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TRACK 1

USING MASS SPECTROMETRY IN PHARMACEUTICAL ANALYSIS

01 November 2010 (Monday)

SESSION CHAIR : **DR. NARAHARA CHARI DINGARI**
Massachusetts Institute of Technology, Cambridge, USA

SESSION CO-CHAIR: DR. BABU SUBRAMANYAM
Bayer HealthCare Pharmaceuticals, CA, USA

SESSION INTRODUCTION

TITLE: RAMAN SPECTROSCOPY: AN EMERGING TOOL FOR CLINICAL DIAGNOSTICS

DR. NARAHARA CHARI DINGARI, Massachusetts Institute of Technology, Cambridge, USA

TITLE: APPLICATION OF LC-MS BASED METHODOLOGIES FOR THE CHARACTERIZATION AND QUANTITATION OF COMPLEX BIOLOGICS-ANTIBODY DRUG CONJUGATES

DR. BABU SUBRAMANYAM, Bayer HealthCare Pharmaceuticals, CA, USA

TITLE: COMBINED LASER INDUCED BREAKDOWN SPECTROSCOPY AND RAMAN SPECTROSCOPY FOR PHARMACEUTICAL APPLICATIONS

DR. G MANOJ KUMAR, Advanced Centre of Research in High Energy Materials (ACRHEM), University of Hyderabad, Hyderabad, India

COFFEE BREAK & POSTER SESSION

TITLE: IDENTIFICATION AND CHARACTERIZATION OF NEW DRUGS USING MASS SPECTROMETRY

DR. K SRINIVASA RAO, Roland Institute of Pharmaceutical Sciences, India

TITLE: APPLICATION OF CURRENT APPROACHES AND NEWER TRENDS IN IDENTIFICATION OF DRUG METABOLITES USING LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

MR. BHAGWAT PRASAD, National Institute of Pharmaceutical Education and Research (NIPER), Punjab, India

TITLE: ROLE OF DRUG METABOLISM AND PHARMACOKINETIC STUDIES IN DRUG DISCOVERY AND DEVELOPMENT

DR. CHANDRA PRAKASH, Senior Director, Dept. of Drug Metabolism and Pharmacokinetics, Biogen Idec, Cambridge, USA

TITLE: DEVELOPMENT AND VALIDATION OF STABILITY INDICATING LC - PDA METHOD FOR MYCOPHENOLATE MOFETIL (MMF) IN PRESENCE OF ITS IMPURITIES AND MAJOR DEGRADATION PRODUCT MYCOPHENOLIC ACID (MPA) USING FACTORIAL DESIGN TOOL AND USE OF MASS SPECTROSCOPY

DR. ANNA PRATIMA NIKALJE, Department of Pharmaceutical Chemistry, Y.B. Chavan College of Pharmacy, India

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Raman Spectroscopy: An Emerging Tool for Clinical Diagnostics

Narahara Chari Dingari*, Ishan Barman, Jeon Woong Kang, Chae-Ryon Kong, Ramachandra R. Dasari, Michael S. Feld

**G. R. Harrison Spectroscopy Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA*

Biomedical applications of lasers and laser spectroscopy are changing the face of medicine as it is currently practiced. Spectroscopy is a promising means of extracting biochemical and morphological information from tissue that is relevant to disease progression and diagnosis. In particular, Raman spectroscopy is a powerful tool for non-invasive and real time diagnosis due to its exquisite molecular specificity and lack of sample preparation requirements. Raman spectroscopy, which measures the molecular vibrations of a sample, is currently being used to study atherosclerosis, measure blood analytes, and detect dysplasia and cancer in various tissues including the breast, cervix, prostate, and skin. In this talk, we present our results on quantitative biological spectroscopy for non-invasive blood analyte detection. Our work in this area is primarily motivated by the necessity for accurate and frequent measurement of blood glucose levels, which is most commonly achieved by withdrawal of blood. Given the inconvenience and invasiveness of this procedure, a non-invasive method would greatly benefit the increasing number of diabetics. Our laboratory has successfully demonstrated the ability to measure glucose, urea and other blood analytes in serum, whole blood and individual human volunteers. In addition, we present our results for turbidity correction and suppression of tissue autofluorescence in biological Raman spectroscopy. We show that correction for these non-analyte specific variances provides a clinically accurate and robust calibration algorithm that can be used for prospective prediction in human population. Finally, we discuss our plans for miniaturization of the device for point of care and commercial applications.

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Application of LC-MS Based Methodologies for the Characterization and Quantitation of Complex Biologics-Antibody Drug Conjugates

Babu Subramanyam*, J. Shen, J-L, Tseng, C. Elango and J-M Gu

Biologics Research US, Global Biologics, Bayer Health Care Pharmaceuticals, Richmond, CA, USA

Though LC-MS based platforms are an integral part of "small molecules"/ Xenobiotic drug discovery/development paradigm, application of these techniques to biologics is still an evolving field. In this paper the application of LC-MS and LC-MS/MS techniques for the characterization and quantitaion of complex biological entities such as antibody drug Conjugates (ADCs) will be discussed. The discussion will highlight how the LC-MS data can complement the more traditional ELISA based assays and what additional information can be gleaned from the MS data regarding the integrity and bioprocessing of these complex drug entities.

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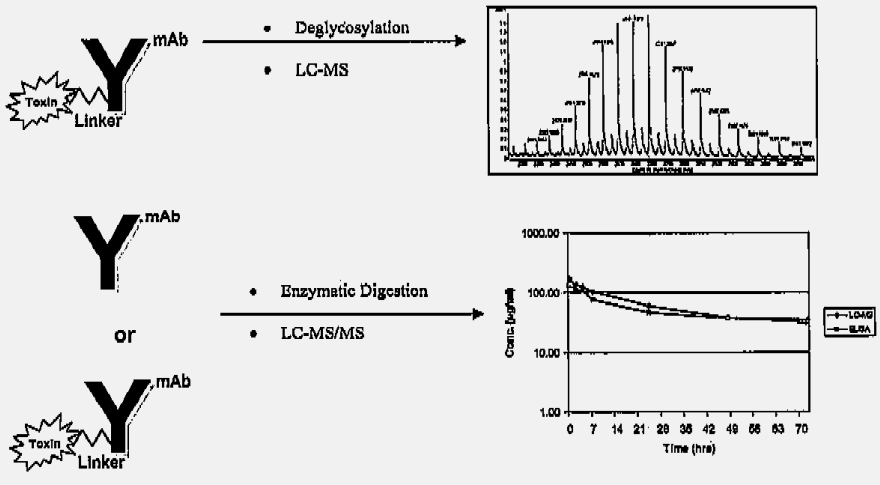

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Combined Laser Induced Breakdown Spectroscopy and Raman Spectroscopy for Pharmaceutical Applications

G Manoj Kumar

Advanced Centre of Research in High Energy Materials (ACRHEM)
University of Hyderabad, Hyderabad, India

I will talk about two complementary spectroscopic techniques, namely Laser Induced Breakdown Spectroscopy (LIBS) and Raman Spectroscopy for the pharmaceutical applications. While Raman spectroscopy which is widely used in the industry to get the molecular information, LIBS is a relatively new technique that gives atomic information. It is an atomic emission based technique involving the interaction of a target with an intense laser pulse which generates plasma. The spectral analysis of the fluorescence emission from the cooling plasma plume contains specific signature of atoms of the material under investigation. The amount of sample and time needed to prepare the sample are far less compared to any other technique used for the elemental analysis such as Absorption, Fluorescence, FT-IR, Raman or Cavity Ring down Spectroscopy. LIBS is particularly attractive for the detection due to its stand-off detection capability, requirement of microscopic quantities of material, and rapid detection. The detection times are very fast which is very ideally suited for the industrial applications. LIBS and Raman studies on pharmaceutical samples have been reported in a recent study. A combined system capable of recording both the Raman and the LIBS spectra can give complementary information.

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Identification and Characterization of New Drugs Using Mass Spectrometry

K Srinivasa Rao

Roland Institute of Pharmaceutical Sciences, Berhampur, Orissa, India

Mass spectrometer can be used in 3 principal ways: firstly, to measure the molecular weights with very high accuracy; from these can be deduced exact molecular formulae. Secondly, to detect within a molecule the places at which it prefers to fragment; from this can be deduced the presence of functional groups within the molecule. And thirdly, as a method for identifying drug molecules by comparison of their mass spectra with libraries of digitized mass spectra of known compounds. Molecular ion gives highly useful information about the identity of the drug molecule. Fragmentation pattern gives further information about the structure of the drug molecule. All fragment ions are, however, not of equal significance to assign the structure to a compound. Intensity of the molecular ion peak in a mass spectrum depends on the type of the compound. Mass spectra cannot be interpreted if they contain any misinformation. Unfortunately, even referred journals and carefully edited collections of the standard spectra sometimes contains spectra that fail to meet this criterion. Some compounds e.g., alcohols may fail to give a visible molecular ion peak. Judging whether or not a mass spectrum is credible is sometimes the most critical step in its interpretation. Most EI mass spectrometers in use today lack sufficient resolving power to provide accurate mass measurement for the determination of elemental composition. However, the elemental composition of an ion can sometimes be determined from the ratios of the peak intensities of the isotope peaks for that ion to the intensity of the nominal mass peak. Some typical features may be helpful i.e., if the M+2 peak of the parent ion looks larger than the M+1 peak, the compound might contain S,Cl or Br. When there is a larger gap and a peak at 127, iodine may be present. The intensity of the M+1 peak can be used to know the number of carbons well as nitrogen atoms. In the absence of nitrogen, the maximum number of carbaon atoms can be calculated by dividing the relative intensity of the M+1 peak by 1.1. Thus, e.g., a molecule with 12 carbon atoms will display a M+1 peak of 13.2 per cent. In case nitrogen is present its contribution to the M+1 peak will amount of 0.4 X number of nitrogen atoms. This quantity must be subtracted from the measured relative intensity of the M+1 peak to know the number of carbon atoms. When in a compound Cl, Br, S or Si is present loss of a proton from the M+2 is likely to enhance the intensity of the M+1 peak. The number of nitrogen atoms present can be deduced with the help of nitrogen rule. In this talk we will address, how to identify different drug molecules using mass spectrophomter with relevant examples.

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Application of Current Approaches and Newer Trends in Identification of Drug Metabolites Using Liquid Chromatography-Mass Spectrometry

Bhagwat Prasad and Saranjit Singh

Department of Pharmaceutical Analysis, National Institute of Pharmaceutical Education and Research (NIPER), Punjab, India

Metabolite identification is amongst the important studies during early stages of drug development because metabolic products may be pharmacologically active or toxic in nature. In the last one decade, there have been revolutionary changes in the way metabolite identification is carried out. This has mainly become possible due to the advent of sophisticated analytical modalities, particularly, hyphenated liquid chromatography-mass spectrometry (LC-MS). There are varieties of LC-MS systems available with difference in their utility in metabolite identification. Particularly, HPLC coupled with high resolution-mass spectrometry (HR-MS) and multiple-stage MS (MS^n) plays a leading role in identification of metabolites (1-2). Sample preparation, mass fragmentation studies, *in silico* metabolite prediction and detection, chromatographic retention, UV spectra matching, determination of molecular formula, and establishment of possible site of metabolism are the important aspects in unequivocal identification of metabolites. In this same context, there have been several recent advancements in metabolite identification. These include approaches for detection of reactive metabolites, new generation LC systems and MS ion sources, isotopic pattern matching, hydrogen/deuterium exchange mass spectrometry, data dependent analyses, MS^E approach, mass defect filter, 2D and 3D approaches for elucidation of molecular formula, polarity switching, background subtraction-noise reduction algorithms (BgS-NoRA), etc. The same will be discussed with case examples, as appropriate.

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Role of Drug Metabolism and Pharmacokinetic Studies in Drug Discovery and Development

Chandra Prakash

Senior Director, Dept. of Drug Metabolism and Pharmacokinetics Biogen Idec, Cambridge, USA

The drug development process is scientifically complex, time consuming and expansive. Recent data indicate that the discovery and development of a new drug costs around 1.0 billion dollars and takes 12-15 years for the drug to reach the marketplace. In addition, 90% of all drugs in clinical development fail to make to the market. Efforts are being made to reduce attrition of drug candidates during the various stages of drug discovery and development, and to bring safer drugs to the market. The major reasons for the attrition and serious side effects are sub-optimal drug metabolism and pharmacokinetic (DMPK) profile, poor clinical efficacy and the formation of reactive metabolites. Given the inherent inefficiency of the development, it is essential to optimize/minimize such factors early in drug discovery process. This has led to greater integration of DMPK functions into early stages of drug discovery process and in addition to potency and selectivity; drug candidates are selected on the basis of DMPK properties, e.g. low clearance, good oral bioavailability, optimum half-life, and an acceptable metabolism profile in preclinical species and humans. This presentation will summarize the in vivo/in vitro techniques used for rapid determination of the DMPK profiles including absorption, metabolic stability, metabolite structures, cytochrome P450 inhibition/induction, and pharmacokinetics and role of these studies in the selection of the drug candidates for further development. Knowledge of metabolic profiles of these candidates in an early stage of drug discovery is essential to select compounds with favorable pharmacokinetic credentials and to aid medicinal chemists for rational drug design.

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Development and Validation of Stability Indicating Lc- Pda Method for Mycophenolate Mofetil (Mmf) in Presence of its Impurities and Major Degradation Product Mycophenolic Acid (Mpa) Using Factorial Design Tool and Use of Mass Spectroscopy

Anna Pratima Nikalje

Department of Pharmaceutical Chemistry, Y.B. Chavan College of Pharmacy, Aurangabad, India

Factorial design tool was applied for development of isocratic reversed-phase stability indicating HPLC method for the analysis of Mycophenolate mofetil (MMF), its degradation products Mycophenolic acid (MPA) and degradation products (DP3). Separation was achieved on a Symmetry C18 (250 mm × 4.6 mm, 5.0 µ) column using a Methanol: acetate buffer (75:25 v/v), pH 6.0 (adjusted with acetic acid), at 0.5 ml flow rate, column maintained at 55 ^{0}C and data was integrated at 251 nm. MMF was subjected to hydrolysis, oxidation, heat degradation, etc. under all these conditions degraded products were well separated. The method validation characteristics included accuracy, precision, linearity, range, specificity, LOD and LOQ. Robustness testing was conducted to evaluate the effect of minor changes to the chromatographic conditions and to establish appropriate system suitability parameters. The proposed method was used to investigate kinetics of acid, alkali hydrolysis and oxidation process. Major degradation product MPA and DP3 were isolated and quantitated. Characterization of MPA by NMR and LC-MS/MS and other degraded products by LC-MS/MS was attempted successfully. The method was used successfully for the quality assessment of three MMF drug products and its acid, alkali and oxidative degradation kinetics study. A simple and efficient stability indicating reverse-phase HPLC method was developed and was found to be accurate, precise and linear across the analytical range and is reported for the first time. The method is simple, fast, sensitive and specific for the determination and quantification of MMF, MPA and DP3.

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Biologic Immunotherapy with Human Intravenous Immunoglobulin Ivig in Inflammatory and Autoimmune Disorders: Experiences During the Last 30 Years

Paul Imbach

University Children's Hospital Basel UKBB, Basel, Switzerland

The first publication on intravenous immunoglobulin (IVIG) administration for children with immune thrombocytopenia ITP (Imbach et al., The Lancet 1981;1:1228-1231) evoked targeted immunomodulation in patients with inflammatory and autoimmune disorders. We update the development of the immunomodulatory effects of IVIG over the last 30 years. The biologic, human IVIG is extracted from the pooled plasma of 10'000 – 60'000 blood or plasma donations. The safety of IVIG is controlled by ongoing careful selection and deferral of donors, by testing and validation of donated blood and plasma as well during the steps of production including the purification process. The key observation/discovery was made in a boy with severe bleeding immune of ITP. Following administration of IVIG the boy's platelet counts started to increase and in a subsequent pilot study the same phenomenon was observed of 12 consecutive children with ITP. Since then, there have been controlled clinical trials of IVIG in patients with ITP as well as in other inflammatory or autoimmune disorders. Examples of documented immunomodulation are today: in hematology: graft versus host disease, allograft recipients, autoimmune lypmphoproliferative syndrome and others, in neurology: Guillain-Barré syndrome, dermatomyositis, myasthenia gravis, multifocal motor neuropathy, remitting-relapsing multiple sclerosis and others, in dermatology: autoimmune mucocutaneous blistering diseases, pemphigus, Stevens-Johnson syndrome and others. Extensive studies on the mechanisms of action of IVIG have documented the immunomodulatory interaction in the disturbed immune response in these patients, although the mechanisms of actions remain far from being clear. Today, the clinical efficacy of IVIG has resulted in high demand for the product. The peer reviewed scientific, original articles on "IVIG" (see PubMed) listed at total of 32'251 publications until 2010. The worldwide annual use of IVIG increased remarkably, from 300 kg to 70,000 kg over the last 30 years. Thus, the human derived product IVIG challenged therapeutic approaches from immunosuppression to biologic immunomodulation in many inflammatory and autoimmune disorders.

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Basic Tetrapeptides as Potent Intracellular Inhibitors of Type A Botulinum Neurotoxin Protease Activity

S. Ashraf Ahmed

Department Cell Biology and Biochemistry, Integrated Toxicology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, USA

Botulinum neurotoxins (BoNT) are the most potent of all toxins. They cause flaccid muscle paralysis leading to death. They are also potential biothreat agents. A systematic investigation of various short peptide inhibitors of the BoNT protease domain with a 17-residue peptide substrate led to arginine-arginine-glycine-cysteine (RRGC) having a basic tetrapeptide structure as the most potent inhibitor. When assayed in the presence of dithiothreitol (DTT), the inhibitory effect was drastically reduced. Replacing the terminal cysteine with one hydrophobic residue eliminated the DTT effect but with two hydrophobic residues made the pentapeptide a poor inhibitor. Replacing the first arginine with cysteine or adding an additional cysteine at the N-terminus did not improve inhibition. When assessed using mouse brain lysates, the tetrapeptides also inhibited BoNT/A cleavage of the endogenous SNAP-25. The peptides penetrated the neuronal cell lines, N2A and M17, without adversely affecting metabolic functions as measured by ATP production, and P-38 phosphorylation. Biological activity of the peptides persisted within cultured chick motor neurons and rat cerebellar neurons for more than 40h, and inhibited BoNT/A protease action inside the neurons in a dose- and time-dependent fashion. Our results define a tetrapetide as the smallest peptide inhibitor in the backdrop of a large substrate protein of 200+ amino acids having multiple interaction regions with its cognate enzyme. The inhibitors should also be valuable candidates for drug development.

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Enhancement of Skin Permeation: the Metabolic Approach

Bijaya Ghosh

KLE University's College of Pharmacy, Bangalore, India

To enhance the skin permeability of a drug, the barrier function of the skin must be overcome at least temporarily. The barrier function of stratum corneum –the rate limiting membrane for skin permeation, depends upon the quality and quantity of its constituent lipids and a decrease in their concentration affects its barrier properties. In general, barrier disruption is followed by quick recovery responses. In the metabolic approach, permeation rate is enhanced by delaying this natural recovery processes by application of chemicals/drugs that interfere with the skin metabolism. However, to use this strategy, one has to have a clear understanding of the constituents of the skin as well as the mechanism of Skin homeostasis. The present article discusses some important aspects related to it. 1) the liquid crystalline nature of stratum corneum (the cholesterol and ceramides) 2) the biophysical aspects of the barrier lipids, 3) the sequence of events at stratum granulosum-stratum corneum interface, 4) role of different enzymes/ drugs (HMGCoA Reductase inhibitors) that interfere with barrier recovery and thereby enhance permeation rate of drugs. Important research on this aspect is also analyzed along with the advantages and limitations of the strategy.

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Biomarkers in Cancer: Recent Advances

Sarasija Suresh

Department of Pharmaceutics, Al- Ameen College of Pharmacy, India

Biomarkers are invaluable tools for cancer detection, diagnosis, patient prognosis and selection of treatment. In cancer research and treatment, assay of biomarker can be used to identify the presence of tumour, determine its stage, subtype and ability to respond to treatment. Further, biomarkers in cancer can be used to better understand tumour formation and to develop new therapeutic approaches. Despite years of intensive analysis only a small number of plasma proteins have been validated as cancer biomarkers such as prostate specific antigen and cancer antigen125. Now with technological advances in gene expression, genomic and proteomic analysis discovery of biomarkers is a fast growing area of cancer research. Development of genomics and proteomics has made it possible to monitor a large number of cellular pathways simultaneously. This has enabled the identification of biomarkers and signalling molecules associated with cell growth, cell death and cellular metabolism. Further, they are facilitating in monitoring the functional disturbances, molecular and cellular damage and damage response. This talk aims to describe some of the development of biomarkers in cancer research and detection with emphasis on different genomic and proteomic tools for the identification and discovery of new biomarkers including gene expression signatures associated with cancer cells which can tell us about cancer pathogenesis, progression and help in identifying patients who are most likely to respond to treatment and 'single cell proteomics' to further understand tumorigenesis and work towards personalized treatment strategies.

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Standardization and Preclinical Studies on 'Kakadshringi': Leaf Galls Used in Ayurvedic System of Medicine

Anuradha S. Upadhye

Plant Sciences Division, Agharkar Research Institute, Pune, India

The growing demand of crude drugs (raw materials and their products) by populations is forcing to develop standards for quality control and to evaluate the health climes. Much more studies in this aspect have been done on plant originated crude drugs/raw materials. Meager evaluation is available considering animal originated raw materials. Use of insect galls as medicine is not new but evaluation of such materials is unique. The ethnobotanical and Ayurvedic literature indicates that 'Kakadshringi' leaf galls are used for the treatment of diarrhoea. The leaf galls occurring on botanically three different plant species viz. *Pistacia integrrima* Stew. ex Brindis, *Terminalia chebula* Retz., and *Garuga pinnata* Roxb. are commerced in trade. These samples were standardized using pharmacognostic and phytochemical parameters to establish identification markers. To investigate the text clime, antidiarrheal activity was evaluated using animal modal. The result suggested that ethanol extract of *P. integerrima* and loperamide, a standard antidiarrheal drug showed significant reduction in fecal output in castor oil and magnesium sulphate induced diarrhoea and castor oil induced intraluminal fluid accumulation. Also it inhibited dose dependently (100-500 mg/kg) the intestinal propulsion of charcoal meal in normal and barium chloride induced changes in gastrointestinal tract. The ethanol extract of *P. integerrima* has antidiarrhoeal, antisecretory and antipropulsive activities and it may be due to their high phenolic and tannin content. Our studies indicated that *P. integerrima* can be equated to 'Kakadshringi' and its indication for the treatment of diarrhoea.