Advance Analytical Course

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Lecture No. # 25

The principle and construction of ICP-MS – now, how does this machine look like? What are the different parameters that have to be kept in mind? The operating conditions are very important.

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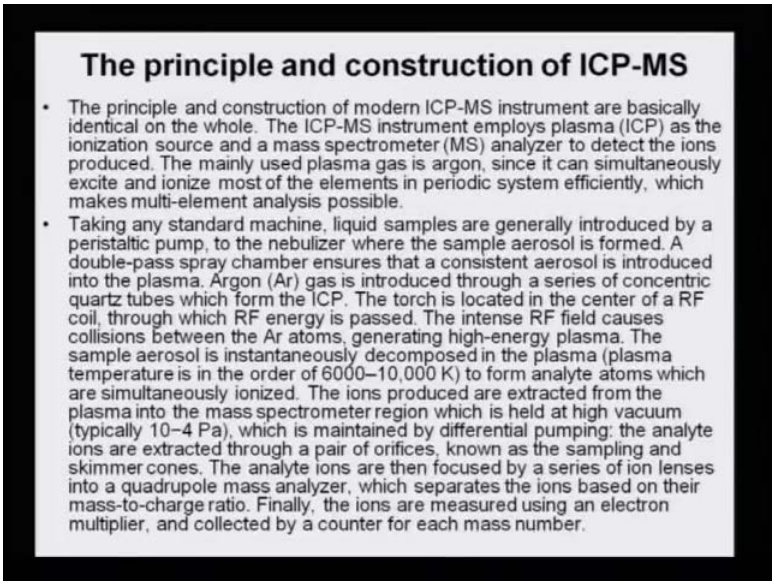

The principle and construction of modern ICP instrument are basically identical on the whole. The ICP-MS instrument employs plasma, that is, the ICP as the ionization source and a mass spectrometer (MS) analyzer to detect the ions produced. So, basically, it is a machine, which has a plasma; that is why the name ICP. And, it has a mass analyzer; that is why the MS nomenclature. It mainly uses plasma gas as argon, since it can simultaneously excite and ionize most of the elements in the periodic system efficiently, which makes multi-element analysis possible. So, what is the advantage of using the argon plasma? It is because it can ionize and excite many elements in a multi-element system very easily and efficiently.

Taking any standard machine, liquid samples are generally introduced by a peristaltic pump to the nebulizer, where the sample aerosol is formed. A double-pass spray chamber ensures that a consistent aerosol is introduced into the plasma. So, what does it mean? That there should be a sample pumping system, preferably a peristaltic pump, which then pushes the solution or the analyte through a nebulizer into a spray chamber. And, this process should be very smooth and efficient. And, thus the sample introduction must be very fissile. Argon gas is introduced through a series of concentric quartz tubes, which form the inductively coupled plasma. There are quartz tube one inside the other. Actually, the diameters are slightly different and three such concentric quartz tubes are put together, through which the argon gas is passed. And, just above these concentric quartz tubes, the flame or the plasma shapes up.

The torch is located in the center of the RF of the quartz tube and the coil, through which the RF energy is passed. The intense RF field causes collisions between the argon atoms, generating high energy plasma. The sample aerosol is instantaneously decomposed in the plasma, because the plasma temperature is of the order of 6000 to 10000 kelvin to form analyte atoms, which are simultaneously ionized. So, you see that all these take place very efficiently, because of such a high temperature that the plasma maintains between 6000 to 10000 kelvin. The ions produced are extracted from the plasma into the mass spectrometer region, which is held at high vacuum (typically at 10 to the power of minus 4 Pa), which is maintained by differential pumping; the analyte ions are extracted through a pair of orifices, known as the sampling and skimmer zones.

The analyte ions are then focused by a series of ion lenses into the quadrupole mass analyzer, which separates the ions based on their mass-to-charge ratio. Finally, the ions are measured using an electron multiplier, and collected by a counter for each mass number. So, the latter part of the mass spectrometer remains the same. There is a quadrupole, where the analyzer acting as an analyzer, and all these analytes when they coming contact with this, are actually made to pass through two types of orifices, that is, two types of apertures. And, these apertures are called the sampling and skimmer cones. And, when these are then focused through lenses into the quadrupole mass analyzer one-by-one as per the mass-by-charge ratio, they are reaching the detector and getting noticed or getting detected.

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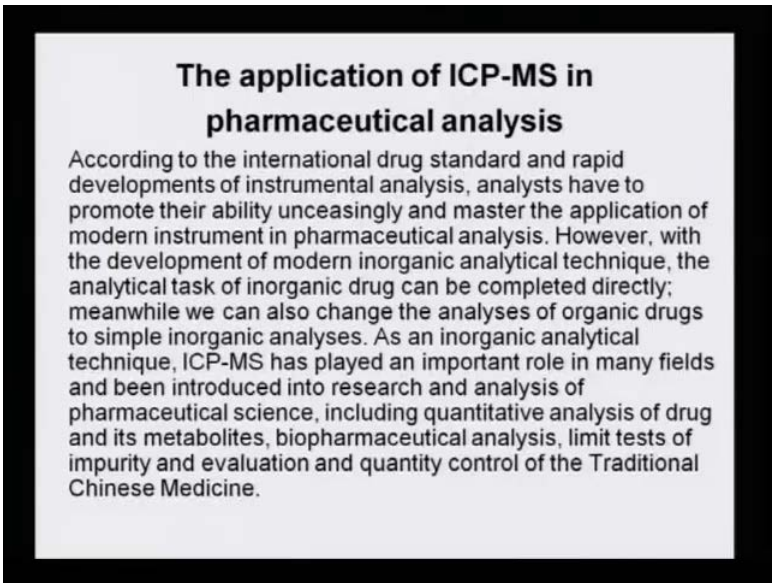

The application of ICP-MS in pharmaceutical analysis – time and again, I have said that ICP-MS is the ultimate machine. Why, because it can do a variety of analysis with very high sensitivity and great precision. According to the international drug standard and rapid developments of instrumental analysis, analysts have to promote their ability unceasingly and master the application of modern instrument in pharmaceutical analysis. However, with the development of modern inorganic analytical technique, the analytical task of inorganic drug can be completed directly; meanwhile, we can also change the analyses of organic drugs to simple inorganic analysis. As an inorganic analytical technique, ICP-MS has played an important role in many fields and has been introduced into research and analysis of pharmaceutical sciences, including quantitative analysis of drug and its metabolites, biopharmaceutical analysis, limits the tests of impurity whether the pharmaceutical preparation is pure or not, and evaluation and quantity control of the Traditional Chinese Medicine, the TCM. Now, it has a varied application. You will see that it not only tries to look at how the drug and its degraded metabolites are in this biological system; and, how these biopharmaceutical analysis can be achieved; then, how it can also help in finding out whether the drug is pure or it has some impurity, and so on and so forth. So, it has a variety of users in pharmaceutical analysis.

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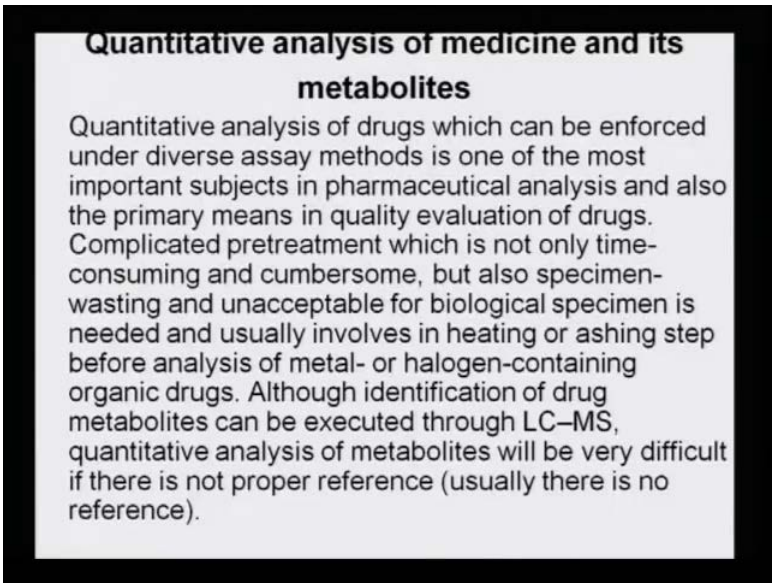

Quantitative analysis of medicines and its metabolites – just now, a while ago, I mentioned that metabolites and the main medicine can all be analyzed from the same sample. Quantitative analysis of drugs, which can be enforced under diverse assay methods is one of the most important subjects in pharmaceutical analysis and also the primary means in quality evaluation of drugs. Complicated pretreatment, which is not only time consuming and cumbersome, but also specimen-wasting and unacceptable for biological specimen is needed and usually involves in heating or ashing step before analysis of metal- or halogen-containing organic drugs. Although identification of drug metabolites can be executed through LC-MS as well, quantitative analysis of metabolites will be very difficult if there is not a proper reference; usually, there is no reference sometimes for these metabolites. So, you see that ICP-MS verses LC-MS – these organic compounds can be analyzed on both, but sometimes there are no reference standards for the metabolites. And then, to identify them becomes a big problem, which can be achieved in a quantitative manner by using ICP-MS. So, there the LCP-MS lags behind.

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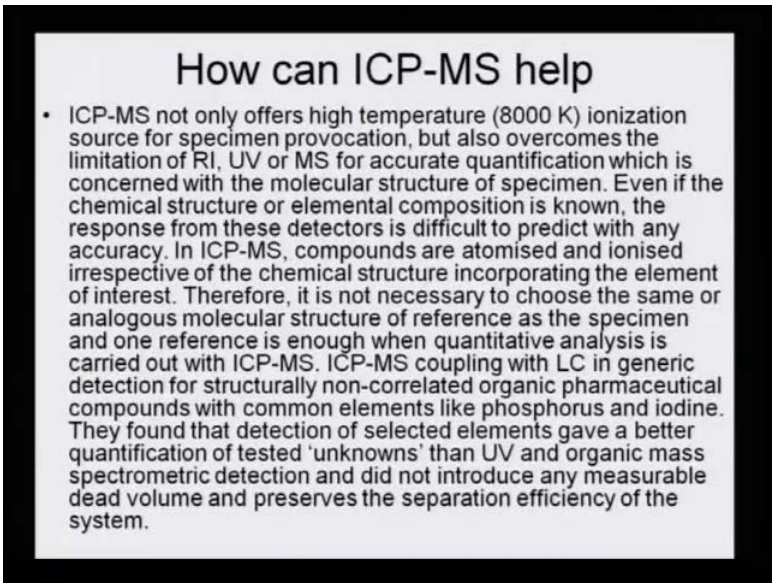

How can ICP-MS help – now, let us try to understand that why the ICP-MS has an edge over LC-MS, and how does it differ in its functionality to be able to quantify the metabolite very appropriately. ICP-MS not only offers high temperature, that is, 8000 kelvin ionization source for specimen provocation, but also overcomes the limitation of RI, UV and MS for accurate quantification, which is concerned with the molecular structure of the specimen. Now, what happens is that some molecules are UV active; so, they will be detected by UV. We are talking in terms of LC-MS detector and the ICP-MS, which has a MS detector anyway. So, in LC, we have RI detector, UV detector and MS detector, but some compounds are not sensitive enough on the RI or the UV. RI is refractive index detector; and, thus molecular structures have to plays a big role. Whereas, ICP-MS is totally independent of whatever be the structure. It is independent for its structural detailing, and therefore, unprecedentedly or without any fail, it can be identified very correctly.

Even if the chemical structure or elemental composition is known, the response from these detectors is difficult to predict with any accuracy. In ICP-MS, compounds are atomized and ionized irrespective of the chemical structure incorporating the element of interest. Therefore, it is not necessary to choose the same or analogous molecular structure of reference as the specimen and one reference is enough when quantitative analysis carried out with ICP-MS. So, it is now very clear – why does LC-MS lag behind and why is ICP step ahead of LC-MS. The simple reason is that here, we are not

concerned with the molecular's entity, what is the structural detail, because whatever be the structural detail, it can identify, quantify and give a very precise data whatsoever be the structure.

The ICP-MS coupling with LC is generic detection for structurally non-correlated organic pharmaceutical compounds with common elements like phosphorus and iodine. They found that detection of selected elements gave a better quantification of tested unknowns than UV or organic mass spectrometric detection and did not introduce any measurable dead volume and preserves the separation efficiency of the system. However, if an LC is attached to IC-MS, that is the best configuration. Why, because the LC does the separation and then it is introduced, and all the element detailing can be carried out even when inorganic compounds are present if they can be analyzed on the ICP-MS, such as phosphorus and iodine, which are coupled some time with the organic pharmaceutical compounds.

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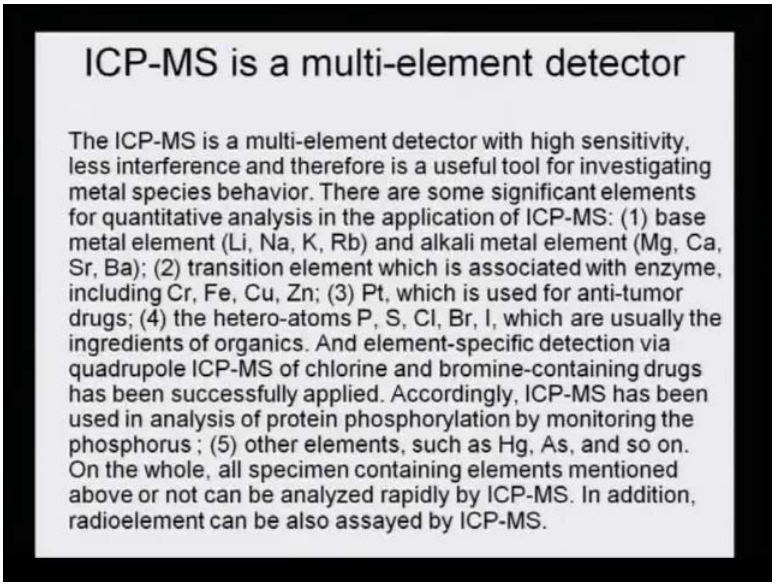

ICP-MS is a multi-element detector – time and again, I have been saying that many metals can be simultaneously analyzed unlike atomic absorption, where it goes one-by-one only. The ICP-MS is a multi-element detector with high sensitivity, less interferences and therefore, is a useful tool for investigating metal species behavior. There are some significant elements for quantitative analysis in the application of ICP-MS. The first is that basic metal elements like lithium, sodium, potassium, rubidium and

alkali metal elements, such as magnesium, calcium, strontium, barium. Then, second class is transition element, which is associated with enzymes including chromium, iron, copper, zinc. Then, the third class is platinum, which is used for anti-tumor drugs. The fourth class is the hetero-atom containing compounds, such as phosphorus, sulfur, chloride, bromide, iodide, which are usually the ingredients of organics. And, element-specific detection via quadrupole ICP-MS of chlorine and bromine-containing drugs has been successfully applied.

Accordingly, ICP-MS has been used in analysis of protein phosphorylation by monitoring the phosphorus; other elements, such as mercury, arsenic, and so on. So, there can be very specific methods by which specific elements like the protein phosphorylation can be monitored, and even the analysis of trace quantities of mercury and arsenic can also be detected by ICP-MS. On the whole, all specimens containing elements mentioned above or not can be analyzed rapidly by ICP-MS. In addition, radioelements can be also assayed by ICP-MS. So, you see, there is a great versatility from every type of samples, from every sphere of life; whatever be the sample type – pharmaceutical sample, biological sample, forensic sample, environmental sample, ceramic sample, alloy sample – all can be detected by ICP-MS very efficiently and precisely.

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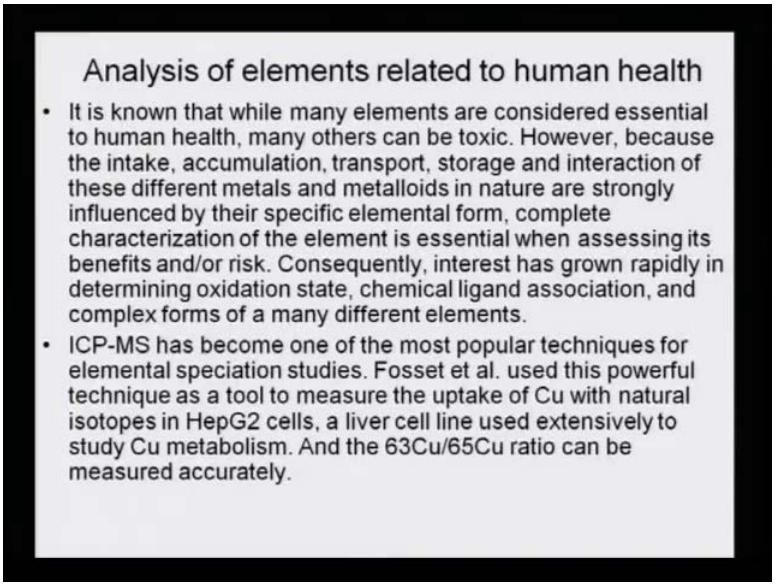

Analysis of elements related to human health – now, therefore, the metals that cause human hazard or health hazard to human beings are of prime importance for their analysis. It is known that while many elements are considered essential to human health, many others can be toxic. So, both essentials as well as toxins can be analyzed on ICP-MS. However, because the intake, accumulation, transport, storage and interaction of these different metals and metalloids in nature are strongly influenced by their specific elemental form, complete characterization of the element is essential when assessing its benefits or/and its risk. So, you see, how do we understand that a particular element is our friend or a particular element is a toxin and is our enemy. It is through this very precise analytical method that one is able to know that a particular quantity is helpful for human health and otherwise, it is a toxin for human health. Consequently, interest has grown rapidly in determining oxidation state, chemical ligand association, and complex forms of many different elements.

ICP-MS has become one of the most popular techniques for elemental speciation studies. Fosset et al. use this powerful technique as a tool to measure the uptake of copper with natural isotopes in HepG2 cells in a liver cell line used extensively to study the copper metabolism. And, it also showed that 63 copper, that is, the isotopic distribution of 63 copper and 65 copper ratios can also be measured accurately. So, you now see that isotopic ratios can be found out and also, certain very precise copper isotopes in liver cells can also be analyzed with high accuracy by this method of ICP-MS.

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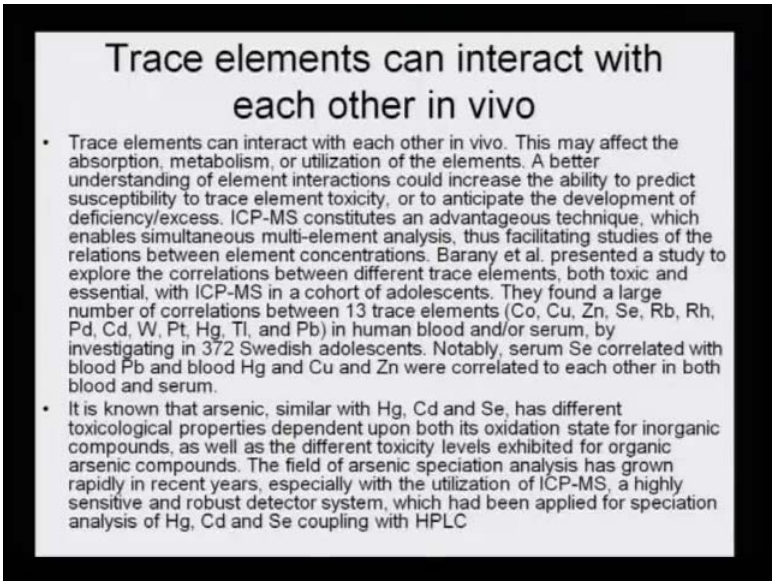

Trace elements can interact with each other in vivo – Trace elements can interact with each other in vivo. This can affect the absorption, metabolism, or utilization of the elements. A better understanding of the element interactions could increase the ability to predict susceptibility to trace element toxicity, or to anticipate the development of deficiency or excess. ICP-MS constitutes an advantageous technique, which enables simultaneous multi-element analysis, thus facilitating studies of the relations between element concentrations. Barany et al. presented a study to explore the correlations between different trace elements, both toxic and essential, with ICP-MS in a cohort of adolescents. They found a large number of correlations between 13 trace elements, such as cobalt, copper, zinc, selenium, rubidium, Rh, palladium, cadmium, tungsten, platinum, mercury, Tl and lead in human blood and/or serum by investigating in 372 Swedish adolescents. Now, they took 372 young people and tried to look at their blood and serum, and analyze these 13 trace elements. Notably, serum selenium correlated with blood lead and blood mercury and copper and zinc were correlated to each other in both blood and serum. So, this kind of correlation studies also could be done with both toxic as well as non-toxic or essential elements in the body fluid.

It is known that arsenic, similar with mercury, cadmium and selenium, has different toxicological properties dependent upon both its oxidation state for inorganic compounds as well as the different toxicity levels exhibited for organic arsenic compounds. So, you see that very intricate analysis of arsenic and its similarity with mercury cadmium and selenium has also been emphasized. The field of arsenic speciation analysis has grown rapidly in recent years – why, because it has been found to be one of the major toxins, and that is why lot of attention has been given for the analysis and very precise analysis of arsenic – especially with the utilization of ICP-MS, a highly sensitive and robust detector system, which has been applied for speciation analysis of mercury, cadmium and selenium coupling with HPLC; so, many methods have either analysis direct on ICP-MS, or it is first separated on LC or HPLC and then analyzed on ICP-MS. So, this kind of in-vivo study also is possible for trace element detection by this versatile machine, which is called ICP-MS.

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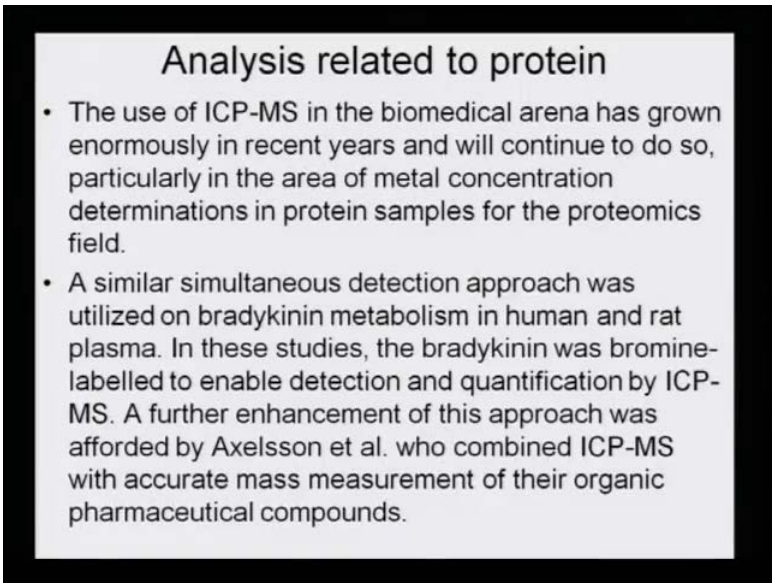

Analysis related to protein – the use of ICP-MS in the biomedical arena has grown enormously in recent years and will continue to do so, particularly in the area of metal concentration, determinations in protein samples for the proteomics field. Now, you see, each field requires specialized analytical method. And, this is one of the methods by which the protein analysis having metal concentrations can also be determined very efficiently by the use of ICP-MS.

A similar and simultaneous detection approach was utilized on bradykinin metabolism in human and rat plasma. In these studies, the bradykinin was bromine-labelled to enable detection and quantification by ICP-MS. A further enhancement of this approach was afforded by Axelsson et al. who combined ICP-MS with accurate mass measurement of their organic pharmaceutical compounds. So, it was possible to do that with the help of ICP-MS. And, such complicated biological systems having such complicated protein and the metal being embedded in that, all could be done very efficiently and precisely.

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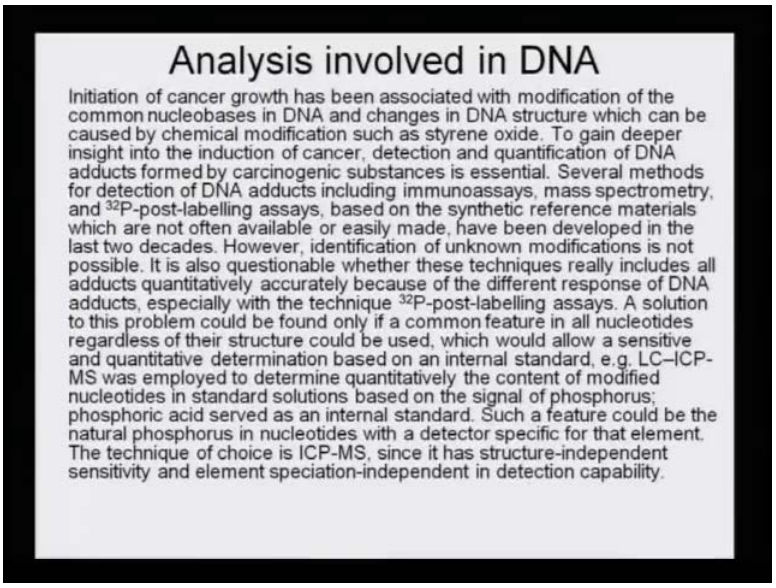

Analysis involved in DNA – even so much so that, it can go down to very small genetic material, the DNA molecule. Initiation of cancer growth has been associated with modification of the common nucleobases in DNA and changes in DNA structure, which can be caused by chemical modification, such as styrene oxide. To gain deeper insight into the induction of cancer, detection and quantification of DNA adducts formed by carcinogenic substances is essential. Several methods for detection of DNA adducts including aminoassay, mass spectrometry and phosphorous 32-post-labelling assays, based on the synthetic reference materials, which are not often available or easily made, have been developed in the last two decades. So, there have been methods for detection, but these are very cumbersome methods or require some very intricate reference standards, which may or may not be available all the time. And, that makes these methods not so popular. So, what is the answer? The answer is that can we use ICP-MS in this case.

However, identification of unknown modification is not possible. It is also questionable whether these techniques really includes all the adducts quantitatively accurately because of the different responses of DNA adducts, especially with the technique 32 phosphorous-post-labelling assays. A solution to this problem could be found only if a common feature in all nucleotides to this problem could be found as a regardless of their structure could be used, which would allow a sensitive and quantitative determination based on an internal standard; that is, liquid chromatography could be coupled with ICP-

MS, and this method can determine quantitatively the content of the modified nucleotides in standard solution based on the signals of the phosphorous; the phosphoric acid served as an internal standard. Such a feature could be natural phosphorous in nucleotides with a detector specific for that element. Thus, this technique is the choice, because ICP-MS what it can do, other machines cannot do such an efficient and precise detection, since it has structure-independent sensitivity and element speciation-independent in detection capability. So, it has this versatility, where it can detect very efficiently even in biological, very intricate molecules of nucleobases of DNA. The actual initiation of cancer growth can be determined due to this butogenic changes or chemical changes that are brought about in the genetic material. So, ICP-MS is the answer for it.

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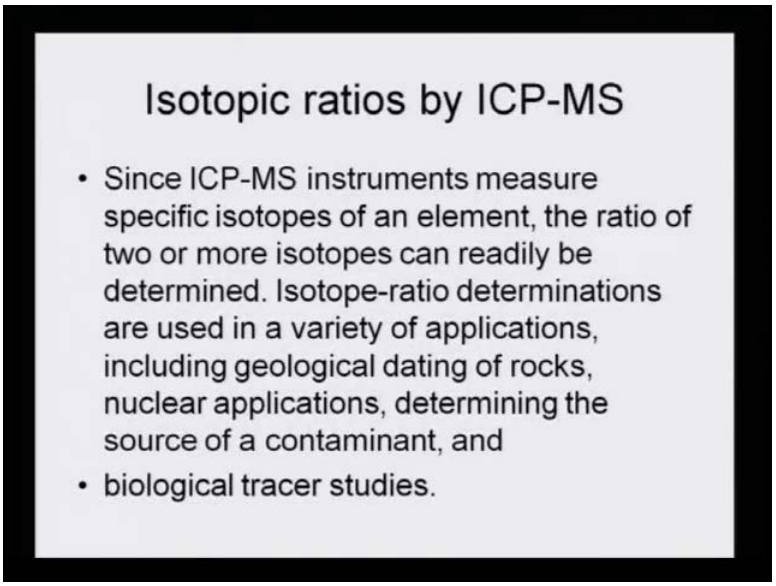

Isotopic ratios by ICP-MS also can be detected. Since ICP-MS instruments measure specific isotope of element, the ratio of two or more isotopes can readily be determined. I just gave you an example of copper 63 and copper 65 being analyzed from a protein system, and that was possible, because both copper 63 and copper 65 coexists in the system, and the isotopic ratio can be determined.

Isotopic ratio determinations are used in a variety of applications; not only in biological system, but also in geological dating of rocks; nuclear applications, that is, when a nuclear material starts emitting alpha, beta, gamma rays, what is its present situation

could also be determined, because of its isotopic ratio analysis possibility by ICP-MS. Determining the source of contaminant and biological tracer studies, all can be covered by this isotopic ratio by ICP-MS machine.

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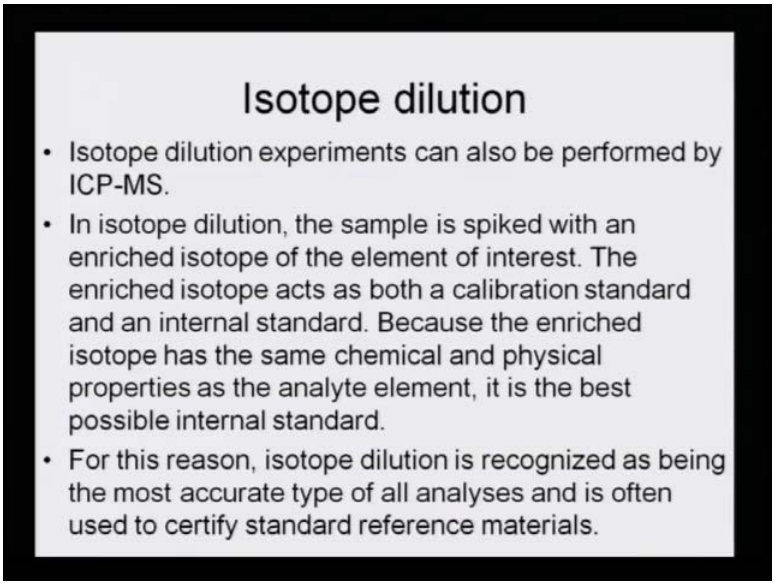

Similarly, there is possibility of even taking care of isotope dilution. Suppose if it is at levels, which are much lower, then how do we detect these isotopes by ICP-MS? The isotope dilution experiments can also be performed by ICP-MS. In isotope dilution, the sample is spiked with an enriched isotope of the element of interest. The enriched isotope acts as both as a calibration standard as well as an internal standard. Because the enriched isotope has the same chemical and physical properties as the analyte element, it is the best possible internal standard. That is the reason why enrichment experiments with an isotopic dilution is possible; why, because one particular isotope is spiked, is added additionally into the sample. So, that particular isotope will get enhanced in its concentration. So, it will behave both like a calibration standard as well as an internal standard. For this reason, isotope dilution is recognized as being most accurate type of all analyses and is often used to certify standard reference materials. So, this is also another very useful methodology, where isotopic dilutions are possible.

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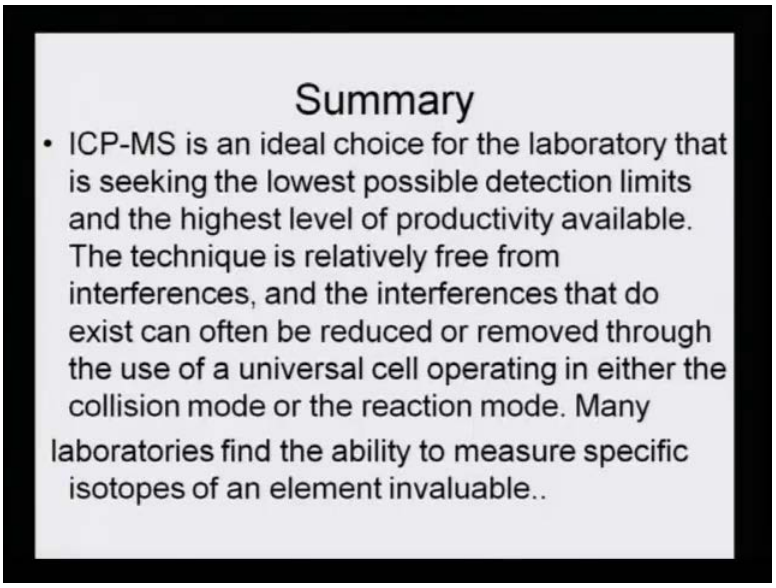

If we have to summarize and if we have to talk highly about this particular instrument, then we may surely be speaking that ICP-MS is an ideal choice for the laboratory that is seeking the lowest possible detection limits and the highest level of productivity available. The technique is relatively free from interferences, and the interferences that do exist can often be reduced or removed through the use of universal cell operating in either the collision mode or in the reaction mode. Many laboratories find the ability to measure specific isotopes of an element invaluable. So, you see, it is such a useful machine that it is recommended that every laboratory should have an ICP-MS, because it not only has the lowest possible detection limit, but it has the highest productively limit; any type of sample from any sphere of life can be analyzed very efficiently.

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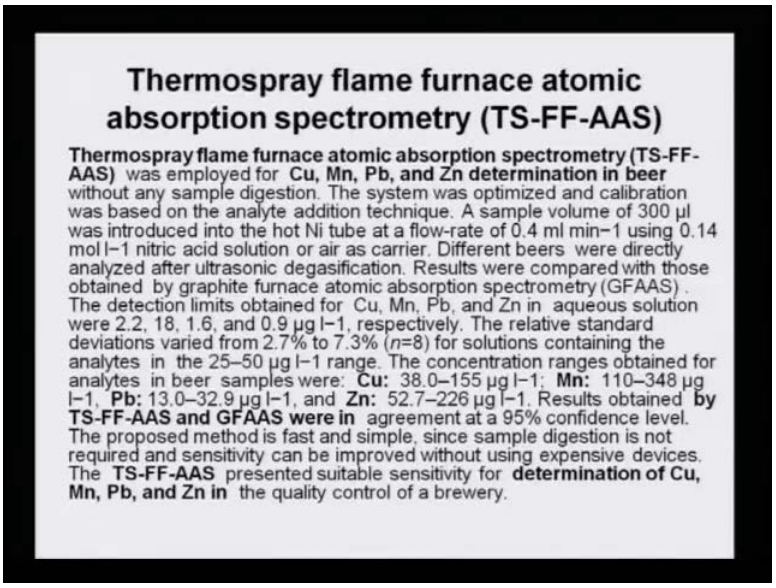

Now, we move on to a very interesting analysis of direct determination of copper, manganese, lead and zinc in drinks by thermospray flame furnace atomic absorption spectrometry. Now, I am giving you another very versatile method; why, because this particular type of thermospray flame was not mentioned or not taught to you in the previous few lectures, which I was covering about the atomic absorption spectrometry or ICP. The TS-FF-AAS (thermospray flame furnace atomic absorption spectrometry) was employed for copper, manganese, lead and zinc determination in beer without any sample digestion. Now, this is a unique case, where the beer sample was not digested, but directly it was analyzed. The system was optimized and calibration was based on the analyte addition technique. A sample volume of 300 microliter was introduced into the hot nickel tube at a flow rate of 0.4 ml per minute using 0.14 mole per liter nitric acid solution or air as carrier. Different beers were directly analyzed using ultrasonic degasification. Results were compared with those obtained by graphite furnace atomic absorption spectrometry, that is, GF-AAS.

The limits of detection obtained for copper, manganese, lead and zinc in aqueous solution were 2.2, 18, 1.6 and 0.0 microgram per liter, respectively. The relative standard deviation varied from 2.7 percent to 7.3 percent for solutions containing the analytes in the 25 to 50 microgram per liter range. The concentration ranges obtained for analytes in beer samples were: for copper, it was 38 to 155 micrograms per liter; manganese was 110 to 348 micrograms per liter; lead was 13 to 32.9 micrograms per liter; and zinc was

52.7 to 227 micrograms per liter. Results obtained by TS-FF-AAS and GF-AAS were in agreement at a 95 percent confidence level. The proposed method is fast and simple, since sample digestion is not required and sensitivity can be improved without using expensive devices. The TS-FF-AAS, that is, the thermo spray flame furnace atomic absorption spectrometry presents suitable sensitivity for the determination of copper, manganese, lead and zinc in quality control of the brewery industry.

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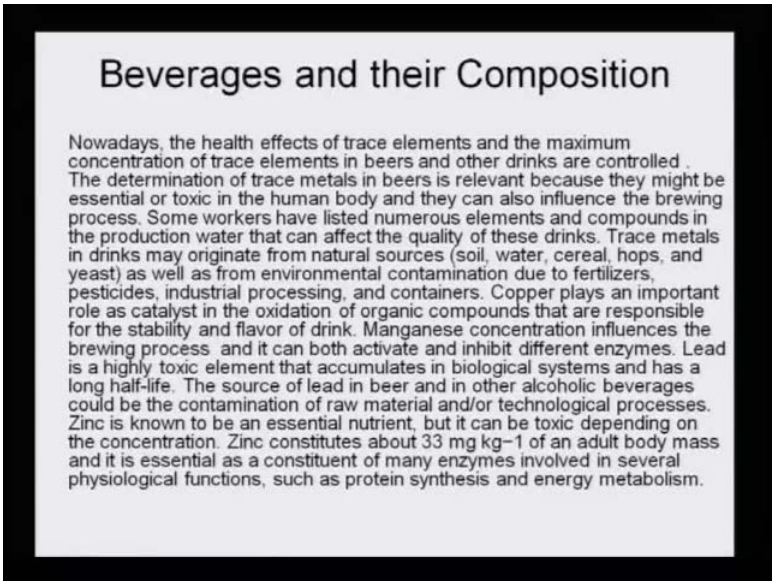

Beverages and their composition – sometimes, these metals can have a very complicated health effect, and these health effects of trace elements and their maximum concentration of trace elements in beer and other drinks are controlled, because brewery is a very important industry in all over the world. And therefore, the determination of trace metals in beers is relevant, because they might be essential or toxic in human body and they can also influence the brewing process. Some workers have listed numerous elements and compound in the production of water that can affect the quality of these drinks. Trace metals in drinks may originate from natural sources, that is, from the soil, water, cereal, hops, and from the pesticide industrial processing, and containers.

Copper plays an important role as catalyst and adds flavor to the drink. Manganese concentration influences the brewery process, and it can both activate as well as inhibit different enzymes. Lead is highly toxic element that accumulates in the biological system and has a long half-life. The source of lead in beer and in other alcoholic beverages could

be the contamination of raw material and/or technological processes. So, it can come from either source. Zinc is known to be an essential nutrient, but it can be toxic depending on the concentration. Zinc constitutes about 33 milligrams per kilogram of an adult body mass and it is essential as a constituent of many enzymes involved in several physiological functions, such as protein synthesis and energy metabolism.

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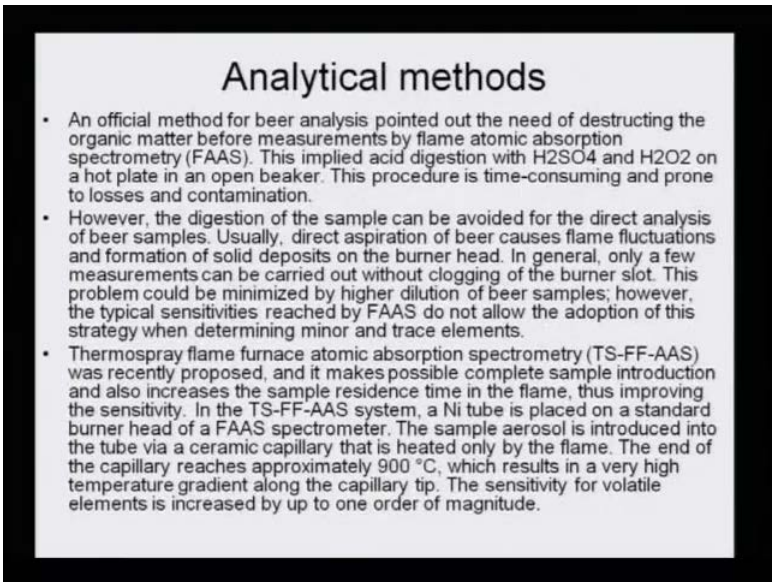

Analytical methods – therefore, has to be very specific in this. And so, how are beer samples analyzed? The thermospray furnaces atomic absorption was recently proposed, and it makes possible complete sample introduction and also increases the sample residence time in the flame, thus improving the sensitivity. In the TS-FF-AAS system, a nickel tube is placed on a standard burner head of the flame atomic absorption, and that is what the new adaptation is. And, this nickel tube can have reached the temperature as high as 900 degrees centigrade. So, that increases the sensitivity of this particular machine to be able to analyze all these three metals together simultaneously without any digestion step.