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SHANGHAI

# POSTER SESSION ABSTRACTS

## Inspiration and Education

POSTER HALL OPEN
Thursday, April 17 9:00 am - 5:00 pm
Friday, April 18 9:00 am - 5:00 pm

Including Submissions for
Young ScientistnExcellence Award
Innovator Award

CPSA Shanghai 2014
April 16 - 19, 2014
Renaissance Shanghai Pudong Hotel
Shanghai

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Young Scientist
Excellence Submission

POSTER ABSTRACT
**#101**

# OATP1A2 and P-gp expression level dependent transport of zolmitriptan and fexofenadine across MDCKII monolayers

Na Yu1, Sijie Lu2, Gang An3, Han Xu3, Chen-bing Guan4, Jasminder Sahi2 and Houfu Liu2,

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OATP1A2 has the highest mRNA expression in human brain as compared with other tissues and is expressed on the luminal membrane of blood-brain barrier (BBB). Zolmitriptan and fexofenadine are substrates for OATP1A2 and P-gp. In the current study, we examined the role of OATP1A2 and P-gp in the transport of zolmitriptan and fexofenadine across the MDCKII monolayers. OATP1A2 spatially localized with P-gp on the apical side of MDCKII-MDR1 monolayer after BacMam2-OATP1A2 transduction. OATP1A2 facilitated apical-to-basolateral (A→B) transport of zolmitriptan and fexofenadine in an OATP1A2 expression level dependent manner in MDCKII-wild type (WT) and MDCKII-MDR1 monolayers. The integrity of monolayers was maintained with increasing multiplicity of infection of BacMam2-OATP1A2 viruses. Endogenous canine P-gp and human P-gp mRNA did not change post BacMam virus transduction. We observed a larger difference in A→B permeability between zolmitriptan and fexofenadine in MDCKII-MDR1 than MDCK-WT cells after OATP1A2 transduction, implying a higher P-gp impact on fexofenadine monolayer transport. In MDCKII-MDR1 cells, A→B permeability of zolmitriptan started to increase when mRNA ratio of OATP1A2/P-gp was larger than 0.4. This implies a potential impact of OATP1A2 on human BBB penetration, as mRNA ratio of OATP1A2/P-gp at human brain cortex and cerebellum are 96 and 25, respectively. Zolmitriptan had higher OATP1A2-mediated transport efficiency, higher passive membrane permeability and lower P-gp liability compared with fexofenadine. Taken together, our data suggests that zolmitriptan is more permeable than fexofenadine at the human BBB.

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Innovator Submission

# POSTER ABSTRACT
**#102**

## a LC/MS based-method combined with data analysis software was developed to evaluate the quality of Pu'er tea

Hui Wang

Waters Corporation

Pu'er tea is widely used in China due to its therapeutic effects and business value, therefore it is of great importance to control the quality of Pu'er tea. However, In this work, a LC/MS based-method combined with data analysis software was developed to evaluate the quality of Pu'er tea and also more than 50 ingredients in Pu'er Tea were identified using a Chinese Medicine Library. 7 control extract of Pu'er tea and 1 blind extract of Pu'er tea were analyzed and the blind Pu'er tea was correctly evaluated. This method was proved to be simple and efficient which can be used in Pu'er tea quality control.

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POSTER ABSTRACT
**#103**

# Quantitative determination of curdione, furanodiene and germacrone in rabbit plasma using liquid chromatography–tandem mass spectrometry

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Department of Drug Metabolism, Beijing City Key Laboratory of Active Substances Discovery and Drug Ability Evaluation, State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China

Objective A rapid and sensitive liquid chromatography –tandem mass spectrometry (LC-MS/MS) method was developed and validated for simultaneous determination of curdione, furanodiene, germacrone, three major active components of Baofukang Suppository in rabbit plasma.

Method Plasma samples containing three analytes and internal standard (IS, costundide) were prepared based on a simple protein precipitation by the addition of acetonitrile. The detection was performed on Finnigan TSQ Quantum Access Mass spectrometer equipped with atmospheric pressure chemical ionization (APCI) in positive ion mode, and operated in the selected reaction monitoring (SRM) of the transitions at m/z 237.2→135.2 for curdione, 217.2→149.2 for furanodiene, 219.3→201.1 for germacrone and 233.2→187.2 for IS. A Zorbax Eclipse Plus-C18 column (3.5 μm, 2.1×100 mm) was used to separate three analytes and IS with mobile phase of methanol/water containing 0.1% formic acid (90:10, v/v) with a isocratic elution at a flow rate of 0.3 mL/min. Result Linear detection responses (R2>0.99) were obtained for curdione and furanodiene ranging from 5 to 5000 ng/mL, and 10 to 5000 ng/mL for germacrone. Intra-day and inter-day precisions (R.S.D. %) were all within 15% and the accuracies (R.E. %) were equal or lower than 8%. The lower limits of quantification (LLOQs) of curdione, furanodiene, germacrone were 5, 5, 10 ng/mL. The recoveries of curdione, furanodiene, germacrone were all greater than 85%, and the matrix effects were 87-90%, 90-95%, 76-82%, respectively. Curdione, furanodiene, germacrone were found to be stable after being placed at room temperature for 48 h, stored at -20°C for 30 days or through three freeze-thaw cycles.

Conclusion The method for simultaneous quantitation of curdione, furanodiene, germacrone was accurate, reliable and reproducible and was successfully applied to the pharmacokinetic studies of three effective components in rabbit plasma.

Keywords Baofukang suppository; curdione; furanodiene; germacrone; LC-MS/MS

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POSTER ABSTRACT
**#104**

# Pharmacokinetic study of TBI-166, a novel antituberculotic drug, in rats

Li Dan, Sheng Li, Li Yan *

(Department of Drug Metabolism, Beijing City Key Laboratory of Active Substances Discovery and Drug Ability Evaluation, State Key Laboratory of Bioactive Substance and Function of Natural Medicines, of Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China)

Objective: A rapid and sensitive LC–MS/MS method was developed and validated for the determination of TBI-166, a novel antituberculotic drug, in rat plasma. The method was applied to the pharmacokinetic and bioavailability studies of TBI-166 in rats.

Methods: Plasma samples containing TBI-166 and propranolol (internal standard) were prepared based on a protein precipitation by the addition of acetonitrile. The separation was performed on a Symmetry C8 column (50mm×2.1mm, 3.5μm), using a mobile phase of acetonitrile containing 0.1% formic acid (v/v) at a flow rate of 0.2 mL/min with gradient elution. The detection was performed on tandem mass spectrometry with positive electrospray ionization (ESI) in multiple reaction monitoring (MRM) mode at the transitions m/z 590 478 for TBI-166, and m/z 260→183 for IS. The present method was applied to the pharmacokinetic studies of TBI-166 in rats after oral (20, 40, 80mg/kg) or intravenous (1mg/kg) administration. Data were analyzed using a non-compartmental approach with WinNonlin software.

Results: The Linear detection response was obtained for TBI-166 ranging from 5 to 2000 ng/mL and the lower limit of quantitation (LLOQ) was 5 ng/mL. Inter- and intra- day precision (RSD) were within 7.2%. The average recovery was greater than 95.2%. After single oral adminitration of TBI-166 at 20, 40, 80mg/kg to rats, the Tmax were 7-9 h with Cmax (0.4±0.2), (0.7±0.2) and (0.7±0.3) μg/mL, respectively. The AUC(0-48h) were (7.7±3.0), (16.9±2.6) and (19.8±11.8) h·μg/mL. The Cmax and the AUC were not dose-dependent, suggesting the saturation of TBI-166 absorption in vivo. The elimination of TBI-166 was slow with MRT 24.4-26.1 h. The bioavailability of TBI-166 was 22.6%. Conclusions ~~A~~ sensitive and accurate LC-MS-MS method was developed for the determination of TBI-166 in rat plasma. TBI-166 exhibited nonlinear pharmacokinetics in rats. The bioavailability of TBI-166 was 22.6%.

Key words: TBI-166; Antituberculotic drug; LC-MS/MS; Pharmacokinetic; Bioavailability

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POSTER ABSTRACT
**#106**

# Quantification of Bradykinin in Human Plasma Using the IonKey/MS System

Hui Wang
Waters Corporation

Highly sensitive microfluidics-liquid chromatography /tandem massspectrometry -based method(LC/MS/MS)has been developed for the quantitative analysis of bradykinin peptide in human plasma which is physiologically and pharmacologically active. Due to low exposure of this peptide, LC/MS/MS based method with lower limit of quantitation was required to support PK study. Compared with small molecules, bradykinin posed significant bioanalytical challenges in the method development due to generation of multiple fragments with low intensities and high level of non-specific bindings throughout sample preparation procedures. In this work, a rapid and holistic LC/MS/MS method was established,which includes a novel solid-phase extraction(SPE) sample preparation method, a microfluidics chromatography separation and a tandem masspectrometor with improved sensitivity detection. A LLOQ of 2.5pg/mL was achieved for this peptide with a linearity of 0.025-8ng/mL(r2>0.99).

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Innovator Submission

ABSTRACT
**#107**

Presentation only - No poster

# Outsourcing and Standardization of Global Studies

Joseph F. Bower Ph.D., MBA

Covance Laboratories, Inc.

The global biologics drug market continues to expand and increase its portion of the global pharmaceutical market. While biologics only accounted for 15% of the global pharmaceutical market's revenue in 2012; the top three drugs in 2012 were all biologics. This portion will rapidly increase as Biosimilars and other new technology platforms (ADC, Gene Therapy) begin to hit the global market. In addition, the regulatory landscape is continuously evolving and becoming much more challenging to navigate. Therefore, it is imperative that companies partner with providers who have experience and expertise in supporting biotherapeutics across multiple global clinical trials as well as those who have a strong global presence. Case studies will be reviewed to highlight challenges faced with cross validating immunoassays across multiple global sites to support clinical trials. These will identify strategic approaches and outcomes and highlight lesson learned precipitating a strategy to ensure your approach consistently delivers quality bioanalytical results from site to site.

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POSTER ABSTRACT
**#108**

# Performance Evaluation of a Flexible, Easy-to-Use Packed-Tip Column Device for Nanospray Enabled LC-MS

Helena Svobodova, Amanda Berg, Gary Valaskovic

New Objective, Inc., Woburn, MA USA

Nanobore liquid chromatography is the method of choice for protein/peptide separation in the life sciences. Despite all the advances in nanospray product development, running experiments in nanospray mode remains challenging and requires specialized training and in-depth knowledge of instrumentation. Here we test the performance of a newly developed integrated nanobore column which combines a packed-tip column, high-voltage liquid-junction, column heater and transfer line into one easy-to-use device with universal connectivity. A self-guided positioning system ensures reproducible tip placement. Leak-free connections inside the integrated nanobore column guarantee good intra- and inter-column reproducibility while maintaining the sensitivity and separation efficiency of packed tip columns.

Tested columns were packed with 1.9, 3.0 and 5.0 µm C18 resins to 10 and 25 cm bed lengths. Using a 1 µl-loop, 300 fmol/µl and 1 pmol/µl bovine serum albumin digests (BSA, Waters) were injected directly onto the column. Spray stability, sample separation and signal intensity were evaluated at different temperature settings. Sample separation was achieved by a 30-min. 2-50% acetonitrile gradient at 300 and 500 nl/min. Analyses were conducted using a nanoLC•2D pump (Eksigent), linear ion trap mass spectrometer (LTQ, Thermo), customized nanospray source and custom packed-tip columns with embedded high-voltage liquid-junction and column heating (New Objective).

The performance of an integrated nanobore column was compared to a standard packed-tip column with the same dimensions. The peak width and peak asymmetry at 13.5% above baseline were calculated for four BSA peptide peaks (m/z 575.5, 569.7, 643.8 and 508.0) extracted from across the gradient elution profile. The peak widths were measured to be between 8.4s and 24s for the targeted peptides. The retention time for m/z 643.8 peak was 15.4 min for column 1, 15.6 min for column 2 and 15.5 for column 3. The peak area for the same peptide was integrated at 13.5 % above baseline and recorded to be 6.19E6, 6.62E6 and 6.57E6 respectively. The resulting RSD is less than 10% among these three tested columns.

Incorporating temperature control into the integrated nanobore column resulted in reduced column backpressures. The column pressure was recorded at 500 nl/min., 0.1% formic acid, 98% water and 2% acetonitrile. The pressure of a 10 cm column packed with 3.5 µm C18 particles decreased from 1575 PSI at 23°C to 1365 PSI at 50°C, a 13% pressure reduction. The pressure difference was more apparent on columns packed with 1.8 µm resin. The column pressure decreased from 4270 PSI to 3170 PSI, a 27% reduction in column pressure. In addition to decreasing the column pressure, heating the column also affected the elution order of some BSA peptides and improved the peak capacity. For example the elution order for peptides with m/z 740.8 and 954.8 was reversed when the temperature was increased from 23°C to 70°C. A 9% improvement in peak capacity was observed when the temperature of the column was increased from 23 to 50 °C. The temperature effect on different types of C18 resin remains under investigation and the data will be presented.

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POSTER ABSTRACT
**#109**

# Evaluating the Ruggedness of Nanospray on a Curtain Gas-Triple Quadrupole MS Equipped with Emitter Rinsing

Amanda Berg, Helena Svobodova, Gary Valaskovic

New Objective, Inc., Woburn, MA USA

Nanospray has become an essential tool in high-sensitivity MS, but limited robustness and reproducibility have historically challenged the adoption of nanospray in quantitative applications with triple quadrupole mass spectrometry. MS-based biomarker quantitation places strict requirements on the analytical performance of nanobore LC-MS not only due to the need for reproducibility and robustness but also the intrinsically complex nature of the samples used in these workflows. Automated emitter positioning with rinsing has been previously demonstrated to improve spray stability and analyte response on a 3D ion trap MS1. Here we investigate the utility of automated tip rinsing to improve emitter spray stability and data quality on a hybrid triple quadrupole/LIT MS equipped with a heated interface blanketed by a laminar flow of nitrogen gas.

Canine plasma was MTBE LLE purified, spin-filtered, evaporated to dryness and reconstituted in 25% MeOH/5%ACN/70% Water. Standards were added at concentrations of 250 ng/µl Caffeine, 5 pmol/µl Angiotensin I, 5 pmol/µl Neurotensin and 5 pmol/µl Bradykinin Fragment 1-7. The standard-spiked plasma was infused at 0.5 µl/min. Q1 MS data (4000 QTRAP/AB SCIEX) was collected in positive ion mode using a mass range of 400 – 1000 Da and a scan time of 0.1801 seconds. Using an uncoated pulled-tip fritted emitter (75 µm ID x 10 µm tip) ten-minute data files were collected using a constant set of parameters for ion-spray voltage, curtain gas and nebulization gas at an inlet temperature of 150°C.

Using a digitally controlled stage positioning system, the emitter was toggled between two sets of XYZ-coordinates functionally defined as the spray position and the wash position. At each sample injection, the emitter is toggled to the wash position diverting the emitter away from the MS inlet where a constant 50 µl/min gravity flow of solvent washes the exterior of the emitter. Upon initiation of the MS data acquisition, the emitter is toggled to the spray position and a spray image file is generated for spray stability validation at the start of each MS run. Comparative analysis of average TIC between data collected with washing and without reveals an obvious discrepancy. Using the RSD of the TIC as an indicator of spray stability, RSDs were calculated for a total of 650 injections for each emitter. Data collected with emitter washing produced consistent RSD values ranging from 4.29% to 8.37% with average TIC values of 1.37E9 and 1.50E9, respectively. Data collected without emitter washing produced RSD values ranging from 4.62% to 132.25% with average TIC values of 2.92E9 and 4.14E7, respectively. The TIC dropped almost two orders of magnitude for data collected without rinsing from matrix-associated particulate accumulation on the emitter. Data collected with washing produced consistent average TIC and RSD values over all 650 injections.

1Berg, AL; Marshall-Waggett, CJ; Valaskovic, GA; Proceedings of the 57th Conference on Mass Spectrometry and Allied Topics, Philadelphia, PA, May 29 - June 5, 2009.

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Innovator Submission

POSTER ABSTRACT

**#110**

# Simultaneous Quantification of Human Insulin and Five Analogs in Human Plasma Using 2D UPLC MS System

Hui Wang

Waters Corporation

Highly sensitive 2D liquid chromatography /tandem massspectrometry(LC/MS/MS) -based method has been developed for the quantitative analysis of Human insulin and five insulin analogs therapeutic peptides in human plasma. Due to low exposure of these peptides, LC/MS/MS based methods with lower limits of quantitation were required to support drug discovery and PK studies. Compared with small molecules, many of these peptides (human insulin, insulin lispro,insulin glargine, insulin aspart, insulin detemir and insulin glulisine) posed significant bioanalytical challenges in the method development due to generation of multiple fragments with low intensities and high level of non-specific bindings throughout sample preparation procedures. In this work, a rapid and holistic 2D LC/MS/MS method was established,which includes a novel solid-phase extraction(SPE) sample preparation method, a 2D ultraperformance chromatography separation and a tandem masspectrometor with improved sensitivity detection. The LLOQ of 50pg/mL was achieved for human insulin,insulin lispro, insulin glargine and insulin glulisine with the linearity of 5-10000g/mL(r2>0.99). And the LOQ of insulin aspart and insulin detemir was 100pg/mL and 200pg/mL with the linearity of 100-10000pg/mL(r2>0.99) and 200-10000pg/mL(r2>0.99)

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POSTER ABSTRACT
**#111**

# Comparative Evaluation of LLDT-8 Biotransformation in vivo in Rats and in vitro in Human Liver Microsomes: Prominent Roles of CYP3A

Jiangbo Du, Jian Meng, Xiaoyan Chen, Dafang Zhong*

Shanghai Institute of Materia Medica, Chinese Academy of Sciences

(5R)-Hydroxytriptolide (LLDT-8) is a potent immunosuppressive and anti-inflammatory triptolide derivative, which is currently undergoing Phase II clinical trials for the treatment of rheumatoid arthritis in China. In this study, the metabolism and excretion of [3H]LLDT-8 were evaluated after oral administration to rats. After a single oral dose of [3H]LLDT-8 (348 μCi/kg, 311 μg/kg) to rats, 86.9% of the administered radioactivity was recovered in 168 h postdose with approximately equal amounts in urine (44.1%) and feces (42.8%). In bile duct-cannulated rats, 45.2% of the dose was recovered in bile, suggesting that the feces-related radioactivity mainly came from biliary excretion. Unchanged parent drug were not detected in urine, bile, and feces. The highest radioactivity in plasma was observed at 0.25 h postdose, indicating rapid absorption of the drug in rats. LLDT-8 along with three hydroxylated metabolites were the prominent drug-related components in the plasma. [3H]LLDT-8 was extensively metabolized in rats, and the prominent metabolites mainly resulted from oxidation (hydroxylation, dehydrogenation) and further phase II conjugation (glucuronidation, sulfation, and GSH conjugation). The metabolism of LLDT-8 was also investigated in vitro in human liver microsomes (HLMs). The metabolic profile in HLMs was qualitatively similar to the in vivo oxidative metabolism of LLDT-8 in rats. In vitro phenotyping study demonstrated that only human recombinant CYP3A4 showed a high metabolic activity to form all LLDT-8 metabolites found in HLMs. Consistent with this, ketoconazole, a typical selective inhibitor for CYP3A, completely inhibited LLDT-8 metabolism in HLMs, whereas other chemical inhibitors (α-naphthoflavone for CYP1A2, quercetin for CYP2C8, sulfaphenazole for CYP2C9, ticlopidine for CYP2B6 and 2C19, quinidine for CYP2D6, and clomethiazole for CYP2E1) had little effect on LLDT-8 metabolism. In a pharmacokinetics study in rats, the $AUC_{0-\infty}$ and $C_{max}$ of LLDT-8 were decreased to 19.7% and 14.2% of the control by 3-day treatment of dexamethasone, an inducer of CYP3A, while coadministration of ketoconazole caused 4.6- and 3.3-fold increase in the $AUC_{0-\infty}$ and Cmax, respectively. Collectively, these results indicated that oxidative metabolism of LLDT-8 is mainly catalysed by CYP3A and the modification of this enzyme activity would affect the pharmacokinetics of LLDT-8 in rats, which highlighted the need for further assessment of the potential CYP3A-mediated drug-drug interactions in humans.

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Innovator Submission

POSTER ABSTRACT

**#112**

# Metabolite Analysis Using A Novel Relationship And Database Driven Software Platform Approch For Screening Metabolism

Hui Wang

Waters Coporation

Current informatics approches for xenobiotic studies limit the progess of scientists to the restrictions of predifined software workflows and the difficulty on passing on one's knowledge to peeers.The ability to share discoveries and knowledge on the biotransformations of drug candidates for universal use in an organization and to feed previously discovered information into today's experiments holds the potential to unlock the potential of historical data.The approch defined here is a system that uses the latest generation hardware and informatics to accurately screen samples ,confidently interrogate datasets and allows users to elucidate in a highly flexble foramt. This system allows users to elucidate metabolic pathways,store,access and interrogate each other's data.This capture and ability to retrieve scientific knowledge allows the organization's best scientists to apply a growing body of work towards future challenges.

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Innovator Submission

POSTER ABSTRACT
**#113**

# Quantification of 5 Steroid Hormones in Human Serum Using the ionKey MS system

Hui Wang

Waters Corporation

Highly sensitive microfluidics-liquid chromatography /tandem massspectrometry -based method (LC/MS/MS) has been developed for the quantitative analysis of steroid hormones in human serum which is important in clinical area. Previous

Methods were used standard flow LC/MS/MS assay which would consume high levels of solvent and often require time-consuming sample extraction procedures such as liquid-liquid or solid phase extraction to achieve biologically relevant sensitivity. In this work, a newly developed microfuidics-liquid/MS(ionKey/MS) system was used to quantify 5 important steroid compounds in human serum: testosterone, dihydrotestosterone, progesterone, cortisone and cortisol. The microfuidics-liquid method results in a 150 fold decrease in solvent consumption and a 100-400 fold increase in on-column sensitivity.

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POSTER ABSTRACT
**#114**

# The Role of Efflux Transporters in Allitinib Disposition and Drug Interaction

Lishan Lin, Xiuli Li, Xiaoyan Chen, Dafang Zhong*

Shanghai Institute of Materia Medica, Shanghai, China

Allitinib, a novel irreversible inhibitor of EGFR and HER2, is currently in clinical trials in China for the treatment of solid tumors. Transporters might play important roles in the disposition of allitinib due to its poor solubility and permeability. In vitro studies showed that allitinib was not only a substrate of P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) but also an mild inhibitor of BCRP ($IC_{50}$ > 100 μM). Since the maximum plasma concentration of allitinib after oral administration of 1000 mg of allitinib tosylate t.i.d was much less than this IC50 value, indicating a minor potential for transporter-mediated drug interactions. Tariquidar and pantoprazole were used as potent inhibitors of P-gp and BCRP, respectively, and the effects of the inhibitors on the plasma concentration, biliary excretion, and distribution to the liver, kidney, and brain of allitinib in rats were examined to demonstrate the contribution of these transporters to the pharmacokinetics of allitinib and potential for drug-drug interaction via these transporters. The AUC0-24 of allitinib administered orally was increased by 60% and 70% by pretreatment with tariquidar and pantoprazole, respectively, whereas the systemic exposure to allitinib administered intravenously did not alter. Neither of them could increase distribution of allitinib in the liver or kidney after intravenous administration of allitinib. Interestingly, the liver distribution in rats was increased 1.9 fold when both transporters were inhibited, whereas the kidney distribution did not alter. In addition, the brain distribution of allitinib was significantly increased by tariquidar but not pantoprazole, and coadministration of both inhibitors further enhanced the accumulation of allitinib in brain. The lack of significant increase in the brain distribution of allitinib in rats pretreated with pantoprazole may be due to the potential compensation by P-gp at the blood-brain barrier. Each inhibitor markedly reduced the biliary excretion of allitinib within 90 minutes after intravenous administration. In conclusion, the plasma concentration, biliary excretion, and accumulation in the liver and brain of allitinib are influenced by inhibitors of P-gp and BCRP.

CPSA SHANGHAI

POSTER ABSTRACT
**#116**

# Identification of Taurine Conjugates and Amino Acid Conjugates of Toosendanin in Rats: Metabolic Activation of Furan Ring with a Strong Preference of Amino Group

Jinghua Yu, Pan Deng, Dafang Zhong, Xiaoyan Chen*

Shanghai Institute of Materia Medica, Shanghai, P. R. China

Toosendanin (TSN), once used as an antiparasite drug in Asia, is a hepatotoxic triterpenoid extracted from Melia toosendan Sieb et Zucc. Although TSN has been used for years, the metabolism and the mechanism of hepatotoxicity of TSN remains unclear. This study aims to characterize the metabolic pathways of TSN in rats and explore the underlying biotransformation and bioactivation mechanism. After a single oral dose of 10 mg/kg TSN to rats, a total of 22, 14, and 30 metabolites were detected in feces, urine and bile, respectively, by UPLC/Q-TOF MS. Esterolysis, taurine conjugation and amino acid conjugation were identified as the main metabolism pathways in rats. Using taurine, GSH, Boc-Lys, NAC, and amino acids as trapping reagents, the corresponding conjugates were found in both HLMs and RLMs incubations supplemented with NADPH. Several isomers of conjugates were observed due to hemiacetal group and different conjugation sites. Comparing remaining amount of the parent drug, the reactivity with reactive metabolites of TSN was sorted in a decreasing order of Boc-Lys > (alanine, lysine, taurine) > (phenylalanine, serine, glutamic acid, glycine, GSH) > cysteine > NAC. The formation of the conjugates was NADPH-dependent, and further recombinant human CYP enzymes and chemical inhibition studies suggested that CYP3A4 was primarily responsible for the metabolic activation of TSN. The standards of taurine conjugates and lysine conjugates were chemically synthesized. The major product of the conjugates was confirmed as 3-substituted 3-pyrroline-2-one adduct while the minor product was confirmed as 4-substituted isomer, implying the metabolic activation in furan ring of TSN. The reactive intermediates of TSN trend to react with amino group considering the nucleophilic group of Boc-Lys. Overall, our study reveals the mechanism for the metabolic activation of TSN as follows. TSN is first oxidized to putative epoxide reactive metabolite which rearranges to butene-1, 4-dial intermediates spontaneously. The aldehyde intermediates react with amino group to form Schiff base followed by ring closure and dehydration to yield 3-pyrroline-2-one adduct. The amino group trends to react with the 4-aldehyde to form 3-substituted products because of the steric hindrance. Due to the high concentration of free amino acids in hepatocytes, different amino acid conjugates (especially taurine conjugates and lysine conjugates) were detected in vivo.

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Innovator Submission

POSTER ABSTRACT
#117

# Prediction of Valsartan Pharmacokinetics in Different Pediatric Age Groups using Physiologically Based Pharmacokinetic (PBPK) Models

Viera Lukacova1, John A. DiBella1, Linda Lin2, Michael B. Bolger1, Walter S. Woltosz1

Institutions: (1) Simulations Plus, Inc., Lancaster, CA, USA ; (2) PharmoGo Co.

A method for transporter-based in vitro-in vivo extrapolation (IVIVE) was previously developed and demonstrated by predicting valsartan PK after i.v. administration in rats and adults [1]. The purpose of this study was to (1) extend the model to describe valsartan PK in human after p.o. administration, and (2) explore the utility of the model to predict valsartan PK in pediatric populations. An absorption/PBPK model for valsartan PK was developed using GastroPlus™ 8.5 (Simulations Plus, Inc.). The program's Advanced Compartmental Absorption and Transit (ACAT™) model described the absorption of the drug, while PK was simulated with its PBPKPlus™ module. Physiologies were generated by the program's internal Population Estimates for Age-Related (PEAR) Physiology™ module. Intestinal absorption and tissue distribution accounted for both passive diffusion and carrier-mediated transport. Total clearance consisted of biliary (major) and renal (minor) secretion. Passive diffusion between the extracellular and intracellular spaces in all tissues was calculated from specific permeability-surface area product (SpecPStc) and tissue cell volumes. SpecPstc along with the carrier-mediated transport kinetics in liver was predicted from previously reported in vitro measurements [2]. Renal secretion was estimated as Fup*GFR. Plasma protein and red blood cell binding was adjusted to account for pediatric plasma protein levels and hematocrit. Effect of intestinal MRP2 on valsartan absorption was included in the model, with differences in regional expression levels scaled based on surface area for the adult and pediatric populations. Model parameters (Vmax for liver and intestinal transporters, and SpecPStc) were also fitted against Cp-time profiles after i.v. and p.o. administration in adults and the refined model was used to predict pediatric PK across several different age groups. The initial model based on PEAR physiologies combined with in vitro estimates of transporter Km and Vmax values for liver transporters and SpecPStc gave reasonable predictions of valsartan exposure in adults and children (Cmax and AUC prediction errors ranged from 20%-120%). A model refined against adult in vivo profiles resulted in much improved prediction of pediatric exposure, with less than 25% prediction error on both Cmax and AUC seen in the 1-4 year-old, 4-6 year-old, 6-12 year-old, and 12-16 year-old age groups. Additional population PBPK simulations were run for the age groups to assess inter-subject variability. The transporter-based IVIVE method showed excellent performance for prediction of pediatric PK, across several different age groups, from adult studies. The method extends the PBPK capabilities to predict pediatric exposure for compounds where PK cannot be described by the simpler, flow-limited, tissue models.

[1] Lukacova V., Poster presentation, 17th North American ISSX Meeting 2011, Atlanta, GA

[2] Poirier A. et al. J Pharmacokinet Pharmacodyn, 2009, 36: 585-611

CPSA SHANGHAI

POSTER ABSTRACT
**#118**

# A New Level of Sensitivity and Standardization: The Promise of Integrated Micro Fluidic Devices Coupled with Mass Spectrometry

Jim Murphy
Waters Corporation

Introduction:

Conventional pneumatically-assisted electrospray ionization mass spectrometry (ESI-MS) has been categorized since its inception as a concentration sensitive technique. As technology evolved commercial ESI-MS sources have become more efficient creating ions by utilizing desolvation heat and gas. ESI-MS can no longer be considered a purely concentration sensitive technique. Sensitivity gains can still be observed at LC flow rates <10 ul/min however as mass spectrometers are more capable of sampling a small electrospray plume. The increased sampling efficiency and ionization efficiency of numerous analytes was determined for flow rates between 0.5 and 600 µL/min using a combination of commercially available UPLC columns and microfluidic devices.

Methods:

Chromatography was performed on a UHPLC and nanoUHPLC system coupled to triple quadrupole mass spectrometer outfitted with the appropriate source. The internal diameters of the chromatographic columns tested include: 2.1 mm , 300 µm, 150 µm and a 75 µm. Furthermore, the load of both a digested protein standard and small molecule pharmaceutical standard were identical on each format columns and only the flow rate was scaled according to the column internal diameter. Accordingly a determination of the sensitivity modification could be quantified and compared to the theoretical gains assuming ESI-MS was in fact concentration sensitive.

Preliminary Data

If the ESI-MS studied was truly concentration sensitive the scale down experiments from the 2.1 mm column should show sensitivity enhancements of 49, 196, and 784 times respectively for each decreasing column diameter. We found that the sensitivity enhancement was always less than the calculated gain in concentration. For example the sensitivity improvement for small molecules ranged from approximately 2.5 to 25 times when comparing 2.1 mm performance to that of the 150 µm ceramic microfluidic device with the average centered around an 8 times improvement. Steps were taken to improve the sensitivity for each column internal diameter through the use of additional heat and gas, as well as different emitter dimensions and orientations with no significant alterations. Accordingly these results suggest that the relationship between the flow rate and sampling efficiency of the ESI-MS are the critical parameters effecting sensitivity not concentration as previously thought in the flow regime between 0.5 and 600 µL/min. We contend that the alterations in sensitivity are due to the improved sampling efficiency of the MS at the lower flow rates resulting from the narrowing of the ESI spray plume. Consistent with this contention we found several small molecules and peptides which showed improvements during the scaling experiments utilizing the 150 µm ceramic microfluidic devices on the order of 20 – 30x compared to the 2.1 mm column. These molecules tended to be more hydrophobic and have a larger molecular volume than their counterparts suggesting a better ionization efficiency leading to them being sampled by the MS more efficiently thereby leading to improved sensitivity.

CPSA SHANGHAI

POSTER ABSTRACT
**#119**

# Altered pharmacokinetics in puromycin aminonucleoside (PAN) induced nephrotic syndrome (NS) model

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NS patients and PAN induced rats are characterized by massive proteinuria, hypoproteinemia and hyperlipidemia. AZ1 (CL(iv)=91.4mL/min/kg), a compound showing efficacy in the PAN model, demonstrated nonlinear PK with dose and dynamic change with days. After single dose of PAN treatment, systemic exposure of AZ1 at 3 and 10mg/kg was similar between PAN and control animals; however, AUC at 30mg/kg was 2.6-fold as much as that in control rats. Maximal AUC for 30mg/kg was observed on day 6 after PAN treatment and return to normal on day13. PAN was neither a reversible nor a time dependent CYP3A inhibitor in rat liver microsomes, and down-regulation of several CYP450 mRNA were observed. Nonlinear PK was not found in rat PAN model for AZ2 a low clearance compound at CL(iv)=6.8mL/min/kg. This data suggest low clearance compound is less affected by PAN mediated CYP450 mRNA repression. Hypoproteinemia secondary to NS is usually associated with an increase in unbound fraction. In rat PAN model, free fraction (Fu) of theophylline with negligible binding to AGP increases and Fu of AGP bound compounds such as imipramine decreases. Understanding altered PK in NS patients and PAN model help interpret PK/PD relationship and predict efficacious dose in human and animal.

CPSA SHANGHAI

POSTER ABSTRACT
**#120**

# Evaluation of a High-Throughput Autosampler (Apricot Design Dual Arm – ADDA) for Discovery ADME Sample Analysis

Haiwei Xiong, Xiaotong Li, Yuanqiang Su, Qiang Liu, Leilei Qin, Wujian Ju, Genfu Chen, Hongshi Yu, Xin Zhang and Yi Tao*

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The ADDA system is a dual-arm autosampler invented recently by Apricot Design Co., and designed especially for high throughput discovery ADME sample analysis when coupling with LC-MS/MS. Similar as Agilent RapidFire, by utilizing on-line solid phase extraction (SPE), ADDA allows for fast screening and can complete one injection within 8~15 second under trap/elute mode. Since on-line SPE without regular column separation is applied, potential matrix effect or metabolite interference leading to data deviation is the major concern of users. In addition, software compatibility, autosampler carryover, data processing, and the throughput of whole procedure are always the key factors and user concerns on the performance of high throughput platform.

In present study, several sets of in vitro ADME samples from microsomal stability, CYP inhibition, and UGT stability assay have been analyzed by using both ADDA/LC-MS/MS and CTC/LC-MS/MS. The obtained data, speed, carryover, sequence edition, and data processing have been compared in between ADDA and CTC system. The results showed that data obtained from ADDA were very close to those from CTC, and ADDA was a suitable high throughput platform when coupling with AB Sciex mass spectrometer on the software integration, hardware configuration, compound tuning, and sample plate layout.

CPSA SHANGHAI

Young Scientist
Excellence Submission

POSTER ABSTRACT
**#121**

# Development and Validation of a UPLC-MS/MS Method for PHOTOCYANINE in Human Plasma to Support in vitro Plasma Protein Binding Assay

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LC-MS/MS determination of metal phthalocyanines (structure shown in Figure 1) or porphyrins in biological samples is always difficult due to the strong binding or even the formation of covalent bond of these compounds with stationary phase of column or protein in biological matrices. To best of our knowledge, this will be the first reported LC-MS/MS method for the determination of metal phthalocyanines in biological samples.

Challenged points:

Bipolar property of PHOTOCYANINE;

Chromatographic optimization regarding peak shape, analyte retention on column, and carryover;

Poor extraction recovery (blue color of PHOTOCYANINE on denatured protein)

Solutions:

Specific column and specific extraction solvent were found to be optimal for PHOTOCYANINE in terms of good calibration curve dynamic range (ranging from 20 nM to 1000 nM), good chromatography and sample extraction recovery (ranging from 102% to 106%).

POSTER ABSTRACT
**#122**

# Mechanism based CYP1A2 inhibition studies of primaquine and 2-tert-butylprimaquine (NP-96)

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Hypothesis: Primaquine (PQ) is an effective antimalarial drug, but it shows clinically relevant drug-drug interactions and related toxicity. PQ is also a potent inhibitor of CYP1A2 and was found to show drug-drug interaction with CYP1A2 substrate (1). 2-tert. butyl primaquine (NP-96), a structural analogue of PQ (developed at NIPER, SAS Nagar, India) was found to be active against blood schizontocides of multidrug-resistant plasmodium and reported to be free from haemoglobin related toxicity (2). The present study was designed to explore the mechanism based inhibition (MBI) potential of NP-96 towards CYP1A2.

Method used: To performed the experiment, PQ and NP-96 (at different selected concentrations) were preincubated with rCYP1A2 in the absence and the presence of a cofactor (NADPH) for 0 min and 30 min, followed by addition of the substrate (BOMCC) and NADPH. BOMCC has converted into fluorescent metabolite (3-CHC), which was analyzed by fluorimeter at emission/excitation wavelength of 409/460 nm and filter cut off at 435 nm. Rofecoxib was used as positive control to check suitability of the system.

Supporting data: IC50 calculated at 0 min time point for rofecoxib (positive control) was 7.03 μM and 6.32 μM with and without NADPH respectively, while the values were 1.39 μM and 4.17 μM at 30 min time point. Similarly, IC50 calculated for PQ was 1.05 μM and 1.16 μM with and without NADPH, respectively, at 0 min time point, while the values were 2.55 μM and 1.12 μM at 30 min time point. The reason for decrease in IC50 of PQ at 30 min in the presence of NADPH was explained using GLIDE docking software, as possible PQ metabolite, had less interaction with the CYP1A2. IC50 calculated for NP-96 was 8.43 μM and 7.98 μM with and without NADPH respectively, at 0 min time point, while the values were 1.06 μM and 4.21 μM at 30 min time point.

Result and discussion: IC50 shift calculated for rofecoxib, PQ and NP-96 was 3.00, 0.44 and 3.77, which indicated that PQ did not show MBI, while NP-96 had high potential to show MBI, meaning that latter had high potential to show drug-drug interaction with CYP1A2 substrate.

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CPSA SHANGHAI

POSTER ABSTRACT
**#123**

# Physiologically Based Pharmacokinetic Modeling to Simulate Transporter-Mediated Disposition of Jatrorrhizine in Rats

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Background

Jatrorrhizine, one of Rhizoma coptidis alkaloids, shows very low plasma levels and extremely high tissue levels. Due to the poor plasma concentration-efficiency relationship of Rhizoma coptidis alkaloids, it is important to monitor the exposure in target organs which may be the major factor determining their pharmacological or toxicological activity.

Objective

To establish and evaluate a physiologically based pharmacokinetic (PBPK) model for monitoring jatrorrhizine tissue concentration after IV administration in rats and understanding the role of transporter in tissue distribution.

Methods

A whole-body PBPK model with all permeability-limited compartments was developed in GastroPlus. The basic assumptions are: 1.influx transporters mediate the uptake in kidney, liver and heart; 2.other tissues are predominantly driven by passive diffusion; 3.hepatic metabolism (CYP3A, UGT1A1) and renal active efflux (MATE1) are the dominant elimination processes. In vitro uptake data in OCT2-MDCK cells and metabolic data in rat liver microsomes were scaled to the corresponding in vivo values. Relative activity factor (RAF) which was based on the activity differences between different systems was manually adjusted. The initial values of extracellular partition coefficient (Kpe) and permeability-surface area product (PSTC) were estimated from in vivo tissue data by area method and nonsaturable uptake clearance in OCT2-MDCK cells respectively and then fitted to observed data. An uncertainty analysis was carried out through varying RAFs of OCT and MATE1 to illustrate the impact of transporter activity on plasma, liver, kidney and urine profiles.

Results

The simulated plasma, tissue and urine profiles and pharmacokinetic parameters (AUC and Cmax) were close to the observed values. Uncertainty analysis showed that the changes of the active uptake ability altered the plasma profiles of jatrorrhizine contrary to the liver profiles, whereas changes in the ability of tubular efflux markedly altered kidney and urine profiles of jatrorrhizine but had a minimal effect on its plasma and liver concentration.

Conclusion

This PBPK model allows the successful prediction of plasma, tissue and urine profiles of jatrorrhizine and the uncertainty analysis furthers the understanding of the role of active transporter in disposition and even suggests potential DDIs. This PBPK model also can be expanded to investigate other Rhizoma coptidis alkaloids.

CPSA SHANGHAI

POSTER ABSTRACT
**#124**

# Pharmacokinetics and Brain penetration of GSK001 in Neonatal and Prenatal Animals: an Exercise of Pharmacokinetics Prediction in Sheep Fetus

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Physiological based pharmacokinetics (PBPK) models have been applied to understand compound absorption, distribution, metabolism and excretion (ADME) properties in maternal body and the placental-fetal unit in pregnant rodents or humans. No model has been developed for pregnant or fetal sheep so far, given the limited physiological data available. We conducted pharmacokinetics (PK) and brain penetration studies for GSK001, in adult C57BL6/J, neonatal CD1 and OF1 mice, respectively. The PK profile in fetal sheep post IV bolus dose was predicted based on the mouse PK data. Fetal sheep clearance ($CL_b$) and volume of distribution ($V_{SS}$) were estimated through allometric scaling and $CL_b$ was further adjusted with assumptions based on literature data. Fetal sheep PK was simulated using Phoenix™. Brain penetration and PK were studied in fetal sheep and a retrospective analysis performed to evaluate the parameters projected earlier. Experimental results showed that GSK001 was a good brain penetrant with excellent free brain-to-blood ratio ($K_{p,uu}$) in neonatal CD1 mice and fetal sheep. In addition, the predicted fetal sheep profile was similar to the observed results, indicating a simple one-compartment model with increased $CL_b$ from allometry might serve as a reasonable preliminary prediction tool for GSK001. More data is needed to increase the predictability of our model.

CPSA SHANGHAI

Young Scientist
Excellence Submission

POSTER ABSTRACT
**#125**

# Two-cycle-one-column derivatization coupled with solid-phase extraction for the high sensitive determination of minodronic acid in human plasma by LC-MS/MS method

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Minodronic acid (MA) is a third-generation bisphosphonates. It has shown great improvement in inhibition activity of bone resorption which resulted in lower dose (1.0 mg) and plasma peak concentration (less than 0.500 ng/mL). As a strong polar compound, MA extends poor retention on reverse-phase chromatography, and the ability to form multiply charged species decreased its MS sensitivity, which made it challenging to quantify MA concentrations in plasma.

In the current study, an offline derivatization with trimethylsilyldiazomethane was employed as the pretreatment approach. However, owing to incomplete reaction, MA, deuterium labeled analog internal standard (IS) and structure analogs IS exhibited unstable and insufficient productivity that eliminated the correcting functions of IS. To overcome this problem a two-cycle-one-column derivatization method was exploited. Plasma samples were derived and eluted on a weak-anion exchange solid phase cartridge with methanol containing trimethylsilyldiazomethane. Eluents were then mixed with 200 μL water and reloaded onto the same cartridge for the second cycle of derivatization. Chromatographic separation was performed on an Eclipse XDB-phenyl column. Detection was carried out on a triple quadrupole mass spectrometry. The method was linear in the range of 10.0–1,000 pg/mL for MA using d4-MA as the internal standard. A lower limit of quantification of 10.0 pg/mL was achieved with acceptable intra and inter-assay precision accuracy. The validated method was successfully applied to characterize the pharmacokinetic profiles of minodronic acid following oral dose of 1.0 mg minodronic acid to healthy volunteers.

CPSA SHANGHAI

POSTER ABSTRACT
**#126**

# Risk analysis of a one directional transport assay to identify P-gp liability using MDCKII-MDR1 cells

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MDCKII-MDR1 monolayers are widely used to predict central nervous system (CNS) accessibility of compounds in drug discovery. Conventionally, we conduct bi-directional transport (ratio of B→A and A→B permeability) in the presence and absence of a P-gp inhibitor to evaluate the passive permeability and calculate the efflux ratio (ER). In order to increase the screening efficiency, a one directional apical-to-basolateral (A→B) transport method was evaluated. This simplified approach could be used to derive the efflux potential of compounds by calculating the ratio of A→B permeability in the presence and absence of a P-gp inhibitor. Based on theoretical analysis, it appeared that the one-directional ratio might be lower than the conventional ER . To test the predictability of this proposed model, we collected bi-directional transport data for 1003 compounds from the GSK database. Of these, 629 were classified as P-gp substrates with ER >=2. We observed a strong correlation between the one- and bi-directional ratios ($R^2$ = 0.73). Classification accuracy was 86% using one-directional ratio >=2 as an indicator for P-gp substrate classification. Among the misclassified compounds, false negative compounds (ER >= 2 and one-directional ratio < 2) accounted for 12% and false positive compounds for 1.8%. Further analysis on the false negative compounds demonstrated that most of these compounds showed poor passive permeability (< 100 nm/s), indicating that these were likely to have poor CNS penetration. In summary, we conclude that one-directional simplified approach had minimal risks to characterize P-gp liability as compared with conventional bidirectional transport assay. This format can be used to screen compounds at early discovery stage with increased efficiency. The false negative risk can be mitigated by overall undesirable low passive permeability and follow-up in vivo or bi-directional transport experiments.

CPSA SHANGHAI

POSTER ABSTRACT
**#127**

# Can Capecitabine and its active metabolite 5-FU achieve sufficient CNS exposure for the treatment of breast cancer brain metastasis?

Jinqiang Zhang, Bonnie Zhang, Yumei Yan, Jerry Li, Liang Xie, Xiuhua Zhang, Yu Bai, Kan Chen, Yuya Wang, Wei Zhong, Zack Cheng

DMPK, Innovation Center China, Asia & Emerging Markets iMed, Astrazeneca

CNS metastasis in patients with Her2 positive metastatic breast cancer is becoming a more frequent diagnosis. Lack of effectiveness in treating brain metastases from current breast cancer drugs, such as Trastuzumab and Lapatinib, is probably due to their inability to cross blood-brain barrier (BBB). It was reported that CNS response rate in breast cancer brain metastases patients improved from 2.6~6% (Lapatinib alone) to 20~65% (Lapatinib in combination with Capecitabine). In this study, we have evaluated the CNS penetration of Capecitabine in preclinical species and hope to shed light of the mechanism of its response in brain metastasis from quantitative pharmacology perspectives. Capecitabine doesn't have antitumor activity and 5-FU is the active metabolite from Capecitabine. Capecitabine was orally administered to naïve mice at the maximal tolerable dose returning $Kp_{,uu,brain}$ (unbound brain-to-blood ratio) at 0.03 and 0.12 and $K_{p,uu,CSF}$ (CSF-to-unbound blood ratio) at 0.23 and 0.27 for Capecitabine and 5-FU, respectively. Neither free brain nor CSF concentration can achieve $GI_{50}$ of antiproliferation in BT474C1 with Her2 amplification cell line. Mice carrying xenograft tumors of Her2-overexpressing BT474C1 (Her2 amplified) cells were treated with Capecitabine as a single agent or in combination with Lapatinib. In consequence, there was no antitumor activity in mice xenograft BT474C1 model as anticipated from mouse brain penetration study. Evaluation of brain penetration of Capecitabine and 5-FU in combination with Lapatinib in xenograft mice is on-going. The CNS penetration of Capecitabine and 5-FU in human was predicted based on their preclinical penetration and human blood PK at the approved clinical dose and the potential for eliciting antitumor activity in human brain metastasis was also explored. These results suggested that CNS penetration of Capecitabine and 5-FU is still not optimal and development of a true CNS penetrable chemoagent will be helpful to treat breast cancer brain metastasis.

CPSA SHANGHAI

POSTER ABSTRACT
**#128**

# Alternative Choice Besides Immunoassays -- Development and Validation of a Highly Sensitive UPLC-MS/MS Method for Exendin-4 in Monkey Plasma

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Abstract:

Exendin-4, a xenobiotic peptide with 39-amino acid length, has been reported widely for the regulation on the blood glucose levels during the treatment of diabetes and obesity. The concentrations of Exendin-4 in preclinical or clinical samples were mostly determined using immunoenzymetric assay (IEMA) or radioimmunoassay (RIA) and the lower limit of quantitation (LLOQ) of these assays can reach into pg/mL level. LC-MS/MS method has been reported in previous for the determination of Exendin-4 and the LLOQ was achieved within ng/mL range, which the assay sensitivity in general could not meet the low-dose design of most pharmacokinetic or pharmacodynamic studies.

In present study, a highly specific and sensitive UPLC-MS/MS method for Exendin-4 in monkey plasma with solid-phase extraction was developed. The LLOQ of the current method was achieved as 10 pmol/L (~40 pg/mL), which was comparable with those obtained from IEMA or RIA. The method was further validated via its linearity, sensitivity, selectivity, within-run accuracy and precision, matrix effect, carryover, and recovery.

Novel Aspect:

To the best of our knowledge, this is the first reported sensitive and specific LC-MS/MS method for the determination of Exendin-4 in monkey plasma, in which the LLOQ is comparable with those from immunoassays. The current method can become an alternative choice for the determination of Exendin-4 in preclinical PK or PD studies, besides immunoassays.

CPSA SHANGHAI

POSTER ABSTRACT
**#129**

# Intellicap, An Intelligent Tool To Accelerate Drug Development

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Rapid evaluation of absorption within regions of the gastro-intestinal tract has great utility for development of drugs for oral administration1. The IntelliCap system comprises an electronic oral drug delivery capsule to accurately delivery drugs in targeted locations. It makes use of a built-in actuator to delivery drug on demand, and a pH sensor for capsule localization and monitoring individual variations in transit and pH2[2,3]. The system has received CE Mark certification and has been used extensively for both clinical and pre-clinical investigation.

Example of IntelliCap utility is illustrated in a case study comprising a two-arm cross-over clinical study, examining the PK data of a commercial modified release formulation (Diltiazem HCl XR 60mg capsules, Mylan; USA) and the same drug released by the IntelliCap system. Release from the IntelliCap capsule is programmed to follow the same in-vitro release profile of the commercial formulation. The results satisfied bio-equivalent criteria, demonstrating the utility of IntelliCap in evaluating and determining the optimum release profiles before developing a solid oral dosage form, a process we call rapid formulation prototyping.

In a second case study, bolus delivery of metoprolol was executed to the ascending colon in dog using a newly developed variant of the IntelliCap system designed for rapid release and wider flexibility of drug payloads. Results allow a clean and simple assessment of colonic absorption, demonstrating the utility of IntelliCap in fast screening of candidate drug for modified release formulations and for evaluating location targeted release strategies for oral therapy

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POSTER ABSTRACT
**#130**

# A new approach to identify major metabolites in liver microsomes for predicting primary metabolic pathways in vivo

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In vitro metabolism studies are routinely conducted in drug discovery and early development for predicting pharmacokinetics in human. For example, in vitro metabolic stability and CYP inhibition data can predict metabolic clearance and CYP-mediated drug-drug interaction in human, respectively. Similarly, in vitro metabolite profiles are often acquired to predict drug biotransformation pathways in vivo. As a common practice, in vitro biotransformation experiments are carried out by incubating a test compound at 10 - 30 μM in human liver microsomes (HLM) for 15 - 45 min followed by metabolite identification using LC/MS. As a consequence, many metabolites are generated, detected and structurally characterized. However, since LC/MS cannot provide good quantitative measurements without standards, major metabolic pathways cannot be readily elucidated based on the LC/MS based metabolite profiling data. The major objective of this study was to develop a simple approach to rapid determination of major metabolites in liver microsomes for predicting primary metabolic clearance pathways in vivo. Model compounds, saquinavir, verapamil and clozapine, were incubated HLM or rat liver microsomes (RLM) at 5 μM that is lower than Km values of most CYP enzymes. Incubation times (4 - 8 min) were selected based on their metabolic stability data to ensure parent disappearance are less than 30%. The test compounds and their metabolites in the incubations were quantitatively estimated by UV detection and structurally characterized by ion trap or Q-TOF mass spectrometers. Although up to nine metabolites were formed from these model compounds in liver microsomal incubations, the LC/UV/MS based method was able to quickly identify two or three major metabolites in HLM or RLM, consistent with primary metabolic pathways in vivo. For example, *N*-demethylation and *N*-dealkylation products of verapamil were quickly determined as major metabolites among eight metabolites formed in HLM, which are in agreement with the primary biotransformation pathways of verapamil in humans. The novel approach can be applied to metabolic soft spot analysis, CYP reaction phenotyping and in vitro metabolism comparison across species. Furthermore, it eliminated efforts in identifying multiple minor metabolites.

CPSA SHANGHAI

Young Scientist Excellence Submission

POSTER ABSTRACT
**#131**

# Improved peptide quantification by hybrid reaction monitoring with ion trapping and switching using the triple quadrupole/linear ion trap mass spectrometry

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Multiple reaction monitoring (MRM) has become a valuable tool in quantitative proteomics field due to the superior sensitivity and selectivity and wide dynamic range. However, in many cases, MRM based peptide quantitation is still difficult due to the complexity of biological samples. Here, we introduce a novel hybrid reaction monitoring (HRM) for improved peptide quantification using the triple quadrupole/linear ion trap (QTRAP®) mass spectrometry. The HRM simultaneously monitors two different transitions in quadrupole and linear ion trap mode and acquires HRMQ for qualification and $HRM^{LIT}$ for quantitation, respectively. A 3~5-fold lower LOQ were observed with both the $HRM^{LIT}$ and $HRM^{Q}$ due to ion trapping compared with the traditional MRM for measurements of tryptic peptides spiked into crude post-synthesis peptide mixture and BSA digest over a range of concentrations. Furthermore, $HRM^{LIT}$ provides better reproducibility because of its significant enhancements (500~1000-fold) in overall signal intensities and better selectivity due to product ion switching. Peptides possessing different fragments in QqQ and LIT mode benefit most from HRM. HRM could also be time scheduled and flexibly incorporated into the traditional multiplexed MRM workflow to specifically enhance the quantification of targeted low abundance peptides. (Supported by the Science and Technology Development Fund of Macao SAR (Ref. No. 043/2011/A2) and the Research Committee of University of Macau (Ref. No. MYRG207).

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POSTER ABSTRACT
**#132**

# HPLC-MS/MS Analysis of Caspofungin in Plasma in Support of Clinical Practice

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3 C & O Pharmaceutical Technology (Holdings) Limited, Nanjing China

BACKGROUND:

Echinocandins is a new class of the antifungal drug, which is approved by the FDA and been widely used for invasive fungal infections in recent years. Caspofungin (CAS NO. 162808-62-0), one of the echinocandins, plays an important role in clinical practice, especially in the treatment of Candida. But it has a high incidence of side effect, and has been proved a change of concentration in hepatic insufficiency patient. As all echinocandins display concentration-dependent fungicidal or fungistatic activity, the therapeutic drug monitoring (TDM) is necessary for its safety and efficacy.

The aim of this study is to develop a HPLC-MS/MS method for quantification of caspofungin serum concentrations, as the basis of TDM and population pharmacokinetic study.

METHODS:

After protein precipitation by acetonitrile, analysis was performed on an Agilent Technologies 1290 Infinity LC system coupled with an Agilent Technologies 6460 Triple Quadrupole MS. Separation with a C18 column (Phenomenex Luna C18 2: 150mm×4.6 5μm) and gradient elution (mobile phase A: 10mM ammonium acetate in 0.1% acetic acid mobile phase B: acetonitrile with 0.1% acetic acid) at 1 mL/min resulted in a 6-min run-time. Detection was performed in dynamic MRM, monitoring 2 ion transitions per compound. Itraconazole was used as internal standard.

RESULTS:

The method was validated in terms of linearity, selectivity, specificity, accuracy, precision, absolute and relative matrix effect and stability.

The method was linear in the range of 1-200 ng/mL, with correlation coefficient values higher than 0.997. Lower limits of quantification (LLOQ) were 5 ng/ml. Mean recovery was 97.3%. Matrix effects were -6.4 to +9.1%. Accuracy ranged between 91.3 and 107.0% at low, medium and high concentrations and between 88.2 and 103.9% at LLOQ. Within and between-run precision was <15% (CV). No interferences were found. No problems of instability were observed. The method was successfully applied on patient samples.

CONCLUSIONS:

The method developed has a straightforward sample preparation and it has been applied successfully for the measurement of caspofungin concentrations in patient samples, both for clinical practice and for research.

CPSA SHANGHAI

Innovator Submission

POSTER ABSTRACT
#133

# A Quantification Study of ZYS016 in Serum by ELISA and Its Pharmacokinetics in Cynomolgues Monkey

HongzhenLia, Conglin Zuo, Xunxia Sun, Yuxia Feng,Yan Xia

Joinn Laboratories

Abstract: A quantitative method of double antibody sandwich ELISA for ZYS016, a IgG-Fc conjugated protein, in cynomolgues monkey serum was developed, and the pharmacokinetics of ZYS016 in cynomolgues monkey was detected with this method.

A total of 18 cynomolgus monkeys in both sexes were randomly assigned into 3 groups (6 animals/group), including low dose of ZYS016 (3μg/kg), middle dose (10 μg/kg) and high dose (30μg/kg ) groups. ZYS016 was i.v. administered once.

The linearity was ranged from 1 to 64 ng/ml (r2 > 0.99), and the low limit of quantification was 1 ng/ml. The recovery and precision met the requirements of bioanalytical method. After a single i.v. dose of 3, 10, 30 μg/kg ZYS016, the mean t1/2 were 195, 126, 151 h, the mean $C_{max}$ were 29, 95, 389 ng/ml, and the AUC were 7, 24, 109 h·μg/mL, respectively. It is concluded that the method we developed is selective and suitable for the quantification of ZYS016 in cynomolgus monkey serum. In dose range of 3 – 30 μg/kg, $C_{max}$ and AUC were positive correlated with dosage.

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POSTER ABSTRACT
**#134**

# A Quantification Study of ZYS016 in Serum by ELISA and Its Pharmacokinetics in Cynomolgues Monkey

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Abstract: A quantitative method of double antibody sandwich ELISA for ZYS016, a IgG-Fc conjugated protein, in cynomolgues monkey serum was developed, and the pharmacokinetics of ZYS016 in cynomolgues monkey was detected with this method.

A total of 18 cynomolgus monkeys in both sexes were randomly assigned into 3 groups (6 animals/group), including low dose of ZYS016 (3μg/kg), middle dose (10 μg/kg) and high dose (30μg/kg ) groups. ZYS016 was i.v. administered once.

The linearity was ranged from 1 to 64 ng/ml (r2 > 0.99), and the low limit of quantification was 1 ng/ml. The recovery and precision met the requirements of bioanalytical method. After a single i.v. dose of 3, 10, 30 μg/kg ZYS016, the mean t1/2 were 195, 126, 151 h, the mean $C_{max}$ were 29, 95, 389 ng/ml, and the AUC were 7, 24, 109 h·μg/mL, respectively. It is concluded that the method we developed is selective and suitable for the quantification of ZYS016 in cynomolgus monkey serum. In dose range of 3 – 30 μg/kg, $C_{max}$ and AUC were positive correlated with dosage.

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CPSA SHANGHAI

POSTER ABSTRACT
**#135**

# Rosiglitazone ethnic diversity: Predicting PK using SymCYP™

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Background: Different pattern of systemic exposure to Rosiglitazone has been observed in Chinese versus Caucasian subjects. Rosiglitazone is extensively metabolized in the liver predominantly by CYP2C8. The enzyme CYP2C8 is genetically polymorphic, and ethnic differences in the CYP2C8 genotype frequencies have been observed between Caucasian and Chinese population. Therefore, the PK difference is assumed to be related with ethnic differences in the CYP2C8 genotype frequencies.

Purpose: SimCYP™, a population based PBPK modeling and simulation tool contains population libraries which include information on the demographics of various ethnic and the associated modifications in physiology and enzymology. This poster highlights an example of how SimCYP™ has been used to predict the impact of ethnic diversity on the exposure of Rosiglitazone.

Methods: Prior in vitro and in vivo information for Rosiglitazone were collated from GSK internal database and literature. Specifically, data on the frequency of the different allelic forms of CYP2C8 and their metabolic activity were incorporated into a physiologically-based pharmacokinetic (PBPK) model within the Simcyp Simulator (V12) to predict differences in the relative exposure of rosiglitazone in a virtual Caucasian and Chinese population.

Results and Conclusions: Following multiple doses of 4 mg rosiglitazone, the predicted mean $C_{max}$ (ng/mL) and AUC0-24 (ng/mL·h) is 241 and 1350 in Caucasian, which was consistent with 260 and 1429 observed in vivo. Predicted mean AUC ratios of rosiglitazone for CYP2C8*1/*3 and *3/*3 versus *1/*1 was 0.77 and 0.58, which was consistent with 0.80 and 0.54 observed in vivo. In Chinese subjects only with the CYP2C8*1/*1 genotype, the predicted $AUC_{0-24}$ ratio compared to that in Caucasian was 1.4, which was aligned with 1.5 observed in vivo.(Kai-Min Chu et al., *J Pharm Pharmaceut Sci*, 2007)

Rosiglitazone case demonstrates the utility of SimCYP™ to predict the relative exposure of a drug as a function of ethnicity. Prior simulation of the potential differences in exposure can support design of clinical studies and help in the selection of a safe and effective dosage regimen.

CPSA SHANGHAI

POSTER ABSTRACT
**#136**

# Development and validation of a sensitive ultra high performance liquid chromatographic/tandem mass spectrometric method to detect amlodipine in human plasma

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Abstract: Amlodipine is a calcium-channel blocking agent of the basic dihdropyridine derivative, inhibits the calcium influx through slow channels in peripheral vascular and coronary smooth muscle cells, and thus is useful in the management of hypertension and angina pectoris. After oral administration, amlodipine has low plasma concentration. In order to satisfy the demand of pharmacokinetic study of amlodipine in human, we developed and validated a sensitive, specific and rapid liquid chromatographic/tandem mass spectrometric (LCMS-8050) method for quantification of amlodipine in human plasma. The isotope internal standard amlodipine-D4 was used for accurate quantification. The linear calibration curves over the concentration range of 50-10000 pg/mL, with correlation coefficient 0.9999. The lower limit of quantification (LLOQ) was 50 pg/mL, and the specificity test demonstrated that there was no interference from the blank matrix. The intra- and inter-day precision was less than 8.99% determined from QC samples at concentrations of 150, 1500 and 8000 pg/mL, and the accuracy was between 87.5~112.1%. With the simple sample treatment of protein precipitation, the method has good recovery for all QC samples which was higher than 85%. The matrix effect factor was higher than 97% and the internal standard normalized matrix effect factor was about 97%. To investigate the carryover of the analytical method, a blank sample was injected after the analysis of upmost concentration of calibration, and there was no interference in the blank sample. Additionally, there was also no interference from the isotope internal standard to the target compound. In conclusion, a rapid, sensitive and reproducible method based on LC-MS/MS was developed and validated for detection of amlodipine in human plasma and could satisfy the demand of pharmacokinetic study of amlodipine in human.

Key words: LC-MS/MS; Amlodipine; Human Plasma

CPSA SHANGHAI

POSTER ABSTRACT
**#137**

# Biomarker of HCC in China

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HCC (hepatocellular carcinoma) is the most common and lethal type of liver cancer mainly caused by hepatitis B or C viruses' infection [1]. Early diagnosis is essential to detect HCC at an earlier stage when curable interventions could be provided to achieve long-term disease-free survival for patients.

The diagnosis of HCC could be achieved by detecting biomarkers. However, since HCC is a complex disease caused by variety of risk factors resulting in multiple pathogenic mechanisms, it is difficult to detect HCC with a single biomarker [2]. Biomarkers from body fluids such as serum, plasma, urine and bile (e.g. Dickkopf-1 and Golgi protein 73) which are easily accessible could be used for early diagnosis of HCC [3]. Dickkopf-1 (DKK1) is a kind of secreted protein which is related to the signaling pathway in HCC cells [4]. However, DKK1 is not overly specific for HCC diagnosis. A recent study showed that serum DKK1 was also elevated in patients with intrahepatic cholangiocarcinoma [5]. Research was conducted in 1284 patients (633 with HCC, 171 with chronic HBV infection, 168 with cirrhosis, and 312 healthy controls) and found that DKK1 has better diagnostic value for HCC than AFP (alpha-fetoprotein), especially for patients with AFP-negative and early stage HCC [6].

The up-regulation of GP73 is also observed in liver diseases such as HCC. The sensitivity and specificity of serum GP73 for HCC were 74.6% and 97.4%, respectively, compared with 58.2% and 85.3% for AFP [7]. However, details of GP73's biochemical function and regulation still need to be studied. The hypothesis of using this new kind of serum marker for detection of HCC in a high-risk population needs to be proved in large cohorts.

MicroRNA (miRNAs) also shows to serve as a biomarker for the detection of HCC in healthy subjects [8]. Up to now, AFP is still the only available marker for HCC diagnosis. The reliability of other biomarkers is debatable.

Among the various biomarkers, the combination of testing des-γ-carboxyprothrombin (DCP) and α-fetoprotein (AFP) shows to achieve a high level of sensitivity and specificity to detect HCC in the early stages in Japan, while AFP is the only serum biomarker widely used to screen for and diagnose HCC in China [9]. Studies have found that the combined test of DCP and AFP has a sensitivity of 84% and a specificity of 83%. The wide use of more efficiency tools and biomarkers to early diagnosis HCC is expected.

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CPSA SHANGHAI

POSTER ABSTRACT
**#138**

# Integrative Pharmacogenomic Analysis of Patient-Derived Xenografts of Chinese Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and the third leading cause of cancer-related deaths. Patient derived xenografts (PDX), generated by direct engraftment of tumor samples from the patients into immunocompromised mice can be used as an in vivo platform that provides the opportunity to test proposed personalized medicine strategies. In this study, comprehensive molecular characterizations of 28 Chinese HCC PDX models were done by both whole genome sequencing (WGS) and RNAseq technology. After removing contaminated murine DNA/RNA reads by a proprietary algorithm (unpublished) to eliminate homology bias, more than 35 million reads per sample were analyzed in the RNAseq experiment, and at least average 40X coverage at each base of DNA in the WGS experiment. In order to find meaningful targets with high confidence, we integrated multiple lines of evidences from both DNA and RNA levels. Out of 346 gene fusion events were identified in 28 HCC PDXs, 51 occurred in more than one sample and 13 were supported by structural variation evidence from WGS dataset.

The same passages (between p3-p5) of 24 PDX tissues were also treated with Sorafenib in the efficacy studies and showed differential responses. The expression of 482 genes (2%) is statistically significantly correlated with drug sensitivity (p-value <0.05), 198 of them (41%) are positively correlated. In the single nucleotide variation (SNV) and indel analysis of WGS dataset, 162 genes were identified as highly mutated genes (p-value <0.05), including classical cancer driver gene TP53. Total 27401 novel SNVs in these 162 genes were identified, 991 (3.6%) of them are nonsynonymous. 557 (2%) SNVs are statistically significantly correlated with drug sensitivity status (p-value<0.05), 28 are nonsynonymous. Pathway analysis shows that these drug responses related genes are enriched in focal adhension formation, induction of apoptosis, P53 signaling pathway, DNA damage response and cell cycle checkpoint.

Comparing to other similar studies, our approach of integrative analysis of WGS (vs. Whole exome sequencing) and RNAseq (vs. microarray) provided the most comprehensive genetic insights/landscapes of HCC patients and genetic alterations' relationship to the drug responses. It also demonstrated that PDX models provide rich information for preclinical studies of experimental therapeutics, and an excellent resource for biomarker discovery and translational research.

POSTER ABSTRACT
**#139**

# Generation and Characterization of a Sorafenib-Resistant Hepatocellular Carcinoma Model from Patient-Derived Tumor Xenografts

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Hepatocellular carcinoma (HCC) is a common malignancy worldwide and sorafenib is a standard of care therapeutics for unresectable HCC. Although sorafenib, a multikinase inhibitor, can suppress both angiogenesis and cell proliferation, its efficacy is hampered by the emergency of drug resistance during the treatment. Thus, it is useful to understand the mechanism underlying sorafenib resistance in an animal model with resemblance of human HCC. Here, we report the generation and characterization of a sorafenib-resistant HCC xenograft model derived from patient tumors. A patient-derived xenograft (PDX) mouse model (LIX004) was treated with sorafenib. While sorafenib inhibited tumor growth at the initial stage of treatment, a few tumors regrew after prolonged sorafenib treatment. A cancer cell line (LIXC-004SR) was generated from a resistant tumor and the xenograft derived from this cell line maintained sorafenib resistance in vivo. On contrary, another cell line (LIXC-004NA) established from the treatment-naïve LIX004 PDX model remained sensitive to sorafenib. We compared the gene expression profiles between these two cell lines using both cDNA microarray and qPCR and found that a class of genes involved in angiogenesis were significantly upregulated in LIXC-004SR cells vs. LIXC-004NA cells, which include those encoding secreted angiogenesis stimulators such as MMP1, FGF-5 and VEGFA. Consistently, increased angiogenesis was detected in sorafenib-resistant LIXC-004SR tumor compared to sorafenib-sensitive LIXC-004NA tumor. Furthermore, the conditional medium from LIXC-004SR cell culture could stimulate HUVEC proliferation as well as MEK,ERK and Akt phosphorylation. These results suggest that sorafenib resistance may arise by bypassing the inhibition of angiogenesis via upgregulation of pro-angiogenesis genes. The generation and molecular characterization of sorafenib-resistant tumor models could shed light on the mechanism of sorafenib resistance which may aid in the development of strategies to overcome drug resistance.

CPSA SHANGHAI

POSTER ABSTRACT
**#140**

# Adaption of Retention Models to Allow Optimisation of Peptide and Protein Separations

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Proteins and peptides are becoming analytes of increasing importance within the pharmaceutical industry. This poster describes the adaption and validation of the retention models necessary to accurately model and optimise analytical scale Reverse Phase Chromatography (RPC) and Ion Exchange Chromatography (IEC) separations of peptides and proteins.

Retention modelling has successfully been used for the optimisation of analytical scale separations of small molecules for approximately 30 years. A large number of articles have been published on this topic by L. Snyder, P. Janderra, P. Schoenmakers et al. and several commercial software are available, i.e., DryLab, ACD/LC Simulator, ChromSword, and Osiris.

When defining a method development strategy for peptides and proteins involving retention modelling, investigated commercially available software packages were not capable of accurately modelling the retention of proteins. This collaboration with ACD/Labs resulted in models that can be utilized within ACD/LC Simulator to accurately model retention times of proteins and peptides.

To create models for proteins we used isocratic retention models in combination with numerical solutions in order to calculate retention times for gradient conditions.

In this study six proteins with a MW of approx. 25 000 Da were chromatographed and modelled by RPC and IEC.

An alpha version of ACD/LC Simulator2 was provided, which allowed gradient modelling employing the RPC/HIC model (Eqn. 2) and the IEC/HILC model (Eqn. 3) in combination with the 2nd order temperature model (Eqn. 5).

It can be concluded that RPC and IEC gradient chromatography at different temperatures can be modeled with the same accuracy for proteins as for small molecules.

Most likely due to the unfolding of proteins at higher temperature, a 2nd order temperature model is needed.

As previously described by Snyder et al.1 also HILIC and HIC should be possible to model using the same models.

CPSA SHANGHAI

POSTER ABSTRACT
**#141**

# Development a sensitive LC/MS/MS method for analysis of a peptide drug

Luke Bi
Covance

LC/MS/MS platform has been utilized to support the clinical development of peptide and protein drugs in the past 10 years. Compare to the traditional immunoassay (ELISA), the LC/MC/MC methodology demonstrated better sensitivity, and less prone to the interference from the endogenous peptides and proteins in the biologic matrix. Most noticeably, LC/MS/MS method has been successfully developed for exenatide, a GLP-1 analogs, and insulin.

In the current research, a LC/MS/MS methodology has been developed for a peptide analog (~5000 dalton). The peptide has multiple charges under the ionization condition and the mass/charge ratio is less than 1000. The plasma samples were extracted with solid phase extraction for clean up and enrichment to achieve high sensitivity. The sample preparation and processing were designed carefully in order to minimize non-specific binding and to achieve high recovery. The HPLC separation was optimized to avoid interference from endogenous peptides and to minimize carryover. The analytical method achieved limit of quantitation of 50 pg/mL.

This method was successfully used in sample analysis for a clinical study. The study results demonstrated that the LC/MS/MS assay has better sensitivity and selectivity and much lower variation than the ELISA assay.

CPSA SHANGHAI

Young Scientist
Excellence Submission

POSTER ABSTRACT
**#142**

# Study on the PK profiles of magnoflorine and its potential interaction in Cortex phellodendri decoction by LC-MS/MS

Xiaoting Tian & Zhixiong Li & Yunfei Lin & Mingcang Chen & Guoyu Pan & Chenggang Huang

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Magnoflorine, an aporphine alkaloid in *Cortex phellodendri*, is increasingly attracting research attention be- cause of its antidiabetic effects. However, at present, little information on its pharmacokinetics (PK) in vivo is available. In this study, a sensitive, rapid, and selective method was developed to determine the magnoflorine content in rat plasma using liquid chromatography–tandem mass spectrometry. Following liquid–liquid extraction, the calibration curve showed good linearity within the concentration range of 2.93 to 1,500 ng $ml^{-1}$. The intra- and inter-day precisions were all below 7.8 %, and the accuracy ranged from 94.9 to 103.4 %. The method was successfully applied in investigat- ing the PK of magnoflorine in rats. The compound had low bioavailability, a high absorption rate, and a high elimination rate. However, area under the curve, $T_{1/2}$, and MRT increased approximately twofold when the same dosage of the com- pound was administered in a C. *phellodendri* decoction (20.8 g $kg^{-1}$). Moreover, $T_{max}$ was prolonged from 0.3 to 3.33 h. Furthermore, a comparison of coadministration of the mixture group, magnoflorine (40 mg $kg^{-1}$) and berberine (696.4 mg $kg^{-1}$), with the *C. phellodendri* decoction group, revealed that no statistical difference ($P > 0.05$) was found in the parameter AUC, and certain similar changes in the PK trend to the herbal medicine group were also observed. These results suggested that oral administration of the herbal medi- cine decreased the absorption and elimination rates of magnoflorine and increased its bioavailability. Berberine played a significant role in interacting with magnoflorine and in affecting the PK profiles of magnoflorine in the *C. phellodendri* decoction group.