CPSA
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POSTER SESSION ABSTRACTS

Inspiration and Education

POSTER HALL OPEN
Thursday, April 26 9:00 am - 5:00 pm
Friday, April 27 9:00 am - 5:00 pm

Including
Student Excellence Award
Innovator Award
Submissions

CPSA Shanghai 2013
April 24 -27, 2013
Renaissance Shanghai Pudong Hotel
Shanghai

Where Technology and Solutions Meet

Where East Meets West

POSTER SESSION ABSTRACTS

POSTER HALL OPEN
Thursday, April 25 9:00 am - 5:00 pm
Friday, April 26 9:00 am - 5:00 pm

POSTER SESSIONS
Thursday, April 25 11:00 am - 12:00 pm
Friday, April 26 11:00 am - 12:00 pm

INNOVATOR SESSION
Friday, April 26 3:30 pm - 5:00 pm

Young Scientist Excellence Submission  DENOTES YOUNG SCIENTIST AWARD SUBMISSION

Innovator Submission  DENOTES INNOVATOR AWARD SUBMISSION

Where Technology and Solutions Meet

Where East Meets West

POSTER ABSTRACT

Bioanlytical

#101

Fluorescent Probes for Highly Selective and Sensitive Detection of Hydrogen Sulfide in living cells and Tissues

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Hydrogen sulfide, H2S, is a colorless gas with unpleasant rotten egg smell that until recently was only considered to be a toxic environmental pollutant with little or no physiological significance. However, the past few years have demonstrated its role in many biological systems and it is becoming increasingly clear that H2S is likely to join nitric oxide (NO) and carbon monoxide (CO) as a major player in mammalian biology, the correlation between H2S and physiological processes has attracted scientists to develop brand new methods to monitor such a gaseous molecule in vitro and in vivo. Herein, we described a coumarin-based fluorescent probe (C-7Az) and resorufamine-based fluorescent probe (C-13Az) that based on azido group reduction for intracellular H2S detection. It were evaluated for the selective and sensitive detection of hydrogen sulfide in degassed PBS buffers and fetal bovine serum. And it gave remarkable changes with 106-fold (C-7Az) and 23-fold(C-13Az) fluorescence intensity enhancement under equiv of H2S (100 μM) added in PBS (10 mM, pH=7.4), and also exhibits a dynamic response range for H2S from 2.5×10-7 to 1.0×10-4 M, with a detection limit of 0.25 μM. The effect of pH on the probes was also studied. In addition, Fluorescence detection of hydrogen sulfide in living cells were also successfully achieved using two-photon confocal fluorescence imaging. Further in situ visualization of endogenous H2S was realized in cardiac tissues of normal rats and atherosclerosis (AS) rats.

POSTER ABSTRACT

ADME

#102

Prediction of human CYP3A-mediated drug metabolism from minipig: Comparation of recombinant minipig CYP3A and human CYP3A

Bian Yicong, Shang Haitao, Wei Hong*, Zeng Su*

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Objective: To express recombinant minipig CYP3A22, CYP3A29 and human CYP3A4, CYP3A5, and to compare the CYP3A metabolic characteration between human and minipig by using the specific probe substrate, so as to evaluate the similarity between minipig and human in drug metabolism.

Methods: Bac-to-bac baculovirus expression system was used to coexpress recombinant CYP3A with POR and CYP b5. The activity of the enzymes was determined by HPLC and HPLC-MS-MS, using testosterone and midazolam as probe substrates.

Results: The recombinant CYP3A4, CYP3A5, CYP3A22 and CYP3A29 were expressed. The same major metabolites were found after incubation with each enzyme. For 6βhydroxylation of testosterone, the Km values of CYP3A4, CYP3A5, CYP3A22 and CYP3A29 were 135.7±9.3 μM, 188.5±24.8 μM, 86.5±4.8 μM and 97.55±4.8 μM. The Vmax were 7.39±0.16 nmol/min/nmol P450, 1.56±0.07 nmol/min/nmol P450, 3.49±0.05 nmol/min/nmol P450, and 6.23±0.09 nmol/min/nmol P450. The Clint were 54.5±2.8 μl/min/nmol P450, 8.3±0.8 μl/min/nmol P450, 40.4±1.8 μl/min/nmol P450 and 63.8±2.4 μl/min/nmol P450. For 1-hydroxylation of midazolam, the Km values of CYP3A4, CYP3A5, CYP3A22 and CYP3A29 were 3.33±0.14 μM, 6.15±0.18 μM, 5.60±0.10 μM and 17.01±0.77 μM. The Vmax were 1.12±0.01 nmol/min/nmol P450, 2.88±0.02 nmol/min/nmol P450, 0.297±0.002 nmol/min/nmol P450, and 0.506±0.010 nmol/min/nmol P450. The Clint were 335.9±11.4 μl/min/nmol P450, 468.5±10.6 μl/min/nmol P450, 53.02±0.74 μl/min/nmol P450 and 29.73±0.83 μl/min/nmol P450.

Conclusion: The results showed that minipig CYP3A had similar metabolic profiles to human CYP3A. The minipig may resemble human in non-clinical studies and can be used to peredict human CYP3A-mediated drug metabolism.

POSTER ABSTRACT

ADME

#103

Characterizing the effect of cytochrome P450 (CYP) 2C8, CYP2C9, and CYP2D6 genetic polymorphisms on stereoselective N-demethylation of fluoxetine

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Abstract: ABSTRACT: OBJECTIVE: To express recombinant human CYPs (2C8*1,*2,*3,*4, 2C9*1,*3,*13,*16, and 2D6*1,*10), determine their expression levels and activity. Then study their enzyme kinetic characteristics on stereoselective N-demethylation of fluoxetine (FLX). METHODS:. Recombinant human CYPs were prepared with the Spodopterafrugiperda Sf9 cell system. The recombinant human CYPs were subjected to Western blot analysis for determining the relative expression levels and their classical in vitro probe substrates were used to validate their metabolic activities. The kinetic parameters of different CYPs on R-, S-, and R/S-FLX N-demethylation were analysed with an LC-MS/MS method. RESULTS: The recombinant CYPs were successfully expressed. The activity assays showed that all the CYPs were active towards their classical substrates. The metabolic profiles of FLX mediated by these CYPs appeared to behave in a classical Michaelis-Menten or a substrate inhibition fashion. For CYP2C8s, CYP2C8*3 showed a higher clearance compared to the wild type. The Km values of R- and S-FLX in CYP2C8*3 and CYP2C8*4 showed significant stereoselectivity. However, only CYP2C8*3 exhibited slight stereoselectivity between R- and S-FLX. For CYP2C9s, the Km value for S-FLX were ranked in order of CYP2C9*1 >> CYP2C9*16 > CYP2C9*3 > CYP2C9*13. All the 4 CYP2C9s showed a self-inhibitory effect only for R-FLX and R/S-FLX. The four allozymes of CYP2C9 were all predominantly toward the R-enantiomer, indicating significant stereoselectivity in CYP2C9-mediated N-demethylation metabolism of FLX. For CYP2D6s, the metabolic of FLX enantiomers obviously appeared stereoselectively catalyzed by CYP2D6*1,*2 and *39. The stereoselectively of CYP2D6*1 (R > S) was the reverse of CYP2D6*2/*39 (S > R). Additionally, CYP2D6*2 and *39 exhibited obvious self-inhibitory effect, which was not shown with CYP2D6*1 and *10. CONCLUSION: The recombinant CYPs were successfully expressed with good enzyme activities. Most of the variants evaluated showed lower catalytic activity towards FLX enantiomers and racemate. CYP2C9*1 and their variants, and CYP2D6*1 may affect the stereoselective N-demethylation to FLX.

KEY WORDS: Fluoxetine; Metabolism; Variant; Substrate inhibition; Stereoselectivity

POSTER ABSTRACT

ADME

#104

Involvement of P-glycoprotein in regulating cellular levels of liensinine and its analogues

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OBJECTIVE: To elucidate the efflux transporters involved in the intestinal permeability of liensinine and its 2 analogues neferine and isoliensinine and to evaluate the role of ABC transporters in limiting their oral absorption.

METHODS: The cytotoxicity of the three alkaloids was evaluated by the MTT assays. The intracellular accumulation study and bi-directional transport experiment were conducted using Caco-2, MDCK, MDCK-MDR1, and MDCK-MRP2 cell models. The content of the individual alkaloid in the donor compartment or cell lysate was determined by LC-MS/MS.

RESULTS: The efflux ratios of liensinine, neferine and isoliensinine in Caco-2 cells were respectively 19.4, 1.8 and 2.2. To further confirm the effect of P-gp on the transport of liensinine, neferine and isoliensinine, MDCK-MDR1/MDCK cells were used to measure the net efflux ratios which were correspondingly 6.7, 3.2 and 6.2. In addition, P-gp inhibitor verapamil significantly decreased the efflux ratio of these alkaloids in Caco-2 cells, but the efflux ratio of liensinine was still higher than 2.0 which indicated other drug transports may participate in the absorption of liensinine.

However, the net efflux ratio of liensinine in MDCK-MRP2/MDCK cells was lower than 1.5 suggested that MRP2 was not participated in the absorption of liensinine.

CONCLUSION: liensinine and its analogues are substrates of P-gp and MRP2 is not involved in the liensinine absorption.

POSTER ABSTRACT

ADME

#105

Establishment of Cell Model Stably Expressed hOAT1 to Screen Potential Inhibitors/Substrates

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OBJECTIVE To establish a cell model stably-expressed hOAT1 in MDCK, then apply this cell model to screen potential inhibitors/substrates of hOAT1 from Chinese herbs.

METHODS Transfected MDCK cells with recombinant plasmid pcDNA3.1(+)-hOAT1, after G418 screening, monoclones were obtained by limiting dilution. Functional monoclones were selected and evaluated by uptake of fluorescent substrate,6-Carboxyfluorescein (6-CFL). The Real-Time PCR Analysis (RT-PCR) was employed to detect the expression of hOAT1 at transcription level. The function of monoclones was evaluated by measuring the accumulation of p-aminohippurate(PAH) an hOAT1 classical substrate.

RESULTS The RT-PCR results showed high hOAT1 mRNA expression in the selected cells compared with MDCK empty vector cells; the uptake of 6-CFL increased up to 15 fold of mock cells. The mRNA level of hOAT1 was significantly higher than that in mock cells; the uptake of PAH was obviously inhibited by probenecid, a known hOAT1 inhibitor. Some chemical ingredients from Chinese herbs inhibited the uptake of 6-CFL using MDCK- hOAT1 cell model.

CONCLUSION A cell model stably-expressed hOAT1 is successfully established, and employed as a high throughput screening assay to identify inhibitors/substrates of hOAT1.

POSTER ABSTRACT

Pharmaceutical Analysis

#106

Simultaneous Determination of Madecassoside, Asiaticoside and their Aglycones in Centella asiatica (L.) urban Extracts by RP-HPLC

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Objective: To establish an RP-HPLC method for simultaneous determination of four major ingredients (madecassoside, asiaticoside, madecassic acid and asiatic acid) in Centella asiatica (L.)urban extracts control the quality of Centella asiatica (L.)urban extracts. Methods: The analysis was performed on a Agilent 1100 HPLC system with a ZORBAX Eclipse XDB-C8 column (4.6×150mm, 5μm). The four major constituents were separated with gradient mobile phase which consists of 1mmol/L potassium dihydrogen phosphate and acetonitrile and determined with UV detector at the wavelength of 205 nm. Results: The four major compounds all had good linear responses over the determined ranges (R2≥0.9998). The average recoveries (n=9) were 97.4%, 93.7%, 97.5%, 99.8% with RSD of 3.4%, 1.4%, 4.7%, 4.4%, respectively. Conclusions: The developed method is sensitive and has good repeatability, which can be used as a reference for quality control of Centella asiatica (L.)urban extracts.

Key words: Centella asiatica (L.)urban extracts; madecassoside; asiaticoside; madecassic acid; asiatic acid; RP-HPLC

POSTER ABSTRACT

Pharmaceutical Development

#107

Analytical Technologies for Determination of Trace Level Genotoxic Impurities in Drug Substances

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The layer of Poria cocos (LPC) is one of traditional Chinese medicines (TCM), which is usually used for treating edema and promoting diuretic process in China, Japan, Korea and other countries. TCM treat many diseases through multi-components, multi-ways and multi-targets. However, the molecular mechanisms of TCM are not yet well understood. In the present work. A metabonomic approach based on ultra performance liquid chromatography coupled with quadrupole time-of-flight high-sensitivity mass spectrometry (UPLC Q-TOF/HSMS) and a mass spectrometry Elevated Energy (MSE) data collection technique was developed to obtained a systematic view of the development and progression of chronic kidney disease (CKD) and mechanism studies of the surface layer of poria cocos. By partial least squares-discriminate analysis analyses, 19 metabolites were identified as potential biomarkers of CKD. Ten biomarkers were reversed to the control level in LPC-treated groups. The study indicates that LPC treatment can ameliorate CKD by intervening in some dominating metabolic pathways, such as phospholipid metabolism, purine metabolism and tryptophan metabolism. This work was for the first time to investigate the LPC therapeutic effect based on metabonomics technology, which is a potentially powerful tool to study the TCM.

POSTER ABSTRACT

Pharmaceutical Analysis

#108

A generic multiple reaction monitoring based approach for flavonoids profiling in plants using a hybrid triple quadrupole linear ion trap mass spectrometry

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The limited quality of data obtained from untargeted metabolomic workflow calls for a shift from the breadth-first to the depth-first screening strategy when a specific biosynthetic pathway was focused on. Here we introduced a generic multiple reaction monitoring (MRM) based approach for flavonoids profiling in plants using a hybrid triple quadrupole linear ion trap (Qtrap) mass spectrometry. The approach includes four steps: (1) Preliminary profiling of major aglycones by multiple ion monitoring triggered enhanced product ion scan (MIM-EPI). (2) Glycones profiling by precursor ion triggered EPI scan (PI-EPI) of major aglycones. (3) Comprehensive aglycones profiling by combining MIM-EPI and neutral loss triggered EPI scan (NL-EPI) of major glycone. Unique neutral loss of one ammoniated sugar unit was observed in flavonoid glycosides and proven to be very informative and confirmative. (4) In-depth flavonoids profiling by MRM-EPI with elaborated MRM transitions and ramped conditions. This approach was applied for profiling flavonoids in Astragali Radix (Huangqi), a famous herb widely used in China for medicinal and nutritional purposes. In total, more than 300 flavonoids were tentatively identified, among which less than 40 have been previously reported in this medicinal plant. This MRM-based approach provides versatility, sensitivity and high through-put that required for flavonoids profiling in plants and serves as a useful tool for plant metabomonics.

POSTER ABSTRACT

Drug Delivery

#109

Lymphatic Absorption of Probucol following Oral Dosing in Three Different Formulations

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Intestinal lymphatic absorption represents an absorption pathway alternative to the absorption via portal vein. This is particularly for the lipophilic compounds. In addition, long-chain triglyceride and some surfactants can stimulate the chylomicrons secretion leading to enhancement of intestinal lymphatic absorption. Probucol is one of the compounds subject to intestinal lymphatic absorption, especially when formulated in oil and Vitamin E-TPGS. On the other hand, the nano-suspension or tablet forms of probucol also led to increased oral absorption of the drug. But the mechanism underlining such improvement remains unknown. In this study, absorption pathway of probucol via lymph duct or portal vein is studied with three different formulations. Nine Sprague Dawley rats were randomly divided into three groups, receiving the probucol oral dosing formulated in pure olive oil (group I), 0.5% Vitmamin E-TPGS in olive oil (group II), and commercial tablet-PBS mixed suspension (group III), respectively. For comparison, the mesenteric lymph duct cannulation surgery was conducted in other nine animals. These animals were divided into three groups, and treated with same three formulations (Group A, B and C). Our data showed that when probcuol was dosed with pure olive oil formulation, the area under the curve (AUC) in Group A and Group I were 17304 hr*ng/mL and 10105 hr*ng/mL, respectively, suggesting the portal uptake contributed 60% absorption (AUCA/AUCI), and lymphatic absorption contributed 40% absorption (AUCI-A/AUCI). Our dose recovery data indicated that lymph sample pretreatment with KOH led to significantly increase in the recovery of probucol, implying the drug may exist as a conjugation form. These experimental observations will be discussed from biotransformation and lymphatic absorption perspective.

POSTER ABSTRACT

ADME

#110

Identification of amlodipine metabolites in human liver microsomes and CYP enzyme involved in the pyridine formation, the major clearance pathway of amlodipine in human

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Amlodipine, a dihydropyridine calcium channel blocker, is one of most commonly prescribed drugs for the treatment of hypertension and ischemic heart disease. The major clearance pathway of amlodipine in human is oxidation to the pyridine metabolite that is further converted to several products. Potent CYP3A4/5 inhibitors, telaprevir and indinavir plus ritonavir, significantly increased the AUC of amlodipine in human. A recent clinical study suggested that CYP3A5 is the key influential factor of the elimination of amlodipine. However, in vitro metabolism of amlodipine has not been reported in the literature. The major objective of this study was to determine metabolite profile of amlodipine in human liver microsomes (HLM) and CYP enzyme(s) that catalyzes the formation of the pyridine metabolite. Metabolite profile of amlodipine in pooled HLM (30 μM, 60 min) was determined by LC/UV/ion trap mass spectrometry. As a result, over 10 oxidative metabolites were detected and characterized. The pyridine product was the single major metabolite. Multiple mono-oxidation products and pyridine sequential metabolites including the products from O-demethylation, O-dealkylation and oxidative deamination were observed as minor metabolites. The metabolite profile in HLM is consistent with metabolism of amlodipine in human. Identification of CYP enzyme(s) responsible for the pyridine formation was conducted using HLM in the presence of chemical inhibitors of various CYP enzymes, and using recombinant human CYP enzymes including CYP3A4 and CYP3A5. The incubations were carried out at 1 uM of amlodipine for 13 min. Under these optimized conditions, the formation of pyridine, but not its sequential metabolites, was observed. Ketoconazole, a potent inhibitor for CYP3A, completely inhibited biotransformation of amlodipine to the pyridine metabolite in HLM. In addition, the pyridine formation was observed in incubation with recombinant CYP3A4, but not with CYP3A5 and other tested CYP enzymes, suggesting CYP3A4 plays the key role of the metabolic clearance of amlodipine in human. Currently, a specific CYP3A4 inhibitor (CYP3cide) and individual HLM from CYP3A5*1/*1 and CYP3A5*3/*3 carriers are employed to confirm the roles of CYP3A4 and CYP3A5 in the amlodipine metabolism. In summary, for the first time, amlodipine metabolites in HLM and the CYP enzyme catalyzed the pyridine formation from amlodipine were identified. The findings provide biochemical basis for better understanding and control of clinical DDI and individual PK variability of amlodipine.

POSTER ABSTRACT

ADME

#111

Involvement of organic cation transporter 2 inhibition in potential mechanisms of antidepressant action

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Novel antidepressants or treatment strategies that may offer a more rapid onset of action, improved efficacy, and greater tolerability are in desperate need. Because prevailing antidepressants, which target high-affinity transporters for serotonin and norepinephrine, fail to provide satisfactory treatment outcomes for 50% patients approximately. In recent investigations, a low-affinity but high-capacity transporter organic cation transporter 2 (OCT2, SLC22A2) has been proposed as an important postsynaptic determinant of aminergic tonus and mood-related behaviors, a complementary system to the high-affinity transporters. In order to evaluate whether OCT2 inhibition may at least in part contribution to the pharmacological effects of antidepressants, several typical front line antidepressants were employed to inhibit OCT2 activity in cells stably over-expressing OCT2. The tested antidepressants included selective serotonin reuptake inhibitors (fluoxetine, sertraline and paroxetine), tricyclic antidepressants (amitriptyline, imipramine, desipramine) and reported antidepressant alkaloids (piperine and jatrorrhizine). The two alkaloids were screened through synaptosome before cell experiments, without the interference of monoamine oxidase. All of them showed strong inhibition effects on OCT2-mediated metformin, serotonin and norepinephrine uptake in physiological condition, with IC50 values below 7.00 μM. Sertraline, desipramine, piperine and jatrorrhizine tended to inhibit OCT2 activity via a competitive mechanism. It remains to be seen whether OCT2 inhibition plays a role to the overall therapeutic effects in clinical practice.

POSTER ABSTRACT
#112

Utilization of MDCKII-MDR1/BCRP Cell Line for Lead Compound Screening in Central Nervous System Drug Discovery

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BCRP is highly expressed at human blood-brain barrier (BBB) and expression is elevated in Alzheimer and Parkinson disease. Along with P-gp, BCRP plays a role in limiting central nervous system (CNS) penetration of substrate drugs. We have transduced MDCKII-MDR1 cells by BacMam2-BCRP virus and used these to screen CNS marketed and discovery compounds. For comparison, MDCKII-wild type, MDCKII-wild type transduced by BacMam2-BCRP virus, and MDCKII-MDR1 cells were screened in parallel. Of more than 30 CNS marketed drugs screened, 4 compounds had efflux ratio (ER) in the range 2 to 5 except for triptans in MDCKII-MDR1/BCRP cells compared with 1 compound in MDCKII-MDR1 cells. Efflux ratios (ER) for all tested CNS drugs were lower than 1.5 in MDCKII-BCRP cells, indicating limited BCRP liability. There is a trend towards higher ER in MDCKII-MDR1/BCRP cells than in MDCKII-MDR1 cells. The mRNA level of Pgp was not changed after BCRP transduction, indicating that the increased efflux observed in the double-transfectants is likely due to coordinated function of the two transporters. Free brain-to-blood ratios were inversely related to ER in MDCKII-MDR1/BCRP cells for a diverse range of discovery compounds with high passive permeability. These results demonstrate the value of MDCKII-MDR1/BCRP cells in helping select brain penetrant compounds in CNS drug discovery.

POSTER ABSTRACT

PK-PD

#113

Simulation of the pharmacokinetics of bisoprolol in healthy adults and patients with impaired renal function using whole-body physiologically based pharmacokinetic modeling

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Aim: To develop and evaluate a whole-body physiologically based pharmacokinetic (WB-PBPK) model of bisoprolol and to simulate its exposure and disposition in healthy adults and patients with renal function impairment.

Methods: Bisoprolol dispositions in 14 tissue compartments were described by perfusion-limited compartments. Based the tissue composition equations and drug-specific properties such as log P, permeability, and plasma protein binding published in literatures, the absorption and whole-body distribution of bisoprolol was predicted using the 'Advanced Compartmental Absorption Transit' (ACAT) model and the whole-body disposition model, respectively. Renal and hepatic clearances were simulated using empirical scaling methods followed by incorporation into the WB-PBPK model. Model refinements were conducted after a comparison of the simulated concentration-time profiles and pharmacokinetic parameters with the observed data in healthy adults following intravenous and oral administration.

Finally, the WB-PBPK model coupled with a Monte Carlo simulation was employed to predict the mean and variability of bisoprolol pharmacokinetics in virtual healthy subjects and patients.

Results: The simulated and observed data after both intravenous and oral dosing showed good agreement for all of the dose levels in the reported normal adult population groups.

The predicted pharmacokinetic parameters (AUC, Cmax, and Tmax) were reasonably consistent (<1.3-fold error) with the observed values after single oral administration of doses ranging from of 5 to 20 mg using the refined WB-PBPK model. The simulated plasma profiles after multiple oral administration of bisoprolol in healthy adults and patient with renal impairment matched well with the observed profiles.

Conclusion: The WB-PBPK model successfully predicts the intravenous and oral pharmacokinetics of bisoprolol across multiple dose levels in diverse normal adult human populations and patients with renal insufficiency.

ABSTRACT
#114

PK-PD

Translating disposition of azithromycin from healthy adults to predict its behavior in children

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Physiologically based pharmacokinetic (PBPK) modeling can assist in the development of drug therapies and regimens suitable for children. This study describes a strategy employing PBPK models to predict the pharmacokinetics of azithromycin following intravenous administration.

A PBPK model of azithromycin was developed and scaled to account for age-related changes in size and composition of tissue compartments, protein binding, and maturation of elimination processes. Dose (milligrams per kilogram) requirements for children aged 0~16 years were calculated based on simulations that achieved targeted exposures based on adult references.

Simulations with a human model were compared with clinical data taken from intravenous studies in healthy adults and children. Finally, exposures after intravenous dosing in age-different group of children were predicted. The simulated and observed data after intravenous dosing showed good agreement for all of the dose levels in healthy adults.

Predictions of the pharmacokinetics in different age groups were also in good agreement with observed data. This work exemplifies the utility of PBPK models in predicting pharmacokinetics in children. Simulations showed agreement with a wide range of observational data, indicating that the processes determining the age-dependent disposition of azithromycin are well described.

ABSTRACT

PK-PD

#115

A mechanistic absorption model to predict the effect of gastric bypass surgery on oral bioavailability of metformin and azithromycin

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The gastrointestinal (GI) tract plays an important role in the absorption of orally administered drugs. However, in some cases the anatomy of the GI tract is changed due to GI surgery, which has the potential of influencing drug bioavailability. To predict the effect of gastric bypass surgery on oral bioavailability of metformin and azithromycin, we were developed a mechanistic absorption model based on 'Advanced Compartmental Absorption Transit' (ACAT) model. Rouxen-Y gastric bypass (RYGB) combines the principle of restriction dramatically decreasing stomach size) with malabsorption (bypassing the entire duodenum and partial jejunum). It may influence gastric emptying, absorptive surface area, first-pass intestinal metabolism, intestinal transit time and intestinal pH environment. These physiological changes have been incorporated into the mechanistic absorption model. Post to bypass surgical simulations were carried out for metformin and azithromycin. The simulated and observed data after oral dosing showed good agreement for both metformin and azithromycin in the gastric bypass patients. Prior to clinical studies, an accurate approach to predict the modifications in oral drug bioavailability would be of significant value in assessing the potential for dose modification of gastric bypass patients.

POSTER ABSTRACT

ADME

#116

The application of fully automated on-line solid-phase extraction in pharmacokinetics

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A fully automated on-line solid phase extraction coupled to high-performance liquid chromatography (on-line-SPE-HPLC) was maked application for pharmacokinetics (PK) of bavachinin, WM-5, and nine cardiovascular drugs. The first, on-line-SPE-HPLC with diode array detection (DAD) method was developed for determination of bavachinin in mouse plasma. Plasma samples were injected directly onto a SPE cartridge and the biological matrix was washed out with the loading solventat. By rotation of the switching valve, bavachinin was eluted from the SPE cartridge in the back-flush mode and transferred to the analytical column. Calibration curve with good linearity (r = 0.9997) was obtained in the range of 20–4000 ng/mL in mouse plasma. The intra-day and inter-day precisions (RSD) of bavachinin were in the range of 0.20–2.32% and the accuracies were between 98.47% and 102.95%. The lower limit of quantification (LLOQ) of the assay was 20 ng/mL. The established method was successfully utilized to quantify bavachinin in mouse plasma to support the PK studies. The next, response surface methodology (RSM) was utilized for rapid and systematic optimization of on-line-SPE parameters to maximize the response and separation of WM-5. The optimization was performed with Box–Behnken designs. Four major parameters were investigated for their contrib-utions to the response and separation of WM-5, with a total of 29 experiments being performed for each instrument, respectively. Quantitative determination of WM-5 in mouse plasma was performed to evalu-ate the statistical significance of the parameters on chromatographic response. On-line-SPE-HPLC-DAD method was developed for the determination of WM-5 in mouse plasma. Calibration curve with good linearity (r = 0.9989) was obtained in the range of 20–4000 ng/mL in mouse plasma. The limit of detection (LOD) and LLOQ of the assay were 6 ng/mL and 20 ng/mL, respectively. The overall intra-day and the inter-day variations were less than 1.90%. The recovery of the method was in the range of 93.74–96.33% with RSD less than 3.06%. The optimized method demonstrated good performance in terms of specificity, LLOQ, linearity, recovery, precision and accuracy, and was successfully applied to quantify WM-5 in mouse plasma to support the PK study.The third, on-line-SPE HPLC–MS/MS method was described for the simultaneous determination of nine cardiovascular drugs in rat plasma. Linear calibration curves were obtained over the range of 0.2-1000 ng/mL, and the method limits ranged from 0.2 to 10 ng/mL. The intra- and inter-day precisions were in the range of 0.57-4.46% and the accuracies were between 93.53% and 102.95%. Excellent recoveries (ranged from 91.15% to 99.75%) from plasma obviate the need for an internal standard. The procedure was easier to execute and required less sample handling than methods currently described in the literature. The correlation study of the physicochemical parameters and the analytical parameters of analytes may provide a solid basis for SPE cartridge selection and method optimization for the therapeutic drug monitoring and PK study of cardiovascular drugs.

POSTER ABSTRACT
#117

A new approach for fast and simultaneous screening of oxidative metabolites and glutathione adducts using triple quadrupole-linear ion trap mass spectrometry

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Application of LC/MS to DMPK research involves both qualitative analysis (metabolite detection and identification) and quantitative analysis (in vitro DDI and bioanalysis). Usually, ion trap and high resolution mass spectrometers are employed for metabolite identification, while triple quadrupole and triple-quardupole-liner ion trap (Qtrap) instruments are routinely used in quantitation. Recently, there is a new trend of using a single LC/MS platform for both qualitative and quantitative analysis. The main objective of this study was to develop and validate a new approach for high throughput analysis of both oxidative metabolites and glutathione (GSH) adducts in vitro using Qtrap 5500. Clozapine (10 μM), a model compound, was incubated in rat liver microsomes in the presence of GSH (5 mM?). Generated oxidative metabolites and GSH-trapped reactive metabolites were analyzed by multiple ion monitoring (MIM)-depended acquisition. Recorded MS/MS dataset was further processed for metabolite detection and MS/MS spectra recovery using extract ion chromatography (EIC) based on predicted molecular ions as well as product ion filter (PIF) and neutral loss filter (NLF) based on predicted fragmentation patterns. As a result, over 10 oxidative metabolites and several GSH adducts of clozapine were detected and structurally characterized, some of which have not been reported in the literature. The clozapine metabolite profiling data was similar to that determined by LTQ ion trap instrument. This Qtrap-based approach has several advantages. (1) A generic data acquisition method is employed for various testing compounds. (2) There is no need to acquire MS/MS spectra and other information of the parent drugs prior to analysis. (3) Unlike precursor ion and neutral loss scanning analysis, detection of drug metabolites is accomplished via post acquisition data mining so that unlimited EIC, PIF and NLF can be applied. (4) Both oxidative metabolites and GSH-trapped reactive metabolites can be analyzed in the same injection. (5) Unlike ion trap, Qtrap generates MS/MS spectra with rich fragments and no mass cut-off. These unique features enable fast and simultaneous screening of metabolic soft spot and reactive intermediates in support of lead optimization. In summary, together with its quantitation capability, Qtrap 5500 is a powerful LC/MS platform well suited for a small DMPK discovery organization or academic research group, where both bioanalysis and metabolite identification are performed with one LC/MS instrument.

POSTER ABSTRACT
#118

Formulation and Evaluation of Poorly Water Soluble Drug

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Solubilization of poorly soluble drugs is a frequently encountered challenge in screening studies of new chemical entities as well as in formulation design and development. Many existing drug molecules are poorly aqueous soluble or lipophilic which limit their potential use. A number of methodologies can be adapted to improve solubilization of poor water soluble drug and further to improve its bioavailability. Orally administered drugs completely absorb only when they show fair solubility in gastric medium and such drugs shows good bioavailability. Bioavailability depends on several factors, drug solubility in an aqueous environment and drug permeability through lipophillic membranes being the important ones. Only solubilized drug molecules can be absorbed by the cellular membranes to subsequently reach the site of drug action (vascular system for instance). Any drug to be absorbed must be present in the form of an aqueous solution at the site of absorption. Poorly soluble compounds belongs to class II of BCS, also present many in vitro formulation obstacles, such as severely limited choices of delivery technologies and increasingly complex dissolution testing with limited or poor correlation to the in vivo absorption. Recently more than 40% NCEs (new chemical entities) developed in Pharmaceutical Industry are practically insoluble in water. These poorly water soluble drugs are allied with slow drug absorption leading to inadequate and variable bioavailability and gastrointestinal mucosal toxicity. The objective of the present study was to enhance the solubility and dissolution rate of poorly water soluble drug using a scalable process. The results from dissolution studies indicated that there was a significant increase in the rate of drug dissolution. The technique is scalable and viable approach for commercial manufacture of drug product with improved therapeutic outcome.

CPSA SHANGHAI

POSTER ABSTRACT
#119

Quantification of Intact Protein Therapeutics Using Bench-top Quadrupole Orbitrap Based LCMS Approach

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Introduction

Antibody-based assays are traditionally the primary tools used to quantify proteins. However, these have a limitation based on the significant time and effort required to generate reagents for each protein studied. LC MS-based approaches have been used to quantify protein therapeutics at the peptide level using specific peptides that are unique to a protein. Quantification of protein therapeutics at the intact level is of increasing interest in the biopharmaceutical industry because proteins may be present in multiple isoforms, therefore accurate identification and quantification of those variants can only be achieved at the intact protein level. Quantification of intact proteins would also eliminate the need for enzymatic digestion of a protein into peptides, which significantly reduces the sample preparation time and complexity of analysis.

Methods

Model molecules from small protein to large therapeutic protein, including insulin (MW 5808 Da), IgG1 light chain (23 KDa), and IgG1 (145 kDa), are used in this study. A Dionex U3000 HPLC system performs a gradient separation. Working concentration calibration curves were prepared across three orders of magnitude. For intact analysis, samples are injected onto a PLRP-S column and then analyzed by QE full MS scan at m/z 800-6000 with resolution ≥15,000. Protein Deconvolution is used for intact MS analysis and LCquan is used for protein quantification.

Results

Insulin, IgG1 light chain and IgG1 were selected as models to demonstrate the quantification of intact therapeutic proteins using Q Exactive MS technology without digestion. For high resolution-based quantification of large molecules and peptides, a generic approach is quanti-fication on parent ion m/z in full-scan mode, then to extract data with a narrow mass window. This methodology takes advantage of high resolution and accurate mass instruments like an Orbitrap FTMS. Our results show that insulin has multiple charged states with +5 and +6 as the most abundant species. The most abundant isotopic peaks of the +5 and +6 charge states were used for quantifi¬cation with a 10ppm window for extracted ion current (XIC). Over four orders of linear dynamic range, a precision of <10% and an accuracy between 85–115% for six injections were achieved. The LLOQ is 10ng/ml and the ULOQ is 1000 ng/ml. h IgG1 light chain and IgG1 have multiple charge states with a mass envelope. Charge states with the intensities over 10% of base peak were summed for quantification, and results show a dynamic range for over two orders with good precision and accuracy. Quantification is based on the sum of multiple abundant isotope peaks of a charge state and then the sum of each charge state. Important parameters, including XIC windows, summing of multiple charge states and isotopic peaks, and ion of interference, are optimized to achieve selectivity, specificity and sensitivity. With an increasing demand in bioanalysis of thera¬peutic protein in the pharmaceuti¬cal industry, the high resolution MS method developed in this study opens new opportunities for reliable quantification.

CPSA SHANGHAI

POSTER ABSTRACT
#120

Establishing translation from in vitro to in vivo, from rat to monkey - how mechanistic PK-PD modeling enabled efficient development of KATII irreversible inhibitors

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When developing irreversible enzyme inhibitors, in addition to drug potency, target turnover rate is another critical component governing the magnitude and duration of in vivo response. In discovering the kynurenine aminotransferase II (KATII) irreversible inhibitors as a potential treatment of schizophrenia, a mechanistic PK-PD model was developed and successfully applied to separate target turnover rates and drug PK-PD parameters based on time course dose response data of four compounds in two species. Separation of system-specific parameters and drug-specific parameters enabled establishment of 1) in vitro-to-in vivo correlation of both drug potency and target turnover measurements as well as 2) allometry-based inter-species scaling of target turnover rates. More importantly, 3) knowledge of slow target turnover enabled advancement of drug candidates with short half lives because the pharmacodynamic response is now more driven by target half life than by drug half life. Quantitative understanding of both drug and biological systems through mechanistic PK-PD modeling lead to efficient development of KATII irreversible inhibitors by lowering chemistry hurdles and providing high confidence prediction of clinical response.

CPSA SHANGHAI

POSTER ABSTRACT
#121

LC/MS analysis of the prodrug and metabolites of a typical antiviral nucleoside inhibitor in various animal studies in support of preclinical development

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Compound X, a prodrug, is being evaluated as a direct acting antiviral drug for the treatment of hepatitis C. It is hypothesized that the prodrug approach increases generation of an intermediate in vivo, which is further metabolized to the active nucleotide.

HPLC/MS methods were developed and qualified/validated to determine the concentration of prodrug and its major metabolites in various animal species and matrices to support different stages of preclinical drug development up to GLP toxicity studies. Three different HPLC separation principles were applied, due to differences in the physicochemical properties of these analytes. Ion-exchange chromatography was used for the nucleotides, while ion-paring chromatography and reverse-phase chromatography were utilized for the nucleoside and the prodrug, respectively. In addition, since the active nucleotide was extremely unstable in the liver, an integrated complex process was developed from liver biopsy, sample preparation, and prompt analysis in order to provide useful information for the selection of the non-rodent toxicity species. Attempt was also made to stabilize the prodrug in the rat plasma before analysis.

In conclusion, the bioanalytical method development and sample analysis played an important role in understanding the deposition of the drug candidate, and provided key information that successfully supported the preclinical development program.

CPSA SHANGHAI

POSTER ABSTRACT
#122

An automatic reporting system for cassette dosing pharmacokinetic studies for high-throughput screening of new chemical entities using LC-MS/MS

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Cassette dosing pharmacokinetic study has been used as a high-throughput and animal saving strategy for the pharmacokinetic evaluation of new chemical entities (NCEs) in the lead optimization phase of drug discovery. The results from this kind of studies might provide information for further structural modification of NCEs, or help with the decision for further activity tests. This paper describes an automatic reporting system for cassette dosing pharmacokinetic studies using LC-MS/MS and the wok flow carried out using this system. The experimental data management was performed using Watson LIMS® software system (version 7.4). The document management system provide by Watson LIMS® software was used to integrate study information (study design, analytes, assay, statistics, time-concentration graphs, pharmacokinetic parameters, etc.) and a soft ware created by our laboratory was employed to transpose the information to the final report in the form of Microsoft Excel® file. This automatic reporting system greatly improved the efficiency of the pharmacokinetic screening for NCEs in our lab.

POSTER ABSTRACT
#123

Characterization of Human Hepatocytes Isolated from the Chinese Population

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In this study, freshly isolated Chinese human hepatocytes were tested in the following assays: (i) Yield and % viability (ii) formation of bile canaliculi network upon culturing in the 2-layer configuration, (iii) Uptake study with plated hepatocytes, (iv) enzymatic activity of suspension and plated hepatocytes, (v) induction activities.

Purpose: To characterize the metabolic capabilities, CYP induction and transporter mediated efflux in human hepatocytes isolated from the Chinese population.

Method: 1) Human Hepatocytes. Human hepatocytes were isolated from liver fragments resected for medical purposes with proper informed consent. A two-step collagenase perfusion was carried out within 4 hours of tissue resection. Cell viability was determined by trypan blue exclusion. 2) Plated Hepatocyte Culture. The isolated hepatocytes were seeded on 24-well or 48-well BD BioCoat™ Collagen I plates at a density of 0.5 million cells/mL. After a 4-hour incubation, the medium was discarded and replaced with new medium with ice cold BD Matrigel™ and the culture media was renewed every 24 hrs. 3) Metabolic Stability Assay. Suspension or Day 2-plated hepatocytes were incubated with 0.2 μM of various substrates at 37°C in William's E Medium. Reaction was terminated with the addition of cold Acetonitrile at 15, 30, 60, 90 and 120 min. 4) CYP Induction Assay. On Day 2 to 4, the plated hepatocytes were treated with vehicle (0.1% DMSO) or various inducers at 10, 20 and 50 μM. Enzyme activities of CYP1A2, 2B6, 2C9 and 3A4 were determined on Day 5 by monitoring the generation of corresponding metabolites. 5) Hepatocyte Transport Study. The uptake activities of plated hepatocytes were examined daily on Day 2 to 5 with the addition of 0.2 μM substrates of OATP and OCT in HBSS buffer at 37°C or 4°C for 1 min.

On Day 5, plated hepatocytes were pretreated for 10 min at 37°C with HBSS or HBSS containing cyclosporin A/ EGTA, followed by assaying the uptake of 1 μM CDCFDA and 1 μM taurocholic acid-d5.

LC-MS/MS Analysis. The LC-MS/MS analysis of substrates and metabolites was conducted with an API4000 connected with a SCL-10A LC system (Shimadzu) and a PAL autosampler (LEAP).

Result: Our results demonstrate that hepatocytes isolated from liver fragments maintain the functionality of major SLC uptake (OATP, OCT) and efflux (MRP2 and BSEP) transporters in 2-layer culture. In addition, the cells are suitable for drug induction as well as metabolic stability studies.

Conclusion: These cells provide a useful tool to characterize the drug-drug interaction or drug induced hepatic toxicity during drug discovery.

POSTER ABSTRACT
#124

Application of Spectral Deconvolution Technique to Improve the Accuracy of Quantitation 1HNMR to Study Reaction Mixture Directly

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The quantitative NMR (qNMR) is accepted as one of the "gold standards" in pharmaceutical, chemistry and biological research with the advantages of high accuracy (better than 1%), absolute quantification (no need of the pure standard of target), easy and quick sample preparation. However, the accuracy and precision of qNMR are limited by the purity of the peak which restricts its application for complex matrix like organic reaction mixture. The crowded spectrum from reaction mixture makes it difficult to select the proper peak for quantitative purpose.

Spectral deconvolution is an analysis algorithm to resolve and interpretation overlapping NMR peaks. In this study, a deconvolution strategy for reaction mixture analysis from 1HNMR spectra was developed and assessed by studying the artifact homologous mixture. It is shown that the peak shaped with Lorentzian and Gaussian equation and line fitting efficiency (evaluated by residual error) are critical for deconvolution processing. The results indicate that our approach significantly improves the purity error of ethyl phenylacetate from 2.44% to 0.27%, as well as relative derivation of ethyl phenylacetate in p-methyl.

POSTER ABSTRACT
#125

A fast and simple LC/MS/MS method for doxorubicin quantification in rat plasma to study the pharmacokinetics of pegylated liposomal doxorubicin formulations

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Doxorubicin is commonly used in the treatment of a wide range of cancers; however, its use is limited by toxicity, Novel drug delivery systems using pegylated liposomal doxorubicin formulations (SPD-05 and SPD-10) have been developed to maintain the clinical utility of conventional doxorubicin while minimizing toxicity. To examine whether a conventional bioequivalence approach is sufficient between different formulation variants of the marketed Lipo-DOX, SPD-05 and SPD-10 formulations, a simple, rapid and accurate LC/MS/MS method for quantification of doxorubicin derived from liposome-encapsulated doxorubicin formulation in rat plasma was developed and validated. The mobile phase consisting of a mixture of water-acetonitrile [containing 0.1% formic acid anhydrous], 75:25, was delivered at a flow rate of 0.8 mL/min. Sample preparation for liposome-encapsulated doxorubicin in rat plasma were achieved directly by protein precipitation with acetonitrile with 70% (w/v) zinc sulfate. The linearity was obtained over the range of 1.0–200.0 µg/mL for doxorubicin and the lower limit of quantitation was 1.0 ng/mL, accuracy and precision at the LOQ was 95.5% and 13.4%, respectively. For each level of quality control samples, the inter-day precision and accuracy ranged from 1.6 to 6.5% and 98.3 to 108.6%, The mean recovery doxorubicin samples was 97.5% in rat plasma. This method was successfully applied to the pharmacokinetics (PK) and biodistribution (BD) of liposomal doxorubicin formulations after i.v. administration to SD rats.

POSTER ABSTRACT
#126

Sensitive quantitation of non-radiolabeled substrates of key hepatic uptake transporters in cells by low flow LC/MS/MS: Method development and evolution

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In vitro transporter inhibition and phenotyping studies have become integral part of DMPK efforts in drug discovery and development. Radiolabeled substrates are commonly employed in the assessment of interaction of drug candidates with transporter to ensure suitable assay sensitivity. Recently, lead compounds that target liver tissues are often required for hepatic uptake transporter phenotyping for optimization and clinical candidate selection. However, due to the lack of radiolabels in drug discovery, the experiments are hardly carried out. The aim of this study was to develop and validate a robust LC/MS/MS methodology for sensitive quantitation of non-radio-labeled substrates of key human hepatic uptake transporter, OATP1B1, OATP1B3 and OATP2B1 in cells used in the transporter phenotpying studies.

Transporter-expressing HEK-293 cells were incubated with atovastatin, [3H]atovastatin, pravastatin, rosuvastatin and OATP inhibitors (BSP and Rifampin). After 3~5 min incubation, the solution was removed. Then cells were washed with ice-cold HBSS and lysed with 0.1% Triton X-100 or a mixture of CH3CN.H2O. Protein concentrations of cellular lysates were determined. For radiolabeled compounds, concentrations were measured based on radioactivity accounting by a liquid scintillation counter. For non-radiolabeled tested chemicals cellular lysates either directly passed through dried filter plate into a 96-well receiver plate by centrifugation or concentrated via N2 gas flow. Analyte concentrations in processed extracts from 1 and 0.1 μM incubations were determined using conventional LC-MS/MS and mirco or nano LC/MS/MS, respectively.

Conventional HPLC column (Atalantic, 5 uM C18 2.1x150 mm) using linear gradient (mobile phase A: 0.1% formic acid in H2O; mobile phase B, 0.1% formic acid in acetonitrile, 0.2 mL/min) was adopted for elution and separation. API 4000 Q TRAP mass spectrometer in MRM positive mode was used for quntitation. Results showed that the uptake ratios of atovastatin in mock cells and transporter-expressing HEK-293 cells with or without BSP were comparable between radioactivity and LC/MS/MS analyses. The IC50 of pravastatin and rosuvastatin in transporter-expressing OATP1B1 cells with rifampin were similar to data reported in literature. However, for the in vitro experiments at 0.1 μM, conventional HPLC was not sensitive enough to detect testing compound. Preliminary data using micro and nano LC/MS/MS showed that analytical sensitivity can be increased to 10- to 100-folds. Based on the results, a tiered approach will be applied to phenotyping of uptake transporters. In lead optimization, assays with 1 uM of incubation followed by conversational LC/MS analysis will be performed for fast screening for a large number of testing compounds. In late discovery and development, more definitive assays with lower incubation concentrations followed by micro or nano LC/MS analysis will be carried out to determine Km values of hepatic uptake transporters.

POSTER ABSTRACT
#127

Drug-drug interactions with traditional Chinese medicines: Predict in vivo interaction using in silico tools

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Background

Due to the wide usage of herbal products, it is conceivable to foresee significant safety and/or efficacy issues arising from the drug-drug interactions (DDI) between medicines and herbal products. This has understandably also aroused the concerns of the regulatory agencies. The cytochrome P450 (CYP) superfamily accounts for much of metabolism endogenous and xenobiotic substrates (including many drugs) and is a major source of pharmacokinetic DDI. Some literature has shown that herbs may affect the activity of CYP enzymes. However, there is still a big knowledge gap regarding herb-drug DDI due in part to the lack of fully characterization of the ingredient(s) and their validated methods and therefore inconsistency of the results. In order to support drug development strategy and provide critical direction to herb-DDI studies, Novartis DMPK has initiated a systematic investigation of traditional Chinese medicine-drug interactions (TCM-DDI), aiming to explore the impact of the TCM widely used in China on the activity of major CYP enzymes and transporters.

Methods and Results

The 10 most popular TCM injectable products in China were selected and screened in vitro to evaluate their effects on the major CYP enzyme activities. Several TCM injectable products showed inhibition potential of one or more CYP enzymes. The likelihood of an in vivo interaction was projected based on the ratio of estimated maximal plasma concentration/IC50 (the maximal plasma concentration after iv administration was estimated based on the volume of TCM administrated in ~2750 mL of plasma volume typical of an adult). Nuo Xin Kang (NXK, Sulfotanshinone sodium injection) showed the highest potential for an in vivo interaction among all the TCM injectables according to this evaluation paradigm. To determine the constituent(s) of NXK that contribute to the inhibitory effect, three major components of NXK, sodium tanshinone IIA sulfonate (STS), an oxidation product of sodium tanshinone IIA sulfonate and sodium tanshinone sulfonate I were purified and characterized by LC/MS and NMR analyses. These were then individually reassessed for CYP inhibition potential. NXK showed the strongest inhibitory potency to CYP2C9. Steps were then taken to predict a DDI with S-warfarin, a CYP2C9 probe drug in human. The human PK profiles for each constituent of NXK were simulated by Simcyp using full PBPK model with human clearance and volume of distribution predicted using single-species scaling from rat (body weight based). The potential of DDI was assessed following a single/multiple doses of S-warfarin (10 mg) and multiple-dose of NXK (80 mg QD) using the Simcyp population-based model. The results showed that it is unlikely to have an in vivo potential interaction with S-warfarin via inhibition by NXK.

Conclusions:

The poster illustrates the interdisciplinary approach. Integrating knowledge of the identity of each inhibitor/ingredient, data from in vitro inhibition studies and available in vivo preclinical (rat) PK allows the prediction of human PK profile and clinical DDI assessment using in silico tools.

POSTER ABSTRACT
#128

Addressing Robustness, Reproducibility, and Throughput in Nanospray LC-MS for Peptide Analysis

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Nanospray is an essential tool in high-sensitivity mass spectrometry, but limited robustness, reproducibility, and ease-of-use have historically challenged the adoption of nanospray in quantitative applications. Recent trends toward MS-based biomarker quantitation have placed strict requirements on the analytical performance of nanobore LC-MS. Nanospray MS and nanobore LC-MS both rely heavily on nanospray source hardware for successful experiments. Nanospray source hardware has matured over the past ten years from simple homemade devices to sophisticated application specific instrumentation featuring stage automation, thermal control and high-resolution imaging. Many of these enhanced features provide robustness (automated tip rinsing, automated emitter change), throughput (multi-channel workflows), ease of use (multi-chip systems) or experimental flexibility. Here we present a novel nanospray source solution which delivers enhanced features of stage automation, multi-channel operation and thermal control. Ease of use has been realized through the incorporation of an integrated nanobore LC-MS consumable (The PicoChip™), on which over 400 replicate injections were collected with no loss of chromatographic performance. A four channel, three column version of the source (The PicoSlide™) enables an MS duty cycle time of 95%, compared to 40% for a single channel system. Throughput for a typical 50 minute LC MS run for peptide analysis (10 min for injection, 25 min of MS acquisition time, 15 minutes for column wash/equilibration) increases from 24 samples/day to 57 samples/day.

POSTER ABSTRACT
#129

Diabetic Genotype and Phenotype in Asian Populations

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Diabetes poses a major global health crisis. Asia is the major site of a rapidly emerging diabetic epidemic. Roughly 80% of people with diabetes are in developing countries and 60% of the world populations with diabetes are from Asia, of which India and China remain the two countries with the highest numbers of people with diabetes. 85-95% of all diabetes cases are of type 2.

Asians have a strong ethnic and genetic predisposition for diabetes and have lower thresholds for the environmental risk factors. Compared with Western populations, Asians develop diabetes at younger ages, at lower degrees of obesity, and at much higher rates given the same amount of weight gain. Asian diabetes also has been associated with greater abdominal and visceral obesity and less muscle mass, rapid nutrition transition, higher intake of trans fatty acids, high intake of foods with a high glycemic index or glycemic load, sedentary lifestyle due to rapid urbanization and inadequate beta cell response to increasing insulin resistance results.

Much progress has been made in our understanding of the genetics of diabetes. Most cases of type 2 diabetes are due to genetic variations that are more common but exert less effect. In the recent years, Genome-wide Association Studies (GWAS) found that genetic variants are associate d type 2 diabetes in different Asia groups. Ethnic differences in their frequencies lead to differences in population-attributable risk, showing the need for population–specific studies.

POSTER ABSTRACT
#130

The utility of simultaneous qualitative and quantitative analysis by HRMS to accelerate drug

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The timely assessment of metabolic stability and identifying metabolic soft spot is critical for accelerating compound structure optimization and enhancing the success rate of drug candidates entering into development. Recent advances in accurate high resolution mass spectrometry (HRMS) which have demonstrated significant improvements in resolution, systemic stability and data quality make it feasible for simultaneous qualitative and quantitative analysis. This work investigates the application of HRMS in in vitro ADME assay and in vivo PK study. The information of clearance data, metabolite profiling and structure elucidation from microsomal incubations can be obtained from a single sample set. The shared soft spot information from a series of compounds is particularly helpful to guild the structure modification. In vivo PK profile of verapamil after oral administration can also be determined using HRMS and the quantitation showed excellent reproducibility, linearity, dynamic range and accuracy. In the meantime, in vivo metabolite profile can be acquired.. The obtained quantitation results showed good accordance with the result analyzed by triple quadrupole MS. In addition, this work also explores the possibility of the integrated utilization of HRMS and triple quadrupole MS to further increase working efficacy while providing valuable guidance for structure modification.

POSTER ABSTRACT
#131

Determination of Acetazolamide in Human K2EDTA blood by DBS- LC-MS/MS

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Acetazolamide is an enzyme inhibitor that acts specifically on carbonic anhydrase, an enzyme that catalyzes the reversible reaction involving the hydration of carbon dioxide and the dehydration of carbonic acid. To support clinical studies, a DBS-LC/MS/MS bioanalytical method was developed for quantization of acetazolamide in human K2EDTA blood. The collection of whole blood samples on a paper card, known as dried blood spot (DBS), has many advantages over conventional blood sampling. DBS requires very small blood volume and can greatly simplify sample collection, storage, shipment and processing. This paper describes the method for Determination of Acetazolamide in human K2EDTA blood by DBS-LC/MS/MS. The method utilized a liquid-liquid extraction with MTBE procedure prior to LC/MS/MS analysis. Acetazolamide-d3 was used as the internal standard. The method has been qualified and used to support clinical sample analysis.

POSTER ABSTRACT
#132

DMPK Tof Screening for Small Molecule Identification and Characterization using UNIFI Scientific Information System

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Utilizing HRMS technologies for metabolic profiling is the de facto standard for qualitative approaches in the modern DMPK laboratory. As HRMS technologies have matured, the quantitative properties, in terms of sensitivity, linear dynamic range, and robustness are approaching levels that rival many of the tandem quadrupole platforms in routine use. This has opened the door for developing and evaluating quan-qual HRMS workflows and broadening the application of these platforms in discovery and development laboratories to complement and harmonize with existing bioanalysis workflows. The major limitations of this approach is clear, how do we (and why would we) manage the large amount of extra data versus a traditional MRM approach. Which assays/workflows would drive programs and derive scientific benefit from the extra HRMS information? Although many software tools have been developed to generate key metabolic information from compounds and equally towards generating bioanalytical results quickly and accurately, few informatics platforms have been developed to look at the data holistically. The UNIFI analytical system is built with an acquisition front end for instrument control, a unified processing system and application specific workflows all built upon an integrated data management system and database to facilitate sharing of data while maintaining security. Organizing large amounts of data electronically is the key to the system, as raw data is linked to processed (summarized) data and to final reports. Conversely, reports from different studies can be combined, searched against and will always maintain their link back to the original data. As an example of the organizational capability, because the data is organized within a database, a metabolite can be tracked across samples and projects, and the assigned metabolite structures are saved to the integrated scientific library allowing for easy access. The processing methods for application specific workflows (such as metabolite identification and screening) also natively support quantification and are not handled by a separate application manager. This allows for simultaneous visualization of both structural and metabolic pathway information alongside the quantitative information, thereby putting the information into context. Waters' UNIFI system provides future proof technology to help labs of any size in the organization, quantification and tagging of data points simplifies the process of handling large volumes of data. This platform has also been designed to handle both tandem quadrupole data and QTof data further harmonizing the data streams being utilized to drive DMPK labs. Typical data from both in vivo (PK data) and in vitro assays will be demonstrated showing the seamless integration between quantitative and qualitative data to support PDM program work.

Generating the additional rich information that HRMS affords without tools to effectively utilize this data and drive decisions with it is wasteful. Informatics tools will be the key to unlocking these workflows in the next generation of DMPK labs.

POSTER ABSTRACT
#133

Measurement of aPTT Clotting Time in Citrated Human Plasma Containing Low Molecular Weight Heparin

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Introduction
The bioanalytical method was developed and validated successfully by Frontage Laboratories (Shanghai) Co., Ltd, for the measurement of plasma recalcification time in presence of standardized aPTT (Activated Partial Thromboplastin Time) reagent (aPTT clotting time reagent) in citrated human plasma containing low molecular weight heparin (LMWH).

Equipment
A Coagulation Analyzer was used for detecting aPTT.

Standard Curve and Quality Control (QC)
Standards and QCs were prepared by using LMWH reference spiked in Normal control plasma according to the activity of Heparin anti-factor Xa.

- Standard Curve: Standard curve was generated by applying linear regression between Log10(Clotting Time (s)) and Concentration of LMWH (IU/mL) . Clotting times prolonged with the increase of LMWH concentrations. The dynamic range of standard curve was from 0.2 to 2.6 IU/mL of LWMH concentration in anti-FXa acivity, and the curve contained clotting time of Normal Control Plasma as 0 IU/mL point.
- QC: Three Quality Controls (LQC, MQC and HQC) are prepared in the concentration of 0.3 IU/mL, 1.3 IU/mL and 2 IU/mL by diluting LMWH reference in Normal Control Plasma.
- ensitivity: Lower detection limit of the assay was 0.2 IU/mL of LMWH present in the Normal Control Plasma.
- Accuracy: Accuracies of standards and QCs were within 72% – 123%, and the coefficient of determination (r2) of the curve greater than 0.990.
- Precision: Intra-day precision of QCs was ≤ 12% and inter-day precision was 7%.

Results
The assay was successfully used on sample analysis

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POSTER ABSTRACT
#134

Measurement of Low Molecular Weight Heparin Concentration in Human Citrated Plasma Using Heparin Anti-Xa/IIa Chromogenic Assays

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Method Principle: The two methods are two-stage chromogenic assays for measuring homogeneously low molecular weight heparin (LMWH) in human citrated plasma for their Anti-Xa/IIa activity. LMWH is a sulphated polysaccharide with high affinity to anti-thrombin. When combined with LMWH, anti-thrombin exhibits a fast acting and potent inhibitory activity for coagulant serine esterase: IXa, Xa and IIa. LMWH and heparin analogues inhibit more efficiently Factor Xa than Factor IIa.

The LMWH Anti-Xa/IIa assays, two-step chromogenic methods, are based on the inhibition of a constant amount of factor Xa/IIa by the tested LMWH in presence of exogenous antithrombin (stage 1) and hydrolysis of a Factor Xa/IIa specific chromogenic substrate by the factor Xa/IIa in excess (stage 2). Then pNA is released from the substrate. The amount of pNA released is related to the residual factor Xa/IIa activity. There is an inverse relationship between the concentration of LMWH and OD values measured at 405 nm.

Standard Curve and Quality Control (QC)

- Standards and QCs were prepared by using LMWH reference spiked in pooled normal human citrated plasma collected in order to avoid any platelet activation according to the anti-FXa/IIa activity of LMWH.
- Standard Curve: "Exponential fit" for Xa assay and "Semi-Log fit" for IIa assay were applied to generate standard curves by using SpectraMax M2 Software. Absorbances (A405) varied inversely with LMWH concentrations. Back-calculate the LMWH concentration for the tested specimen directly against the standard curve (concentration corresponding to the measured A405), by using the SpectraMax M2 software.
- In Xa assay, standard curve dynamic range was from 0.02 to 0.7 IU/mL before 1:10 dilution in buffer, and 0 IU/mL and 1 IU/mL were added in standard curve as Anchor 1 and Anchor 2 to stabilize standard curve at low and high concentrations.
- In IIa assay, standard curve dynamic range was from 0.01 to 0.15 IU/ml before 1:5 dilution.
- QC: In Xa assay, three Quality Controls (LQC, MQC and HQC) were prepared in the concentration of 0.05 IU/mL, 0.12 IU/mL and 0.55 IU/mL. And they were 0.025 IU/mL, 0.06 IU/mL and 0.12 IU/mL in IIa assay.
- Sensitivity: LLOQ in Xa assay was 0.02 IU/mL of LMWH present in human citrated plasma, and in IIa assay was 0.01 IU/mL, which was much better than 0.1 IU/mL, the sensitivity of original method.
- Accuracy: In Xa assay, values of accuracy for standards were within 70% – 125%, and the coefficients of determination (r2) of curves were > 0.990; in IIa assay, they are within 77% – 130% (accuracy), and the coefficients of determination (r2) of the curves r2 > 0.960, respectively.
- Precision: In Xa assay, intra-run precision of QCs was ≤ 7% and inter-run precision < 13%.
- In IIa assay, intra-run precision ≤ 5% and inter-run precision <11%.

Results

These two assays were successfully used on analysis of samples from clinical study in measurement of LMWH concentration.

*The first two authors contributed equally to the work.

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POSTER ABSTRACT
#135

A Complete Workflow Solution for Monoclonal Antibody Glycoform Characterization Combining a Novel Glycan Column Technology and Bench-Top Orbitrap LC-MS/MS

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Introduction

Because glycosylation is so critical to the efficacy of antibody therapeutics, the FDA requires that a consistent human-type glycosylation be maintained for recombinant monoclonal antibodies, irrespective of the system in which they are produced. The complex branching and the isomeric nature of glycans pose significant analytical challenges to the identification and characterization of these structures. Liquid chromatography (LC) coupled to mass spectrometry (MS) has emerged as one of the most powerful tools for the structural characterization of glycans. We characterized the N-linked glycans released from a monoclonal antibody by LC-MS/MS methods using a new column technology and a bench-top quadrupole-orbitrap mass spectrometer.

Methods

All the glycans were separated using a recently developed high-performance HPLC/UHPLC column, GlycanPac AXH-1, utilizing an Ultimate 3000 UHPLC system. The GlycanPac AXH-1 column is based on advanced mixed-mode chromatography technology. Unlike other columns, this separates glycans based on charge, size and polarity. Mass spectroscopic analysis was performed using a Q Exactive bench-top quadrupole Orbitrap instrument. A data-dependant high energy collision dissociation (HCD) method was performed in negative ion mode to analyze the glycans. SimGlycan software from PREMIER Biosoft was used for data analysis for glycan identification and structural elucidation.

Results

Previous intact mass measurement of a monoclonal antibody identified glycoforms derived from the combination of any two of the three N-glycans, G0F, G1F and G2F. However, the mass errors for some of the intact glycoforms of this antibody ranged from 20-60 ppm which is larger than the expected 10 ppm for such measurement using Orbitrap FTMS. Furthermore, the intact mass error for the deglycosylated form of this antibody was within 10 ppm, suggesting that some minor glycosylation forms of this molecule that were not detected at intact level had interfered with the observed intact mass of the major glycoforms. To further characterize this protein, released glycans from this protein were separated using the GlycanPac AXH-1 column. The separation and elution of glycans from GlycanPac AXH-1 column are based on charge with neutral glycans eluting first, followed by the acidic sialylated species. Glycans of each charge state are further separated based on their size and polarity. Characterization of glycans in each peak was performed by Full MS and data dependent MS/MS usingHCD. These information rich spectra contain fragment ions that were generated from both cross-ring and glycosidic bond fragmentations. Three different types of glycans were found from this monoclonal antibody, the majority of glycans identified were neutral, including G0F, G1F and G2F which were also the major glycoforms identified at intact protein level for this antibody. Also identified were less abundant, non-fucosylated forms of G0, G1 and G2 as well as minor amounts of mono-sialylated and di-sialylated species with and without fucosylation that were not identified at the intact protein level. These results explain that the mass error observed previously is due to the overlapping glycoforms that are close in molecular mass. Rapid and sensitive antibody glycan profiling can be achieved using GlycanPac AXH-1 column and HR/AM Orbitrap LC MS/MS.

POSTER ABSTRACT
#136

A flexible SPE/LC/MS/MS platform for the simultaneous quantitation of multiple amyloid peptides in cerebrospinal fluid

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Highly sensitive liquid chromatography /tandem mass spectrometry (LC/MS/MS)-based method had been developed for the quantitative analysis of amyloid beta peptides in cerebrospinal fluid(CSF) which are diagnostic biomarkers of Alzheimer's Disease. Due to low abundance of these peptides, LC/MS/MS based methods with lower limit of quantitation were required to support drug discovery and biomarker studies. Compared with small molecules, many of these peptides (amyloid beta 1-38, 1-40 and 1-42) 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 platform was established, which included a novel solid-phase extraction (SPE) sample preparation method, a ultra-performance chromatography separation and a tandem mass spectrometer with improved sensitivity detection. A LLOQ of 50pg/mL was achieved for all the investigated peptides with a linearity of 0.05-10ng/mL (r2>0.99).

POSTER ABSTRACT
#137

A system for reliable ultra-low flow nanospray: Breaking barriers to enable metabolite quantitation

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Drug metabolite analysis places high demands on analytical mass spectrometry to obtain the accurate measurement - qualitative and quantitative - of specific drug related molecules in a timely manner. Previous work at ultra-low flow rates (< 10 nL/min) using non-pumped, off-line nanospray, has demonstrated unique applications of nanospray methods for small molecule analysis that deal with the quantitation of xenobiotic metabolites in the absence of true analytical standards. Unfortunately much of the significant analytical benefit of low-flow nanospray (efficiency, ionization response, matrix effect reduction) is lost even at "modest" low flow rates of ≥ 25 nL/min. As a result, control of flow rate is a critical parameter for successful ionization. A robust, closed-loop control pump capable of directly generating and delivering flow rates in the range from 1-100 nl/min has been developed. This pump is used in conjunction with a unique nanospray emitter geometry designed to maximize productivity at flow rates below 5 nL/min. A custom software package in communication with a pressure cell and flow sensor enables the calibration and control of the pump and emitter at flow rates with an uncertainty that is typically ± 1 nL/min at an absolute flow rate of 4 nL/min. The system enables > 15 minutes of acquisition time of a single sample at the target flow rate. This presentation will focus on recent technological developments that enable the relative quantitation of drug metabolites by the low flow system. Future opportunities and present challenges in the analysis of structural isomers will also be addressed.

POSTER ABSTRACT
#138

A generic multiple reaction monitoring based approach for flavonoids profiling in plants using a hybrid triple quadrupole linear ion trap mass spectrometry

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The limited quality of data obtained from untargeted metabolomic workflow calls for a shift from the breadth-first to the depth-first screening strategy when a specific biosynthetic pathway was focused on. Here we introduced a generic multiple reaction monitoring (MRM) based approach for flavonoids profiling in plants using a hybrid triple quadrupole linear ion trap (Qtrap) mass spectrometry. The approach includes four steps: (1) Preliminary profiling of major aglycones by multiple ion monitoring triggered enhanced product ion scan (MIM-EPI). (2) Glycones profiling by precursor ion triggered EPI scan (PI-EPI) of major aglycones. (3) Comprehensive aglycones profiling by combining MIM-EPI and neutral loss triggered EPI scan (NL-EPI) of major glycone. Unique neutral loss of one ammoniated sugar unit was observed in flavonoid glycosides and proven to be very informative and confirmative. (4) In-depth flavonoids profiling by MRM-EPI with elaborated MRM transitions and ramped conditions. This approach was applied for profiling flavonoids in Astragali Radix (Huangqi), a famous herb widely used in China for medicinal and nutritional purposes. In total, more than 300 flavonoids were tentatively identified, among which less than 40 have been previously reported in this medicinal plant. This MRM-based approach provides versatility, sensitivity and high through-put that required for flavonoids profiling in plants and serves as a useful tool for plant metabomonics.

POSTER ABSTRACT
#139

Simultaneous Determination of Four Endogenous Phytosterol Biomarkers in Human Serum by LC/MS/MS

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Phytosterols have been proposed as endogenous biomarkers for many diseases. A simple and rapid method for accurate quantification of Cholestanol, Cholestenol, Campesterol and ß-Sitosterol in human serum was developed and qualified in our lab. After saponification with potassium hydroxide and liquid-liquid extraction from human serum, phytosterols and IS were derivatized with p-toluene sulfonyl isocynate into p-toluene sulfonyl carbamate. The derivatization procedure was optimized and reaction could be completed in 10 minutes at room temperature, which is much faster and more convenient than other derivatization procedures in literature. Due to all of the 4 phytosterols were endogenous biomarkers, working standards, LLOQ and LQC samples were prepared in surrogated matrix, while MQC and HQC samples were prepared in human serum by spiking known amount of sterols after the baseline level was established in the pooled serum. The method was qualified to be highly selective, precise and accurate, and has been applied to clinical research studies.

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