ELSEVIER

J. Pharm. Biomed. Anal. 22 (2000) 527–540

JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS

www.elsevier.com/locate/jpba

Determination of the kinetics of rat UDP-glucuronosyltransferases (UGTs) in liver and intestine using HPLC

Rangaraj Narayanan, Barbara LeDuc, David A. Williams *

Division of Pharmaceutical Sciences, Massachusetts College of Pharmacy and Allied Health Sciences, 179 Longwood Avenue, Boston, MA 02115, USA

Received 10 May 1999; received in revised form 16 September 1999; accepted 19 September 1999

Abstract

Uridinediphosphoglucuronosyl transferases (UGTs) are a group of membrane bound proteins which catalyze the transfer of glucuronic acid from UDP-glucuronic acid to a wide variety of xenobiotics and drug molecules enabling them to be eliminated. The major UGT isoforms found in the rat are 1A1, 1A6, 2B1 and 2B12. Conventional methods for the assay of glucuronides (GLs) include TLC, extraction and colorimetry or quantification of the aglycone, liberated after hydrolyzing the GL with β-glucuronidase. However these techniques cannot distinguish between isomeric GLs or GLs of multiple acceptor site substrates. Therefore the purpose of this study was to develop simple and sensitive HPLC methods for the direct and simultaneous analysis of the GL(s) and their aglycones without the drawbacks of the conventional methods. The three classical substrates we chose were 4-methylumbelliferone (4MU), testosterone (TES) and 8-hydroxyquinoline (8HOQ) representing UGT isoforms 1A6, 2B1 and 2B12 of the rat family, respectively. Here we report the validated HPLC conditions, for the detection and separation of 4-methylumbelliferone glucuronide (4MUG), testosterone glucuronide (TESG) and 8-hydroxyquinoline glucuronide (8HOQG) and their aglycones in incubation media containing male Sprague–Dawley rat liver and intestinal microsomal preparations. The separations were achieved on a Zorbax SB-CN column (150×4.6 mm, 5 μ). The analysis time for the separation of TES, 8HOQ and 4MU and their glucuronides were 17, 12 and 30 min, respectively. The methods showed excellent linearity ($r^2 > 0.99$) over the concentration ranges tested (0.25–5.0 nmoles of TESG; 0.125–18.75 nmoles of 8HOQG and 0.125–12.5 nmoles of 4MUG), good precision and accuracy (RSD $<$ 2.5%). Inter-day variability studies ($n = 3$) showed no significant difference between the regression lines obtained on the three days. Recoveries were good ($>$ 90%) at all three points (low, mid-point, high) of the standard curve. The limits of detection were 0.125, 0.1 and 0.1 nmole for TESG, 8HOQG and 4MUG, respectively. The above methods were used to estimate kinetic parameters such as V_{max} and K_m for the GLs of the three substrates in both liver and intestinal tissue preparations and the values were comparable with previously reported results. UGT2B1 was found primarily

* Correponding author. Fax: +1-617-7322737.

E-mail address: dwilliams@mcp.edu (D.A. Williams)

0731-7085/00/$ - see front matter © 2000 Elsevier Science B.V. All rights reserved.

PII: S0731-7085(00)00241-7

in the liver while UGTs 1A6 and 2B12 were present in comparable amounts in both tissues. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Testosterone; 8-Hydroxyquinoline; 4-Methylumbelliferone; Uridine diphosphoglucuronosyl transferases (UGTs); Glucuronidation; Method validation; Glucuronidation kinetics

1. Introduction

Uridinediphosphoglucuronosyl transferases (UGTs, EC 2.4.1.17) constitute a major class of Phase II enzymes that are involved in the transfer of glucuronic acid from UDP-glucuronic acid (UDPGA) to a variety of acceptor groups like phenols, alcohols, aliphatic amines, carboxylic acids and acidic carbon atoms found in a wide range of drugs and xenobiotics [1]. This represents a major step in the detoxification and elimination of many potentially toxic compounds [1,2]. The UGTs are primarily classified into families 1 and 2, in both rats and humans and their nomenclature has been assigned based on evolutionary divergence [3]. In rat family 1, UGTs 1A1 and 1A6 are two important isoforms involved in the glucuronidation of bilirubin and small planar phenols respectively [3]. In family 2, UGTs 2B1 and 2B2 are involved in the glucuronidation of steroids and UGT 2B12 glucuronidates bulky phenols and mono-terpenoid alcohols [3]. Characterization of these isoforms based on substrate specificity is essential to understand their role in drug metabolism. The model substrates of the isoforms 1A6, 2B1 and 2B12 whose glucuronidation we decided to study were testosterone (TES) for rat UGT2B1 [4], 8-hydroxyquinoline (8HOQ) for rat UGT2B12 [5] and 4-methylumbelliferone (4MU) for rat UGT1A6 [4]. It was therefore necessary to develop bio-assays to quantify the glucuronides. Looking into literature, the most commonly used assay was the universal TLC method which used the incorporation of (^{14}C)-UDPGA into the acceptor site(s) of a substrate to form radio-labeled glucuronide(s) which can be quantified using a radio TLC scanner [6]. The problem with this method is the lack of specificity, sensitivity and the inability to distinguish between glucuronides of substrates with multiple acceptor sites or between isomeric glucuronides. Alternatively quantification of glucuronides, for e.g. 4MU glucuronide (4MUG) is commonly done through indirect means involving extraction of the resultant glucuronide, hydrolysis with acid or β-glucuronidase and then assaying for the liberated aglycone using fluorescence [7]. This method has problems of sensitivity, recovery, incomplete hydrolysis and instability of the aglycone in acid media. Other investigators have separated 4MU from 4MUG in various tissues using open column chromatography with Dowex AG-50W resins [8]. Methods not involving such complex extractions or non-specificity and those which quantify the glucuronide directly in the incubation mixture would be preferred. HPLC would then be an obvious choice. In the case of TES and 8HOQ, previously reported HPLC methods are radiometric assays utilizing either radiolabeled UDPGA or substrate and are gradient elutions [9,10]. Gradient separations have problems with baseline drifting and reproducibilty. Radiometric HPLC assays have problems of availability of labeled substrate or co-factor and have high background noise due to the scintillant thereby requiring the use of high concentration of labeled material which makes routine analysis expensive. HPLC methods for the assay of 4MU and 4MUG have been reported and involve either the use of ion-pair reagents [11] or are gradient elutions with a run time of 40 min [12].

This paper describes simple isocratic methods for the direct quantification of the glucuronides of TES, 8HOQ and 4MU in incubation mixtures which contained their respective aglycones and all the separations were achieved on the same stationary phase. Using these methods enzyme kinetic parameters like V_{max} and K_m were calculated for these substrates in rat liver and intestinal microsomal preparations.

2. Experimental

2.1. Chemicals

TES, 8HOQ, 4MU and their glucuronides were obtained from Sigma Chemical (St Louis, MO). All other chemicals were of the highest purity available and were also obtained from Sigma. HPLC grade acetonitrile and ammonium phosphate monobasic were from Fisher Scientific (Pittsburg, PA). Filtered (0.22 μm filter) deionized water was used for all preparations.

2.2. Chromatography

The HPLC system consisted of a Perkin Elmer Series 410 LC Bio Pump (Norwalk, CT), a SIL-6B auto sampler (Shimadzu, Japan) and a Spectra 100 variable wavelength detector (Thermo Separation Products, CA) set at 220, 250 and 235 nm for the detection of 4MUG, TESG and 8HOQG, respectively. Data was analyzed using WinFlow™ chromatography software from IN/US (Tampa, FL). Separation was achieved on a Zorbax SB-CN column (150 × 4.6 mm I.D, 5 μm, Mac-Mod Analytical, PA, USA) with a Zorbax SB-CN guard column. The mobile phase consisted of acetonitrile-50 mM ammonium phosphate buffer, pH 4.50 (30:70, v/v for TES and TESG), acetonitrile–20 mM ammonium phosphate buffer, pH 4.50 (10:90, v/v for 4MU and 4MUG) and methanol–20 mM ammonium phosphate buffer, pH 3.00 (5:95, v/v for 8HOQ and 8HOQG). The flow rate was 1.0 ml/min in all three cases.

2.3. Preparation of microsomes

Male Sprague–Dawley rats (200–250 g, Charles River, Wilmington, MA) were used in these studies. The animals were housed in stainless steel cages and were allowed food (Purina rodent chow, Purina, St Louis, MO) and water ad libitum. The animals were deprived of food the night before they were sacrificed. The rats were killed by cervical decapitation and their livers removed. A 30 cm long segment of the intestine (starting from the pylorus) was also cut and used for the intestinal microsomal preparation. All steps in the microsomal preparation were done on ice (0–4°C). The liver microsomes were then isolated by differential centrifugation according to the procedure outlined in [13] and stored as pellets at −80°C. The intestinal microsomes were prepared as described in [14]. The protein content was assayed using the BCA protein assay kit (Pierce, Rockford, IL).

2.4. Sample preparation

Incubation samples contained the following: microsomal protein (0.2 mg/ml), $MgCl_2$ (5 mM), Brij 58 (0.05%), TRIS (50 mM) and standards (TESG, 8HOQG and 4MUG in various amounts). The final volume of this mixture was 250 (TESG) and 125 μl (8HOQG, 4MUG). Acetonitrile (100 μl for TESG and 50 μl for 8HOQG and 4MUG) was added to precipitate the protein. The internal standard (20 μl of 40 mM diphenhydramine (DPH) for TESG, 10 μl of 2 mM acetaminophen (APAP) for 8HOQG and 10 μl of 1 mM lidocaine (LIDO) for 4MUG) was added to the above mixture, which was vortex mixed and centrifuged at 14 000 rpm for 5 min. All the internal standard solutions were prepared in methanol. The supernatant was removed and injected into the LC. Injections of 20 (TESG) and 10 μl (8HOQG, 4MUG) were made. All analyses were performed in triplicate. Peak area ratios (glucuronide standard to its internal standard) were calculated for all samples injected.

2.5. Standard solution preparation for linearity

To perform linearity for the standards, stock solutions of TESG, 8HOQG and 4MUG containing 2.5 μmoles each were prepared (500 μM in 5 ml of 50 mM TRIS, pH 7.40). The stock solutions were stored at approximately 4°C when they were not in use. Under these conditions, the stock solutions were stable for 15 days. A series of dilutions were made to obtain working solutions containing the following amounts of each standard in the final mixture (0.25–5.0 nmoles of TESG, 0.125–18.75 nmoles of 8HOQG and 0.125–12.5 nmoles of 4MUG). The standard solution mixture was then prepared as described in

Section 2.4. Blanks were prepared in the same manner except that they did not contain any standard or internal standard. Inter-day variability was determined by making fresh calibration sets of standards on days 1, 5 and 10. The linear regression line was plotted between the peak area ratio and the known amount of each glucuronide using Microsoft Excel®. The regression equation and the regression coefficient (r^2) values were obtained. For verifying inter-day variability, the back calculated amounts of each standard solution, obtained by entering the respective peak area ratios into the corresponding regression equations, were determined on all three days. These values were then compared using a single factor repeated measures analysis of variance (ANOVA) (Sigma Stat®, SPSS, Chicago, IL).

2.6. Precision and accuracy

Precision was determined at the top, mid point and bottom of the calibration curve (5, 2.5 and 0.25 nmoles for TESG; 18.75, 6.25 and 0.125 nmoles for 8HOQG; 12.5, 3.125 and 0.125 nmoles for 4MUG). Six injections were made for each standard solution mixture. Precision was expressed as % relative standard deviation (% RSD). Accuracy was determined in the following manner: low, medium and high amounts of each standard (0.5, 3 and 4.5 nmoles for TESG; 0.25, 5 and 17.5 nmoles for 8HOQG; 0.25, 3.75 and 11.25 nmoles for 4MUG) were added to a blank matrix and from the response obtained, the measured amount of those standards were calculated using their respective calibration curves (three injections were made for each standard). The percentage bias was calculated using the expression:

$$\%\ \text{Bias} = \frac{\text{Measured amount} - \text{True amount}}{\text{True amount}} \times 100$$

2.7. Recovery

Absolute recovery has been defined as a measure of efficiency of extraction of the analyte from the sample matrix. Recoveries were calculated at the extremes and mid-point of the calibration curve (0.25, 2.5 and 5 nmoles for TESG; 0.125, 6.25 and 18.75 nmoles for 8HOQG; 0.125, 3.125 and 12.5 nmoles for 4MUG). Recovery experiments were repeated 5 times and in each experiment samples were analyzed in triplicate. The absolute recovery was calculated using the formula:

$$\%\ \text{Recovery} = \frac{\text{Amount of standard recovered}}{\text{Amount of standard added}} \times 100$$

2.8. Limit of quantification and detection

The lowest amount of standard which could be quantified with reasonable precision and accuracy (% RSD $< 3.0\%$) was deemed lower limit of quantification (LLOQ) [15]. The amount of standard which could be detected with a signal to noise ratio ≥ 3 was considered to be limit of detection (LOD).

2.9. Determination of UGT activities

Enzyme assays were carried out in 2 ml microcentrifuge tubes. All kinetic experiments were performed using optimal conditions of detergent activation. Linear dependencies of enzyme activity on incubation time and protein concentration were established in initial experiments. A typical incubation mixture contained 50 mM TRIS–HCl buffer (pH = 7.40), 5 mM $MgCl_2$, 0.05% Brij 58, 0.20 mg/ml of microsomal protein and substrate

Table 1
Chromatographic retention values

Compound name	Retention time (t_r) (min)	Capacity factor (k')
TESG	3.30	1.36
DPH	7.30	4.21
TES	14.60	9.43
8HOQG	4.30	1.68
8HOQ	5.90	2.68
APAP	9.30	4.81
4MUG	4.70	1.94
LIDO	7.40	3.63
4MU	28.10	16.56

Table 2
Linearity for TESG, 8HOQG and 4MUG in incubation mixtures ($n = 3$)

TESG			8HOQG			4MUG		
Amount added (nmoles)	Peak area ratio (mean ± S.D)	R.S.D (%)	Amount added (nmoles)	Peak area ratio (mean ± S.D)	R.S.D (%)	Amount added (nmoles)	Peak area ratio (mean ± S.D)	R.S.D (%)
0.25	0.03 ± 0.00	5.73	0.125	0.01 ± 0.00	9.49	0.125	0.02 ± 0.01	21.74
1.25	0.12 ± 0.00	0.00	1.25	0.10 ± 0.00	2.03	0.625	0.12 ± 0.00	3.36
2.50	0.23 ± 0.00	0.00	6.25	0.48 ± 0.01	1.33	3.125	0.58 ± 0.01	0.84
3.75	0.33 ± 0.01	1.55	12.50	0.99 ± 0.01	1.15	6.25	1.13 ± 0.01	1.17
5.00	0.45 ± 0.00	0.97	18.75	1.45 ± 0.01	0.38	12.50	2.29 ± 0.01	0.48

in a total volume of 112.50 μl (4MU, 8HOQ) or 225 μl (TES). The concentration ranges of the three substrates were 5–250 μM (TES), 10–1250 μM (4MU, 8HOQ). These solutions were then pre-incubated at 37°C in a shaking water bath for 3 min. Reactions were initiated by the addition of 12.50 μl (4MU, 8HOQ) or 25 μl (TES) of 50 mM UDPGA and allowed to proceed for 10 min (TES, 8HOQ) and 5 min (4MU). Reactions were then stopped by the addition of acetonitrile (100 μl for TES, 50 μl for 4MU and 8HOQ). Internal standard (20 μl of 40 mM diphenhydramine (DPH) for TESG, 10 μl of 2 mM acetaminophen (APAP) for 8HOQG and 10 μl of 1 mM lidocaine (LIDO) for 4MUG) was added to the above mixture, which was vortexed and centrifuged at 14 000 rpm for 5 min. All the internal standard solutions were prepared in methanol. The supernatant was removed and injected into the LC. Injections of 20 μl (TESG) and 10 μl (8OHQG, 4MUG) were made.

Peak area ratios (glucuronide to internal standard) were calculated and the amount of glucuronide formed was determined using the standard curve. For each kinetic experiment, a calibration curve was prepared using the pure glucuronide standard solutions. Enzyme activity was expressed as reaction velocity by dividing the amount of product formed by the incubation time and microsomal protein content (nmoles/min per mg).

Table 3
One way repeated measures ANOVA results for determining inter-day variation[d]

Compound name	Fitted least square regression equation	r^2
TESG	[a] $y = 0.0877 * x + 0.0067$	0.99
	[a] $y = 0.0946 * x - 0.0047$	0.99
	[a] $y = 0.0872 * x + 0.0049$	0.99
8HOQG	[b] $y = 0.0776 * x + 0.0031$	0.99
	[b] $y = 0.0773 * x + 0.0007$	0.99
	[b] $y = 0.0853 * x - 0.0039$	0.99
4MUG	[c] $y = 0.1825 * x + 0.0044$	0.99
	[c] $y = 0.1788 * x - 0.0027$	0.99
	[c] $y = 0.1704 * x + 0.0266$	0.99

[a,b,c] Not statistically significant, single factor repeated measures ANOVA ($P > 0.05$).

[d] y, peak area ratio; x, amount of standard added (nmole).

3. Results and discussion

The representative chromatograms showing the separation between the glucuronides and the aglycones along with their respective internal standards are shown in Fig. 1C–Fig. 3C. The retention times and capacity factor (k') values are listed in Table 1. Chromatograms of blank incubations are shown in Fig. 1A–Fig. 3A. These chromatograms demonstrate the specificity of the assays by the absence of endogenous substances in drug and internal standard free matrices, which may have interfered with the quantitation of TESG, 8-HOQG and 4MUG. The chromatograms also show no interference from the solvent front and between the individual peaks.

The data for linearity is presented in Table 2. The peak area ratios (glucuronide to internal standard) were linearly related to the amounts of standard present ($r^2 > 0.99$). Inter-day variability was analyzed using single factor repeated measures ANOVA and the results are shown in Table 3. There was no statistically significant difference between the calibration curves on three different days for the three glucuronide standards ($P > 0.05$) demonstrating the reproduciblity of the methods and stability of the stock solutions. The stock solutions were stable during the course of the validation study. The working standards were made fresh on each day from these stock solutions. No degradation of the aglycones, glucuronides and the internal standards was observed while the samples were in the autosampler waiting to be injected (up to 24 h) or during incubations at 37°C (up to 30 min).

The method was precise at all three amounts of standards analyzed as shown in Table 4 and the % R.S.Ds were within 2.5% in all cases except for 0.25 nmole of TESG where it was 7.20% which is still within the range usually allowed [15]. Accuracy data is shown in Table 5 and the percent biases were all within 15%. Recoveries of the glucuronides from their matrices were efficient as shown in Table 6, except for 0.25 nmole of TESG where it was 136.00%.

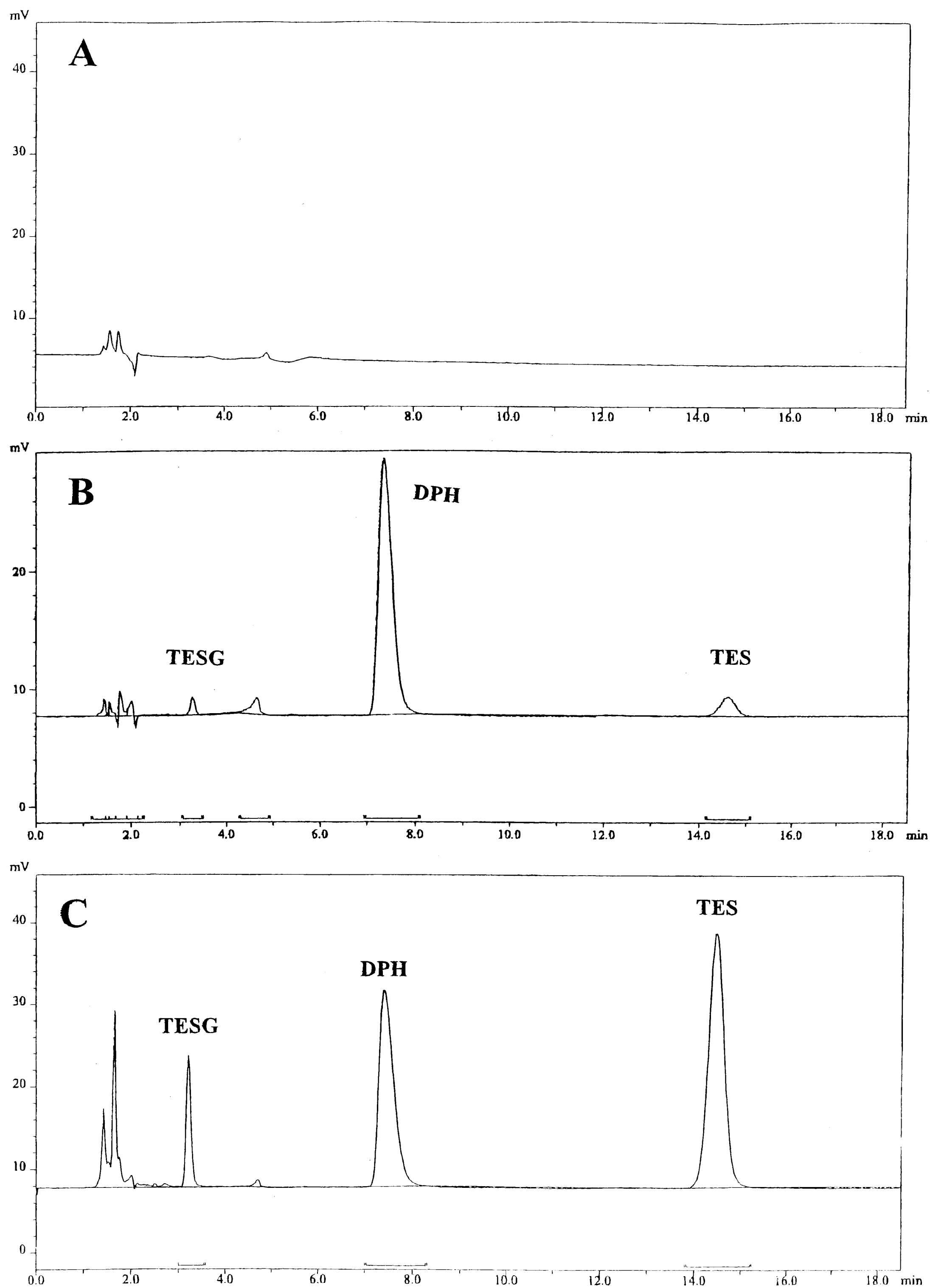

Fig. 1. Representative chromatograms of testosterone microsomal incubation sample: (A) blank incubation sample; (B) TESG (0.25 nmole) at LLOQ and TES (1.0 nmole) with internal standard (DPH); (C) TESG (2.5 nmole), TES (25.0 nmole) with DPH. Chromatography conditions as described in Section 2.2.

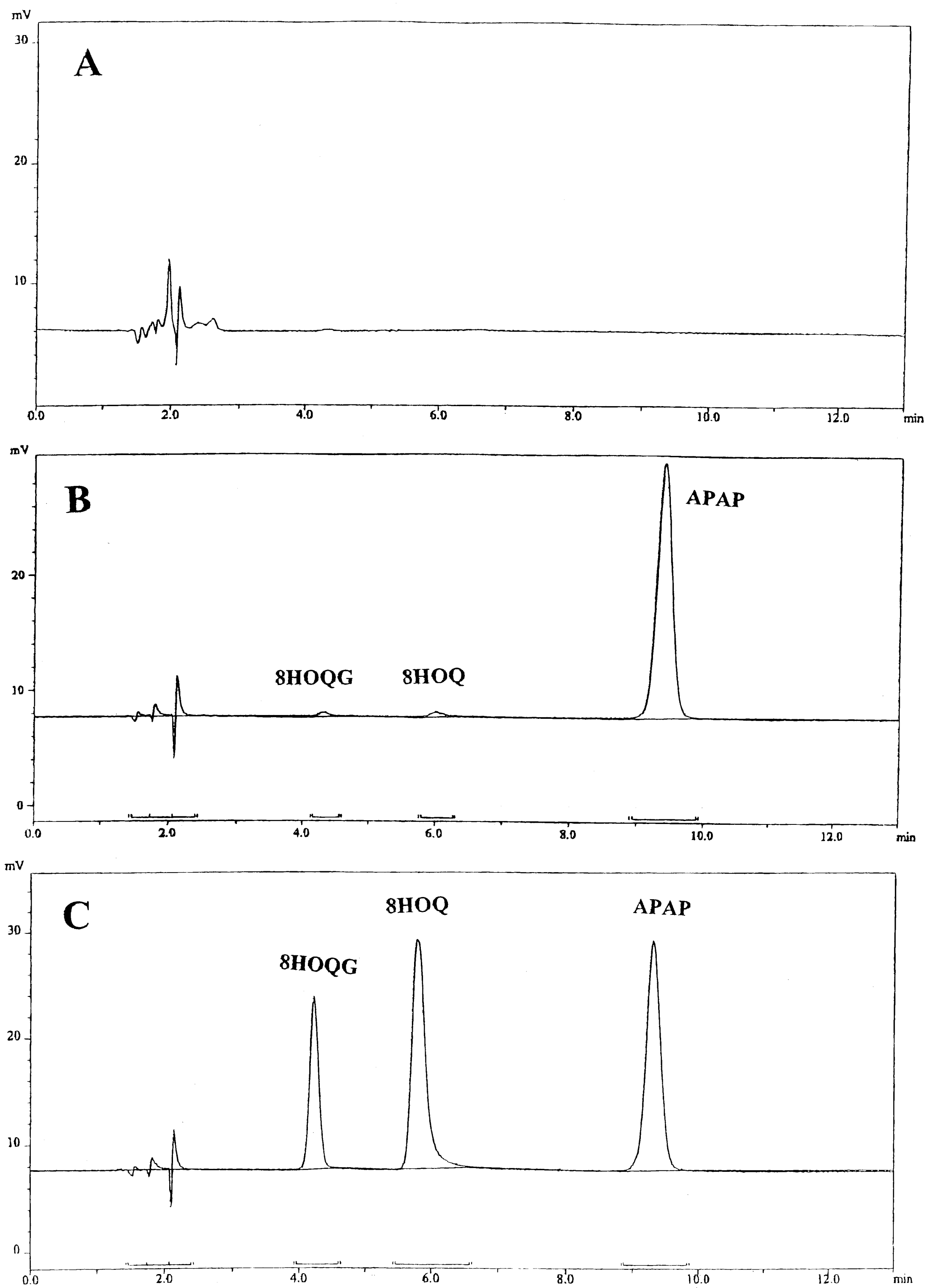

Fig. 2. Representative chromatograms of 8-hydroxyquinoline microsomal incubation sample: (A) blank incubation sample; (B) 8HOQG (0.125 nmole) at LLOQ and 8HOQ (1.25 nmole) with internal standard (APAP); (C) 8HOQG (6.25 nmole), 8HOQ (31.25 nmole) with APAP. Chromatography conditions as described in Section 2.2.

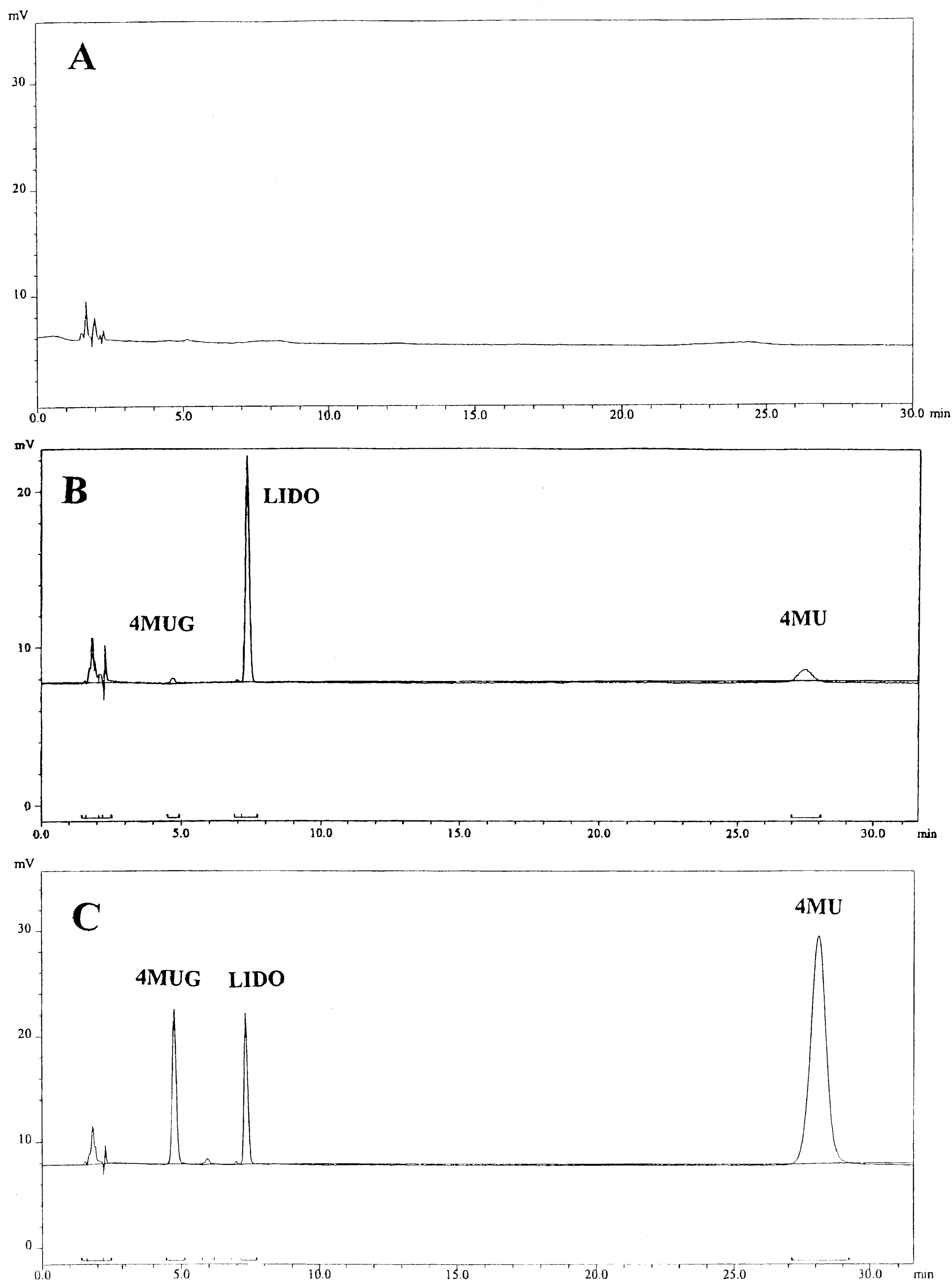

Fig. 3. Representative chromatograms of 4-methylumbelliferone microsomal incubation sample: (A) blank incubation sample; (B) 4MUG (0.125 nmole) at LLOQ and 4MU (1.25 nmole) with internal standard (LIDO); (C) 4MUG (6.25 nmole), 4MU (31.25 nmole) with LIDO. Chromatography conditions as described in Section 2.2.

Table 4
Precision determination for TESG, 8HOQG and 4MUG ($n = 6$)

TESG			8HOQG			4MUG		
Amount added (nmole)	Peak area ratio (mean ± S.D)	R.S.D (%)	Amount added (nmole)	Peak area ratio (mean ± S.D)	R.S.D (%)	Amount added (nmole)	Peak area ratio (mean ± S.D)	R.S.D (%)
0.25	0.02 ± 0.00	7.20	0.125	0.01 ± 0.00	1.76	0.125	0.02 ± 0.01	1.98
2.50	0.23 ± 0.00	0.95	6.25	0.48 ± 0.01	1.16	3.125	0.59 ± 0.01	1.49
5.00	0.49 ± 0.00	0.89	18.75	1.44 ± 0.01	0.57	12.50	2.27 ± 0.02	1.21

Table 5
Accuracy determination for TESG, 8HOQG and 4MUG ($n = 3$)

TESG				8HOQG				4MUG			
Amount added (nmoles)	Amount found (nmoles) (mean ± S.D)	R.S.D (%)	Bias (%)	Amount added (nmoles)	Amount found (nmoles) (mean ± S.D)	R.S.D (%)	Bias (%)	Amount added (nmoles)	Amount found (nmoles) (mean ± S.D)	R.S.D (%)	Bias (%)
0.50	0.57 ± 0.00	0.00	+13.53	0.25	0.23 ± 0.02	8.66	−8.75	0.25	0.25 ± 0.01	2.56	+0.75
3.00	2.95 ± 0.03	0.97	−1.57	5.00	4.69 ± 0.07	1.45	−6.22	3.75	3.76 ± 0.07	1.92	+0.32
4.50	4.31 ± 0.03	0.61	−4.31	17.50	17.69 ± 0.19	1.12	+1.13	11.25	11.17 ± 0.03	0.28	−0.68

Table 6
Recovery of TESG, 8HOQG and 4MUG from incubation mixtures ($n = 5$)

TESG			8HOQG			4MUG		
Intestine	Amount recovered (nmole) (mean ± S.D)	Recovery (%)	Amount added (nmole)	Amount recovered (nmole) (mean ± S.D)	Recovery (%)	Amount added (nmole)	Amount recovered (nmole) (mean ± S.D)	Recovery (%)
0.25	0.34 ± 0.06	136.00	0.125	0.11 ± 0.01	88.00	0.125	0.12 ± 0.06	96.00
2.50	2.57 ± 0.00	102.80	6.25	6.11 ± 0.11	97.76	3.125	3.14 ± 0.05	100.48
5.00	4.86 ± 0.17	97.2	18.75	19.28 ± 0.55	102.83	12.50	12.13 ± 0.12	97.04

Table 7
V_{max} and K_m data for Liver and Intestinal UGTs[a]

	Liver		Intestine	
	V_{max} (nmole/min/mg)	K_m (μM)	V_{max} (nmole/min/mg)	K_m (μM)
4MU	80.25 ± 1.90	183.38 ± 14.71	61.20 ± 2.44	97.32 ± 15.43
TES	6.47 ± 0.18	22.26 ± 2.38	0.12 ± 0.01	8.75 ± 2.74
8HOQ ($n=3$)	68.87 ± 3.58	388.14 ± 39.56	28.41 ± 1.17	302.9 ± 36.47

[a] All values are expressed as mean $\pm$ S.E of two experiments except as noted.

Representative chromatograms at the lower limit of quantitation (LLOQ) are shown in Fig. 1B–Fig. 3B. The LLOQ was 0.25 nmole for TESG and 0.125 nmole for 8HOQG and 4MUG. The limit of detection (LOD) was 0.125 nmole for TESG and 0.10 nmole for both 8HOQG and 4MUG.

The kinetic parameters V_{max} and K_m for each substrate were calculated using the non-linear regression analysis program from Sigma Plot®. These are shown in Table 7. The V_{max} values for testosterone in both tissues were comparable to those obtained previously by TLC [6,16]. The V_{max} for 8-hydroxyquinoline in the liver is comparable with that obtained by using ^{14}C-UDPGA and gradient elution [9]. The estimates for 4-methyumbelliferone are also close to what was determined previously using ^{14}C-UDPGA and autoradiography [16].

4. Conclusions

Isocratic HPLC methods were developed and validated for the direct analysis of the glucuronides of testosterone, 8-hydroxyquinoline and 4-methylumbelliferone present in incubation mixtures and have shown excellent reproducibility, linearity and sensitivity. The recoveries obtained were much greater than those obtained with the reported TLC method and were $> 90\%$. Testosterone glucuronide formation has been mostly studied using radio labeled substrate and TLC, which is expensive and needs a TLC plate scanner to quantitate the data. Our results for testosterone glucuronide show a LOQ of 0.25 nmoles and a recovery greater than 90%. With this method analysis of 4-methylumbelliferone glucuronide can be performed without extraction and hydrolysis. To our knowledge there has been no previously reported isocratic HPLC method describing the simultaneous separation of 8-hydroxyquinoline and its glucuronide. These methods are simple to adapt and we think can be reproduced in any lab under the conditions specified. From the kinectic parameters estimated using these methods, testosterone glucuronidation (UGT2B1) is primarily hepatic and is almost absent in the intestine whereas 4-methylumbelliferone (UGT1A6) and 8-hydroxyquinoline (UGT2B12) get glucuronidated to a considerable extent in the intestine, although the liver is still the major organ in glucuronidating all the three substrates.

Acknowledgements

The authors would like to thank Gary R. Tataronis for his valuable advice in the statistical analysis.

References

[1] G.J. Dutton, Glucuronidation of Drugs and Other Compounds, CRC Press, Boca Raton, FL, 1980.
[2] B. Burchell, Rev. Biochem. Tox. 3 (1981) 1–39.
[3] P.I. Mackenzie, et al., Pharmacogenetics 7 (1997) 255–269.
[4] C.N. Falany, M.D. Green, E. Swain, T.R. Tephly, Biochem. J. 238 (1986) 65–73.
[5] M.D. Green, et al., Arch. Biochem. Biophy. 322 (1995) 460–468.
[6] S. Bansal, T. Gessner, Anal. Biochem. 109 (1980) 321–329.

[7] W. Lilienblum, K.W. Bock, Biochem. Pharmacol. 33 (1984) 2041–2046.
[8] G.M.J. van Kempen, G.S.I.M. Jansen, Anal. Biochem. 46 (1972) 438–442.
[9] M.W.H. Coughtrie, B. Burchell, J.R. Bend, Anal. Biochem. 159 (1986) 198–205.
[10] B.T. Ethell, G.D. Anderson, K. Beaumont, D.J. Rance, B. Burchell, Anal. Biochem. 255 (1998) 142–147.
[11] U. Femfert, H.D. Kuntz, B. May, J. Chromatogr. 278 (1983) 452–457.
[12] C.L. Zimmerman, S. Ratna, E. Lebouef, K.S. Pang, J. Chromatogr. 563 (1991) 83–94.
[13] G.W. Lucier, O.S. McDaniel, J.R. Bend, E. Faeder, J. Pharmacol. Exp. Ther. 186 (1973) 416–424.
[14] P.B. Watkins, S.A. Wrighton, E.G. Schuetz, D.T. Molowa, P.S. Guzelian, J. Clin. Invest. 80 (1987) 1029–1036.
[15] S. Braggio, R.J. Barnaby, P. Grossi, M. Cugola, J. Pharm. Biomed. Anal. 14 (1996) 375.
[16] M. Vargas, M.R. Franklin, Xenobiotica 27 (1997) 413–421.