

Mass Spectrometry

www.anilmishra.name

1

Mass Spectrometry (MS)

- An analytical technique for measuring the mass-to-charge ratio (m/z) of ions in the gas phase
 - Mass spectrometry is our most valuable analytical tool for determining accurate molecular masses
 - Also can give information about structure
 - Proteins can now be sequenced by MS

www.anilmishra.name

2

Mass Spectrometry (MS)

- A technique for measuring and analyzing molecules, that involves introducing enough energy into a (neutral) target molecule to cause its ionization and disintegration.
- The resulting primary ions and their fragments are then analyzed, based on their mass/ charge ratios, to produce a "molecular fingerprint."

www.anilmishra.name

3

Mass Spectrometry (MS)

- Ionization implies a chemical process induced by physical methods.
- The sample is consumed during the measurement.
- There is no defined stimulation of molecular energy levels through interaction with electromagnetic radiation, where you can get the sample back without modification.

www.anilmishra.name

4

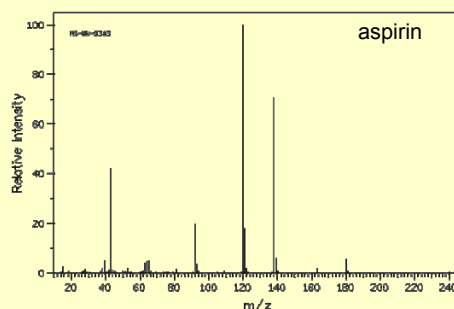
Mass Spectrometry (MS)

- A mass spectrometer bombards a substance under investigation with an electron beam and quantitatively records the result as a spectrum of positive ion fragments. This record is a Mass Spectrum.
- A mass spectrum is a presentation of the masses of the positively charged fragments vs. their relative concentration.
- Separation of the positive charge ion fragment is on the basis of mass. (Mass/Charge)

www.anilmishra.name

5

Typical Mass Spectrum



www.anilmishra.name

6

Mass Spectrometry

- Molecular weight can be obtained from a very small sample.
- It does not involve the absorption or emission of light.
- A beam of high-energy electrons breaks the molecule apart.
- The masses of the fragments and their relative abundance reveal information about the structure of the molecule.

www.anilmishra.name

7

MS How It Works

- The physics behind mass spectrometry is that a charged particle passing through a magnetic field is deflected along a circular path on a radius that is proportional to the mass to charge ratio, m/e .

www.anilmishra.name

8

MS How It Works

- A high energy beam of electrons is used to displace an electron from the organic molecule to form a **radical cation** known as the **molecular ion**.
- If the molecular ion is too unstable then it can **fragment** to give other smaller ions.

www.anilmishra.name

9

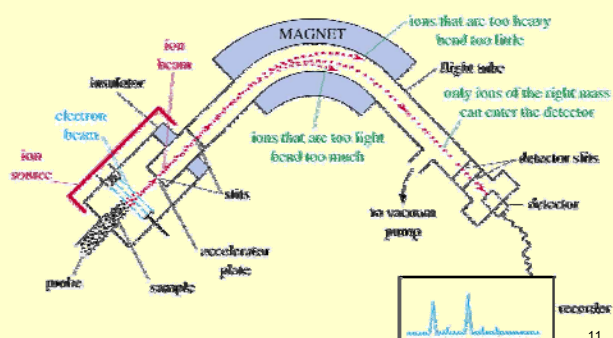
MS How It Works

- The collection of ions is then focused into a beam and accelerated into the magnetic field and deflected along circular paths according to the masses of the ions.
- By adjusting the magnetic field, the ions can be focused on the detector and recorded.

www.anilmishra.name

10

Mass Spectrometer



www.anilmishra.name

11

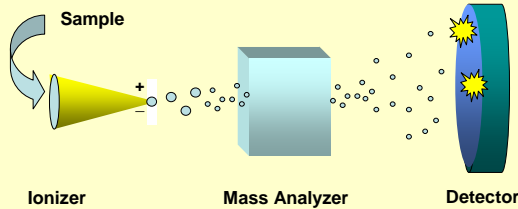
Mass Spec Principles

- Find a way to “charge” an atom or molecule (**ionization**)
- Place charged atom or molecule in a magnetic field or subject it to an electric field and measure its speed or radius of curvature relative to its mass-to-charge ratio (**mass analyzer**)
- **Detect** ions using microchannel plate or photomultiplier tube

www.anilmishra.name

12

Mass Spec Principles



www.anilmishra.name

13

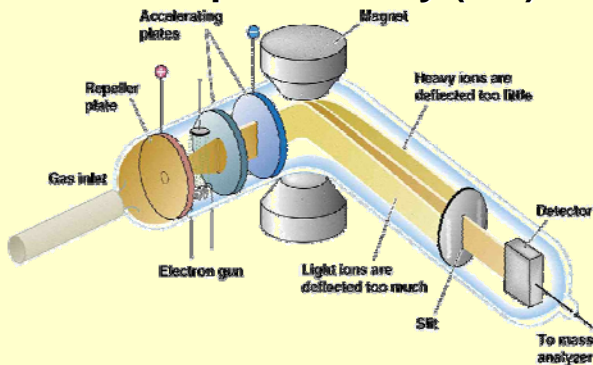
A Mass Spectrometer

- A mass spectrometer is designed to do three things
 - Convert neutral atoms or molecules into a beam of positive (or negative) ions
 - Separate the ions on the basis of their mass-to-charge (m/z) ratio
 - Measure the relative abundance of each ion

www.anilmishra.name

14

Mass Spectrometry (MS)



www.anilmishra.name

15

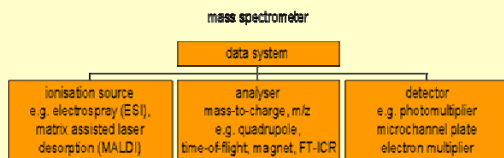
How does a Mass Spectrometer work?

- 3 fundamental parts:
 - Ionisation Source,
 - Analyser
 - Detector
- Samples easier to manipulate if ionised
- Separation in analyser according to mass-to-charge ratios (m/z)
- Detection of separated ions and their relative abundance
- Signals sent to data system and formatted in a m/z spectrum

www.anilmishra.name

16

Simplified Schematic

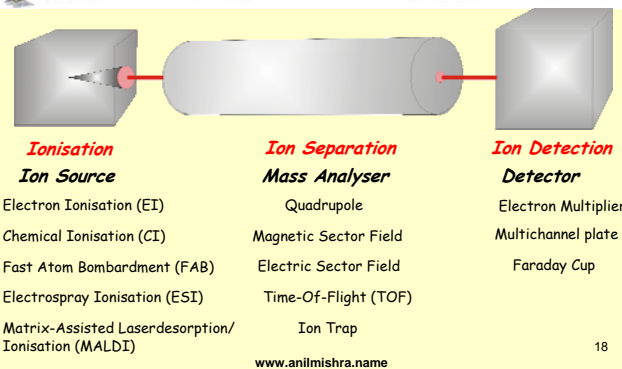


- The analyser, detector and ionisation source are under high vacuum to allow unhindered movement of ions
- Operation is under complete data system control

www.anilmishra.name

17

Components Of A Mass Spectrometer



www.anilmishra.name

18

Typical Mass Spectrometer



www.anilmishra.name

19

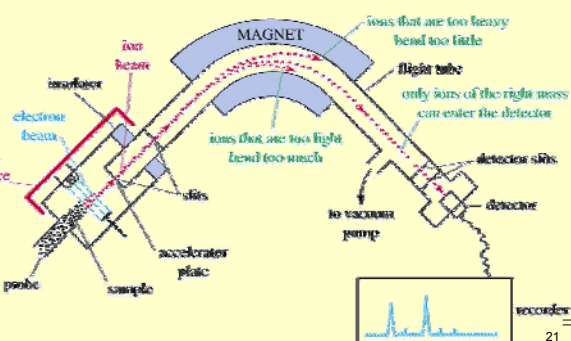
Separation of Ions

- Only the cations are deflected by the magnetic field.
- Amount of deflection depends on m/z .
- The detector signal is proportional to the number of ions hitting it.
- By varying the magnetic field, ions of all masses are collected and counted.

www.anilmishra.name

20

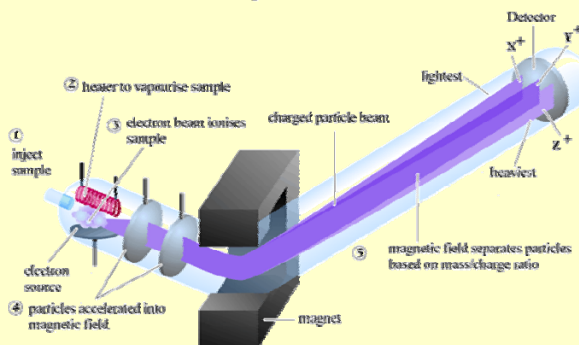
Mass Spectrometer



www.anilmishra.name

21

Mass Spectrometer



www.anilmishra.name

22

Typical Mass Spectrum

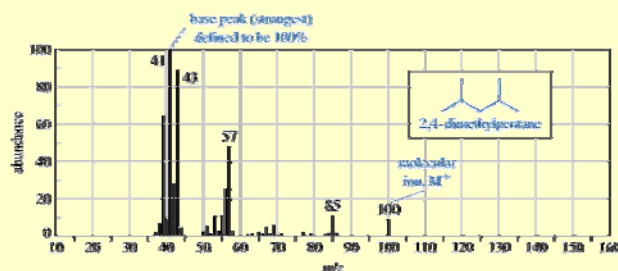
- Characterized by sharp, narrow peaks
- X-axis position indicates the m/z ratio of a given ion (for singly charged ions this corresponds to the mass of the ion)
- Height of peak indicates the relative abundance of a given ion (not reliable for quantitation)
- Peak intensity indicates the ion's ability to desorb or "fly" (some fly better than others)

www.anilmishra.name

23

Typical Mass Spectrum

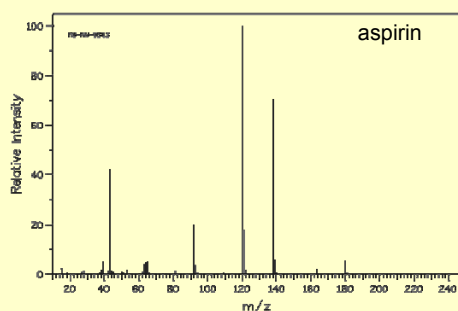
Masses are graphed or tabulated according to their relative abundance.



www.anilmishra.name

24

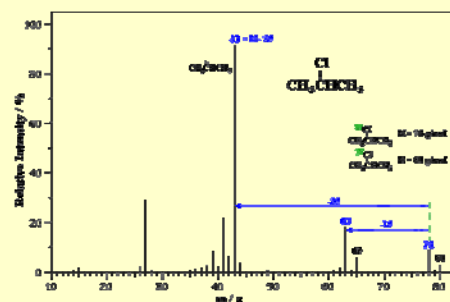
Typical Mass Spectrum



www.anilmishra.name

25

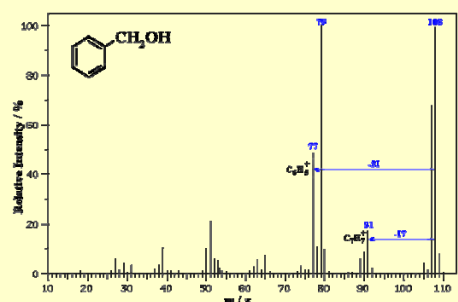
Typical Mass Spectrum



www.anilmishra.name

26

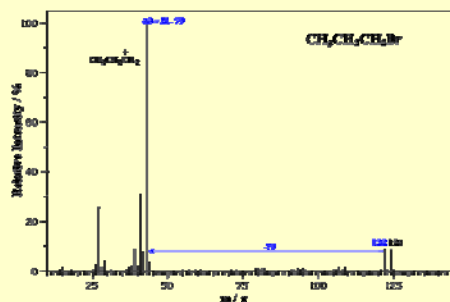
Typical Mass Spectrum



www.anilmishra.name

27

Typical Mass Spectrum



www.anilmishra.name

28

Mass Terminology

Molecular ion	The ion obtained by the loss of an electron from the molecule
Base peak	The most intense peak in the MS, assigned 100% intensity
M⁺	Symbol often given to the molecular ion
Radical cation	+ve charged species with an odd number of electrons
Fragment ions	Lighter cations formed by the decomposition of the molecular ion. These often correspond to stable carbocations.

www.anilmishra.name

29

Molecular Ion

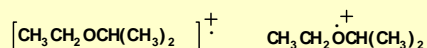
- **Molecular ion (M):** a radical cation formed by removal of a single electron from a parent molecule in a mass spectrometer.
- Molecules containing only majority isotopes and having lost or gained electrons without bond break-age.
- Normally designated as M⁺ or M^{•+}. The term also denotes a type of molecular-related ion.
- In order to unify the symbols with multiply charged ions, represented by M²⁺ and M³⁺, the symbol is also expressed as M^{•+}.

www.anilmishra.name

30

Molecular Ion

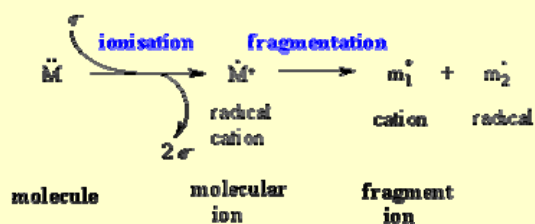
- It does not matter which electron is lost; radical cation character is delocalized throughout the molecule; therefore, we write the molecular formula of the parent molecule in brackets with
 - a plus sign to show that it is a cation
 - a dot to show that it has an odd number of electrons
- At times, however, we find it useful to depict the radical cation at a certain position in order to better understand its reactions



www.anilmishra.name

31

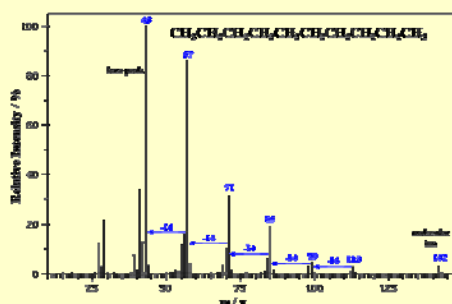
Molecular Ion



www.anilmishra.name

32

Typical Mass Spectrum



www.anilmishra.name

33

Molecular Ion

- Not necessary the strongest peak
- May not be visible
- Can be increased by reducing the energy of the electron beam
- With ionization methods other than electron impact (particular laser desorption), the molecular ions are much prominent

www.anilmishra.name

34

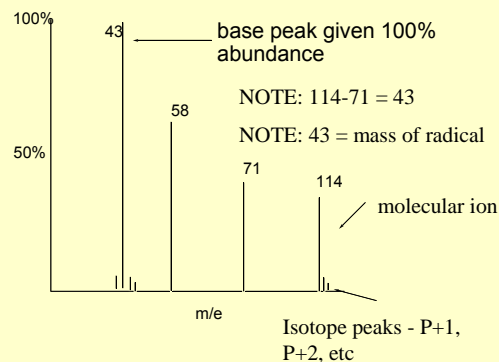
Mass Spectrum

- Mass spectrum:** a plot of the relative abundance of ions versus their mass-to-charge ratio
- Base peak:** the most abundant peak
 - assigned an arbitrary intensity of 100
- The relative abundance of all other ions is reported as a % of abundance of the base peak

www.anilmishra.name

35

Typical Mass Spectrum

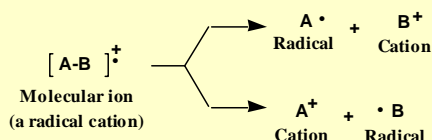


www.anilmishra.name

36

Fragmentation of M

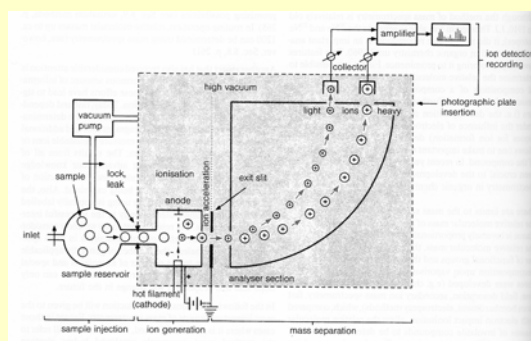
- Fragmentation of a molecular ion, M, produces a radical and a cation
 - only the cation is detected by MS



www.anilmishra.name

37

Metastable Peak



www.anilmishra.name

38

Metastable Peak

- Some ions have so short lifetimes that they dissociate while moving through the spectrometer.
- They undergo secondary fragmentations in the analyzer tube of the mass spectrometer; the resulting "signals" or peaks represent neither the m/e of the first ion nor that of the second ion
- This fragment ion is called the metastable ion.

www.anilmishra.name

39

Metastable Peak

- A metastable ion peak is a broad peak which appears at non-integral values of m/e in a mass spectrum.
- It is formed by a fragment ion.
 - An unstable molecule such as an alcohol can undergo fragmentation during ionization (collision with high energy electrons in ionization chamber of the mass spectrometer) to give rise to a fragment ion.
- This fragment ion is called the metastable ion.

www.anilmishra.name

40

Metastable Peak

- Suppose M_1^+ is the parent ion & m_1^+ is the daughter ion and if the reaction $M_1^+ \rightarrow m_1^+$ takes place in the source, then the daughter ion, m_1^+ may travel the whole analyzer region & is recorded as m_1^+ ion.
- On the other hand, if the transition M_1^+ to m_1^+ occurs after the source exist and before arrival at the collector, then m_1^+ is called a metastable ion.

www.anilmishra.name

41

Metastable ion

- A fragmentation reaction may be written:

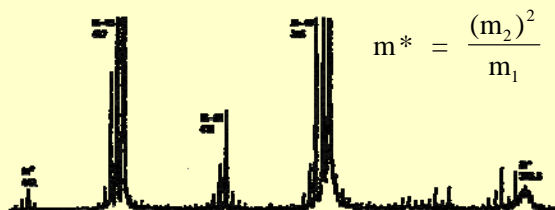
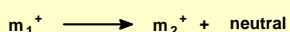
$$M_1^+ \longrightarrow M_2^+ + M_3$$
- The decomposition of a metastable ion of mass-to-charge ratio m_1/z_1 into an ion of mass-to-charge ratio m_2/z_2 after electric acceleration of the metastable ion and before magnetic deflection gives rise to a peak in the mass spectrum at an apparent mass,

$$m^* = m_2^2 z_1 / (m_1 z_2^2) = m_2^2 / m_1$$
- The symbol m^* should be used to indicate the apparent mass of the product ions giving rise to this peak.
- Traditionally, the peak itself has been called a **metastable peak** and this should preferably be expressed as **metastable ion peak**.

www.anilmishra.name

42

Metastable Peak



www.anilmishra.name

43

Metastable Peak

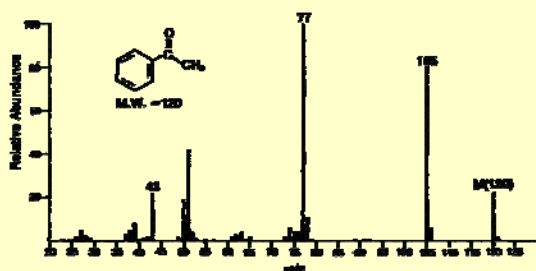
- *Characteristics of metastable peaks are:*
 - These peaks are much broader, i.e., they spread over several mass units.
 - These peaks appear in the mass spectrum usually at non-integral m/e values.
 - These peaks are of relatively low abundance or low intensity.
 - The metastable ions can be detected by a double focusing mass spectrometer.

www.anilmishra.name

44

Metastable Peak

Acetophenone

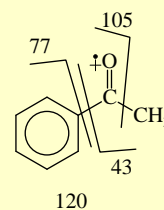


www.anilmishra.name

45

Metastable Peak

Acetophenone



metastable peaks at 91.9, 56.4, 33.8

www.anilmishra.name

46

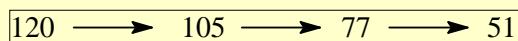
Metastable Peak

$$m^* = \frac{(m_2)^2}{m_1}$$

$$m^* = \frac{(105)^2}{120} = 91.875 \quad m^* = \frac{(77)^2}{120} = 49.4$$

$$m^* = \frac{(77)^2}{105} = 56.47 \quad m^* = \frac{(51)^2}{77} = 33.8$$

timing of fragmentation



www.anilmishra.name

47

Resolution

- **Resolution:** a measure of how well a mass spectrometer separates ions of different mass
 - **low resolution:** refers to instruments capable of separating only ions that differ in nominal mass; that is ions that differ by at least 1 or more atomic mass units
 - **high resolution:** refers to instruments capable of separating ions that differ in mass by as little as 0.0001 atomic mass unit

www.anilmishra.name

48

Resolution & Resolving Power

- Width of peak indicates the resolution of the MS instrument
- The better the resolution or resolving power, the better the instrument and the better the mass accuracy
- Resolving power is defined as:

$$\frac{\Delta M}{M}$$

- M is the mass number of the observed mass (ΔM) is the difference between two masses that can be separated

www.anilmishra.name

49

Resolution

- C_3H_6O and C_3H_8O have nominal masses of 58 and 60, and can be distinguished by low-resolution MS
- C_3H_8O and $C_2H_4O_2$ have nominal masses of 60
- distinguish between them by high-resolution MS

Molecular Formula	Nominal Mass	Precise Mass
C_3H_8O	60	60.05754
$C_2H_4O_2$	60	60.02112

www.anilmishra.name

50

Isotopes

- virtually all elements common to organic compounds are mixtures of isotopes

Element	Atomic weight	Isotope	Mass (amu)	Relative Abundance
hydrogen	1.0079	1H	1.00783	100
		2H	2.01410	0.016
carbon	12.011	^{12}C	12.0000	100
		^{13}C	13.0034	1.11
nitrogen	14.007	^{14}N	14.0031	100
		^{15}N	15.0001	0.38
oxygen	15.999	^{16}O	15.9949	100
		^{18}O	17.9992	0.20
sulfur	32.066	^{32}S	31.9721	100
		^{34}S	33.9679	4.40
chlorine	35.453	^{35}Cl	34.9689	100
		^{37}Cl	36.9659	32.5
bromine	79.904	^{79}Br	78.9183	100
		^{81}Br	80.9163	98.0

www.anilmishra.name

51

Molecules with Heteroatoms

- Isotopes: present in their usual abundance.
- Hydrocarbons contain 1.1% C-13, so there will be a small M+1 peak.
 - If Br is present, M+2 is equal to M⁺.
 - If Cl is present, M+2 is one-third of M⁺.
 - If iodine is present, peak at 127, large gap.
 - If N is present, M⁺ will be an odd number.
 - If S is present, M+2 will be 4% of M⁺.

www.anilmishra.name

52

Alkyl Halides

the M + 2 Peak

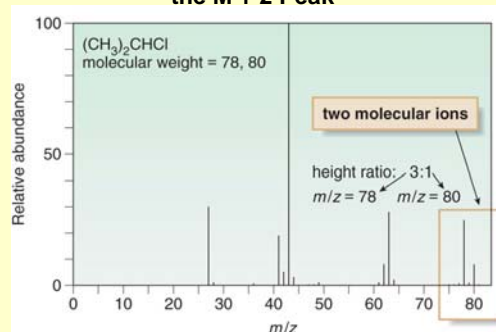
- Most elements have one major isotope.
- Chlorine has two common isotopes, ^{35}Cl and ^{37}Cl , which occur naturally in a 3:1 ratio.
 - Thus, there are two peaks in a 3:1 ratio for the molecular ion of an alkyl chloride.
 - The larger peak, the M peak, corresponds to the compound containing the ^{35}Cl .
 - The smaller peak, the M + 2 peak, corresponds to the compound containing ^{37}Cl .
 - Thus, when the molecular ion consists of two peaks (M and M + 2) in a 3:1 ratio, a Cl atom is present.

www.anilmishra.name

53

Alkyl Halides

the M + 2 Peak



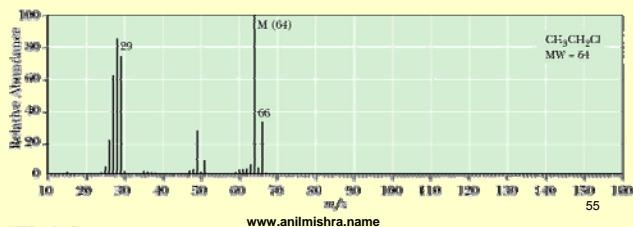
Mass spectrum of 2-chloropropane [(CH₃)₂CHCl]

www.anilmishra.name

54

M+2 and M+1 Peaks

- The most common elements giving rise to significant M + 2 peaks are chlorine and bromine
 - chlorine in nature is 75.77% ^{35}Cl and 24.23% ^{37}Cl
 - a ratio of M to M + 2 of approximately 3:1 indicates the presence of a single chlorine in a compound



Alkyl Halides

the M + 2 Peak

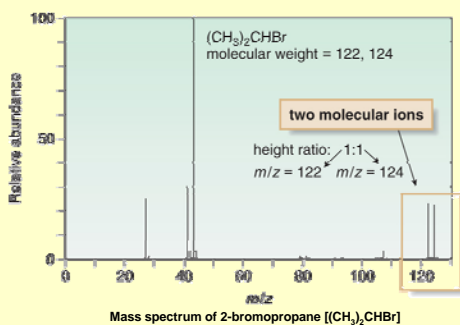
- Br has two isotopes— ^{79}Br and ^{81}Br , in a ratio of ~1:1. Thus, when the molecular ion consists of two peaks (M and M + 2) in a 1:1 ratio, a Br atom is present.

www.anilmishra.name

56

Alkyl Halides

the M + 2 Peak

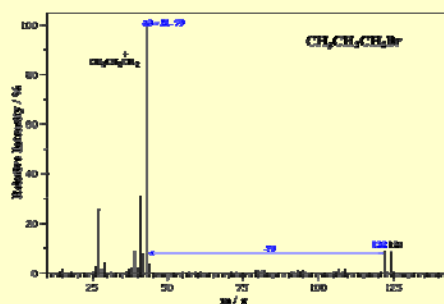


www.anilmishra.name

57

Alkyl Halides

the M + 2 Peak



www.anilmishra.name

58

M+2 and M+1 Peaks

- Sulfur is the only other element common to organic compounds that gives a significant M + 2 peak

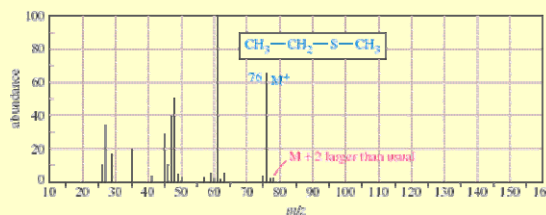
$^{32}\text{S} = 95.02\%$ and $^{34}\text{S} = 4.21\%$

- Because M + 1 peaks are relatively low in intensity compared to the molecular ion and often difficult to measure with any precision, they are generally not useful for accurate determinations of molecular weight

www.anilmishra.name

59

Mass Spectrum with Sulfur



www.anilmishra.name

60

Nitrogen Rule

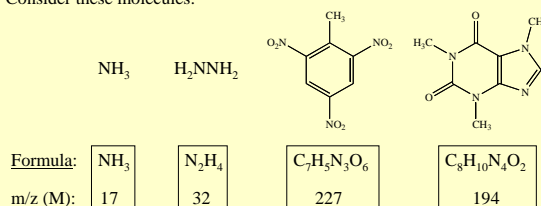
- If a compound has
 - **Zero or an even number** of nitrogen atoms, its molecular ion will have an **even *m/z*** value
 - An **odd** number of nitrogen atoms, its molecular ion will have an **odd *m/z*** value

www.anilmishra.name

61

How Many Nitrogen Atoms?

Consider these molecules:



Conclusion

- When *m/z* (M) = **even**, number of N in formula is **even**
- When *m/z* (M) = **odd**, number of N in formula is **odd** } **The Nitrogen Rule**

www.anilmishra.name

62

Nitrogen Rule

- If the molecular mass of an unknown compound to the nearest integer value is an odd number, the compound contains an odd number of nitrogens in its molecular formula.
- Correspondingly, if the molecular mass is an even number, the compound contains zero or an even number of nitrogens in its molecular formula.
- This rule results from nitrogen having a valence of three and an even atomic mass.
 - Consistent with the nitrogen rule, a correct molecular formula for a molecule with an odd molecular mass in the nearest integer value will have the sum of the number of hydrogens plus halides as an odd number.
- Correspondingly, a correct molecular formula for a molecule with an even molecular mass will have the sum of the number of hydrogens plus halides as an even number.

www.anilmishra.name

63

Nitrogen Rule

Examples

- The molecular ion for aminoethane (ethylamine), $[\text{CH}_3\text{CH}_2\text{NH}_2]^+$, is *m/z*=45 amu, an odd number; the number of hydrogens is five, also an odd number.
- The molecular ion for 1,2-diaminoethane, $[\text{NH}_2\text{CH}_2\text{CH}_2\text{NH}_2]^+$, is *m/z*= 60 amu, an even number; the number of hydrogens is eight, also an even number.
 - if we know a compound is free of nitrogen and gives an ion at *m/z*=201, then that peak cannot be the molecular ion.

www.anilmishra.name

64

Fragmentation of M

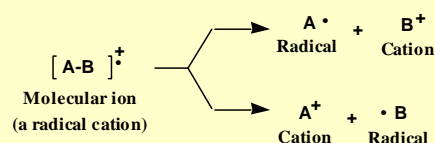
- To attain high efficiency of molecular ion formation and give reproducible mass spectra, it is common to use electrons with energies of approximately 70 eV [6750 kJ (1600 kcal)/mol]
 - this energy is sufficient not only to dislodge one or more electrons from a molecule, but also to cause extensive fragmentation
 - these fragments may be unstable as well and, in turn, break apart to even smaller fragments.

www.anilmishra.name

65

Fragmentation of M

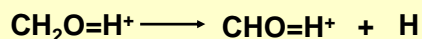
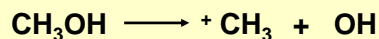
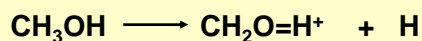
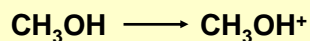
- Fragmentation of a molecular ion, M, produces a radical and a cation
 - only the cation is detected by MS



www.anilmishra.name

66

Fragmentation of CH₃OH

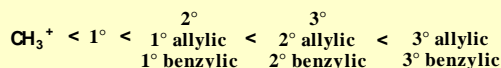


www.anilmishra.name

67

Fragmentation of M

- A great deal of the chemistry of ion fragmentation can be understood in terms of the formation and relative stabilities of carbocations in solution
 - where fragmentation occurs to form new cations, the mode that gives the most stable cation is favored
 - the probability of fragmentation to form new carbocations increases in the order



www.anilmishra.name

68

Different Ionization Methods

- Electron Impact (EI - Hard method)
 - small molecules, 1-1000 Daltons, structure
- Fast Atom Bombardment (FAB - Semi-hard)
 - peptides, sugars, up to 6000 Daltons
- Electrospray Ionization (ESI - Soft)
 - peptides, proteins, up to 200,000 Daltons
- Matrix Assisted Laser Desorption (MALDI-Soft)
 - peptides, proteins, DNA, up to 500 kD

www.anilmishra.name

69

Electron Impact Ionization

- Electron Impact (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.

www.anilmishra.name

70

Electron Impact Ionization

(low picomole)

Advantages

- Well-Established
- Fragmentation Libraries
- No Suppression
- Insoluble Samples
- Interface to GC
- Non-Polar Samples

Disadvantages

- Parent Identification
- Need Volatile Sample
- Need Thermal Stability
- No Interface to LC
- Low Mass Compounds (<1000 amu)
- Solids Probe Requires Skilled Operator

www.anilmishra.name

71

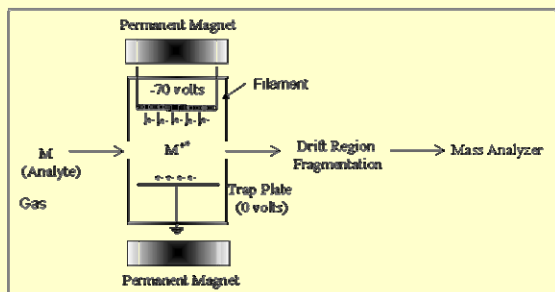
Electron Impact Ionization

- Sample introduced into instrument by heating it until it evaporates
- Gas phase sample is bombarded with electrons coming from rhenium or tungsten filament (energy = 70 eV)
- Molecule is "shattered" into fragments (70 eV >> 5 eV bonds)
- Fragments sent to mass analyzer

www.anilmishra.name

72

Electron Impact Ionization

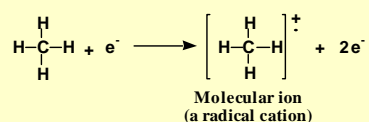


www.anilmishra.name

73

Electron Impact Ionization

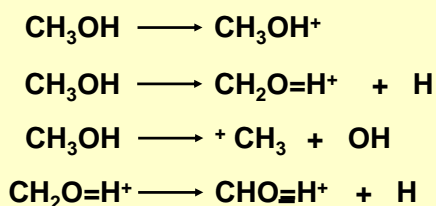
- Electron Ionization MS
 - in the ionization chamber, the sample is bombarded with a beam of high-energy electrons
 - collisions between these electrons and the sample result in loss of electrons from sample molecules and formation of positive ions



www.anilmishra.name

74

EI Fragmentation of CH₃OH

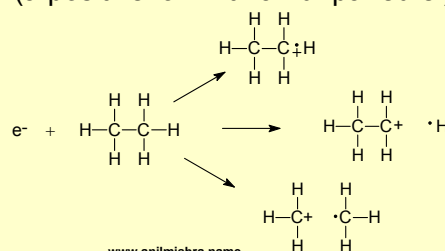


www.anilmishra.name

75

Electron Impact Ionization

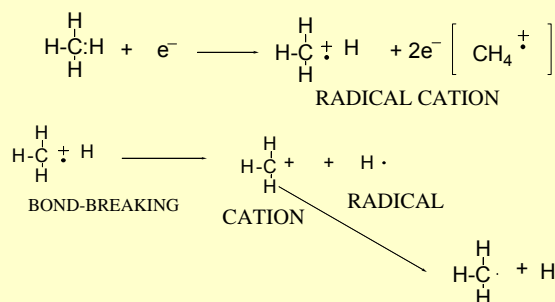
- A high-energy electron can dislodge an electron from a bond, creating a radical cation (a positive ion with an unpaired e⁻).



www.anilmishra.name

76

ELECTRON IMPACT

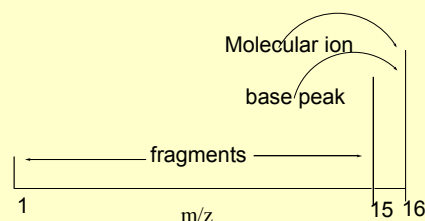


ONLY CATIONS ARE CARRIED TO DETECTOR

www.anilmishra.name

77

MS of METHANE



mass measurement takes about 20 μsecs
many fragmentations occur

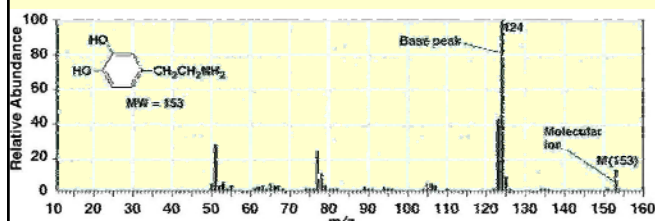
So what does a typical ms looks like????

www.anilmishra.name

78

MS of dopamine

- a partial MS of dopamine showing all peaks with intensity equal to or greater than 0.5% of base peak



www.anilmishra.name

79

Chemical Ionization

- EI is not appropriate for some molecules (it causes too much fragmentation)
- Instead, ionize a reagent gas (by EI) then react it with a analyte molecules
- Typically use methane or ammonia for reagent gas

www.anilmishra.name

80

Chemical Ionization

- CI is a "soft" ionization technique
 - Leaves the molecular ion intact
- Ionization occurs due to collisions of ionized gases with the target analyte

www.anilmishra.name

81

Chemical Ionization

- CI is easiest to envisage as a two stage process.
- In the first step a reagent gas at relatively high pressure (~ 1 Torr) is subjected to electron impact ionization.
- The products of the EI process at high pressure can be complex mixtures of ions, radicals and neutrals, but some gases produce major products that are favorable for the CI process.

www.anilmishra.name

82

Chemical Ionization

- In a CI experiment, ions are produced through the collision of the analyte with ions of a reagent gas that are present in the ion source.
- Some common reagent gases include:
 - Methane
 - Ammonia
 - Isobutane
- Inside the ion source, the reagent gas is present in large excess compared to the analyte.
- Electrons entering the source will preferentially ionize the reagent gas.
- The resultant collisions with other reagent gas molecules will create ionization plasma.
- Positive and negative ions of the analyte are formed by reactions with this plasma

www.anilmishra.name

83

CI: Form Reagent Ions First

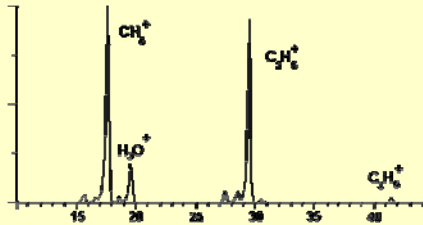
- For Example - Methane CI
 1. Electron ionization of CH₄:
 - $\text{CH}_4 + e^- \rightarrow \text{CH}_4^+ + 2e^-$
 - Fragmentation forms CH_3^+ , CH_2^+ , CH^+
 2. Ion-molecule reactions create stable reagent ions:
 - $\text{CH}_4^+ + \text{CH}_4 \rightarrow \text{CH}_3 + \text{CH}_5^+$
 - $\text{CH}_3^+ + \text{CH}_4 \rightarrow \text{H}_2 + \text{C}_2\text{H}_5^+$
 - CH_5^+ and C_2H_5^+ are the dominant methane CI reagent ions

www.anilmishra.name

84

Methane CI Reagent Ions

- Ions at m/z 17, 29, and 41 are from methane;
- H_3O^+ is also formed from water vapor in the vacuum system



www.anilmishra.name

85

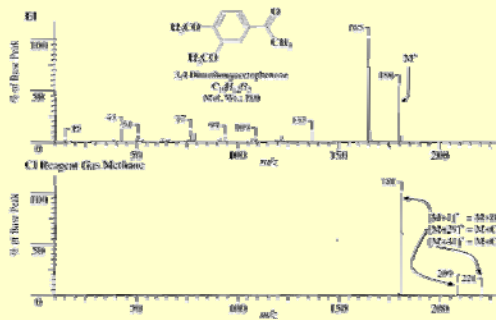
Reagent Ions React with Analytes

- Several Types of Reactions May Occur
 - Form Pseudomolecular Ions (M+1)
 - $\text{CH}_5^+ + \text{M} \rightarrow \text{CH}_4 + \text{MH}^+$
 - M+1 Ions Can Fragment Further to Produce a Complex CI Mass Spectrum
 - Form Adduct Ions
 - $\text{C}_2\text{H}_5^+ + \text{M} \rightarrow [\text{M} + \text{C}_2\text{H}_5]^+$ M+29 Adduct
 - $\text{C}_3\text{H}_7^+ + \text{M} \rightarrow [\text{M} + \text{C}_3\text{H}_7]^+$ M+41 Adduct
 - Molecular Ion by Charge Transfer
 - $\text{CH}_4^+ + \text{M} \rightarrow \text{M}^+ + \text{CH}_4$
 - Hydride Abstraction (M-1)
 - $\text{C}_3\text{H}_5^+ + \text{M} \rightarrow \text{C}_3\text{H}_6 + [\text{M}-\text{H}]^+$
 - » Common for saturated hydrocarbons

www.anilmishra.name

86

Chemical Ionization

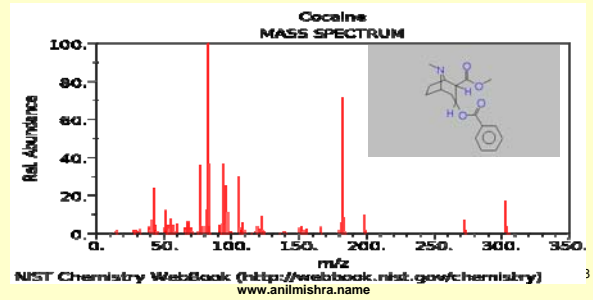


www.anilmishra.name

87

EI Spectrum of Cocaine

- Extensive Fragmentation
- Molecular Ion is Weak at m/z 303

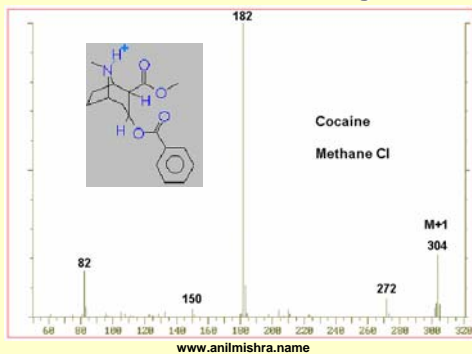


NIST Chemistry WebBook (<http://webbook.nist.gov/chemistry>)

www.anilmishra.name

88

Methane CI of Cocaine Pseudo molecular Ion and Fragment Ions

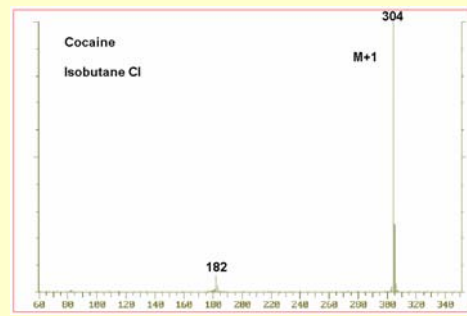


www.anilmishra.name

89

Isobutane CI of Cocaine

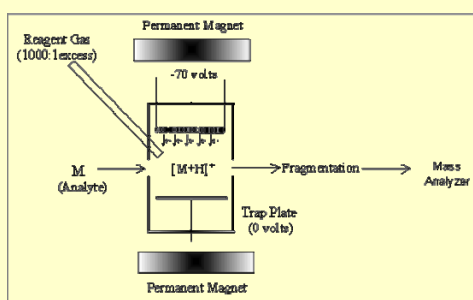
- Soft Reagent - Less Fragmentation



www.anilmishra.name

90

Chemical Ionization



www.anilmishra.name

91

Chemical Ionization

(low picomole)

Advantages

- Parent Ion
- Interface to GC
- Insoluble Samples

Disadvantages

- No Fragment Library
- Need Volatile Sample
- Need Thermal Stability
- Quantitation Difficult
- Low Mass Compounds (<1000 amu)
- Solids Probe Requires Skilled Operator

www.anilmishra.name

92

Ionization Methods in MS

- **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.

www.anilmishra.name

93

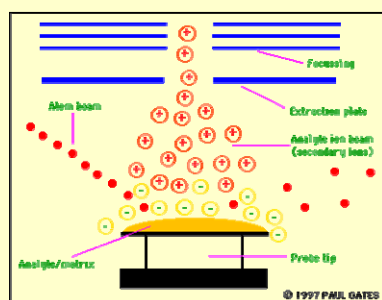
Fast Atom Bombardment

- Ionization method whereby a solution prepared by dissolving sample compounds in a liquid matrix, such as glycerin, is spread on a metallic target.
- The target is then bombarded by neutral atoms (Ar, Xe, Cs) accelerated to 2kV ~ 30kV to generate ions.
- The method is further categorized as solid FAB when the sample is a solid, matrix FAB when a matrix solution is used, and gas-phase FAB when the sample is a gas.

www.anilmishra.name

94

Fast Atom Bombardment



www.anilmishra.name

95

Fast Atom Bombardment

(nanomole)

Advantages

- Parent Ion
- High Mass Compounds (10,000 amu)
- Thermally Labile Compounds (R.T.)

Disadvantages

- No Fragment Library
- Solubility in Matrix (MNBA, Glycerol)
- Quantitation Difficult
- Needs Highly Skilled Operator
- Relatively Low Sensitivity

www.anilmishra.name

96

MALDI

- Identity of suitable matrix compounds is determined using specific molecular design considerations
 - fairly low molecular weight (to allow facile vaporization)
 - large enough (with a high enough vapor pressure) not to evaporate during sample preparation or while standing in the spectrometer
 - are acidic / act as a proton source to encourage ionization of the analyte
 - have strong absorption in the UV so they rapidly and efficiently absorb the laser irradiation
 - functionalized with polar groups - allowing use in aqueous solutions

www.anilmishra.name

103

MALDI

- MALDI is based on the bombardment of sample molecules with a laser light to bring about sample ionization.
- The sample is pre-mixed with a highly absorbing matrix compound for the most consistent and reliable results and a low concentration of sample to matrix works best.
- The matrix transforms the laser energy into excitation energy for the sample, which leads to sputtering of analyte and matrix ions from the surface of the mixture.
- In this way energy transfer is efficient and also the analyte molecules are spared excessive direct energy that may otherwise cause decomposition.
- Most commercially available MALDI mass spectrometers now have a pulsed nitrogen laser of wavelength 337 nm.

www.anilmishra.name

104

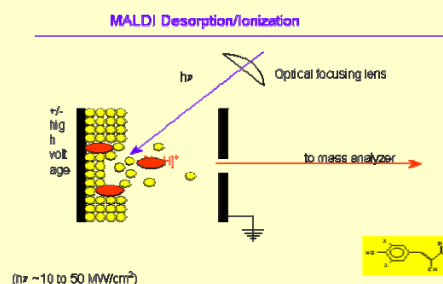
MALDI

- The sample to be analysed is dissolved in an appropriate volatile solvent, usually with a trace of trifluoroacetic acid if positive ionisation is being used, an aliquot of this removed and mixed with an equal volume of a solution containing a vast excess of a matrix.
- A range of compounds is suitable for use as matrices:
 - Sinapinic acid is a common one for protein analysis
 - Alpha-cyano-4-hydroxycinnamic acid is often used for peptide analysis.
- An aliquot of the final solution is applied to the sample target which is allowed to dry prior to insertion into the high vacuum of the mass spectrometer.
- The laser is fired, the energy arriving at the sample/matrix surface optimized, and data accumulated until a m/z spectrum of reasonable intensity has been amassed.
- The time-of-flight analyser separates ions according to their **mass(m)-to-charge(z) (m/z)** ratios by measuring the time it takes for ions to travel through a field free region known as the flight, or drift, tube. The heavier ions are slower than the lighter ones.

www.anilmishra.name

105

MALDI



www.anilmishra.name

106

MALDI

- Matrix solution is mixed with the analyte (e.g. protein-sample)
 - organic solvent allows hydrophobic molecules to dissolve
 - water allows for hydrophilic molecules to do the same
- Solution is spotted onto a MALDI plate
 - solvents vaporize, leaving only the recrystallized matrix
 - analyte molecules spread throughout the crystals in co-crystallized MALDI spot

www.anilmishra.name

107

MALDI

(low femtomole)

Advantages

- Parent Ion
- High Mass Compounds (>100,000 amu)
- Thermally Labile Compounds (R.T.)
- Easy to Operate

Disadvantages

- No Fragment Library
- Wide variety of matrices
- Quantitation Difficult

www.anilmishra.name

108

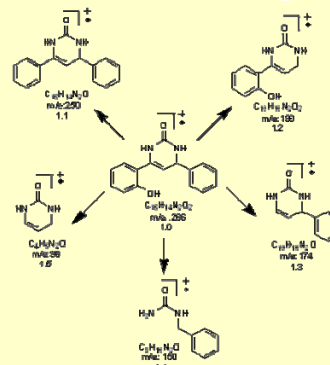
Interpretation of Mass Spectra

- Most mass spectra are not trivial to decipher due to:
 - interferences
 - complexities of fragmentation
 - (exception: some elemental analysis cases)
- Instead, MS Interpretation is problem solving, 'playing chess', or 'cracking a code'

www.anilmishra.name

109

Representation of Fragmentation



www.anilmishra.name

110

Calculation of Molecular Formula

- The "Rule of 13" as an aid to guessing a molecular Formula
 - Consider CH unit \rightarrow 13 amu
 - Take the Weight of ion, divide by 13
 - Example:
 - Molecular ion \Rightarrow 152
 - $152 / 13 = \# \text{ carbons} = 11$
 - Mass = $12 * 11 = 132$
 - therefore H = $152 - 132 = 20$
- Basic formula $C_{11}H_{20}$

www.anilmishra.name

111

Calculation of Molecular Formula

- After establishing the basic formula with only Carbon/hydrogen,
- Other element can be introduced by subtracting the proper hydrocarbon value

Hetero substitution	CH replacement	Hetero substitution	CH replacement
O	CH ₄	P	C ₂ H ₇
N	CH ₂	S	C ₂ H ₈
O+N	C ₂ H ₆	O+S	C ₄
F	CH ₇	I	C ₁₀ H ₇
Si	C ₂ H ₄	Cl, Br	(use isotopes)

www.anilmishra.name

112

Calculation of Molecular Formula

- If Oxygen is present : mass = 16 \rightarrow remove CH₄
 - If one oxygen :
 - $C_{11}H_{20} - CH_4 + O = C_{10}H_{16}O$
 - If second oxygen :
 - $C_{10}H_{16}O - CH_4 + O = C_9H_{12}O_2$
 - If third oxygen :
 - $C_9H_{12}O_2 - CH_4 + O = C_8H_8O_3$
- If Nitrogen is present : mass = 14 \rightarrow remove CH₂

www.anilmishra.name

113

Interpretation of Mass Spectra

- Select a candidate peak for the molecular ion (M^+)
- Examine spectrum for peak clusters of characteristic isotopic patterns
- Test (M^+) peak candidate by searching for other peaks correspond to reasonable losses
- Look for characteristic low-mass fragment ions
- Compare spectrum to reference spectra

www.anilmishra.name

114

Alkanes

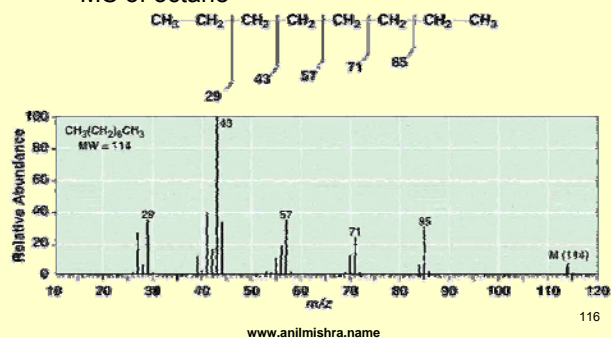
- Fragmentation tends to occur in the middle of unbranched chains rather than at the ends
- The difference in energy among allylic, benzylic, 3°, 2°, 1°, and methyl cations is much greater than the difference among comparable radicals
 - where alternative modes of fragmentation are possible, the more stable carbocation tends to form in preference to the more stable radical

www.anilmishra.name

115

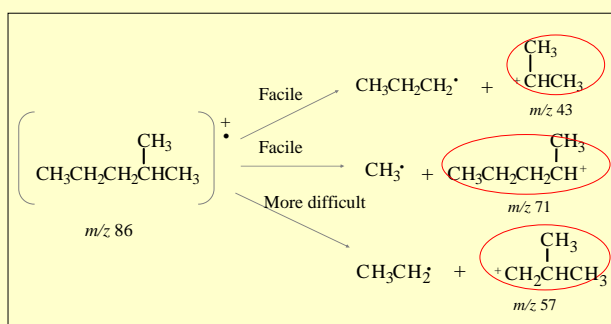
Alkanes

– MS of octane



116

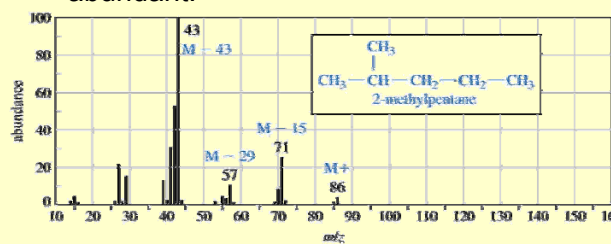
Fragmentation of 2-Methylpentane



117

Alkanes

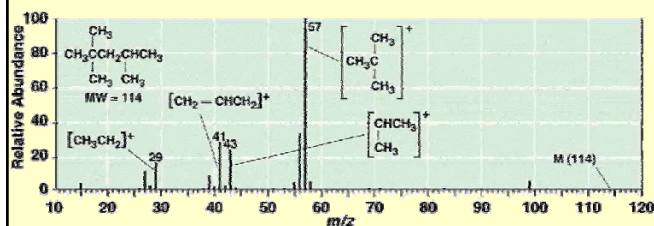
More stable carbocations will be more abundant.



118

Alkanes

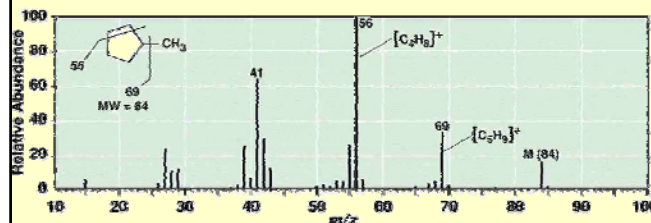
– MS of 2,2,4-trimethylpentane



119

Alkanes

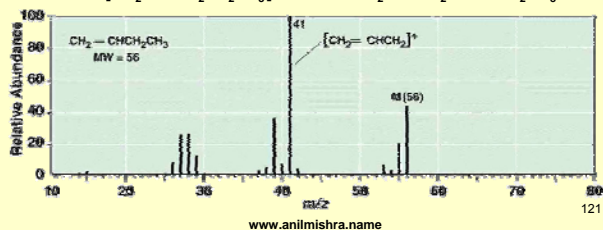
– MS of methylcyclopentane



120

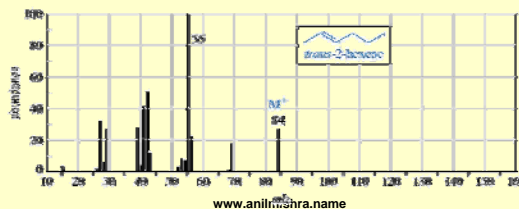
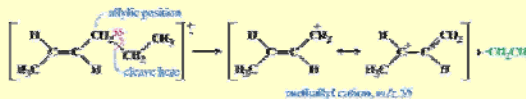
Alkenes

- Alkenes characteristically
 - show a strong molecular ion peak
 - cleave readily to form resonance-stabilized allylic cations
- $$[\text{CH}_2=\text{CHCH}_2\text{CH}_2\text{CH}_3]^+ \longrightarrow \text{CH}_2=\text{CHCH}_2^+ + \cdot\text{CH}_2\text{CH}_3$$



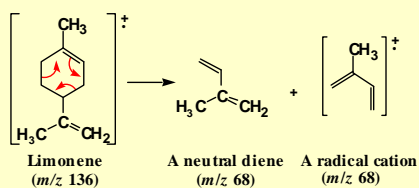
Alkenes

Resonance-stabilized cations favored.



Cyclohexenes

- Cyclohexenes give a 1,3-diene and an alkene, a process that is the reverse of a Diels-Alder reaction

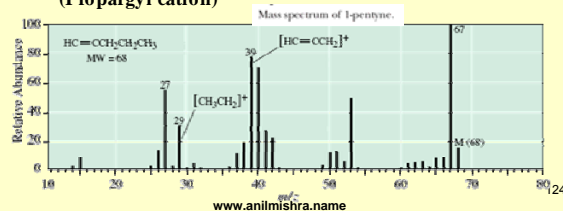
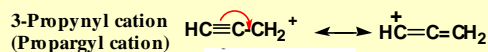


www.anilmishra.name

123

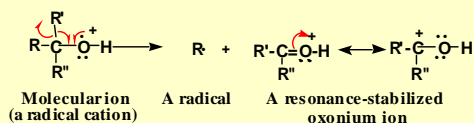
Alkynes

- Alkynes typically
 - Show a strong molecular ion peak
 - Cleave readily to form the resonance-stabilized propargyl cation or a substituted propargyl cation



Alcohols

- One of the most common fragmentation patterns of alcohols is loss of H_2O to give a peak which corresponds to M-18
- Another common pattern is loss of an alkyl group from the carbon bearing the OH to give a resonance-stabilized oxonium ion and an alkyl radical

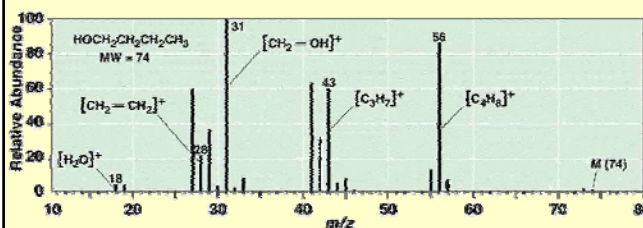


www.anilmishra.name

125

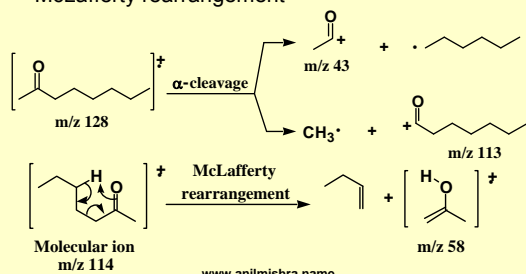
Alcohols

– MS of 1-butanol



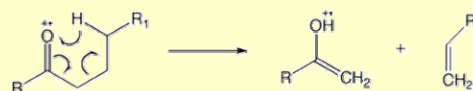
Aldehydes and Ketones

- Characteristic fragmentation patterns are
 - cleavage of a bond to the carbonyl group (α -cleavage)
 - McLafferty rearrangement



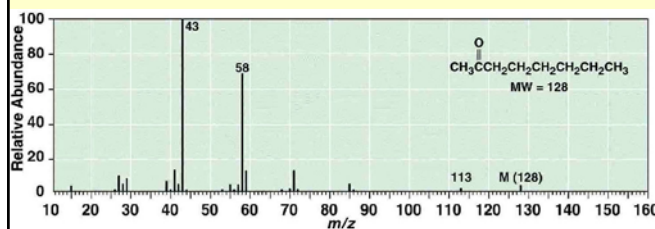
McLafferty rearrangement

- The **McLafferty rearrangement** is a phenomenon observed in mass spectrometry. Usually, it is observed that a molecule containing a keto-group undergoes β -cleavage, with the gain of the γ -hydrogen atom. This rearrangement may take place by a radical or ionic mechanism.



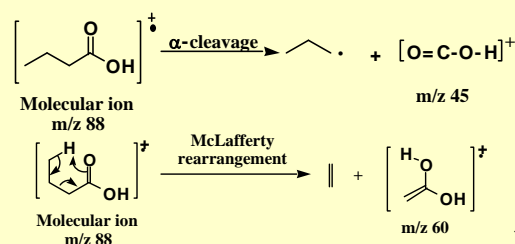
Aldehydes and Ketones

– MS of 2-octanone



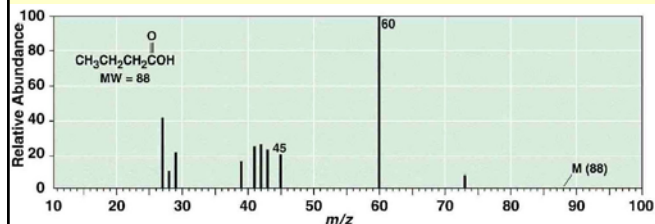
Carboxylic Acids

- Characteristic fragmentation patterns are
 - α -cleavage to give the ion $[\text{CO}_2\text{H}]^+$ of m/z 45
 - McLafferty rearrangement



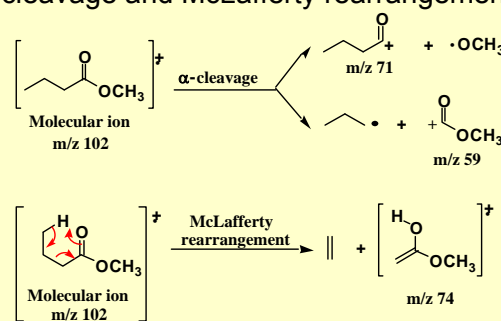
Carboxylic Acids

– MS of butanoic acid



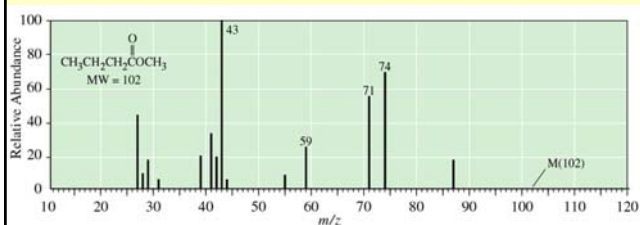
Esters

α -cleavage and McLafferty rearrangement



Esters

– MS of methyl butanoate

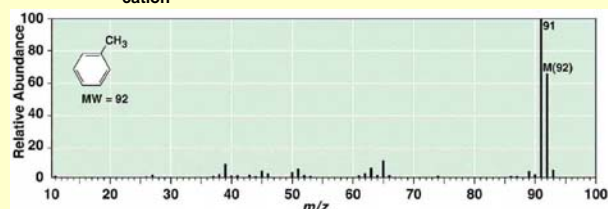
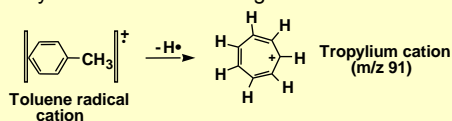


www.anilmishra.name

133

Aromatic Hydrocarbons

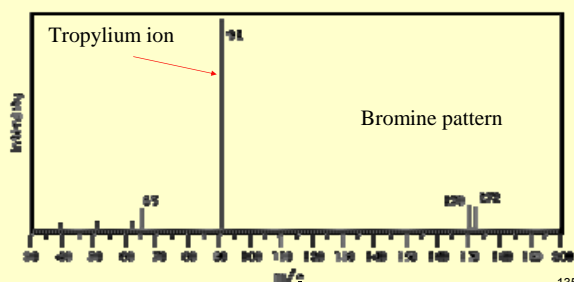
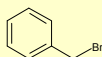
- most show an intense molecular ion peak
- most alkylbenzenes show a fragment ion of m/z 91



www.anilmishra.name

134

Aromatic Hydrocarbons

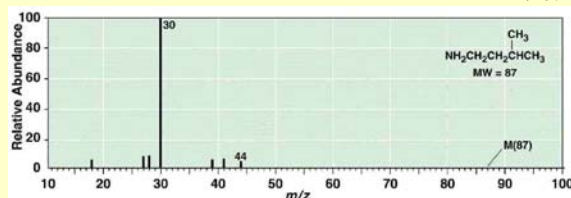
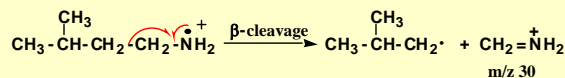


www.anilmishra.name

135

Amines

- The most characteristic fragmentation pattern of 1°, 2°, and 3° aliphatic amines is β -cleavage



www.anilmishra.name

136

Summary

Molecular Ion Peak

- The molecular ion peak in aromatic compounds is relatively much intense due to the presence of π -electron system
- Conjugated olefins show more intense molecular ion peak as compared to the corresponding non-conjugated olefins with the same number of unsaturation. Conjugated olefins are more stable than the corresponding non-conjugated olefins.
- Unsaturated compounds give more intense peak as compared to the saturated or the cyclic molecule.
- The relative abundance of the saturated hydrocarbon is more than the corresponding branched chain compound with the same number of carbon atoms.

www.anilmishra.name

137

www.anilmishra.name

138

Summary

- The substitute groups like -OH, -OR, -NH₂, etc., which lower the ionization potential increase the relative abundance in case of aromatic compounds.
- Also groups like -NO₂, -CN, etc., which increase ionization potential, decrease the relative abundance of the aromatic compounds.
- Absence of molecular ion peak in the mass spectrum means that the compound under examination is highly branched or tertiary alcohols. Primary and secondary alcohols give very small molecular ion peaks.
- In case of Chloro or Bromo compounds, isotope peaks are also formed along with the molecular ion peak. In case of Bromo compounds, M⁺ & (M⁺+2) peaks are formed in the intensity ratio 1:3.

Summary

Fragment ion Peak

- When potential and energy is given to the molecular ion during electron impact, further cleavage takes place and ions of lower mass number (fragment ions) are produced which gives the fragment peak.
 $M^+ \rightarrow M^+1 + M^+2$
- Many of the peaks in the mass spectrum are due to fragment ions. Fragment peaks in the spectrum give valuable information regarding molecular structure, because fragmentation is specific to the structure of the molecule.
- The greater the fragmentation of the molecular ion or parent ion, the greater is the loss in its intensity.
 - *The probability of fragmentation decreases in the following order:*
ALCOHOLS > BRANCHED HYDROCARBONS > CARBOXYLIC ACIDS > ETHERS > ESTERS > AMINES > KETONS > THIOLS > STRAIGHT CHAIN HYDROCARBONS > SULPHIDES > ALICYCLIC COMPOUNDS > CONJUGATED DIENES > AROMATIC COMPOUNDS.
- Fragmentation is initiated by electron impact. Only a small part of the driving force for fragmentation is energy transferred as the result of the impact.