# AN INTRODUCTION TO MASS SPECTROMETRY

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# 1: INTRODUCTION

Mass Spectrometry is a powerful technique for identifying unknowns, studying molecular structure, and probing the fundamental principles of chemistry. Applications of mass spectrometry include identifying and quantitating pesticides in water samples, identifying steroids in athletes, determining metals at ppq (Parts Per Quadrillion) levels in water samples, carbon-14 dating the Shroud of Turin using only 40 mg of sample (1), looking for life on Mars, determining the mass of an $^{28}Si$ atom with an accuracy of 70 ppt (2), and studying the effect of molecular collision angle on reaction mechanisms.

Mass spectrometry is essentially a technique for finding the mass by weighing molecules. Obviously, this is not done with a conventional balance or scale. Instead, mass spectrometry is based up on the motion of a charged particle, called an ion, in an electric or magnetic field. The mass-to-charge ratio $m/z^*$ of the ion effects this motion and it is actually the mass-to-charge ratio that is determined by the experiment. Since the charge of an electron is known, the mass to charge ratio a measurement of an ion's mass. Typical mass spectrometry research focuses on the formation of gas phase ions, the chemistry of ions, and applications of mass spectrometry.

This paper covers the basics of mass spectrometry instrumentation and introduces the interpretation of mass spectra. It is only an introduction and interested readers are encouraged to consult more specialized books and journal articles for additional details. The articles and books referenced in this paper should be available at most college and university libraries.

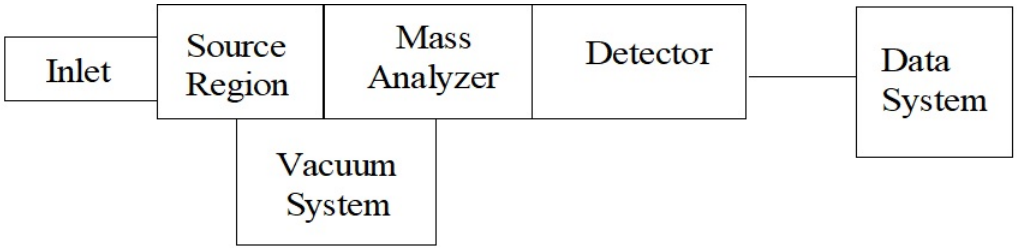


Figure 1.1: Mass Spectrometer Block Diagram

Figure 1.1 is a block diagram that shows the basic parts of a mass spectrometer. The inlet transfers the sample into the vacuum of the mass spectrometer. In the source region, neutral sample molecules are ionized and then accelerated into the mass analyzer. The mass analyzer is the heart of the mass spectrometer. This section separates ions, either in space or in time, according to their mass to charge ratio. After the ions are separated, they are detected and the signal is transferred to a data system for analysis. All mass spectrometers also have a vacuum system to maintain the low pressure, which is also called high vacuum, required for operation. High vacuum minimizes ion-molecule reactions, scattering, and neutralization of the ions. In some experiments, the pressure in the source region or a part of the mass spectrometer is intentionally increased to study these ion-molecule reactions. Under normal operation, however, any collisions will interfere with the analysis.

* The mass to charge ratio, $m/z$, is used to describe ions observed in mass spectrometry. By convention, $m$ is the numerical value for the mass of the ion and $z$ is the numerical value for the charge of the ion. The unified atomic mass (u) and the elementary charge units $e$ are used for these values. The unified atomic mass is defined as $1/12$ the mass of an atom of $^{12}C$. Note: the amu is no longer an accepted term because there are conflicting definitions. Another unit, the dalton, is frequently used for polymers, peptides and other large molecules.

The elementary charge unit is defined as $z$ is an integer equal to the number of electrons lost (or gained for negative ions). For many experiments one electron is lost during ionization so $z$ is +1 and the $m/z$ value is equivalent to the relative molecular mass of the ion. Because the unified atomic mass and the charge number are pure numbers the mass-to-charge ratio is a number and does not have any units. For calculations of the physical behavior of ions it is often necessary to use the actual mass (SI units of kilogram) and charge (SI units of coulomb).

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# CHAPTER OVERVIEW

## 2: SAMPLE INTRODUCTION

The selection of a sample inlet depends up on the sample and the sample matrix. Most ionization techniques are designed for gas phase molecules so the inlet must transfer the analyte into the source as a gas phase molecule. If the analyte is sufficiently volatile and thermally stable, a variety of inlets are available. Gases and samples with high vapor pressure are introduced directly into the source region. Liquids and solids are usually heated to increase the vapor pressure for analysis. If the analyte is thermally labile (it decomposes at high temperatures) or if it does not have a sufficient vapor pressure, the sample must be directly ionized from the condensed phase. These direct ionization techniques require special instrumentation and are more difficult to use. However, they greatly extend the range of compounds that may be analyzed by mass spectrometry. Commercial instruments are available that use direct ionization techniques to routinely analyze proteins and polymers with molecular weights greater than 100,000 dalton.

# 2.1: Direct Vapor Inlet

Direct Vapor Inlet. The simplest sample introduction method is a direct vapor inlet. The gas phase analyte is introduced directly into the source region of the mass spectrometer through a needle valve. Pump out lines are usually included to remove air from the sample. This inlet works well for gases, liquids, or solids with a high vapor pressure. Samples with low vapor pressure are heated to increase the vapor pressure. Since this inlet is limited to stable compounds and modest temperatures, it only works for some samples.

# 2.2: Gas Chromatography

Gas chromatography is probably the most common technique for introducing samples into a mass spectrometer. Complex mixtures are routinely separated by gas chromatography and mass spectrometry is used to identify and quantitate the individual components. Several different interface designs are used to connect these two instruments. The most significant characteristics of the inlets are the amount of GC carrier gas that enters the mass spectrometer and the amount of analyte that enters the mass spectrometer. If a large flow of GC carrier gas enters the mass spectrometer it will increase the pressure in the source region.

Probably the most common GC/MS interface uses a capillary GC column. Since the carrier gas flow rate is very small for these columns, the end of the capillary is inserted directly into the source region of the mass spectrometer. The entire flow from the GC enters the mass spectrometer. Since capillary columns are now very common, this inlet is widely used. However this design is not well suited for experiments with wide bore capillaries and packed GC columns which have higher flow rates. The increase in the flow rate significantly increases the pressure in the mass spectrometer and maintaining the required source pressure will require larger and more expensive vacuum pumps. Several inlet designs are available to reduce the gas flow into the source. The simplest design splits the GC effluent so that only a small portion of the total flow enters the mass spectrometer. Although this inlet reduces the gas load on the vacuum system, it also reduces the amount of analyte and thus the sensitivity. Effusive separators and membrane inlets are more selective and transport a higher fraction of the analyte into the source region. Each of these methods has efficiency and resolution drawbacks but they are necessary for some experiments.

# 2.3: Liquid Chromatography

Liquid Chromatography. Liquid chromatography inlets are used to introduce thermally labile compounds not easily separated by gas chromatography. These inlets have undergone considerable development and LC/MS is now fairly routine. Because these inlets are used for temperature sensitive compounds, the sample is ionized directly from the condensed phase. These inlets are discussed in greater detail in the section on ionization techniques.

# 2.4: Direct Insertion Probe

Direct Insertion Probe. The Direct Insertion Probe (DIP) is widely used to introduce low vapor pressure liquids and solids into the mass spectrometer. The sample is loaded into a short capillary tube at the end of a heated sleeve. This sleeve is then inserted through a vacuum lock so the sample is inside the source region. After the probe is positioned, the temperature of the capillary tube is increased to vaporize the sample. This probe design allows higher temperatures than are possible with a direct vapor inlet. In addition, the sample is under vacuum and located close to the source so that lower temperatures are required for analysis. This is important for analyzing temperature sensitive compounds. Although the direct insertion probe is more cumbersome than the direct vapor inlet, it is useful for a wider range of samples.

# 2.5: Direct Ionization of Sample

Direct Ionization of Sample. Unfortunately, some compounds either decompose when heated or have no significant vapor pressure. These samples may be introduced to the mass spectrometer by direct ionization from the condensed phase. These direct ionization techniques include electrospray, matrix assisted laser desorption (MALDI), glow discharge mass spectrometry, fast atom bombardment and laser ablation. The development of new ionization techniques is an active research area and these techniques are rapidly evolving. Direct ionization is discussed in greater detail in the next section.

# CHAPTER OVERVIEW

## 3: IONIZATION TECHNIQUES

A variety of ionization techniques are used for mass spectrometry. Most ionization techniques excite the neutral analyte molecule which then ejects an electron to form a radical cation $M^{\bullet+}$. This radical cation is the molecular ion and is produced by removing a single electron from from a neutral molecule. Other ionization techniques involve ion molecule reactions between an ion and a neutral molecule that produce an adduct ion like $[M+H]^+$. Many of these reactions cause the addition of a proton $H^+$ to the analyte molecule but other ions can also be formed. The most important considerations for selecting an ionization technique are the physical state of the analyte and the ionization energy needed. Electron ionization and chemical ionization are only suitable for gas phase ionization. Fast atom bombardment, secondary ion mass spectrometry, electrospray, and matrix assisted laser desorption are used to ionize condensed phase samples. The ionization energy is significant because it controls the amount of fragmentation observed in the mass spectrum. Although this fragmentation complicates the mass spectrum it provides structural information for the identification of unknown compounds. Some ionization techniques are very soft and only produce molecular ions, the intact ionized analyte molecule, while other techniques are very energetic and cause ions to undergo extensive fragmentation.

# 3.1: Electron Ionization

## Electron Ionization

Electron Ionization (EI) is the most common ionization technique used for mass spectrometry.* EI works well for many gas phase molecules, but it does have some limitations. Although the mass spectra are very reproducible and are widely used for spectral libraries, EI causes extensive fragmentation so that the molecular ion is not observed for many compounds. The fragmentation is useful because it provides structural information for interpreting unknown spectra. Fragmentation patterns are discussed in more detail in the chapter on Interpretation.

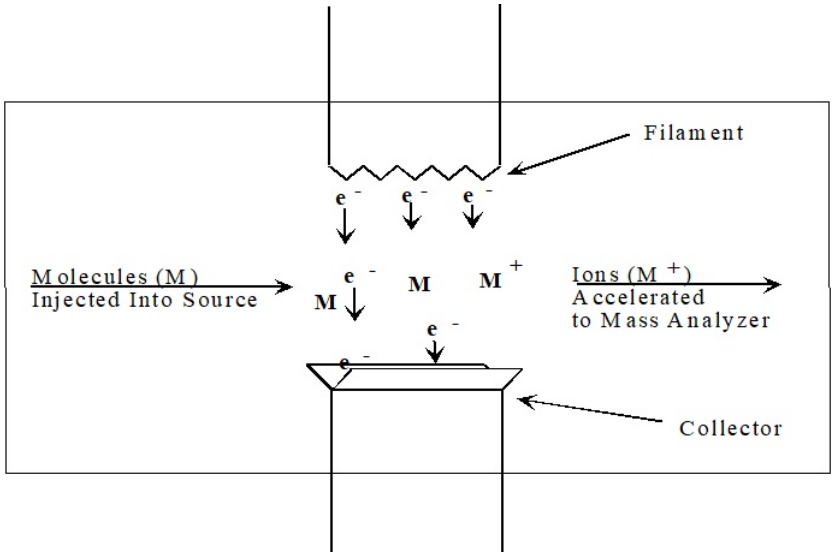


Figure 3.1.1: Electron Ionization Source

The electrons used for ionization are produced by passing a current through a wire filament (Figure 3.1.1). The amount of current controls the number of electrons emitted by the filament. An electric field accelerates these electrons across the source region to produce a beam of high energy electrons. When an analyte molecule passes through this electron beam, a valence shell electron can be removed from the molecule to produce an ion.

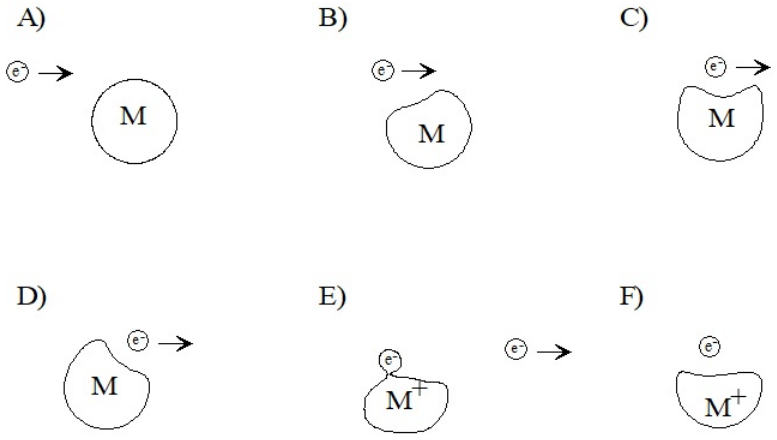


Figure 3.1.2: Electron Ionization Process. A) Ionizing electron approaches the electron cloud of molecule; B) Electron cloud distorted by ionizing electron; C) Electron cloud further distorted by ionizing electron; D) Ionizing electron passes by the molecule; E) Electron cloud of molecule ejecting an electron; F) Molecular ion and ejected electron.

Ionization does not occur by electron capture, which is highly dependent upon molecular structure. Instead, EI produces positive ions by knocking a valence electron off the analyte molecule (Figure 3.1.2). As the electron passes close to the molecule the negative charge of the electron repels and distorts the electron cloud surrounding the molecule. This distortion transfers kinetic

energy from the fast-moving electron to the electron cloud of the molecule. If enough energy is transferred by the process, the molecule will eject a valence electron and form a radical cation $M^{\bullet+}$.

Since the ionization is produced by a single electron that is accelerated to 70 V, this is commonly referred to as 70 eV EI.** This is enough energy to cause extensive fragmentation, and at this level small changes in the electron energy do not significantly effect the fragmentation patterns. The amount of energy transferred during this process depends up on how fast the electron is traveling and how close it passes to the molecule. In most 70 eV EI experiments, approximately 1400 kJ (15 eV) of energy is transferred during the ionization process. There is, however, a distribution of energy and as much as 2800 kJ (30 eV) is transferred to some molecules. Since approximately 960 kJ/mole (10 eV) of energy is required to ionize most organic compounds and a typical chemical bond energy is 290 kJ/mole (3 eV), extensive fragmentation is often observed in 70 eV EI mass spectra. The distribution of energy transferred during ionization and the large number of fragmentation pathways results in a variety of products for a given analyte. Other electron voltages may be used to vary the amount of fragmentation produced during ionization. For most organic compounds the threshold energy for EI is about 20 eV.

Because a mass spectrum is produced by ionizing many molecules, the spectrum is a distribution of the possible product ions. Intact molecular ions are observed from ions produced with little excess energy. Other molecular ions are formed with more energy and undergo fragmentation in the source region. The abundance of the resulting fragments, often called product ions, is determined by the kinetics of the fragmentation pathways and the ionization energy. Changing the ionization energy changes the observed distribution of fragment ions. This distribution provides the structural information for interpreting mass spectra and is discussed in detail in the section on interpretation.

* Some older literature will refer to EI as electron impact, but this term is not considered accurate. Electron Ionization is the currently accepted term.

** The SI unit for energy is the Joule. The energetics of chemical reactions are typically expressed in kilojoules per mole. In many gas phase experiments (like mass spectrometry), the mole is not a convenient unit. The electron volt is frequently used as an energy unit for single molecules or atoms. 1 eV = 1.60217733(49) x $10^{-19}$ J. So that: 1 eV (per molecule or atom) = 96.4152206 kJ/mole.

# 3.2: Chemical Ionization

## Chemical Ionization (1, 2)

Chemical Ionization (CI) is a soft ionization technique that produces ions with little excess energy. As a result, less fragmentation is observed in the mass spectrum. Since this increases the abundance of the molecular ion, the technique is complimentary to 70 eV EI. CI is often used to verify the molecular mass of an unknown. Only slight modifications of an EI source region are required for CI experiments.

In Chemical Ionization the source is enclosed in a small cell with openings for the electron beam, the reagent gas and the sample. The reagent gas is added to this cell at a pressure of approximately 10 Pa (0.1 torr). This is higher than the pressure of $10^{-3}$ Pa ($10^{-5}$ torr) typical for a mass spectrometer source. At $10^{-3}$ Pa the mean free path between collisions is approximately 2 meters and ion-molecule reactions are unlikely. In the CI source, however, the mean free path between collisions is only $10^{-4}$ meters and analyte molecules undergo many collisions with the reagent gas. The reagent gas in the CI source is ionized with an electron beam to produce a cloud of ions. The reagent gas ions in this cloud react and produce adduct ions like $CH_5^+$ (Figure 3.2.1), which are excellent proton donors.

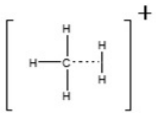

Figure 3.2.1: $CH5^+$ Ion

When analyte molecules are introduced to a source region with this cloud of ions, the reagent gas ions donate a proton to the analyte molecule and produce adduct ions, $[M+H]^+$. The energetics of the proton transfer is controlled by using different reagent gases. The most common reagent gases are methane, isobutane and ammonia. Methane is the strongest proton donor commonly used with a proton affinity (PA) of 5.7 eV. For softer ionization, isobutane (PA 8.5 eV) and ammonia (PA 9.0 eV) are frequently used. Acid base chemistry useful for describing these chemical ionization reactions. The reagent gas must be a strong enough Brønsted acid to transfer a proton to the analyte. Fragmentation is minimized in CI by reducing the amount of excess energy produced by the reaction. Because the adduct ion have little excess energy and are relatively stable, CI is very useful for molecular mass determination. Some typical reactions in a CI source are shown in Figure 3.2.2.

A) EI of reagent gas to form ions:

$$CH_4 + e^- \rightarrow CH_4^+ + 2e^-$$

B) Reaction of reagent gas ions to form adducts:

$$CH_4^+ + CH_4 \rightarrow CH_3 + CH_5^+$$

OR

$$CH_4^+ \rightarrow CH_3^+ + H$$
$$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2$$

C) Reaction of Reagent Gas Ions with analyte molecules:

$$CH_5^+ + M \rightarrow CH_4 + MH^+$$
$$C_2H_5^+ + M \rightarrow C_2H_4 + MH^+$$
$$CH_3^+ + M \rightarrow CH_4 + (M-H)^+$$

Figure 3.2.2: Chemical Ionization Reactions

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# 3.3: Atmospheric Pressure Ionization and Electrospray Ionization

## Atmospheric Pressure Ionization and Electrospray Ionization. (1, 2, 3)

Atmospheric Pressure Ionization (API) sources ionize the sample at atmospheric pressure and then transfer the ions into the mass spectrometer. These techniques are used to ionize thermally labile samples such as peptides, proteins and polymers directly from the condensed phase. The sample is dissolved in an appropriate solvent and this solution is introduced into the mass spectrometer. With conventional inlets the solvent increases the pressure in the source region of the mass spectrometer. In addition, Joule-Thompson cooling of the liquid as it enters the vacuum causes the solvent droplets to freeze. The frozen clusters trap analyte molecules and reduce the sensitivity of the experiment. No matrix is used and the ionizing beam is focused directly on the sample. Although this makes sampling more difficult, it is useful for studying surface chemistry.

API sources introduce the sample through a series of differentially pumped stages. This maintains the large pressure difference between the ion source and the mass spectrometer (Figure 3.3.1) without using extremely large vacuum pumps. In addition a drying gas is used to break up the clusters that form as the solvent evaporates. Because the analyte molecules have more momentum than the solvent and air molecules, they travel through the pumping stages to the mass analyzer.

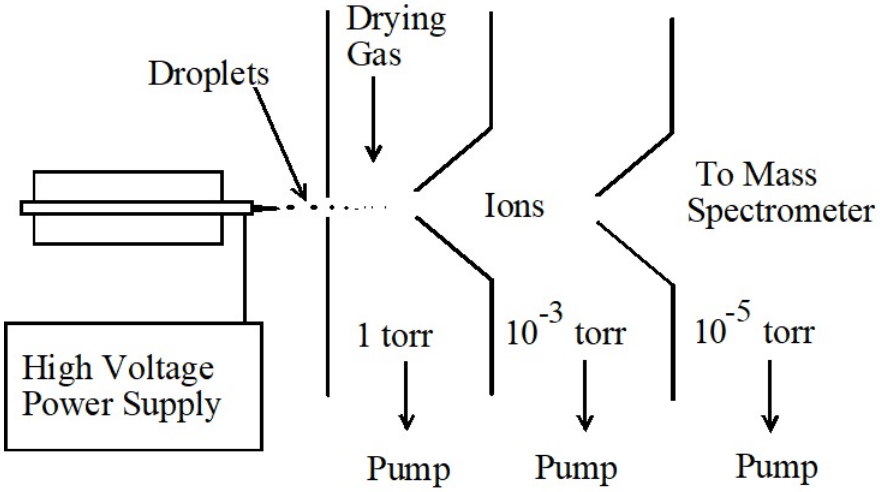


Figure 3.3.1: Electrospray Ionization Source

**ElectroSpray Ionization** (ESI) is the most common API application. It has undergone remarkable growth in recent y ears and is frequently used for LC/MS of thermally labile and high molecular weight compounds. The electrospray is created by apply ing a large potential between the metal inlet needle and the first skimmer in an API source (Figure 3.3.1). The mechanism for the ionization process is not well understood and there are several different theories that explain this ionization process. One theory is that as the liquid leaves the nozzle, the electric field induces a net charge on the small droplets. As the solvent evaporates, the droplet shrinks and the charge density at the surface of the droplet increases. The droplet finally reaches a point where the coulombic repulsion from this electric charge is greater than the surface tension holding it together. This causes the droplet to explode and produce multiply charged analyte ions. A typical ESI spectrum shows a distribution of molecular ions with different charge numbers.

Because electrospray produces multiply charged ions, high molecular weight compounds are observed at lower *m/z* value. This increases the mass range of the analyzer so that higher molecular weight compounds may be analyzed with a less expensive mass spectrometer. An ion with a mass of 5000 *u* and a charge of $+10$ is observed at 500 *m/*z and is easily analyzed with an inexpensive quadrupole analyzer.

API Sources are also used for Inductively Coupled Plasma Mass Spectrometry (ICP/MS) and glow discharge experiments (4, 5, 6). In ICP/MS a nebulizer is used to introduce liquid samples into a high temperature plasma. The temperature of the plasma is high enough to efficiently ionize most elements. These ions are introduced to the mass spectrometer using an series of differentially

pumped regions similar to the electrospray source discussed above. Glow discharge experiments are similar, but used for solid samples. The high sensitivity and selectivity of the mass spectrometer provides rapid multi-element detection at very low levels. Because the high temperature of the plasma destroys any chemical bonds, these techniques are used for elemental analysis.

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# 3.4: Matrix Assisted Laser Desorption/Ionization

## Matrix Assisted Laser Desorption/Ionization. (1,2)

Matrix Assisted Laser Desorption/Ionization (MALDI) is used to analyze extremely large molecules. This technique directly ionizes and vaporizes the analyte from the condensed phase. MALDI is often used for the analysis of synthetic and natural polymers, proteins, and peptides. Analysis of compounds with molecular weights up to 200,000 dalton is possible and this high mass limit is continually increasing.

In MALDI, both desorption and ionization are induced by a single laser pulse (Figure 3.4.1 ). The sample is prepared by mixing the analyte and a matrix compound chosen to absorb the laser wavelength. This is placed on a probe tip and dried. A vacuum lock is used to insert the probe into the source region of the mass spectrometer. A laser beam is then focused on this dried mixture and the energy from a laser pulse is absorbed by the matrix. This energy ejects analyte ions from the surface so that a mass spectrum is acquired for each laser pulse. The mechanism for this process is not well understood and is the subject of much controversy in the literature. This technique is more universal (works with more compounds) than other laser ionization techniques because the matrix absorbs the laser pulse. With other laser ionization techniques, the analyte must absorb at the laser wavelength. Typical MALDI spectra include the molecular ion, some multiply charged ions, and very few fragments.

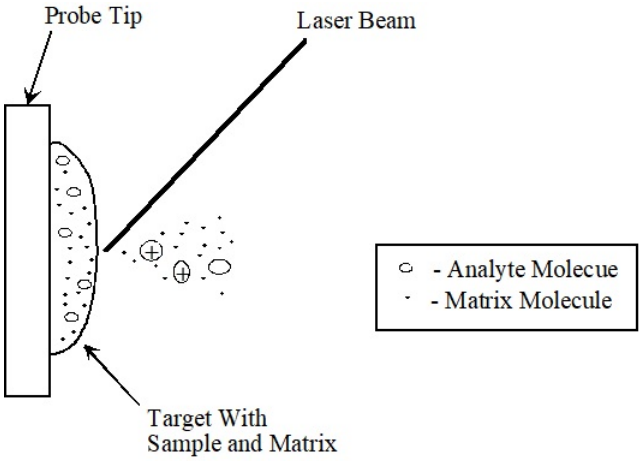


Figure 3.4.1 : MALDI Ionization Target with Sample and Matrix

Other Ionization Methods. There are several other ionization methods used for mass spectrometry and interested readers are referred to the chemical literature for additional information about other techniques. Field Desorption (3) was used for ionization and vaporization of moderate sized molecules before the development of FAB, electrospray, and MALDI. It is still an important technique for some analysis and is typically used for non-polar polymers and petroleum samples. Plasma Desorption (PD) (4, 5) is a technique used to analyze high molecular weight compounds before the development of MALDI and electrospray. However, it is very complex and has not found widespread application. Resonance Ionization Mass Spectrometry (RIMS) is used for selective atomic and molecular ionization. (6) Photoionization with lasers, lamps, and synchrotron sources is used to study the photochemistry and energetics of many compounds. (7) Lasers are used to ionize surface samples with Laser Microprobe Mass Analysis (LAMMA). (8, 9)

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# 3.5: Fast Atom Bombardment and Secondary Ion Mass Spectrometry

## Fast Atom Bombardment and Secondary Ion Mass Spectrometry. (1)

Fast Atom Bombardment (FAB) and Secondary Ion Mass Spectrometry (SIMS) both use high energy atoms to sputter and ionize the sample in a single step. In these techniques, a beam of rare gas neutrals (FAB) or ions (SIMS) is focused on the liquid or solid sample. The impact of this high energy beam causes the analyte molecules to sputter into the gas phase and ionize in a single step (Figure 3.5.1: Fast Atom Bombardment Source. ). The exact mechanism of this process is not well understood, but these techniques work well for compounds with molecular weights up to a few thousand dalton. Since no heating is required, sputtering techniques (especially FAB) are useful for study ing thermally labile compounds that decompose in conventional inlets (2, 3).

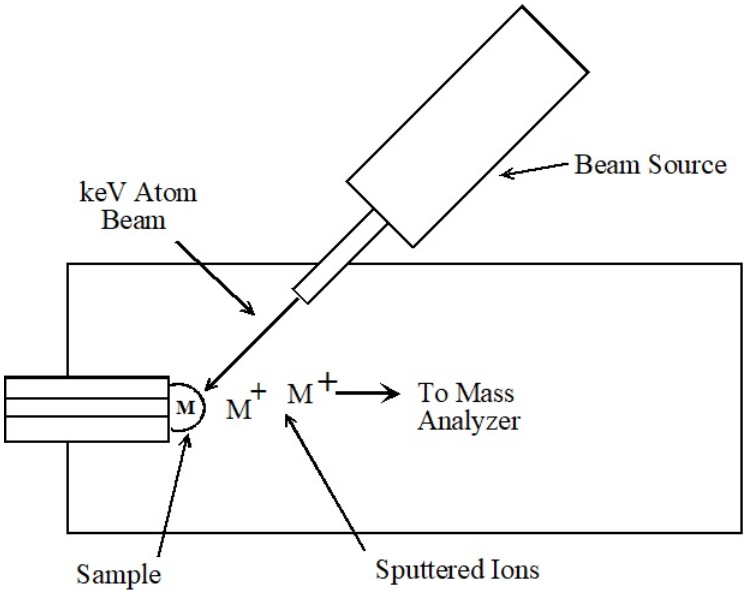


Figure 3.5.1: Fast Atom Bombardment Source.

The most significant difference between FAB and SIMS is the sample preparation. In FAB the analyte is dissolved in a liquid matrix. A drop of the sample/matrix mixture is placed at the end of an insertion probe and introduced to the source region. The fast atom beam is focused on this droplet to produce analyte ions. Glycerol or similar low vapor pressure liquids are typically used for the matrix. Ideally, the analyte is soluble in the liquid matrix and a monolayer of analyte forms on the surface of the droplet. According to one theory, this monolayer concentrates the analyte while the dissolved sample provides a reservoir to replenish the monolayer as the analyte is depleted. Without this constant replenishment from the bulk solution, the ionizing beam will rapidly deplete the analyte and the signal is difficult to observe.

SIMS experiments(4) are used to study surface species and solid samples. Liquid SIMS (LSIMS) is very similar to FAB except cesium ions are used for higher energy collisions. No matrix is used and the ionizing beam is focused directly on the sample. Although this makes sampling more difficult, it is useful for studying surface chemistry. High resolution chemical maps are produced by scanning a tightly focused ionizing beam across the surface and depth profiles are produced by probing a single location(5,6). Although SIMS is a very sensitive and powerful technique for surface chemistry and materials analysis, the results are often difficult to quantitate.

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# 3.6: Inductively Coupled Plasma

In addition to use for atomic emission spectroscopy, Inductively Coupled Plasma (ICP) is also used as an ionization method for elemental analysis. A liquid or slurry sample is introduced into an inductively coupled plasma torch and the ions produced are extracted and analyzed by mass spectrometry. These instruments are capable of extremely low detection limits and simultaneous detection of multiple elements.

# 3.7: Self-Test #1

**Exercise 3.7.1**

What ionization technique would be appropriate for analyzing the following compounds:

1. gasoline fractions,
2. pesticide residue,
3. ibuprofen and acetaminophen,
4. insulin,
5. tripeptides,
6. heavy metals in water.

**Answer**

1) Gasoline fractions. Since these are very volatile, EI would be very easy to use and would provide abundant fragment information. CI may help to identify the molecular ions.

2) Pesticide residue. These are usually volatile enough to use with EI. Once again CI may provide some useful information that would compliment the fragmentation in the EI spectrum. If the pesticide is thermally labile it may be appropriate to use electrospray to avoid sample decomposition.

3) Ibuprofen and acetaminophen. These pharmaceutics are often analyzed by liquid chromatography, so electrospray would be an ideal interface for ionization.

4) Insulin. This is a large protein molecule. MALDI is probably required.

5) Tripeptides. These are generally small enough to be readily ionized by FAB.

6) Heavy metals in water. Atmospheric pressure ionization in a ICP torch will provide very low limits of detection.

# CHAPTER OVERVIEW

## 4: MASS ANALYZERS

After ions are formed in the source region they are accelerated into the mass analyzer by an electric field. The mass analyzer separates these ions according to their *m/z* value. Each analyzer design has very different operating characteristics and the selection of an instrument involves important tradeoffs.The selection of a mass analyzer depends up on the resolution, (1) mass range, scan rate and detection limits required for an application.

- Resolution in mass spectrometry refers to the separation of two ions where $\mathrm{R} = \mathrm{m}/\Delta\mathrm{m}$. These terms are defined several different ways. The most common are the $10\%$ valley definition "Let two peaks of equal height in a mass spectrum at masses $\mathrm{m}$ and $\Delta\mathrm{m}$ be separated by a valley that at its lowest point is just $10\%$ of the height of either peak." and the peak width definition "For a single peak made up of singly charged ions at mass m in a mass spectrum, the resolution may be expressed as $\mathrm{m}/\Delta\mathrm{m}$, where $\Delta\mathrm{m}$ is the width of the peak at a height that is a specified fraction of the maximum peak height. It is recommended that one of three values $50\%$, $5\%$ or $0.5\%$ be used."(2)
- Mass range refers to the highest mass to charge ratio transmitted by the mass spectrometer.
- The scan rate of a mass spectrometer refers to how fast it scans a mass spectrum. This is important for chromatography applications where the entire mass spectrum must be scanned faster than the elution time of the chromatographic peak. Ideally, a minimum of ten complete mass spectra are acquired for a single chromatographic peak

Analyzers are typically described as either continuous or pulsed. Continuous analyzers include quadrupole filters and magnetic sectors. These analyzers are similar to a filter or monochromator used for optical spectroscopy. They transmit a single selected *m/z* to the detector and the mass spectrum is obtained by scanning the analyzer so that different mass to charge ratio ions are detected. While a certain *m/z* is selected, any ions at other *m/z* ratios are lost, reducing the S/N for continuous analyzers. Single Ion Monitoring(SIM) enhances the S/N by setting the mass spectrometer at the *m/z* for an ion of interest. Since the instrument is not scanned the S/N improves, but any information about other ions is lost. Pulsed mass analyzers are the other major class of mass analyzer. These are less common but they have some distinct advantages. These instruments collect an entire mass spectrum from a single pulse of ions. This results in a signal to noise advantage similar to Fourier transform or multichannel spectroscopic techniques. Pulsed analyzers include time-of-flight, ion cyclotron resonance, and quadrupole ion trap mass spectrometers.



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# 4.1: Quadrupole

The quadrupole mass spectrometer (1) is the most common mass analyzer. Its compact size, fast scan rate, high transmission efficiency,* and modest vacuum requirements are ideal for small inexpensive instruments. Most quadrupole instruments are limited to unit *m/z* resolution** and have a mass range of 1000 *m/z* . Many bench-top instruments have a mass range of 500 *m/z* but research instruments are available with mass range up to 4000 *m/z*.

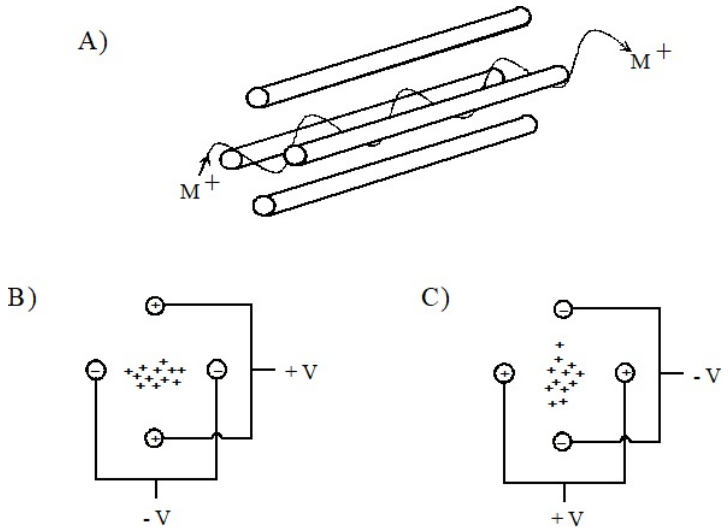


Figure 4.1.1: Quadrupole Mass Analyzer. A) Ion trajectory through the quadrupole, B) Ion focusing during first half of RF cycle, C) Ion focusing during second half of RF cycle.

In the mass spectrometer, an electric field accelerates ions out of the source region and into the quadrupole analyzer. The analyzer consists of four rods or electrodes arranged across from each other ( Figure 4.1.1). As the ions travel through the quadrupole they are filtered according to their *m/z* value so that only a single *m/z* value ion can strike the detector. The *m/z* value transmitted by the quadrupole is determined by the Radio Frequency (RF) and Direct Current (DC) voltages applied to the electrodes. These voltages produce an oscillating electric field that functions as a bandpass filter to transmit the selected mass to charge value.

The RF voltage rejects or transmits ions according to their *m/z* value by alternately focusing them in different planes (Figure 9). The four electrodes are connected in pairs and the RF potential is applied between these two pairs of electrodes. During the first part of the RF cycle the top and bottom rods are at a positive potential and the left and right rods are at a negative potential. This squeezes positive ions into the horizontal plane. During the second half of the RF cy cle the polarity of the rods is reversed. This changes the electric field and focuses the ions in the vertical plane. The quadrupole field continues to alternate as the ions travel through the mass analyzer. This causes the ions to undergo a complex set of motions that produces a three-dimensional wave.

The quadrupole field transmits selected ions because the amplitude of this three dimensional wave dep ends up on the *m/z* value of the ion, the potentials applied, and the RF frequency. By selecting an appropriate RF frequency and potential, the quadrupole acts like a high pass filter, transmitting high *m/z* ions and rejecting low *m/z* ions. The low *m/z* ions have a greater acceleration rate so the wave for these ions has a greater amplitude. If this amplitude is great enough the ions will collide with the electrodes and can not reach the detector. The low *m/z* value cutoff of the quadrupole is changed by adjusting the RF potential or the RF frequency. Any ions with a *m/z* greater than this cutoff are transmitted by the quadrupole.

A DC voltage is also applied across the rods of the analyzer. This potential combined with the RF potential acts like a low pass filter to reject high *m/z* ions. Because they respond quickly to the changing RF field the motion of the low *m/z* ions is dominated by the RF potential. This motion stabilizes their trajectory by refocusing each time the RF potential changes polarity. Because low *m/z* ions are quickly refocused, the DC potential does not affect these ions. High *m/z* ions, however, do not refocus as quickly during the RF cycle. The DC potential has a greater influence on their trajectory and they slowly drift away from the center of the quadrupole. At the end of the analyzer, they are too far off-axis to strike the detector.

The combination of high and low pass filters produced by the RF and DC potentials is adjusted to only transmit the selected *m/z* value. All ions above or below the set *m/z* value are rejected by the quadrupole filter. The RF and DC fields are scanned (either by potential or frequency) to collect a complete mass spectrum. Quadrupole mass analyzers are often called mass filters because of the

similarity between *m/z* selection by a quadrupole and wavelength selection by an optical filter or frequency selection by an electronic filter.

*Transmission efficiency refers to how many of the ions produced in the source region actually reach the detector. This is an important measure of sensitivity for mass spectrometers.

**Unit resolution (or low resolution) mass spectra distinguish between ions separated by $1\ \mathrm{m/z}$ unit. The spectra, like those presented here, are commonly displayed as histograms. This is a common method for presenting spectra because it results in much smaller data file size. Some mass analyzers can obtain spectra at much higher resolution. This is discussed in detail in the interpretation section.

References

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# 4.2: Magnetic Sector

Magnetic Sector. The first mass spectrometer, built by J.J. Thomson in 1897 , used a magnet to measure the *m/z* value of an electron. Magnetic sector instruments have evolved from this same concept. Sector instruments have higher resolution and greater mass range than quadrupole instruments, but they require larger vacuum pumps and often scan more slowly. The typical mass range is to 5000 *m/z*, but this may be extended to 30,000 *m/z*. Magnetic sector instruments are often used in series with an electric sector, described below, for high resolution and tandem mass spectrometry experiments.

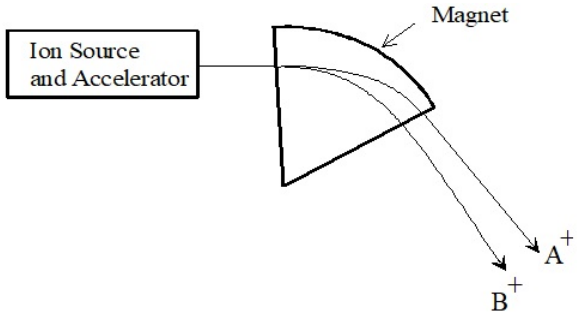


Figure 4.2.1: Magnetic Sector Mass Spectrometer. The low $m/z$ ion $(B^+)$is separated from the high $m/z$ ion $\left(\mathrm{A}^+\right)$.

Magnetic sector instruments (Figure 4.2.1) separate ions in a magnetic field according to the momentum and charge of the ion. Ions are accelerated from the source region into the magnetic sector by a 1 to 10 kV electric field. This acceleration is significantly greater than the 100 V acceleration typical for a quadrupole instrument. Since the ions are charged, as they move through the magnetic sector, the magnetic field bends the ion beam in an arc. This is the same principal that causes electric motors to turn. The radius of this arc (r) depends upon the momentum of the ion $\mu$, the charge of the ion (C) and the magnetic field strength (B) according to Equation 4.2.1.

$$r = \frac{\mu}{C \times B}$$

Ions with greater momentum will follow an arc with a larger radius. This separates ions according to their momentum, so magnetic sectors are often called momentum analyzers. The momentum of the ion is the product of the mass $(m)$ and the velocity $(v)$. The charge of the ion is the product of the charge number of the ion (z) and the charge of an electron (e). Substituting these variables into Equation 4.2.1 yields:

$$r = \frac{m/z \times v}{\mathrm{B} \times \mathrm{e}}$$

The velocity of an ion is determined by the acceleration voltage in the source region (V) and the mass to charge ratio (*m/z*) of the ion. Equation 4.2.2 rearranges to give the *m/z* ion transmitted for a given radius, magnetic field, and acceleration voltage as:

$$m/z = \frac{r^2\ \mathrm{B}^2\mathrm{e}}{2\ \mathrm{V}}$$

Only one *m/z* value will satisfy Equation 4.2.3 for a given radius, magnetic field, and acceleration voltage. Other *m/z* ions will travel a different radius in the magnetic sector.

Older magnetic sector instruments use a photographic plate to simultaneously detect ions at different radii. Since each *m/z* has a different radius, they strike the photographic plate at a different location. Modern instruments have a set of slits at a fixed radius to transmit a single *m/z* to the detector. The mass spectrum is scanned by changing the magnetic field or the acceleration voltage to transmit different *m/z* ions. Some new instruments use multichannel diode array detectors to simultaneously detect ions over a range of *m/z* values.

# 4.3: Electric Sector/Double Focusing Mass Spectrometers

Electric Sector/Double Focusing Mass Spectrometers (1). An electric sector consists of two concentric curved plates. A voltage is applied across these plates to bend the ion beam as it travels through the analyzer. The voltage is set so that the beam follows the curve of the analyzer. The radius of the ion trajectory (r) depends up on the kinetic energy of the ion (V) and the potential field (E) applied across the plates.

$$r = \frac{2V}{E}$$

Equation $4.3.1$ shows that an electric sector will not separate ions accelerated to a uniform kinetic energy. The radius of the ion beam is indep endent of the ion's mass to charge ratio so the electric sector is not useful as a standalone mass analyzer.* An electric sector is, however, useful in series with a magnetic sector. The mass resolution of a magnetic sector is limited by the kinetic energy distribution ( V ) of the ion beam. This kinetic energy distribution results from variations in the acceleration of ions produced at different locations in the source and from the initial kinetic energy distribution of the molecules. An electric sector significantly imp roves the resolution of the magnetic sector by reducing the kinetic energy distribution of the ions**. These high resolutions experiments are discussed in the section on mass spectral interpretation. The effect of the electric sector is shown in Figure $4.3.1$ for a reverse geometry (BE) instrument with the magnetic sector $(B)$ located before the electric sector $(\mathrm{E})$.

*The electric sector is a kinetic energy analyzer. In the source region of the mass spectrometer all ions are accelerated to the same kinetic energy. Because they have the same kinetic energy, they are not separated by an electric sector. A magnetic sector will resolve different mass ions accelerated to a uniform kinetic energy because it separates ions based upon their momentum (See 4.2: Magnetic Sector).

**Ion optics are complex and interested readers are referred to the literature for more detail. The model presented here provides a framework for understanding many high resolution and tandem mass spectrometry experiments. The article by Nier (1) provides an excellent introduction, a historical perspective, and many references for the development and theory of these instruments.

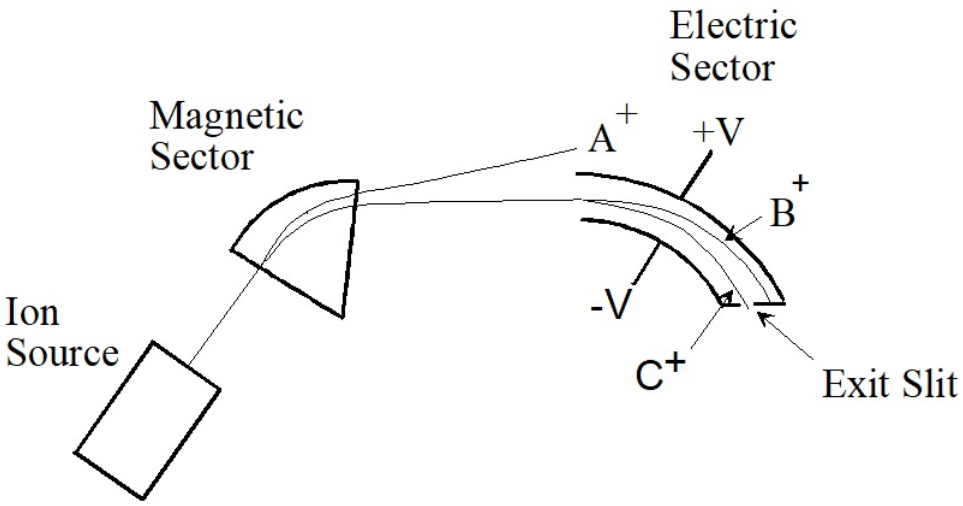


Figure \(\PageIndex{1}\): Reverse Geometry Double Focusing Mass Spectrometer. $A^+$ is a 100.00 *m/z* ion, $B^+$ is a 50.00 *m/z* ion and $C^+$ is a 50.01 *m/z* ion. $A^+$ is rejected by the magnetic sector because of it's greater momentum. $B^+$ and $C^+$ are not resolved by the magnetic sector because they have the same momentum. To have the same momentum, however, $B^+$ must have a more kinetic energy than $C^+$. As a result the electric sector separates these two ions.

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# 4.4: Time-of-Flight

Time-of-Flight. The time-of-flight (TOF) mass analyzer separates ions in time as they travel down a flight tube (Figure 4.4.1). This is a very simple mass spectrometer that uses fixed voltages and does not require a magnetic field. The greatest drawback is that TOF instruments have poor mass resolution, usually less than 500 . These instruments have high transmission efficiency, no upper *m/z* limit, very low detection limits, and fast scan rates. For some applications these advantages outweigh the low resolution. Recent developments in pulsed ionization techniques and new instrument designs with improved resolution have renewed interest in TOF-MS. (1)

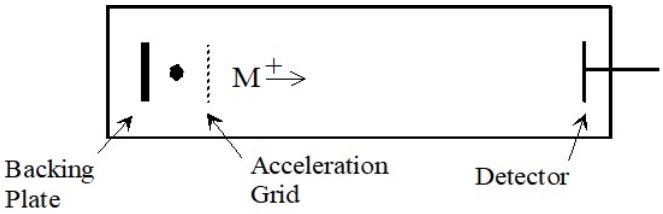


Figure 4.4.1: Time-of-Flight Mass Spectrometer.

In the source of a TOF analyzer, a packet of ions is formed by a very fast (ns) ionization pulse. These ions are accelerated into the flight tube by an electric field (typically 2-25 kV) applied between the backing plate and the acceleration grid. Since all the ions are accelerated across the same distance by the same force, they have the same kinetic energy. Because velocity $(v)$ is dependent upon the kinetic energy, Equation 4.4.1 shows $(\mathrm{E_{kinetic}})$ and mass $(m)$ lighter ions will travel faster.

$$\mathrm{E_{kinetic}} = \frac{1}{2}mv^2$$

$\mathrm{E_{kinetic}}$ is determined by the acceleration voltage of the instrument $(\mathrm{V})$ and the charge of the ion $(\mathrm{e} \times z)$. Equation 4.4.2 rearranges to give the velocity of an ion $(v)$ as a function of acceleration voltage and *m/z* value.

$$v = \sqrt{\frac{2\,\mathrm{V} \times \mathrm{e}}{m/z}}$$

After the ions accelerate, they enter a 1 to 2 meter flight tube. The ions drift through this field free region at the velocity reached during acceleration. At the end of the flight tube they strike a detector. The time delay ( t ) from the formation of the ions to the time they reach the detector dependents up on the length of the drift region (L), the mass to charge ratio of the ion, and the acceleration voltage in the source.

$$\mathrm{t} = \frac{\mathrm{L}}{\sqrt{\sqrt{2 \times \mathrm{C} \times}}}\sqrt{m/z}$$

Equation 4.4.3 shows that low *m/z* ions will reach the detector first. The mass spectrum is obtained by measuring the detector signal as a function of time for each pulse of ions produced in the source region. Because all the ions are detected, TOF instruments have very high transmission efficiency which increases the $\mathrm{S/N}$ level.

References

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# 4.5: Quadrupole Ion Trap

## Quadrupole Ion Trap. (1, 2, 3)

The Quadrupole ion storage trap mass spectrometer (QUISTOR) is a recently developed mass analyzer with some special capabilities. Several commercial instruments are available and this analyzer is becoming more popular. QUISTORs are very sensitive, relatively inexpensive, and scan fast enough for GC/MS exp eriments. The sensitivity of the QUISTOR results from trapping and then analyzing all the ions produced in the source. Since all the ions are detected, the $S/N$ is high.

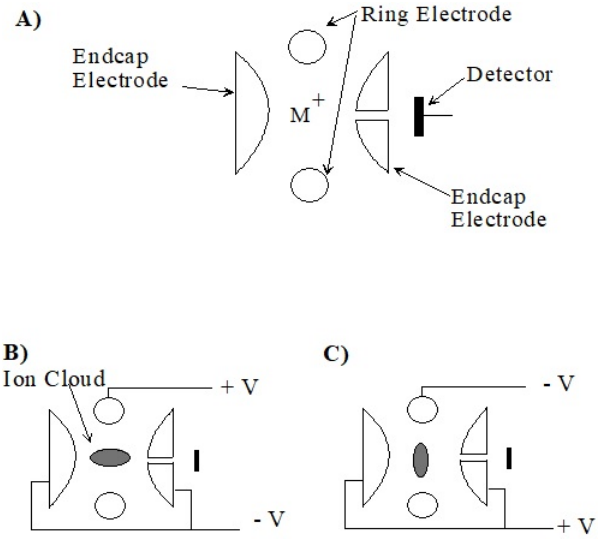


Figure 4.5.1: Quadrupole Ion Trap Mass Spectrometer. A) shows the major components. B) and C) ion response to the applied RF field.

The QUISTOR consists of a doughnut shaped ring electrode and two endcap electrodes. A cutaway view of this arrangement is shown in Figure 4.5.1. A combination of RF and DC voltages is applied to the electrodes to create a quadrup ole electric field similar to the electric field for the quadrupole mass analyzer. This electric field traps ions in a potential energy well at the center of the analyzer. The mass spectrum is acquired by scanning the RF and DC fields to destabilize low mass to charge ions. These destabilized ions are ejected through a hole in one endcap electrode and strike a detector. The mass spectrum is generated by scanning the fields so that ions of increasing $m/z$ value are ejected from the cell and detected. The trap is then refilled with a new batch of ions to acquire the next mass spectrum. The mass resolution of the ion trap is increased by adding a small amount $0.1$ Pa $\left(10^{-3}\right.$ torr $\left.\right)$ of Helium as a bath gas. Collisions between the analyte ions and the inert bath gas dampen the motion of the ions and increases the trapping efficiency of the analyzer.

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# 4.6: Ion Cyclotron Resonance

Ion Cyclotron Resonance. (1, 2, 3) The Ion Cy clotron Resonance (ICR) mass spectrometer uses a superconducting magnet to trap ions in a small sample cell. This type of mass analyzer has extremely high mass resolution (ca. $10^9$ ) and is also useful for tandem mass spectrometry experiments. These instruments are very expensive and are typically used for specialized research applications. The ICR traps ions in a magnetic field that causes ions travel in a circular path (Figure $4.6.1$). This is similar to the path of an ion in a magnetic sector, but the ions are not traveling as fast and the magnetic field is stronger. As a result the ions are contained in the small volume of the trap.

The ion's cyclotron frequency $(\omega)$, is the angular frequency* of an ion's orbit. Equation $4.6.1$ shows this frequency is determined by the magnetic field strength $(B)$ and the *m/z* value of the ion.

$$\omega = \frac{\mathrm{B} \times \mathrm{e}}{m/z}$$

After ions are trapped in this cell they are detected by measuring the signal at this cyclotron frequency. This signal is measured by placing electrodes on each side of the ions circular orbit. An RF voltage is applied to the transmitter electrodes at the cyclotron frequency of the ion of interest. This RF energy moves ions at the applied frequency to a larger orbit. As these ions travel around the ICR cell they are close enough to the receiver electrodes to induce a capacitive current. This capacitive current oscillates at the cyclotron frequency and is detected as the signal.

The ICR is also used as a Fourier Transform Mass Spectrometer (FT-MS). Instead of using a single excitation frequency, a fast RF pulse is applied to the transmitter electrodes. This simultaneously excites all the ions and produces a signal at the cyclotron frequency of each *m/z* ion present. This signal is similar to the Free Induction Decay (FID) produced in an FT-NMR experiment. A complete mass spectrum is obtained by using the Fourier transform to convert this signal from the time domain to the frequency domain.

*The angular frequency $(\omega)$ is in radians per second. The unit Hertz (Hz) is in cycles per second where there are $2\pi$ radians per cycle.

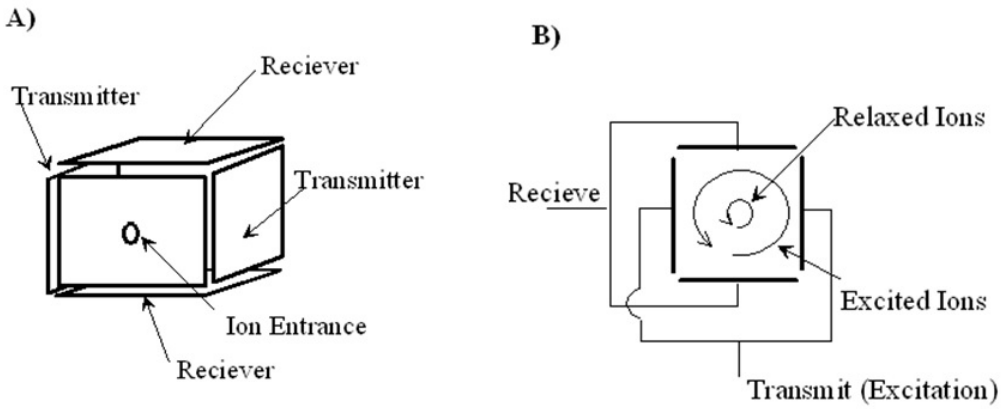


Figure 4.6.1: Ion Cyclotron Mass Spectrometer. A)major components, B) ion motion within the trap.

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# 4.7: Self-Test #2

**Exercise 4.7.1**

Self-Test #2: Which mass analyzer is appropriate for the following analysis:

1. Routine analysis of drug testing samples
2. Analysis of small, 2000 dalton, peptides
3. Analysis of 50,000 dalton polymers
4. High sensitivity for detecting chemical warfare agents
5. High resolution analysis.

**Answer**

Self-Test #2: Which mass analyzer would be appropriate for the following analysis:

1) Routine analysis of drug testing samples. A quadrupole mass analyzer would provide the necessary mass range and resolution. It is also fast enough for use with high resolution chromatography.

2) Analysis of small, 2000 dalton, proteins. This will push the limits of a quadrupole (unless electrospray ionization is used to create multiply charged ions). A sector instrument with FAB ionization would work well.

3) Analysis of polymers up to 50,000 dalton. The value of singly charged ions is probably to high for a sector instrument (It might work with electrospray ionization to form multiply charged ions). A TOF analyzer does not have any mass limit so it would be ideal for this analysis.

4) High sensitivity testing for chemical warfare agents. For this experiment the high sensitivity of a QUISTOR would be beneficial.

5) High resolution analysis. This is usually done with a double focusing sector instrument, although even higher resolution is possible with an ICR.

# CHAPTER OVERVIEW

## 5: MASS SPECTROMETER SYSTEMS

Deciding the appropriate sample introduction system, ionization technique, and mass analyzer are the major variables in deciding what type of mass spectrometer to use. Operating a mass spectrometer also requires understanding how these are integrated into an operating spectrometer system for acquiring and processing mass spectra. The details of how the data acquisition system work will vary from instrument to instrument and from one manufacturer to another. However, the basics will include control over the vacuum system, the source region, the mass analyzer and the detector.

# 5.1: Vacuum System

All mass spectrometers operate at very low pressure (high vacuum). This reduces the chance of ions colliding with other molecules in the mass analyzer. Any collision can cause the ions to react, neutralize, scatter, or fragment. All these processes will interfere with the mass spectrum. To minimize collisions, experiments are conducted under high vacuum conditions, typically $10^{-2}$ to $10^{-5}$ Pa ($10^{-4}$ to $10^{-7}$ torr) depending up on the geometry of the instrument. This high vacuum requires two pumping stages. The first stage is a mechanical pump that provides rough vacuum down to 0.1 Pa ($10^{-3}$ torr). The second stage uses diffusion pumps or turbomolecular pump s to provide high vacuum. ICR instruments have even higher vacuum requirements and often include a cryogenic pump for a third pumping stage.

The pumping system is an important part of any mass spectrometer and the control software will allow the user to turn the pumps off and on and monitor the pressure in different parts of the spectrometer. The pumpdown sequence for turning on a spectrometer starts by operating the roughing pumps to establish the initial vacuum and check for major leaks. After the roughing pumps get the system down to a pressure of approximately 0.1 Pa the high vacuum pumps are turned on to establish operating pressure. This sequence is more important with diffusion pumps for the high vacuum system because they do not tolerate atmospheric pressure.

The vacuum system will also include different types of gauges for measuring pressure in different parts of the system. Thermocouple or convectron gauges are used with the roughing pumps to measure pressure down to 0.01 Pa. Ion gauges are used to measure high vacuum down to 10-8 Pa but they cannot be used above 0.1 Pa. To protect the ion gauges and other high voltage electronics the instrument will typically include an interlock system that does not allow power to these components until the roughing pumps have reduced the pressure below a certain threshold. If there is a leak or loss of vacuum the interlock will also turn off power to these systems to protect the components. The thermocouple gauges are normally located at the entrance to any mechanical pumps and ion gauges are normally located in the source and analyzer regions. Depending on the ionization method additional pressure gauges may also be used to monitor the ionization system or any collision regions.

## 5.2: Source Region Control

The mass spectrometer system will also include controls for the source region. These controls will vary depending upon the ionization technique being used for analysis. For GC/MS and LC/MS systems this software will also control the chromatography system. In general, this requires setting parameters that control the temperature of the sample inlet, determine the ionization energy and efficiency along with parameters that control the efficiency of extracting ions from the source region and transferring them into the analyzer region. These parameters often interact with each other so acquiring spectra with good signal to noise levels requires careful optimization. Typically, this is done using a reference sample and an automated tuning program. The automated tuning program allow the user to set some parameters, like the ionization energy, and the software then varies the other parameters, including voltages on the ion extraction lens systems, to get the best signal possible. The source region for an electrospray mass spectrometer is shown in Figure 5.2.1


Figure 5.2.1: Electrospray Source

The reference sample is often a fluorinated compound used for calibration and tuning since fluorine has a single isotope, which simplifies the spectra, and they have relatively high vapor pressure for their mass. Perfluorotributylamine and prefluorokerosene are two common reference standards for gas phase samples. Ultramark 1621, a mixture of fluorinated compounds, is often used for electrospray and FAB.

# 5.3: Mass Analyzer Control

Control of the mass analyzer requires adjustment of voltages and currents described for the operation of the analyzer and control over voltages on the ion lenses that focus and direct the ions to the detector. The user will need to set the mass range for the analysis. The mass range is limited by the design of the instrument and is an important specification. The range scanned and the speed of the scan are typically adjusted for an experiment. The mass range should cover the expected range for the analyte but scanning a larger range than needed should be avoided. Scanning outside the range needed will either slow down the analysis or just results in the collection of background noise. The scanning speed should be adjusted to balance the speed of the experiment with the signal to noise. Scanning too fast may distort the spectra and reduce S/N levels. Scanning too slow is a significant problem with chromatography or other experiments where the signal is transient. For GC/MS in particular the chromatography peak may only be several seconds wide. The mass spectrum needs to be acquired quickly enough that the analyte concentration is constant for the entire scan. If the concentration varies during the scan it will distort the relative intensity of ions collected at different times.

The mass analyzer needs to be tuned to optimize the efficiency of ion transmission and calibrated to determine the mass scale. This is typically done using a reference sample as part of the tuning process described for the source region.

# 5.4: Detector Control

Detection of ions is based up on their charge or momentum. For large signals a faraday cup is used to collect ions and measure the current. Older instruments used photographic plates to measure the ion abundance at each mass to charge ratio. Most detectors currently used amplify the ion signal using a collector similar to a photomultiplier tube. These amplifying detectors include: electron multipliers (shown in Figure 5.4.1), channeltrons and multichannel plates. The gain is controlled by changing the high voltage applied to the detector. A detector is selected for it's speed, dynamic range, gain, and geometry. Some detectors are sensitive enough to detect single ions.

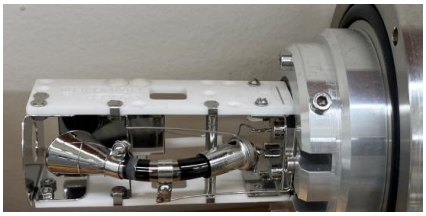

Figure 5.4.1: Electron Multiplier.

The mass spectrometer system will include controls for the gain of the detector. Typically, the gain is adjusted by changing the potential applied to the detector. These voltages are controlled by the software and care should be taken to balance the sensitivity required for the analysis. If the gain is set too low, signal will not be detected, if the gain is set too high the signal will include a lot of noise, the response may not be linear, and the detector life will be shortened.

For GC/MS systems it is typical to use a solvent delay so that the detector is turned off at the start of a run. After the solvent has gone through the system the detector is turned on. This protects the detector from being overloaded by the signal from the solvent but set the gain high enough to see analytes at very low concentration.

# 5.5: Data System

The final component of a mass spectrometer is the data system. This part of the instrument has undergone revolutionary changes. It has evolved from photographic plates and strip chart recorders to data systems that control the instrument, acquire hundreds of spectra in a minute and search tens of thousands of reference spectra to identify an unknown. Important features of the data system include control over data acquisition and effective data processing.

Critical features for data processing include averaging, subtracting, and deconvolution of spectra. Figure 5.5.1 shows the background spectra for a mass spectrometer using 70 eV electron ionization. The peak at 44 m/z corresponds to carbon dioxide. Water (18 m/z), nitrogen (28 m/z), and oxygen (32 m/z) are normally observed if they are included in the mass range scanned by the spectrometer. The peak at 207 m/z is from a siloxane compound that is commonly observed in mass spectra and is likely caused by the GC septum used for injection. Other background peaks may be carryover from previous experiments or from the vacuum pump oil. It is a good idea to be familiar with the background peaks and levels for an instrument since changes in the background often indicate possible problems with the instrument.

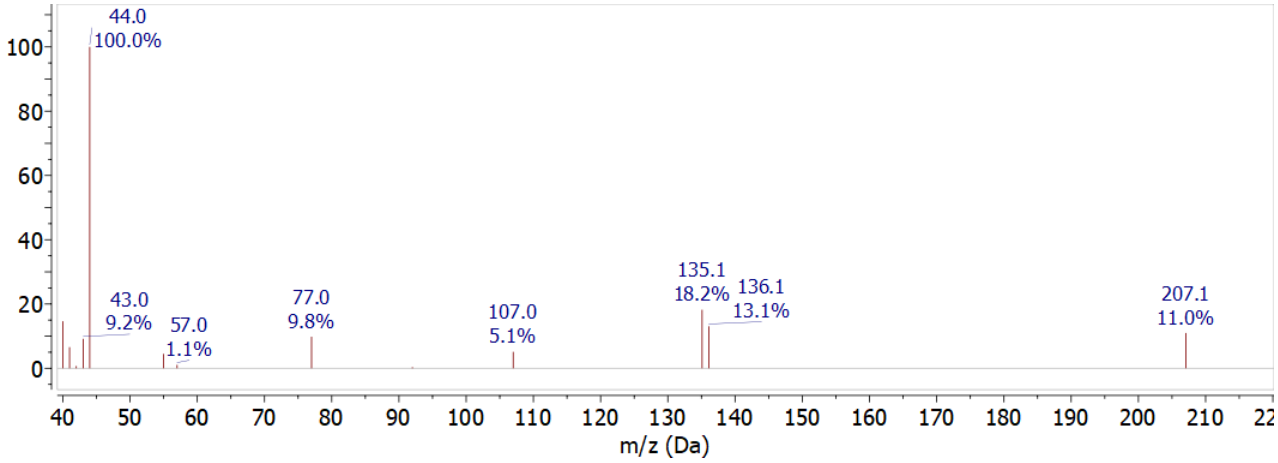


Figure 5.5.1: Background Mass Spectrum. (Copyright; Van Bramer via MestReC)

The data processing software for the system can be used to reduce the background signal in mass spectrum. Figure 5.5.2 shows two mass spectra from the same time in the chromatogram. The top spectrum is the raw data from the spectrometer. In the bottom spectrum the background signal was subtracted. The background peaks at 77 m/z and 207 m/z are removed and a large number of smaller peaks are also eliminated.

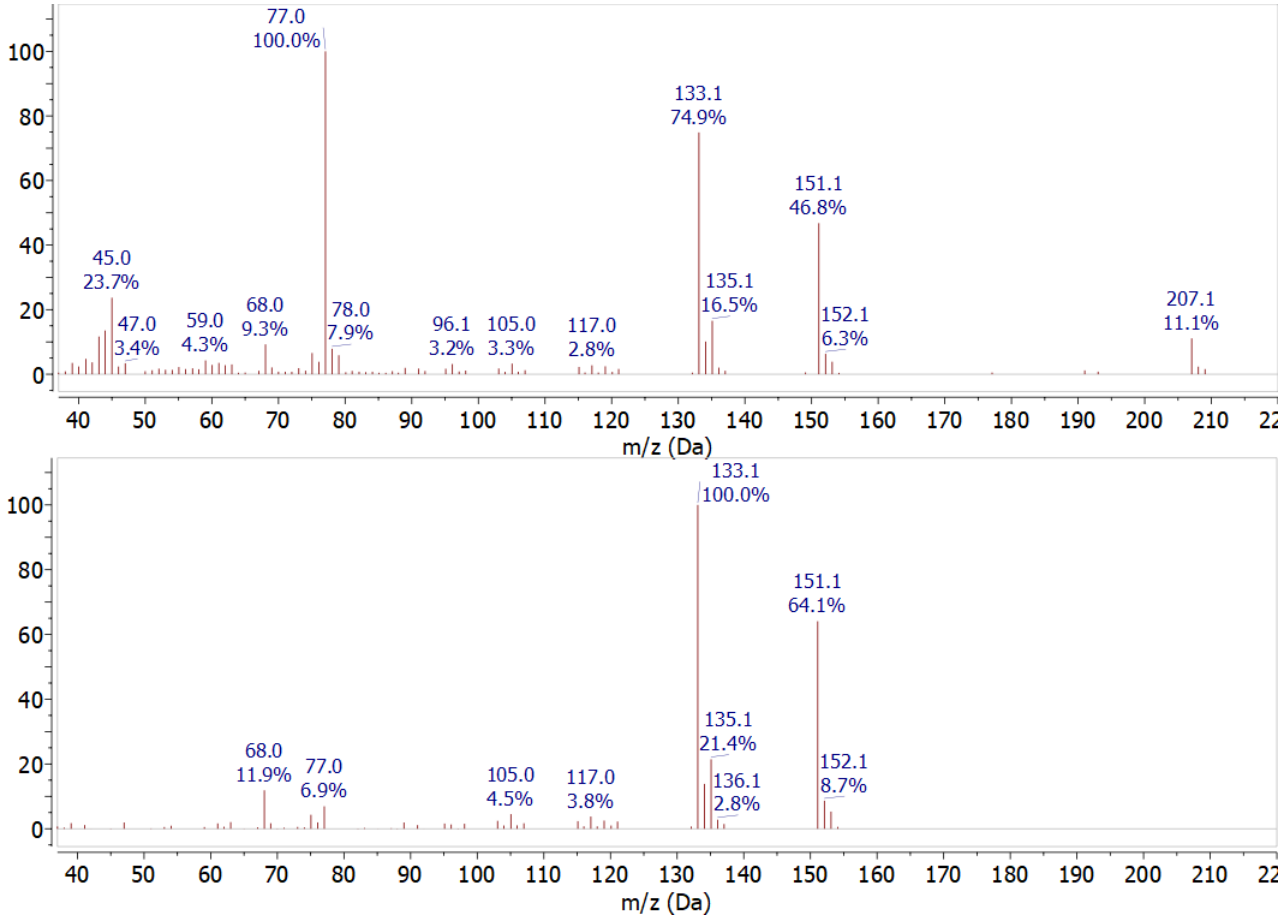


Figure 5.5.2: Demonstration of background subtraction in mass spectrum. (Copyright; Van Bramer via MestReC)

Another important data processing feature is shown in Figure 5.5.3. This figure shows data for the analysis of caffeine by GC/MS. The top trace is the total ion chromatogram – the sum of the intensity for all masses as a function of time. The bottom trace is the extracted ion chromatograph that only shows the intensity of the 194 m/z signal as a function of time. Since caffeine is the only compound with an ion observed at 194 m/z this is the only peak in the chromatogram. This chromatogram shows a significant reduction in the background noise. This chromatogram was extracted from a full scan at each time in the chromatogram. It is also possible to set up the spectrometer to only monitor a single ion, this is called selective ion monitoring and the technique can significantly enhance the sensitivity of a mass spectrum analysis.

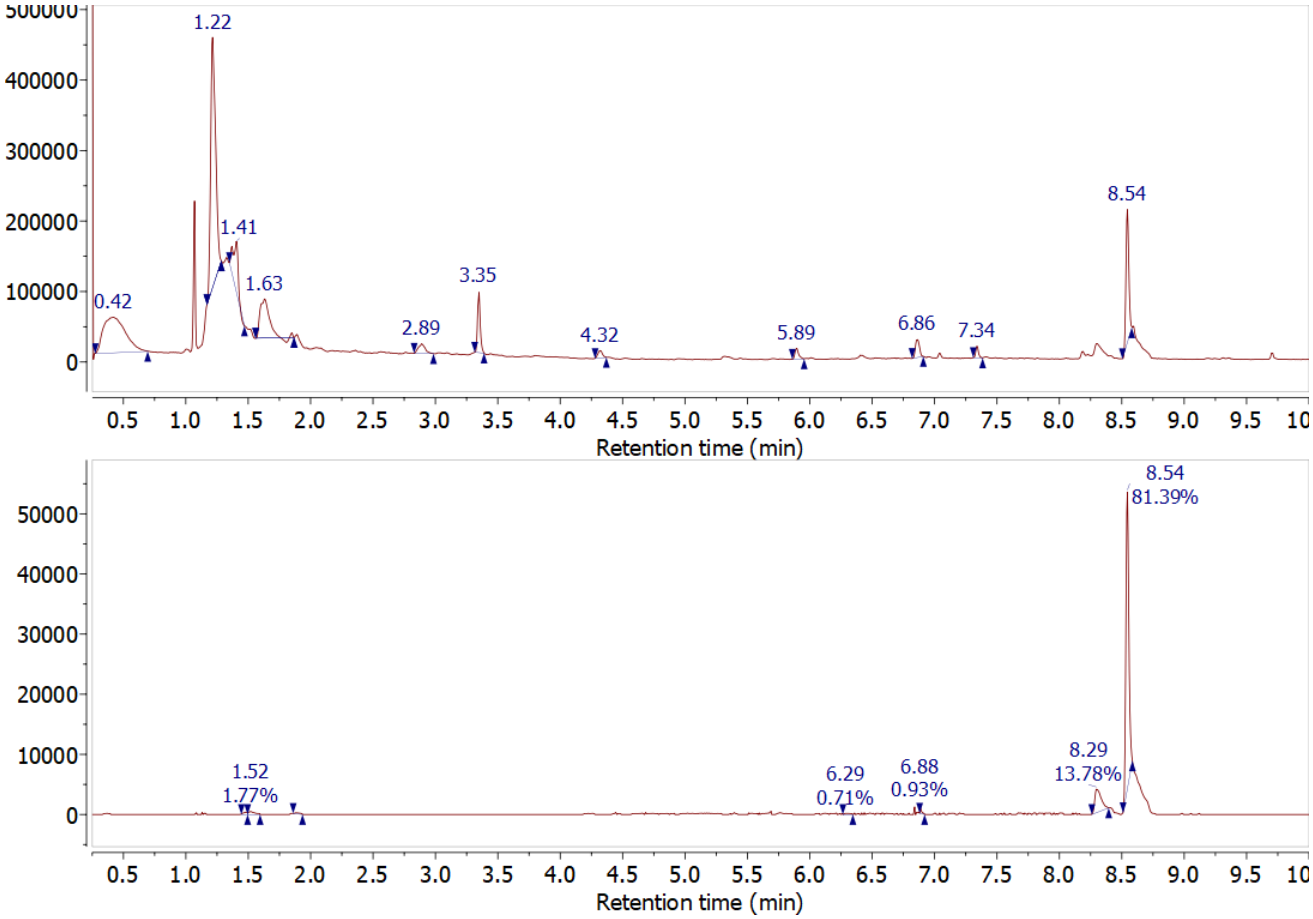


Figure 5.5.3: Comparison of total ion chromatagram (top) and the extracted ion chromatagram for 194 m/z (bottom). (Copyright; Van Bramer via source)

The final data processing technique for discussion here is database searching. Figure 5.5.4 shows the library search results using NIST MS Search 2.0 for the caffeine peak at 8.54 minutes in the chromatogram. After sending the mass spectrum to the search routine, the program displays likely matches and shows the reference spectra for easy comparison. Search routines like this make it possible to compare an unknown with a large database of target compounds for quick identification.

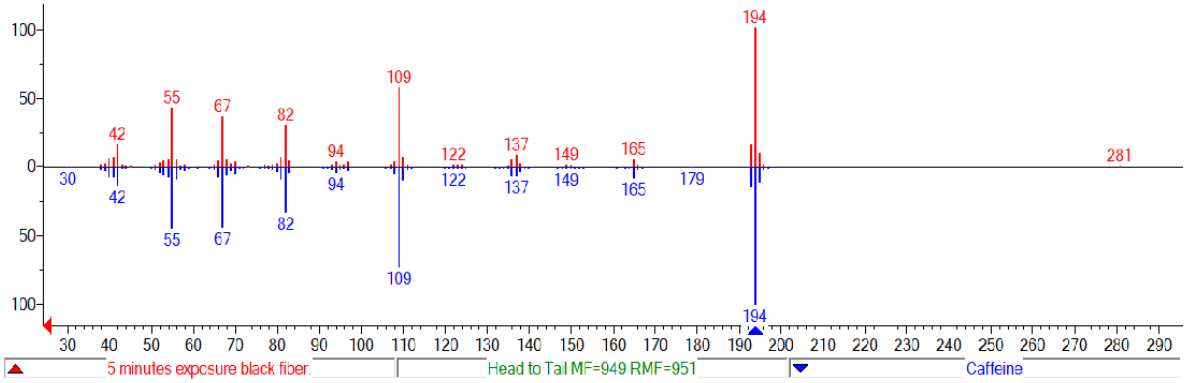


Figure 5.5.4: Mass Spectra search results for caffeine, the red trace on the top is the experimental data the blue trace on the bottom is the reference spectrum from the NIST MS database. (Copyright; Van Bramer via NIST MS Search 2.0)

# CHAPTER OVERVIEW

## 6: INTERPRETATION

Although mass spectrometry is a very sensitive instrumental technique, there are other techniques with picogram detection limits. In addition to sensitivity, however, mass spectrometry also is also useful for identifying the chemical structure of this picogram sample. Since the mass spectrum is a fingerprint of the molecular structure, comparison to a computer databases can be used to identify an unknown compound. This is often done using Probability Based Matching (PBM), a popular pattern recognition technique. Although these computer searches are convenient and powerful, it is important to understand how to interpret a mass spectrum. A computer only compares the unknown spectrum to the library spectra and offers a selection of compounds in the database that produce similar spectra. This computer search is very useful and it makes interpretation much easier, but there are limits to the computer search.

Molecular structure is important for understanding mass spectral interpretation. To get the most from this section, draw out the structures of the molecules discussed. During the discussion find which bonds break and calculate the mass of the fragments. Actively reading this section will result in a much greater understanding of and appreciation for mass spectrometry. You can interpret the spectrum but it will take some effort. One common mistake made in mass spectrometry is to blindly trust the results of a computer library match. You need to learn how to interpret and understand the mass spectrum to effectively use these computer searches. This section should help you get started.

# 6.1: Molecular Ion

## Molecular Ion

The molecular ion provides the molecular mass of the analyte and is the first clue used to interpret a mass spectrum. The mass to charge ratio of the molecular ion is based up on the mass of the most abundant isotope for each element in the molecule. This is not the relative atomic mass from the periodic table. Since many mass spectrometers have unit mass resolution, the isotope mass is normally rounded to the nearest whole number, this is called the nominal mass. For example the molecular ion for $CHBr_3$ is observed at 250 *m/z*;(12 + 1 + 3 $\times$ 79)=250), not at the relative molecular weight of 253. The mass of the molecular ion is based upon the mass of the isotope with the highest natural abundance. The most common bromine isotope is $^{79}Br$. Do not use the weighted average atomic weight for Br (79.9) which is based upon the natural abundance of $^{79}Br$ and $^{81}Br$. The mass spectrum of $CHBr_3$ includes ions for all the naturally occurring isotopes and the intensity of each peak depends upon the probability for that combination of isotopes. These patterns are discussed in detail in the section on isotope abundance.

In many mass spectra, the molecular ion is easily identified as the ion with the highest mass to charge ratio. However, this assignment should be made with caution because the highest mass to charge ion may be an impurity, an isotope of the molecular ion, or a fragment. Many compounds fragment easily and no molecular ion is observed in the 70 eV EI spectrum. It is important to clarify that the molecular ion IS NOT necessarily the ion with the greatest abundance, the ion with the greatest abundance is called the base peak. The base peak is the peak with the greatest abundance. The mass spectrum is usually normalized so that this peak has an intensity of 100.

A list of molecular ion characteristics are in Table 6.1.1 to help you identify them in a mass spectrum. Low energy EI, where the ionization energy is reduced, often increases in intensity of the molecular ion. Chemical Ionization, CI, is also useful for identifying the molecular ion since the the adduct ion is often more stable than the radical cation produced by electron ionization. The adduct ion is often formed by protonating the analyte to form $(M+H)$ and is observed at a mass to charge ratio of M+1.

Table 6.1.1: Characteristics of Molecular Ions

| |
|---|
| The mass to charge ratio must correspond to a reasonable molecular formula with the proper isotope abundance. |
| Most compounds have an even molecular mass. The one common exception to this is the "Nitrogen Rule" discussed below. |
| The Nitrogen Rule: Any compound with an odd number of nitrogen atoms will have an odd molecular mass. Any compound with an even number of nitrogen atoms (including zero) will have an even molecular mass. This is because nitrogen is the only common atom where the most common isotope has an odd valence and an even mass. For example: the molecular ion for $CH_4$ is observed at 16 *m/z*, the molecular ion for $NH_3$ is observed at 17 *m/z*, and the molecular ion for $N_2H_4$ is observed at 32 *m/z*. |
| If a peak is the molecular ion, the next highest mass fragment must correspond to the loss of a possible neutral fragment. For example, a peak that corresponds to loss of 5 u from the molecular ion is highly unlikely |

Figure 6.1.1 shows the mass spectrum of acetone ($CH_3COCH_3$). The molecular ion is clearly shown at 58 *m/z* (12 x 3 + 6 x 1 + 16 = 58). The base peak is at 43 *m/z* and corresponds to loss of 15 *m/z* from the intact molecule, this is caused by breaking a C-C bond for loss of a $CH_3^{\bullet}$ radical to give $CH_3CO^+$ at 43 *m/z* (12 x 2 + 3 x 1 + 16 = 43). The mass spectrum also includes several other minor peaks - the peak at 59 *m/z* is caused by the small abundance of C-13 that gives a small fraction of the acetone molecules a mass of 59; the peak at 15 *m/z* is caused by the $CH_3$ fragment retaining the charge when the C-C bond breaks. These fragmentation and isotope patterns are discussed in more detail in the following sections.

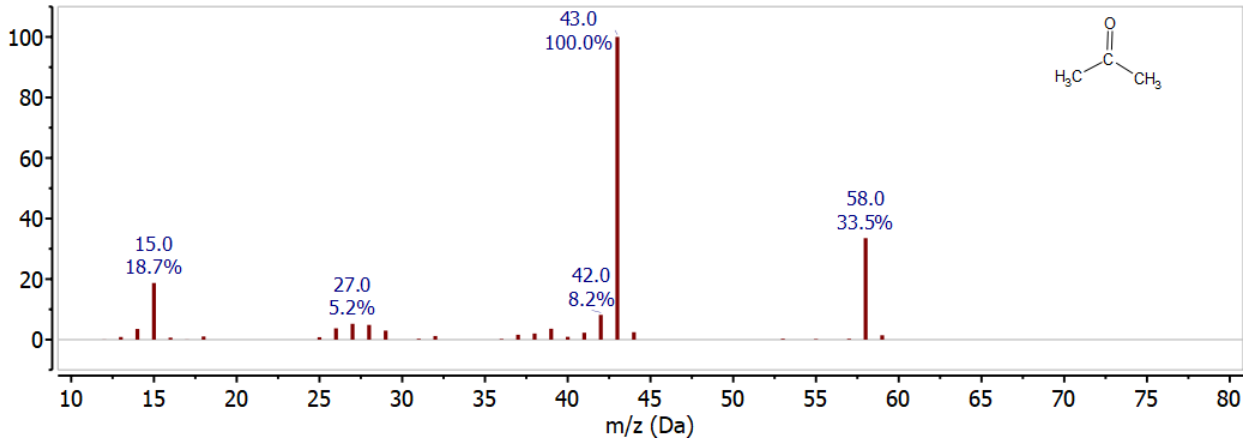


Figure 6.1.1: Mass Spectrum of Acetone $CH_3COCH_3$.

# 6.2: Fragmentation

## Fragmentation

Although the molecular ion is useful for identification, it does not provide any structural information about an unknown. The structural information is obtained from the fragmentation patterns of the mass spectrum. Identifying an unknown without analyzing the fragmentation patterns is like putting together a jigsaw puzzle without the picture. Fragmentation patterns are often complex, but they fit together like pieces of the puzzle to identify the structure of the molecule.

After a molecule is ionized, the molecular ion retains the excess ionization energy. If this excess energy is greater than the energy required to break a chemical bond, the molecule can fragment. The fragmentation processes are typically categorized as direct cleavage where a single bond is broken or rearrangement where bonds are broken and created simultaneously (Figure 6.2.1).

1. Electron Ionization: $ABCD + e^- \rightarrow ABCD^{+\bullet} + 2e^-$
2. Fragmentation:
    1. Direct Cleavage $ABCD^{+\bullet} \rightarrow AB^+ + CD^\bullet$
    2. Rearrangement $ABCD^{+\bullet} \rightarrow AD^{+\bullet} + B{=}C$

Figure 6.2.1: Fragmentation Mechanisms

The molecular ion formed by electron ionization is an odd electron ion, a radical species with an unpaired electron. These ions are formed by removing a lone pair electron or a bonding electron from a molecule during ionization. For example, water is ionized by removing a non-bonding electron from oxygen to produces $H_2O^{+1}$. This is an example of an odd electron ion. Odd electron ions have an even mass to charge value. The exception to this is if the ion has an odd number of nitrogen atoms. Calculate the mass to charge value for some molecular ions to verify this statement.

When a molecular ion fragments by direct cleavage a single bond is broken to produce two fragments. This usually separates the charge and the radical of the molecular ion. Direct cleavage produces an even electron ion, $AB^+$, and a neutral odd electron radical, $CD^\bullet$. The even electron ion is detected at an odd mass to charge value (assuming no nitrogen) and since the radical is a neutral fragment it is not observed in the mass spectrum. Even electron ions have all paired electrons. An example of this was shown in the mass spectrum of acetone where the molecular ion, $CH_3–CO–CH_3^{+\bullet}$, fragments to form $CO–CH_3^+$ - an even electron ion observed at an 43 *m/z*. The radical $CH_3^\bullet$ has an odd mass but since it is neutral this fragment is not observed in the mass spectrum. It is possible for the charge and radical species to switch. As a result, cleavage of $CH_3–CO–CH_3^{+\bullet}$ can also form $CH_3^+$ which is an even electron ion observed at 15 *m/z*, an odd mass. The radical formed by this cleavage, $CO–CH_3^\bullet$, would not be observed. In the mass spectrum of acetone the 43 *m/z* peak is much larger than the 15 *m/z* peak, so the formation of $CO–CH_3^+$ at 43 *m/z* is clearly favored. When you are interpreting mass spectra look for possible cleavage fragments but keep in mind that either or both of the fragments may be observed in the mass spectrum.

Rearrangements are more complex reactions that involve both making and breaking bonds. These reactions are thermodynamically favorable because they require less energy. However they also require a concerted mechanism that is not as kinetically favorable when compared to a simple cleavage reaction. Rearrangement ions are easily identified because they are observed as odd electron ions with an even *m/z* value. These fragments often provide important clues about the location and identity of certain functional groups. Rearrangements are discussed in more detail in the next section.

The mass spectra of 4 different $C_4H_{10}O$ isomers are shown in Figures 16 - 19. These spectra show how cleavage patterns help to identify the structure of a compound. It is important to remember that determining the molecular formula is just the first step in interpretation of mass spectra.

## 1-Butanol

At this point get a piece of scratch paper, draw a Lewis dot structure for 1-butanol, find the mass of the molecular ion, break some bonds and find the mass of some possible fragments. Then look to see which of these fragments are observed in the mass spectrum. This exercise will take some time, but the practice will help you learn how to interpret mass spectra. After you have some possibilities, take a look at Figure 6.2.2 and see what you can find.

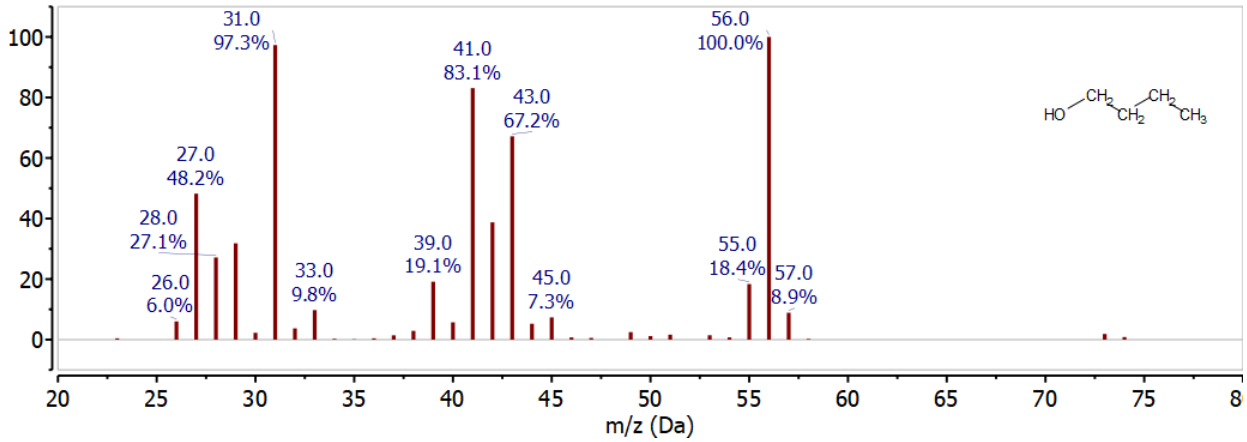


Figure 6.2.2 : Mass Spectrum of 1-butanol.

For 1-butanol the molecular ion should be observed at 74 *m/z* (4x12 + 10x1 + 1x16 = 74). There is a very small peak at this location, which is not unusual alcohols - and many other compound classes. If you look at the mass spectra for a large number of alcohols you will notice that they often show little or no molecular ion intensity. This makes interpreting their spectra challenging and IR spectra - which very clearly show OH functional groups - compliment mass spectra by helping to identify these functional groups. If you know from the IR that a compound is an alcohol you can be careful about identifying the molecular ion, knowing that it may not be observed.

Next look at possible cleavage fragments from the molecular ion. One possibility is loss of a hydrogen to give 73 *m/z*. There is a small peak at 73 *m/z* in the mass spectrum - that indicates that this fragmentation is possible but it is is not common. The same is also true for loss of $OH^{\bullet}$ - which is observed with a small peak at 57 *m/z* (74 - 17). The next loss is alpha-clevage, breaking the C-C bond next to the OH functional group, to form $CH_2OH^+$, observed at 31 *m/z* ($CH_2OH^+$) or the compliment, $CH_3CH_2CH_2^+$, observed at 43 *m/z*. Since the charge could be retained by either fragment both are observed in the spectrum. Alpha-cleavage is a common fragmentation pattern for alcohols, so observing a peak at 31 *m/z* is useful for identifying primary alcohols.

The 1-butanol spectrum also has a major peak at 56 *m/z*. This is an even mass ion so it is not formed by breaking a single bond. Looking at the loss from the molecular ion to this fragment (74 - 56 = 18) is a clue to the identity. Alcohols often undergo loss of water ($H_2O$ - 18 *m/z*, so 56 *m/z* is a likely peak for 1-butanol. This rearrangement is favorable because water is very stable and the resulting radical ion, $CH_2$=CH-$CH_2$-$CH_3^+$, has the same structure as an alkene. Rearrangements are much more likely when they create a stable species. The other significant peak in this mass spectrum is at 41 *m/z*. It is not possible to get this mass from breaking a single bond so it must also involve some sort of rearrangement. It is not unusual for fragmentation and loss of $H_2$ to occur so this ion could be formed by alpha-cleavage followed by $H_2$ loss. Since $H_2$ is an intact molecule, this fragmentation is energetically favorable although it also requires some rearrangement.

## 2-Butanol

The next spectrum to examine is 2-butanol. Before looking at the mass spectrum draw the Lewis dot structure for 2-butanol and determine the mass of the possible alpha-cleavage fragments. Then compare your results with the spectrum in Figure 6.2.3.

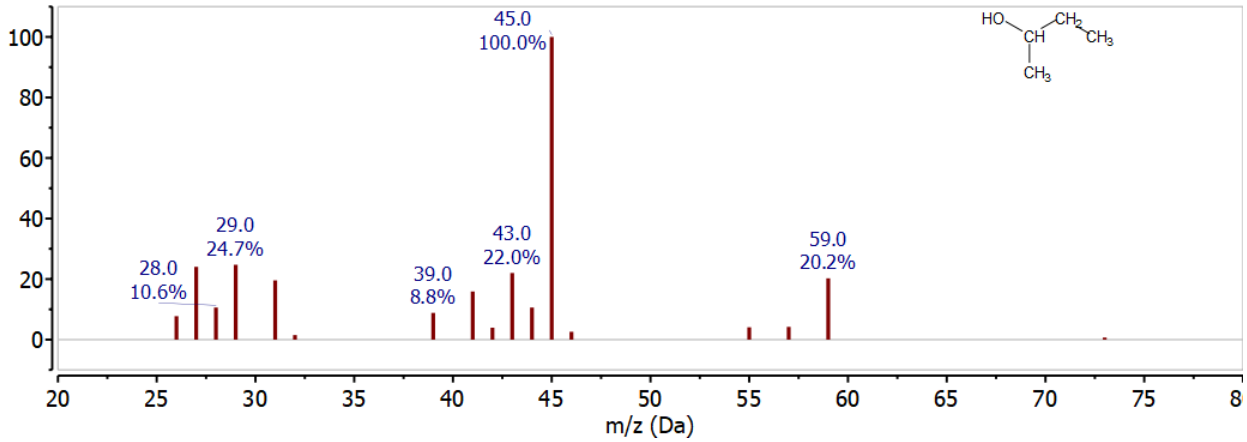


Figure 6.2.3: Mass Spectrum of 2-butanol

Since 2-butanol has the same molecular formula as 1-butanol, $C_4H_{10}O$, it also has the same molecular ion at 74 *m/z*. The molecular ion is not seen in Figure 17 but there are several very informative fragment ions observed in the mass spectrum. From the analysis of 1-butanol, it is reasonable to look for alpha cleavage fragments. Since this is a secondary alcohol, there are two possible alpha-cleavage locations for 2-butanol. Alpha-cleavage could result in loss of $CH_3^{\bullet}$ or $C_2H_5^{\bullet}$ to produce ions observed at 59 *m/z* (74 - 15) and 45 *m/z* (74 - 29) respectively. Both of these peaks are observed in Figure 17 and their high intensity clearly distinguish this mass spectrum from 1-butanol. The compliment ions, $CH_3^+$ or $C_2H_5^+$ are observed at 15 *m/z* and 29 *m/z* but are not particularly useful for identification since they are present in almost all organic mass spectra.

## 2-Methyl-1-Propanol

The next spectrum to examine is 2-methyl-1-propanol. Before looking at the mass spectrum in Figure 6.2.4, draw the Lewis dot structure for 2-methyl-1-propanol and determine the mass of the possible alpha cleavage fragments. Then compare what you find with the spectrum below.

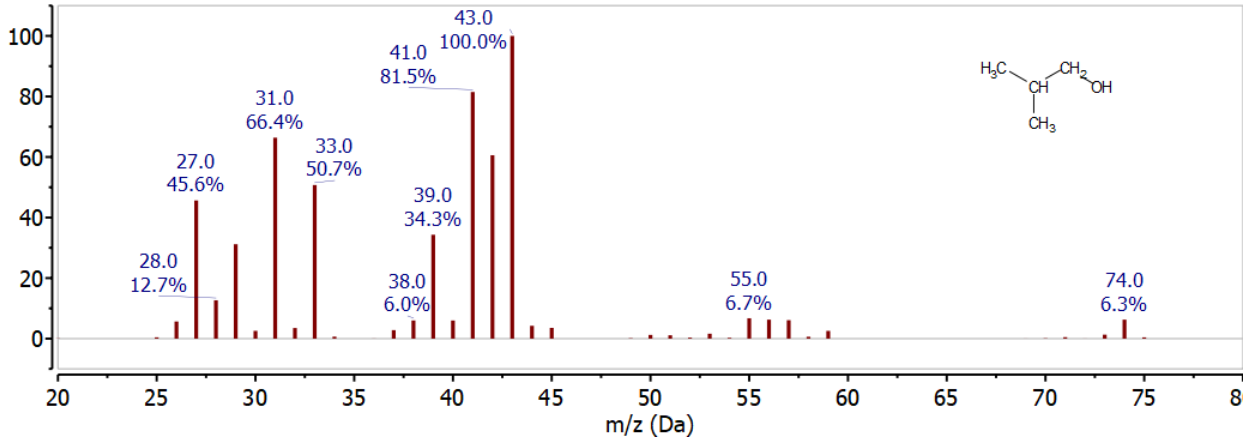


Figure 6.2.4: Mass Spectrum of 2-methyl-1-propanol

Since 2-methyl-1-propanol has the same molecular formula as 1-butanol and 2-butanol, $C_4H_{10}O$, it also has the same molecular ion at 74 *m/z*. Although the molecular ion at 74 *m/z* was not readily observed in the previous two spectra, it is clearly seen for 2-methyl-1-propanol in Figure 18. Based on the discussion of the previous two spectra we should also look for alpha cleavage fragments. Based on the structure for 2-methyl-1-propanol alpha-cleavage would result in loss of $C_3H_7^{\bullet}$ to form $CH2OH^+$ which is observed at 31 *m/z* and is characteristic of a primary alcohol. The complement ion $C_3H_7^+$ at 43 *m/z* is also observed in Figure 18. Since the $C_3H_7^+$ at 43 *m/z* is a secondary carbocation it is more stable than the $C_3H_7^+$ ion formed from the fragmentation of 1-butanol. As a result the peak at 43 *m/z* is the base peak in the spectum of 2-methyl-1-propanol. The relative intensity of peaks like this is very important for distinguishing the mass spectra of similar compounds. You can also compare the relative intensity of the peaks at 31 *m/z* and 43 *m/z* in the spectra of 1-butanol and 2-methyl-1-propanol.

## 2-Methyl-2-Propanol

The next spectrum to examine is 2-methyl-2-propanol. Before looking at the mass spectrum draw the Lewis dot structure for 2-methyl-2-propanol and determine the mass of the possible alpha cleavage fragments. Then compare what you find with the spectrum in Figure 6.2.5.

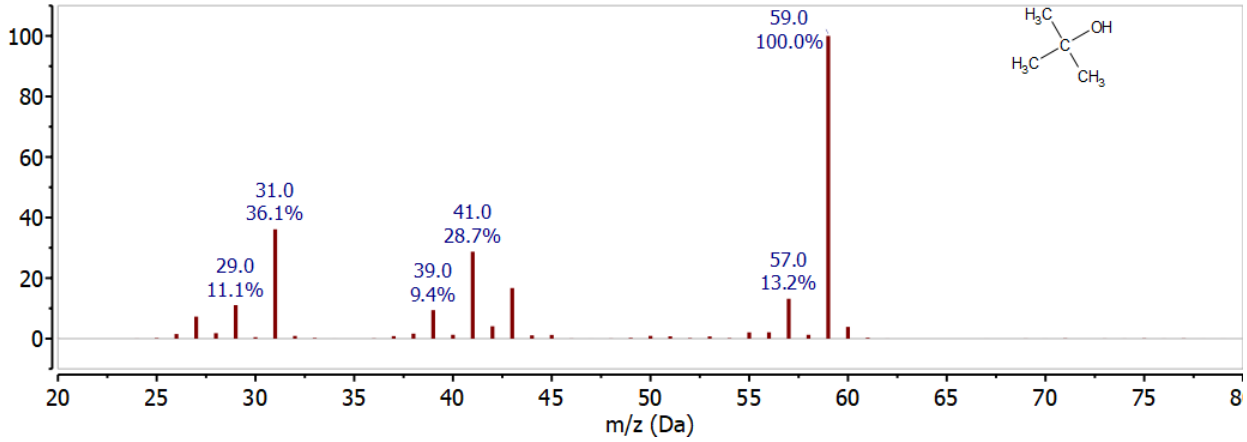


Figure 6.2.5: Mass Spectrum of 2-methyl-2-propanol

The molecular ion is not observed for 2-methyl-1-propanol in Figure 19. However, the alpha-cleavage peak showing loss of $CH_3^{\bullet}$ at 59 *m/z* is the base peak and is far more abundant than any other ion in the spectrum. There are two reasons for this, the first is that there are three different locations in the structure where alpha-cleavage results in loss of $CH_3^{\bullet}$. This alone would increase the probability of forming 59 *m/z* but the additional consideration is that the $C_3H_7O^+$ carbocation produced by alpha-cleavage is a tertiary carbocation. As a result it is much more stable and therefore less likely to undergo further fragmentation.

## $C_4H_{10}O$ Summary

It is clear from the spectra shown in Figures 16-19 that the mass spectra for these four different $C_4H_{10}O$ structures are readily distinguished based on the alpha-cleavage patterns. Learning common fragmentation patterns for different functional groups is very helpful for identifying unknowns and for distinguishing the spectra for similar compounds.

## Toluene

The mass spectrum for toluene is shown in Figure 6.2.6. Given the stability of aromatic compounds it should not be surprising that the molecular ion at 92 *m/z* has a high intensity. The base peak observed at 91 *m/z* is interesting because loss of $H^{\bullet}$ is not typically this intense. It turns out that the tropylium ion, $C_7H_7^+$, is also aromatic and so this fragment is very stable and often has a high intensity.

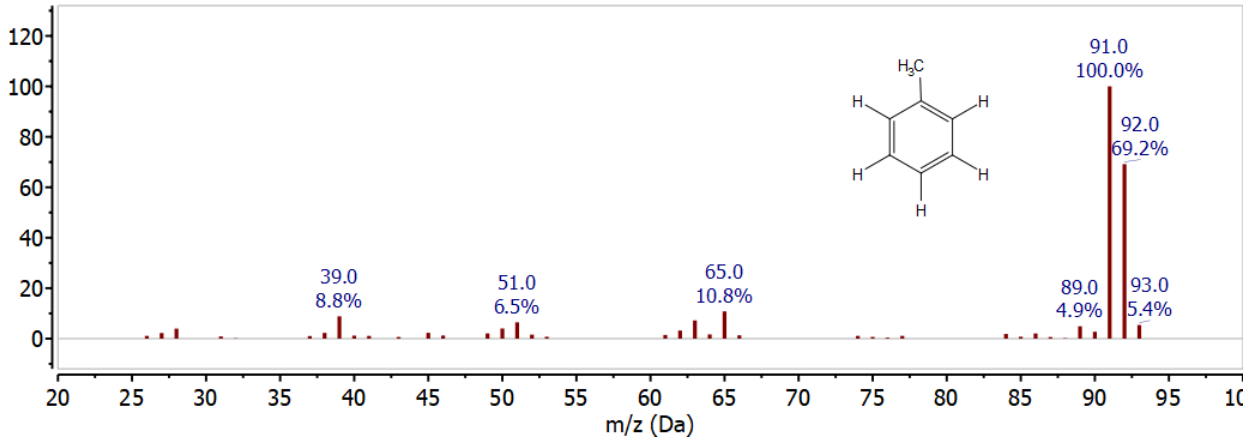


Figure 6.2.6: Mass Spectrum of Toluene

# 6.3: Rearrangement

## Heptane

The mass spectrum of heptane is shown in Figure 6.3.1. This mass spectrum is consistent with the fragmentation patterns discussed in the previous section. The molecular ion, $C_7H_{16}^{\bullet+}$ is observed at 100 *m/z* and a series of cleavage peaks are observed for loss of $CH_3^{\bullet}$ (M - 15), $C_2H_5^{\bullet}$ (M - 29), and $C_3H_7^{\bullet}$ (M - 43). These peaks are observed at 85 *m/z* , 71 *m/z* , and 57 *m/z* respectively. This fragmentation is characteristic for a linear hydrocarbon.

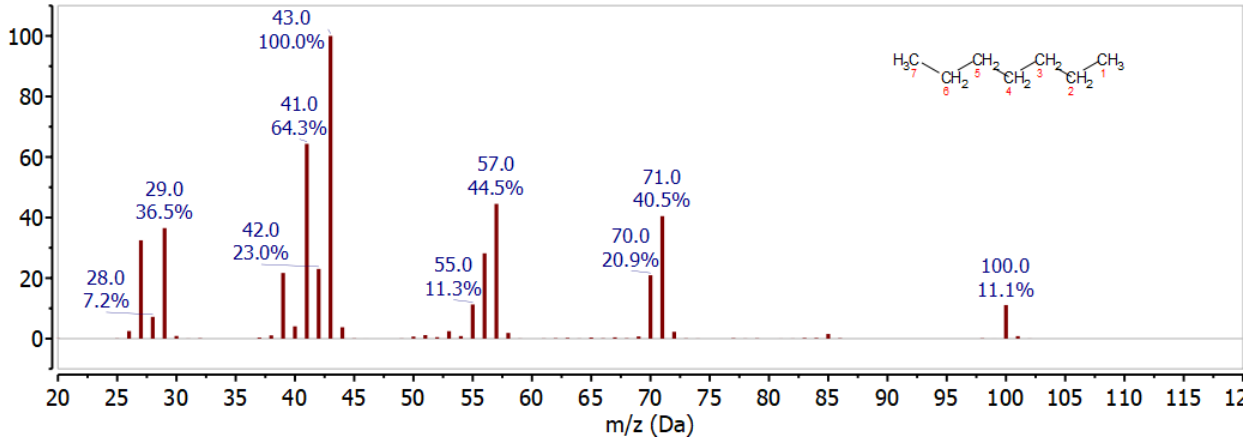


Figure 6.3.1: Mass Spectrum of heptane.

## McLafferty Rearrangement

Some functional groups, however, can undergo very different fragmentation processes than the direct cleavage discussed so far. One common example is the McLafferty rearrangement (Figure 6.3.2) which results in formation of an intact neutral molecule and a radical ion, both with an even mass to charge ratio. Since the most intense direct cleavage fragments have odd mass to charge ratios, this fragmentation pattern is very useful for identifying carbonyl compounds and for determining their structure. The McLafferty rearrangement is often observed for carbonyl compounds that contain a linear alkyl chain. If this alkyl chain is long enough, a six-membered ring forms from the carbonyl oxygen to the hydrogen on the fourth carbon. This spacing allows the hydrogen to transfer to the carbonyl oxygen via a six membered ring. This is followed by a rearrangement of the electrons to break the beta C-C bond, the second bond from the carbonyl carbon, to form an alkene and resonance stabilized radical with the carbonyl group. The McLafferty rearrangement is energetically favorable because it results in loss of a neutral alkene and formation of a resonance stabilized radical. Both these fragments may be observed in the mass spectrum, depending upon which fragment retains the charge. Figure 6.3.2 shows the charge on the resonance stabilized radical, this is the McLafferty ion. The alkene is referred to as the McLafferty compliment.

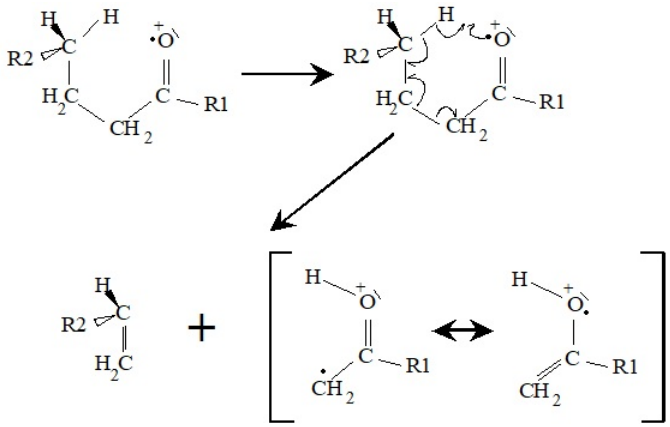


Figure 6.3.2: McLafferty rearrangement mechanism.

The products from the McLafferty rearrangement are observed in the mass spectra of C-7 carbonyl compounds shown in Figures 22-26. Draw structures for the following compounds and use the mechanism shown in Figure 21 to predict the mass of the two fragments formed by the McLafferty rearrangement. Then compare these predictions with the mass spectra shown in Figures 6.3.3 - 6.3.7.

- heptanal
- 2-heptanone
- 3-heptanone
- 4-heptanone
- heptanoic acid

## Heptanal

The mass spectrum of heptanal shown in Figure 6.3.3 contains two even mass ions. $C_2H_4O^+$ *m/z* 44 is produced by the McLafferty rearrangement of an aldehyde and is a characteristic peak that is very useful for interpretation of aldehydes. The McLafferty compliment, $C_5H_{10}^+$, is observed at 70 *m/z* . The McLafferty compliment is produced when the charge is transferred to the alkene fragment during the rearrangement.

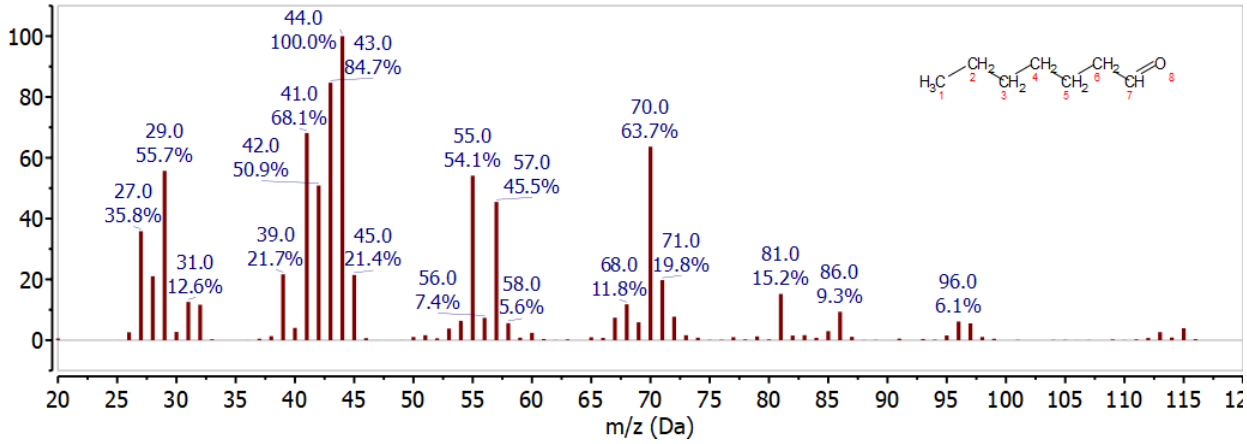


Figure 6.3.3: Mass Spectrum of heptanal.

## 2-Heptanone

The mass spectrum of 2-hepanone shown in Figure 6.3.4 is easily distinguished from heptanal because the McLafferty rearrangement breaks the C-C bond between C-3 and C-4. This results in loss $C_4H_8$ to give the McLafferty ion for a 2-ketone, $C_3H_6O^+$, at 58 *m/z*. The McLafferty compliment, $C_4H_8^+$ (56 *m/z*) is not observed for 2-heptanone.

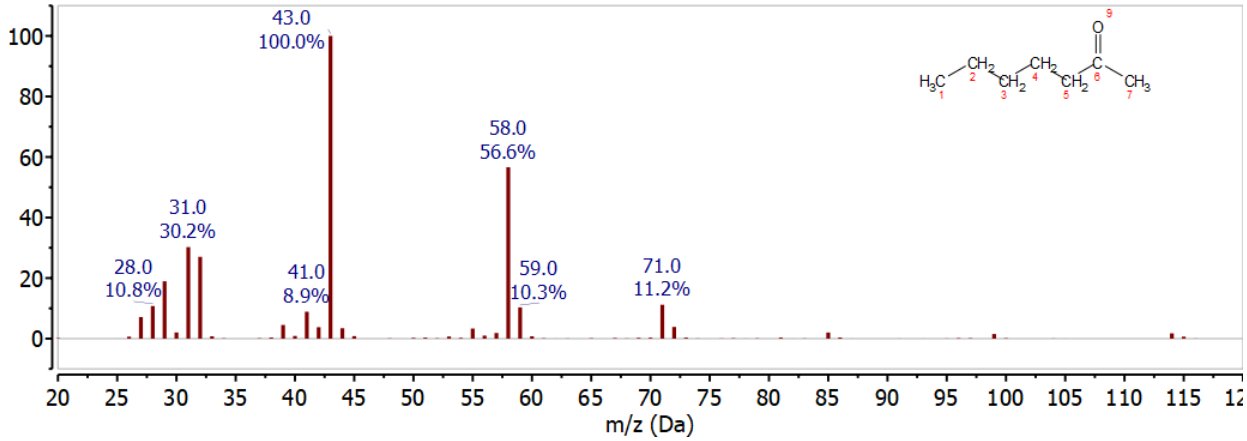


Figure 6.3.4: Mass Spectrum of 2-heptanone

## 3-Heptanone

The mass spectrum of 3-hepanone in Figure 6.3.5 is easily distinguished from heptanal and 2-heptanone because the McLafferty rearrangement breaks the C-C bond between C-4 and C-5. This results in loss $C_3H_6$ to give the McLafferty ion for a 3-ketone, $C_4H_8O^+$, at 72 *m/z*. The McLafferty compliment, $C_3H_6^+$ (42 *m/z*) is not observed for 3-heptanone.

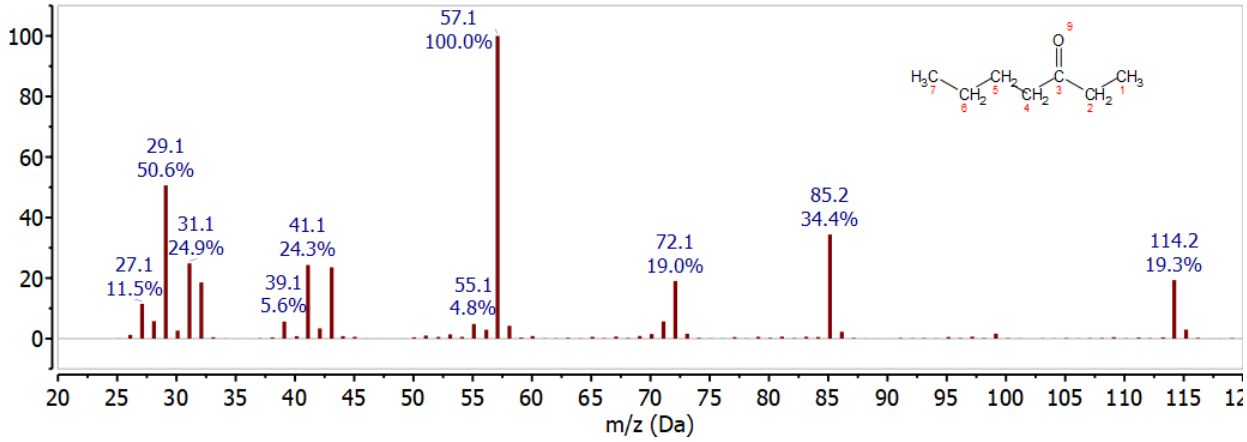


Figure 6.3.5: Mass Spectrum of 3-heptanone.

## 4-Heptanone

The mass spectrum of 4-hepanone shown in Figure 6.3.6 is easily distinguished from heptanal, 2-heptanone, and 3-heptanone. The McLafferty rearrangement would break the C-C bond between C-2 and C-3. This results in loss $C_2H_4$ to give the McLafferty ion for a 4-ketone, $C_5H_{10}O^+$, at 86 *m/z* - which has a very low intensity in the mass spectrum of 4-heptanone shown in figure 25. The two major peaks in this spectrum 43 *m/z* and 71 *m/z* correspond to alpha cleavage to produce $C_3H_7$ and $C_4H_7O$ which would be observed at 43 *m/z* and 71 *m/z* respectively. In this molecule the direct cleavage is highly favored over the McLafferty rearrangement. In this case the faster kinetics of the direct cleavage are favored over the concerted mechanism required for the rearrangement.

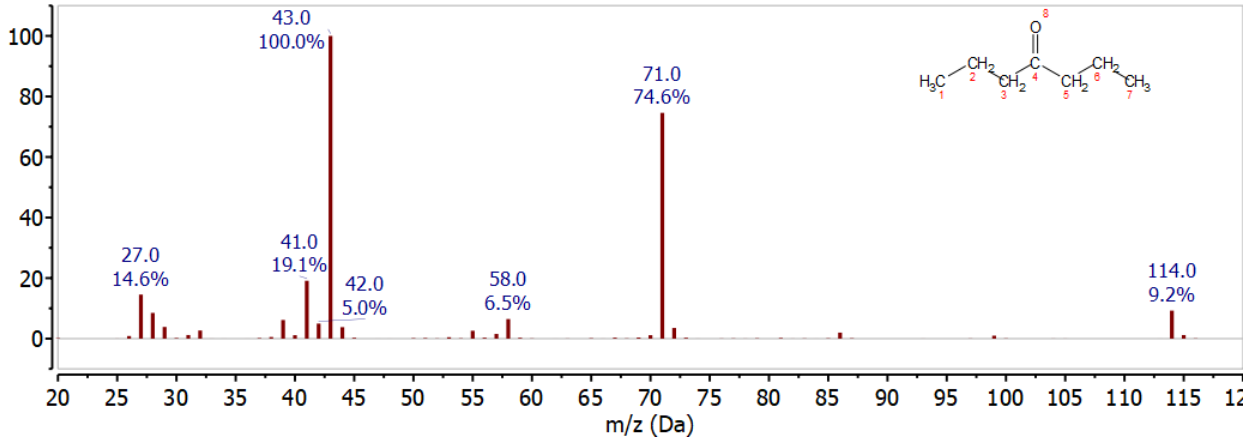


Figure 6.3.6: Mass Spectrum of 4-heptanone.

## Heptanoic Acid

The mass spectrum of heptanoic acid shown in Figure 6.3.7 is easily distinguished from heptanal, 2-heptanone, 3-heptanone, and 4-heptanone because the McLafferty rearrangement produces $C_2H_4O_2^+$ observed at 60 *m/z* and characteristic of a carboxylic acid. In this case the McLafferty compliment, $C_5H_8^+$, is not observed in the mass spectrum.

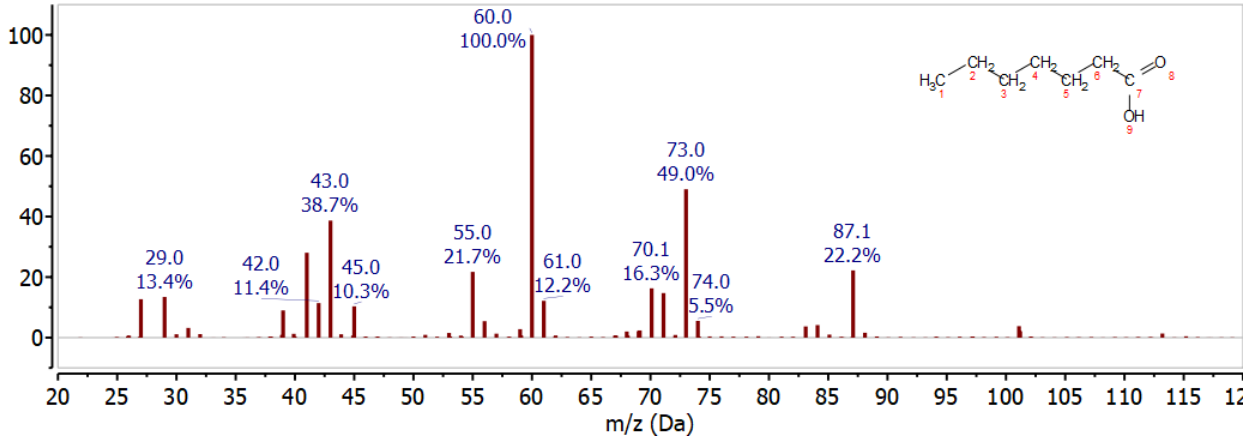


Figure 6.3.7: Mass Spectrum of heptanoic acid.

Based up on the discussion so far you should be able to identify many of the other fragments in these three mass spectra. Spend some time with a piece of scratch paper and see what you come up with.

---

# 6.4: Isotope Abundance

## Isotope Abundance.

The existence of isotopes was first observed by Aston using a mass spectrometer to study neon ions. When interpreting mass spectra it is important to remember that the relative atomic mass or atomic weight of an element is a weighted average of the naturally occurring isotopes. Mass spectrometers separate these isotopes and they are each observed at their respective mass to charge ratio. The relative abundance used to determine the relative atomic mass is determined using mass spectrometry. Although this complicates the mass spectrum, it also provides useful information for identifying the elements in an ion. Chlorine is an excellent example of how isotope distributions are useful for interpretation. The molecular weight of chlorine is $35.45u$ This is calculated from the natural abundance of $^{35}Cl(75\%)$ and $^{37}Cl(25\%)$. To avoid ambiguity the molecular ion is defined as the ion with the most commonly occurring isotopes. For $CH_3Cl$ the molecular ion is $^{12}C^{1}H_3{}^{35}Cl$ at 50 *m/z* .

## Chlorine Isotope Abundance

The natural abundance of these two isotopes is observed in the mass spectrum as two peaks separated by 2 *m/z* with a relative intensity of $3:1$. The mass spectrum of chlorobenzene $C_6H_5Cl$ in Figure $6.4.1$ clearly shows the chlorine isotope distribution at 112 *m/z* and 114 *m/z* . These peaks correspond to the molecular ion - the molecular ion has the most abundant isotope for each element - at 112 *m/z* (6x12 + 5x1 + 35) and the $^{37}Cl$ isotope peak at 114 *m/z* (6x12 + 5x1 + 37) and the relative intensity is determined by the natural abundance of the $^{37}Cl$ isotope. The other major peak in this spectrum at 77 *m/z* corresponds to the loss of chlorine from the molecular ion or the $^{37}Cl$ isotope peak to give $C_6H_5{}^+$ (112 - 35 = 77 OR 114 - 37 = 77).

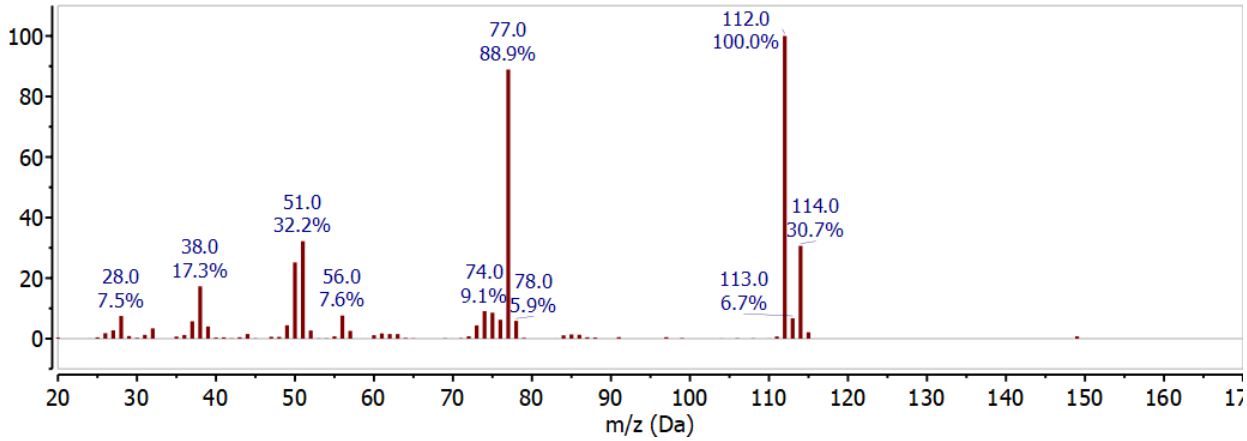


Figure 6.4.1: Mass Spectrum of chlorobenzene.

If more than one chlorine atom is present, the isotope abundance is more complex. An ion with two chlorine atoms has three possible isotope combinations. This pattern is apparent in the mass spectrum of $CH_2Cl_2$ shown in Figure $6.4.2$. Ions are observed for $CH_2{}^{35}Cl_2^+$ at 84 m/z, $CH_2{}^{35}Cl{}^{37}Cl^+$ at 86 *m/z* , and $CH_2{}^{37}Cl_2^+$ at 88 *m/z* . Based up on the probability of each combination of isotopes, the relative intensity of these peaks is $10:6:1$. The $3:1$ isotope ratio for an ion with a single chlorine atom is observed at 49 *m/z* and 51 *m/z* . This corresponds to $CH_2{}^{35}Cl^+$ and $CH_2{}^{37}Cl^+$ fragments formed by loss of $Cl$ from the molecular ion. Careful examination of the spectrum also shows ions produced by loss of $H^{\bullet}$ and $H_2$.

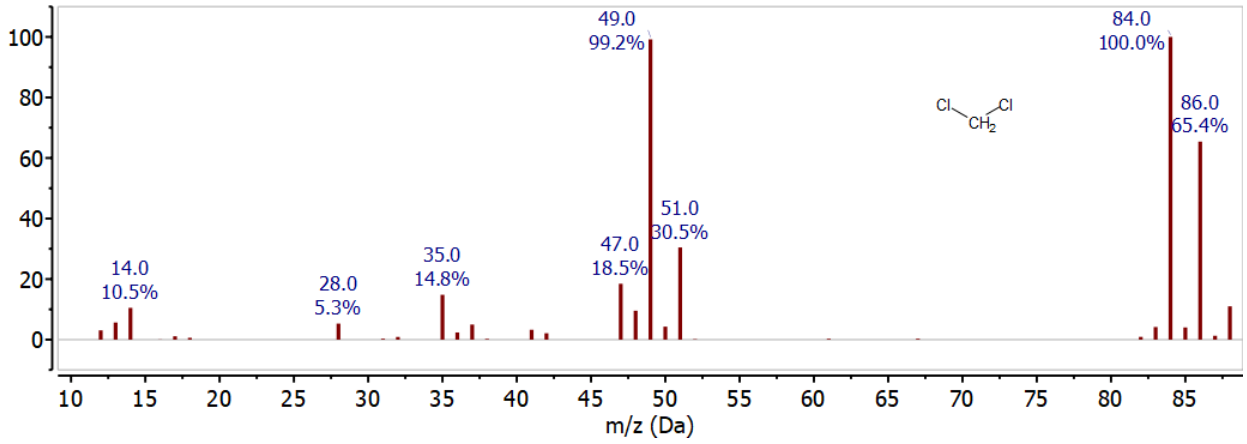


Figure 6.4.2: Mass Spectrum of dichloromethane.

## Bromine Isotope Abundance

Bromine also has two naturally occurring isotopes, $^{79}$Br is the most abundant and $^{81}$Br has a relative abundance of 98% which results in a relative intensity for these two peaks of 1:1. This is observed in the mass spectrum of bromobenzene shown in Figure 6.4.3. The bromine isotope pattern is seen in the peaks at 156 *m/z* and 158 *m/z* which have the 1:1 relative abundance characteristic of bromine. These two peaks correspond to the molecular ion $C_6H_5{}^{79}Br$ at 156 m/z and $C_6H_5{}^{81}Br$ at 158 *m/z* . The base peak in this spectrum is from loss of Br to form $C_6H_5$ observed at 77 *m/z* .

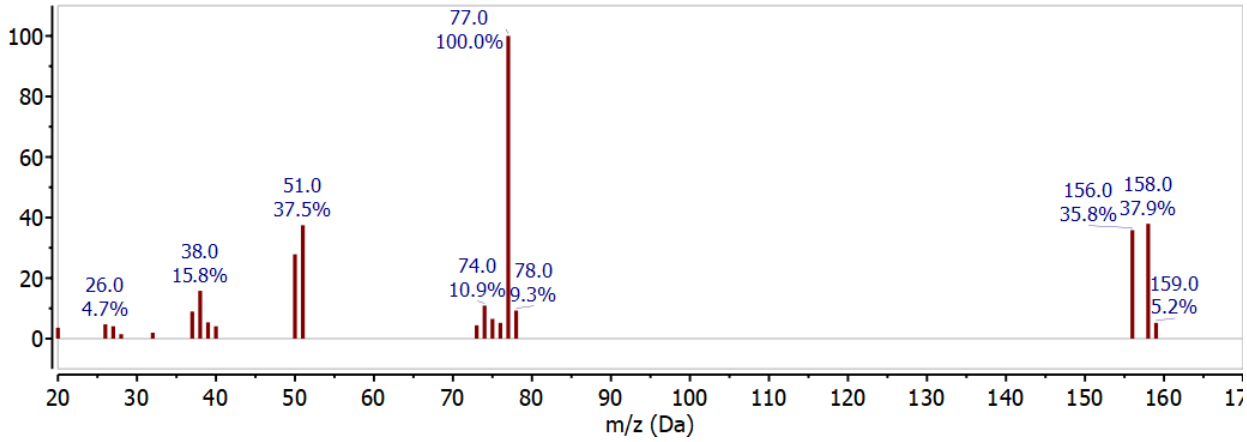


Figure 6.4.3: Mass Spectrum of bromobenzene.

## Carbon 13 isotope peak

The 1.1% of natural abundance of $^{13}$C is another useful tool for interpreting mass spectra. The abundance of a peak one *m/z* value higher, where a single $^{12}C$ is replaced by a $^{13}C$, is determined by the number of carbons in the ion. The rule of thumb for small compounds is that each carbon atom in the ion increases the abundance of the $M+1$ peak by 1%. This effect is seen in all the spectra discussed in this paper. For example, in the *n*-decane mass spectrum (Figure 6.4.4 ) compare the peak for $^{12}C_9{}^{13}C^1H_{22}$ at 143 *m/z* (0.38 % relative abundance) to the peak for $^{12}C_{10}{}^1H_{22}$ at 142 *m/z* (3.96% relative abundance). The abundance of the 13C peak is 10% the abundance of the $^{12}$C peak, consistent with a compound containing 10 carbon atoms. Now look at some previous spectra to find more examples of this pattern. Be aware that for compounds with low molecular ion abundances the uncertainty in measuring this ratio may be +/- several carbon atoms.

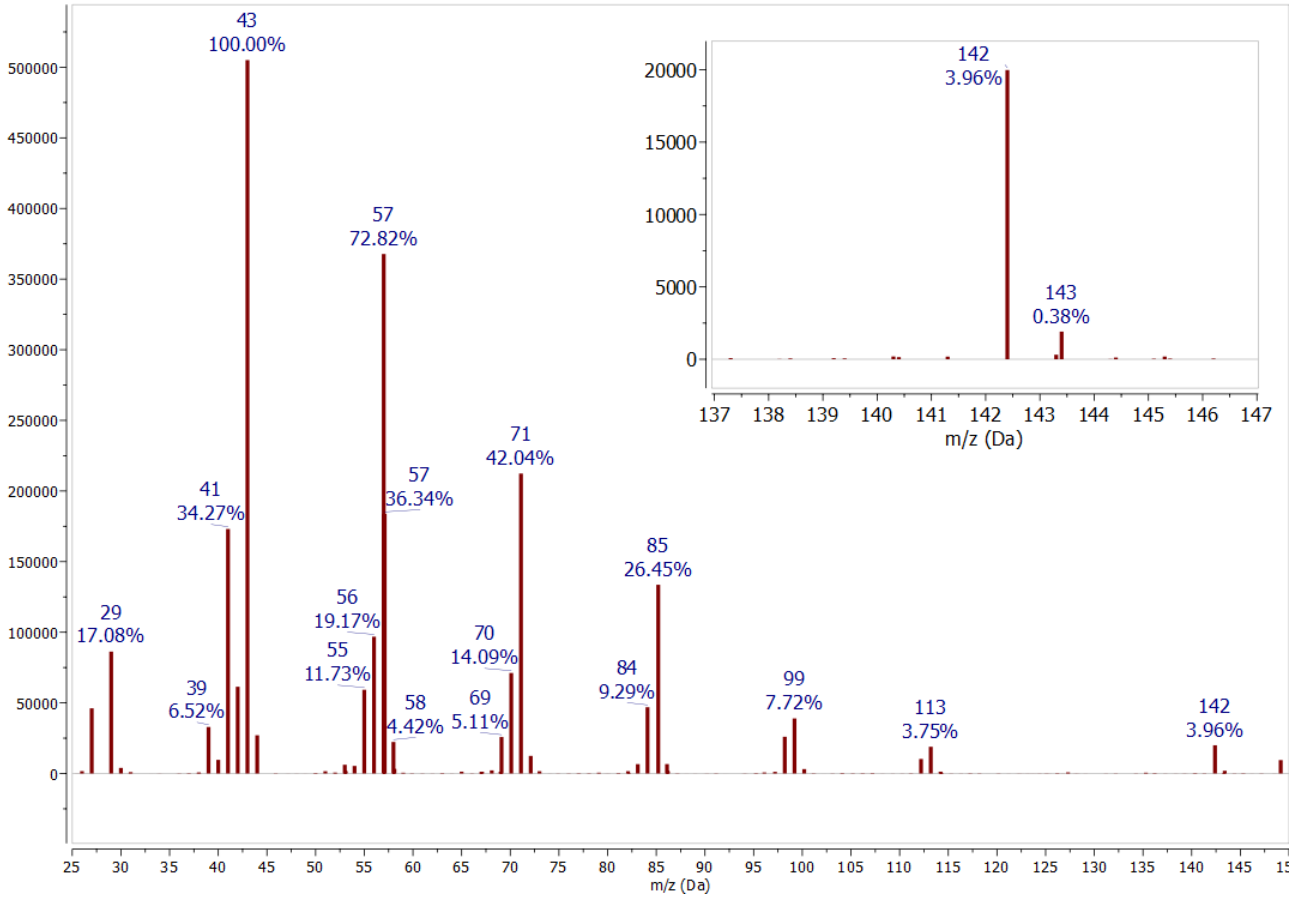


Figure 6.4.4: Mass Spectrum of Decane.

## Isotope Abundances

Because all atoms have several naturally occurring isotopes, the patterns discussed here become more complex. Fortunately, most elements common in organic mass spectrometry have one predominant isotope. The high abundance of the two chlorine isotopes is unusual, so they are easy to identify. The relative abundances for isotopes of frequently encountered elements are given in Table 6.4.1. For molecules with more complex isotope patterns there are a number of programs and websites available for modeling the distributions. The calculator provided by Scientific Instrument Services is available at: https://www.sisweb.com/mstools/isotope.htm.

Table 6.4.1: Isotope Abundances. Adapted from McLafferty, F. Interpretation of Mass Spectra (University Science, Mill Valley CA: 1980.

| Atom | Isotope A | | | Istope A+1 | | | Isotope A+2 | |
|---|---|---|---|---|---|---|---|---|
| | mass | % | | mass | % | | mass | % |
| H | 1 | 100 | | 2 | 0.015 | | | |
| C | 12 | 100 | | 13 | 1.1 | | | |
| N | 14 | 100 | | 15 | 0.37 | | | |
| O | 16 | 100 | | 17 | 0.04 | | 18 | 0.20 |
| F | 19 | 100 | | | | | | |
| Si | 28 | 100 | | 29 | 5.1 | | 30 | 3.4 |
| P | 31 | 100 | | | | | | |
| S | 32 | 100 | | 33 | 0.80 | | 34 | 4.4 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Cl | 35 | 100 | | | | | 37 | 32.5 |
| Br | 79 | 100 | | | | | 81 | 98.0 |
| I | 127 | 100 | | | | | | |

# 6.5: Amine Fragmentation

Functional groups can have a significant effect the fragmentation patterns observed in mass spectrometry and textbooks on mass spectrometry cover a large range of common fragmentation patters for different functional groups. For a detailed discussion of this, interested readers are encouraged to look at any of the following books:

As one final example aliphatic amines often undergo cleavage at the $\alpha \mathrm{C}-\mathrm{C}$ bond to produce a relatively stable $\mathrm{CH_2NH_2}^+$ ion (Figure $6.5.1$). The resulting fragments distinguish primary, secondary, and tertiary amines.

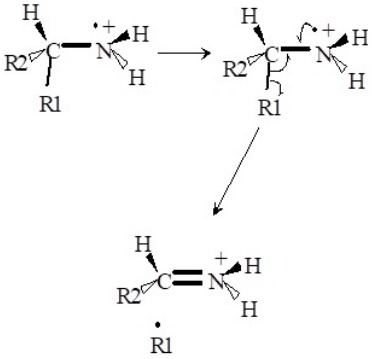


Figure $6.5.1$: $\alpha$-Cleavage fragmentation of an amine.

This fragmentation is useful for distinguishing mass spectra of the three different $\mathrm{C_4H_{11}N}$ isomers. Draw the structure of 1-butanamine, 2-butanamine, 2-methyl-1-propanamine, and 2-methyl-2-propanamine. Determine the mass to charge ratio for the molecular ion, identify the site for alpha-cleavage for each molecule, and determine the mass to charge ratio for the expected fragments. After you have done this, look up the mass spectra for these four compounds in the NIST Chemistry WebBook (https://webbook.nist.gov/) which contains mass spectra for a large number of compounds.

All four compounds have the same molecular formula, $\mathrm{C_4H_{11}N}$ with an odd number of nitrogen atoms so the molecular ion has an odd mass to charge ratio. The molecular ion is observed for all four compounds at 73 *m/z* .

1-butanamine. The $\alpha$-cleavage fragment for 1-butanamine produces $\mathrm{CH_2NH_2}^+$ at 30 *m/z* and $\mathrm{C_3H_7}^{\bullet}$. The $\mathrm{C_3H_7}$ fragment has a very low intensity in the mass spectrum because since the charge is retained by the nitrogen containing fragment. See NIST Webbook for the mass spectrum of 1-butanamine.

2-butanamine. There are two $\alpha$-cleavage sites for 2-butanamine. Loss of $\mathrm{CH_3'}$ produces $\mathrm{C_3H_6NH_2}^+$ (58 *m/z*) and loss of $\mathrm{C_2H_5'}$ produces $\mathrm{C_2H_4NH_2}^+$ (44 *m/z*). Both of these ions are observed but the greater abundance of the 44 *m/z* signal indicates that loss of $\mathrm{C_2H_5''}$ is favored. See NIST Webbook for the mass spectrum of 2-butanamine.

2-methyl-1-propanaimne. The $\alpha$-cleavage fragment for 2-methyl-1-propanamine produces $\mathrm{CH_2NH_2}^+$ at 30 *m/z* and $\mathrm{C_3H_7}^{\bullet}$. The $\mathrm{C_3H_7}$ fragment has a very low intensity in the mass spectrum because since the charge is retained by the nitrogen containing fragment. The resulting mass spectrum is very similar to 1-butanamine and distinguishing these two isomers by mass spectrometry will depend on careful comparison of the relative intensity of the molecular ion and other fragments observed in the mass spectrum. The See NIST Webbook for the mass spectrum of 2-methyl-1-propanamine.

2-methyl-2-propanaimne. The $\alpha$-cleavage fragment for 2-methyl-2-propanaimne produces $\mathrm{C_3H_6NH_2}^+$ at 58 *m/z* and $\mathrm{CH_3}^{\bullet}$. The $\mathrm{CH_3}$ fragment has a very low intensity in the mass spectrum because since the charge is retained by the nitrogen containing fragment. See NIST Webbook for the mass spectrum of 1-butanamine.

# 6.6: Exact Mass

Exact Mass. In most mass spectrometry experiments the nominal mass is used and the mass to charge ratio of an ion is rounded to the nearest whole number. High resolution instruments, including double focusing and FT-ICR mass spectrometers, are capable of determining the "exact mass" of an ion. This is useful for interpretation because each element has a slightly different mass defect. This "mass defect" is the difference between the mass of the isotope and the nominal mass (which is equivalent to the number of protons and neutrons).

Recall that the atomic mass scale is defined by carbon-12 with a mass of exactly $12.0000\,\text{u}$. The exact mass of a specific isotope is determined relative to $^{12}C$ by high resolution mass spectrometry (see Table $6.6.1$). High resolution mass spectrometry can distinguish compounds with the same nominal mass but different exact mass caused by different elemental composition.

For example, $C_2H_6$, $CH_2O$, and $NO$ all have a nominal mass of 30 u. Because they have the same nominal mass, a mass spectrometer with unit mass resolution can not distinguish these three ions. However, the exact masses for $C_2H_6(30.04695039)$, $CH_2O(30.01056487)$ and $NO^2$ (29.99798882) are different and a high resolution mass spectrometer can distinguish these three compounds.

Table $6.6.1$ lists the exact mass for the most abundant isotopes of several common elements. The Isotope Distribution Calculator on the SIS website will also calculate the exact mass for any chemical formula. This is available online at: https://www.sisweb.com/mstools/isotope.htm

Table $6.6.1$: Adopted from DiFlippo, F.; et. al. Phys Rev Lett. 1994, 73, 1482 .

| Element | Isotoope | Mass |
|---|---|---|
| H | $^{1}H$ | 1.007 825 031 6 (5) |
| | $^{2}H$ | 2.014 101 777 9 (5) |
| He | $^{4}He$ | 4.002 603 36 |
| | $^{3}He$ | 3.016 0 |
| C | $^{12}C$ | 12.000 000 000 0 (0) |
| | $^{13}C$ | 13.003 354 838 1 (10) |
| N | $^{14}N$ | 14.003 074 004 0 (12) |
| | $^{15}N$ | 15.000 108 897 7 (11) |
| O | $^{16}O$ | 15.994 914 619 5 (21) |
| | $^{17}O$ | 17.999 2 |
| P | $^{31}P$ | 30.973 763 3 |
| S | $^{32}S$ | 31.972 072 8 |
| | $^{34}S$ | 33.967 9 |

Values in parentheses indicate error in last digit.

This section is only an introduction to the interpretation of mass spectra. A full analysis of fragmentation patterns is beyond the scope of this text but with practice interpretation becomes much easier. Several excellent references include McLafferty's book (35) and the ACOL book on mass spectrometry (36). These contain additional information on mass spectral interpretation and many more practice problems.

# 7: END OF CHAPTER QUESTIONS-

1. You work for a mass spectrometer vendor who sells all the instruments described here. Make a product table that shows all the different options for each major part of the mass spectrometer.
2. Using thermochemical data, find the energy required to remove an electron from the following species: $H_2$, Na, C, $CH_4$, and Fe. Express this energy in $kJ/mole$ and eV (per atom).
3. Using thermochemical data, determine the energy required to break the following bonds: $H-H$, $C-C$, $C=C$, $C-H$ , and $C-O$. Express this energy in $kJ/mole$ and $eV$ (per atom).
4. Determine the kinetic energy, velocity, and momentum of the following ions: $m/z10, 50$, 100 , and 1000. The ions are formed in the center of the source region, which is $10.0$ mm long and has a $5000$ V potential applied across the two ends.
5. How long would it take each of the ions from question 4 to travel through a 1 meter flight tube in a TOF mass spectrometer?
6. What magnetic field is required to select each of the ions from question 4 in a magnetic sector with a radius of 1 meter?
7. What is the cy clotron frequency of each ion from question 4 in an ICR with a 3 T magnetic field?
8. What electric field strength is required for each of the ions from question 4 to be selected by an electric sector with a radius of $0.5$ meter?
9. What is the $m/z$ value for the molecular ion produced by EI of the following molecules: A) benzene, B) octane, C) trinitrotoluene, D) acetone, E) t-butyl amine.
10. The highest mass ion observed in a mass spectrum is at $m/z127$. If the compound contains a single N atom, could this be the molecular ion? If the compound contains four N atoms, could this be the molecular ion? Why?
11. The molecular ion in a high resolution mass spectrometry experiment is observed at $m/z$ 58.0055. What is the molecular formula for this compound? If this was a low resolution mass spectrometer, what other molecular formula's are possible?

# 8: ACKNOWLEDGMENTS

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