RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Validated assay for the evaluation of multiple glucuronidation activities in human liver microsomes via liquid chromatography-tandem mass spectrometry

Rong Shi, Yuanyuan Yang, Jie Zhong, Tianming Wang, Yueming Ma*

Department of Pharmacology, Shanghai University of Traditional Chinese Medicine, Shanghai, China

ABSTRACT:

A sensitive and high-throughput liquid chromatography-tandem mass spectrometry system was developed and validated for the simultaneous determination of major human hepatic UDP-glucuronyltransferase forms in human liver microsomes. The analytes were detected using a triple-quadrupole mass spectrometer equipped with an electrospray ionization source in the negative ion and selected reaction monitoring modes. The method provided satisfactory linear concentration range, accuracy, precision, and stability. The developed method was successfully applied to the enzyme kinetic study of estradiol 3-O-glucuronidation, 4-methylumbelliferone O-glucuronidation, propofol O-glucuronidation, and 3-azido-3-deoxythymidine glucuronidation in human liver microsomes.

Key words: probe substrate; glucuronidation activities; LC-MS/MS; human liver microsomes

INTRODUCTION

Phase two metabolism of the primary conjugation reaction with glucuronic acid is considered as a fundamental mechanism to detoxify and eliminate lipophilic waste chemicals from the body. UDP-glucuronyltransferase (UGT) enzymes are metabolic enzymes that catalyze glucuronic acid transfer from cofactor UDP-glucuronic acid to

* Correspondence to: Professor Yueming Ma (Tel: +86-21-51322386, Fax: +86-21-51322386, E-mail: mayueming_117@hotmail.com)

particular endogenous substances and exogenous drugs; thus, β-D-glucopyranosiduronic acids (glucuronides) were formed [1–3]. Predicting potential drug–drug interactions is important and is done by studying the effect of drugs on UGT activities.

Methods based on determining metabolic reactions by detecting metabolite formation of probe substrate via liquid chromatography (LC) [4] or LC-tandem mass spectrum (LC-MS/MS) have been used for the evaluation of UGT activities [5–8]. In these methods, the activity of one UGT subtype was measured once [4–6], whereas the other was measured at a long-run time, which lasted for 15 min [7]. Alternatively, a relative quantification was performed using the calibration curves of the probe parent through the activity of several UGT subtypes that was measured one at a time [8]. The limitations of these methods hindered their application for the quick evaluation of multiple UGT-subtype activities. Therefore, a selective and efficient method should be established to determine the activity of multiple UGT subtypes.

In human liver, two families of UGT (UGT1 and UGT2) are known. In the human UGT1 family, UGT1A1 is an important isoform for the glucuronidation of endogenous compounds (such as estradiol), whereas UGT1A9 is involved in the glucuronidation of many drugs, including bulky phenols, flavonoids, and anthraxquinones. UGT2B7 from the human UGT2 family is an important isoform involved in the glucuronidation of steroids and has been regarded as one of the most important UGT isoforms. It participates in the glucuronidation of various compounds, including different steroid hormones (androsterone, epitestosterone), carboxylic–nonsteroidal anti-inflammatory drugs, fatty acid, and anticarcinogens (all-trans retinoic acid) [9, 10]. In the present study, we investigated a battery of validated assays for the simultaneous assessment of multiple UGT activities expressed in human liver microsomes. We succeeded in its application to studies regarding the enzyme kinetics of estradiol 3-O-glucuronidation (for UGT1A1), propofol O -glucuronidation (for UGT1A9), 3-azido-3-deoxythymidine glucuronidation (for UGT2B7), and 4-methylumbelliferone O-glucuronidation (for UGT isoforms) in HLMs.

EXPERIMENT

Chemicals

Estradiol was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Propofol, 4-methylumbelliferone (4-MU), 3-azido-3-deoxythymidine (AZT), estradiol 3-glucuronide (3-EG), uridine diphosphate glucuronic acid (UDPGA), coumarin-3-carboxylic acid, D-glucaric acid 1,4-lactone monohydrate, and alamethicin were supplied by Sigma Chemical Co. (St. Louis, MO, USA). Propofol glucuronide (PG), 3-azido-3-deoxythymidine glucuronide (AZTG) and 4-methylumbelliferone glucuronide (4-MUG) was obtained from Toronto Research Chemicals (North York, Ontario, Canada). Chromatogram-grade acetonitrile was purchased from Merck (Darmstadt, Germany). HPLC water was purified using a MilliQ water system (Millipore, Bedford, MA, USA). Chromatogram-grade acetic acid was provided by Tedia Company Inc. (USA). Chromatogram-grade aceticacid and ammonium acetate were provided by Tedia Company Inc. (USA). Other chemical reagents were analytically pure. Pooled HLMs were purchased from the Research Institute for Liver Diseases (Shanghai, China).

Microsomal incubation

Incubation was done in linear form with respect to time and protein concentration. The incubation mixtures contained HLMs, $MgCl_2$ (10 mM), alamethicin (25 μg/mL), saccharic acid-1,4-lactone (5 mM), tris–HCl buffer (50 mM, pH 7.4), and individual substrate (estradiol, propofol, AZT, or 4-MU). The reaction was initiated by adding UDPGA. After a few minutes of incubation at 37 °C, the reaction was terminated by adding the same volume of cold methanol containing an internal standard (IS, coumarin-3-carboxylic acid, 100 ng/mL). Each individual incubation medium (in the same volume) was pooled and centrifuged at 17,000 g for 10 min. The supernatant was diluted with the same amount of pure water for LC-MS/MS analyses.

LC-MS/MS condition

An LC-MS/MS system consisted of a Shimadzu Prominence UFLCXR system

(Shimadzu, Japan) coupled to a Thermo Scientific TSQ Quantum Ultra triple-quadrupole mass spectrometer controlled by Xcalibur software (Version 1.0.2.65 SP2, Thermofisher Scientific, San Jose, CA, USA). LC separations were achieved using a Hypersil Gold C_{18} (100 mm × 2.1 mm, 5 μm) with a guard column (10 mm × 2.1 mm, 5 μm) under a binary gradient, which were (A) 0.1% acetic acid, and 0.5 mM ammonium acetate in water and (B) acetonitrile (0→1.0 min: 20% B→20% B; 1.0 min→5.0 min: 20% B→90% B; 5.1 min→7.0 min: 20% B→20% B) at a flow rate of 0.25 mL/min and at a temperature of 30 °C.

The mass spectrometer was operated using a heated electrospray ionization source in the negative ion mode with selective reaction monitoring (SRM). The spray voltage was 3500 V, and the vaporizer and capillary temperatures were both 300 °C. Sheath and auxiliary gas pressures were 40 and 5 arb, respectively. The collision gas was argon, and the collision pressure was set at 1.5 mTorr. The peak widths of Q1 and Q3 were 0.7 Da. The instrumental parameters of LC-MS/MS analysis of the four analytes and IS were shown in Table 1.

Table 1 Instrument method of LC–MS/MS analyses for the analytes

Compound	Precursor ion	Product ion	SRM Collision Energy	Retention time	Tube lens
3-EG	447.0	271.3	48	4.3	158
4-MUG	351.3	175.1	28	3.1	93
PG	353.2	177.2	26	4.9	93
AZTG	442.0	125.0	25	2.2	99
IS	189.1	117.3	20	3.7	46

3-EG: estradiol 3-glucuronide; 4-MUG: 4-methylumbelliferone glucuronide; PG: propofol glucuronide; AZTG: 3-azido-3-deoxythymidine glucuronide; IS: internal standard.

Method validation

Specificity

The specificity of the method was tested using six different lots of incubation matrix,

which were compared with the control incubation to ensure that analyte and IS did not been introduced any interference.

Calibration curve, precision, and accuracy

A known amount of individual analyte (3-EG, 4-MUG, PG, or AZTG) was added to the incubation system. After sample preparation, each individual sample of the same volume was pooled and centrifuged at 17,000 g for 10 min. The lowest concentration in the calibration curve was defined as the lower limit of quantification (LLOQ) with an accuracy of 80% to 120% and a precision of no more than 20%, which was determined at a signal-to-noise ratio of more than 10. The highest concentration in the calibration curve was defined as the upper limit of quantification (ULOQ) with an accuracy of 85% to 115%, and a precision of no more than 15% was set based on the highest possible detecting concentration of the sample and the quantitative linear range of the instrument. The correlation coefficient ($R \geq 0.99$) was necessary. Calibration curves ($y = a + bx$) were established using weighted linear least-square regression of peak area ratios (y) of the analyte to their IS versus the analyte in seven different concentrations (x) of the standard samples.

The quality control (QC) samples at three different concentration levels (0.02, 0.08, and 3.58 μM for 3-EG; 0.06, 0.24, and 10.75 μM for 4-MUG; 0.02, 0.08, and 3.58 μM for PG; 0.16, 0.64, and 28.67 μM for AZTG, $n = 5$ for each concentration) were used to determine the intra-day and inter-day precision and accuracy of the method in five replicates within the same day or over three consecutive days. Relative standard deviation (RSD) and relative error (RE) were set to calculate the accuracy and the precision of the method required within 15%.

Extraction recovery and matrix effect

Extraction recovery and matrix effect were determined at QC levels. The percentage recovery of each analyte was estimated as the ratio of analyte/IS peak area spiked before the extraction to analyte/IS spiked post-extraction. The matrix effect was investigated by comparing the peak response of the blank incubation system spiked with analytes (A) with that of pure standard solution containing equivalent amounts of

the compounds (B). The ratio (A/B×100)% was used to evaluate the matrix effect.

Dilution integrity experiment

Dilution integrity experiment was performed with five replicate samples prepared at nominal concentrations of ULOQ in an incubated system. These samples were diluted 16- and 64-fold by using a blank matrix. The diluted samples were analyzed, and the mean concentrations were compared with the nominal value after the dilution factor was applied.

Stability

Stability experiments were performed on five replicates to evaluate post-extracted stability at an autosampler condition (4 °C for 24 h) and long-term stability (-80 °C for 14 d) at QC levels. To meet the acceptance criteria, RE (%) should be within ± 15%.

Method application

The enzyme kinetics of estradiol 3-O-glucuronidation, 4-methylumbelliferone O-glucuronidation, propofol O-glucuronidation, and 3-azido-3-deoxythymidine glucuronidation were determined in HLMs. Incubations of the mixture containing each substrate (8 μM to 160 μM for estradiol, 10 μM to 640 μM for 4-MU, 1.5 μM to 600 μM for propofol, or 0.375 μM to 4 mM for AZT), HLMs (0.1 mg/mL for estradiol, 0.1 mg/mL for 4-MU, 0.5 mg/mL for propofol, or 0.1 mg/mL for AZT) were performed as previously described. After preincubation at 37 °C for 5 min, the reaction was initiated by the addition of UDPGA (7 mM for estradiol, 3 mM for 4-MU, 5 mM for propofol or AZT). The mixture was incubated at 37 °C for a few minutes (30 min for estradiol, 15 min for 4-MU, 20 min for propofol or AZT), and then the reaction was terminated and analyzed. The kinetic parameters of glucuronidation by HLMs were obtained by fitting the data to a Michaelis–Menten model or to substrate inhibition models using a GraphPad Prism software (version 5.00, GraphPad Software, San Diego, CA, USA) based on the Akaike information criterion.

Results

Validation of the method

Specificity

Typical SRM chromatograms of blank incubation matrix spiked with standards and IS, a blank incubation matrix, and a pooled incubation sample are shown in Fig. 1. No significant endogenous interferences were observed at retention times of the analytes and IS.

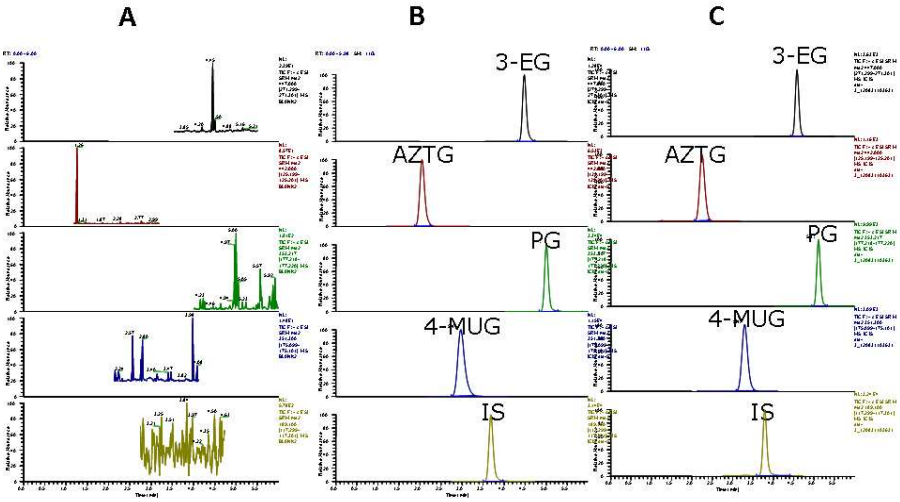


Fig. 1. LC-MS/MS chromatogram of estradiol-3-glucuronide (3-EG), 4-methylumbelliferone glucuronide (4-MUG), propofol glucuronide (PG), 3-azido-3-deoxythymidine glucuronide (AZTG), and internal standard (IS) in the liver microsomal sample: (A) a drug-free blank sample, (B) a blank sample spiked with standards, and (C) a practice sample after incubation of estradiol, 4-methylumbelliferone, propofol, and 3-azido-3-deoxythymidine

Calibration curve and LLOQ

The calibration curves, correlation coefficients, linear ranges, and LLOQ of 3-EG, 4-MUG, PG, and AZTG in HLMs are listed in Table 2.

Table 2 Calibration curve, linear range, and LLOQ for 3-EG, 4-MUG, PG, and AZTG

Compound	Calibration curve	*r*	Linear range(μM)	LLOQ(μM)

3-EG	y=-0.00142+0.378x	0.996	0.01–5.12	0.01
4-MUG	y=-0.00285+0.208x	0.997	0.08–40.96	0.08
PG	y=-0.00112+1.062x	0.996	0.01–5.12	0.01
AZTG	y=-0.00043+0.030x	0.996	0.03–15.36	0.03

3-EG: estradiol 3-glucuronide; 4-MUG: 4-methylumbelliferone glucuronide; PG: propofol glucuronide; AZTG: 3-azido-3-deoxythymidine glucuronide.

Precision and accuracy

Tables 3 and 4 present the results for intra- and inter-day precision and accuracy of the method for 3-EG, 4-MUG, PG, and AZTG. These results reveal that the precision and accuracy of the assay were within the acceptable range.

Table 3 Intra-day precision and accuracy for 3-EG, 4-MUG, PG, and AZTG from QC samples. (n = 5, mean ± sd)

Compounds	Norminal concentration (μM)	Measured concentration (μM)	Precision RSD (%)	Accuracy percent error (%)
3-EG	0.02	0.02 ± 0.002	11.2	1.70
	0.08	0.08 ± 0.005	6.67	-3.92
	3.58	3.63 ± 0.152	4.17	1.82
4-MUG	0.06	0.059 ± 0.005	7.77	-2.36
	0.24	0.24 ± 0.012	5.15	0.95
	10.75	10.69 ± 0.402	3.76	-0.59
PG	0.02	0.019 ± 0.002	10.0	-5.99
	0.08	0.082 ± 0.006	7.17	-3.53
	3.58	3.69 ± 0.088	2.38	7.02
AZTG	0.16	0.16 ± 0.014	9.07	-0.95
	0.64	0.62 ± 0.025	4.12	-11.5
	28.67	29.7 ± 1.126	3.79	7.14

3-EG: estradiol 3-glucuronide;4-MUG: 4-methylumbelliferone glucuronide; PG: propofol glucuronide; AZTG: 3-azido-3-deoxythymidine glucuronide; IS: internal standard

Table 4 Inter-day precision and accuracy for 3-EG, 4-MUG, PG, and AZTG from QC

samples. (n = 5×3, mean ± sd)

Compounds	Norminal concentration (μM)	Measured concentration (μM)	Precision RSD (%)	Accuracy percent error (%)
3-EG	0.02	0.019 ± 0.001	5.31	3.28
	0.08	0.085 ± 0.004	4.54	5.83
	3.58	3.49 ± 0.095	2.71	-2.62
4-MUG	0.06	0.058 ± 0.003	4.83	-3.19
	0.24	0.24 ± 0.016	6.89	-0.64
	10.75	10.55 ± 0.37	3.46	-0.64
PG	0.02	0.020 ± 0.002	11.7	10.1
	0.08	0.080 ± 0.003	3.50	-0.01
	3.58	3.49 ± 0.205	5.87	-2.73
AZTG	0.16	0.15 ± 0.015	9.56	-4.09
	0.64	0.64 ± 0.047	7.33	-0.71
	28.67	28.1 ± 0.91	3.24	-1.97

3-EG: estradiol 3-glucuronide; 4-MUG: 4-methylumbelliferone glucuronide; PG: propofol glucuronide; AZTG: 3-azido-3-deoxythymidine glucuronide.

Extraction recovery and matrix effect

The extraction recoveries and matrix effects determined for 3-EG, 4-MUG, PG, and AZTG are shown in Table 5. The average recoveries of the investigated targets ranged from 55.7% to 102.3%, and the RSD values were all 11.2% (n = 5). The matrix effect of all analytes was found to be within the range of 93.1% to 107%, and the RSD values were all below 9.6% (n = 5). These results showed that ion suppression or enhancement from HLMs matrix was negligible for the present condition.

Table 5 Recovery and matrix effect for 3-EG, 4-MUG, PG, and AZTG from QC samples. (n = 5, mean ± sd)

Compounds	Nominal concentration (μM)	Recovery (%) (mean ± sd)	Matrix effect (%) (mean ± sd)
3-EG	0.02	64.5 ± 6.23	93.6 ± 8.96

	0.08	81.4 ± 6.44	105 ± 8.21
	3.58	77.7 ± 0.77	94.4 ± 6.61
	0.06	102.3 ± 9.10	93.2 ± 5.82
4-MUG	0.24	97.6 ± 1.79	107 ± 4.46
	10.75	97.3 ± 2.35	95.4 ± 7.28
	0.02	55.7 ± 6.25	99.9 ± 6.89
PG	0.08	57.8 ± 1.89	98.1 ± 5.13
	3.58	59.9 ± 2.46	102 ± 7.06
	0.16	64.5 ± 6.23	94.7 ± 4.82
AZTG	0.64	81.4 ± 6.44	105 ± 7.71
	28.67	77.7 ± 0.77	95.3 ± 7.45

3-EG: estradiol 3-glucuronide; 4-MUG: 4-methylumbelliferone glucuronide; PG: propofol glucuronide; AZTG: 3-azido-3-deoxythymidine glucuronide.

Dilution integrity

Dilution integrity experiments were carried out in five replicates by 16- and 64-fold dilutions with blank incubation matrix, and the assay precision and accuracy were tested. The accuracy was within ± 7.65%, and the precision was less than 9.4%. The determined concentration of the sample after 16- or 64-fold dilution was still in the range of quantification.

Stability

Stability tests were carried out at various conditions that the samples may experience. Results demonstrated that 3-EG, 4-MUG, PG, and AZTG were stable at the autosampler condition for 24 h and at -80 °C for 14 d after post extract. Data are not shown.

Method application

The developed method was applied to study the enzyme kinetics of estradiol 3-O-glucuronidation, 4-methylumbelliferone O-glucuronidation, propofol O-glucuronidation, and 3-azido-3-deoxythymidine glucuronidation in HLMs. The concentration-velocity of 4-MU, propofol, and AZT adheres to Michaelis–Menten

kinetics. However, estradiol exhibited substrate-inhibition kinetics (Fig. 2). Apparent K_m for 4-MUG, PG, and AZTG were 96.37 ± 12.4, 92.0 ± 9.05, and 646 ± 55.5 μM, respectively. V_{max} for 4-MUG, PG, and AZTG were 348.4±15.1, 228.6 ± 6.3, and 1.18±0.03 nmol/min/mg protein, respectively. Apparent K_m, V_{max}, and Ki for estradiol were 43.74±18.36 μM, 2707±781.7 pmol/min/mg protein, and 40.19±16.0 μM, respectively.

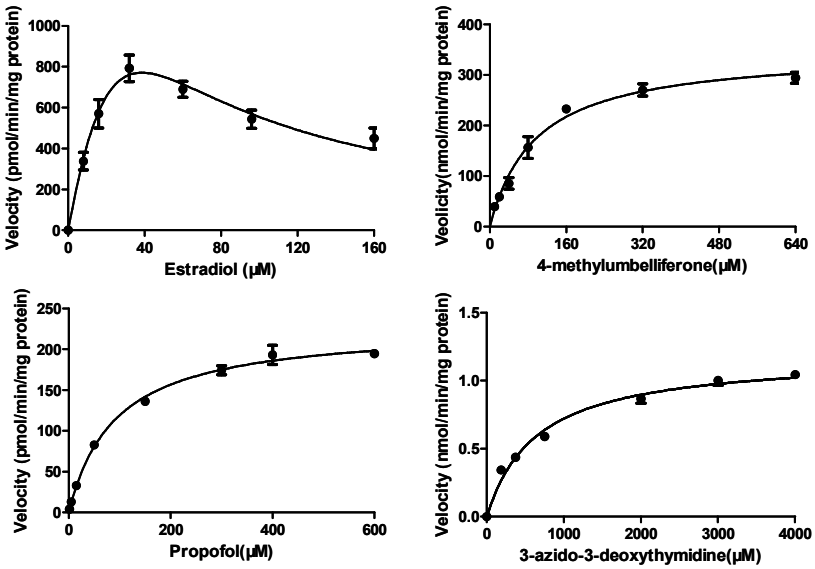


Fig. 2. Kinetics of estradiol 3-O-glucuronidation, 4-methylumbelliferone O-glucuronidation, propofol O-glucuronidation, 3-azido-3-deoxythymidine glucuronidation. (n = 3, mean ± sd).

DISCUSSION

Physiological and pathological factors and concomitant drugs can significantly contribute to the changes in UGT activities and can further influence the elimination of drugs in vivo. Therefore, a highly efficient method should be used to evaluate UGT activities in biological systems (e.g., hepatocytes, liver slices, or microsomes) to study changes in phase two metabolism more effectively.

The method for quantification of probe substrate metabolites to evaluate the activity of metabolic enzymes had been widely used in drug metabolism. Several methods, such as LC and LC-MS/MS, are currently used to determine UGT activities.

Moreover, several methods were reported for the determination of glucuronidation of estradiol, 4-MU, propofol, and AZT [4–6]. An assay for the quantification of glucuronides formed from four probe substrates was used to detect the activities of UGT, but the complete run lasted for 15 min [7]. Another reported method showed that the simultaneous determination of glucuronides formed from six probe substrates was used to detect activities of UGT. However, the quantitative accuracy of glucuronides was limited because of the relative quantification by the calibration curves of their respective parent probe [8]. These problems limit the application of these methods in a high-throughput study of the activities of multiple UGT isoforms.

Estradiol is considered an excellent marker of UGT1A1 activity in the liver [12], and propofol has been proposed as a suitable probe for human UGT1A9 [6, 13]. Moreover, AZT is primarily catalyzed by UGT2B7 [14]; meanwhile, 4-MU, a nonspecific probe substrate for UGT isoforms, is often used to investigate multiple UGT activities [7, 15]. Therefore, estradiol, propofol, AZT, and 4-MU were used to evaluate the multiple glucuronidation activities in HLMs for this experiment.

In this study, we established a method for the evaluation of UGT isoform activities wherein the substrates were separately incubated, and the mixed detection of glucuronidation products was done via LC-MS/MS analysis. During the development of this method, chromatographic conditions, particularly the composition of the mobile phase, were optimized to achieve good sensitivity and peak shape, as well as a relatively short run time. Acetonitrile provided better separation than methanol and thus selected as the organic phase. A good peak shape could be achieved by adding 0.5 mmol/L ammonium acetate into the mobile phase. The use of acetic acid could achieve higher response signals for AZTG than formic acid. Finally, a mobile phase consisting of acetonitrile, 0.5 mmol/L ammonium acetate, and 0.1% acetic acid (gradient elution) was used in this experiment. To optimize ESI conditions for 3-EG, 4-MUG, PG, AZTG, and IS, quadrupole full scan ESI mass spectra were obtained in the positive and negative modes by infusing approximately 200 ng/mL solution of the analytes in acetonitrile/water (50/50, v/v). ESI in positive and negative ion modes were tested, and the results showed that these compounds conferred predominant

single-charged deprotonated precursor $[M-H]^-$ in negative ions mode (ESI^-). Compared with other reported methods [7, 8], our method can simultaneously evaluate major UGT isoform activities within a short run time (7 min) and can be carried out in a high-throughput study of the activities of multiple UGT isoforms in HLMs. The procedure may also be useful for screening potential UGT inducers or inhibitors as well as for evaluating the UGT activities by using other models. The established method was applied to the kinetic investigation of estradiol 3-O-glucuronidation, 4-methylumbelliferone O-glucuronidation, propofol O-glucuronidation, and 3-azido-3-deoxythymidine glucuronidation. In the present study, the kinetic experimental results were in accordance with previous reports [6, 9].

CONCLUSIONS

In this research, we established a method for the simultaneous quantification of glucuronidation products of four substrates, which provided satisfactory linear concentration range, accuracy, precision, and stability. The current assay can be effectively used to rapidly assess the activities of multiple UGT subtypes in HLMs. These findings indicate the possibility of further increasing the analytical throughput.

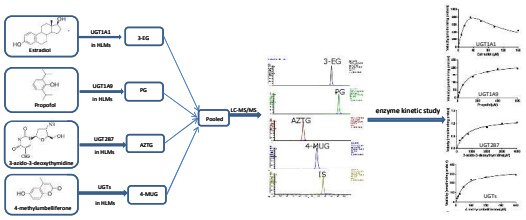
Acknowledgements

The authors are grateful for the financial support from Program for Shanghai Innovative Research Team in University (2009), “085” First-Class Discipline Construction of Science and Technology Innovation (085ZY1205) and Xinglin scholar (2013).

Reference

1) Miners JO, Mackenzie PI, Knights KM. The prediction of drug-glucuronidation parameters in humans: UDP-glucuronosyltransferase enzyme-selective substrate and inhibitor probes for reaction phenotyping and in vitro-in vivo extrapolation of drug clearance and drug-drug interaction potential. Drug Metab Rev. 42(20110) 196–208.
2) Di Marco A, D'Antoni M, Attaccalite S, Carotenuto P, Laufer R. Determination of drug glucuronidation and UDP-glucuronosyltransferase selectivity using a 96-well radiometric assay. Drug Metab Dispos. 33(2005) 812–819.
3) Kiang TK, Ensom MH, Chang TK. UDP-glucuronosyltransferases and clinical drug-drug interactions. Pharmacol Ther. 106(2005) 97–132.
4) Yu C, Ritter JK, Krieg RJ, Rege B, Karnes TH, Sarkar MA. Effect of chronic renal insufficiency on hepatic and renal udp-glucuronyltransferases in rats. Drug Metab Dispos. 34(2006): 621–627.
5) Mano Y, Usui T, Kamimura H. Comparison of inhibition potentials of drug against zidovudine glucuronidation Drug Metab Dispos. 35(2007) 602–606.
6) Liang SC, Ge GB, Liu HX, Shang HT, Wei H, Fang ZZ, Zhu LL, Mao YX, Yang L. Determination of propofol UDP-glucuronosyltransferase (UGT) activities in hepatic microsomes from different species by UFLC-ESI-MS. J Pharm Biomed Anal. 54(2011) 236–241.
7) Donato MT, Montero S, Castell JV, Gómez-Lechón MJ, Lahoz A. Validated assay for studying activity profiles of human liver UGTs after drug exposure: inhibition and induction studies. Anal Bioanal Chem. 396(2010) 2251–2263.
8) Gagez AL, Rouguieg-Malki K, Sauvage FL, Marquet P, Picard N. Simultaneous evaluation of six human glucuronidation activities in liver microsomes using liquid chromatography-tandem mass spectrometry. Anal Biochem. 427(2012) 52–59.
9) Liu HX, He YQ, Hu Y, Liu Y, Zhang JW, Li W, Wang ZT, Yang L. Determination of UDP-glucuronosyltransferase UGT2B7 activity in human liver microsomes by ultra-performance liquid chromatography with MS detection. J Chromatogr B Analyt Technol Biomed Life Sci. 870(2008) 84–90.
10) Mackenzie P, Little JM, Radominska-Pandya A. Glucosidation of hyodeoxycholic acid by UDP-glucuronosyltransferase 2B7. Biochem Pharmacol. 65(2003) 417–421.
11) Ji HY, Lee H, Lim SR, Kim JH, Lee HS. Effect of efavirenz on UDP-glucuronosyltransferase 1A1, 1A4, 1A6, and 1A9 activities in human liver microsomes. Molecules. 17(2012) 851–860.
12) Sato Y, Nagata M, Kawamura A, Miyashita A, Usui T. Protein quantification of UDP-glucuronosyltransferases 1A1 and 2B7 in human liver microsomes by LC-MS/MS and correlation with glucuronidation activities. Xenobiotica. 42(2012) 823–829.
13) Shimizu M, Matsumoto Y, Tatsuno M, Fukuoka M. Glucuronidation of propofol and its analogs by human and rat liver microsomes. Biol Pharm Bull. 26(2003) 216–219.
14) Williams JA, Hyland R, Jones BC, Smith DA, Hurst S, Goosen TC, Peterkin V, Koup JR, Ball SE. Drug-drug interactions for UDP-glucuronosyltransferase substrates: a pharmacokinetic explanation for typically observed low exposure (AUCi/AUC) ratios. Drug Metab Dispos. 32(2004) 1201–1208.

15) Zhao JW, Wang GH, Chen M, Cheng LH, Ji XQ. Demethylzeylasteral exhibits strong inhibition towards UDP-glucuronosyltransferase (UGT) 1A6 and 2B7. Molecules. 17(2012)9469–9475.



A faster and more accurate LC-MS/MS method was established for the activity determination of multiple UGT isoforms in HLMs.