POSTER SESSION ABSTRACTS

Inspiration and Education

POSTER HALL OPEN
Thursday, April 13 9:00 am - 5:00 pm
Friday, April 14 9:00 am - 5:00 pm
Yangtze Ballroom

Including Submissions for Young Scientist Excellence Awards

CPSA Shanghai 2017
April 12-14, 2017
Renaissance Shanghai Pudong Hotel
Shanghai, China

Where Technology and Solutions Meet

Where East Meets West

POSTER ABSTRACT

Young Scientist Excellence Submission #101

Clinical and Pharmaceutical Aspects of Intraocular Lens Manufacturers: Economic Considerations for Public Healthcare Policies in Cataract Task Forces

Camila R. K Pena, Priscilla A. Jorge, Delano Jorge, Newton Kara-Junior

USP- University of São Paulo

Abstract

Objective: Evaluate the effectiveness and efficiency of cataract task forces for underprivileged communities, in a follow-up of 4 years, with the use of low price intraocular lens.

Methods: Retrospective study, based on a database of a sample of 58 patients randomly selected, submitted to a cataract task force in a rural area of Pernambuco, Brazil. All surgeries were performed by the same surgeon using the same surgical technique and equipment. The data collected included pre-surgical visual acuity, postoperative visual acuity and late post-surgical visual acuity, biomicroscopy slitlamp and fundoscopy evaluation. Patients with visual loss related to posterior capsule opacification was referred to Nd:YAG laser. For comparative purposes, it was used literature data from a hydrophobic intraocular lens. Cost of intraocular lens material and Nd:YAG laser capsulotomy for posterior capsule opacification was then evaluated and compared between the intraocular lenses.

Results: The mean age of patients with Ioflex was 72 ± 10.2 years. Four years after surgery,24 eyes (41.3%) had decreased visual acuity due to posterior capsule opacification. Two patients with posterior capsule opacification had decreased visual acuity due to intraocular lens opacification. Twelve eyes (20.7%) presented mild posterior capsule opacification with unchanged visual acuity. The total cost of the post-surgical procedures represents 74.5% more in the initial budget. The total cost of hydrophilic intraocular lens implantation and the postoperative complications per 100 was USD 7283.23. Based in the literature data, the hydrophobic intraocular lens had 7.47% of Nd:YAG laser treatment for posterior capsule opacification, and the total cost of hydrophobic intraocular lens implantation and its postsurgical complications per 100 was USD 10130.21.

Conclusions: Ioflex intraocular lens is efficient in cataract task forces in short term, however, long-term scheduled follow-up of operated patients is required, with an easier access to avoid low visual acuity again and loss of project efficiency.

POSTER ABSTRACT

Young Scientist Excellence Submission #102

In Situ Single Pass Intestinal Perfusion in Rats

Yangshun Tang, Honglei Ma, Xiaorong Lu, Quanli Feng, Cheng Tang, Sharon Tong, Xin Zhang*

Department of DMPK Service, WuXi AppTec, 90 Delin Rd, Waigaoqiao Free Trade Zone, Shanghai, P.R. China

Abstract

Intestinal drug permeability has been recognized as a critical determinant of the fraction dose absorption, with direct influence on bioavailability, bioequivalence and biowaiver, respectively. The purpose of the research is to validate the permeability of *in situ* Single-Pass Intestinal Perfusion (SPIP) model with the literature data and the correlation with human permeability. In this study, we assessed the rat intestinal permeability of 8 model drugs with different permeability characteristic (high, moderate and low). The effective permeability coefficients in intestinal lumen (P_{eff}) of the 8 model drugs were consistent with literature data. Moreover, excellent correlation was obtained between the P_{eff} from WuXi SPIP model with the P_{eff} values in humans. The P_{eff} values obtained from WuXi SPIP model also well correlates with the P_{app} (A to B) values from WuXi in-house Caco-2 model. In conclusion, the SPIP model is useful for obtaining intestinal permeability values that can be further used for predicting the intestinal permeability in humans.

POSTER ABSTRACT

#103

Microdose Carbon-14 Analyzing Systems

Stephen R. Dueker[1], HeeJoo Lee[1], Sookie La[1], Ted Ognibene[2], A. Daniel McCartt, Feng Dong[3]

[1]BioCore, Seoul Korea
[2]Lawrence Livermore National Lab, Livermore California
[3]Picarro, Santa Clara California

Novel Aspect: Low background 14C tagged molecules provides high specificity detection with minimal sample preparation. Newer AMS and Laser based analysis systems are democratizing access to low level radiocarbon detection to support human microdosing, microtracing, and even animal ADME studies. The lower cost Laser systems are expected to displace LSC as the method of choice for 14C radioanalysis.

Abstract: Radioisotopes such as radiocarbon are distinctive and specific because they are very rare in natural materials. Any radioisotope-labeled compound has a high signal to noise ratio in the biological system (but the poor signal to noise of a detector may mask this fact). For example, 14C has a natural level of abundance due to cosmic radiation at 1.2 parts per trillion. The “rare” stable isotope of carbon, 13C, is naturally 1.1%. A part per million concentration of a 13C-labeled compound (assume 200 g/mol) will change the concentration of 13C by only 0.3 per mil, measurable under good conditions using an excellent mass spectrometer. The same material labeled with 14C changes the concentration of that isotope in the biological sample by a factor of 3 million. Efficient detection of radioisotopes is a key to using this specificity.

AMS is the current method of choice for efficient detection of radiocarbon, but its availability and utility has been hampered by instrument size and cost, operational complexity, and a lack of automation for sample preparation and loading. The barriers are rapidly falling and new Laser based methods may soon replace AMS and LSC for many applications.

Methods: We review and present data taken on recent advances in AMS interface automation driven by the development of gas-accepting ion sources. These advances have led to fully automated samples loading, LC interfacing, and the ability to work with ever smaller sample masses. Systems discussed are the gas accepting ion source from ETH Zurich, the fully automated sample combustion and sample feed system at TNO (the Netherlands), and the LC moving wire interface developed at Lawrence Livermore National Lab. We then step away from AMS into CRDS (Cavity-Ring-Down Spectroscopy) - laser-based spectrometry for 14C quantitation being developed by LLNL and Picarro Inc (Santa Clara, CA). The CRDS system is

rapidly becoming competitive with AMS in its performance, but will be similar to LSC in terms of cost and ease of use.

Analysis: We will compare and contrast the current state of the art AMS and CRDS systems. The primary variables are presented in the below table.

Variable	CURRENT GRAPHITE AMS (MOST AMS FACILITIES)	AUTOMATED GAS ACCEPTING AMS (ETH ZURICH, TNO)	LC-AMS (MOVING WIRE, LLNL)	CRDS LASER (LLNL, PICARRO)
Size	Facility	Facility	Table Top	Tabletop
Capital & Facility Costs (Millions USD)	2 MM	2.5 MM	2.5MM (LC +AMS)	0.25MM
Expertise required	Ph.D.	Ph.D.	Ph.D.	Lab Tech
Sample turnaround	Days	Minutes	Seconds	Minutes
Carryover	<0.1%	1%	Variable	1%
Dynamic range (Modern)	0.01 - 300	0.06-80	0.01 -600	0.7 – 20,000
Sensitivity (Mol)	10^{-18}	10^{-18}	10^{-21}	10^{-15}
Precision	0.1%	1-5%	1-5%	5%
Sample Mass	1 mg	5 ug	50 ng	<1 mg-C
Analysis Costs* (USD)	~200	<100	1000 per metabolite radiochromatogram	<50 USD

* Analysis costs are presented based upon the author's experience, costs of consumables, staffing and throughput considerations.

POSTER ABSTRACT

#104

Matrix Independent Quantification of Proteins to Attomole Levels in Milligram-sized Samples

Stephen Dueker, HeeJoo Lee, Sookie La

BioCore, Seoul Korea

Novel Aspect: Combustive AMS solves many of the quantitative problems associated with protein or biologics analysis and complements existing MS Identification methods

Abstract: Mass Spectrometry has become the tool of choice for quantification of peptides and proteins. This tool is applied for intact protein analysis, pharmacokinetic profiling and determination of degradation products. Variation in ionization, fragmentation and detector response across the protein mass range hamper the quantitative power of MS. Combustive isotope ratio Accelerator Mass Spectrometry solves many of quantitative issues by providing standard free quantitation of lightly 14C-labeled large molecules without confounding matrix interferences. We illustrate the process for matrix independent quantification of proteins or protein-small molecule conjugates using combustive AMS on a 14C labeled antibody injected into three test rats.

Methods: A 150,000 kDa antibody labeled in a sulfhydryl position(s) using 14C radiolabeled N-ethylmaleimide. The resultant specific activity was 55 mCi/mol, showing almost complete labeling at this single reactive site (1 14C per molecule = 62 Ci/mol). After cleanup by multiple dialysis steps, the purified antibody was delivered byn intravenous bolus injection into three rats (10 nCi per animal). Whole blood was serially harvested for 24 hr post dose by tail bleed into K3EDTA tubes. 15 uL aliquots were removed, dried, and combusted to gaseous CO2 in sealed quartz tubes using CuO as a solid oxygen source. The CO2 was reduced to graphite over an iron group catalyst and analyzed for 14C contents by AMS.

Data: The 14C counts were normalized to the stable carbon-12 macroscopic current which in essence serves as the internal standard. Results are reported as pg-antibody equivalents per mL after factoring in the blood total carbon concentration and the specific activity of the antibody. Blood pharmacokinetics displayed a biphasic disappearance that closely mimicked a parallel dataset generated using established ELISA method. Limits of detection were order of magnitude greater for the AMS method (low femotmolar) than the ELISA method and repeatability was <3% CV on independently processed replicates. No method specific development was required.

POSTER ABSTRACT

#105

Highly Sensitive Quantitation of Oxidative Biomarker 3-nitrotyrosine in Rat Plasma by Coupling μElution SPE with Microflow LC-MS/MS

Ping Xu, Marc H Cao

Lilly China Research and Development Center, Shanghai, China

3-Nitrotyrosine (3-NT) is an endogenous oxidative biomarker exhibiting elevated levels in diverse pathological conditions, such as the inflammation, vascular disease, diabetes and neurodegenerative disorders. Even though numerous analytical methodologies, i.e. ELISA, GC-MS, HPLC-UV/FL/ECD, LC-MS, had been developed to quantify free 3-NT in different biological fluids and tissues, the reported concentration varied considerably, underlying special analytical challenges and problems associated with its accurate determination. This confounded the interpretation and significance of 3-nitrotyrosine as in vivo oxidative biomarker. In this presentation, we demonstrated a high throughput and ultra-sensitive LC-MS/MS method to accurately quantify free 3-NT using only 50 μL of rat plasma. By combining mixed-mode cation exchange micro-elution SPE (μMCX) extraction and fast separation on a Kinetex F5 column (2.1x 100 mm, 2.6 μm), we eliminated strong matrix effect, endogenous interference and several redundant steps causing artificial oxidation of highly abundant free tyrosine to 3-nitrotyrosine during sample preparation procedure, which had plagued other methods required harsh condition, such as GC-MS. 0.2% BAS was used as surrogate matrix to construct calibration curve after establishing the parallelism with that in rat plasma. The assay was validated from 0.1-50 nM with good linearity, precision, accuracy, specificity and minimal matrix effect. We also demonstrated the lower limit of quantification (LLOQ) can be further lowered by about three-folds when utilizing micro-flow LC as front-end chromatographic platform. Artifactual 3-NT formation was observed when using isotope-labeled tyrosine as a tracer, but was less than 6% of endogenous basal level. The addition of antioxidants could not significantly inhibit the oxidation process. The method was applied to quantify 3-NT in a preliminary rodent study. This novel method is one of the most sensitive assay for fast detection of free 3-nitrotyrosine in biological samples by LC–MS/MS.

POSTER ABSTRACT

#106

Development of Human MATEs-mediated Uptake and Inhibition Assay System

Ting Zhang, Hui Dai, Hongxia Zhang, Haiwei Xiong, Wujian Ju, Genfu Chen

Department of DMPK Service, Lab Testing Division, WuXi AppTec Co. Ltd., 90 Delin Rd, Waigaoqiao Free Trade Zone, Shanghai, 200131, P.R. China

ABSTRATE: The kidney is one of the main organs responsible for the excretion of drugs and xenobiotics. Human multidrug and toxin extrusion proteins (MATEs), e.g., MATE1 (SLC47A1) and MATE2-k (SLC47A2), are one of the major transporters for the secretion of cationic drugs into the urine. Because the widespread distribution of MATE proteins in various living organisms, and their capacity to transport wide variety of endo-/exogenous substrates, the importance of these transporters in physiological and pharmacological processes are recommended in DDI studies.

In this study, WuXi AppTec utilized the human embryonic kidney cell line (HEK293), stably transfected with human uptake transporter genes (MATE1, MATE2-k) (licensed from GenoMembrane, Kanagawa, Japan) to evaluate the inhibition and substrate potential of the test compound on the transporters, individually.
The known substrate, metformin exhibited pH-dependent, time-dependent and concentration-dependent MATE1 and MATE2-k uptake, respectively, and statistically significant differences were observed at each concentration between HEK293-MATE1 and HEK293-MOCK, HEK293-MATE2-k and HEK293-MOCK cells of uptake folds.
The inhibitory parameters of known inhibitors for MATEs, pyrimthamine, cimetidine were determined in the study. These data are comparable to literature reports.

INTRODUCTION: The MATE transporters are primarily expressed in the kidney and liver, and they are localized at the apical membranes of the renal tubules and bile canaliculi. Though MATEs' function as efflux transporters pumping their substrates out of cells, they belong to the family of solute carriers (SLC). The driving force for MATE-mediated transport is provided by oppositely directed pro-ton gradient; MATEs are, therefore, considered secondary active transporters.

MATE1 and MATE2-k are able to transport metformin utilizing an oppositely directed H+ gradient as a driving force, these indicate that MATE1 and MATE2-k are H+/organic cation antipoters. WuXi AppTec utilized the human embryonic kidney cell line (HEK-293), stably transfected with human uptake transporter genes (MATE1 and MATE2-k) (licensed from GenoMembrane, Kanagawa, Japan) to evaluate the inhibition and substrate potential of the test compound on the transporters, individually.

POSTER ABSTRACT

#107

In Vitro Evaluation of OATP1B1- and OATP1B3-mediated drug-drug interactions using statins as probe substrates

Beáta Tóth, Viktória Juhász, Ildikó Nagy, Zsolt Fekete, Zsuzsanna Gáborik, Emese Kis and Erzsébet Beéry

SOLVO Biotechnology, Gyár utca 2, 2040 Budaors, Hungary.

HMG-CoA inhibitors (statins) are among the most prescribed medications. As a result, it is almost unavoidable that drugs in development will ultimately be co-administered with statins. Statins are generally well tolerated in humans, but adverse effects associated with myopathy have been reported, and range from muscle pain to fatal rhabdomyolysis. Because of this risk, any drug-drug interaction (DDI) that might cause an increase in statin systemic exposure is of particular clinical importance, and should be identified early in the drug development process. There are numerous reports of clinically relevant DDIs involving statins, the majority of which are ascribed to OATP interactions, with or without contributions from other transporters and drug metabolizing enzymes. OATPs are also among the most often cited transporters mediating clinically relevant DDIs, often due to interaction with statins.

Preclinical evaluation of the potential DDI risk between statins and drugs in development is therefore of particular importance. In order to further characterize the current *in vitro* tools used to assess the risk of OATP-mediated DDIs, we have validated the use of stable OATP1B1- and OATP1B3-expressing HEK293 cell lines using the range of commercially available statins.

Methods: Uptake of selected statins (atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin and simvastatin) was studied in HEK293-OATP1B1 and HEK293-OATP1B3 cell lines, as well as in a mock-transduced HEK293 (control) cell line. Initially, time dependent and transporter-specific transport was evaluated. Furthermore, concentration-dependent transport kinetics as well as the inhibiting effect of selected inhibitors were studied for statins showing detectable active transport. The accumulated amount of each statin was analysed using LC/MS.

Results: Time-dependency assays revealed excellent signal to noise ratios for transport of atorvastatin, pitavastatin, pravastatin and rosuvastatin. Due to the higher lipophilicity fluvastatin transporters-specific transport was lower, yet still yielded acceptable signal to noise ratios. On the contrary, the signal to noise ratio for highly lipophilic statins lovastatin and simvastatin was below acceptable levels. Based upon these initial results only atorvastatin, fluvastatin, pitavastatin and pravastatin

were selected for further characterizing transporter kinetics in concentration-dependent experiments to determine K_m/V_{max} values. Subsequently atorvastatin and pitavastatin were selected as probe substrates to determine IC_{50} values of high- and low-affinity inhibitors.

Conclusions: In summary, the use of one or more statins as probe substrates in the OATP1B1 and OATP1B3 transporter inhibition assay can be a useful tool for the *in vitro* study of potential drug-drug interactions and substrate-dependent inhibition involving this clinically important class of drugs.

POSTER ABSTRACT

#108

Bioanalytical Validation for the Determination of Erythropoietin (EPO) in Human Plasma Using ELISA

Ji Hee Yun, Sookie La, Hee Joo Lee

Drug Development Supporting Service Division, BioCore Co., Ltd., Korea

Abstract

Erythropoietin (EPO) a glycoprotein produced primarily by the kidney, is the principal factor regulating red blood cell production (erythropoiesis) in mammals. EPO is used to treat anemias. In this study, the methods for the quantification of EPO in human plasma samples had been developed and validated by Enzyme linked immunosorbent assay (ELISA). Quantikine IVD Human Erythropoietin ELISA, based on a sandwich format utilizing a monoclonal antibody and polyclonal antibody, raised against recombinant human EPO, will be used. The color intensity will be measured at 450 nm with reference wavelength at 570 nm using a microplate reader. All absorbance levels will be corrected for non-specific background reading. The simplest model that adequately describes the optical density-concentration relationship will be used. The equation for this calibration curves will be used to calculate the EPO concentration in plasma. The standard curves was linear (r^2>0.9600) over the concentration range of 2.5-200 mIU/ml for EPO with acceptable accuracy and precision, respectively.

The each within- and between-batch precision and accuracy was less than 20% (LLOQ < 25%) and 80-120% (LLOQ 75-125%) of the relative standard deviation. And the selectivity, sensitivity, dilution linearity, parallelism and stability were validated and the method was successfully applied to the pharmacokinetic study of EPO.

POSTER ABSTRACT

#109

Bioanalytical Validation for the Determination of Risedronate and Cholecalciferol in Human Plasma Using LC/MS/MS

Jin Hyun Park, Sun Koung Joung, Kyung Hee Cho, Sookie La, Hee Joo Lee

Drug Development Supporting Service Division, BioCore Co., Ltd., Korea

Risedronate and cholecalciferol combinations are used to treat osteoporosis. In this study, the methods for the quantification of risedronate and cholecalciferol in human plasma samples had been developed and validated by liquid chromatography-tandem mass spectrometry. The lower limit quantifications (LLOQ) were 0.2 ng/mL and 0.3 ng/mL for risedronate and cholecalciferol respectively. Risedronate was extracted by derivatization reaction and solid phase extraction using 200 µL plasma and analyzed by LC/MS/MS systems. For the quantification of risedronate in human plasma, the separation was performed on a cation exchange column (150 X 2.0 mm i.d., 5 μm) and the flow rate was 0.2 mL/min. The LC-MS/MS system was equipped with an ESI source operating in the positive ion mode. Since cholecalciferol is an endogenous substance, a surrogate matrix was used for quantitative analysis. Cholecalciferol was extracted by protein precipitation and solid phase extraction using 200 µL plasma and analyzed by LC/MS/MS systems. For the quantification of cholecalciferol in human plasma, the separation was performed on a C_{18} column (50×2.0 mm, 3 µm) using column-switching system and the flow rate was 0.3 mL/min. The LC-MS/MS system was equipped with an APCI source operating in the positive ion mode. The standard curves of all compounds were linear (r>0.9950) over the concentration range of 0.2-100 ng/ml for risedronate and 0.3-25 ng/mL for cholecalciferol with acceptable accuracy and precision, respectively. The each within- and between-batch precision and accuracy was less than 15% (LLOQ < 20%) and 85-115% (LLOQ 80-120%) of the relative standard deviation. And the sensitivity, specificity, recovery, matrix effect, carry-over, effect of concomitant-drug and stability of these methods were validated and these methods were successfully applied to the pharmacokinetic study of combination of risedronate and cholecalciferol.

POSTER ABSTRACT

#110

Elimination the Interference of Rheumatoid Factors (RFs) in Immunogenicity Assay

Chenpu Zhang, BAS, LTD, WuXi AppTec

Immunochemistry Team, Bioanalytical Service, WuXi AppTec

Abstract

Rheumatoid factors (RFs) are endogenous human antibodies. RFs demonstrate preferential binding to aggregated gamma globulins and are involved in the clearing mechanism of immune complexes. The potential impact exists in the traditional immuno-bridge-assay of the immunogenicity assay in monoclonal antibody therapeutics in clinical BA studies. In the immunogenicity assay, the human anti-human antibodies (HAHA, the analyte in this assay) after administration of monoclonal antibody therapeutics are thus vulnerable to interference by RFs. When using the electrochemiluminescent (ECL) bridge immunoassay, pre-dose samples of the patients from Rheumatoid arthritis populations were measured much higher baseline reactivity than healthy subjects. In the pre-established immune-bridging assay, the aggregation of the Biotinylated monoclonal antibody therapeutics has been observed when it was stored for more than 8 months. The aggregated therapeutic antibody (after Biotinylation and Ruthenylation) conjugated with the RFs instead of the HAHA. When only 1% high molecular weight aggregates (>600 kDa) exists, it is sufficient to lead to increased ECL values, which have been observed in the immunogenicity assay for human monoclonal antibody therapeutics in clinical BA studies.

Optimize and establish an immune-bridge-assay on MSD platform for the immunogenicity assay to detect human monoclonal antibody therapeutics for clinical studies with RA patients.

- Human gamma globulins from Cohn Fractions II and III were cross-linked with glutaraldehyde and allowed to from a gel.
- After dispersal, centrifuge, wash, neutralize glutaraldehyde activity and final centrifuge and wash, the aggregated IgG (AIgG) formed a pellet with a clear supernatant.
- RFs have natural affinity to the Fc region of IgGs. A sample pre-treatment with the AIgG was added to the original sample analytical method.
- The test samples will be mixed with the equal volume of the AIgG pellet solution in the PP tube and 37°C incubated with shaking at 500 rpm.

- After pre-treatment and centrifuge, the supernatant of the samples will be transferred to a new dilution plate and do the remaining assay steps.

The condition was optimized successfully, and can be used for sample pre-treatment.
The AIgGs decrease the RFs interference on the ECL signal to 10% of the AIgG untreated.
58 pre-dose samples from RA populations were measured in the optimized assay and generated the acceptable SCPF = 1.82.

POSTER ABSTRACT

#111

Integrated Capability of DMPK for Preclinical Drug Discovery of Antibodies, Antibody Drug Conjugates and Peptides

Weihua Gu, Hua Zhong, Qi Gu, Rong Yang, David Xie, Jih-Lie Tseng, Lilly Xu, Wei Tang

Department of DMPK, Shanghai ChemPartner Co. Ltd

ChemPartner DMPK department started to provide integrated PK/PD/BA service for large molecule drug discovery from 2012. In the past five years, we have accumulated deep and wide knowledge in antibody drug, antibody drug conjugates (ADC) and peptide drug PK studies in rodents and monkey.

Here we presented several T-DM1 PK studies in rodents and Cynomolgus monkeys. T-DM1 was made by ChemPartner. The PK studies include: 1) T-DM1 single iv dose (6 mg/kg) PK study in SD rat. 2) T-DM1 (0.3, 1 or 10 mg/kg) and Kadcyla® (1 mg/kg) single iv dose or repeat iv dose (1 mg/kg, Q3W) monkey PK study. Total antibody and ADC were quantiatated using ELISA or LC-MS/MS, while MCC-DM1 was quantitated using LC-MS/MS. A generic hIgG ELISA method was also used to quantitate total antibody in monkey serum and the PK curve completely overlaps with the curve obtained by HER2 specific ELISA data. The PK parameters of ChemPartner manufactured T-DM1 are similar to that of Kadcyla® in the same study, and similar to the data disclosed by Genetech. Immunogenicity of T-DM1 in repeat dose PK study was tested using a bridging ELISA method, with a sensitivity of 50 ng/mL, when using a commercial anti-trastuzumab antibody as positive control. The occurrence of ADA can correlate well with the PK data.

Besides antibody, peptide PK capability has also been well set up, with LC-MS/MS method being the primary assay format, and ELISA or bioassay being the secondary assay format.
For mouse PK study, one mouse, one PK can be achieved through microsampling technique and high sensitive ELISA quantitation method.

Biomarkers, such as CTX-I, Amyloid β peptide, c-peptide, triglyceride, etc., can be tested using ELISA, ECL, or biochemical method. Modeling of PK/PD can be applied and used for predicting clinical use of drugs.

To conclude, ChemPartner has developed integrated capability of DMPK for preclinical drug discovery of antibodies, antibody drug conjugates and peptides.

POSTER ABSTRACT

#112

Development and Validation of a High Sensitivity LC-MS/MS Method to Quantify Hepalatide in Human Plasma and Urine for a Phase I Clinical Trial

Rong Yang[1], Weihua Gu[1], Quanfei Zou[1], Xiaofang Li[1], Jih-Lie Tseng[1], Lilly Xu[1], Wei Tang[1]
Cuili Fan[2], Hongli Liu[2]

[1]GLP BA center, DMPK, Shanghai ChemPartner Co. Ltd
[2]Shanghai Hep pharmaceuticals Co., Ltd.

Abstract

Quantification of peptides by LC-MS/MS continues to be a challenge regarding to sample stability and processing, method sensitivity and robustness, etc. We developed and validated a simple, rapid, sensitive and specific liquid chromatography-tandem mass spectrometry method for the quantification of Hepalatide, a 5,000 Da peptide, in human plasma and urine. The method has been used in the sample analysis of a randomized, double-blind, and dose-escalation Phase Ia and Ib Clinical Trial conducted by Shanghai Hep pharmaceuticals. CFDA GLP regulation was followed during both method validation and sample analysis.

After optimizing LC and MS conditions, and sample extraction procedures, the method was finalized. An isotope labeled peptide was used as internal standard. Proteins in plasma or urine samples were precipitated. The supernatant was dried and reconstituted. C18 column was used in reverse-phase HPLC. The protonated analyte was quantitated in positive ionization by multiple reaction monitoring with a mass spectrometer.

The analytical method was validated with respects to selectivity, dynamic range, determination of the lower limit of quantification, intra-assay and inter-assay accuracy and precision, carryover, analyte stability, recovery, matrix effect, and analyte interference. Current FDA and CFDA guidelines on bioanalytical method validation were followed. The standard curve was over the range of 0.20 to 100 ng/mL. The method demonstrated good accuracy and precision, the intra-run and inter-run precision for the analysis of plasma and urine are within 4.6~15.2% and 2.3~7.6% respectively, and the corresponding accuracies are within -4.6~8.5% and -13.5~7.7%, respectively.

The method has been successfully applied in the sample analysis of more than 2000 clinical samples in 25 analytical runs, with only 1 run failed, and more than 95% of ISR sample meet requirements.

POSTER ABSTRACT

#113

Quantification of Insulin Lispro in Human Plasma using UPLC-MS/MS

Hui Hong, Chenglin He, Jieqiong Sun, Jifa Zheng, Chao Bian, Kaisheng Jiao, Baomin Xin, Xinping Fang

WuXi AppTec Co., Department of Bioanalytical Services, 288 Fute Zhong Rd, Waigaoqiao Free Trade Zone, Shanghai, P.R. China, 200131

Abstract:

Insulin lispro is a recombinant human insulin analog in which the primary sequence has been altered by the inversion of amino acids at positions 28 and 29 of the B chain. Insulin lispro is equipotent to regular human insulin but has faster onset of action and shorter duration of activity. It is considered more efficient in the controlling of postprandial blood glucose. Now it is indicated for the treatment of type 1 diabetes mellitus in many countries worldwide.

The small structural difference between insulin lispro and human insulin has brought challenges to the effort of specifically measuring insulin lispro in the presence of endogenous insulin. Traditionally, early assay of insulin lispro have been carried out by radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA). However, these analytical techniques have many shortcomings such as lack of standardization and specific antibodies, cross-reactivity, limited linear dynamic range, long sample preparation time and complicated procedure.

Due to these disadvantages, LC-MS methods have been gaining popularity in the quantitation of lispro in recent years following the emergence of high sensitivity mass spectrometers. However, establishing a reproducible quantification method of lispro is still a big challenge in most domestic bioanalytical laboratories. In our lab, a robust and convenient UPLC-MS/MS method based on API6500 was developed and validated to support a clinical PK study to quantify intact insulin lispro in human plasma. In present study, the achieved LLOQ of validated method was 400 pg/mL, meeting the requirement of this PK study. The total run time was 5 min. During method development, we optimized not only the MS parameters and LC condition, but also utilized two steps of sample treatment procedures to significantly remove the majority of the endogenous plasma protein. A cleaner sample was obtained before injected into LC-MS/MS system. It is effectively avoided the potential pollution to quadrupole mass spectrometer, thus ensured a consistent and sufficient S/N ratio of the desired LLOQ.

POSTER ABSTRACT

#114

Bioanalytical Services on Large Molecules for TK/PK/PD/BE/Biosimilars/Biomarker Studies

Bo Li, Song Zhao, Xiaojuan Wang, Xirong Li, Yuxuan Liu, Wanxin Wu, Dongjun Li, Pin Ye, Jing Jia, Tianyi Zhang

Department of Biologics Services, Frontage Laboratories (Shanghai) Co., Ltd.

Up to Mar. 2017, the Department of Bioanalytical Services for Large Molecule, Frontage Laboratories (Shanghai) Co., Ltd. conducted nearly 100 projects (including method validation and sample analysis for preclinical or clinical studies on TK/PK/PD/BE/Biosimilars/Biomarker evaluations) by using immunoassays and biochemical assays on the platforms of SpectraMax M2 and MSD Sector Imager 6000.

During last year, we developed new immunoassays (including bridging immunoassays for detections on Anti-drug antibodies) for sponsors when sponsor did not have methods or commercial kits on market did not work. These new methods were validated and used on sample analysis for clinical studies successfully.

The team by using LC/MS/MS platform for large molecule services was established after Dr. Zhao joined Frontage at the beginning of this year. This will strongly enhance our capability on bioanalytical services for large molecules.

In addition, new model of instrument MESO Sector S 600 (MSD) was sent to our lab and will be used soon after IQ/OQ/PQ/software validation.

POSTER ABSTRACT

#115

Method Validation for the Quantitative Determination of c-Met in Human Serum by ELISA

Han Li, Ruili He, Guoqing Hu, Xueying Guo

WuXi AppTec Co., Immunochemistry Team, Department of Bioanalytical Services, 288 Fute Zhong Rd, Waigaoqiao Free Trade Zone,Shanghai, P.R. China, 200131

Introduction

c-Met, a member of the tyrosine kinase superfamily in human serum, is the receptor for hepatocyte growth factor(HGF, also known as scatter factor). Soluble c-Met is a truncated form of the c-Met membrane receptor. It can be cleaved by proteases and released from the lipid bilayer in a process known as ectodomain shedding. Many transmembrane proteins are released through this shedding process and it is a normal part of development which when defective can cause a number of pathologies. The soluble form of the c-Met receptor is smaller than the membrane bound receptor, contains the extracellular region of the receptor, and is able to bind the HGF ligand. As an important biomarker for drug development, investigation in C-met can be significant in some clinical study area, such as cancer, autism and heart function. In this study, we validated a method by commercial kit for the quantitative determination of c-Met in human serum in support of clinical study.

Methods

The assay for the quantification of c-Met in human serum was developed at WuXi AppTec., Shanghai, China, using commercial kit “Human c-Met (soluble)” (invitrogen, catalog number: KHO2031 (96 tests). The Quantikine c-Met assay is a sandwich enzyme immunoassay in a microtiter plate format utilizing an anti-c-Met antibody, a biotinylated secondary antibody, a Streptavidin-Peroxidase (enzyme), and a TMB sbustrate to quantify c-Met in human serum.

Results and Discussion

- No apparent effect of dilution was observed for human serum diluted from 1:50 to 1:400 and high selectivity in spiked human serum diluted from 1:50 to 1:200
- Assay working range is validated from 71.42 to 1914.31 ng/mL
- The Intra- and inter-run accuracy and precision are within the following range: 78.8- 125.3% and 1.3 – 11.5%; 103.6 – 113.3% and 3.6- 17.9%, respectively.
- c-Met is stable in human serum at RT up to 14 days, at 2-8°C up to 31 days, at nominal -20°C for 185 days and at nominal -80°C up to 381 days. c-Met is stable in human serum after 5 freeze-thaw cycles at nominal -20°C and nominal -80°C.

Conclusion

The assay was successfully validated for the determination of c-met in human serum.

POSTER ABSTRACT

#116

Method for Charactering Neutralizing Antibodies (NAb) with a Functional Cell Based Assay

Immunochemistry Team, Bioanalytical Service, WuXi AppTec

Qikuan Huang, Chenpu Zhang, Jia Fu, Lan Yang, Huan Wang, Xiaoqiu He, Yinqiao Chen, Jingjing Ji

Introduction:

NAb assays are an important part of safety assessment during clinical drug development and postmarketing approval. A functional NAb assay (thus having an actual biological readout), is most commonly used and can take the form of either a cell-based functional assay or non-cell-based functional assay. A cell-based NAb assay is recommended for agonistic therapeutics that interact directly with cellular receptors, since it is appropriate to reflect the drug's mechanism of action (MoA). Here we present two forms of functional cell-based NAb assay.

A cell-based assay with competitive Homogenous Time-resolved Fluorescence Resonance Energy Transfer (HTRF)

The method utilises a competitive Homogenous Time-resolved Förster Resonance Energy Transfer (FRET; HTRF) immunoassay to assess intracellular cyclic adenosine monophosphate (cAMP). Drug binds to the surface of THP-1 cells and initiates an intracellular signaling pathway, the initial step being increased production of cyclic adenosine monophosphate (cAMP). cAMP labelled with the HTRF acceptor fluorophore d2 (cAMP-d2) is then added together with cryptate-labelled anti-cAMP (anti-cAMP-K) in cellular lysis buffer, facilitating cell lysis and the release of intracellular cAMP. Upon excitation at 337 nm, fluorescence at 620 nm is emitted from the cryptate molecule, leading to excitation of d2 and emission of fluorescence at 665 nm. Emission is detected at both 620 nm and 665 nm. Calculation of the processed HTRF ratio ((emission at 665 nm / emission at 620 nm) x10,000) facilitates determination of the ratio of anti-cAMP-K bound to labelled or unlabelled cAMP. The HTRF ratio value is inversely proportional to the concentration of unlabelled cAMP. Presence of neutralising antibodies in the samples result in a drop in the level of THP-1 derived cAMP. The ability of the sample to inhibit cAMP production in response to drug is analyzed to determine the presence of NAb within the sample.

A cell -based assay with Luciferase Reporter Gene Assay System

The bioassay utilizes the physiological function of CTLA-4 to inhibit T cell activation

and IL-2 production by measuring IL-2-luciferase reporter gene expression. A T-cell line was engineered to over-express CTLA-4 receptor and also contains a luciferase reporter gene driven by IL-2 promoter. Agonistic anti-CD3 and B7-expressing B-cell are also added to the assay, providing essential activation signals through TCR/CD3 complex and CD28-B7 interaction, respectively. In the absence of drug, high levels of CTLA-4 on the surface of T cell will compete for B7 present on B cell, inhibiting CD28-B7 interaction and IL-2 luciferase reporter expression. Addition of drug will prevent the inhibition by CTLA-4, and lead to high luciferase production in a dose dependent manner.

Thus in the cell based assay, samples without neutralizing antibodies have high levels of IL-2 luciferase expression. And samples with neutralizing antibodies have lower level of IL-2 luciferase expression.

POSTER ABSTRACT

#117

Elimination the Interference of Rheumatoid Factors (RFs) in Immunogenicity Assay

Qikuan Huang, Chenpu Zhang, BAS, LTD, WuXi AppTec.

Immunochemistry Team, Bioanalytical Service, WuXi AppTec

Abstract:

Rheumatoid factors (RFs) are endogenous human antibodies. RFs demonstrate preferential binding to aggregated gamma globulins and are involved in the clearing mechanism of immune complexes. The potential impact exists in the traditional immuno-bridge-assay of the immunogenicity assay in monoclonal antibody therapeutics in clinical BA studies. In the immunogenicity assay, the human anti-human antibodies (HAHA, the analyte in this assay) after administration of monoclonal antibody therapeutics are thus vulnerable to interference by RFs. When using the electrochemiluminescent (ECL) bridge immunoassay, pre-dose samples of the patients from Rheumatoid arthritis populations were measured much higher baseline reactivity than healthy subjects. In the pre-established immune-bridging assay, the aggregation of the Biotinylated monoclonal antibody therapeutics has been observed when it was stored for more than 8 months. The aggregated therapeutic antibody (after Biotinylation and Ruthenylation) conjugated with the RFs instead of the HAHA. When only 1% high molecular weight aggregates (>600 kDa) exists, it is sufficient to lead to increased ECL values, which have been observed in the immunogenicity assay for human monoclonal antibody therapeutics in clinical BA studies.

Optimize and establish an immune-bridge-assay on MSD platform for the immunogenicity assay to detect human monoclonal antibody therapeutics for clinical studies with RA patients.

- Human gamma globulins from Cohn Fractions II and III were cross-linked with glutaraldehyde and allowed to from a gel.
- After dispersal, centrifuge, wash, neutralize glutaraldehyde activity and final centrifuge and wash, the aggregated IgG (AIgG) formed a pellet with a clear supernatant.
- RFs have natural affinity to the Fc region of IgGs. A sample pre-treatment with the AIgG was added to the original sample analytical method.
- The test samples will be mixed with the equal volume of the AIgG pellet solution in the PP tube and 37°C incubated with shaking at 500 rpm.

- After pre-treatment and centrifuge, the supernatant of the samples will be transferred to a new dilution plate and do the remaining assay steps.

The condition was optimized successfully, and can be used for sample pre-treatment.

The AIgGs decrease the RFs interference on the ECL signal to 10% of the AIgG untreated.

58 pre-dose samples from RA populations were measured in the optimized assay and generated the acceptable SCPF = 1.82.

POSTER ABSTRACT

#118

A New Automated Solution for Targeted and Untargeted Metabolite Identification in Drug Discovery

Richard Lee, Vitaly Lashin, Alexandre Sakarov

Advanced Chemistry Development

In early drug discovery and development, information acquired from metabolism studies plays a critical role to determine the viability of the new chemical entity. The site of biotransformation or “hotspots” are recognized through interpretation of mass spectrometric data, which ultimately leads to the elucidation of the biotransformation pathway. In the last 10 -15 years there has been a number of technological breakthroughs in both LCMS hardware and the software which handles the data from the instruments. However, there still lies challenges for structure elucidation of metabolites from the parent structures. In this work, we describe a new automated software protocol for detecting potential metabolites and their structure elucidation, which combines batch processing, prediction and data driven analysis through mass spectral investigation.

The new software algorithm developed by ACD\Labs, was intended to be a vendor neutral platform to investigate batch processing mass spectrometry data. The implemented workflow was designed to work with several mass analyzers including various orbitrap and Q-Tof mass analyzers. Post-acquisition data processing was performed on a set of high resolution LC/MSn data files, representing a complete study of several incubation time points. The data and its associated parent structure file were automatically process within the new software routine. Possible phase 1 and phase 2 metabolite structures were predicted and generated from an assembly based metabolism model. Potential metabolites were detected based on the predicted list, and as a complement, a non-targeted unexpected metabolite extraction process was combined into the overall processing routine which employs a fractional mass filter within the component detection algorithm.

Initially, metabolites were identified based on their accurate mass and theoretical isotopic distribution calculated from molecular formulae. Subsequently, their MS2 and MS3 spectra were extracted where available and later used to verify the metabolite structures. As part of spectral interpretation, the algorithm was able to assign fragment ions of the parent and metabolites to their respective MS2 spectra. Structures of metabolites were verified and scores were provided by comparing the assigned fragment pairs. For cases where a discrete structure was not provided, Markush notations were used, until further manual curating was

performed to allow for changes to the substructure. Upon completion, both predicted and unexpected metabolites were combined into a single biotransformation map, where all related mass spectra were associated to each element in the map, and uploaded to a knowledge management system for easy data review. As an added benefit, all peak areas from their respective XICs across the incubation study were tabulated in a summary table and graphically displayed as a stability/kinetic plot. In addition a java script based web interface was developed for dissemination of results from the processing routine to supporting groups or collaborators. This web interface is browser independent provides an interactive interface and allows for easy navigation of the results.