LECTURE-23

HYBRID-MS/MS CONFIGURATIONS

TRANSCRIPT

Welcome to proteomics course. Today we will talk about Hybrid-MS/MS configurations. As we have discussed that there are different types of mass spectrometers available and depending upon the mass analyzers how can they be used together? Different type of MS/MS configurations hybrid-MS/MS have emerged. So we have discussed that different type of hybrid MS or tandem MS can be used for various proteomic applications.

There are several new advancements which have happened in this field and to keep up the pace for recent advancements, in today's lecture I thought to involve a discussion on hybrid MS/MS configurations and talk with two leading companies one with Agilent technologies for Q-TOF and QQQ (triple quadrupole) as well as chip technology and then with ThermoFisher about orbitrap technology.

So we will discuss about quadrupole-time of flight and triple quadrupole and chip technology. What are the advantages of using hybrid configurations? What is the latest chip technology? How it can be used to overcome several limitations of HPLC-based method, liquid chromatography which is used prior to the ionization method? So I will discuss these things with one of the leading application expert from Agilent Mr. Abhijeet and during the short interview we will try to provide you an overview of different type of latest configurations available and what are their advantages?

A- Prof. Sanjeeva Srivastava   B- Mr. Abhijeet

A- It is my pleasure to introduce Mr. Abhijeet who is product specialist in the mass spectrometry division of Agilent technologies in India. So welcome Abhijeet for this brief discussion session on mass spectrometry and your experience in this area. So first of all, how long have you been working mass spectrometry division of Agilent?

B- I have spent almost 9 years in MS field, out of which I am completing 4 year in Agilent technologies to take care of Agilent MS based products line to support entire sales team for technical support.

A- Can you briefly tell us about your education background and what motivated you to work in the area of MS?

B- I have done my graduation and post-graduation from Mumbai University and immediately after finishing my PG as a technical person in one of the leading instrument industry. I was responsible for spectroscopic product line, later on I got the responsibility for MS and at the same time MS was one of the my favorite subject in my college days because of the I got the flavor for MS and that is why I entered in this field and continuing the same.

A- Can you mention what are the major applications of MS on both small molecules and large molecules such as proteomics?

B- If you ask me, MS is wonderful technique. I majorly classify two type of applications one is small molecule application and large molecule applications.

Small molecules applications involve many many applications starting from drug discovery, drug development, forensic applications, food analysis and many other applications are available but when you look large biomolecular applications proteomics is one of the application where you really need to explore the possibilities and features what MS can support for this application. If you look at starting from peptide mass fingerprinting or post-translational modification or drug discovery or in terms of biomarker discovery or protein-protein interactions, drug protein interactions all such applications are very much possible with MS based solutions.

A- But in some of the major shortcomings you foresee in MS based applications which are currently being used?

B- If you ask me in principle MS based proteomics, particularly liquid chromatography coupled to electron spray ionization technique has provide very high throughput application, unbiased identification and characterization of proteins in biological samples. Moreover multiple techniques are available today to monitor these changes in protein expression as well as PTM studies however people think that MS suffers from

limited dynamic range or finite acquisition rate but it is not true, it is not really true. Many new innovations has been implemented to overcome all these issue and all these innovations with the goal to improve detection of low abundance proteins and rare PTM studies.

A- So what MS based instruments and technologies Agilent is able to provide is the field of MS?

B- The field of proteomics is rapidly expanding and it's just about every aspect, any scientist we are looking for each and every aspect which protein research involves from detection and characterization or to the biomarker discovery and the quantitation studies. Protein analysis has many challenges, so Agilent has a complete solution if you look at from Agilent offers a chip based Q-TOF proteomics integrated proteomics solutions. Integrated proteomics solutions include advanced LC-MS platforms with unprecedented plug and play flexibility. If you look at it is not only instrument you have to take care of many things, starting from best of MS detector, the software which assist you to get the desired information and the sample preparation so Agilent has complete end to end solutions for all your proteomics analysis.

A- So what is this chip based technology which you just mentioned?

B- If you ask me about chip based technique, the very biggest problem comes when you work with the protein sample is you have very low concentration and low volume of samples and to work with all these low volume, low concentration samples you have to work with the nano-HPLCs and traditional or conventional nano-LCs has a biggest problem of the leakage. As soon as the leaks is out it's very difficult to identify where the leakage happened and worse why the leakage cannot be detected the reason is that it has lot of nuts, tubings, fittings so you cannot detect leakages. In fact I am carrying a chip with me if you look at conventional nano-LCs you see a lot of fittings, columns, consumables because of that leakages are there you cannot detect the leakages. So Agilent has come up with excellent solution, this is the chip technology where you have sample enrichment capability here so once your sample is enriched by the capillary pump then the capillary nano-flow pump comes and takes the samples to the nano-columns and in fact you have ionization source itself on the chip. So it's completely

integrated to avoid all the complications of conventional nano-LC. This chip technology has the best solution available today and it is one of the best available for any of the proteomics lab.

A- So now as MS technology is very much revolutionizing all the aspects of life science research and it is heavily used in the clinical proteomics and clinical research. How do you foresee, why still the MS is not so much used in the clinical hospitals?

B- In fact I think the MS is waiting to make changes in the industry. It will have a huge impact on the infectious diseases if you look at instead of many biological methods one should used MS. MS is very very simple, it gives you information in a minute and saves time and money and more accurate results. MS is basically a transformative technique but the only question is how fast it will be adopted by the scientific community that is the one thing. Most of the analysts have the perception that this instrument has some limitations but it is not true, it is not user friendly technique but it is not true this instrument is very simple easy to use, get result in minute and more accurate results.

A- You rightly mentioned and I will mention here that although cost is one of the limiting factor for adopting these MS based technologies in the hospitals and clinical settings but nevertheless even in Mumbai different hospitals I visited, I saw lot of these MSs are actually been integrated for various type of diagnosis so that is actually I see a very good change in terms of using the technology and directly providing the result for the deciding what type of treatment, drug and dose patients should get.

B- I totally agree with you. Even I have seen most of the hospitals nowadays having MS for the clinical applications and majorly I have seen for the small molecules application but nowadays they are looking for proteomics based clinical trials and applications and it starts from various research institutes most of the research institutes those of the doing research on the clinical based proteomics they started using MS.

A- Right. Can you briefly describe some of these technologies which you mentioned triple quad and chip based technology?

B- We Agilent have very good solution and if you look at the point of triple quadrupole in fact I have some videos to show you. Here this video explains you what all techniques are available and how it is useful for proteomics applications.

Whether you quantitate drug metabolites, measure herbicide level in food or determine contaminant level in ground water the triple quadrupole MS is unequal for quantitating organic trace compounds in complex matrices. The Agilent triple quadrupole LC/MS has outstanding sensitivity great ease of use and legendary Agilent reliability, all at very attractive price. Great sensitivity starts with superior ionization technology. Agilent's LC-MS ion sources use our patented nebulization technology and high volume counter flow drying gas. Together they reduce noise related to incomplete drying of sample droplets and keep the sample capillary and ion optics cleaner to reduce maintain. A thin skimmer aperture carefully matched hole-size and short capillary to skimmer distance reduce beam broadening. An octapole ion guide provides better ion transmission over a wider mass range. Patented lenses enhance high mass ion transmission and increase sensitivity over a wider mass range.

The first quadrupole mass filter allows only ions of the target mass to pass through. The hyperbolic shape of the rod enhances ion transmission and spectral resolution. In the hexapole collision cell precursor ions strike collision gas molecules generating product ions and neutral fragments. Linear axial acceleration and high collision gas pressure simplify operation and ensure fast sensitive MS/MS without cross experiment memory effect. The second quadrupole serves as a mass filter for the products ions produced in the collision cell. For quantitative analysis of a target compound, the second mass filter is operated in a selected ion monitoring mode. In the detector, the conversion dynode operates at 10000 Volts to improve sensitivity, because the conversion dynode is off the main access of ion path, neutral molecules miss the dynode, eliminating neutral noise. Secondary dynode helps to extend the useful life of the electron multiplier. The electron multiplier has a long life but it is also easily replaced.

The Agilent 6410 triple quadrupole LC-MS establishes a new standard value in a triple quadrupole MS. It delivers outstanding sensitivity great ease of use and legendary Agilent reliability all at a very attractive price.

Now we have seen how triple quadrupole is best technique for the quantitative application. Now let's look at the Q-TOF technology, how it is useful for the proteomics applications.

Whether you are indentifying proteins and characterizing PTMs, searching for metabolite biomarkers or finding impurities in pharmaceuticals or food the Agilent 6510 quadrupole time of flight LC-MS is an outstanding choice. It delivers more than 2 PPM mass accuracy for MS and more than 5 PPM mass accuracy for MS/MS. It also delivers wide spectrum dynamic range and unsurpassed Q-TOF sensitivity, all in reliable and easy to use system. . Great sensitivity starts with superior ionization technology. Agilent's LC-MS ion sources use our patented nebulization technology produces finer droplets and delivers more ions to the MS. A second nebulizer introduces reference mass solution that ensures continual mass access correction for the best possible mass accuracy. High volume counter flow drying gas reduces noise related to incomplete drying of solid droplets and keeps the sample capillary and ion optics cleaner to reduce maintain. A thin skimmer aperture carefully matched hole-size and short capillary to skimmer distance reduce beam broadening. An octapole ion guide provides nearly 100% ion transmission over a wider mass range. Patented lenses enhance high mass ion transmission and increase sensitivity over a wider mass range.

The quadrupole mass filter allows only ions of the target mass to pass through. The hyperbolic shape of the rods enhances ion transmission and spectral resolution. In the hexapole collision cell precursor ions strike collision gas molecules generating product ions and neutral fragments. Linear axial acceleration and high collision gas pressure ensure that all ions exit the collision cell with nearly identical energy this allows the same mass calibration factors to be applied to MS and MS/MS ions, result is better than 5 PPM mass accuracy for MS/MS ions. Another octopole ion guide keeps the ions together while allowing excess collision gas to be out of the way. A quadrupole ion guide flattens the stream of ions for better transmission through the slicer. The slicer reduces variations in the vertical momentum of the ions. Ions with too much vertical momentum do not reach the pulser, this impulse mass accuracy for all ions.   fast

sensitive MS/MS without cross experiment memory effect. The flight tube is constructed of a special material with very low coefficients of thermal expansion so it is less sensitive to temperature changes. The reflectron compensates for minor velocity differences improving resolving power of 6510. The microchannel-plate detector converts the ion signal from electrons to photons and back to electrons, this electrically isolates the high voltage flight tube and front of the detector from a signal passed to the electronics. The 6510 offers outstanding in spectrum dynamic range for a time of flight instrument when you need the ultimate LC/MSMS power versatility the Agilent 6510 Q-TOF provides it with ease of use and reliability.

Mr. Abhijeet- Q-TOF technology has lot of tremendous advantages for proteomics application. We have seen how triple quadrupole and Q-TOF works for your application but what about chromatography? I do have some videos how chromatography which explain how chromatography- the conventional chromatography and how CHIP technology has advantageous features? Let's look at the video-

It's time to prepare for a new generation LC-MS technology from Agilent, HPLC-Chip/MS. The Agilent 1200 series HPLC-Chip/MS takes you to a new level of nano-flow LC-MS performance by combining micro-fluidics with an easy to use plug in play interface that lets you to focus on your results. The Agilent HPLC-Chip/MS platform is based on the Agilent HPLC Chip and Agilent HPLC Chip/MS interface that is designed for use with all Agilent 6000 series MSs. The Agilent HPLC-Chip integrates enrichment and analytical columns, micro-valve connections and nano-electro spray tip on an inert multilayer polymide film. A closer look at HPLC-Chip reveals that sample enrichment and separation columns of a nano-flow LC system are integrated with intricate connections and nano-electro spray tip for compound ionization in MS. This eliminates 50% of the traditional fittings and connections typically required in a nano-flow LC/MS system which dramatically reduces the possibility of leaks and dead volumes and significantly increase ease of use, sensitivity, productivity and reliability. The HPLC chip also incorporates all electrical contacts with a nano-electro spray tip and features

embedded in radio frequency ID tag that tracks the usage and operating parameters of the chip. The HPLC-Chips are housed in the Agilent 1200 series HPLC-Chip MS interface the Chip cue. The Chip cue includes an electro-spray, ion source with optics for spray visualization, HPLC-Chip loading and ejection mechanism. The HPLC-Chip loading mechanism precisely and optimally positions the electro spray tip orthogonal to MS inlet for maximum sensitivity and robustness day in day out. The Agilent 1200 nano-LC system including microvalve plate, auto sample and loading pump connected directly to Chip cue and HPLC Chip is loaded and connections are established automatically by sandwiching the chip between the rotor and the stator. Fast movement of the rotor ensures reliable switching between sample loading and sample analysis positions on the HPLC-Chip. Replacement of the HPLC-Chip is simple and can be completed in a few seconds.

Let's look at how Agilent 1200 HPLC-Chip MS system can be applied to a typical protein identification analysis. The Agilent microvalve plate auto sampler loads the digested proteins. A solvent flow moves the peptides into the trapping column. The microvalve changes the flow path. The gradient flow from the nano-flow pump takes the enriched sample from the trapping column to the separation column. The peptides are separated just like on a conventional nano-flow column reduced peak dispersion yields better separation efficiency and sensitivity. The integrated nano spray tip ensures reproducible nebulization of the effluent vital for optimum ionization of compounds and best results. Proven nano-flow LC-MS technology and the new and exciting capabilities of micro-fluidics combined to form a system that is easy to set up and easy to maintain, scientists can get more results faster. The flexibility of the HPLC-Chip design and the HPLC-Chip MS interface, microvalve technology in integrating additional chemistries and operation strategies opens up a wide range of potential solutions for many research challenges. Arm chip, multi-dimensional nano LC is one of many possible new applications by adding more layers to the HPLC chip, additional capabilities such as 2-D HPLC affinity chromatography and on chip chemistries such as on chip protein digestion are possible. These new applications and many others such as chips with different column lengths and packing material are part of Agilent's exciting custom HPLC chip

portfolio. Moving beyond protein identification the new phospho-chip with sandwiched reversed phase titanium dioxide trapping column provides researchers working on post translational modification with a convenient tool targeted at phosphorylated peptides. Pushing beyond proteomics the new ultra high capacity chip with a 500 nano liter trapping column facilitate analysis of pharmaceuticals such as drug metabolism pharmako-kinetics with better sensitivity and much lower sample requirement, this will be extremely attractive when the single animal testing model is implemented in pharmaceutical analysis. The HPLC-chip MS interface is a standard module within the Agilent 1200 series LC portfolio and is fully controllable through the Agilent cam station or Agilent mass hunter software. Step by step, chip by chip Agilent facilitates new applications in life science, pharmaceutical and chemical analysis. HPLC-Chip MS a growing trend in LC-MS technology.

A- It was very good to see the recent advancements in the field of MS and what new technologies Agilent is offering. My final question to you is what will be your advice to our students or users and how do you like to conclude this session for the MS?

B- That is very good question, actually. MS has tremendous potential in the field of analytical as well as research application but we need to explore the possibilities what all we can do? It's all in our hand, instrument generates data but how actually we can utilize information and how we can use this information for our purpose we need to explore the possibility and I should say this is one of the best technology available today in this world and any proteomics lab should have mass based solution for their applications. Thank you.

C- Thank you very much for discussion.

So after discussion on Q-TOF as well as triple quadrupoles now let's move on to another latest configuration the Orbitrap.

It has very much similarity with Ion trap but it is one of the very latest additions to this proteomics workflow where people are applying it for various type of biomarker discovery and other applications. So I have invited one of the application experts from

ThermoFisher to discuss about what is the Orbitrap technology, it principle and how it can be applied for different type of applications so I will have a discussion with Mr. Sangram PattanaiK from ThermoFisher.

This is my pleasure to introduce Mr. Sangram Pattanaik, project manager LCMS division of ThermoFisher scientific. He is working in the mass spectrometry area.

A- Prof. Sanjeeva Srivastava   B- Mr. Sangram Pattanaik

A- Hello Sangram

B- Hello Dr. Srivastava

A- Good to see you for this short conversation about MS and some of the latest advancements happening. I thought it would be a good idea to seek your expertise about the Orbitrap and MS applications available. Before I start this conversation I would like to know little bit about your educational background and may be about your experience in MS field.

B- For the last six years I am looking at the MS divisions of different platforms and ThermoFisher for last two years, here I am handling the Orbitrap technologies and we have all Ion trap and triple quadrupole systems. Prior to that last 4-5 years handling the Q-TOF system. Altogether I am here for the last 15 years in analytical industry.

A- Great. So you have long experience in this field and you have seen different type of advancements in the field. So with that experience, can you share what types of major applications of MS are currently being used in proteomics area?

B- Yes. In proteomics area mostly there is two basic applications area. People are looking at the discovery and the targeted quantification. Biological discovery, people are talking about comprehensive proteomics and targeted people are more talking about biomarker validations. In comprehensive proteomics peoples talk would about identifying and wants to quantifying proteins all in the same sample as well what are the post-translational modifications in the whole proteome. So these are the new areas where research going on currently.

A- Right. I think you rightly mentioned that emphasis is moving more towards quantification of those proteins rather than identifying them and leaving only their abundance level. So

in that light can you brief us about shortcomings of currently available MSs and what challenges we have to overcome to have a really comprehensive coverage of proteome and to do various applications including the PTM and targeted quantification and biomarker discovery?

B- As you have mentioned correctly that biological matter is itself complex in nature. When you different kind of approach or workflow of applications you look out the analytical solutions which can fulfill that demand. In most of the areas people are more talking about sensitivity, resolution and mass accuracy these are the areas now. New areas are coming what kind of fragmentation pattern and fragmenting capabilities are the areas people are now looking at.

A- So proteomics is quite competitive and challenging field and right now lot of emphasis from all the companies are in this field for the mass spectrometry, How to provide good solutions for analyzing the complex proteome? So what types of major MS instrumentations are available from ThermoFisher currently?

B- As ThermoFisher, we have different technologies starting from Iontrap to Orbitrap but for proteomics platforms we normally try to provide them Orbitrap so Orbitrap is main choice of scientist now if you see globally.

A- If you don't mind, can you just give some overview about the Orbitrap technology currently available?

B- Currently we have three different platforms of Orbitrap. To look at orbitrap, Orbitrap is nothing but one kind of Iontrap where ions move in a orbit. To give a better idea it's better to have a presentation.

A- Please go ahead.

B- This is how this system looks like.

If you look at this slide there is ionization source and two MSs are in combination with each other that's why it's called hybrid technology. The front hand is a linear Iontrap, second part is an Orbitrap. First ions are generated from the source goes to linear iontrap, it is being trapped there. Once it has been trapped, it is axially ejected and then goes to the C-trap, the C-trap squeezes the ion into the #### and eject that ion into

Orbitrap. Once the ion gets ejected into the Orbitrap, it goes into axial motion as well as radial motion; we measure the axial frequency of ion. The frequency which is being measured is transferred into mass/charge ratio. If you look at there is an end electrode which is connected to frequency domain which measures frequency. This is how Orbitrap works.

A- I think you mentioned that you want to show some more details for the path.

B- I have a small video which we can see.

Orbitrap elite- The LTQ Orbitrap is a hybrid system, it has two MS, the MS which is sitting front is a linear trap and on the back hand is orbitrap elite. If you look at the schematic of the system, at the front part of the system is ionization source then you have the transfer optics and in the transfer optics we have S lens which is the newest generation then we have transfer tube and we have neutral blockers which blocks neutrals which is coming from the source then you have the octapole and then the ion trap. In the ion trap we have two regions high pressure cell and low pressure cell then there are two detectors- detector 1 and detector 2 when you walk with the linear trap, detectors can use for the detections of ion from the linear trap. From the linear trap it goes to transfer optics then you C-TRAP which dynamically squeezes the ion into packet and allows that ion to go into high field Orbitrap.

Coming back to Ionization source, the ions generated from the source and goes into the transfer optics and after going from the transfer optics it goes to the linear trap and the linear trap in full scan mode ions are shoved into the high pressure cell. After this, ions are then sent to C-TRAP through the ion transfer optics. C-TRAP does cooling of the ions and mixes it into the packet of ion so that packet of ion is injected into Orbitrap. Because the orbitrap is itself is a static so we have to inject the ions tangent to #####. As the ion goes into the Orbitrap, it moves into the orbit and the frequency is recorded which gives you in turn MS.

If you want to walk the linear trap and the Orbitrap at the same time you can run both at the same time. The linear trap the ions get shoved and fragmented and then sent into the Orbitrap. We can have the full scan MS as well as full scan MS/MS on the same

time. Apart from that in addition to full scan MS and MS/MS you can have also low resolution scan from the linear trap. So this is what we can obtain from Orbitrap.

A- It is very good to learn about Orbitrap technology and how it works. Finally I would like to ask you what is your recommendation for MS users? What are different challenges occuruing in this field and may your message to the MS users.

B- It's very difficult to answer but in a simplest way I can tell you it is all depend on the applications area. The selection of an instrument depends upon what kind of applications people are looking at. It's more of a thing which people should look at more global scenario how people are doing applications in those areas. If you have a instrument you can generate lot of data but it depends upon interpretation of the data and it has to be application oriented.

A- So you mentioned very rightly that MS has infinite possibilities. It has tremendous potential and depending upon your application one can explore possibilities and how one can interpret those data is actually becoming more challenging and people are coming up with very ways of analyzing the data for different applications. So with that I will conclude the interview. I will thank Mr. Sangram for sharing his experience with us. Thank you.

B- Thank you very much.

All right so as you have seen two interviews and discussion on different type of hybrid MS configurations, we talked about Q-TOF, triple Quad and Orbitrap technology. We have also seen the latest advancements in the field, the direction of chip based technology. These are just few examples; there are many other good configurations available from various manufacturers in the field. These two interviews were mainly intended to showcase different type of hybrid configurations available and latest technologies which are trying to overcome HPLC based methods and their limitations. There are many other good manufacturers available. Now we will continue our discussion on different type of MS based applications in the next lecture. Thank you.