HANDOUT

LECTURE-23

HYBRID-MS/MS CONFIGURATIONS

This lecture will cover Hybrid-MS/MS configurations, with a summary of the input from application specialists from two leading companies- ThermoFisher, to talk about Orbitrap technology and Agilent, to discuss Q-TOF and QQQ(triple-quadrapole) configurations as well as chip technology.

In Proteomics research, there are two basic applications areas, namely, biological discovery and targeted quantification. Biological discovery involves biomarker discovery and validation. Comprehensive proteomics, on the other hand, focusses on quantification and characterization of the total protein content of a proteome, with an intent to identify proteome changes such as post-translational modifications. Mass spectrometry technologies that fulfill these needs need to deliver high sensitivity, resolution and mass accuracy. In this module, specific examples of such instruments will be discussed.

Summary of discussion with Mr. Sangram Pattanaik, Project Manager, LCMS division of ThermoFisher Scientific.

Thermofisher offers instruments that incorporate a range of mass analyzers, starting from Iontrap to Orbitrap, but for proteomics platforms, Orbitrap remains the globally preferred mass analyzer configuration.

Orbittrap Mass analyzer:

Three different platforms of Orbitrap are offered by Thermofisher. Orbitrap is nothing but one type of Iontrap where ions move in an elliptical orbit. It consists of an outer and inner electrode that work together to generate an electric field with quado-logrithamic potential distribution, in which the ions oscillate around the inner electrode in an elliptical trajectory. This movement of the ions only is determined by their mass/charge ratio. The

image current generated by these trapped ions is first transformed into a frequency and finally to a mass spectrum.

Hybrid technology used in these MS instruments combine an ionization source with 2 mass analyzers, namely a liner iontrap and an orbitrap. Ion injection is carried out via is curved linear trap called the 'C-trap'. The ions generated from the source first move into the linear iontrap, and are trapped there. Once they have been trapped, they are axially ejected and then go to the C-trap, which eject them in to the Orbitrap. Once the ion gets ejected into the Orbitrap, it goes into axial motion as well as radial motion. The current produced by the axial oscillation is converted by a Fourier transform into frequency, which is then converted into a mass spectrum which plots intensity vs mass/charge ratio of the ion.

Overview of the LTQ Orbitrap elite

This is a hybrid system, consisting of 2 mass analyzers, with a linear trap followed by the orbitrap elite.

Product Features:

- An examination of the schematics of the system shows an ionization source followed by the transfer optics, which contains a S lens followed by the transfer tube and a Neutral blocker which blocks neutrals generated by the ionization source, followed by the octapole and then the ion trap.
- The ion trap contains two regions, a high pressure cell and low pressure cell and two detectors.
- The linear trap is followed by transfer optics that lead to the previously mentioned C-trap, which dynamically squeezes the ion into a packet and allows that ion to go into high electric field of the Orbitrap mass analyser.
- In this system, the ions generated from the source enter the transfer optics and then the linear trap.
- Here, in the 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 cools the ions and injects them into Orbitrap. After the ion goes into the Orbitrap, it oscillates around the spindle shaped inner electrode of this system. The image current produced by the axial oscillations are converted, through a series of steps, to a mass spectrum.

The next section will be an overview of mass spectrometry applications, discussed with Mr, Abhijeet, a product specialist in the mass spectrometry division of Agilent technologies in India He first offers an overview of MS applications followed by instrument discussions.

Summary of discussion with Mr. Abhijeet,from Agilent Technologies

Applications of Mass Spectrometry:

Small molecule applications: These cover many applications starting from drug discovery, drug development, forensic applications, food analysis and others.
Large biomolecule applications: This includes various proteomics applications, starting from peptide mass fingerprinting or characterization of post-translational modification, drug discovery or in terms of biomarker discovery or protein-protein interactions, drug protein interactions etc.
MS in clinical settings: While this technology originated in the academic and industrial setting, it is increasingly used in a clinical setting and should have a huge impact on clinical diagnostic methods. While a limiting factor is cost, it should be valuable to information for deciding what type of treatment/ drug dose patients should receive, after using MS in proteomics based clinical trials and small molecule proteomic applications.

In principle, MS-based proteomics, particularly liquid chromatography coupled to electron spray ionization techniques provides high throughput analysis, allowing 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 PTMs. While people think that MS suffers from limited dynamic range or finite acquisition rate,that may not be accurate. Many new innovations have been implemented to overcome all these issues, with the goal to improve detection of low abundance proteins and rare PTMs.

MS Instrumentation offered by Agilent:

The field of proteomics is rapidly expanding, and to accommodate the wide variety of applications studied, Agilent offers a wide variety of instruments to provide a complete solution. Agilent offers a chip based Q-TOF Integrated proteomics solutions include advanced LC-MS platforms with unprecedented plug and play flexibility.

This company also offers end-to-end solutions, with a range of MS detectors, and software tailored to meet the entire range of proteomics analyses.

Agilent Triple Qudrupole mass analyzers:

Whether you quantitate drug metabolites, measure herbicide level in food or determine contaminant level in ground water the triple quadrupole MS is very useful for quantitating organic trace compounds in complex matrices.

The Agilent 6410 triple quadrupole LC-MS

Product Features:

- Great sensitivity starts with superior ionization technology. Agilent's LC-MS ion sources use their 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 clean, helping to reduce maintenance.
- 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 any 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.
- The 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.

Agilent 6510 quadrupole TOF LC-MS

Whether you are identifying proteins and characterizing PTMs or searching for metabolite biomarkers or finding impurities in pharmaceuticals or food the Agilent 6510 quadrupole time of flight LC-MS is an outstanding choice.

Product Features:

- It delivers more than 2 PPM mass accuracy for MS and more than 5 PPM mass accuracy for MS/MS.
- It also provides a wide spectrum dynamic range and unsurpassed Q-TOF sensitivity, all in a reliable and easy to use system.

- Great sensitivity starts with superior ionization technology. Agilent's LC-MS ion sources use patented nebulization technology produces finer droplets and delivers more ions to the MS. A second nebulizer introduces a reference mass solution that ensures continual mass access correction for the best possible mass accuracy.
- A 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 maintenance.
- A thin skimmer aperture carefully matches 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, resulting in better than 5 PPM mass accuracy for MS/MS ions. Another octopole ion guide keeps the ions together while allowing for 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, increasing impulse mass accuracy for all ions and allowing 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 the 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 inversetality the Agilent 6510 Q-TOF provides it with ease of use and reliability.

Agilent HPLC-Chip/MS (Product descriprion, Courtesy Mr. Abhijeet, Agilent Technologies).

The chip-based technique has evolved to address the limitations of the previously available solutions for samples of low volume and concentration. Such samples were studied using nano-HPLC techniques, in which leakage from the system posed a major issue, which were often difficult to pinpoint given the size and the bulkiness of the system. In comparison, chip technology is developed by Agilent offers an excellent solution with enhanced sample enrichment capability. The sample is enriched by the capillary pump then enters the nano-columns, with ionization occurring on the chip itself. The chip thus offers a completely integrated technology to avoid all the complications of conventional nano-LC. 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.

Product features:

- 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, allowing faster obtainment of data .
- 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 produced 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.
- 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.

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. Step by step, chip by chip Agilent facilitates new applications in life science, pharmaceutical and chemical analysis.

In summary, today, MS has tremendous potential in analytical and well as basic research areas, but the list of its potential applications need to explored and expanded. This lecture has discussed Orbitrap, Q-TOF, triple Quad and technology, as well as the newest advancements in the field, namely, chip based technology. These discussions are mainly intended to showcase different type of hybrid configurations available and latest technologies which are trying to overcome limitations of HPLC based methods.