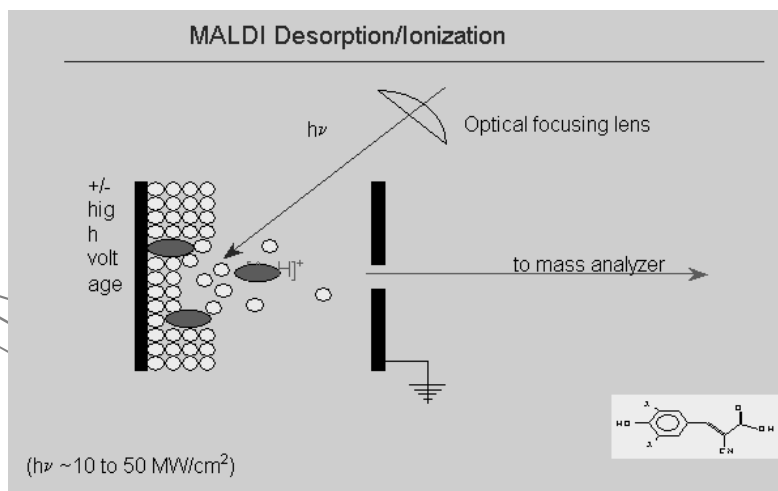


Image from <http://www.noble.org/Plantbio/MS/iontech.maldi.html>

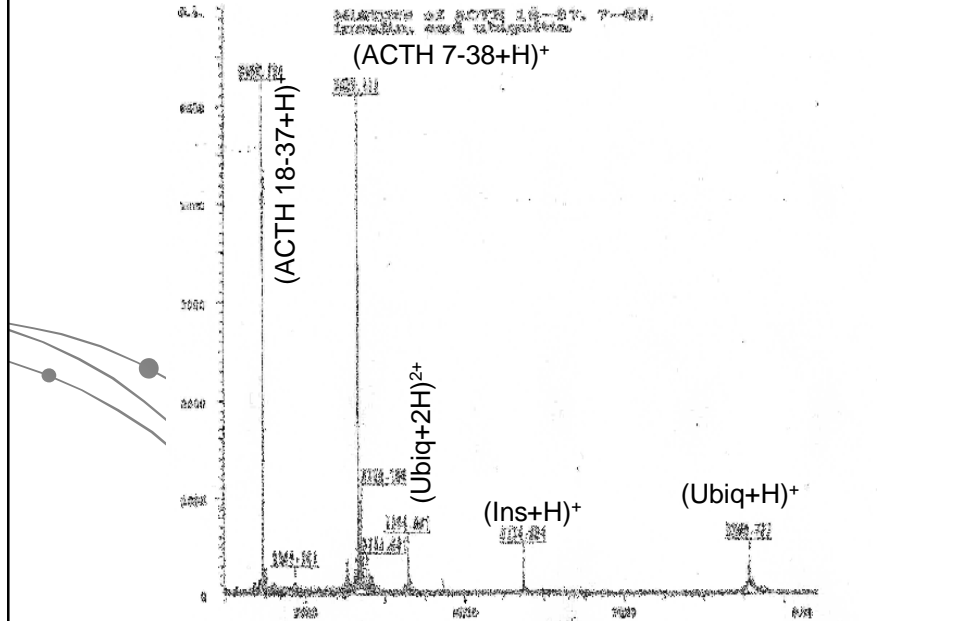
## MALDI Advantages

- Relatively gentle ionization technique
- Very high MW species can be ionized
- Molecule need not be volatile
- Very easy to get sub-picomole sensitivity
- Usually 1-3 charge states, even for very high MW species
- Positive or negative ions from same spot
- Wide array of matrices available

## MALDI Disadvantages

- MALDI matrix cluster ions obscure low  $m/z$  (<600) range
- Analyte must have very low vapor pressure
- Pulsed nature of source limits compatibility with many mass analyzers
- Coupling MALDI with chromatography can be difficult
- Analytes that absorb the laser can be problematic
  - Fluorescein-labeled peptides

## MALDI Example

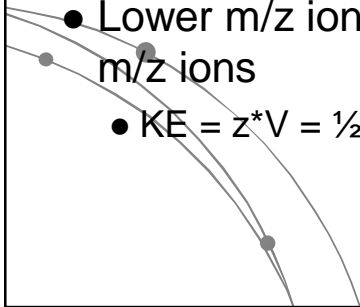


## Types of Mass Analyzers

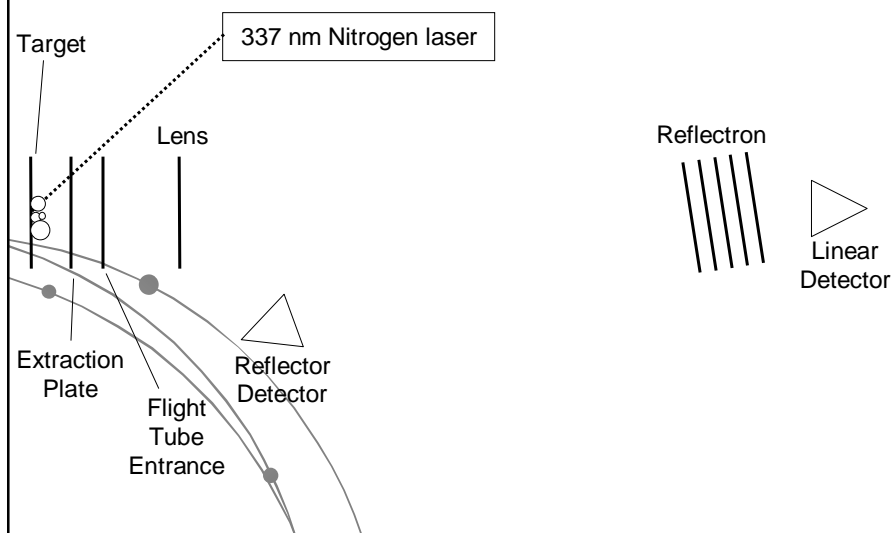
- Scanning: only one  $m/z$  ratio measured at a time (cf grating spectrophotometer)
  - Quadrupole mass filter
  - Magnetic/electric sector
- Multiplexing: all  $m/z$  ratios analyzed simultaneously (cf FTIR or PDA)
  - Time-of-flight
  - Ion trap
  - Fourier transform ion cyclotron resonance

## Time-of-Flight (TOF)

- All ions simultaneously accelerated through the same voltage
  - Excellent choice for MALDI
- Ions drift through a field-free region
- Lower  $m/z$  ions travel faster than higher  $m/z$  ions
  - $KE = z \cdot V = \frac{1}{2} m \cdot v^2 \rightarrow TOF \propto (m/z)^{\frac{1}{2}}$



## MALDI-TOF Diagram



## TOF Advantages

- All ions detected at once (multiplexing)
- High mass accuracy and resolving power possible
- Reasonable performance for cost
  - <5 ppm mass accuracy and >20,000 resolving power commercially available (\$150k-\$300k)
- High mass, low charge ions not a problem
  - Theoretically unlimited mass range
  - +1 Ion > 1,000,000 Th by MALDI-TOF

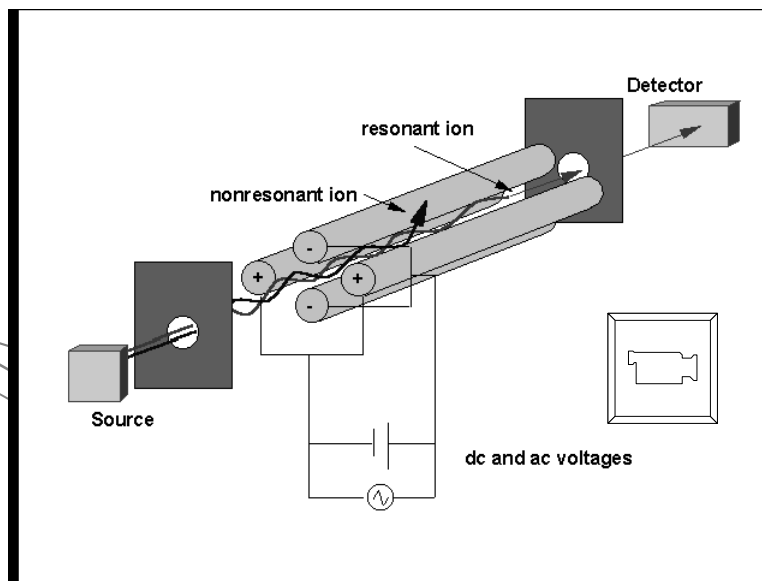
## TOF Disadvantages

- High vacuum required for resolution and accuracy (<10<sup>-7</sup> torr)
  - Complex vacuum system necessary
- Must be recalibrated often
  - Temperature and voltage fluctuations alter flight times
- Fast detectors prone to saturation
- Long flight tubes for high resolving power can make instruments large

## Quadrupole Mass Filter (QMF)

- QMF has radio frequency (RF) and DC field between 4 rods
  - Rods can be cylindrical or hyperbolic
  - Ion motions governed by set of Mathieu equations (2<sup>nd</sup> order differential equations)
- A narrow range of  $m/z$ 's have stable trajectories through the quadrupole (usually 0.7 Th FWHM)
- Scanning the quadrupole generates the mass spectrum
  - 50.0, 50.2, 50.4, 50.6,  $\rightarrow$  399.6, 399.8, 400.0 (repeat)

## Quadrupole Diagram



Movie URL: <http://www.youtube.com/watch?v=8AQaFd1Yow%20%20mode=related%20%20search=>

## QMF Advantages

- Very simple to implement
- Low cost (<\$100k)
- Moderate vacuum required ( $\sim 10^{-5}$  torr)
- Small size
- Very robust
- Most common MS in use

## QMF Disadvantages

- Limited mass range (up to  $m/z$  4,000)
- Limited resolving power and mass accuracy
  - Unit mass accuracy ( $\pm 0.2$  Th for all ions)
  - Unit resolution (0.5 Th wide) peak
    - Cannot resolve isotopes on multiply charged ions
    - High resolving power, less sensitivity
- Scanning limits sensitivity and speed
  - Quad can rapidly jump between select  $m/z$  ratios for increased speed & sensitivity

## Walk-up Instruments in the MSF

- Agilent 6890n/5973i GC-MS
  - EI QMF instrument
  - 10-800 m/z range
  - All analytes MUST pass through GC column
- Waters LCT Classic (2 in lab)
  - ESI-TOF instrument
  - One is set up for flow injection analysis of small molecules (no LC column)
  - The other is set up for LC-MS of biomolecules
- Bruker Biflex III
  - MALDI-TOF instrument

## Upcoming Application Seminars in Ballantine Hall 006

- Analyzing small molecules by ESI-TOF
  - Monday July 28 @ 1:30 noon
- Analyzing proteins/peptides by MALDI-TOF
  - Tuesday July 29 @ 1:30 noon
- Analyzing semi-volatiles by GC-MS
  - Thursday July 31 @ 1:30 noon
- Analyzing proteins/peptides by ESI-TOF
  - Monday Aug. 4 @ 1:30 noon)
- Please indicate which ones you want to attend on the sign-up sheet