Today, if one 'Googles' the internet, no day goes by without a conference or workshop on mass spectrometry at one corner or the other in the world. The conferences, workshops, short term courses and trainings on focused applications on mass spectrometry and its hyphenated techniques have become order of the day because mass spectrometry has now invaded all branches of science. As everyday dawns for a scientist with a meeting on mass spectrometry, it is in no way less in India too!

Indian Institute of Chromatography & Mass Spectrometry (IICMS), a dedicated institute for mass spectrometry and first of its kind, has now become a year old 'adult'. IICMS has successfully conducted the second one day national conference on 'Frontiers in Mass Spectrometry & Emerging Applications' unifying all stalwarts of mass spectrometry under one roof at Chennai on November, 19, 2012. It has been further decorated by the presence of renowned mass spectrometry scientist Prof. Newman Siu Kwan SZE from Nanyang Technological University, Singapore.

2nd National Conference on

'Frontiers in MS Technology and Emerging Applications'

Prof. Newman Siu Kwan SZE
Director of Proteomics and
Mass Spectrometry Facility
Nanyang Technological University,
Singapore

Protein Post-translational-modifications in blood is an unexploited source of disease biomarkers

Among the different strategies for disease diagnosis and prognosis, the detection of protein biomarkers in blood is the most ideal and practical clinical method. Thus, numerous studies have used proteomics strategies to discover biomarkers from blood plasma. However, this previous proteomics driven biomarker research have achieved only modest success. Using whole plasma limited the detection sensitivity for low abundant proteins such as cancer biomarkers.

Protein post-translational modifications can be the cause or effect of a specific local pathophysiological process. It has been proposed that the age-related decline in capacity and function of tissues comes about, in part, from progressive accumulation of posttranslational modifications (PTM) of long-lived proteins. Thus, identification of the PTMs may reveal a unique disease-specific biochemical marker. However, a protein with a PTM is usually present in very low abundance compared with other proteins in the sample.

Instead of using whole plasma and to overcome the challenge of identifying low abundant PTM, we have developed novel proteomic strategy to selectively enrich low abundant proteins that potentially include disease biomarkers in blood plasma by targeting post-translationally modified proteins such as glyco-proteins, phosphorylated proteins, deamidated proteins and proteins enclosed in plasma microvesicles. This strategy has been successfully applied to identify and validate plasma biomarkers for cardiovascular disorder and stroke. Some of the potential biomarkers are proteins that are post-translationally modified by pathophysiology of the diseases produced by the ischemic heart tissue. This biomarkers have the potential for earlier detection of myocardial infarction than cardiac troponin. As protein PTMs in blood is an unexploited source of disease biomarkers, we believe the same strategy can be applied to identify biomarkers for other diseases, such as cancer and infectious disease.

Inauguration

It was a conglomeration of more than 180 scientists where experience and exposure of mass spectrometry had become the 'talk of the town' through invited talks and dialogues in the form of questions and answers. It was like 'West meeting the East' where invited speakers, who had imbibed mass spectrometry over couple of decades, enlightened the young minds such as research scholars or the young scientists who have started practicing mass spectrometry. This overview will present some of the highlights of the conference to those who could not be present on that day.

Arvind Thyagarajan, Principal Scientist, IICMS was the host of the conference. He began the proceedings with a 90 second video clip where the audience travelled through some of the highlights of our first national conference on mass spectrometry in 2011.

Following our great Indian tradition, the conference was inaugurated through lighting the ceremonial lamp by our guest of honors Dr. A. V. Rama Rao, Chairman & Managing Director, AVRA Laboratories Pvt Ltd, Prof. S. P. Thyagarajan, Pro Chancellor Sri Ramachandra University, Dr. M. D. Nair, renowned Pharma Consultant, Mr. S. Imamichi, Managing Director, Shimadzu Analytical India Pvt Ltd, Dr. S. Y. Pandey, Director Global Chemical Research, Jai Research Foundation, Mr. S. Thyagarajan Chairman, Spinco Biotech Pvt Ltd and Dr. Venkat Manohar, Director, IICMS.

Dr. Venkat Manohar welcomed the Chief Guest Padmasri Dr. A. V. Rama Rao, and other distinguished speakers like Dr. M. Vairamani, Dean, School of Bioengineering, SRM University, Prof Newman Siu Kwan SZE, Director of Proteomics and Mass Spectrometry facility, Nanyang Technological University, Singapore, besides a host of other invited speakers. The participants came from various industries like pharmaceuticals, CRO, Food Safety laboratories, R&D & Testing centers, National laboratories and Universities.

Mass Spectrometry: Indian Scenario & Keynote address

Dr. M. Vairamani, one of the pioneers in mass spectrometry of our country, presented a 'bird's eye view' of the growth of mass spectrometry and its applications under the context of Indian scenario. He elaborated how mass spectrometry and its hyphenated tools were initially used by many researchers in national laboratories and subsequently how it became one of the routine analytical tools, extensively used also in industries. He recalled the recent applications and the importance of mass spectrometry in the area of proteomics. He appreciated the efforts of IICMS in bringing all the mass spectrometry users under one roof through this kind of conference to further introduce newer applications of mass spectrometry, as well as emerging technological advancements in this area of science.

Padmasri Dr A V Rama Rao delivered his key note address sharing his early days of synthesizing very complex natural products and how he used the first mass spectrometer installed at NCL, Pune to unravel structure of those complex molecules such as Morellin(1974). In fact, he shared a couple of the very early publications (revisiting structure of Gardenin) in mass spectrometry in the Journal of Organic Mass Spectrometry. He also cited some of novel inventions that led to Nobel Prize in early 80s and 90s where mass spectrometry played a major role. He made special mention on how the work of Koichi Tanaka of Shimadzu corporation, presented through a poster in a scientific conference, fetched him the Noble prize. Some of the presentations reflecting his unconventional approach in solving structure of difficult molecules were really a source of inspiration for many scientists practicing mass spectrometry. He also showed how mass spectrometry was used as a confirmatory technique to understand the structure of small molecules. His key note address was not only a source of inspiration to young scientists but it also a 'call for' mass spectrometry manufacturers to come up with newer technologies to meet the challenges of emerging applications in the area of chemistry as well as life science.

Dr. V. Meenakumari
Global Technical Manager,
Softlines & Chemicals TÜV SÜD
South Asia, Bangalore

New challenges in Food Safety and the advantages of Mass Spectrometry

Food safety deals with identification of processes through HACCP and then finally confirming using the analytical techniques to identify and quantify the amount of various chemical toxins present in a given product. Are we really sure that the identification and quantification of such substances is always right, if not what is the probability of that being correct. A big challenge that the food industry is facing as more & more new substances have been suspected to be or proven as hazardous. In general the problems could be in the area of identification through false positive or negative or the actual amount that can be identified where the technique employed has the direct impact. Can Mass spectrometry help us in this respect.

Dr. A. T. Bapuji
Vice President - Research Center
Aurobindo Pharma Ltd.,
Hyderabad

Role of Mass Spectrometry in BA/BE studies

Analytical techniques applied to measure/ quantify the drug concentrations in biological matrices/Specimens is called bioanalysis. Bioanalysis assumes significance at all stages of drug Discovery and development. LCMSMS is the mainstay for all Bioanalysis. All kinds of Human clinical pharmacology studies utilize mass spectrometry. Pharmacokinetics is the time course of Absorption, Distribution, Metabolism, and Excretion (ADME) of drugs in the body. In many cases, pharmacological action, as well as toxicological action, is related to plasma concentration of drugs. The present talk focus is on Bioavailability and Bioequivalence studies where the mass spectrometry is extensively used in the determination of plasma concentrations. Consequently, determination of plasma concentrations assumes importance. By developing good bioanalytical methods and subsequent validation and further quantitation of drugs from the Biological matrices provide the support for pharmacokinetic analysis. Blood level studies are the most common type of human bioavailability studies and are based on the assumption that there is a direct relationship between the concentration of drug in blood or plasma and the concentration of drug at the site of action. By monitoring the concentration in the blood, it is thus possible to obtain an indirect measure of drug response. Selective and sensitive analytical methods for the quantitative evaluation of drugs and their metabolites (analytes) are critical for the successful conduct of pharmacokinetic studies. Developing sensitive, rugged, cost-effective and robust bioanalytical methods is challenging and time consuming. This is particularly challenging when these methods are used for assessing the pharmacokinetics by using advanced analytical instrumentation like LCMS/MS. A regulatory aspect of method validation is also discussed.

Mass spectrometry: Proteomics, Foods & Bioanalysis

Having set the tone to understand the newer challenges in the application of mass spectrometry by Dr. Rama Rao, Prof Newman from Nanyang Technological University, Singapore gave an impressive talk on how proteomics strategies could be applied through mass spectrometry to 'pick up' the clinically relevant biomarker proteins in blood. Introducing proteomics based mass spectrometric technique, he explained Proteome Analysis (Qualitative and Quantitative) could be carried out using multi-dimensional HPLC and tandem MS. He emphasized that post translational modification-(PTM) of long lived proteins, normally present in low abundance in blood might reveal disease specific biomarkers. He demonstrated through couple of examples how his laboratory had developed novel methods to enrich the low abundant PTM to identify the possible biomarkers for cardiovascular disorder and stroke in blood samples. He indicated that the same strategy could be expanded to other areas such as cancer and infectious diseases. Thus an emerging application of mass spectrometry was shared to participants by Prof Newman Siu.

The awareness of Food Safety is fast emerging in our country and newer developments that have occurred in food processing and packaging have led to increased application of mass spectrometry. Dr. V. Meenakumari, Global Technical Manager - TUV SUD South Asia, shared her two decades of experience in the area of food safety and use of mass spectrometry. She educated the participants in terms of sample preparation, choice of appropriate technique in terms of selectivity, sensitivity and robustness of the analysis. Some of the examples that she showed on 'single shot' multi-residue analysis through LCMSMS, multi-screening for antibiotic residues and advances in bromine speciation through HPLC-ICPMS were well received by participants in understanding the power of

mass spectrometry towards safety of foods. The awareness created by her presentation could pave the way for increased utilization of mass spectrometry in food science.

Bioanalytical application of mass spectrometry is reasonably well known in India over the past eight to ten years. However, newer regulatory requirements continuously require revisit of existing bioanalytical methods in the context of ADME of drugs in the body. Dr. A. T. Babuji, Vice President - Research Center, Aurobindo Pharma Ltd who has been practicing bioanalysis over the past two decades has explained the role of mass spectrometry in BA/BE studies.

Dr. Bapuji initially gave an overview of the elements of bioanalysis and its basic requirements. He stressed the importance of sample preparation from different matrices and showed a few examples on how the newer technologies in mass spectrometry could lead to sensitivity down to 2.0 pg/ml to 5.0 pg/ml. He demonstrated the power of LCMSMS analysis at ng/ml concentration through a case study on pharmacokinetics (PK) of an extended drug product. The participants extensively interacted with him during the question-answer sessions. Dr. Manish SinghYadav, Associated Vice President and Head of Clinical Research and Bioanalysis, Alkem Laboratories Ltd, explained what was 'dynamism' in bioanalytical science. He highlighted the importance of matrix effects and explained the causes for contamination along with remedies to overcome the challenges in bioanalytical methods. He stressed the importance of the 'method's ability to discrete and quantify targeted analyte in the presence of other components'. He concluded his presentation by commenting that LCMSMS was able to address most of the current issues due to its speed, inherent selectivity, sensitivity and adaptability. Other aspect of bioanalysis, namely, role of mass spectrometry in bioanalytical method for low dose drugs was highlighted by Dr Vijaya Bharathi, Head, Bioanalytical-R&D - IPDO, Dr. Reddy's Laboratories. She discussed some of the issues relating to sample preparation, co-related method sensitivity and how to improve upon S/N ratio so that the overall sensitivity of the LCMSMS method

Dr. Manish Singh Yadav
AVP & Head
Clinical Research & Bioanalysis
Alkem labs Ltd, Navi Mumbai

LC/MS/MS technique a 'Dynamic analytical tool' to meet 'dynamic regulatory requirements'

Bioanalytical science progressed noticeably from last two and half decades and the advancement, transformations and learning out of progressive and purposeful innovations is invaluable. Likewise, at least 20-30% advancement in Bioanalysis and associated analytical tools has been envisioned by almost each decade, such level of transformations signify the highly dynamic nature of regulated Bioanalysis hence necessitates sound scientific knowledge, extended and continuous efforts of bioscientists, and an analytical technique which can facilitate bioanalysts to cope with the dynamism of RegBio. Coupling of HPLC with Mass Spec took almost 3 decades, and in 1990 first bench top LC/MS/MS has been introduced, there after a bioscientist can't imagine their analytical life without LC/MS/MS due to flawless contribution of this analytical tool to meet not only scientific requirements of RegBio but also highly demanding and rapidly transforming Quality standards. On the whole, LC/MS/MS has become an indispensible element of Bioanalysis required to support the development of NCE and generic drugs. We feel, sound scientific knowledge of chromatography along with keen understanding of mass spectrometry enables bioanalytical scientists to make use of most admired, efficient and dynamic analytical tool named as LC/MS/MS to complement the highly dynamic nature of Regulated Bioanalysis.

Dr. D. Vijaya Bharathi
Bioanalytical - R & D - IPDO
Dr.Reddys Laboratories Limited
Hyderabad

Role of mass spectrometry in developing ultra sensitive bioanalytical methods for low dose drugs.

The use of LC/MS/MS has grown exponentially in last decade due to its unequaled sensitivity, extraordinary selectivity and rapid rate of analysis. Mass spectrometry has enabled researchers and pharmaceutical companies to discover and develop novel therapeutic compounds and generic drugs in a rapid manner. Mass spectrometry now plays a vital role in pharmacokinetic studies. Improved chro-matographic techniques which are hyphenated with mass spectrometry have further helped to develope ultra sensitive methods. Mass spectrometry along with innovative chromatography and clean sample extraction has allowed researchers to develop ultra sensitive LC/MS/MS methods for low systemically available levels of potent drugs namely, hormones, steroids, immuno-suppressive agents and cytotoxic drugs etc.

could remain 'intact' for trace level bioanalysis ≤ 2.0pg/ml! Some of the examples shown by her amply illustrated the power LCMSMS for such trace level bioanalysis.

Mass Spectrometry: Impurity analysis, Characterization & AMS

Having seen the application of mass spectrometry in the area of life science, the afternoon session mainly focused on impurity characterisation, Design of Experiments as a tool, role of Accelerator Mass Spectrometry (AMS) in drug development, an emerging area such as process chemistry through mass spectrometry.

Dr Jyothi Ganti, Vice President - Analytical R&D, Hetero Research Foundation gave an overview of genotoxic impurities and discussed both EMEA as well as US FDA regulatory guidelines of genotoxic impurities. She further shared the FDA structural alerts on genotoxic impurities and explained a stepwise approach towards quantification of these impurities through mass spectrometry methods with a couple of case studies. She brought to the attention of the participants the analytical challenges in this kind of impurity analysis and how LCMSMS technique could successfully be used to overcome the challenges of quantifying genotoxic impurities at every stage of a drug substance /drug product.

Accelerator Mass Spectrometry is a well known technique for measuring long-lived radionuclides that occur naturally in our environment, an indispensable tool in archeology. Of late, it has found increased application in drug discovery too. AMS, being an emerging science in mass spectrometry, Dr Meenakshi Sivakumar educated the participants on the basics of this technique as well as some of its unique advantages in drug discovery. 14C labeled molecules could be analysed by this technique at attomole sensitivity. She explained that such radio labeled compounds do not require sample preparation techniques and also shared how safe the analysis can be in clinical trials. She demonstrated the power of this technique using some of the recently published preclinical data. There were lot of discussions on this emerging topic and the participants appreciated our efforts for bringing into their attention these kind of newer MS techniques.

Characterisation of process related impurities are no way less important when compared to other kind of related impurity analyses. Dr. H. Sivaramakrishnan, President - Research, Nicholas Primal Lifesciences, being a synthetic and process chemist, shared his three decades experience in understanding characterization process related impurities at very low level concentration using mass spectrometry. He elaborated on the formation of these impurities and how difficult it was to char-

acterize them. He stressed how indispensible LCMSMS was for understanding and controlling process related impurities.

Design of Experiments (DoE) that shortened time to develop bioanalytical methods was discussed by Dr. Sandeep Sharma, Director BA/BE, Jubilant Clinsys Ltd. The statistical tools that he showed enables the user to design minimum set of experiments to accelerate method optimization to save time. He demonstrated the method using 'Cause and Effect diagram', known as Fishbone or Ishikawa diagram.

Panel discussion

Having listened to experts on various aspects of mass spectrometry, a panel discussion was set up as a concluding session on 'Applications in Mass Spectrometry: Future Perspectives'. The panelists comprising of Dr Vairamani, Dr Meenakumari, Dr Sivaramakrishnan, Dr Babuji and Sandhya, Senior Product Speacilist - LC/LCMS, Shimadzu Asia Pacific, Singapore moderated by Dr Venkat Manohar. Each one of them shared their thoughts on future applications in foods, impurity profiling and pharmaceutical analysis. There were a number of questions from the audience and the panel members provided elaborate clarifications for each of them.

One of the highlights of this year's conference was the introduction of a poster session. There were 15 posters presented by scientists from IICMS and Shimadzu application laboratories. They shared a few posters presented by them earlier during American Society of Mass Spectrometry in Canada (2012) and Internationa Mass Spectrometry Conference in Japan (2012). There was also posters displayed by NIPER and Hetero Drugs, Hyderabad. Poster session drew continuous stream of scientists throughout the day. Number of participants and active discussions during the poster sessions were very encouraging to consider larger poster session in the coming years.

Conclusion

The 2nd National Conference on 'Frontiers in MS Technology and Emerging Applications', truly brought the MS community from all over India to a new platform to interact and exchange ideas with each other under one roof. The support by Shimadzu and Spinco Biotech were excellent which enabled the participants not only to enjoy good science but memorable hospitality too!

It was once again a successful event to 'Keep Learning' mass spectrometry.

Dr. Jyothi Ganti
Vice President, Analytical - R & D
Hetero Research Foundation
Hyderabad

Control of Genotoxic Impurities- Strategies, Pharmaceutical Industry and Regulatory Perspective

Genotoxic impurities (GTIs) in pharmaceuticals at trace levels are of increasing concerns to both pharmaceutical industries and regulatory agencies due to their potentials for human carcinogenesis. Pharmaceutical genotoxic impurities (GTIs) may induce genetic mutations, chromosomal breaks or chromosomal rearrangements and have the potential to cause cancer in human. Therefore, exposure to even low levels of such impurities present in final active pharmaceutical ingredient (API) may be of significant toxicological concern. Therefore, it is important to explore possible opportunities to avoid the use and generation of these genotoxic materials in the manufacturing process. However, completely eliminating the use of such chemicals or preventing the generation of DNA-reactive impurities is not always guaranteed. Although present at trace levels, GTIs can be critical in drug development and if not addressed correctly, could lead to a delayed development and or approval from regulatory agencies. This poses an imperative challenge on analytical scientists to develop appropriate analytical methodologies to accurately measure and control the levels of GTIs in pharmaceuticals. Adequate analytical methods are not only important for ensuring patient safety but also for the development of a robust manufacturing process.

Dr. Sandeep Sharma
Director - BA/BE
Jubilant Clinsys Ltd,
Noida

Design of experiments as a tool for LC/MS/MS method development

Bioanalytical methods are developed at various stages of the drug development process. Due to the inherent nature of the method development process, redundant efforts take place across an organization, resulting in costly and time-consuming activities. Different approaches are used to develop LC/MS/MS methods, including trial and error, method/column scouting and software approaches. Design of Experiment (DoE) is a system of experimental conduct with two important benefits: more information about process output and impact of variables which is available without additional experiments/ resources and; compared to One Factor at a Time (OFAT), fewer experiments generate the same amount of data. DoE system output is in the form of mathematical equation where Output is function of input variables like, Y (Output) = f (X1,X2,X3…Xn). A cause and effect diagram, also known as a Fishbone or Ishikawa diagram, is an effective tool for identifying potential variables for method performance criteria. A decision need regarding the Constant (C) that must be controlled, which are potential Noise (N) factors and which Variables need to be experimental (X). If a potential Noise (N) is affecting the process output then it should become a Constant (C) or a Variable (X). The QbD approach with DoE system was used to develop a LC/MS/MS method for quantitating drug molecule in plasma for pharmacokinetic analysis.

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Accelerator Mass Spectrometry and Drug Development

Accelerator Mass Spectrometry (AMS) is an ultra-sensitive tracer technique. AMS has some distinct advantages over conventional assay techniques, such as LC/MS/MS and also complements conventional techniques, facilitating innovative, cost-effective app-lications. One of the major advantages of AMS is that the technique is independent of compound structure and non-susceptible to matrix effects. AMS established itself as an indispensable tool in archeology and radiocarbon dating in the 1980's. With proven sensitivity and robustness for quantification of rare isotopes in biological systems and with 14C analysis becoming more accessible, AMS has found increasing applications in many areas of drug development. Some of these include Phase 0 microdosing, phar-macokinetics, metabolism and per-sonalizing medicines. The use of AMS has made it possible to conduct radiotracer studies in humans, with the administration of such low levels of 14C that the radiation exposures are below commonly accepted risks.