Mass Spectrometry & Purification Techniques

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Ionization Techniques in Mass Spectrometry: A Review

Sharad Medhe*

Department of Chemistry, Educational Institute, Dayalbagh, Agra-282005, India

***Corresponding author:** Sharad Medhe, Department of Chemistry, Educational Institute, Dayalbagh, Agra-282005, India, Tel: 0562 280 1545; E-mail: sharadmedhe@gmail.com

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Abstract

Mass Spectrometry- It is an analytical technique that generates charged particles in the form of ions from the substance to be analysed to measure its mass to charge ratio. Ion Source plays an important role for generation of charged ions which further travels through analyser and ends at detector. The production of intact molecular ions can be achieved under adequate experimental conditions, with minimal fragmentation known as soft ionization method. Neutral species either loss or gain of charge to generates ions. This paper covers an information about various type of mass ion Source (Electron impact, Chemical ionization and field ionization, Desorption-Field desorption, Electro spray ionization, matrix assisted desorption ionization, Plasma desorption) are in use for mass spectroscopy. Ions get affected by change in electrical, magnetic and radiofrequency effect as they containing either positive or negative charges this leads to better resolution of ions.

Keywords: Mass spectroscopy; Ion source, Ionization, Desorption

Introduction

An analytical technique which defines the chemical composition of compound or sample with reference to their mass to charge ratio is known as mass spectroscopy [1]. After ionization of sample molecule it gets converted to charged species which is permitted to enter in mass spectrometer. This charged species is in the gaseous form used for analysis, fragmentation and detection [2]. Proteins or peptides or oligosaccharide are the common compounds that get analysed by mass spectrometer. Mass spectrometer instrument get separates into six divisions (Table 1).

Sr. No.	Division of Mass spectrometer	Type
1	Inlet system	Solid, Liquid and Gas
2	Ion Source	Gas Phase-Electron impact, Chemical ionization and field ionization
		Desorption-Field desorption, Electro spray ionization, matrix assisted desorption ionization, Plasma desorption, Fast atom bombardment, Secondary ion mass spectrometry and thermo spray ionization
3	Mass Analyser	Quadrupole, TOF, Ion Trap, FTICR
4	Ion Detector	Electron Multiplier, Faraday cups, Photographic plates, Scintillation counter, channel electron Multipliers, Resistive anode Encoder image Detectors, High mass detection detectors
		Conversion Dynodes- Helium leak detectors, Advanced detectors
		Cryogenic Detectors- Multi Pixel Photon counter
		Other Detectors-TQD Tandem Quadrupole MS Detector, Photonics BI Polar Maldi TOf Detector, Flexar SQ 300 MS Detector
5	Vacuum system	-
6	Recorder	-

Table 1: Divisions of mass Spectrometer.

Ionization source is a mechanical device which allows ionization. Various methods of ion generation are protonation (Positive ions), Cationization (Positive ions), Deprotonation (Negative ions), Transfer of charged molecules to gas phase (which generate positive and negative ions), Electron ejection (Positive ions) and electron capture (for the generation of negative ions). The production of intact molecular ions can be achieved under adequate experimental conditions, with minimal fragmentation known as soft ionization method. Ion source in mass spectroscopy plays a vital role for the generation of ions.

As neutral species either loss or gain the charges to convert in to ionic form [3]. The generated ion species forwarded into mass analyzer system of the mass spectrometer and detection occurs on the basis of mass to charge ratio.

Ion Source

Gas phase

Electron Impact: Electron Impact (EI) is the simple and very much common method of ionisation. During world war II Arthur Jeffrey Dempster an American-Canadian physicist developed and used the term Electron Impact.

The introduction of sample in ion source by solid inlet or a gas Chromatography column. The most important thing about analysed sample is its entry is in gaseous phase for that heating of solid probe and source is required.

Heated filament made up of either Tungsten or Rhenium metal collides sample molecules which are in gaseous form which generates positively charged ions after removal of electron. The generated ion concurs to the molecular mass of analyzing sample [4]. The original molecule gets converted into fragments by this energetic ionisation technique.

For the purpose of focused beam the ions travel through source to a series of slits. This ionic beam passes through a flight tube situated in between the poles of an electromagnet, where the separation of ions takes place on the basis of their mass/charge ratio after scanning in magnetic field. The plot of intensity versus mass/charge ratio is based on the ions are detected by electron or photomultipliers which further represents the analysed sample in the form of mass spectrum.

In Electron Impact thermally volatile and stable compounds of mass range 500 Da are introduced. A beam of electron at a pressure of greater than 10-3 Torr passes through a gas phase sample and collides with neutral analyte molecules (M) which produces charged ion or a fragment ion. 70 eV electron energy is required to form fragment ions. By collecting positive ions in focusing plates they are passed to mass analyzer (Figure 1). Although EI has some limitation, EI causes extensive fragmentation so that the molecular ion is not observed for many compounds.

Figure 1: Electron Impact source by Kore Technology Ltd UK.

Chemical ionization: Chemical ionization (CI) is a soft ionization technique which is useful when no molecular ion is observed in Electron Impact mass spectrum. Burnaby Munson and Frank H Field in 1966 [5] introduced chemical ionization for first time. The regular difference of this technique is Charge-exchange chemical ionization and atmospheric-pressure chemical ionization (APCI). Comparatively lower energy requires to this process than Electron Ionizations. Sometimes a lower energy corresponds to no fragmentation and or usually a simpler spectrum. As softer ionization technique it gives less fragmentation and easier to find molecular ions (Figure 2a).

It involves the ionization of methane a reagent gas at comparatively high pressure (~1 mbar) in a common electron impact source. The gas phase reaction processes occurs by colliding produced reagent ion gas with the analytic molecule where the proton transfer process takes place. Generally methane, Isobutene and ammonia are used as reagent gases.

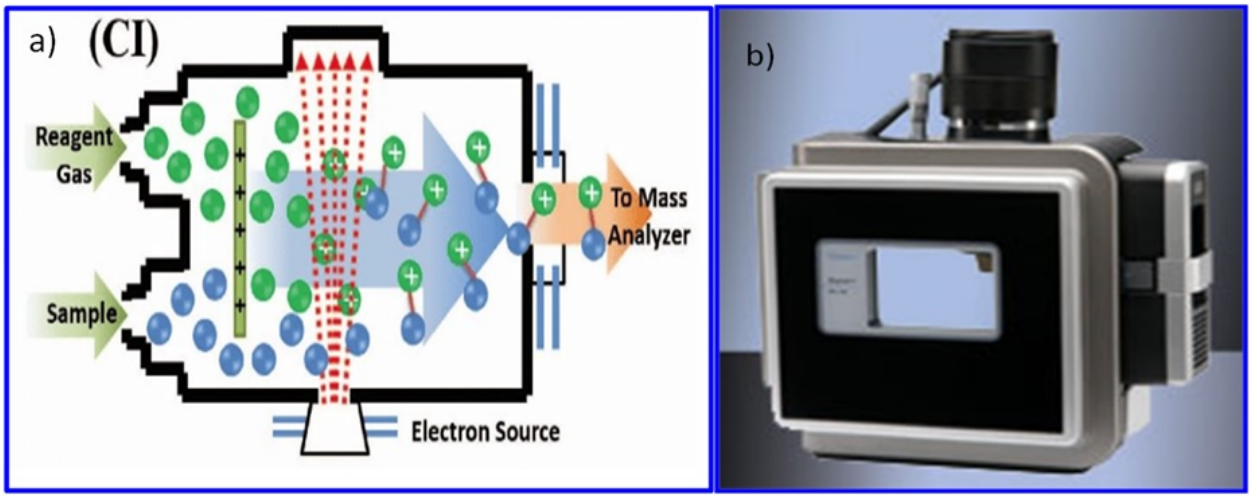

Figure 2: a) Chemical ionization [6]. b) Atmospheric Pressure Chemical ionization Source by Waters UK.

Reagent gas is directly releases in to ionization chamber and electrons from filament ionize the reagent gas. Oxygen and hydrogen are used in negative ion chemical ionization [7]. In APCI the charged ions are generated using voltage 2 to 4 KV by using corona discharge needle. Wherein chemical ionization it is produced by applying 70 eV through filament (Figure 2b). There is a disadvantage for this technique. As there is no fragmentation it is less informative.

Field Ionization: In this by applying very strong electric fields (20 KV) to the emitters devised by thin tungsten wire to heat the samples which volatilize it onto ionization surface to generate ions from gas phase molecules in a vacuum. For effective ionization the anode to be activated by growing micro needles or whiskers by applying high potential gradients. The length of whiskers are 10 micrometers and of diameter greater than 1 micrometer [8].

The valence electrons of organic molecule have been removed by whisker through quantum mechanical tunneling mechanism. Owing to the high concentration of the sample molecules at the anode, ion molecule reactions, both M^+ and $(M+H)^+$ is ascertain in field spectrum (Figure 3).

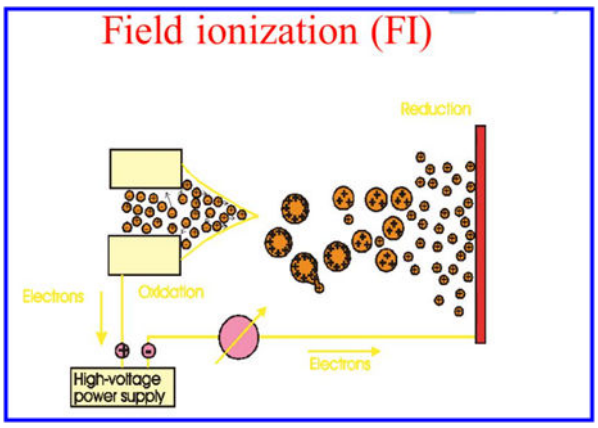

Figure 3: Field Ionization, (By presentation of Yonghai Chai, School of Chemistry and Material science, Bilingual Chemistry Education).

Field ionization is suited for use with volatile thermally stable compounds. It generates molecular ions barely there is no fragments. A sample molecule at its vapor phase is brought in between closely spaced two electrodes of high electric field (10^7-10^8 V/Cm).

By experiencing electrostatic force and a metal surface has a proper geometry (a pointed tip, cluster of tips or a thin wire) is under vacuum (10-6 torr), without transmitting much surplus energy electrons ejects from the sample molecules. Relative molecular mass and empirical formula get determined as there is less fragmentation and profusion of molecular ions $(M)^+$.

There are some disadvantages for this technique. It is not suitable for thermally unstable and the samples those are non-volatile. It has less sensitivity compared to electron impact ion source and there is no structural information gets generated as very less fragmentation occurs.

Desorption

Field desorption: Field Desorption is developed by HD Bakey in 1969. By applying high electrical field gas phase ion formation achieved from a material deposited on solid surface (a multi-tipped emitter where a carbon or silicon whiskers grown on tungsten wire).

Basically it is used for thermally labial substances or non-volatile compounds. Either by inserting the emitters into solution of an analyte or with the help of a micro syringe the sample solution is sprayed on the tip of the emitter whiskers after that the probe is then introduced into the sample compartment. This process is like a chemical ionization or electrical ionization unit. In this the ionization takes place by quantum mechanical tunnelling mechanism, where there is a shifting of ions to the anode (emitter) from the sample molecule. It leads to the formation of positive ions (M^+) and cation attached species like $(M+Na)^+$. $(M+Na)^+$ get produced while desorption process because of the trace alkali metal ions is present in analyte. This ion source is good to small organic molecules, low molecular weight polymers and for petrochemical fractions [9] but for alkali metal contamination it is very sensitive. So the precaution should be there for sample that it should completely soluble in a solvent, and also it is unsuitable for thermally not suitable and non-volatile samples. The disadvantage is that in this technique as there is very little fragmentation occurs, and no structural information generated (Figure 4).

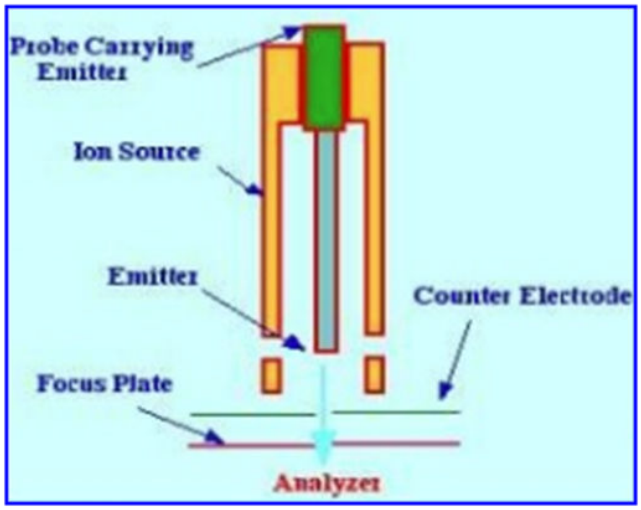

Figure 4: Field Desorption (by A. Solairajan Presentation).

Electrospray ionization: Fenn and his group discovered the generation of multiply charged ions from proteins allowing their molecular weight to be determined with instruments of low mass range using Electrospray ionization. After protein it is used for polymers co polymers and smaller molecules. In this technique to produce charged aerosol droplet, liquid analyte passes through a metal capillary which has maintained at high voltage (Capillary voltage-around 4 Kv) in a chamber heated near atmospheric pressure. It produces ion sample solution by producing fine spray charged droplets. In this a sample solution is pumped constantly from a fine, charged stainless steel capillary needle has low rate and needle is maintained at a high electric field concern to cylindrical electrode.

The capillary produces the mist or aerosol of fine charged droplets from the liquid. This leads to the production of Taylor cone and a jet from the tip of this cone. The charged droplets then travelling through desolvating capillary where there is attachment of charge by the evaporation of solvent. This process carried out in a vacuum.

Warm nitrogen a nebulising gas is used by desolvating capillary which is kept under high pressure. After the evaporation process of droplet the distance between the analyte molecules get closer. As the similarly charged molecules come closer together these molecules become unstable and get explodes once again. This is referred as columbic fission. The process continuously going on till the analyte is with no solvent and it became lone ion. Then the journey of this ion starts towards the mass analyzer [10].

With the help of electrospray ionization technique macromolecules such as protein, polypeptides and oligo-nucleotides (m/z 10000 Da) converted in to ions. This is due to the Proneness of these molecules to fragment when ionized. ESI works by online HPLC MS/MS and capillary electrophoresis for positive and negative modes (Figure 5).

Figure 5: Electrospray Ionization source (by Agilent US).

Matrix assisted desorption ionization: The term MALDI is used in 1985 by Franz Hillenkamp Michael karas and their colleagues, a soft ionization technique used for peptide mass fingerprinting. Particularly for synthetic polymers which are not soluble in the standard solvents, a solvent less method is developed.

In this method there is mixing of matrix and analyte powder together with a mortar. By applying the mixture to MALDI target support and the spectrum is observed. For this particular method has mass limit of 30 to 55 kDa. Matrix sample absorbs the UV laser light with ablation of the top layer of the matrix this leads to a hot plume containing many molecules.

In MALDI matrix is used for the absorption of laser energy, prevent analyte agglomeration, and protect analyte from being from destroying by direct laser beam [11].

Matrix is in crystallized formed molecule of which, the most generally used are 3,5 dimethoxy-4-hydroxy cinnamic acid (sinapinic acid), alpha–Cyano-4-cinnamic acid (alpha-matrix) and 2,5 dihydroxy benzoic acid (DHB).

Preparation of a matrix: For the preparation of matrix solution a mixture of organic compound (ACN or EtOH), highly purified water, and Trifluro acetic acid (TFA) is added. If matrix is changed to a sinapinic acid then the preparation of solution is by adding 20 mg/ml of sinapinic acid, Water: Acetonitrile: TFA (50:50:0.1), after that the matrix solution is mixed properly and uniformly with the analyte to be studied. Acetonitrile has an important role of dissolving hydrophobic proteins which present in the sample and water to dissolve the hydrophilic proteins. Then the solution is spotted in an air tight chamber on the tip of sample probe. Vacuum is created by using vacuum pump to remove the air this leads to the evaporation of solvent and living back a layer of recrystallized matrix carrying analyte molecules. Matrix assisted Laser desorption ionization: In this technique pulsed laser beam strikes on the solid mixture.

Sublimation of sample molecules occurs by absorbing the laser energy and transferring some of it to the analyte molecules of matrix. In this process by absorbing the laser energy, ionization of sample took place easily [12]. Nitrogen (337 nm) and carbon dioxide (10600 nm) laser are most commonly used. In this process there is generation of ions which are recognized as quassimolecular ions that gate ionized by addition of proton $(M+H)^+$ or cation like sodium $(M+Na)^+$ or ejection of proton (M-H). By this process single charged or in some cases doubly charged ions such as $(M+2H)^{2+}$ are also getting produced. In this the chamber which is made up of two electrode and the production of ions are occurs in between these electrodes. As the polymers from cations the cathode is located at right behind the sample and anode from the front. The cations attracts in the direction of negatively charged anode. Movement of ions towards detector is occurs due to the acceleration process. When the polymer forms anions the electrodes are interchanged (Figure 6).

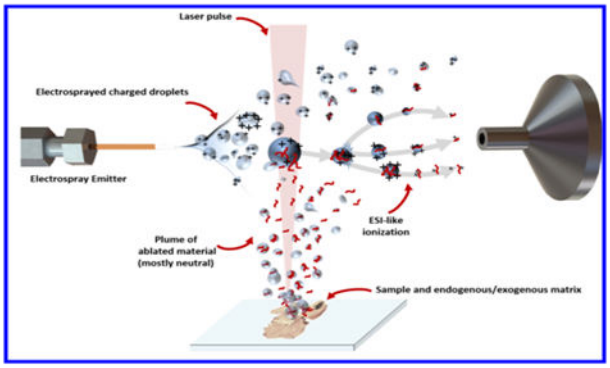

Figure 6: Matrix assisted Laser desorption ionization (by-The schematic of IR-MALDESI imaging source, Wikipedia).

Plasma desorption: Generation of Molecular ions from sample took place in plasma desorption. In this the sample is coated on thin foil and from the opposite side of the foil a highly energetic fission fragments from the californium-252 blast. A highly exothermic fission process of Californium-252 nucleolus releases energy which is carried away by a wide range of fission fragments having heavy atomic ion pairs. The process of ion pair fission fragments depart always in opposite directions [13]. Each and every fission of this radioactive nucleus generates two fragments which travels in to an opposite directions (This is due to necessity of momentum conversation). A normal pair of fission fragments is 142Ba18+ and 106TC22+, with kinetic energies around 79 and 104 MeV individually. As high energy

fission fragments running through the sample foil, highly rapid localized heating occurs, which produces the temperature around 10,000 K. It makes the molecules in this plasma zone are desorbed with the generation of both positive and negative ions. These ions then start their journey by accelerating from source to the analyzer system (Figure 7).

Figure 7: Plasma desorption source (by 35 years of H-ions at Fermi lab)

Fast atom bombardment: In 1980 Michael Barber, University of Manchester develops a new technique known as Fast atom bombardment (FAB) for the purpose of mass ionization. Where in this technique beam of Ar and Xe is focused on to an analyte and non-volatile liquid matrix mixture. High molecular weight and polar compounds likes peptides with a molecular weight up to 10000 Da can be analysed with this technique [14]. This is a soft ionization technique and generally matrices used are Glycerol, monothioglycerol, carbowax 2,4 dipentyl phenol and 3 nitrobenzyal alcohol (3-NBA). In this process organic compounds get easily dissolve in solvent and do not evaporate in vacuum. In this Xe and Ar get ionized first, with a beam of high translational energy to give Xe or Ar radical cations. Xe+e-=Xe ++2e- The Acceleration (6-10 KeV) of radical cations to produce radical cations with high translational energy (Xe)++, these are then runn through a chamber containing Xe atoms at 10-5 Torr pressure. By the process of electrostatic deflector the lower energy ions are get removed.

$(Xe)^{++}=Xe^{++}Xe$

$(Xe)^{++}\ Xe=(Xe)^{+}Xe^{+}$

While preparing matrix the analyte is dissolved in liquid matrix compound like glycerol and coated in the form of thin uniform layer on the sample probe shaft. High energy beam of Xenon atoms bombarded on this mixture. Xe ionizes to the glycerol molecule to produce glycerol ions. As the glycerol ions are very unstable and get react with the surrounding glycerol molecules which undergoes transfer of proton or hydride transfer or ion pair interaction with reactant ions to generate quassimolecular ions like $(M+H)^{+}$, $(M-H)^{-}$ or $(M+G+H)^{+}$. These ions then obtained from slit lens system which designed for collection of ions and direct them to mass analyzer for further investigation. FAB technique is best for fast heating of samples and slow down the sample fragmentation and also good for rapid ionization. Disadvantage of this technique is there is difficulty in tracing and distinguishing between low molecular weight compounds. Also as the compound which should be soluble in liquid matrix, this is not good for multiply charged compounds (Figure 8a and Figure 8b).

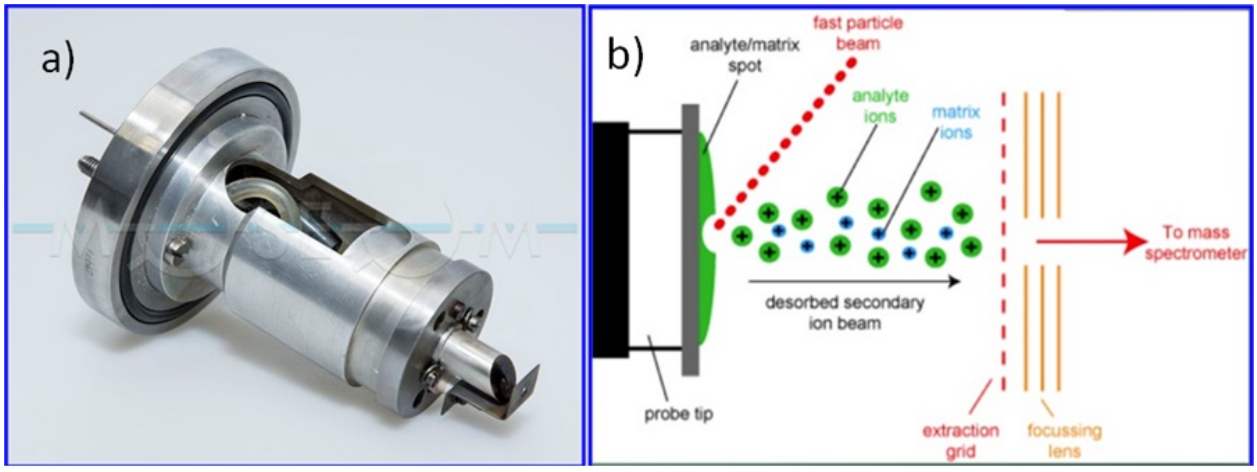

Figure 8: (a) Fast Atom Bombardment Gun by the Czech Museum of Mass Spectrometry, Czech Republic. b) Fast Atom Bombardment process (by Presentation of B. Keerthana, SPSP)

Secondary ion mass spectrometry: With a high energy of 1-25 kV primary ions beam of Cesium or Argon bombarded on solid analyte, followed the mass spectrometry of emitted secondary ions (positive, negative and neutral). In this, the source composed of cylindrical grid and vertically places ion gun or filament. By heating a filament Argon or cesium gas get ionized and generates mono energetic noble gas ions. The ion gun capable to produce a beam of 0.1 to 1 mm diameter with potential of 300 to 3000 eV which then bombarded on the surface of the sample. This bombardment generates secondary sample ions by the process charge transfer interaction between sample molecules and the

primary gas ions. Using the process of electrostatic lens system the ions ready in the cylindrical grid obtained from one end and concentrate on the target or mass analyzer.

SIMS consists of three analysis modes that is static, Imaging and Dynamic. The technique has higher sensitivity [15]. Level of detection is less than 1 ppm or 1 ppb. Isotopic ratios measured normally up to the precision of 0.5 to 0.05%. 2-D ion images acquisition also possible. For this a very little or sometimes sample preparation is not required. The interesting thing of this technique is it measures in depth profile of all elements with high sensitivity large dynamic range and in depth resolution. The technique has also an application in measuring dilute element profile in speciality glasses, ceramics and metal alloys. Measurement of isotope ratios in art history geology is an extra-terrestrial research.

Thermospray ionization: In the University of Houston (TX), Vestal, et al. developed Thermospray interface and which later get commercialized by Vestal in the company Vestec (Houston, TX). This is a soft ionization technique. It is a type of atmospheric pressure ionization in mass spectrometry which shifts ions from one phase to the other that is from liquid phase to the gaseous phase for analysis purpose. It generally useful in liquid chromatography-mass spectrometry as an interface. The flow rate maintained 1.5 ml/min, at this flow rate eluent like polar sample and ammonium acetate forms a jet of vapours and small droplets is formed due to heating the column effluent of an LC column or by any other continuous liquid stream in heated vaporizer tube. Vacuum expelling the charged ions from the surface produces M+H+ or M-H- ions [16] (Figure 9).

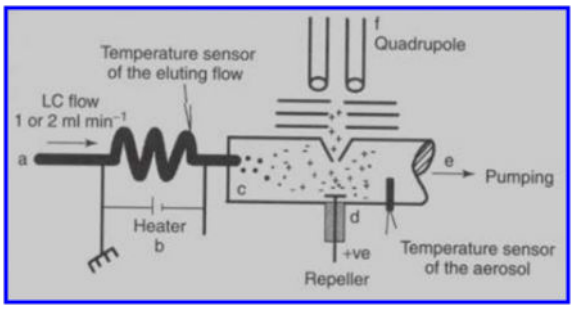

Figure 9: Thermospray Ionizer [17].

Conclusion

Ion source is an important part of mass spectroscopy. This article focuses on general study of ion source. With change in design to generate charged ion to define mass to charge ratio is possible. Now a day's vast research going for development of more advanced and compatible mass ion source which maps all the field of analysis, this makes mass instruments advance journey.

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