Comprehensive Molecular Diagnostics and Advanced Gene Expression Analysis

Prof. Arindam Ghosh

Dr. B.C. Roy Multi-Speciality Medical Research Centre

Indian Institute of Technology Kharagpur

Lecture 28 : Proteomic Techniques - Mass Spectrometry

Namaskar. Hello students welcome back to the lecture series on comprehensive molecular diagnostics and advanced gene expression analysis. We are in module 6 of proteomics where we are discussing proteomic techniques. Now we already discussed our workflow the basic steps in proteomics workflow and in the last lecture we discussed up to liquid chromatography and high performance liquid chromatography that is HPLC. So now we will continue our discussion in the next phase which deals with techniques that are useful in protein identification and characterization. So we will be starting with mass spectrometry in this class and the concepts that will be covered is the principle of mass spectrometry how under what principle does the mass spectrometry equipment work.

So we will discuss the instrumentation we will be discussing in brief elaborately about various terminologies like ESI which stands for electro spray ionization MALDI which stands for matrix assisted laser desorption ionization time of flight TOF quadruple analyzer. So these are the terms we will be discussing in details. Next we will be discussing the applications we will be discussing what is tandem mass spectrometry and how mass spectrometry can also be combined and coupled with liquid chromatography. So there is a lot to discuss this is going to be very interesting discussion.

So let us start with the very definition now whenever we use the word acronym MS it stands for mass spectrometry in proteomics and in general diagnostic diagnostics alright. So mass spectrometry what is it is an analytical chemistry technique that helps to identify and as well as quantify it will tell you how much it will tell you what are the compound that are present in a sample. Based on what based on the charge to mass ratio and an abundance of gas phase ions it is a technique where somehow we split the compound that analyte or the mixture into various ions in the gaseous phase and then we detect the charge to mass ratio in the form of a mass spectrum it will give us a picture based on which we can diagnose alright. So this is the definition or definition is the idea there is no definition when it comes to a technique if you understand the technique you can write two lines in your own language as well right. So what is the principle of mass

spectrometry will be you can get acquainted with this figure in this slide, but we will be discussing it over and over again various parts in the this presentation right.

So you can see there is a sample there are something vaporizer there are electron beams there are processes of accelerating there are processes of some there is a bending in the pathway right there is a detector there is a magnetic field so much is going on in this equipment alright. So basic principle is the molecular targets are subjected to a high beam of electrons energetic electrons alright. So what will happen that will fragment the molecule well they are being fragmented there will be a lot of positive ions right. Now every ion has got its distinct every ion has got a mass every ion has got a charge right and we can actually make a ratio that is known as m by z ratio it is a specific value for a specific ion right this is very useful parameter which is useful in mass spectrometry. Now it is to be noted then while this procedure the majority of the ions that are generated have got a charge of 1.

So generally the m by z ratio which can also be denoted as m by e ratio deliberately used two terminologies because from text book to text book from article to article you may find one or the other both are same so mass by charge ratio. So if the charge is more or less 1 right if in general if it is 1 so the m by z ratio generally corresponds to the molecular mass so that is one thing we need to remember. Now after all these things after all the intricacies that are going on the these via detector I mean via electric fields via magnetic fields they are recorded they reach a detector but the signals are recorded in order to generate a mass spectra. This is the basic principle of mass spectrometry I have made it very try to make it very lucid so that it is I mean easy to grasp for all categories of learners right. So how does a mass spectra look like so this is how it looks like we have got an m by z ratio in the x axis and relative abundance in the y axis.

So if suppose we just try to analyze pentane very simple compound right it will have a specific peaks across various m by z numbers all right. So based on that based on our previous data which is already standardized we can know that what compound is there simply by looking at the mass spectra it can be more than one compound it can be a mixture it can be some compound which is more or less depending on the quantity so it all can be done. So this is the basic principle of mass spectrometry right. So what are the steps if we know the principle we can just implement it in an elaborate manner and we will be easily able to figure out the working steps. So if you have followed the previous slide you can predict what are the working steps.

So let us see so a sample first we will need to start with sample which can be a solid or liquid or gas ok this is our sample. First step this is ionized in a standard procedure there are multiple procedures of ionization which will be discussing very soon. So the first step is ionization of the sample all right. What happens next a portion of the sample

molecule may be subsequently fragmented into charged pieces all right they are fragmented broken down and then these ions are separated or I mean detected according to the mass to charge ratio right. How do we achieve that as stated in the as shown in the diagram we pass them through either or on the combination of an electric field or a magnetic field all right.

So what will happen then the ions that are identical that have got an identical mass to charge ratio will definitely experience an equivalent degrees of deflection. So we can easily figure out so this peak is for this group of ion this peak is for that group of ion based on the mass spectra. So first ionization next step we are fragmenting and detecting we are fragmenting that so that they can be detected all right this is hypothesis. Next so once the ions I mean they are fragmented what happens we can actually multiply the signal. So multiplication can be done by many mechanism for example, electron multiplier it is an inherent mechanism that is technology that is built in the mass spectrometer for proteomic technique we do not need to know the intricacies and details of the machine I mean mechanism, but still what are the essential things we should will need to discuss over here right.

So that electron multiplier actually helps us to detect the relative abundance of the ion depending on the mass to charge ratio as shown in the mass spectrum all right. Now it is to be noted that every compound have got their characteristic fragmentation pattern very very very important. For example, I showed the molecule of pentane it will have its signature peaks that is known as molecular pattern which is already known. So if for example, I know the pattern of pentane I know the pattern of hexane for I use a I am giving you simple example or we know the pattern of multiple amino acids. If we give a mixture seeing those three characteristic pattern we can easily figure out that all right this sample has got a mixture of these amino acids or any compound for that matter all right.

So it is possible to identify the atoms or molecules in the sample with these principles. So next so we now know the principle and basically how this principle is implemented in working of the mass spectrometer right. They are more or less the same thing that we have discussed. Now we should have some brief idea about the equipment or the parts that are present or how what is the flow of the sample that goes from in to out all right. So these are more or less the important parts of the mass spectrometer.

So the sample inlet there is a mechanism of ionization chamber in that then we need to accelerate then there is a mechanism of deflecting those accelerated ions and finally, there is a detector which will detect the signals to generate a mass spectra all right in the form of a diagram. Now the sample inlet definitely the the sample what happens is kept a large reservoir all right this is a big depot of sample. From that the samples are

continuously streamed in to the ionization chamber. It the the whole thing inside is under very very very low pressure almost near vacuum all right through a pin hole and this phenomena is known as molecular leak all right. So very small inlet from a large reservoir through a very small inlet the sample is continuously streamed inside a small amount of sample is continuously flowing into the equipment all right.

So after the sample has flown in the next step is ionization. So the main principle that we discussed what we do with the sample we ionize the sample. So how does it happen? They are done by knocking the sample by striking the sample or bombarding the sample with electrons one of the standard procedure is this all right. Now mind it one of the standard procedure of ionization is bombarding the sample with electrons so that they can be positive ions. However ionization can be achieved by multiple procedures.

For example, one we discussed electron ionization there are methods of chemical ionization fast atom bombardment of an abbreviated as FAB these all have got their abbreviations. So EICI, FAB electro spray ionization is actually ESI and matrix assisted laser desorption ionization this is abbreviated as MALDI all right. We will be discussing these two in detail because they are the most preferred method of ionization when it comes to proteomic experiment detection of proteomic diseases I mean diseases that are I mean detectable in our diagnostic field medical science. So actually you should know ionization techniques can be categorized into two parts. Number one the terminologies that we use are hard ionization and soft ionization.

So what do you mean by hard ionization? Hard ionization H for H wherever there is hard ionization there will be a high energy involved all right. The sample is subjected to a high amount of energy in any form that will lead to fragmentation of the sample in numerous parts. There will be immense number of fragments even the sample will be degraded at an atomic level right. Each single substance will be broken apart right. So the ion can be in form of an atom ion can be formed from molecular radical right.

So in case of hard ionization the number of molecular ion will be low right. For example, what do we mean by molecular ion? I am just giving an example an amino acid can be present in an ionic form positive charge that is molecular ion whereas, we can break apart that amino acid into carbon, hydrogen, oxygen and nitrogen. So that will not be a molecular ion anymore right. So hard ionization does what it breaks apart the entire sample into multiple small atoms, but we do not prefer that. We prefer so that the molecule is minimally broken because we need to detect the molecular signals in proteomics all right.

So we prefer soft ionization techniques where low energy will be used, the amount of fragmentation of the entire molecule will be low and hence it will lead to much more

high number of molecular ions. Suppose it is a big complex molecule it will be broken into smaller molecules ok, but not at an atomic level. So this is very important principle that we should understand before going forward. So what is the basic difference between hard and soft? So this soft ionization is our method of choice for detecting in proteomic techniques. So we will be learning two important ionization method that is electro spray ionization or ESI and the next one is MALDI.

So electro spray ionization is one of the most widely used technique ok, very very very popular and very very very important and commonly used. So what happens in ESI? A liquid sample is introduced into a nebulizing chamber mind it. It is sample in a liquid form is directly introduced into a nebulizing chamber where it is sprayed as fine droplets ok. You just focus on the diagram not in the text right now. So directly we are letting in the sample in a form of spray where they are broken into fine droplets right.

Next what happens this nebulized droplet very small particle they gain charge through application of a high voltage ok. There is a high voltage that we are placing across this field and then they will gain charge. So it will become charged droplets right. So what happens? As the charged droplets move towards the downstream I mean components of the mass spectrometer solvent evaporation occurs for resulting the formation of gas phase ions that is a Rayleigh effect ok. Now we should know that electro spray ionization you see what is happening over here? You see under high voltage Taylor cone the after the discoverer Taylor which who worked who is a pioneer in figuring out this electro spray ionization technique this cone is known as Taylor cone already is a historical is of historical importance emits jet of liquid drops.

So when the solvent drops become progressively evaporates leaving them more and more charged. Now there is an amount of charge which can be held on by a droplet. For example, droplets are very slow and they are becoming charged, but there is a maximum limit right. After which what happens the drop will explode it will explosively dissociate and what will happen it will lead to production of positive ions charged positive ions ok. So again it is also after the discover of Lord Rayleigh who discovered it in 18 I mean early 19th century or late 18th century all right.

So the thing is when it cross it is Rayleigh limit the droplet the liquid sample becomes a gaseous ion and we have achieved our ionization all right. So our step our motto over here was to discuss how we are achieving this ionization phases. So next very important it is considered as a soft ionization technique right why because I am what does it mean? It means minimal fragmentation right therefore, it is suitable for analysis of fragile or large biomolecules such as proteins and peptides which are our area of interest in proteomics that we are discussing all right. So this is about electro spray ionization. Now what is the next method? Matrix assisted laser desorption ionization MALG again

whenever we are I mean you read this term MALG it is referring to this technique.

So this is a mass spectrometric ionization technique commonly used for analysis of large biomolecules. So we prefer ESI or MALG depending on our experimental setup. How it is different? In ESI we were using directly streaming liquid sample into the ionization chamber. What happens here? In MALG the sample is mixed with a crystalline matrix semisolid compound crystalline compound before the analysis forming a matrix sample co-crystal. So there is a crystalline matrix a base on which the sample is mixed right.

Then the sample matrix sample co-crystal now it is behaving as a single compound the crystal or single unit right is irradiated with laser fine. So we are now treating the matrix sample co-crystal with laser energy right. What happens? It leads to absorption of energy by the matrix. Then it transfers it to the sample molecule leading to the desorption ionization. Now very important fundamental thing you should know what is desorption right.

Now you have I mean I hope you know the term adsorption right. Even if you do not know adsorption I hope you know absorption right where I gave an example where if you dip a sponge into a bucket of water the entire water molecule I mean the sponge will become wet. Not only that when you lift it up it is the interior of the sponge is filled with water right. So if you press it all the water molecule will come on this is known as absorption. Now adsorption what is adsorption? It is interaction of a molecule on the surface right.

We have where we have read the term adsorption again in adsorption chromatography right. So whenever there are multiple compounds that you have I believe you must have read in your chemical chemistry lessons that are getting adsorbed on the surface. They are not going inside they are adsorbed in the surface interacting in the surface. So this is adsorption whenever compound gets interact or tightly bound in the surface right.

Desorption is just the opposite. So whatever is present in the surface is now being emitted ok. So this is a sample matrix co-crystal right and when we irradiate the mixture with laser matrix absorb energy it is transferred to the sample it dissolves and then it leads to dissociation of molecular ions ok. So this is the basic principle of MALDI. This is much you need to know. So again it is considered a soft dilation technique preserving the integrity of large biomolecule during ionization alright.

We do not want to fragment because we want to detect the molecule as it is. So making it again making it suitable for peptides, proteins and other macromolecules right. For example, lipidomics polymer analysis ability to analyze large complex molecule with

high sensitivity very very very important. Soft dilation technique ability to detect large complex biomolecules. So these are the two method of choice for ionization when it comes to mass spectrometry in proteomic with respect to proteomic techniques.

So now when we have the ions the next thing we need to do is they are accelerated right. They are accelerated so that they all have the same kinetic energy because you know when we either by electro span ionization when it explodes or by desorption all the ions are going in every side like a divergent stream. We need to make sure that they are directed in the same way right. So vectors direct in the same way. So they are being subjected to multiple fields electric field, magnetic field etcetera etcetera.

So first in acceleration phase they are passing through three slits of voltage in decreasing order right. So first a high voltage gate, then a second medium voltage gate and finally almost at zero voltage gate. So they are passing through three voltage gates where they are accelerated and all the ions are getting or gaining a single direction of movement right. The next as it is clear by the diagram is deflection. So now what happens? Since the charge is almost the same for example, they are mostly positive the amount of interaction by the electric field will be similar right.

However when we are applying a magnetic field to the charge molecule then they will be deflected based on the mass or m by z ratio which is actually corresponding to the mass right. So how it is deflected the lighter the mass the more they will be deflected the heavier there will be less deflection right. So what happens? Now since we have got multiple molecules and they are travelling in different direction towards the detector right they will be detected. Not only that considering we have got molecules which have got different charge as well that will also be deflected in different way the characteristic will be different for example, number of positive charge and the it is carrying positive charge the more it will be deflected all right. So based on the characteristic or difference in mass based on the difference in charge everything with a different characteristic property different m by z ratio even if the charge is same they will have different mass.

So they will be detected and deflected and detected which can be easily caught by the detector right. So now we are in a phase of discussing about the detection method ok. So again as stated they are detected on the basis of m by z ratio. So what happens actually when an ion hits the metal there is a metal box that will the ion will hit right the charge is neutralized right by electron jumping from the metal on to the ion.

So that is a basic principle that you need to know. Now the main thing is this m by z ratio this deflecting ions that are coming in towards the detector again there are multiple mechanisms by which they can be detected each have got their own proprietary technology and again we do not need to know all of them. We should know the names

for example, there are magnetic sector mass analyzer, double focusing analyzer, quadruple mass analyzer, time of flight, TUF analyzer, ion trap, ion cyclotron there are many variety right. But some of them for example, are more applicable in synthetic chemistry for example, detection of multiple crystal compound synthetic elements some of them are very important drug discovery and proteomic techniques. For our interest the most important one that are used are time of flight analyzer and in some cases quadruple analyzer. So we will be discussing these two quadruple and TUF right.

So the time of flight analyzer the name over here will suggest how it work right. So it is a technique by which it measures the time taken by the ion to travel a fixed distance in an electric field right. So here you see the ions are travelling in an electric field right from here right to the detector and every ion will take different time because of the different m by z ratio right. So time of flight analyzer will detect that based on the time taken right because the distance is fixed. So ions are accelerated into a flight tube right the one we saw and the time of flight is proportional to the m by z ratio the mass to charge ratio right and this principle is what is used by the time of flight analyzer.

Now time of flight produces very high mass resolution allowing for detection of accurate m by z ratio which are actually crucial for precise detection of ions right. Basically it is one of the most I mean best methods that is why we are using in medical diagnostics right. So what happens and what is another benefit? So time of flight it is known for its not only rapid data acquisition capability means we can generate a rapid amount of data in a very short time therefore, it helps in analyzing multiple samples in a unit time that is known as throughput. So it is also suitable for high throughput analysis for example, there are multiple samples from multiple patients we can analyze all of them in one go. So therefore, very important and useful in proteomics and metabolomics alright and we can now amalgamate the concept that we learnt earlier.

So time of flight mass spectrometers are versatile and can be coupled with various ionization techniques such as MALDI and electro spray ionization depending on their applicability alright. So here we see a diagram for example, we are pre-pairsing a sample via matrix. So this is a MALDI target plate. So now we are irradiating it with the laser beam then it goes I mean via multiple voltage gate there are equations that we do not need to know in detail alright. However we will see that the time taken will greatly vary and that will be characteristically that will generate a mass spectra which will help us to detect the various components of the molecule right.

So time of flight concept is very simple the time taken by the molecular ions to travel a fixed distance across the electric field and that will be detected. Quadruple analyzer. So quadruple analyzer definitely there are something 4 that are involved right. So what they use radio frequency and direct current these are often abbreviated an RF and DC alright.

They use the RF and DC electric fields that are applied to 4 rods to create a quadruple electric field and in this quadruple electric field the ions will different ions will behave in a different way.

They can be filtered based on their properties right. So we can target selective ions we can target multiple ions right allowing a precise mass analysis. So we can regulate the nature of the electric field and radio frequency that is present in the poles so that we can specifically transmit one ion at a time and they can be detected by an ion detector. Again they offer moderate resolution, but high sensitivity. What do you mean by that? High sensitivity means if the disease is if the disease is present or if a sample is present it will give a signal, but the cons of high sensitivity is it can sometime give a false positive signal as well, but it is very important to have high sensitivity especially in screening test alright. So compared to MALDI quadruple analyzer have got a moderate resolution.

I mean time of flight I am sorry compared to TOF quadruple analyzer have got moderate time of flight analyzer have got a very high resolution alright. Again there are multiple scan modes which can be implemented so at the cost of moderate resolution we are gaining multiple advantage for example, single ion monitoring multiple ion monitoring those are abbreviated as SIM, MIM alright. What happens these equipment which have got quadruple analyzer they can be integrated into something known as tandem mass spectrometry. What is tandem mass spectrometry? We will be discussing them that is coupling of two mass spectrometer one after another.

So they will behave both as a separator as well as a detector right. So both compound identification and structural elucidation right. So before discussing tandem mass spectrometry let us briefly discuss what are the applications of mass spectrometry number one since we are studying proteomics one thing very important detection of any peptides proteins anything. We are also studying I mean with respect to our course is advanced gene expression analysis right. So nucleotide is very important again can be detected by mass spectrometry right. Biopolymers, proteins, oligosaccharide different biomolecules not only proteins, carbohydrate, nucleic acid, lipids everything can be detected.

Beyond this biology and medical science again environmental monitoring soil, water, air pollutant, geochemistry, petrochemical industry, quality control again analysis of breath that is for I mean mass spectrometry very important all the compounds that are coming out in a volatile form in breath can be analyzed by mass spectrometry right. So for example, there are multiple compounds that are excreted or inhaled in breath whenever the patient is unconscious and that gives us some idea about what are the biological changes that are going on inside can be detected by mass spectrometry right. So again coming back to medical science for example, excess of any metabolic

substance, drug abuse, any abnormal metabolic that are excreted in any body fluid, blood, urine, saliva, aerosol particle, any can be detected, any pesticides in food everything I mean this has got a such a wide spectrum of application you simply can think of anything mass spectrometry can be utilized in detecting that. But we will narrow our discussion in detection of proteomic samples relating to proteomic experiments.

So, we shall discuss LCMS. Now what is LCMS? Now from the diagram itself it is quite clear that two things are going on over here. The first phase the one on the left side is what we discussed in the last class that is HPLC right. It is coupled with mass spectrometry van interface ok. So, LCMS combination of liquid chromatography and mass spectrometry liquid chromatography is HPLC. So, HPLC with mass spectrometry it offers high sensitivity and specificity for detecting compounds with low concentration and has got a diverse application.

For example, pharmaceutical environment analysis, clinical research, metabolomics, proteomics everything alright. So, why? Why it is so much advantageous? It integrates the separation power of liquid chromatography. So, HPLC has very high very efficient in separating all the complex mixture and mass spectrometry is very efficient in detecting individual components of the mixtures. If we combine those we can achieve wonders, we can achieve identification, we can achieve quantification which is very effective for proteomic experiment right. So, it provides precise mass information for confident compound identification right for I mean for precise specific compound identification and differentiation of closely related molecules.

Even if two compounds are very closely related right, proper LCMS analysis can differentiate between them ok. So, what is tandem mass spectrometry that we discussed a few slide before? It is coupling of two mass spectrometer. How does it happen? So, for initial ionization and separation so, molecules are ionized. So, fast mass spectrometer separate ions based on their charge mass ratio.

So, it can be again m by z m q m e alright. Next they are subjected to a fragmentation process right by some type of collision. For example, collision induced dissociation ion molecule reaction photo dissociation with the help of light causes these ions to split into smaller fragments ok whenever favorable. Generally we discuss for complex molecule we do not want to fragment them, but there are situation definitely we when we want to fragment them alright. So, generally MS MS or tandem mass spectrometry is also used for detection of multiple metabolites where we know that the final mass spectrometer spectrogram or mass spectrum can be extrapolated back to the known compound right. So, next for first ionization separates them based on m by z ratio, second a photo collision or ion induced collision breaks them into multiple fragments and then those small

fragments are now entering the second mass spectrometer alright.

So, these MS2 separates the fragment ions based on their m by z ratio right and then again they are detected and analyzed in the separate fragments are detected and analyzed and then the whole pieces of puzzle put together with the help of multiple softwares and softwares and bioinformatic tools and then we get the final spectrum of the original compound right. Why it is used? Why do we need fragmentation? For example, identification separation of ion with very similar m by z ratio right. If we have two ions that have got a very similar m by z ratio if we just run one MS they will be traveling the same way they will be detected deflecting the same way by single magnetic field will not be able to detect right. So, first if we know what are the compounds, what are the fragments right and then if we I mean if you know the component ions in the initial mass spectrometer then after fragmentation if we can further analyze them then we will have a clear picture of the bigger picture of the entire compound alright.

So, what does the enhance is the precision and specificity of the analysis. So, we discussed LCMS, we discussed MS-MS can we combine them somehow to achieve both. Now before going there MS-MS also have got advantages just like LCMS we can I mean for selective cases MS-MS is the method of choice I already discussed for closely related compounds for quantification and confirmation it is used in diverse fields. For example, pharmaceutical environment clinical research alright. Again multiple reaction monitoring can be done alright for example, quadruple MS-MS for targeted analysis they enhance the sensitivity and specificity of the reaction very important for proteomic experiment ok. Remember whenever there are multiple reaction multiple components are being fragmented tandem mass spectrometry is very good right.

So, what I was talking about combining all the three combining tandem mass spectrometry with HPLC. So, what happens over here? So, HPLC separates and concentrates the sample right and mass spectrometer with quadruples they are now analyzing the sample. So, we are getting highest level of separation and analysis of the sample at the molecular level right. So, basically this tandem mass spectrometer has got a triple quadruple configuration quadruple is the analyzer alright then if we use triple quadruple it must sound something eerie right no quadruple is actually quadruple analyzer. So, we can say three quadruple analyzer configuration commonly we are saying triple quadruple alright.

So, what it does? First it first one isolates analyte molecular ions the second quadruple analyzer based on RF alright radio frequency and direct current field they actually fragments the ions and again third isolates the fragments for measurement alright. Basically even in spite of having a triple quadruple it is actually a tandem mass spectrometer the concept is same first ions next fragmenting and then again we are

detecting the fragments. So, this is what is happening. So, first HPLC separation then ionization with the help of either for example, we are doing electrospray ionization because HPLC liquid chromatography the sample is liquid I hope you are clear with this point. So, with the help of electrospray ionization whenever we are doing LCMS the ionization method of choice has to be electrospray ionization for separate sample which are for example, serum sample we can dry we can use MALDI TOF alright very important again mixture of liquid sample HPLC or LCMS or LCMS ESI for example, serum sample that we can dry or any we use MALDI TOF ok.

For serum we can also use tandem mass spectrometry alright. We can couple two time of flight analyzer alright for better detection resolution. So, then it will be referred to as MALDI TOF TOF. Once you know what a module does we can combine one after another serially for greater resolution right. So, here LCMS what is doing with the help of electrospray ionization it is subjected to ions then is a triple array of quadruple analyzer right. So, based on the first analyzer it is getting separated the ions second they are being fragmented by the second quadruple array and the third one it is separating and it is going into the detector alright very very very useful for proteomic experiment and as the technology is developing we are combining more and more analyzer in series.

For example, LCMS MS if it is available definitely it is might be costly it will be the method of choice over simple LCMS ok. So, what are the benefits of LCMS MS? It is both structural information with I mean not only it will help to separate alright it gives us detail about very at the structural information at minute level right. It has got very high sensitivity and specificity needless to say because we are achieving a very high resolution we are now able to monitor what is happening at a single atomic level alright. Again it ensures a robust and clean signal for example, if as I discussed in if one of the we have got two molecule with similar M by Z ratio that there will be might be an overlapping peak in the mass spectrogram, but if we are somehow separating and getting a higher resolution the signal of each peak will be very clean ok. Again in some cases you might be asked orally that can we use single versus triple quadruple analyzer definitely we can use based on our specification experimental setup laboratory I mean standardization of experiment as well as budget.

Single quadruple select specific masses for clean sample if we have got clean sample there is no mixture if we know that the sample is clean ok, there is no contaminant we can simply use a one quadruple analyzer that will be an excellent result right. However for complex mixture sample at different masses and if we know that some things will be unpredictable right then it is must I mean always preferable to utilize triple quadruple setup for better resolution and better analysis right. So, to summarize we have read mass spectrometry again analytical technique detecting useful for detecting compound based on the charge to mass ratio abundance of gas ions what happens in them for example, in

one case electron bombardment it is transferred to ions right. Then under help of electric field they are separated right then they form a mass spectrum based on how there is the detector all right. And with the help of this spectra with the help of characteristic signals electrical signals elemental signals or isotopic signals of a given compound we can clarify the chemical nature of the molecule and other compounds all right.

So, these are my references for today's class and I thank you for your kind attention. Thank you.