

## POSTERS

Wednesday, October 24 – Boardroom Lobby

### **WP-1: Qualitative and Quantitative Analysis at Microdosing Levels in Vivo Rat-Plasma Samples using LC-MS/MS Technique**

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The concept of microdosing or Phase 0 was introduced by FDA in early 2006 to conduct a full metabolism study after administration a dose less than 1/100th the dose of a test substance with a maximum dose level of 100 µg. This strategy has been adopted in Exploratory Investigational New Drug Guideline to offer drug developers more innovative and fast ways to investigate their candidate molecules. The main advantage of microdosing studies is the early ADME-PK information to ensure that drugs do not have to be dropped later down the development pathway because of inappropriate metabolism, too short a half-life, or poor bioavailability. This information is then used as part of the decision tree to select which of the microdosed drugs has the appropriate PK parameters to take further. To detect such low levels of the target compound in biological matrices the FDA suggests the use of Accelerator Mass Spectrometry (AMS), although the cost per analysis is extremely high and the throughput is very low. The goal of this work is to show the potential of LC-ESI/MS/MS technique to identify metabolites and quantify (pharmacokinetics) the proprietary compound X in vivo rat plasma samples at microdosing levels (30 µg/kg and 100 µg/kg). The hybrid triple quadrupole linear ion trap 4000 QTRAP™ and high sensitive triple quadrupole API 5000™ were used for qualitative and quantitative analysis, respectively. The strategy for sample preparation applied was a simple plasma dilution by a factor of 10 with and direct injection, which showed good sensitivity, reproducibility, low matrix effects, resulting in a less time consuming and lower cost per analysis. A total of 15 metabolites were detected in the qualitative strategy and a LOQ of 30 pg mL<sup>-1</sup> were achieved in the quantitative one.

### **WP-2: Increased Throughput of *In Vitro* GSH Conjugate Screening of Drugs in Discovery with the Use of Principal Component Analysis**

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It is generally accepted that reactive drug metabolites may cause drug toxicity. Increasingly, in the drug discovery process, efforts are focused on the early detection of biologically active intermediates (BRI) to minimize potential toxic liability of drug candidates. A common approach to identify BRIs is to trap them with glutathione (GSH) *in vitro*. Characterization of the GSH conjugates reveals important information about the functional group(s) in small molecules that may be of potential toxic risk. Liquid chromatography in combination with tandem mass spectrometry (LC/MS/MS) is one of the best methods of detecting and characterizing trace levels of these conjugates. The workflow usually requires the analysis and comparison of parallel sets of incubates in the absence and in the presence of GSH to confirm GSH conjugate formation. Traditionally, the LC/MS/MS software allows for comparison of only a single sample and control, which is time consuming. In this case, all of this data is loaded into the PCA matrix simultaneously to allow for a comprehensive analysis. Furthermore, the use of a hybrid triple quadrupole/linear ion trap LC/MS/MS system, in combination with Information Dependent Acquisition (IDA), provides high sensitivity detection via: (1) neutral loss of 129 amu and (2) parent drug fragment precursor scans. One important feature of PCA is the ability of the software to process both of these scans in a combined fashion, further increasing throughput of analysis. This approach was applied to evaluate discovery compounds A and B and allowed for fast characterization of their GSH-conjugated metabolites. The results indicated significant formation of GSH-reactive metabolites predominantly in human microsomes for Compound A. In contrast, Compound B did not show any GSH-conjugated metabolites. This data illustrates the ability of PCA to facilitate the comparison of a large array of samples.

### **WP-3: Nanospray ionization combined with a Nanobore Column Switching Chromatography Format for the Quantitative Analysis of Paclitaxel in Plasma**

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The quantitative analysis of drugs in plasma is a well-accepted surrogate marker for drug exposure. Quantitative analysis of plasma has been performed with conventional liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Drug discovery demands fast method development and high-throughput; column-switching strategies have met these needs. As potency increases and dosages decrease, there is a need to increase sensitivity for quantitative analysis. Previous studies have demonstrated that off-line nanospray provides unique advantages for sensitive analysis

of small molecules in prepared plasma samples. On-line nanospray has similar potential, but there are challenges in sample prep, loading, and analytical cycle time. A novel nanobore chromatography column-switching device has been developed for quantitation in an attempt to meet these challenges. Paclitaxel was used as a model compound in this study. Samples were prepared by spiking paclitaxel directly into human plasma. The plasma samples were protein precipitated (50/50 ACN) and injected directly onto the nanobore column switching system. The system was comprised of dual gradient HPLC pumps and dual 6-port valves. A trap column (25mm x 100-150µm, C18 or polymer) is used to desalt samples and bump elute to a (10cm x 75µm C18) packed-tip column. The first valve was used to switch the sample trap column out of the flow path; the second to switch the analytical column from the trap directly to the second LC. Quantitation was performed on a triple quad instrument equipped with a nanospray interface.

#### **WP-4: An Interweaved multi-Algorithm Approach for Computer-Assisted Identification of Drug Metabolites**

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In modern pharmaceutical drug discovery it is of crucial importance to identify all possible metabolites of a new chemical entity because of their possible toxic effects on humans. Today, high resolution and high mass accuracy MS and MS/MS data, which are acquired from in vitro as well as in vivo metabolism experiments, are used for metabolite identification in the different stages of the drug discovery and development process. To make use of all potential information contained in such data it is essential to use different efficient computer algorithms for data analysis. For analysis of the high resolution high mass accuracy MS and MS/MS data gained from the metabolite experiments the data are extracted from a metabolite sample file and a parent drug control sample file. After extraction all molecular information such as isotopes and adducts they are grouped together, chemical noise is removed and after comparison only new emerging compounds from the metabolite sample file are processed further. For this processing different algorithms are used depending on the information that will be extracted. Each algorithm is able to identify new metabolites individually or can use or modify results of another algorithm to identify a metabolite. The results of all metabolite identification algorithms are weighted and a final identification score is created. With the described computer-assisted, multi-algorithm approach various pharmaceutical drugs from in vivo as well as in vitro experiments were tested to prove the concept. Data is presented that supports the suitability of this computer-assisted approach for the identification of metabolites from complex samples. An example is discussed, which shows the entire workflow from data extraction through to identification of the metabolizing reaction and elucidation of the metabolite structure.

#### **WP-5: Profiling Natural Lipid Mixtures to the Specificity Level of Individual Molecular Structures: Determining the Location of Double-Bonds on the Extended Hydrophobic Chain**

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Here we report an LC/MS/MS method for determining the location of the double-bond on unsaturated lipid species which can be very useful in identification of individual lipids. The LC/MS/MS experiment involves the use of a hybrid experiment whereby a full-scan survey experiment triggers a dependent product ion scan experiment. The resulting spectra of specific fragment ions contains information which is interpreted by our method. Our non-aqueous reverse phase variation allows for very high chromatographic resolution over a wide range of lipid types in a single injection. Nearly any lipid molecule and all of its metabolically related variations can be individually detected in a single injection. Here we interpret lipid fragmentation data including C24:1 ceramide, C18:1 ceramide, C16:0-18:1 PC (palmitoyl-2-oleoyl-sn-glycero-phosphocholine), 18:0- 18:1-S-O-G-P –serine, 16:0-18:1 PE (POPE) and 18:0-18:1-PC (1-steroyl-2-oleoyl-sn-glycero-phosphocholine). Fragmentation data were collected on species in positive and negative ionization modes for comparison.

#### **WP-6: Accelerating Component Identification in Global Metabolite Profiling Studies Using a Quadrupole Ion Trap Time of Flight Mass Spectrometer**

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Identifying and quantifying endogenous components in biological fluids is a critical factor in assessing target effects (efficacy markers) and off target effects (toxicity/liability markers). Identification of markers not only provides insight into the mechanism of toxicity but may result in the early evaluation of potential clinical markers. In this study a quadrupole ion trap-time of flight mass spectrometer was used to generate high accuracy MS<sup>n</sup> data on rat plasma samples taken from two strains of Zucker rat (the Zucker (fa/fa) obese, which is a leptin deficient mutant, and the wild type, which is normal) to determine metabolite profiles between the two strains of rat and to identify specific endogenous components. The study focused on the distribution of phospholipids in rat plasma and the identification of specific components using high mass accuracy MS<sup>n</sup> analysis with formula prediction software.

## **WP-7: Integrated Radioprofiling and LC/MS Data for Quantitative and Qualitative Drug Metabolism Profiling all from a Single Injection**

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The gold standard for the detection and quantitation of metabolites of a compound in a drug metabolism study is by radioactivity detection after incubation or administration of radiolabeled compound. Quantitative and qualitative metabolite data are usually obtained in parallel by post column splitting of the HPLC effluent between a radioflow detector and a mass spectrometer equipped with an electrospray source. More sensitive radiodetection may be achieved by fraction collecting into 96-well plates containing solid scintillant, evaporating to dryness and reading with a plate based radioactivity detector. Aligning the LC/MS data with the radioactivity "histograms" reconstructed from plates is not integrated. LC/MS/MS data collected on low level metabolites often provides incomplete data for structure elucidation requiring reinjection with targeted MS/MS. This approach has successfully been used to identify and characterize metabolites of diclofenac formed during rat and human liver microsomal incubations in the presence and absence of glutathione. The custom software package provided a simple and robust method for converting the microscintillation plate data into a reconstructed radio-chromatogram which is correctly aligned with the LC/MS data. This allowed for the accurate automated selection of fractions for nanoelectrospray infusion, and the automated collection of an MSn tree for each metabolite based on both the in line LC/MS data and the radioactivity data. The infusion MSn trees provided greater structural information of higher spectral quality and depth than can be obtained from online techniques alone. The ability to obtain the MSn data subsequent to the LC/MS chromatogram produced a higher quality LC/MS chromatogram for alignment with the radioactivity data as the duty cycle was not disrupted by attempting to obtain MSn spectra in a data dependent fashion during the chromatographic timescale. The reproducibility of MSn trees created by the automated infusion of collected fractions was found to be greater than those obtained by in-line data dependent techniques and provided much greater flexibility in allowing manual creation of MSn trees under operator defined parameters as required.

## **WP-8: An Integrated Method for Metabolite Detection and Identification Using High Resolution Mass Spectrometry and Multiple Data Processing Techniques**

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## **WP-9: Detailed Investigation of the Fragmentation of Aminopyridinyl-Imidazole-Based MAPKinase Inhibitors Using Accurate MS(n) and Isotopic Pattern Data**

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Inhibitors of the p38 MAP kinase family have demonstrated anti-inflammatory effects in preclinical disease models. Inhibitors based on aminopyridinyl-imidazole core structures (see Fig. 1) have been investigated concerning their fragmentation behaviour upon various chemical modifications by medicinal chemistry. Elemental composition of those fragmentation products can effectively determined with accurate mass data. The unequivocal identification is however dependent on the absolute m/z value and the number of elements present in the compound. A novel software tool to accelerate the elucidation of fragmentation pathways allowing the combined determination of formulae from precursor and its product ions is applied. In this study, we describe the elucidation of fragmentations of potential new drugs.

## **WP-10: Eliminating Sample Prep Time for Bioanalysis Using Automated ITSP SPE**

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Solid Phase Extraction (SPE) is well established as a preferred tool for extracting small organic molecules from complex biological matrices (e.g. plasma) because of efficient matrix removal. This paper details the use of a new SPE device for quantification of steroids in plasma using on-line automated SPE LC/MS. For decades SPE cartridges have been used to enrich or extract target molecules from complex matrices. Because of the efficiency in sample preparation, SPE is the "gold standard" method for many sample preparation procedures. However, traditional SPE cartridge and plate implementation suffers from difficult automation and extensive method development requirements. So, while SPE is generally regarded as the "best" method of sample prep, it is often the last choice because of the extensive labor requirements. To bypass the labor requirements of traditional SPE, on-line concentration and extraction approaches have gained popularity in recent years. These on-line "trap and elute" systems are fully automated and have demonstrated efficient performance for many traditional SPE applications. However, these on-line approaches suffer many shortcomings including: complex hardware and software setup; expensive "up-front" costs; and extensive effort to validate trap column regeneration. The ITSP (Instrument Top Sample Prep) format was developed to overcome these shortcomings by facilitating automated sample prep (SPE, filtration) using common laboratory robots (e.g. CTC PAL).

Using the device it is possible to perform SPE on the next sample while the current sample is being analyzed. This paper will describe our successful implementation of the ITSP device in quantitative bioanalysis.

## **WP-11: Method Development and Validation for Quantitative Analysis of a NCE and Its Isobaric metabolites in Plasma by Direct-Injection Turbulent Flow LC-MS/MS**

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A high throughput bioanalytical method using turbulent flow column switching liquid chromatography/tandem mass spectrometry has been developed and validated for simultaneous analysis of a new chemical entity (NCE) and its active metabolites. Two of three metabolites were isobaric compounds, which posed a great challenge to LC-MS/MS analysis. Therefore, chromatographic separation of the isobaric metabolites was essential. The Aria TX2\_HTLIC system coupled with an API-4000 mass spectrometer was used for the analysis. A Cohesive Cyclone extraction column and an Agilent SB C18 analytical column were selected. Chromatography was optimized to achieve sufficient separation of the isobaric metabolites. The calibration curves were from 10-1000 ng/mL for the NCE parent and M2; and 5-500 ng/mL for the M1 and M3 in plasma. Validation results showed that all criteria (precision, accuracy, specificity, matrix effects, stability and carryover etc.) met our laboratory SOP requirements. Advantages of on-line technology include cost-saving on SPE plates and elimination of labor-intensive and time-consuming sample extraction processes. This validated method has been successfully used to analyze NCE and its metabolites in several toxicokinetic (TK) studies with biological different matrices including rat, dog, mouse and monkey plasma.

## **WP-12 Human Serum and Plasma Protein Depletion – Novel High Capacity Affinity Column for the Removal of the ‘Top 14’ Abundant Proteins**

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The serum and plasma proteome are desirable biological samples due to their accessibility and representative complexity. There is great hope that the investigation of these samples will lead to the discovery of new protein markers for disease diagnosis and therapeutic monitoring and novel drug targets. The tremendous complexity of the plasma proteome presents extreme analytical challenges in proteome characterization. Depletion of high-abundant proteins in serum and plasma has become routine and an accepted technique. These high-abundant protein components interfere with identification and characterization of important low-abundant proteins by limiting the dynamic range for mass spectral and electrophoretic analyses. We are presenting the results on a new device for the specific depletion of the 14 high abundant proteins from serum and plasma. By depleting these 14 high-abundant proteins we are removing ~95% of the total protein mass. This depletion column has the same specificity, reproducibility, and high capacity as the industry standard Top-7 device. The device depletes the 14 targeted-proteins with robust performance for over 200 runs and excellent depletion efficiency as determined by ELISA. The depletion results in an improved dynamic range for proteomic analysis. Furthermore, the removal of the high-abundant proteins improves loading capacity on 2DGE and LC/MS, which simplifies a complex system in the goal of discovering biomarkers.