Advance Analytical Course

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Module No. # 01
Lecture No. # 06

In the previous lecture, we had learnt about gas chromatography. This is a new lecture related to gas chromatography with a dedicated detector called the mass detector. In this particular machine, we are able to get more information about a sample gas chromatography mass spectrometry or G C M S.

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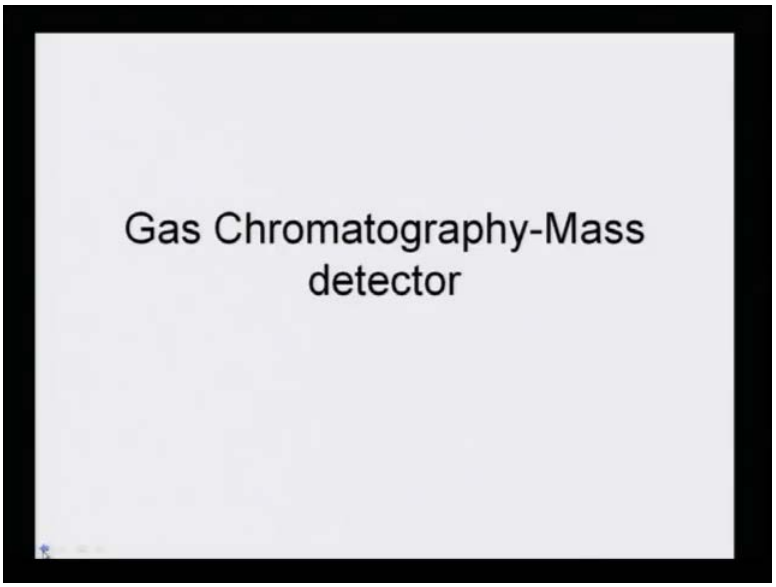

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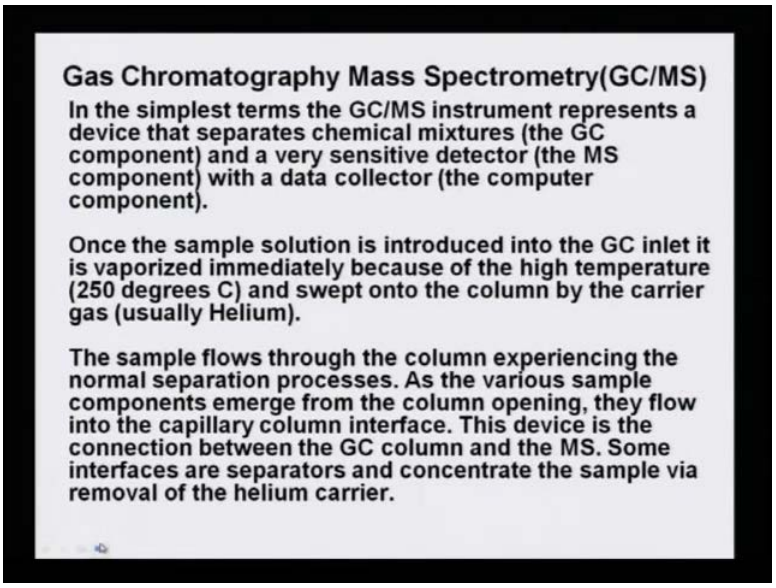

In the simplest term, the G C M S instrument represents a device that separates chemical mixtures; that is through the G C component and a very sensitive detector that is the mass component with a data collector which is the computer component. So, basically it is made up of 3 components.

We had already learnt about G C, that is the gas chromatography. Now, today we will learn a little more about the detector which is called the mass detector and how this data is collected and interpreted. Once the sample solution is introduced into the G C Inlet, it is vaporized immediately because of the high temperature that is about 250 degrees centigrade and swept onto the column by the carrier gas which is usually helium. Of course, there could be other non-reacting gases like nitrogen and organ but, helium is the one which is the lightest and it is more prevalent to be used. The sample flows through the column experiencing the normal separation processes we have already learnt about the G C the column and the separation processes that take place in a column of the G C. As the various sample components emerge from the column opening, they flow into the capillary column interface. This device is the connection between the G C column and the m s that is the mass detector.

So, the point at which the G C outlet and the mass detector meet, that is called the Interface. It should be very clear because, these terms will be continuously used and you

should be aware of what each term means. Some interfaces are separators and concentrate the sample via removal of helium carrier gas.

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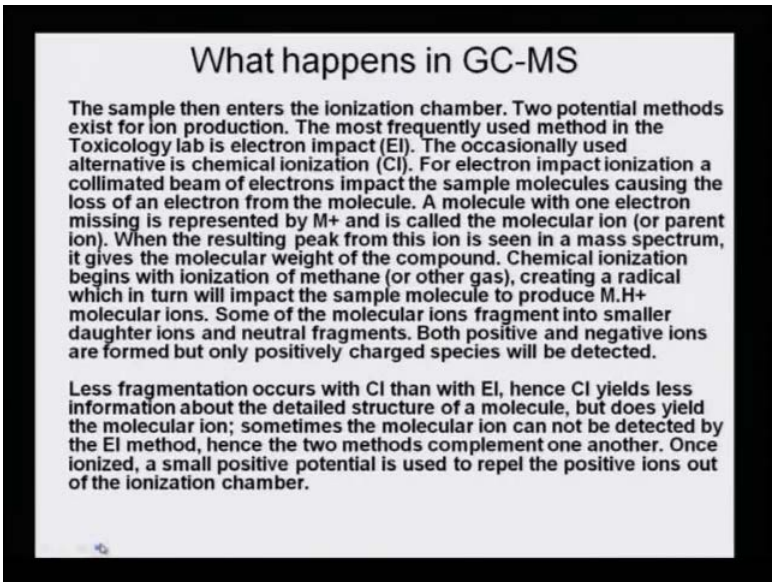

There are other devices also but however, we will go slowly into the detail of G C M S. The sample then enters the ionization chamber; two potential methods exist for ion production. The most frequently used method in the toxicological laboratories is the electron impact. The toxicological laboratories use electron impact method; more prevalently, the occasionally used alternative is the chemical ionization or the C I method for electron impact ionization. In abbreviation, we call it E I method; a collimated beam of electron impacts the sample molecules causing loss of an electron from the molecule. A molecule with one electron missing is represented by M plus dot and is called the molecular ion or the parent ion. When the resulting peak from this ion is seen in a mass spectrum, it gives an idea about the molecular weight of the compound. Chemical ionization begins with ionization of methane or any other gas which is used, creating a radical which in turn will impact the sample molecule to produce M.H plus molecular ion.

Some of the ions fragment into smaller daughter ions and neutral fragments. Both positive and negative ions are formed but, only positively charged species will be detected. So, that is the beauty of this machine; that it can only identify the positively charged species and this whole process actually occurs with the first collimated beam of

electron impact. So, the molecule first is impacted and molecular ion is generated and that is the starting point of a molecules fragmentation and then subsequently, the molecule breaks up into daughter ions or daughter nuclei. When we try to correlate the molecular structure, we try to look at the fragmentation pattern of these molecules and then try to derive as to what must have been the structural design of the molecule.

Now here, I would like to bring to your notice that every organic or inorganic molecule breaks up in a very definite manner. It is not that today one bond will break, tomorrow some other bond will break and so, the molecular fragmentation will differ from time to time, from machine to machine. Always the most labile bond will break first because, that will be most acceptable for this electronic bombardment and that is how the fragmentation pattern remains the same in, under whichever condition we try to fragment. So, whether we are using G C M S of a brand a or brand b, the fragmentation pattern of a compound remains the same; so this should be absolutely clear.

Second point that needs to be learnt is that. less fragmentations occurs when C I that is the chemical ionization method is used as compared to the electron impact or the E I method. Hence, C I yields less information about the detailed structure of a molecule but does yield the molecular ion. Sometimes, the molecular ion cannot be detected by the E I method. Hence, the two methods complement each other and it should be used in, if and when required. It is always more prevalent to use the E I method but C I should be available that under conditions where the information about the fragmentation or the molecular ion peak cannot be detected, there the C I method should be adapted. Once ionized, a small positive potential is used to repel the positive ion out of the ionization chamber.

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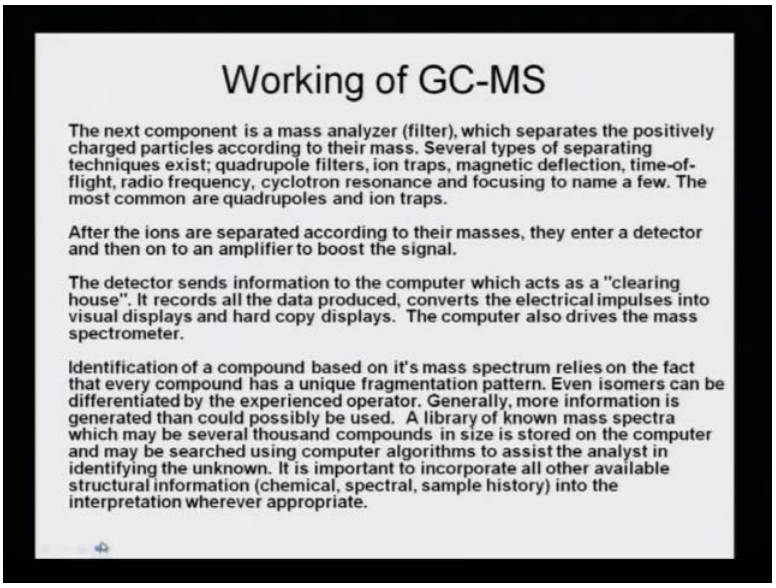

So, it needs that there should be a driving force for these ions to be traversed from the ionization chamber to the outside fragmentation situation. So, let us now look at the working of G C M S. The next component in a mass detector is the mass analyzer or the filter which separates the positively charged particles according to their masses. As the name suggests, it is a mass analyzer; that means, the smaller fragments will reach faster, the medium fragments will come a little later and the highest fragments will come much later.

Several types of separating techniques exist. Quadrupole filters, ion trap, magnetic deflection, time-of-flight or torr, radio frequency, cyclotron resonance and focusing to name a few. The most common are the quadrupoles and the ion traps. So, if we try to look at a common G C M S machine, it would either have a quadrupole as a mass analyzer or it will have an ion trap after the ions are separated according to their masses. They enter a detector and then on to an amplifier to boost the signal; so as what we had seen in the case of G C as well as in the case of H P L C, the signals need to be amplified. Whether they are mass detector signals or they are chromatographic signals, they need to be amplified to be able to see on the screen or to be able to take the print out of the chromatogram.

The detector sends information to the computer which acts as a clearing house. It records all data produced converts the electrical impulses into visual displays and hard copy

displays the computer; also drives the mass spectrometer. So, it is as what you would understand from this above paragraph that I read out. It means that the whole machine is computer driven it is not a manual machine and secondly, all the commands can be given on the computer and the machine can run exactly on the commands that have been given on the machine.

Identification of compound is based on its mass spectrum and it totally relies on the fact that every compound has a unique fragmentation pattern. Just a while ago I was telling you that, whether it is an organic compound, whether it is an organ or metallic compound, whether it is an inorganic compound having an organic Allegan, it will always fragment in a set pattern. The pattern never changes because, the labiality of the bonds never changes and that is the reason why mass fragmentation gives us a good idea of the molecular structure.

Even isomers can be differentiated by the experienced operator. Generally, more information is generated then could possibly be used. There is enormous amount of information but, what an analysis needs to do is to take the crux of the chromatogram or the spectrum and that itself will give us an idea about what is the structure of the compound. There is an advantage that there is a library of known mass spectra which may be several thousand compounds in size and is stored in the computer data and may be searched using computer algorithm to assist the analyst in identifying an unknown compound. It is important to incorporate all other available structural information, chemical spectral sample history, into the interpretation wherever appropriate.

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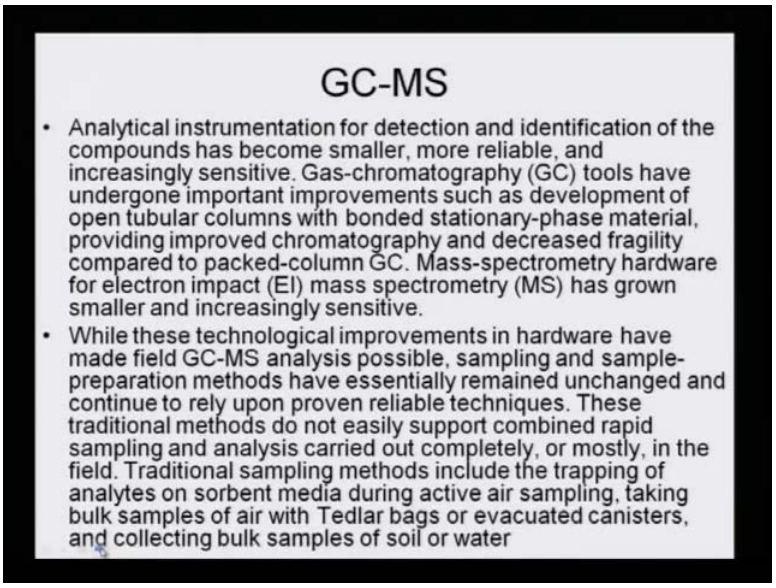

Analytical instrumentation for detection and identification of the compounds has become smaller, more reliable and increasingly sensitive with the use of G C M S. now, what does this mean? That we are able to identify an structurally unknown compound by a very precise method and it is a very sensitive method. Gas chromatography that is the G C, is a tool which has undergone important improvement such as development of open tubular columns with bonded stationary phase material providing improved chromatography. Because, as what I have emphasized last time when I was talking about lectures related to G C that gas chromatography separates the column. There is a mixture which needs to be separated and unless and until the separation is complete and the separation is really marked out, the compounds are separated from a mixture. We will not be able to identify the structure completely. So, in order to have a good separation, the chromatographic technique should be very efficient. The first thing is and so, lot of G C development has taken place and many improvements in the chromatographic technique have come about and with decreased fragmentation and a pattern can be identified.

Mass spectrometry hardware for electron impact that is, the mass spectrometry method which includes electron impact, has become more and more sensitive and it has become increasingly more important. While these technological improvements in the hardware have made the field G C M S analysis possible, sampling and sample preparation methods have essentially remained unchanged and continue to rely upon proven reliable

techniques. These traditional methods do not easily support combined rapid sampling and analysis carried out completely or mostly in the field traditional sampling methods include the trapping of analytes on sorbent media during active air sampling taking bulk samples of air with Tedlar bags or evacuated canisters and collecting bulk samples of soil and water.

Now, one needs to reevaluate where all the innovations and newness has come and what is still existing; as what we had learnt in the past few lectures, sampling methods and preparation of sample are two very important aspects. If we make any mistake in either of these two areas, we are bound to make analytical mistakes in the next few steps. So, one has to keep in mind that sampling of procedure or sampling of samples should follow a protocolic procedure and not in any and every method.

Secondly, we should follow the preparation of G C M S sample in the prescribed manner not by a using our own whims and fancy. Why? Because, a G C M S is a very sensitive machine and we cannot afford to have any contaminant flowing into the G C and then affecting the mass detector. So, we have to, utmost care of taking care of this sample procedure. But, all kinds of samples can be analyzed; be it an air sample or a soil sample or water sample, proper extraction methods have to be devised for these different types of samples and then only we can analyze them on G C M S machines.

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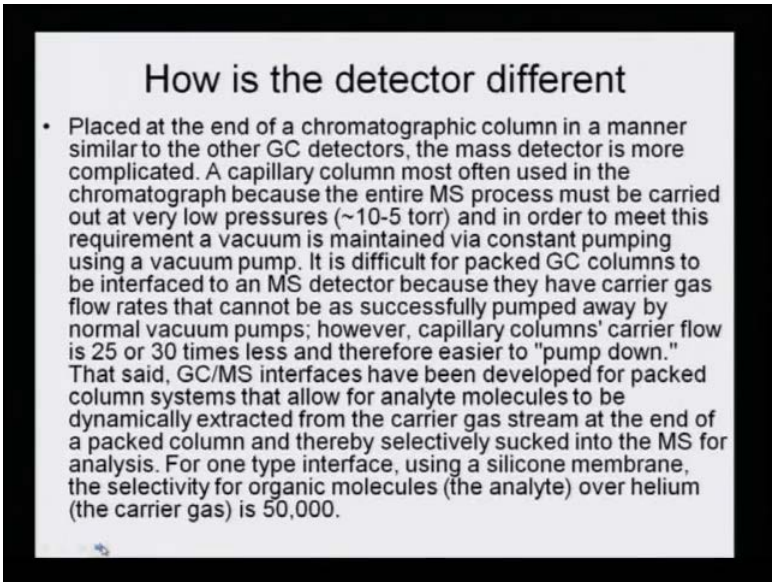

How is the detector different? We had seen that in G C we could use different types of detectors like E C D detector, F I D detector, N P D detector. How is this these detectors different from the mass detector which has now been newly attached to the G C and the new machine is now known as G C M S machine, placed at the end of the chromatographic column in a manner similar to other G C detectors? The mass detector is more complicated and more sensitive. So, one has to keep in mind that once we have the mass detector in the machine, the machine automatically becomes more sensitive and is to be dealt with utmost care. A capillary column most often used in the chromatograph because, the entire m s process must be carried out at very low pressure, that is between 10.5 torr or less than that and in order to meet this requirement, a vacuum is maintained via constant pumping using a vacuum pump. So, the entire process of mass detector and its placement in the machine configuration, has to be such that it is under high vacuum and then only it works efficiently.

It is difficult for packed G C columns to be interfaced to an m s detector because they have carrier gases flow rates that cannot be successfully pumped away by the normal vacuum pumps and that is precisely why I mentioned a little while ago that only capillary columns are attached to the mass detector and not the packed columns so one must go along the line to understand what is the configuration of the machine and what are the do is and what are the do not is in this machine because it is a sensitive machine.

Now because the however capillary column and the carrier flow is about 25 to 30 times less. Therefore, it is easier to pump down the gases that are flowing through the capillary columns and as said, G C M S interfaces have been developed by for packed column systems also that allow the analyst molecule to be dynamically extracted from the carrier gas stream at the end of the packed column. Thereby, selectively it is sucked into the m s for analysis but of course, this arrangement is not always available in every machine. It is possible to do that and that is why I am a making you aware that there is a possibility that packed columns can also be used; but, that configuration of machine is slightly different from the usual G C M S machine.

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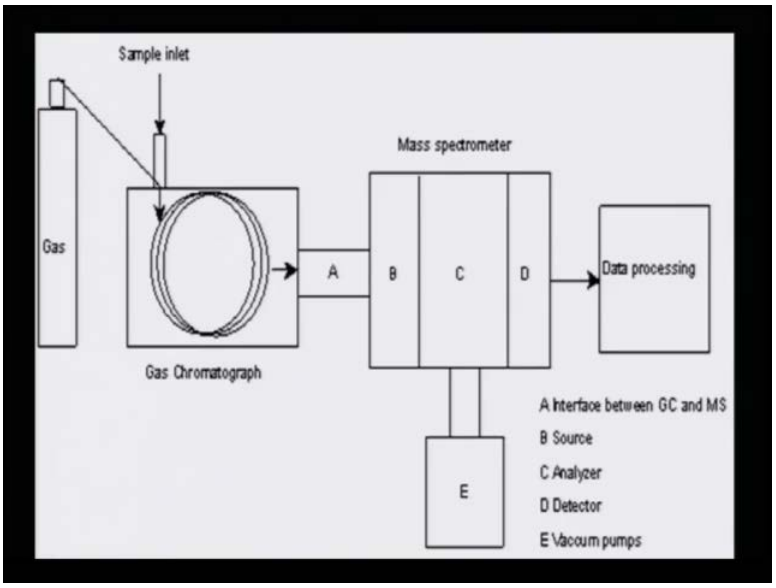

However, there is a possibility of even using packed columns but, normally only the capillary columns are the ones which are used in G C M S for one type of interface using a silicon membrane. The selectivity for organic molecules that is, the analyte over helium that is, the carrier gas is about 50000 times. This is how is the layout of a G C M S machine you will see that there is a gas cylinder which is connected to the gas chromatogram and there is an inlets sample inlet. This is a very pictorial description of the machine. The machine is not like this, in a very geometrical shape as what it shows but this is a pictorial description of a machine. There is a sample inlet passing through the column and then entering into the ionization chamber so you will see that a is the interface between the G C and the m s and in the mass spectrometer there are three regions B C D. B is the source at which the Ionization takes place. C is where these masses are analyzed; so it the analyzer and D is the detector and then from the detector d the information is passed to the data processing and E shows that it the whole system of mass spectrometer is under the vacuum pump condition. So, this is a very pictorial description of the machine.

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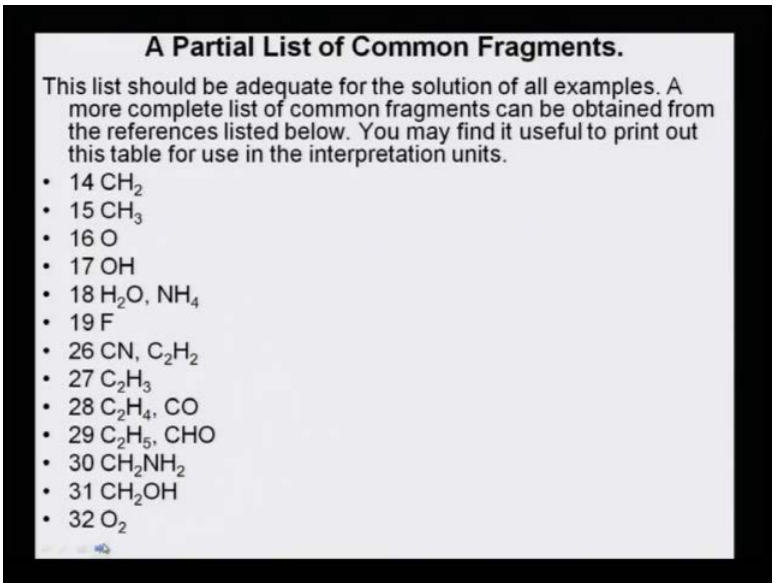

Now, I would just like to draw your attention towards some common fragments and I list, a very partial list of some common fragments which means, that if you see an ion peak at 14 15 16 17 18 19 and so on, these are the molecular parts that you should expect or atoms should be present in the structural detailing of a compound. Now, when we try to look at different fragments at different A M U that is, atomic mass units this 14 15 16 and so on depicts the atomic mas unit of a part of a molecule it does not describe a molecule completely. So, if one finds that there is a loss of a fragment of 18, one could expect that either ammonium has been lost from the molecule or water must have come out of the molecule. Similarly, if there is a loss of 26 one could expect a cyanide loss or a acetylenic loss and so on.

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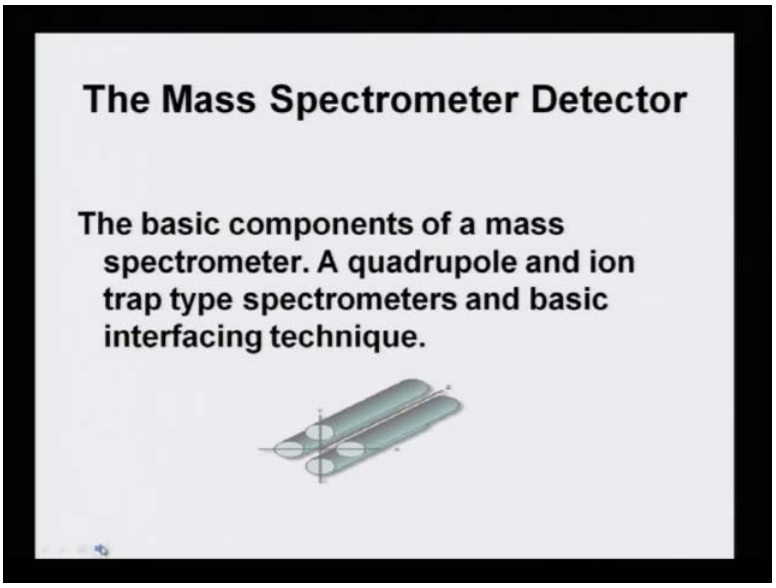

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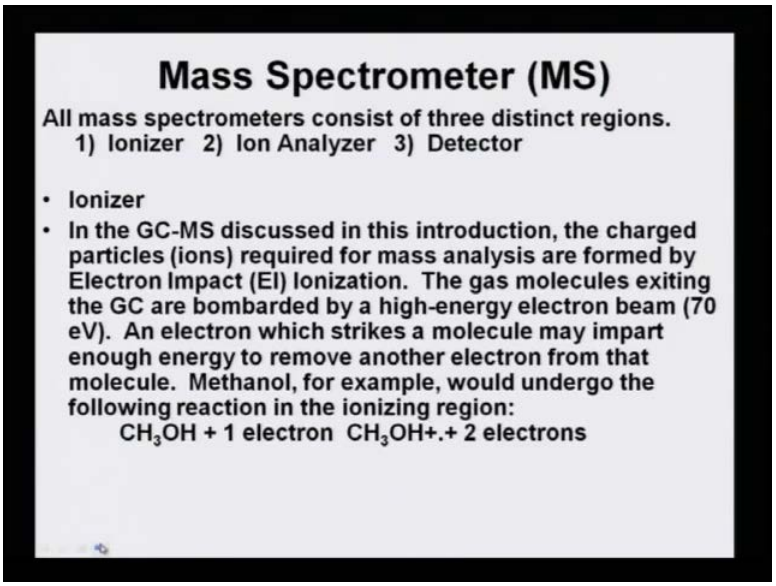

Now, the partial list can go on and on but, to bring to your attention I would just spend a little time on the mass spectrometer detector. The basic component of the mass spectrometer is that it is made up of a quadrupole and either it will be quadrupole or an ion trap which means, that the beam of fragments must be detected at some level and this detection is carried out either by a quadrupole or an ion trap. As the name suggests that means, it has the capability to trap these ions and to identify them the mass spectrometer. All mass spectrometers consist of 3 distinct regions as what I had shown the B C D region in the pictorial description.

One, the first part is the ionizer were the ionization of the molecule starts. The second part is the analyzer where the ions that have been formed must be identified and the third is the detector which actually traps one by one according to the m by z ratio. As and when they reach the detector, they are identified. So, ionizer the first part in the G C M S as discussed earlier, there is the charged ions are produced by the electron impact and it is called electron impact ionization or E I ionization.

The gas molecules existing in the G C are bombarded by a high energy electron beam. Usually, this electron beam is at 70 electron volt; an electron which strikes a molecule may impart enough energy to remove another electron from that molecule; so, that is how. There is a small example that I have taken; methanol for example, would undergo the following reaction during the ionizing procedure. Methanol, when it is impacted with 1 electron will form C H3 O H plus dot which is a radical cation and 2 electrons will come out of it and that is how this is the molecular ion peak of methanol.

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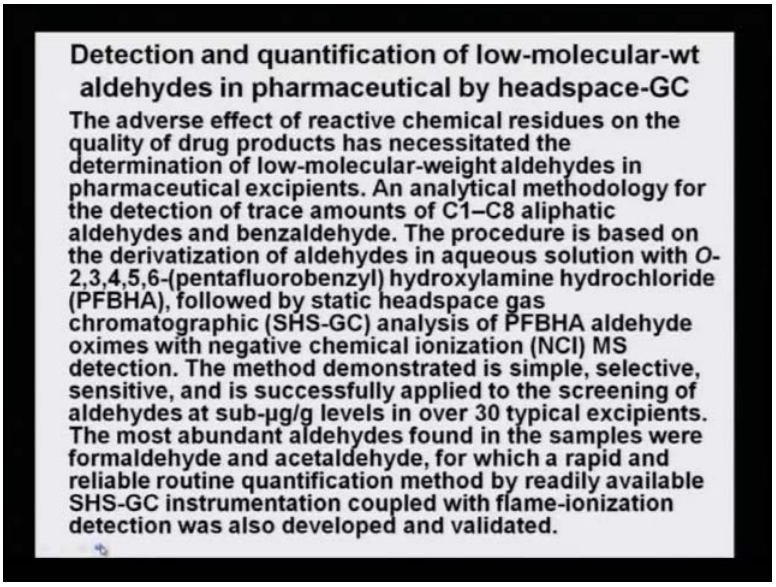

Detection and quantification of low molecular weight aldehydes in pharmaceuticals by headspace G C: now, when we come down to certain examples we have to understand how these compounds vary. Typical organic compounds are analyzed in a G C machine or a G C M S machine. The adverse effect of reactive chemical residues on the quality of drug products has necessitated the determination of low molecular weight aldehydes in pharmaceutical excipients. An analytical methodology for the detection of trace amounts

of C 1to C 8 aliphatic aldehydes and benzaldehyde. It is not easy to detect them otherwise. The procedure is based on the derivatization of aldehydes in an aqueous solution with 2 3 4 5 6 pentafluorobenzyl hydroxylamine hydrochloride that is P F B H A followed by a static headspace gas chromatographic S H S G C analysis of the P F B H A aldehyde oximes with negative chemical ionization mass detection.

Now, you see we have gone into a little specified technique because, these aldehydes are low boiling and also they do not give a molecular ion peak at the required time. it a and At the required length it is important to be able to find a new method; to be able to analyze these very specific aldehydes, they are very sensitive. The method that is demonstrated is simple, selective sensitive and is successfully applied to the screening of aldehydes even at micro gram per gram levels in over 30 typical examples. The most abundant aldehyde found in the samples were formaldehyde and acetaldehyde for which a rapid and reliable routine quantification method by readily available the S H S G C instrumentation coupled with flame ionization detection was also developed and validated. Always, two methods are put hand in hand to verify the results and to validate the results.

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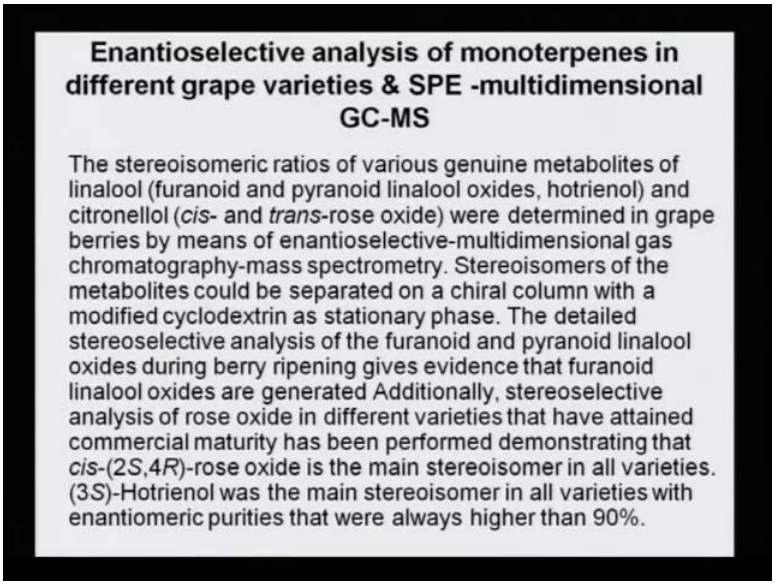

Enantioselective analysis of monoterpenes in different grape varieties and S P E multidimension G C M S: this is another example were G C M S was modified according to the requirement of the sample. The stereoisomeric ratios of various genuine

metabolites of linalool that is, a furanoid and pyranoid linalool oxides and citronellol which is the cis and trans rose oxide were determined in grape berries by means of enantioselective multidimensional gas chromatography with mass spectrometry stereoiosomers are not easy to detect. Let me tell you by a simple G C M S and so modifications had to be done according to the requirement.

Stereoisomeric isomers of the metabolites could be separated on a chiral column with a modified cyclodextrin as stationary phase. The detailed stereoselective analysis of the furanoid and the pyranoid linalool oxides during berry ripening, gives evidence that furanoid linalool oxides are generated additionally. Stereoselective analysis of rose oxide in different varieties that have attained commercial maturity have been performed demonstrating that the cis rose oxide is the main stereoisomer in all varieties. The hotrienol was the main stereoisomer in all varieties with enantiomeric purities that were always higher than 90 percent. So that, in order for the brewery to find out whether the grapes have ripened or not there should be a technique to find out whether the required compounds have formed in 90 percent and above and whether the stereoisomers which are most likely to be present in the ripened variety of grapes are present or not and that is how they will understand that whether it should be taken for the brewery exercise or not.

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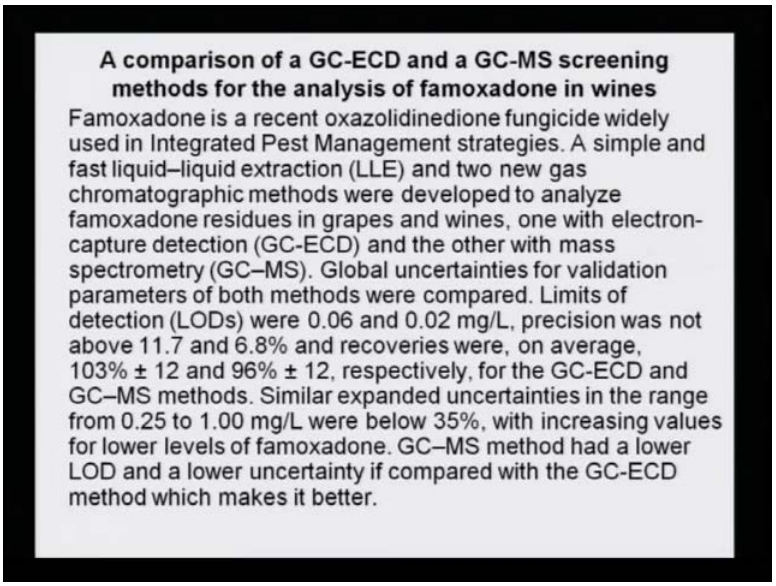

Similarly, a comparison of G C E C D and G C M S screening methods for the analysis of famoxadone in wines has been carried out. Now, unless and until we make two

methods simultaneously, we will not be able to say that G C M S is more superior to G C E C D. and so there was a method that I have chosen here particularly a compound called famoxadone. It is a recent oxazolidinedione fungicide widely used in integrated pest management strategies. A simple and fast liquid liquid extraction that is the L L E and the two new gas chromatographic methods were developed to analyze famoxadone residues in grapes and wines. It is important because, if pesticides are found in residues and if these grapes are used for making wines it is going to make a contaminated wine rather than pure wine. One with electron capture detection that is simultaneous analysis was carried out by G C E C D and the other with mass spectrometry that is the G C M S.

Global uncertainties for validation parameters of both methods were compared; limits of detection that is the L O Ds were 0.06 and 0.02 milligram per litre precision was not above 11.7 and 6.8 percent and recoveries were on an average 103 percent plus minus 12 and 96 percent plus minus 12, respectively, in the two methods. So, you see that they are almost comparable; however, G C M S is definitely more superior in terms of limiting the uncertainties for G C E C D and G C M S methods.

Similarly, expanded uncertainties in the range of 0.25 to 1.00 milligram per litre were below 35 percent with increasing values for lower levels of famoxadone. G C M S method had a lower limit of detection and lower uncertainty as you could see from the results given above. If compared with the G C E C D method which makes it better; so we do not just come to a conclusion by saying this is better because it is like this. It is better because analytical data speak for themselves.

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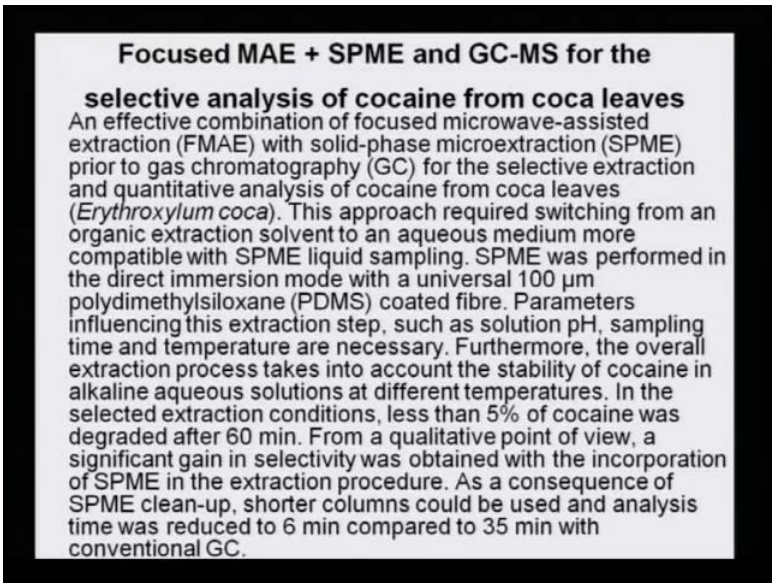

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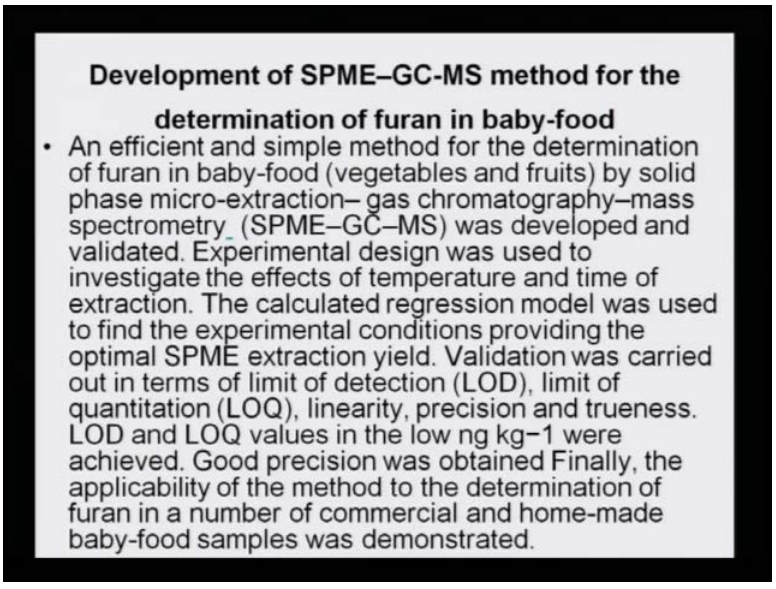

Now, when we come to another method of using focused M A E and using S P M E methods of extraction and when it is connected to G C M S, it definitely has a more advantageous situation selective analysis of cocaine from coca leaves and effective combination of focused microwave assisted extraction with solid phase micro extraction. And then, G C M S of the cocaine from coca leaves has been done in order to get very good results as compared to the conventional methods development of s p m e with G C M S method for the determination of furan in baby food. Again, G C M S when it is coupled with a special technique of extraction that is, the solid phase micro extraction S

P M E, it makes the process more and more sensitive and the extraction process is very effective and it was developed primarily because the amount of furan in baby food is very small or rather in trace quantity.

Experimental design was used to investigate the effects of temperature and time of extraction. The calculated regression model was used to find the experimental condition providing the optimal S P M E. Extraction yield validation was carried out in terms of limit of detection limit of quantitation, linearity precision and trueness and it speaks for itself; that as low as nano gram per kilogram could also be detected. So, we know that good precision was obtained finally the application of this method was the ideal for determining furan in baby food.