

## Review Article on: Mass Spectrometry

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### ABSTRACT:

Mass spectroscopy is an analytical technique that identifies biomolecules or proteins present in biological samples and is also useful for studies on protein-protein interactions. The basic principle involves the fragmentation of a compound or molecule into charged species, which are accelerated, deflected, and finally focused on a detector according to their mass and charge ratio. Ion deflection is based on charge, mass, and velocity, ions separation is based on mass to charge ( $m/z$ ) ratio, and detection is proportional to abundance of ions. This chapter further discusses the anomalies involved in the raw data acquisition, and further processing and interpreting the mass spectrum. For data acquisition, peaks are identified based on preset values from a survey scan and are further taken in for MS/MS analysis. Peptide fragment ion mass is determined for each peak obtained from MS/MS spectrum and subjected to peptide scoring through database search. Protein is identified from the correctly identified peptide scoring.

**Keywords:** Mass, Charge, Velocity, Ion, Fragment, Protein.

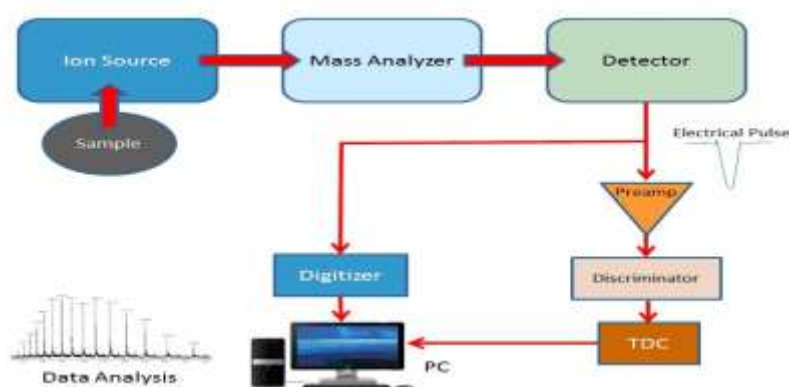
### I. INTROCTION:

- Analytical tool measuring of molecular weight of sample.
- Only picomolar concentrations required.
- With an high accuracy and within 5 ppm of std.error for small organic molecules.
- It can detect amino acid substitutions/post translational modification in biological systems.
- In this technique,molecules are bombarded with a beam of energetic electrons.
- The molecules are ionized and broken up into many fragments.
- Each kind of ion has a particular ratio of mass to charge.
- That is  $m/e$  ratio (value).
- $m/e$  ratio is simply the molecular mass of the ion.

### Principle:

- ❑ Mass spectroscopy is most accurate method for determining the molecular mass of the compound and its elemental composition.
- ❑ In this technique, molecules are bombarded with a beam of energetic electrons.
- ❑ The molecules are ionized and broken up into fragments,some of which of positive ions.
- ❑ Each kind of ion has particular ratio of mass charges that is  $m/e$  ratio (value).

### Instrumentation



### Intel system /sample handling system

- ❖ In mass spectrometer should have a vapoursample and to ensure that the sample enters the ionization chamber.
- ❖ Sample is converted into gaseous state.
- ❖ Inlet system also heated.
- ❖ Less volatile sample may be heated in the flask before introduce into ionization chamber.
- ❖ In this case,it should be remembered that no sample is thermally stable .
- ❖ Less volatile liquids and solids may be vapourised directly within the ionization chamber.
- ❖ Only a few percent of this will actually enter the ionization chamber and only about 0.1 % is ionized in the ionosotion chamber.

### Ion source

- ❖ From the inlet system sample introduce in the ionization chamber.
- ❖ Beam of electrons is put across the molecules of sample.molecule become ionized.
- ❖ Ion sources :
  - 1.Knudsen cell
  - 2.Surface ionization
  - 3.Spark source ionization
  - 4.Chemical ionization

#### 1.Knudsen cell

- The Knudsen cell employs the thermal and electron bombardment excitation.
- The cell is heated by either radiation or electron bombardment to attain temperature continuously adjustable from ambient to 2500 degree celcius.

#### 2.Surface ionization.

- In this method solid sample to be studied by mass spectrometer is coated on a ribbon filament.
- When filament is heated to 2000 degree celcius.
- There are chances of positive ion may evaporate instead of neutral molecule.

- This techquie is very useful for inorganic material.

### 3.Spark source ionization.

- In this positive ion beam is very broad and therefore it is controlled by the ion optic.
- Advantages of spark source,its detection sensitivity is very high.

### 4. Chemical ionization

- In is techquie a reaction gas like methane is introduced along the sample to be analysed.
- Reaction gas (methane) undergoes ionization to produce ions which react further with neutral molecules to form product.
- The products so formed are chemical reactive species and can interact with the sample molecules to form positive ions.

### Magnetic Field

- As The accelerated particles from the electric field enter the magnetic field.
- The force of the magnetic field requires them to move in a curved path.

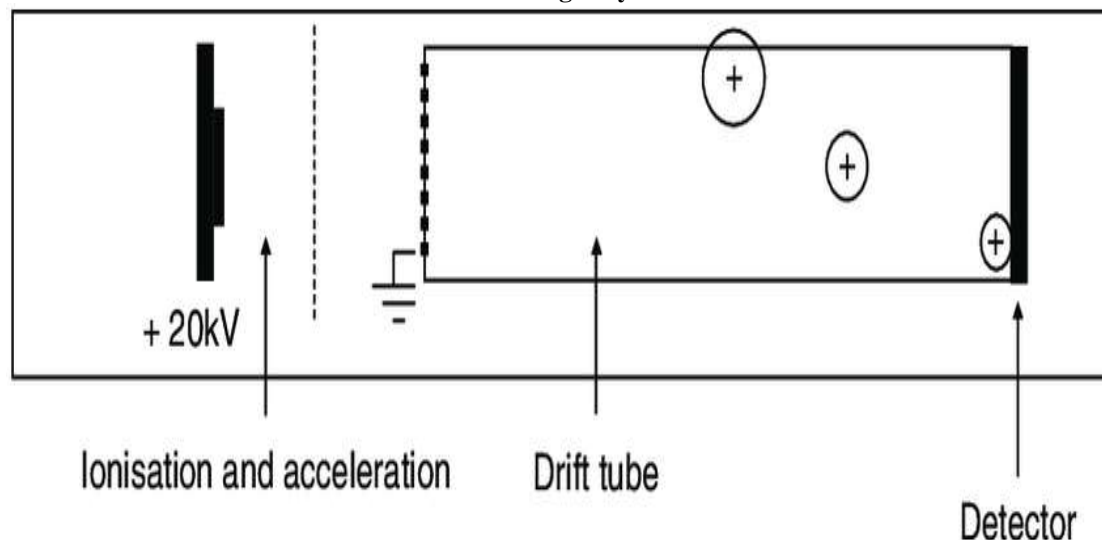
### Analyzer:

- In that separate the ions according to their masses.
- It must have a high rate of transmission ions.
- It should have high resolution.

### Detector:

1. The ion collection system is measures the relative abundance of ion fragments of each mass.
2. Several types of detectors are available for mass spectrometers. The detector used for most routine experiments is the electron multiplier.
3. Another type of detector is photographic plates coated with a silver bromide emulsion, it is sensitive to energetic ions.
4. A photographic plate can give a higher resolution than an electrical detector.

### Time of Flight System



#### Rule of Interpretation of mass spectra

1. The exact molecular weight
2. The Isotop effects.
3. Nitrogen Rule.
4. Ring rule

#### 1. The exact molecular weight

- Mass spectrometer can be used to determine the molecular weight of pure compound from the identification of the parent peak.
- From the molecular weight one can determine molecular formula.

#### 2. Isotop effect

- Compounds containing halogens (especially chlorine and bromine) can produce very distinct isotope peaks.
- Even when compounds only contain elements with less intense isotope peaks (carbon or oxygen), the distribution of these peaks can be used to assign the spectrum to the correct compound.

#### 3. Nitrogen rule

1. The nitrogen rule states that organic molecules that contain hydrogen, carbon, nitrogen, oxygen, silicon, phosphorus, sulfur, or the halogens have an odd nominal mass if they have an odd number of nitrogen atoms or an even mass if they have an even number of nitrogen atoms present.
2. The nitrogen rule is true for structures in which all of the atoms in the molecule have a number of covalent bonds equal to their standard valency, counting each sigma bond and pi bond as a separate covalent bond.

#### 4. Ring Rule

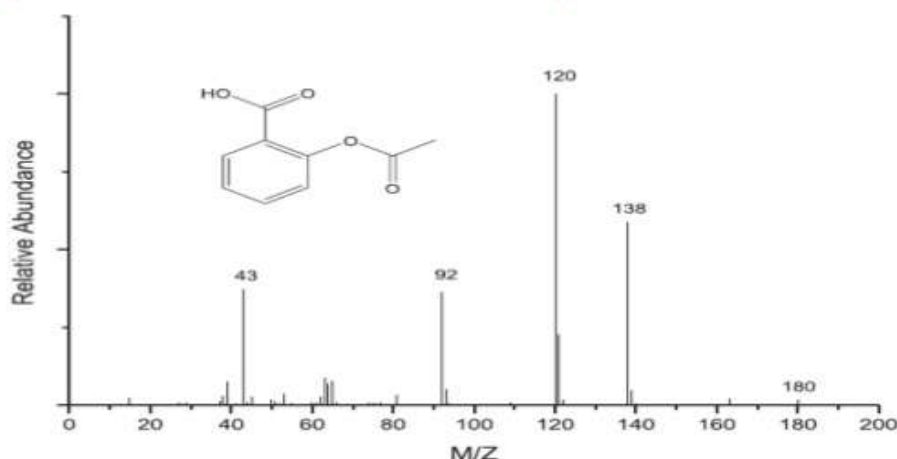
- molecules containing only carbon, hydrogen, halogens, nitrogen, and oxygen follow the formula.
- $\text{Ring} + \text{pi Bonds} = \text{u} = \text{c} - \text{H}/2 - \text{X}/2 + \text{N}/2 + 1$
- where C is the number of carbons, H is the number of hydrogens, X is the number of halogens, and N is the number of nitrogen.

### Interpretation of mass spectra

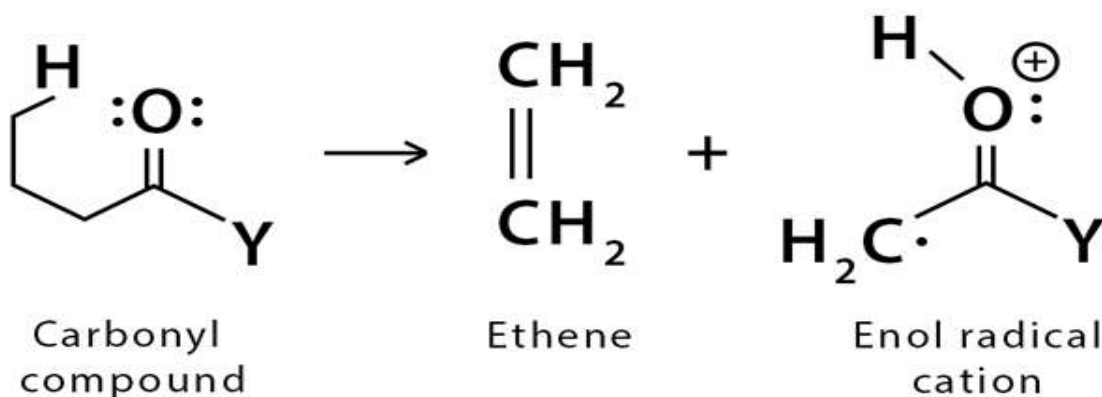
## ASPIRIN (or) SALICYLICACID(m.wt-180)

Mass Spectrum

Empirical Formula C<sub>9</sub>H<sub>8</sub>O<sub>4</sub>



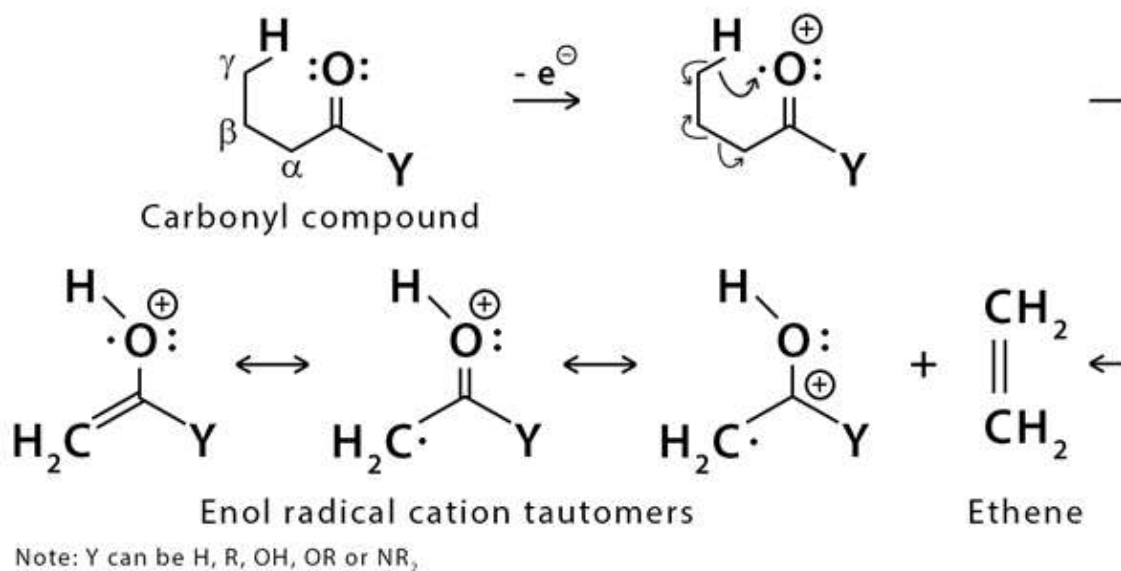
## McLafferty Rearrangement



Note: Y can be H, R, OH, OR or NR<sub>2</sub>

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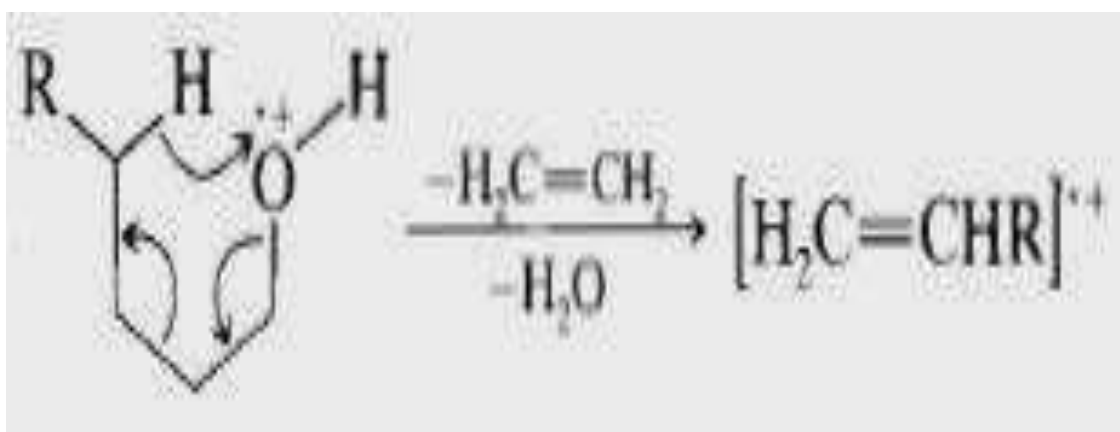
## Mechanism of McLafferty Rearrangement



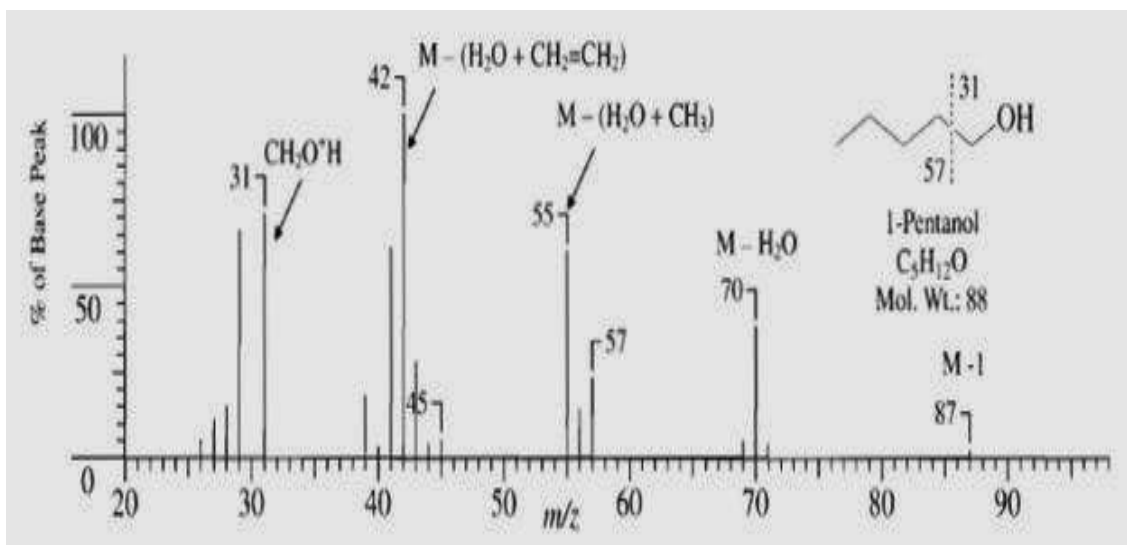
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### Fragmentation of alcohol

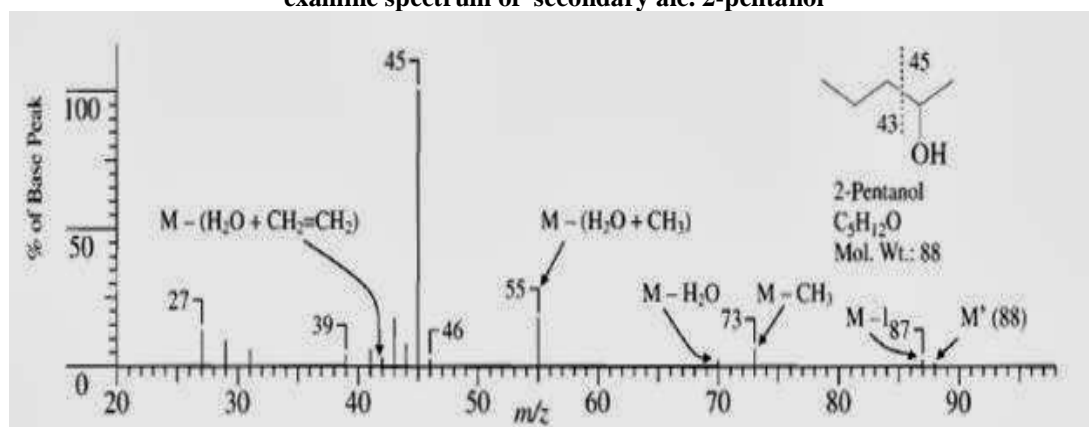
- Molecular ion peak of primary and secondary alcohol is quite small and for tertiary alcohol is undetectable.
- Molecular ion peak is formed by the removal of one electron from the lone pairs on the oxygen atom of primary and secondary alcohol.
- Cleavage of C-C bond next to the oxygen ( $\alpha$  cleavage) is of general occurrence.
- Thus primary alcohol show a prominent peak resulting from oxoniumion ( $m/z$  31)
- secondary alcohol show a prominent peak resulting from ( $m/z$  45, 59, 73 etc)
- tertiary alcohol show a prominent peak resulting from ( $m/z$  59, 73, 87 etc)
- A distinct and sometimes prominent peak can usually be found at  $M-18$  from loss of  $\text{H}_2\text{O}$ .
- In primary alcohol elimination of water, together with elimination of alkene, accounts for the presence of a peak at  $M-(\text{alkene} + \text{H}_2\text{O})$  ie ( $m/z$  46, 74, 102 etc).



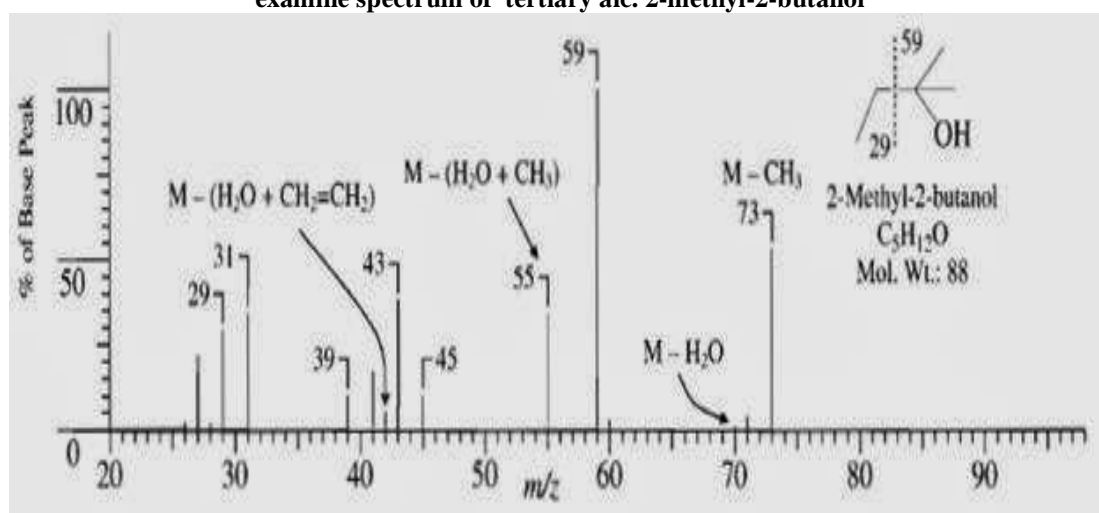
examine spectrum of 1-pentanol



examine spectrum of secondary alc. 2-pentanol



examine spectrum of tertiary alc. 2-methyl-2-butanol



### Application

Mass spectrometry, coupled with different chromatography techniques (GC, IC and LC), can

routinely analyze hundreds of compounds in a single sample and run, making it a very powerful,

high-throughput process for metabolomics and lipidomics

- Environmental toxins in air, water and soil are routinely analyzed via mass spectrometry because this technology offers unprecedented resolution and detection of trace contaminants.
  
- **Pharmaceuticals and biopharmaceuticals must undergo rigorous processes of testing, production and verification**
- Molecular Mass Determination
- Isotopic abundance
- Isotopic dilution method
- Quantitative analysis of mixtures
- Distinction between cis and trans isomers
- Evaluation of heat of sublimation
- Bonding
- Determination of bond dissociation energies
- Reaction kinetics
- Impurity detection
- Identification of an unknown compound
- Characterisation of polymers.

## II. CONCLUSION

- Advanced mass spectrometry methods can unambiguously identify more than 2,000 proteins in a single proteome. a single proteome.
- Complex mixture analysis is not limited by sensitivity but by a combination of dynamic range (high abundance peptides preventing sequencing of low abundance ones) and by effective sequencing speed.

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