Journal of Chromatography B, 870 (2008) 84–90

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

# Determination of UDP-glucuronosyltransferase UGT2B7 activity in human liver microsomes by ultra-performance liquid chromatography with MS detection

Hui-Xin Liu[a,c], Yu-Qi He[b], Ying Hu[a,c], Yong Liu[a], Jiang-Wei Zhang[a,c], Wei Li[a,c], Zheng-Tao Wang[b], Ling Yang[a,*]

[a] Laboratory of Pharmaceutical Resource Discovery, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 457, Zhong-shan Road, Dalian 116023, China
[b] Shanghai University of Traditional Chinese Medicine, Shanghai, China
[c] Graduate University of Chinese Academy of Sciences, Beijing, China

ARTICLE INFO

*Article history:*
Received 1 March 2008
Accepted 4 June 2008
Available online 11 June 2008

*Keywords:*
Ultra-performance liquid chromatography
UGT2B7
Human liver microsomes

ABSTRACT

A rapid and specific ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS) method was developed for the qualitative and quantitative determination of UGT2B7 activity using 3′-azido-3′-deoxythymidine (AZT) as probe substrate in human liver microsomes (HLMs). The method was validated for the determination of AZT glucuronidation (AZTG) with respect to specificity, linearity, detection limit, recovery, stability, precision and accuracy. The chromatographic separation was achieved on a UPLC BEH $C_{18}$ column (50 mm × 2.1 mm i.d., 1.7 μm), with phase of acetonitrile–water (ratio 6:94). Selective ion reaction (SIR) monitor was specific for AZT, AZTG and I.S. The method was linear over the concentration range 0.5–500 μM for AZTG in spiked HLMs. Good precision and accuracy were obtained for concentrations over the standard curve range. AZTG was stable at 4 °C for at least 72 h in spiked liver microsomes samples. The method was successfully used to determine the kinetics of UGT activities toward AZT in HLMs. In addition, the method could determine the effects of fluconazole, a known UGT2B7 selective inhibitor, on AZTG in HLMs. Therefore, this method is suitable for *in vitro* studies using AZTG formation as an index reaction for UGT2B7 activity.



## 1. Introduction

UDP-glucuronosyltransferases (UGTs, EC 2.4.1.17), a superfamily located in the endoplasmic reticulum of liver, play an important role in the glucuronidation, a major metabolic pathway that facilitates efficient detoxification and elimination, of potentially harmful xenobiotics (e.g. drugs, toxins, carcinogens) and endobiotics (e.g. bile acid, bilirubin, steroids) [1–3]. Among the more than a dozen UGT isoforms that have been identified in humans [4], UGT2B7, one member of UGT2 family, accounts for 40% of hepatic glucuronidated drugs based on data reported by Williams et al. [5]. In considering the clinical relevance of pharmacokinetic drug–drug interactions (DDI) mediated by drug-metabolizing enzymes, cytochrome P450 is usually thought to be most important. However, numerous examples primarily cleared by UGT2B7 exist of DDI have been described [6–8]. Therefore, the evaluation of UGT2B7 activity and kinetics is important to prediction of the metabolic fate and clearance of a drug in human and also the magnitude and likehood of any clinically important interactions in drug discovery and development.

For the interaction assessment, a common strategy is to monitor the effect of compounds on the metabolism of UGT probe substrates. Morphine and AZT are commonly used drugs that could potentially serve as UGT2B7 probes [9,10]. However, morphine has two sites (3-OH and 6-OH) that could be catalyzed by UGT2B7 [11]. Furthermore, AZT has been shown to be most selective for UGT2B7 [12]. In addition, AZT is an ideal substrate to work with because it is cleared almost completely via hepatic metabolism and not appreciably bound in plasma or liver tissue [13], suggesting that AZTG formation could be used as an *in vitro* and *in vivo* probe for this isoform [12] (Fig. 1).

Several *in vitro* methods have been reported for the detection of UGT2B7 activities [9,14–17]. Most of them used a broadly substrate to monitor UGT isoforms activities including UGT2B7, which is unselective and relatively insensitive [9,14,15,17]. Some adopted complex elution systems and added an additional solid-phase extraction step to enrichment sample before analyzed, which increases potential of inaccuracy and is time-consuming [9,16]. Others employed the radioactive [$C^{14}$] UDPGA [9,17], which is expensive and difficult to handle. Boase and Miners [18] recently

* Corresponding author. Tel.: +86 411 84379317; fax: +86 411 84676961.
*E-mail address:* yling@dicp.ac.cn (L. Yang).

1570-0232/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2008.06.009

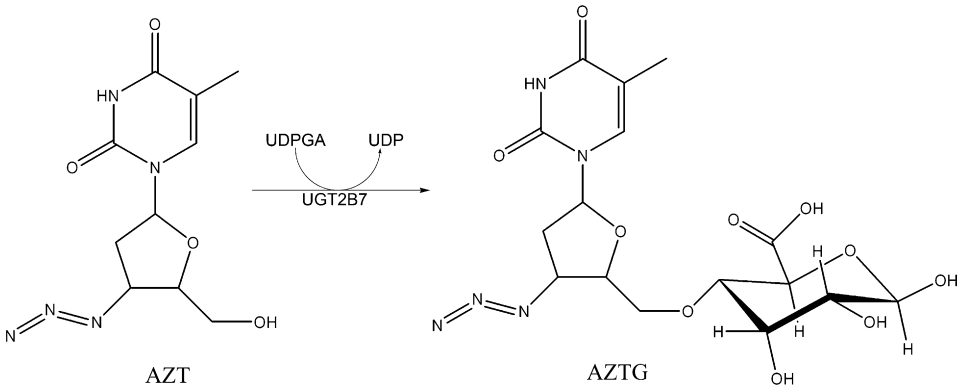


**Fig. 1.** Structures of AZT and AZTG.

reported a HPLC method, which is relatively higher in detection limit and not ideally suited for the determination of lower UGT2B7 activities from small amounts of biological samples. Therefore, there is a need for more powerful techniques that can be used for UGT2B7 activity screening research.

Recently, UPLC was introduced as commercially available instrument, which has been applied for the pharmaceutical, toxicological and biochemical analysis [19–23]. It has the advantages of the fast analysis, high peak capacity, good sensitivity and low consumption of samples [24]. Furthermore, MS techniques play an important role in the metabolism study, because the high sensitivity of MS as an LC detector facilitates the detection of metabolites which are difficult to obtain by conventional means.

The aim of this study is to develop a rapid and sensitive UPLC–MS method for the determination of UGT2B7 activity toward AZT in HLMs. This method provides a fast and sensitive qualitative and quantitative detection of AZTG by validation with respect to specificity, linearity, detection limit, recovery, stability, precision and accuracy.

## 2. Experimental

### 2.1. Reagent and chemicals

AZT, alamethicin, uridine-5′-diphosphoglucuronic acid (UDPGA), magnesium chloride, and D-saccharic acid 1,4-lactone were purchased from Sigma (St. Louis, MO, USA). AZTG was obtained from Toronto Research Chemicals (North York, Ontario, Canada). Human livers were obtained from autopsy samples ($n = 8$, male Chinese, ages from 27 to 48) from Dalian Medical University, with the approval of the ethics committee of Dalian Medical University. The medication history of the donors was not known. Research involving human subjects was done under full compliance with government policies and the Helsinki Declaration. Liver specimens were stored in liquid nitrogen until preparation of microsomes. Microsomes were prepared from liver tissue by differential ultracentrifugation as described previously [25]. The microsomal protein content was determined according to Lowry et al. [26] using bovine serum albumin as standard. The microsomal samples were stored at −80 °C until use. All other materials were of the highest quality commercially available.

### 2.2. UPLC–MS conditions

All analyses were performed on Waters Acquity UPLC system (Waters, MA, USA), including binary solvent manager, autosampler manager, column compartment, single quadrupole mass spectrometry, connected with Waters MassLynx V4.1 software. An Acquity UPLC BEH $C_{18}$ column (50 mm × 2.1 mm i.d., 1.7 μm) was used. The column temperature was maintained at 45 °C. The isocratic mobile phase composition was a mixture of water–acetonitrile (96:4, v/v) The flow rate was set at 0.3 mL/min and the injection volume was 10 μL. Positive ion of $[M+Na]^+$ of AZTG which was 466 and $[M+H]^+$ of I.S. which was 152 were used for determination with the SIR mode. The full-scan mass spectra are shown in Fig. 2. In order to optimize the MS parameters, a standard solution (40 μM) of AZTG was infused into the mass spectrometer. For AZTG, the following optimized parameters were obtained: capilary voltage: 3.2 kV; cone voltage: 40 V; extractor voltage: 1 V; RF lens: 0.5 V; source temperature: 100 °C; desolvation temperature: 350 °C; desolvation gas flow: 550 (L/h); cone gas flow: 50 (L/h).

### 2.3. Standards

Stock solutions at a concentration of 50 mM in 50 mM Tris–HCl buffer (pH 7.4) were prepared for AZTG. Different volume of stock solution was used to mix with 10 μL HLMs, 10 μL alamethicin, 20 μL UDPGA and 20 μL $MgCl_2$, and different volume of Tris–HCl buffer was added to make the system volume be 200 μL, and made the concentration of AZTG be 0.5–500 μM in 200 μL system, then 100 μL stop solution containing internal standard was added. After centrifugation, the solution were used to prepare the calibration curve and quality control (QC) samples. These solutions were stored at −20 °C and were stable for at least 4 weeks. Working solutions were freshly prepared each day and were obtained by dilution from the stock solutions as stated above.

### 2.4. Method validation

The specificity of the method was assessed to evaluate the influence of the matrix and non-enzymatic reactions in blank samples (Fig. 3).

A calibration curve for AZTG was prepared at 11 concentrations (0.5, 1, 2, 5, 10, 20, 60, 80, 100, 300, and 500 μM). This was based on a preliminary kinetic experiment which gave an approximation of the concentration range to be used. The linear relationship was demonstrated by the statistical analysis of linear regression model of $y = ax + b$ (where $x$ represents concentration of AZTG and $y$ represents the peak area ratio of AZTG to I.S., 3-acetamidophenol). The lower limit of detection (LLOD) for AZTG was defined as the quantity of AZTG corresponding to three times the baseline noise (signal-to-noise ratio of 3). The lower limit of quantitation (LLOQ) of the assay, defined as the lowest concentration on the calibration curve. The calibration curve had to have a correlation coefficient ($r$) of 0.99 or better. The acceptance criterion for each back-calculated standard

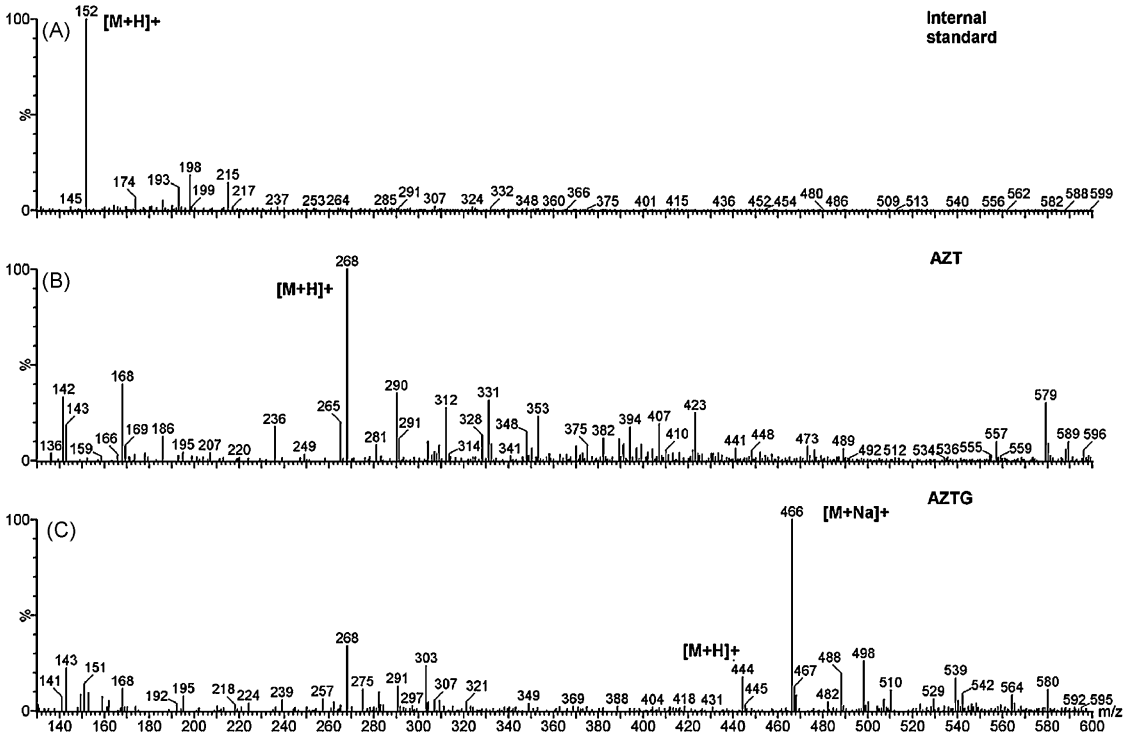


**Fig. 2.** Positive ion spectra of $[M+H]^+$ of I.S. (A), $[M+H]^+$ of AZT (B), and $[M+Na]^+$ of AZTG.

concentration was 15% deviation from the nominal value except at LLOQ, which was set at 20%.

The intra- and inter-day precision and accuracy of the method were assessed by analyses of AZTG in QC samples of low, middle, and high concentrations (0.5, 40, and 400 μM). Concentrations in QC samples were calculated from the resulting peak area ratios and the regression equation of the calibration curve. The intra-day precision and accuracy were determined by analyzing six times in sextuplicate on the same day. The inter-day precision and accuracy were determined by analyzing once in sextupli-

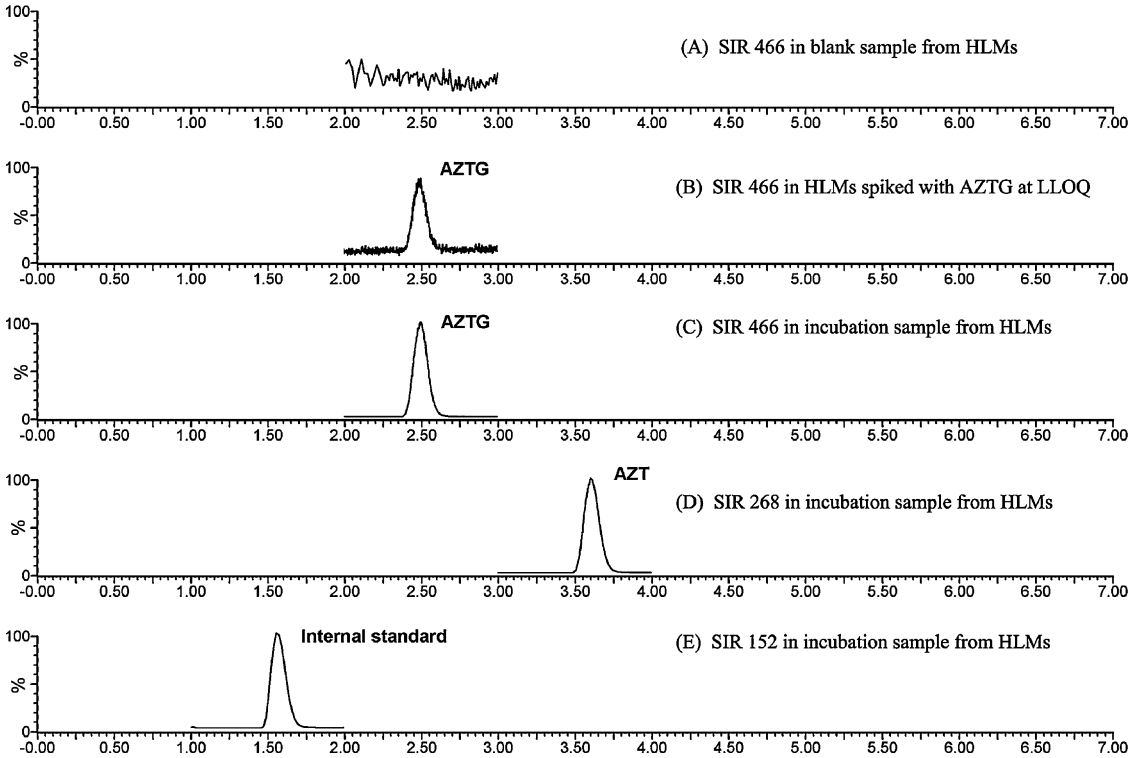


**Fig. 3.** UPLC analysis of UGT activity toward AZT in HLMs. Reactions were performed in the presence of AZT (500 μM) incubated with HLMs (20 μg) in a total volume of 200 μL for 30 min at 37 °C. The method of sample preparation and the UPLC conditions are described in Section 2.

**Table 1**
Precision and accuracy of AZTG calibration standards and quality control samples ($n = 6$)

| Nominal concentration (μM) | Mean calculated concentration (μM) | Accuracy (RME%) | Precision (RSD%) |
|---|---|---|---|
| Intra-day | | | |
| Calibration standards | | | |
| 0.50 | 0.48 | −3.61 | 2.59 |
| 1.00 | 0.98 | −1.62 | 3.21 |
| 2.00 | 1.94 | −2.83 | 3.72 |
| 5.00 | 5.15 | 3.13 | 5.43 |
| 10.00 | 9.59 | −4.08 | 1.91 |
| 20.00 | 19.32 | 3.37 | 1.79 |
| 60.00 | 63.41 | 5.66 | 2.21 |
| 80.00 | 84.14 | 5.21 | 0.88 |
| 100.00 | 100.85 | 0.82 | 2.57 |
| 300.00 | 301.36 | 0.49 | 0.63 |
| 500.00 | 511.43 | 2.33 | 2.61 |
| Quality control samples | | | |
| 0.50 | 0.48 | −4.41 | 2.34 |
| 40.00 | 41.10 | 2.82 | 1.32 |
| 400.00 | 407.11 | 1.81 | 2.04 |
| Inter-day | | | |
| Calibration standards | | | |
| 0.50 | 0.47 | −6.86 | 0.43 |
| 1.00 | 0.98 | −1.57 | 1.61 |
| 2.00 | 2.01 | 0.68 | 2.12 |
| 5.00 | 4.99 | −0.21 | 2.24 |
| 10.00 | 9.76 | −2.43 | 3.86 |
| 20.00 | 19.45 | −2.69 | 1.32 |
| 60.00 | 63.25 | 5.36 | 2.33 |
| 80.00 | 84.64 | 5.78 | 1.64 |
| 100.00 | 99.18 | −0.83 | 0.61 |
| 300.00 | 303.03 | 1.03 | 0.62 |
| 500.00 | 510.41 | 2.14 | 3.04 |
| Quality control samples | | | |
| 0.50 | 0.48 | −4.12 | 2.32 |
| 40.00 | 41.04 | 2.57 | 1.47 |
| 400.00 | 410.61 | 2.61 | 2.32 |

cate daily for 6 days. The precision was assessed by relative standard deviation (RSD), while the accuracy was calculated as relative mean error of calculated concentrations from nominal concentrations [RME = (calculated concentration − nominal concentration)/nominal concentration × 100%].

To check the stability of the biological matrix, HLMs were spiked with AZTG at three concentrations (0.5, 40, and 400 μM). The stability was assessed after storage in a refrigerator (4 °C) and at room temperature (25 °C) for various intervals (8, 24, and 72 h), by comparing peak areas from initial and subsequent determinations. The precision was assessed by mean ± SD%, while the accuracy was calculated as RME.

### 2.5. Incubations

UGT activities toward AZT were determined by quantification of the AZTG production from glucuronidation by liver microsomes. Incubations were performed in 1.5-mL glass test tubes. The standard incubation mixture contained AZT (25–2000 μM) as substrate, liver microsomal proteins from humans (50 μg), 8 mM $MgCl_2$, 25 μg/mL alamethicin, and 5 mM UDPGA in a final volume of 200 μL of 50 mM Tris–HCl buffer (pH 7.4). After preincubation at 37 °C for 3 min, the reaction was started by the addition of UDPGA. The mixture was incubated at 37 °C for 0–120 min and the reaction was terminated by the addition of 100 μL 10% trichloroacetic acid containing I.S., 3-acetamidophenol (final concentration 10 μM). Deproteined samples were centrifuged at 14,300 rpm for 10 min at 4 °C to obtain the supernatant. The supernatant was transferred to an auto injector vial and 10 μL was used for UPLC analysis within 8 h. Blank samples contained all components except the UDPGA which was added after termination of the reaction.

### 2.6. Kinetic analysis

Incubation conditions were chosen such that product formation was linear with respect to both microsomal protein amount and incubation time for the determination of UGT activities toward AZT in HLMs. The microsomal protein amounts and incubation time for the determination of UGT activities were 50 μg and 30 min, respectively. Substrate concentrations for the determination of UGT activities were 25 to 2000 μM. The Michaelis–Menten parameters, such as $K_m$ and $V_{max}$, were estimated by analyzing Eadie–Hofstee plots.

### 2.7. Inhibition of AZTG activity

A total volume of 200 μL incubation was performed in a 50 mM Tris–HCl buffer (pH 7.4), UDPGA, 500 μM AZT and a wide range of fluconazole (0, 100, 500, 750, 1000, 1250, 1500, and 2000 μM). There was a 3 min preincubation step at 37 °C before the reaction was started by the addition of AZT. Fluconazole were previously dissolved in acetonitrile, and the final concentration of acetonitrile was <1% (v/v). After 10 min incubation, the reactions were quenched by adding 100 μL 10% trichloroacetic acid containing I.S., 3-acetamidophenol (final concentration 10 μM). The incubation mixtures were then centrifuged at 14300 rpm for 10 min at 4 °C. Finally, 10 μL supernatant was used for UPLC analysis.

## 3. Results

### 3.1. Method validation

Under the UPLC–MS conditions described above, peaks were adequately separated. AZTG were detected with retention times of 2.5 min. The specificity of the method was assessed to evaluate the influence of the matrix from liver microsomes and non-enzymatic reactions. The interfering peaks for the determination of AZTG were not detected in blank samples from HLMs (Fig. 3).

**Table 2**
Stability data of AZTG in spiked HLMs under various conditions ($n = 3$)

| Storage conditions | | 0.5 μM | | 40 μM | | 400 μM | |
|---|---|---|---|---|---|---|---|
| | | (Mean ± SD%) | RME (%) | (Mean ± SD%) | RME (%) | (Mean ± SD%) | RME (%) |
| 4 °C | 8 h | 96.97 ± 1.89 | −3.02 | 101.71 ± 1.49 | 1.71 | 100.10 ± 1.04 | 0.10 |
| | 24 h | 98.27 ± 2.39 | −1.73 | 101.83 ± 2.19 | 1.83 | 99.27 ± 2.06 | −0.73 |
| | 72 h | 97.94 ± 1.96 | −2.06 | 101.92 ± 2.05 | 1.92 | 99.92 ± 3.67 | 0.08 |
| 25 °C | 8 h | 97.51 ± 1.63 | −2.49 | 101.66 ± 2.15 | 1.66 | 98.27 ± 3.25 | −1.73 |
| | 24 h | 96.98 ± 1.18 | −3.02 | 102.01 ± 2.11 | 2.01 | 98.09 ± 3.44 | −1.91 |
| | 72 h | 95.13 ± 2.33 | −6.87 | 99.92 ± 2.72 | −0.08 | 96.67 ± 4.14 | −3.33 |

**Table 3**
Intra- and inter-day duplicability of the assay for UGT2B7 activities toward AZT in HLMs[a] ($n = 6$)

| Substrate concentration (μM) | Activity (mean ± SD%) (pmol/min/mg protein) | RSD (%) |
|---|---|---|
| Intra-day | | |
| 50.00 | 94.24 ± 4.09 | 4.34 |
| 200.00 | 313.6 ± 12.58 | 4.01 |
| 500.00 | 658.7 ± 11.02 | 1.67 |
| Inter-day | | |
| 50.00 | 92.57 ± 4.56 | 4.92 |
| 200.00 | 303.3 ± 13.58 | 4.48 |
| 500.00 | 652.3 ± 11.50 | 1.76 |

[a] Reactions were performed in the presence of AZT (50, 200, and 500 μM) and human liver microsomal protein (50 μg) in a total volume of 200 μL for 30 min at 37 °C. The method of sample preparation and the UPLC–MS conditions are described in Section 2.

Calibration curves ($y = ax + b$) were prepared from solution to which AZTG had been added in the range of 0.5–500 μM. A high correlation was found between the amount of AZTG and the peak area. The means of their calibration curves yielded the following equations ($n = 6$): $y = 0.002x + 0.0015$ ($r^2 = 0.9996$) for AZTG. The coefficients of variations of slope for AZTG were found to be <15%, which indicate a high precision of the present assay. Recoveries from HLMs spiked with AZTG at concentrations of 0.5, 40 and 400 μM were 98.5%, 99.9%, and 100.8%, respectively. The QC samples were prepared by spiking liver microsomes with AZTG at three different concentrations (0.5, 40 and 500 μM), and were analyzed with the calibration standards (0.5–500 μM). The results are shown in Table 1. LLOD was assessed using the 0.5 μM standard solutions, the detection limit for AZTG was 0.05 μM.

Intra- and inter-day precision was <5.4% for all concentrations of AZTG in HLMs. It was demonstrated that the UPLC–MS method for the determination of AZTG was reliable and reproducible since both precision and accuracy were below 10% for all estimated concentrations of AZTG (Table 1).

### 3.2. Stability and duplicability studies

AZTG was stable for 72 h at 4 °C and at 25 °C in HLMs. The percentage recoveries were similar at all concentrations (Table 2). The duplicability of the assay was determined by analysis of UGT activities toward AZT of microsomal proteins from human liver samples. Three concentrations (50, 200, and 500 μM) were chosen. The results are summarized in Table 3. Intra- and inter-day duplicabilities (expressed as RSD) were better than 4.9% at all substrate concentrations.

### 3.3. Optimization of reaction condition

For these studies, substrate concentrations of 500 μM for AZT was used. Fig. 4 shows time-dependent formation of AZTG by HLMs (20 μg of microsomal protein). The AZTG formation was linear for at least 60 min in HLMs ($r^2 = 0.997$). Similarly, in assays of UGT activities toward AZT in HLMs, the formation of AZTG was found to be linear up to 125 μg of microsomal protein ($r^2 = 0.999$) (Fig. 5).

### 3.4. Enzyme kinetics and inhibitory of AZTG

This enzyme kinetics study of AZTG showed that $K_m$ was 550.6 ± 32.7 μM and $V_{max}$ was 2.07 ± 0.06 nmol/min/mg of protein (mean ± SD%). The curves for the enzyme kinetics are shown in Fig. 6. This enzyme kinetics study of AZTG followed single enzyme

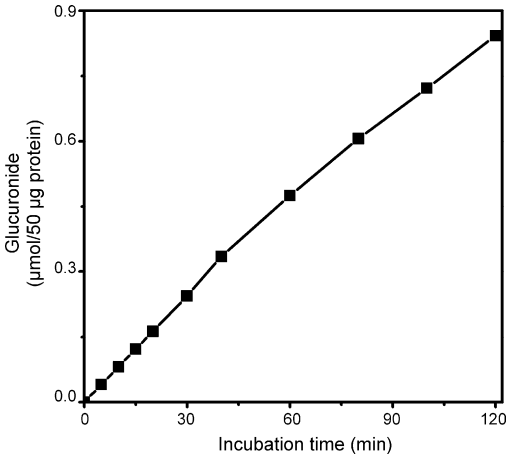


**Fig. 4.** Dependence on incubation time of AZTG activity in human liver microsomes. Reactions were performed in the presence of AZT (500 μM) and human liver microsomal proteins (20 μg) in a total volume of 200 μL for 0–120 min at 37 °C. Each point represents the mean of three separate experiments performed in duplicate.

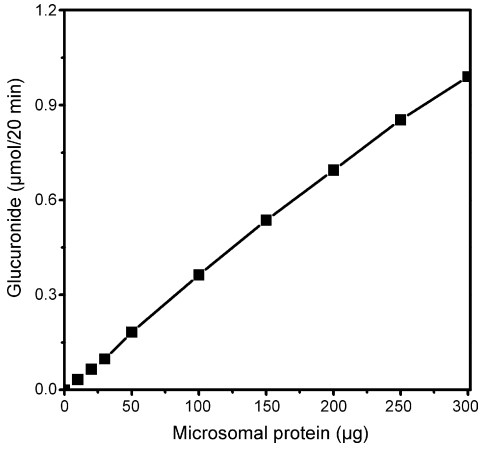


**Fig. 5.** Dependence on microsomes protein concentration of AZTG activity in human liver microsomes. Reactions were performed in the presence of AZT (500 μM) and human liver microsomal proteins (0–300 μg) in a total volume of 200 μL for 20 min at 37 °C. Each point represents the mean of three separate experiments performed in duplicate.

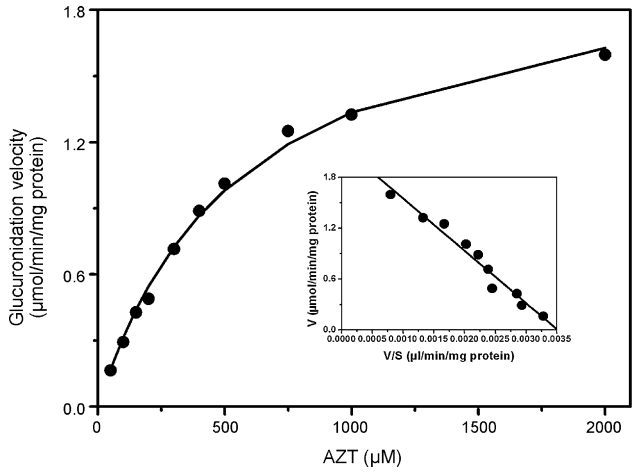


**Fig. 6.** Enzyme kinetics of AZTG in human liver microsomes. Reactions were performed in the presence of AZT (25–2000 μM) and human liver microsomal proteins (50 μg) in a total volume of 200 μL for 20 min at 37 °C. Each point represents the mean of three separate experiments performed in duplicate.

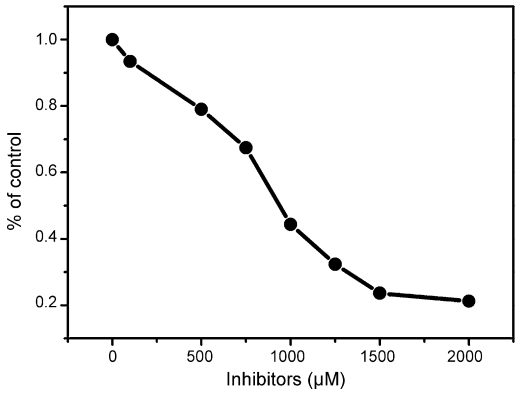


**Fig. 7.** Inhibitory effects of fluconazole on AZTG in human liver microsomes. Reaction were performed in the presence of AZT (500 μM), fluconazole (0–2000 μM), and human liver microsomal proteins (50 μg) in a total volume of 200 μL for 10 min at 37 °C. Each point represents the mean of three separate experiments performed in duplicate.

Michaelis–Menten kinetics as described previously [12,18]. The effects of fluconazole, a known UGT2B7 selective inhibitor [27], on AZTG was determined using this method. As shown in Fig. 7, AZTG was inhibited by fluconazole in a concentration-dependent manner. The $IC_{50}$ value was calculated to be 980 ± 50 μM (mean ± SD%).

## 4. Discussion

Glucuronidation is an important route of drug metabolism in humans, and many drugs are cleared by this pathway. UGT2B7 is involved in catalyzing 40% of hepatic glucuronidated drugs [5], and drug interactions involving UGT2B7 have been demonstrated both *in vitro* and *in vivo* [6–8]. Therefore, the valuation of effect on the activity of UGT2B7 is vital in drug discovery and the prediction of potential of DDI.

Due to the consideration of high throughout, the shorter time cost in the samples preparation, more selectivity and higher sensitivity is firstly needed. There are some methods for the determination of UGT2B7 activity adopting solid-phase extraction step or improper substrate, which is time-consuming and decreases accuracy [9,14–17]. With the great resolution and expeditiousness of UPLC, the problem could be resolved under appropriate conditions. To clean the incubation sample, a step of high-speed centrifugation was adopted, and the time of sample preparation needed was finished within 1 h. The column temperature was maintained at 45 °C because of two reasons. First, retention time would be different under different column temperature, and the column temperature higher than room temperature would be easy to control; second, higher column temperature was used so as to decrease the pressure at higher flow rate, which could improve the separation and peak shape. To elevate the reproducibility of separation and simplify the experiment, a simple elution system was selected, and the run time was less than 3 min. Then under similar sensitivity comparing with HPLC-based methods, the preparation and separation of per sample using this UPLC-based method was efficiently shorten, which favored the need of high throughout.

AZT was employed as the model substrate in this work since it is extensively glucuronidated and identified as a probe of UGT2B7 [12]. It is necessary to use an I.S. to obtain good accuracy and precision when a mass spectrometer is used as the LC detector. 3-Acetamidophenol was adopted as I.S. because of the similarity of its retention to AZTG and good ionization characteristics under the MS conditions. The ESI in positive ion mode was adopted for the MS determination of AZTG. The SIR mode was selected. The test results showed that the base peak in the mass spectra of AZTG was at $m/z$ 466, which was the ion $[M+Na]^+$ of AZTG. The intensity of the $[M+Na]^+$ was stable and reproducible. Therefore, it was selected as the target ion for AZTG. In the same condition, the base peak in the mass spectrum of the I.S. was at $m/z$ 152, which was the protonated molecule $[M+H]^+$ of the I.S. Therefore, the ion at $m/z$ 152 was selected as the target ion for the I.S. Under the optimized UPLC–MS conditions, no interfering peaks were detected for the AZTG or I.S. in blank samples from HLMs.

For enzyme assays in biological experiments, the mean values of intra- and inter-day precision and accuracy found should be <15% of the nominal values, except for the lower concentration, at which the deviation should be <20% in biological samples [28]. The described UPLC–MS method displayed excellent precision and accuracy over a linear range of 0.5–500 μM for AZTG. LLOD obtained by this UPLC–MS method was about 20 times lower than previous results obtained using the HPLC method [18] and allowed UGT activities toward AZT as low as 6.7 pmol/min per miligram protein to be determined.

Usually, the samples for *in vitro* kinetic study using liver microsomes should be analyzed as soon as possible or within short-term storage. Thus, we assessed samples at three concentrations after storage in a refrigerator (4 °C) and at room temperature (25 °C) for various intervals (8, 24, and 72 h), which could reflect situations likely to be encountered during actual sample handling and analysis. The results showed in Table 2 suggested that HLMs containing AZTG can be handled under normal laboratory conditions without significant loss of compound.

The activity range of UGT2B7 in human is very broad because of its extensive tissue distribution and polymorphism [12] and high sensitivity is needed to determine UGT2B7 activity from small amounts of biological samples. Therefore, the enzyme reaction conditions were optimized in incubation time and microsomal protein concentration. The UPLC–MS method developed was successfully used to the determination of lower UGT2B7 activities, such as kinetic and inhibition studies within a total run time of 3 min. Even though the HLMs were prepared from cadavers' livers with no detailed medication history of the donors, the kinetic parameters obtained in this study were still within reported ranges.

With the method described here, the kinetics and inhibition of UGT activities toward AZT in human liver were rapidly and precisely determined using small amounts of microsomal proteins.

## 5. Conclusion

A UPLC–MS method was developed for the first time for the identification of UGT2B7 activity in HLMs using AZT as selective substrate. The proposed method was simple, robust and sensitive, revealing that it was appropriate for rapid screening UGT2B7 activity in HLMs. Furthermore, with this improved sensitivity and specificity, the kinetics of UGT activities toward AZT in HLMs could be determined more precisely. Therefore, this method is suitable for *in vitro* studies using AZTG formation as an index reaction for UGT2B7 activity.

## Acknowledgement

This work was supported by the National Natural Science Foundation of China (30630075, 30772608), DICP Innovation Fund of Chinese Academy of Sciences and Ph.D Exploring Fund (S200628).

## References

[1] B. Burchell, M.W. Coughtrie, Pharmacol. Ther. 43 (1989) 261.
[2] S.N. de Wildt, G.L. Kearns, J.S. Leeder, J.N. van den Anker, Clin. Pharmacokinet. 36 (1999) 439.

[3] M.B. Fisher, M.F. Paine, T.J. Strelevitz, S.A. Wrighton, Drug metab. Rev. 33 (2001) 273.
[4] R.H. Tukey, C.P. Strassburg, Mol. Pharmacol. 59 (2001) 405.
[5] J.A. Williams, R. Hyland, B.C. Jones, D.A. Smith, S. Hurst, T.C. Goosen, V. Peterkin, J.R. Koup, S.E. Ball, Drug Metab. Dispos. 32 (2004) 1201.
[6] R.S. Addison, S.L. Parker-Scott, W.D. Hooper, M.J. Eadie, R.G. Dickinson, Biopharm. Drug Dispos. 21 (2000) 235.
[7] T.K. Kiang, M.H. Ensom, T.K. Chang, Pharmacol. Ther. 106 (2005) 97.
[8] J. Sahai, K. Gallicano, A. Pakuts, D.W. Cameron, J. Infect. Dis. 169 (1994) 1103.
[9] B.L. Coffman, G.R. Rios, C.D. King, T.R. Tephly, Drug Metab. Dispos. 25 (1997) 1.
[10] O. Barbier, D. Turgeon, C. Girard, M.D. Green, T.R. Tephly, D.W. Hum, A. Bélanger, Drug Metab. Dispos. 28 (2000) 497.
[11] A.N. Stone, P.I. Mackenzie, A. Galetin, J.B. Houston, J.O. Miners, Drug Metab. Dispos. 31 (2003) 1086.
[12] M.H. Court, S. Krishnaswamy, Q. Hao, S.X. Duan, C.J. Patten, L.L. Von Moltke, D.J. Greenblatt, Drug Metab. Dispos. 31 (2003) 1125.
[13] M.A. Quevedo, G.N. Moroni, M.C. Briñón, Biochem. Biophys. Res. Commun. 288 (2001) 954.
[14] S. Merchant, J.J. Jin, E.H.L. Tai, Drug Metab. Rev. 36 (2004) 288.
[15] P. Baranczewski, A. Kallin, A. Andersson, S. Hagigi, M. Aberg, H. Postlind, L. Mankowitz, Drug Metab. Rev. 36 (2004) 291.
[16] M.H. Court, Methods Enzymol. 400 (2005) 104.
[17] A. Di Marco, M. D'Antoni, S. Attaccalite, P. Carotenuto, R. Laufer, Drug Metab. Dispos. 33 (2005) 812.
[18] S. Boase, J.O. Miners, Br. J. Clin. Pharmacol. 54 (2002) 493.
[19] L.G. Apollonio, D.J. Pianca, I.R. Whittall, W.A. Maher, J.M. Kyd, J. Chromatogr. B 836 (2006) 111.
[20] X.J. Li, A. Fekete, M. Englmann, C. Gotz, M. Rothballer, M. Frommberger, K. Buddrus, J. Fekete, C.P. Cai, P. Schroder, A. Hartmann, G. Chena, P. Schmitt-Kopplin, J. Chromatogr. A 1134 (2006) 186.
[21] D. O'Connor, R. Mortishire-Smith, D. Morrison, A. Davies, M. Dominguez, Rapid Commun. Mass Spectrom. 20 (2006) 851.
[22] M. Kalovidouris, S. Michalea, N. Robola, M. Koutsopoulou, I. Panderi, Rapid Commun. Mass Spectrom. 20 (2006) 2939.
[23] E.C.Y. Chan, S.L. Yap, A.J. Lau, P.C. Leow, D.F. Toh, H.L. Koh, Rapid Commun. Mass Spectrom. 21 (2007) 519.
[24] L. Novakova, L. Matysova, P. Solich, Talanta 68 (2006) 908.
[25] G.J. Sanderink, B. Bournique, J. Stevens, M. Petry, M. Martient, J. Pharmmacol. Exp. Ther. 282 (1997) 1465.
[26] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, J. Biol. Chem. 193 (1951) 265.
[27] V. Uchaipichat, L.K. Winner, P.I. Mackenzie, D.J. Elliot, J.A. Williams, J.O. Miners, Br. J. Clin. Pharmacol. 61 (2006) 427.
[28] N. Hanioka, Y. Saito, A. Soyama, M. Ando, S. Ozawa, J. Sawada, J. Chromatogr. B 774 (1) (2002) 105.