Advance Analytical Course

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Lecture No. # 07

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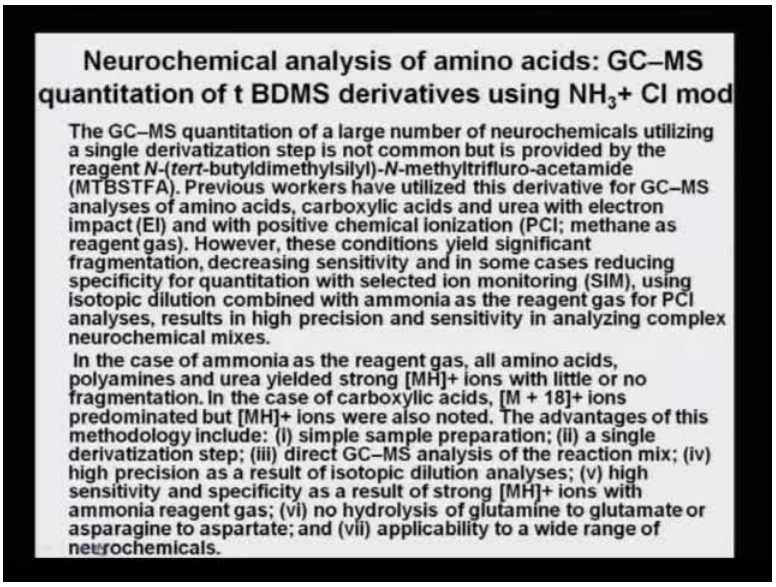

Another example, where more adaptation of GC-MS and a very specified use of GC-MS has been brought into notice is the neurochemical analysis of amino acids. And in many cases, derivatization are carried out, because the compound per say is not really a suitable material to be detected by GC-MS alone, and so, I have taken this particular analysis or example of the analysis of amino acids by quantification of its derivatives, that is the tertiary BDMS derivative using ammonia and CI mode.

We have seen so far that examples of EI methods were taken into consideration. So, where all are EI methods used? EI methods are used in most of the known compounds, unknown compounds, simple compounds, but under very special conditions, where very small quantity has to be analysed or if the analyte is not sensitive enough to generate molecular ion peak, the CI method must be used. And this is one such example, where I have brought to your notice, that even derivatization is a very crucial step in order to carry out specific GC-MS analysis.

The GC-MS quantitation for a large number of neurochemicals utilizing a single derivatization step is not a very common method, but it provides us by a reagent called N tertiary-butyldimethylsilyl N-methyltrifluro-acetamide that is MTBSTFA. Previous workers have utilized this derivative for GC-MS analysis of amino acids, carboxylic acids and urea with electron impact and with positive chemical ionization using methane as the reagent gas. However, these conditions yield significant fragmentation, decreasing sensitivity, and in some cases, reducing specificity for quantitation with selected ion monitoring, that is the SIM method, using isotopic dilution combined with ammonia as the reagent gas for PCI analyses, results in high precision and sensitivity in analyzing complex neurochemical mixes.

In the case of ammonia as the reagent gas, all amino acids, polyamines and urea yielded strong MH plus ion with little or no fragmentation. In the case of carboxylic acid, M plus 18 plus ions predominated, but MH plus ions were also noted. The advantage of this derivatization method includes that it is a simple sample preparation method, a single derivatization step is required, and thirdly, direct GC-MS analysis of the reaction mixture can be carried out, and fourthly, it has very high precision as a result of isotopic dilution analyses and finally, and fifthly, it is high sensitivity and specificity as a result of the strong MH ions with ammonia reagent gas.

So, one sees that there is no hydrolysis of glutamine to glutamate or no asparagine to aspartate and it is applicable to a wide range of neurochemicals. So, when it comes to analysis of neurochemicals, this particular method was developed precisely to get correct information about the sequencing of amino acids, and so, this tertiary BDMS derivatization was a very, very typical case in the case of amino acid analyses by GC-MS.

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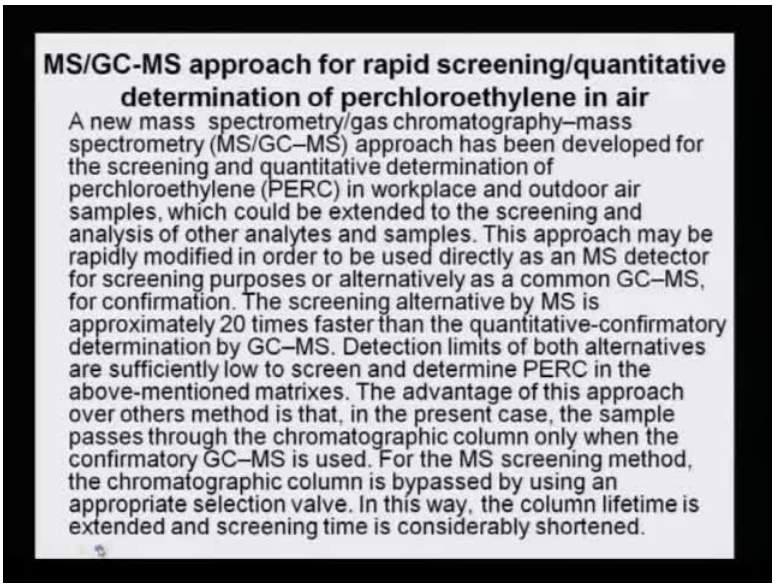

Similarly, another method that I have selected, where an MS and GC-MS approach for rapid screening and quantitative determination of perchloroethylene in air has been shown - a new mass spectrometry/gas chromatography - mass spectrometry. Now, you see MS and GC-MS have been hyphenated or connected. When we write it with this slash sign, it means that two machines have been combined. Approach has been developed for the screening and quantitative determination of perchloroethylene, that is, PERC in workplace, outdoor air samples, which could be extended to the screening of analysis of other analytes and other samples also.

It is not that only this compound can be screened and others cannot be screened by this method, but largely it has been developed for the screening of perchloroethylene. That is in abbreviation it is known as PERC and whether it is a workplace or an outdoor air, these samples can be collected and analyzed by this method.

This approach may be rapidly modified in order to be used directly as an MS detector for screening purposes or alternatively, as a common GC-MS method for just confirmation of the presence of this compound called the perchloroethylene. The screening alternative by MS is approximately 20 times faster than the quantitative confirmatory determination of the GC-MS method.

Detection limits of both alternatives are sufficiently low to screen and determine the perchloroethylene compound in the above-mentioned matrices. The advantage of this

approach over other method is that in the present case, the sample passes through the gas chromatographic column only, when the confirmatory GC-MS is used.

For the MS screening method, the chromatographic column is bypassed by using an appropriate selection valve. In this way, the column lifetime is extended and screening time is considerably shortened.

Again, there is a comparative method, whether a direct MS could be used for this perchloroethylene compound or whether a GC-MS method should be used for the analysis of this particular compound, which is very, very crucial in either indoor air or outdoor air, and the analysis must give confirmatory results; and it was found that both the methods are equivalent and are comparable, and one can carry out by either of them.

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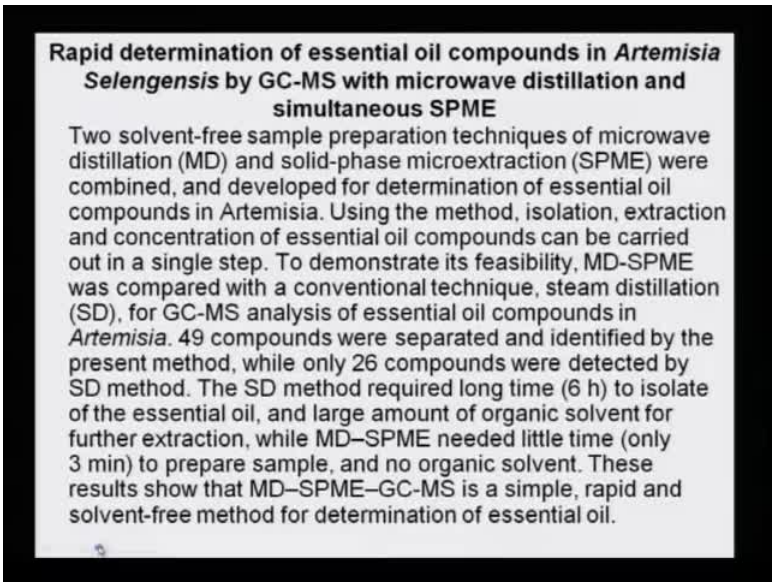

Similarly, when very rapid determination of essential compounds in Artemisia Selengensis by GC-MS had to be carried out, microwave distillation and simultaneous solid phase microwave extraction was carried out.

Now, you will see that in the previous lectures, when I was talking about different methods of extraction, we had taken into consideration and we had learned about the SPME method. So, without going into the details of the methodology of SPME, I would now just directly refer to this method, and why and how it was used in this particular essential oil determination.

Two solvent-free sample preparation techniques of the microwave distillation, that is, the MD, and the solid phase microwave extraction, SPME were combined and developed for determination of essential oil compounds in Artemisia. Using the method, isolation extraction and concentration of essential oil compounds can be carried out in just one single step. To demonstrate its feasibility, the MD-SPME was compared with a conventional technique. Why is it superior to the conventional technique? Unless and until as I told earlier also, we make a comparative data whether conventional is better method or whether this MD-SPME method is better, we will not be able to conclude which is superior. So, one needs to make this comparative data and to see which method is more applicable in the determination of essential oil in Artemisia.

To demonstrate its feasibility, the MD-SPME was compared with conventional technique steam distillation, for GC-MS analysis of essential oil compounds in Artemisia was carried out. 49 compounds were separated identified by the present method, while only 26 compounds were detected by the SD method; that is, the steam distillation method could only be able to recover 26 compounds whereas, the new method that is the microwave distillation followed by solid phase microwave extraction gave 49 compounds.

So, obviously the number of components that came out of simple, simple steam distillation was much higher as compared to the number of compounds, which were yielded by the MD-SPME method and so, the s d method required long time, 6 hours to isolate the essential oil, large amount of organic solvent was required because it is a steam distillation process it takes longer time, more solvent for further and further extraction and even then it was able to give only 26 compounds detailing and whereas, the other method gave us the detailing of 49 components.

Therefore, the MD-SPME needed only very little time, 3 minutes were required to extract and the sample preparation method was also not very difficult, no organic solvent was used and so, in all these parameters, MD-SPME method is much superior extraction method for essential oils of Artemisia as compared to the conventional method and the steam distillation method

The results show that the MD-SPME when connected to GC-MS is a simple, rapid and solvent free method for determination of essential oils. So, you have seen that how from

one method to another method, we are trying to progress and learn more and more intricacies of using the GC-MS and modifying the GC-MS, either from the extraction point of view or from the analysis point of view, where we are changing the EI or the CI mode.

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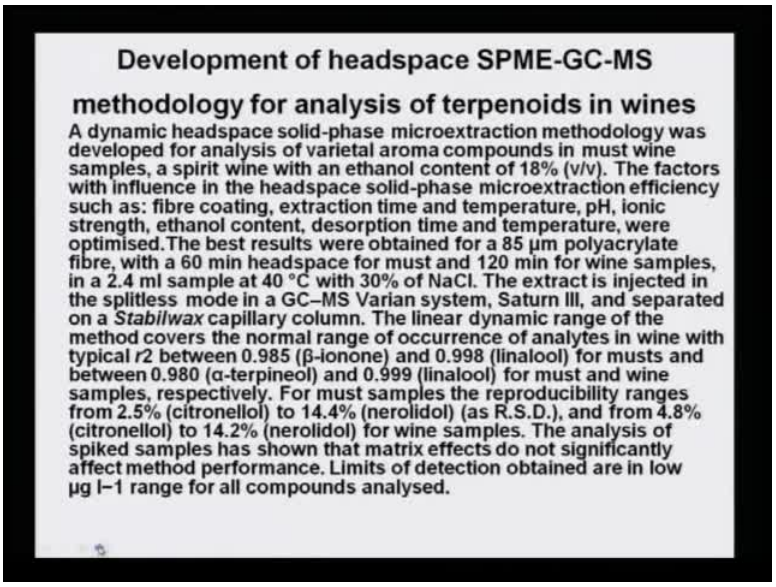

Development of headspace: SPME, when it is coupled with GC-MS, this is a very, very typical method for the analysis of terpenoids in wines. It is important to be able to know the exact quantitative description of wines, in terms of its terpenoid content and that is why a dynamic headspace solid-phase microwave extraction methodology was developed for analysis of varietal aroma compounds and it is a must in the case of wines because a spirit wine with an ethanolic content of 18 percent volume by volume, must have an edge over the terpenoid analysis.

The factors, which influence in the headspace solid-phase microextraction efficiency are the fibre coating, extraction time and temperature, pH, ionic strength, ethanolic content, desorption time and temperature. These parameters were all optimized. You see there are so many parameters: what is the fiber coating; what is the extraction time that is required; what is the temperature at which it is to be done; what is the pH of the wine at which this should be carried out; what is the ionic strength of the wine and how much is the ethanolic content; how much time does it take to desorb and at what temperature, the desorption takes place; then only, this method would be effectively used with GC-MS.

The best results were obtained for 85 micro polyacrylate fiber, with a 60 minutes headspace for must and a 120 minutes for wine samples, in a 2.4 ml sample at 40 degrees with 30 percent sodium chloride.

The extract is injected in the splitless mode in the GC-MS Varian system and was separated on the Stabilwax capillary column. These are the details of the machine on which the system was the analysis was carried out and Stabilwax is the name of that particular capillary column. The linear dynamic range of the method covers the normal range of occurrence of analytes in wine with typical r2 between 0.98 and 0.99 for musts and between 0.98 for alpha terpineol and linalool.

So, you can see that for most of these samples, the range was that it had 2.5 percent of citronellol to 14.4 percent of nerolidol and so on and therefore, one could find out whether this wine sample has enough terpenoids to be able to be used as good wines. Limits of detection obtained are in low range, that is, from micro gram per gram range for all compounds that are analysed.

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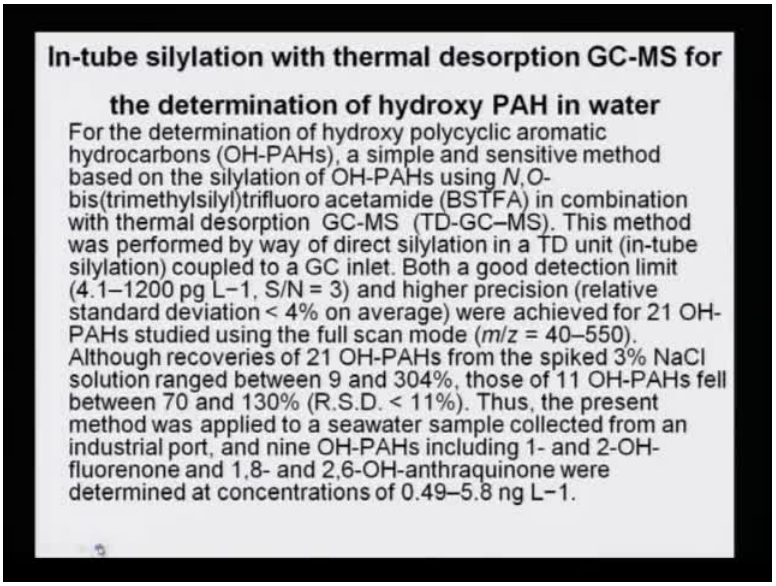

Another very typical case that I have selected, where GC-MS has been used extensively and that method is in-tube silylation with thermal desorption or in the GC-MS machine, for the determination of hydroxy PAH in water.

Now, what are PAH? PAH are poly hydrocarbons polycyclic aromatic hydrocarbons. Now, they are very notorious compounds because they are persistent organic pollutants. For the determination of hydroxy polycyclic aromatic hydrocarbons, that is OH-PAHs, a simple and sensitive method based on the silylation of OH part of the PAH using N, O-bis trimethylsilyl trifluoro acetamide - BSTFA in combination with thermal desorption at the GC-MS was carried out.

This method was performed by way of dirite I am sorry This method was performed by way of direct silylation in a TD unit, that is, in-tube silylation is carried out coupled with that GC inlet. So, everything is being done in a very compact and inserted manner. There is no big reaction flask, which are attached to the GC inlet. There is a small tube and there, the silylation reaction is carried out and that is connected because all the silyl compounds are very, very volatile. So, they come into the GC inlet very readily. Both a good detection limit, that is, 4.1 pico gram per litre sensitivity and very high precision was observed and achieved and almost 21 such hydroxy polycyclic aromatic hydrocarbons was studied using a full scan mode; that is, the m by z from 40 to 550 amu was covered by this particular machine organization.

Although recoveries of 21 hydroxy PAH from the spiked 3 percent sodium chloride solution ranged between 9 to 304 percent, those of 11 hydroxy PAH fell between 70 to 130 percent. Thus, the present method was applied to a seawater sample collected from an industrial port and 9 OH-PAH including 1 and 2 OH fluorenone and 1, 8 and 2, 6 hydroxy anthraquinone were determined at concentrations as low as 0.49 to 5.8 nano gram per litre. So, you can see that how precise is the silylation method and how accurately the hydroxy polycyclic aromatic hydrocarbons can be analysed through this specialized technique of GC-MS.

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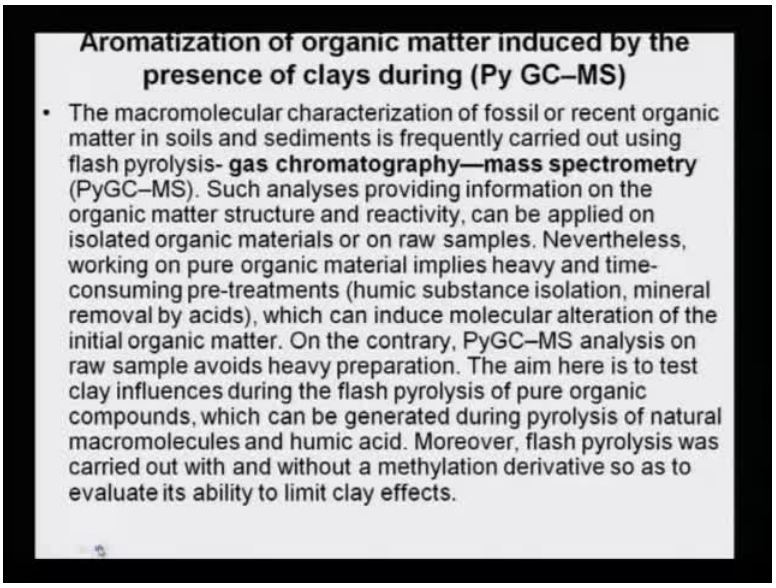

Aromatization of organic matter induced by the presence of clays during the pyrolysis GC-MS: Now, I am giving you the varieties of GC-MS and its adaptation for different samples so that you are aware of the fact that so many variations can be carried out by this particular method, which is broadly called the gas chromatography mass detector.

The macromolecule characterization of fossils or recent organic matters in soils and sediment is frequently carried out using flash pyrolysis gas chromatography mass spectrometry - that is PyGC-MS. Such analyses provide information on the organic matter structure and reactivity and can be applied on isolated organic materials or on raw samples. Nevertheless, working on pure organic material implies heavy and time consuming pre-treatments such as humic substance isolation, mineral removal by acids, which can induce molecular alteration of the initial organic matter.

Now, if all these procedures have to be carried out simultaneously, it is bound to make differences in the original material and to be able to avoid this, new method of pyrolysis GC-MS was introduced. On the contrary, the PyGC-MS analysis on the raw sample, it avoids heavy preparation time and difficult preparation. The aim here is to test the clay influences during the flash pyrolysis of pure organic compounds, which can be generated during pyrolysis of natural macromolecules and humic acid. Moreover, flash pyrolysis was carried out with and without a methylation derivative so as to evaluate its ability to limit clay effects.

Now, one can see that these methods that are actually devised are very, very specific and they have been carried out or they have been devised for a specific reason and for a specific compound, but this does not limits its use for only that compound. Although it was initially designed for that particular compound, but it can be used for similar types of compounds from time onwards.

So, methods that are developed from time to time are generally focused methods, very specific methods, but these methods can now be kind of an array of analysis methods, which could be also used for similar compounds. That is how research goes on because when the GC machine was first devised, nobody had any clue about any kind of machine that would come, with the passage of time, from the GC-MS machine, but there was a necessity which arose to find the new compounds and therefore, a mass detector was attached, instead of another detector like ECD or FID detector and it gave more information about the analyte. So, similarly, these advances in technology and in methodology are brought about for the ease of the analyst.

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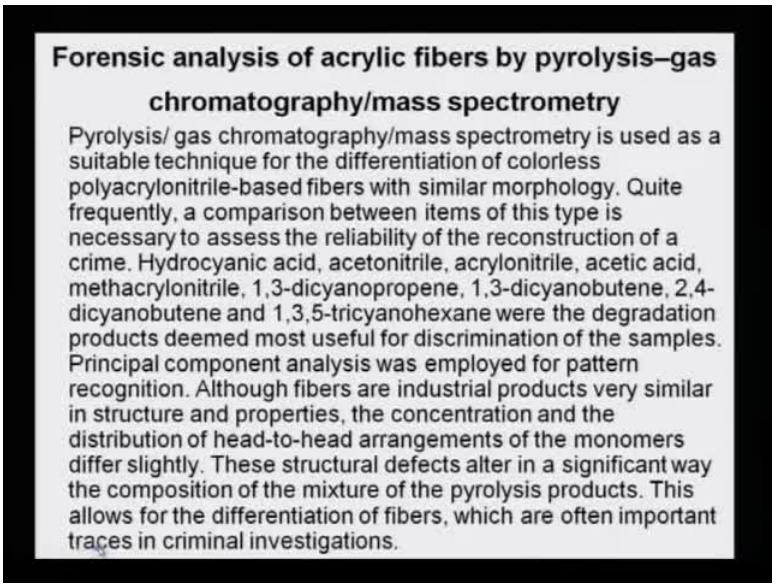

Another example that I have chosen is the forensic analysis of acrylic fiber by pyrolysis gas chromatography and mass spectrometry. Pyrolysis gas chromatography mass spectrometry is not only used as what I showed in the clay samples, but it is also a suitable technique for the differentiation of colourless polyacrylonitrile-based fibers with

similar morphology. Quite frequently, a comparison between items of this type is necessary to assess the reliability of the reconstruction of a crime.

Now, when we look at the forensic analysis, it has to be related to some kind of crime, where some sample has been procured and analysis of this particular forensic sample can give some clue as to how the crime has taken place, but I will not go into the detail of that part, but we will concentrate on the chemical analysis.

The comparison of hydrocyanic acid, acetonitrile, acrylonitrile, acetic acid, methacrylonitrile, 1, 3-dicyanopropene, 1, 3-dicyanobutene, 2, 4-dicyanobutene and 1, 3, 5-tricyanohexane were the degradation products deemed most useful for discrimination of the samples.

Now, when we have samples of acrylic fibers and a pyrolytic gas chromatographic analysis is carried out, these are the compounds like hydrocyanide - HCA or CH 3 CN – acrylonitrile, acetic acid, these are various components that would be deriving from a big macromolecule of acrylonitrile fiber and if these are present, that means it is a clue that it is an acrylonitrile fiber and that these components have come out of degradation.

Principle component analysis was employed for pattern recognition. Although fibers are industrial products, very similar in structure and properties, the concentration and the distribution of head to head arrangements of the monomers differ slightly. Now, when we have a macromolecule, it always is not that all the monomers are aligned in one particular manner. There could be some alteration in the arrangement of different monomers and that is what we get idea from the pyrolytic gas chromatography mass detector.

These structural defects alter in a significant way and the composition of the mixture of the pyrolysis products. This allows for the differentiation of fibers, which are often important traces in criminal investigation. So, depending on whether the sample was from the same origin or was from different origin and whether the monomers have a different pattern or the same pattern, gives a lot of clue and that is why it is used in forensic analysis.

So, you see, the versatility of GC-MS machine, be it clay, be it forensic samples, be it wine samples, be it terpenoid analysis various modifications and various adaptations help

us to use this particular universal machine for different types of samples and gives us very sensitive and very accurate results to be able to interpret the required information. So, with his we have come to an end of a very important detection machine which is called GC-MS. I would now like to draw your attention to the fact that GC-MS machine.

Let us now just re capture what we have learnt in this lecture. How is the GC machine connected to the MS; why is this detector more sensitive; what are the different types of compounds that can be analysed and what are the different types of adaptations in extractions, which need to be taken care in terms of doing a very specific GC-MS analysis.

So, we go one by one. GC-MS is a machine, which is a higher and more sensitive machine than the GC with a FID or ECD detector. This we have seen by making comparative data that the sensitivity results of GC-MS is far more higher than the GC simple with that ECD or FID detector.

Now, mass detector, where it joins the GC machine is called the interface and the mass detector is the most sensitive part of this machine. The GC part remains as common, whether it is our GC with the FID or ECD detector or a GC with the MS detector, the GC part remains the same. There is an inlet, there is a column, there is an oven and the compound is made to go through this column with the help of a carrier gas so that there is proper separation on the column; that part remains the same.

Once the compounds that are separated on the column enter the mass detector, that point is called the interface and when it enters the mass detector, there are three discrete regions in the mass detector: one is the analyzer, the first part is the ionizer, second is the analyzer and the third part is the detector. That means, the separated compound, when it enters the ionizer, there is a beam of electron of 70 electro volts, which is continuously bombarding that compound and because of this, the molecular ion peak is generated and when the molecule is then further on bombarded, the daughter nuclei or the daughter ions are formed and this fragmentation pattern for a compound is always the same. It will not choose to break from anywhere and everywhere according to this electron impact method.

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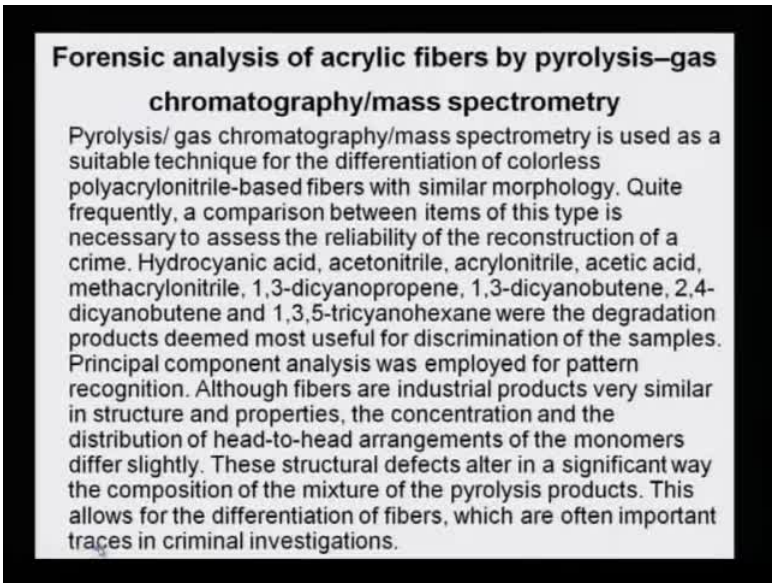

However, in some cases as what I mentioned that electron impact may not give the molecular ion peak. There it is necessary to have another alternated mode and that is called chemical ionization method, where methane gas or ammonia gas are used and then molecular ion peak can be traced, but the CI method is less sensitive method as what I mentioned and so, it is just a supplementary or a complementary method.

However, we should mostly follow the EI method. Now, after the ions are formed, the ions are made to move towards the detector and by the sizes, the smallest will reach first, then medium size ion will reach and then the larger ions will reach the detector and then this detector, then amplifies the signals and gives us the spectrum of the compound.

Now, when we go backward, that is, molecular ion peak is the stand point. From there, we move on and see how the fragments had broken. That gives us an idea about the molecular structure of the compound and depending on different types of compounds that need to be analysed by the GC machine, their extraction methods need to be varied so that most and more compounds can be extracted. The better the extraction, the better would be the analytical result and so, it always must be chosen that the extraction method must go hand in hand with the analytical tool that we are using.

Just the way, we cannot use very readily packed columns of the GC, in a GC-MS machine, only capillary columns are recommended. Similarly, we need to make certain adaptations when we are analyzing compounds on GC-MS. So, these were certain points

that I thought would be of reference so that you know what GC-MS machine looks like and works like.