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Fully automated liquid–liquid extraction for the determination of a novel insulin sensitizer in human plasma by heated nebulizer and turbo ionspray liquid chromatography-tandem mass spectrometry

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Abstract

I, 2-{[5,7-dipropyl-3-(trifluoromethyl)-1,2-benzisoxazol-6-yl]oxy}-2-methyl propionic acid is an α peroxisome proliferator-activated receptor (PPAR) agonist with some γ activity being investigated for potential use in the treatment of Type II diabetes mellitus and dyslipidemia. Two automated liquid–liquid extraction methods were developed and validated for the determination of **I** in human plasma. Concentrations of **I** were determined over a wide range of clinical doses. For Method A, plasma was acidified and extracted with ethyl acetate using a fully automated procedure. Analysis was performed by LC-MS/MS with a turbo ionspray source in negative ion mode. For Method B, a larger volume of plasma was extracted and a heated nebulizer source was used on the mass spectrometer. Method A was linear from 0.05 to 50 ng/mL and Method B from 0.2 to 1000 ng/mL. Validation procedures showed that both methods were robust, specific and reproducible.

Keywords: Insulin sensitizer; PPAR; LC-MS/MS; Human plasma; Automated liquid–liquid extraction

1. Introduction

I, 2-{[5,7-dipropyl-3-(trifluoromethyl)-1,2-benzisoxazol-6-yl]oxy}-2-methyl propionic acid is an α peroxisome proliferator-activated receptor (PPAR) agonist with some γ activity being investigated for potential use in the treatment of Type II diabetes mellitus and dyslipidemia. **I** is an insulin sensitizer that differs from more recently marketed PPAR agonists such as Avandia® and Actos® in that it does not contain a thiazolidinedione (TZD)-related structure. The novel structure of **I** led to the application of general principles of extraction and quantitative analysis by liquid chromatography-tandem mass spectrometry. Initially, a very sensitive bioanalytical method was required to support the analysis of clinical samples from patients receiving sub-mg doses of **I**. As doses were escalated in the clinic, a second method, with a wider linear dynamic range, was required to support the analysis of samples.

Liquid–liquid extraction (LLE) is a favorable extraction technique for biological samples because of its tendency to result in cleaner extracts. In the past, LLE was not amenable to automation because it was generally carried out in larger test tubes using several milliliters of solvent for extraction. A number of papers have demonstrated the effectiveness of semi-automated liquid–liquid extraction techniques using 96-well plates. Zhang et al. reported the semi-automated liquid–liquid extraction of four compounds (diphenhydramine, chlorpheniramine, desipramine, and trimipramine) in rat plasma using a Tomtec Quadra 96 with analysis by LC-MS/MS [1]. A Quadra 96 was also used to perform the liquid–liquid extraction of methotrexate and its metabolite in human plasma. Steinborner and Henion reported that four 96-well plates could be prepared in 90 min by one person with analysis by LC/MS [2]. Ramos et al. reported the semi-automated 96-well liquid–liquid extraction of Ritalin® in

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human plasma [3]. Jemal et al. performed a comparison of manual LLE, automated LLE and automated solid phase extraction (SPE) for a carboxylic acid containing analyte in human plasma. The results showed that the automated LLE and SPE reduced sample preparation time by almost one third [4]. A Packard Multiprobe II was used to partially automate the liquid–liquid extraction of paclitaxel in human plasma [5].

This paper describes the development, validation, and application of two fully automated liquid–liquid extraction methods for the determination of **I** in human plasma. The first method (A) has a linear range of 0.05–50 ng/mL in plasma to support the analysis of samples from low dose treatments. The second method (B) has a wider linear dynamic range of 0.2–1000 ng/mL to support the analysis of plasma samples from subjects receiving increasing doses of **I**.

2. Experimental

2.1. Reagents and materials

I and the internal standard (**II**) were obtained from Merck Research Laboratories (Rahway, NJ, USA, Fig. 1). Optima grade methanol, acetonitrile, ethyl acetate, and glacial acetic acid were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Formic acid (99%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Heparinized control human plasma was obtained from Biological Specialty Corporation (Lansdale, PA, USA). Milli-Q filtered water (18 ohm) was obtained at Merck Research Laboratories.

2.2. Equipment

Automated liquid–liquid extraction was performed using a combination of a Tecan Genesis RSP 150 (Research Triangle Park, NC, USA) and a Tomtec Quadra 96, Model 196–320 (Hamden, CT, USA). The LC-MS/MS system consisted of a PE Sciex (Thornhill, Ont., Canada) API 3000 with either a turbo ionspray or a heated nebulizer source and two Perkin-Elmer (Norwalk, CT, USA) Series 200 high pressure mixing pumps. A CTC PAL Leap (Leap Technologies, Carrboro, NC, USA) autosampler was used with a temperature-controlled cooling stack set to 4 °C. A model 7990 Jones Chromatography (Hegoed, Wales, UK) column heater was set to 40 °C. Data was processed using PE Sciex Analyst software (version 1.1) on a Windows NT platform.

2.3. Instrumental conditions

2.3.1. Method A

The analytical separation was performed on a Waters Xterra RP8 (2.1 mm × 50 mm, 3.5 μm) column at 40 °C. The mobile phase consisted of 0.1% formic acid (A) and acetonitrile (B). A gradient was employed to improve the separation of the analyte (**I**), internal standard (**II**) and an acylglucuronide metabolite not quantitated by this method. The gradient consisted of a starting mobile phase composition of A/B (55:45, v/v), performing a linear gradient to A/B (10:90, v/v) over 3.5 min, and holding at A/B (10:90, v/v) for 1 min. The column was then re-equilibrated at the starting conditions for 4 min. The analytes were detected by tandem mass spectrometry with a turbo ionspray interface in negative ion mode. Precursor → product ion combinations (*m/z* 372.2 → *m/z* 286.2 for **I** and *m/z* 288.2 → *m/z* 202.0 for **II**) were monitored in multiple reaction monitoring (MRM) mode. The negative ion product spectra for **I** and **II** are shown in Fig. 1. Data were collected for 6 min and the total run time was 8.5 min, including re-equilibration. The autosampler stack holding the sample plate(s) was held at 4 °C.

2.3.2. Method B

The above method was modified to allow for a wider linear dynamic range. The conditions were the same as for Method A, with the following exceptions: the LC-MS/MS interface was changed to heated nebulizer; and the mobile phase hold time at A/B (10:90, v/v) was 1.5 min. The same precursor → product ion combinations were used to detect the analytes. Data was collected for 6.5 min and the overall run time was 9 min.

2.4. Plasma standard preparation

2.4.1. Method A

Primary stock solutions for **I** and **II** were prepared at 100 μg/mL in methanol. Stock solutions for **I** were further diluted in methanol:water (50:50, v/v) to make a series of working standard solutions at the following concentrations: 0.1, 0.2, 0.8, 2, 8, 20, 40 and 100 ng/mL. Plasma standards were prepared daily by addition of each working standard to control human plasma containing 1% formic acid, resulting in plasma concentrations ranging from 0.05 to 50 ng/mL. The stock solution for **II** was further diluted in methanol:water (50:50, v/v) to 5.0 ng /mL. This solution was aliquotted to each plasma standard and sample prior to extraction.

2.4.2. Method B

The primary stock solution for **I** was prepared at 500 μg/mL in methanol. The solution was further diluted in methanol:water (50:50, v/v) to make a series of working standard solutions at the following concentrations: 0.4, 0.8, 4, 16, 50, 200, 1000 and 2000 ng/mL. Plasma standards were prepared daily by addition of each standard to control human plasma containing 1% formic acid. Plasma concentrations were 0.2–1000 ng/mL. The primary stock solution for **II** was prepared at 100 μg/mL in methanol. The solution was further diluted in methanol:water (50:50, v/v) to 10 ng/mL and aliquotted to each plasma standard and sample.

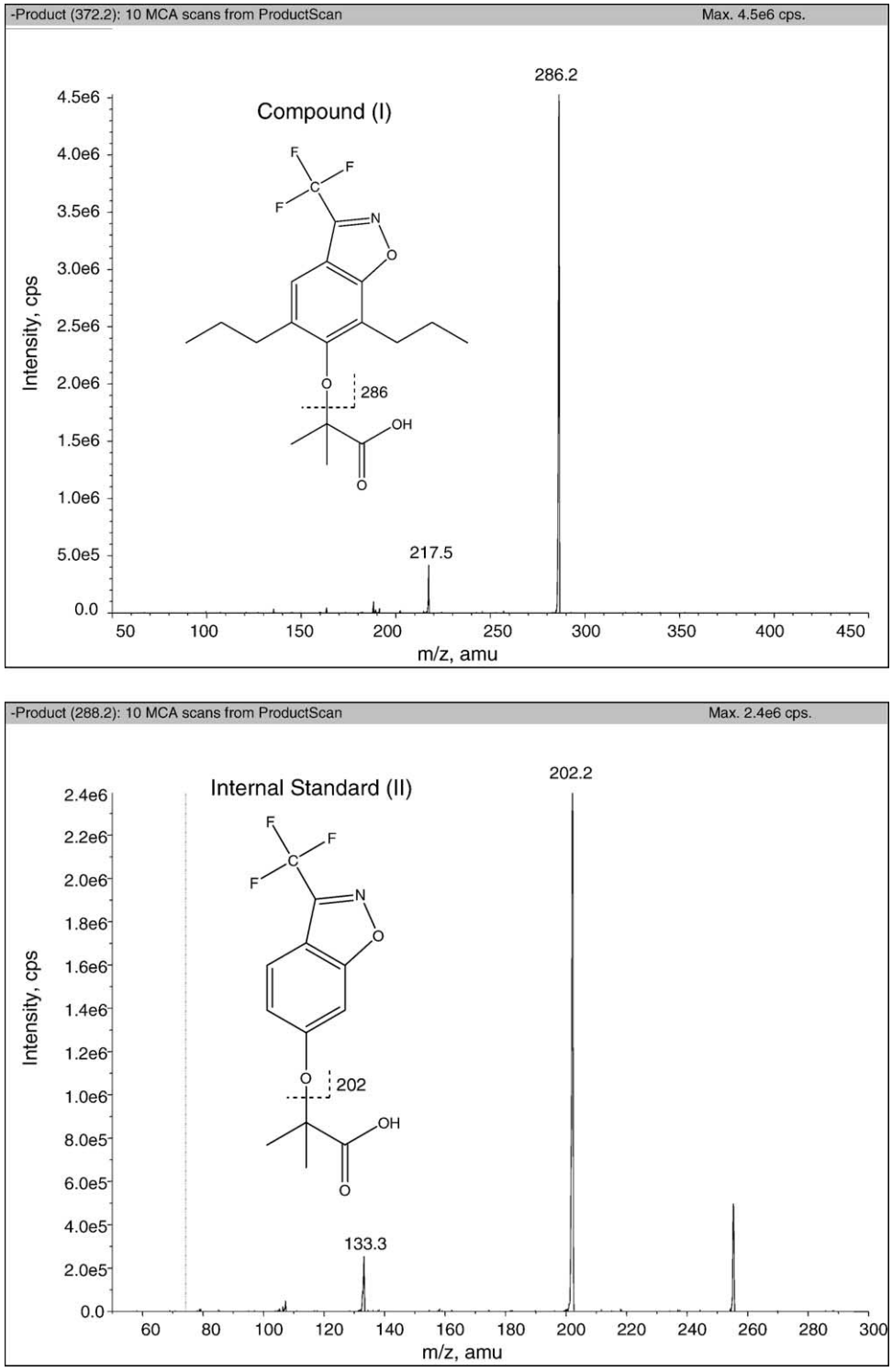

Fig. 1. Structures of the compound and internal standard investigated in this study. Negative ion product scan mass spectra for **I** and **II** obtained on a PE Sciex API 3000 mass spectrometer with a turbo ionspray source.

2.5. Quality control sample preparation

2.5.1. Method A

Primary quality control (QC) standard solutions for **I** was prepared at 100 μg/mL in methanol. The stock solution was diluted in methanol:water (50:50, v/v) to concentrations of 10 μg/mL, 1 μg/mL and 30 ng/mL for preparation of high, medium and low QC samples. QC samples were prepared by addition of the appropriate standard solution to control human plasma. In order to mimic the conditions at clinical

sites, formic acid was added to the plasma to equal 1% of the plasma volume. QC plasma concentrations were 40, 4 and 0.12 ng/mL. QC samples stored at −70 °C until assayed.

2.5.2. Method B

Primary quality control standard solutions for **I** was prepared at 500 μg/mL in methanol. The stock solution was diluted in methanol:water (50:50, v/v) to concentrations of 80 μg/mL, 2 μg/mL and 50 ng/mL for preparation of high, medium and low QC samples. QC samples were prepared by addition of the appropriate standard solution to control human plasma. Formic acid was added to mimic the conditions at clinical sites. QC plasma concentrations were 800, 20 and 0.5 ng/mL. QC samples were stored at −70 °C until assayed.

2.6. Extraction procedure

2.6.1. Method A

Frozen plasma samples were thawed at room temperature, vortexed for 1 min and centrifuged at 2060 × *g* for 5 min. Using a Tecan Genesis RSP 150, 0.05 mL of each plasma sample was aliquotted to a single well of a 2-mL 96-well polypropylene plate, followed by addition of the internal standard (0.025 mL). The plate was placed onto the deck of a Tomtec Quadra 96 (Model 196–320), where acetic acid:acetonitrile (0.5:99.5, v/v, 0.1 mL) was added to each sample, followed by 1 mL of ethyl acetate. The plate was covered with a Webseal mat, sonicated for 15 min, and centrifuged at 2060 × *g* for 5 min. Approximately 1 mL of the organic layer was transferred by the Tomtec Quadra 96 to a clean polypropylene 1-mL 96-well plate. The plate was placed on a SPE Dry 96 (Argonaut Technologies, Foster City, CA, USA) and the ethyl acetate was evaporated to dryness. The dry plate was returned to the Tomtec Quadra 96, and 0.15 mL of methanol: 0.1% formic acid (50:50, v/v) was added to each well. The plate was covered with a clean Webseal mat, the samples were sonicated for 5 min, and 10–50 μL of sample was injected on the LC-MS/MS system for analysis.

2.6.2. Method B

The following modifications to Method A were used: a 0.1 mL aliquot of plasma sample was treated with 0.2 mL of the acetic acid:acetonitrile (0.5:99.5, v/v) solution; the samples were reconstituted in 0.2 mL methanol:0.1% formic acid (50:50, v/v). Method B was automated in the same manner as Method A.

2.7. Quantitation

For both methods, plasma standards were prepared daily to construct the calibration curve. Concentrations were determined from the linear least-squares fitted regression of the peak area ratios of **I** to the internal standard (**II**) versus the concentration of **I** with reciprocal weighting (1/*x*) on the concentration. Standards were prepared and assayed daily with quality control and unknown samples.

3. Results and discussion

3.1. LC-MS/MS conditions

Drug metabolism studies showed that an acylglucuronide metabolite of **I** was circulating in the plasma of several animal species (data not published). Although present at low amounts in animals (approximately 3–4% of the concentration of **I**), predictions of levels in human samples were unclear. Therefore, storage, extraction and LC-MS/MS conditions were developed for Method A as though an acylglucuronide metabolite would be present in human samples. Under neutral or slightly alkaline conditions, acylglucuronides are known to undergo hydrolysis to parent drug, which could lead to inaccurately high concentrations of the parent compound [6–15]. All clinical samples were acidified with formic acid at the time of collection to prevent hydrolysis of the acylglucuronide during storage and analysis. On the mass spectrometer, in-source decomposition of the acylglucuronide was also observed using both the turbo ionspray and heated nebulizer sources. As a result, a signal due to the acylglucuronide was observed on the ionization channel for the parent compound (**I**). Therefore, the acylglucuronide and the parent compound were separated chromatographically using the previously described gradients. Fig. 2 illustrates the separation of the acylglucuronide metabolite from parent compound in a plasma sample from a subject treated with 80 mg of **I**. The sample was analyzed using Method B.

The use of the two methods illustrates the advantages of both the turbo ionspray and heated nebulizer sources that are available with PE Sciex API mass spectrometers. A low limit of detection was required during assay development because anticipated doses in human clinical studies were low (0.5 mg). One of the main advantages of the turbo ionspray source is the ability to achieve low limits of detection as was possible with Method A. The limit of quantitation (LOQ), or the lowest concentration on the standard curve that could be detected with acceptable precision and accuracy, was 0.05 ng/mL of **I** in 0.05 mL of human plasma. The LOQ for Method A was sufficient to detect concentrations of **I** in subjects given sub-mg doses in the first clinical study up to 24 h post-dose. However, as doses were escalated in the initial human clinical study, the need for a wider linear dynamic range became more important, leading to the development of Method B.

Method B was developed using a heated nebulizer source on a PE Sciex API 3000 mass spectrometer. One of the main advantages of the heated nebulizer source is the ability to obtain wider linear dynamic ranges, often at the expense of sensitivity. For Method B, 0.1 mL of plasma was extracted in order to accommodate the lower sensitivity achieved with the heated nebulizer source. The linear range for Method B was 0.2–1000 ng/mL. As doses were increased during the study, the 5000-fold linear range allowed for less dilution of clinical samples and for detection of **I** at later time points. For both methods, the correlation coefficient for all standard curves

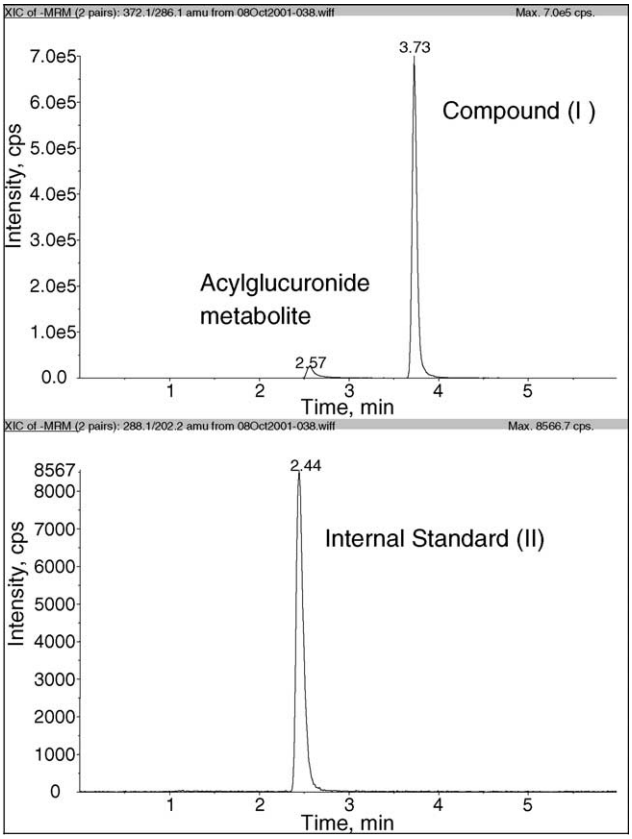

Fig. 2. Representative chromatogram from a subject receiving a single oral dose of 80 mg **I**. Sample was collected 0.5 h post-dose. Analyzed concentration of **I** using Method B: 2464.71 ng/mL (after 4× dilution). Ions monitored were *m/z* 372.2 → *m/z* 286.1 for **I** and *m/z* 288.1 → *m/z* 202.2 for **II**.

analyzed was >0.999 for compound **I** using a weighted (1/*x*) linear least-squares regression.

3.2. Accuracy and precision

Intraday precision and accuracy for each method was determined from the analysis of five standard curves containing **I**. Peak area ratios of **I** to the internal standard (**II**) were used to determine the coefficient of variation (CV%). CVs were <10% for both methods. Accuracy was determined by the comparison of mean back-calculated concentrations to nominal concentrations. Accuracy was within ±10% for both methods. Intraday precision and accuracy for both methods are summarized in Table 1.

Intra-assay precision and accuracy were also evaluated by the replicate ($n = 5$) analysis of quality control samples. Accuracy for the intraday analysis of QC samples was evaluated by comparison of the mean calculated concentrations to nominal concentrations. For Method A, accuracy was within ±15% for high, medium and low QCs, respectively. Precision, measured as the coefficient of variation (CV%), was determined from calculated concentrations of the QC samples. Precision was <10% for high, medium and low QCs, respectively. For Method B, accuracy was within ±2% for high, medium and low QCs, respectively. CVs for high, medium and low QCs were <10%. Intra-assay precision and accuracy for QC samples for both Methods A and B are summarized in Table 2.

3.3. Sample stability

Stability of **I** in human plasma was evaluated under a number of conditions, including extract, freeze–thaw, and room temperature stability. Stability of **I** after exposure to three freeze cycles was evaluated for both methods using replicates ($n = 4$) of QC samples. One freeze–thaw cycle consisted of removing the QCs from a −70 °C freezer and thawing unassisted at room temperature for up to 4 h. No effect was observed for the QC samples prepared using Method A after three freeze–thaw cycles.

For Method B, the freeze–thaw experiment was carried out in the presence of the acyl-glucuronide metabolite not quantitated with this method. Aliquots of the QC samples were analyzed after each freeze–thaw cycle. The results indicated that when the metabolite was present in the QC samples at concentrations similar to those found in human clinical samples, the concentration of **I** decreased with increasing freeze–thaw

Table 1
Intraday precision and accuracy for the replicate ($n = 5$) analysis of calibration curves for **I** in human plasma using both Methods A and B

Method A				Method B			
Nominal conc. (ng/mL)	Mean conc. (ng/mL)	% Accuracy†	Precision‡	Nominal conc. (ng/mL)	Mean conc. (ng/mL)	% Accuracy†	Precision‡
0.05	0.045 (0.004)	90.0	4.3	0.20	0.21 (0.02)	105.0	5.27
0.1	0.093 (0.007)	93.0	4.4	0.40	0.39 (0.04)	97.5	8.71
0.4	0.402 (0.027)	100.5	5.8	2.00	1.92 (0.19)	96.0	9.67
1	1.055 (0.099)	105.5	8.9	8.00	8.60 (0.50)	107.5	5.81
4	4.331 (0.222)	108.3	5.1	25.00	23.95 (1.05)	95.8	4.37
10	10.228 (1.018)	102.3	9.9	100.00	99.60 (7.85)	99.6	7.88
20	20.588 (1.548)	102.9	7.5	500.00	490.60 (18.87)	98.1	3.85
50	48.810 (0.839)	97.6	1.7	1000.00	1010.34 (89.20)	101.0	8.83

† % Accuracy = mean calculated concentration/nominal concentration × 100%.
‡ Precision is expressed as CV% as determined from the analyte to internal standard peak area ratios.

Table 2
Intra-assay precision and accuracy for the replicate ($n = 5$) analysis human plasma QC samples containing **I**

	Method A				Method B			
	Nominal conc. (ng/mL)	Mean (ng/mL)	% Accuracy[†]	Precision[‡]	Nominal conc. (ng/mL)	Mean (ng/mL)	% Accuracy[†]	Precision[‡]
High	40	35.170 (1.856)	87.9	5.28	800	788.52 (78.15)	98.6	9.91
Medium	4	3.582 (0.318)	89.6	8.88	20	19.53 (1.88)	97.7	9.60
Low	0.12	0.116 (0.009)	96.7	7.76	0.5	0.49 (0.04)	98.0	8.03

[†] % Accuracy = mean calculated concentration/nominal concentration × 100%.
[‡] Precision is expressed as CV% as determined from calculated concentrations.

cycles. The recommendation was made that samples should be thawed no more than two times prior to analysis.

Stability of **I** was assessed in human plasma after storage at room temperature for 24 h. Replicates ($n = 4$) of QC samples from Method A were used to perform the experiment. The samples analyzed using Method A showed good stability in plasma at room temperature after 24 h.

Stability of **I** and the internal standard in the final extract was also evaluated in the event that samples are extracted and not immediately analyzed. Extract stability was evaluated using both methods. QC samples were extracted and analyzed on the same day. The samples remained on the cooled (4 °C) autosampler tray for 48 h prior to re-analysis against a new standard curve. The results indicate that for both methods, **I** and **II** were not stable in the final extract after 48 h and should be analyzed within 24 h of extraction.

3.4. Recovery and matrix effect

Extraction recovery was determined by analyzing extracts of **I** at three different concentrations (0.1, 4 and 50 ng/mL for Method A and 0.4, 25 and 1000 ng/mL for Method B). The internal standard was evaluated at 5 ng/mL (Method A) and 10 ng/mL (Method B). Five different lots of human plasma were used to prepare the replicates at each concentration level. For comparison, neat standard solutions were prepared at the same concentrations as the final extracted concentrations. The neat standards were used to reconstitute extracted blank control plasma. Recovery was determined by comparing the mean chromatographic peak areas of extracted plasma samples with mean peak areas of the corresponding spiked extracts. Recoveries of **I** using Method A were 85.9, 76.7 and 78.4% at 0.1, 4 and 50 ng/mL, respectively, averaging 80.3% over the three concentrations. Recovery of the internal standard (**II**) was 87.7% at the concentration used in the assay. Extraction recovery for Method B was 49.0, 40.6 and 45.9% at 0.4, 25 and 1000 ng/mL, respectively, averaging 45.4% for the three concentrations. Recovery of the internal standard was 58.9% at 5 ng/mL. Although recovery for Method B was low, there was adequate sensitivity using the stated LC-MS/MS conditions to accurately determine concentrations of **I**.

Competition between ionization of the analyte and ionization of co-eluents may result in signal enhancement or suppression by the mass spectrometer [16–18]. This matrix effect was evaluated by comparing chromatographic peak areas for analytes from neat standard added to control blank plasma extracts ($n = 5$ lots of control plasma) to peak areas of the same neat standards. Matrix effect was evaluated using the same concentrations as the recovery experiments. For Method A, ion suppression averaged 12.0% for compound **I**. Signal enhancement of 21.7% was observed for the internal standard (**II**). For Method B, ion suppression averaged 2.2% for compound **I** with signal enhancement of 20.6% for the internal standard. Although signal enhancement for the internal standard was high, the precision (CV%) of the measurements was low (5.1% for Method A and 6.1% for Method B), indicating reliable detection and reproducibility. Analyses of clinical samples showed consistent measurements for the internal standard both within-run and between days. Furthermore, slopes of the standard curves for both methods were consistent between analytical runs. Although a matrix effect was present for these methods, it did not effect the accurate and precise quantitation of **I**.

3.5. Specificity

Specificity of the methods was determined by extracting and analyzing control human plasma treated with 1% formic acid from at least five different sources. Blank samples containing no analyte or internal standard and blank samples containing only the internal standard (**II**) were analyzed. Chromatograms obtained using each method indicated that the assays were selective and specific for both **I** and **II**. There was no detectable interference in any of the samples at the retention times of the analytes. The acyl glucuronide metabolite was not analyzed using these methods. Representative chromatograms of blank control plasma and plasma containing **I** at the LOQ and the internal standard are shown for both methods in Fig. 3.

3.6. Application of the methods

The methods were used to support the analysis of samples from a clinical study. Fig. 4 shows mean concentration plots for **I** following single oral doses ranging from 0.5 to 40 mg. Plasma samples from subjects ($n = 6$) receiving the doses in Fig. 4 were analyzed using Method A. Results from

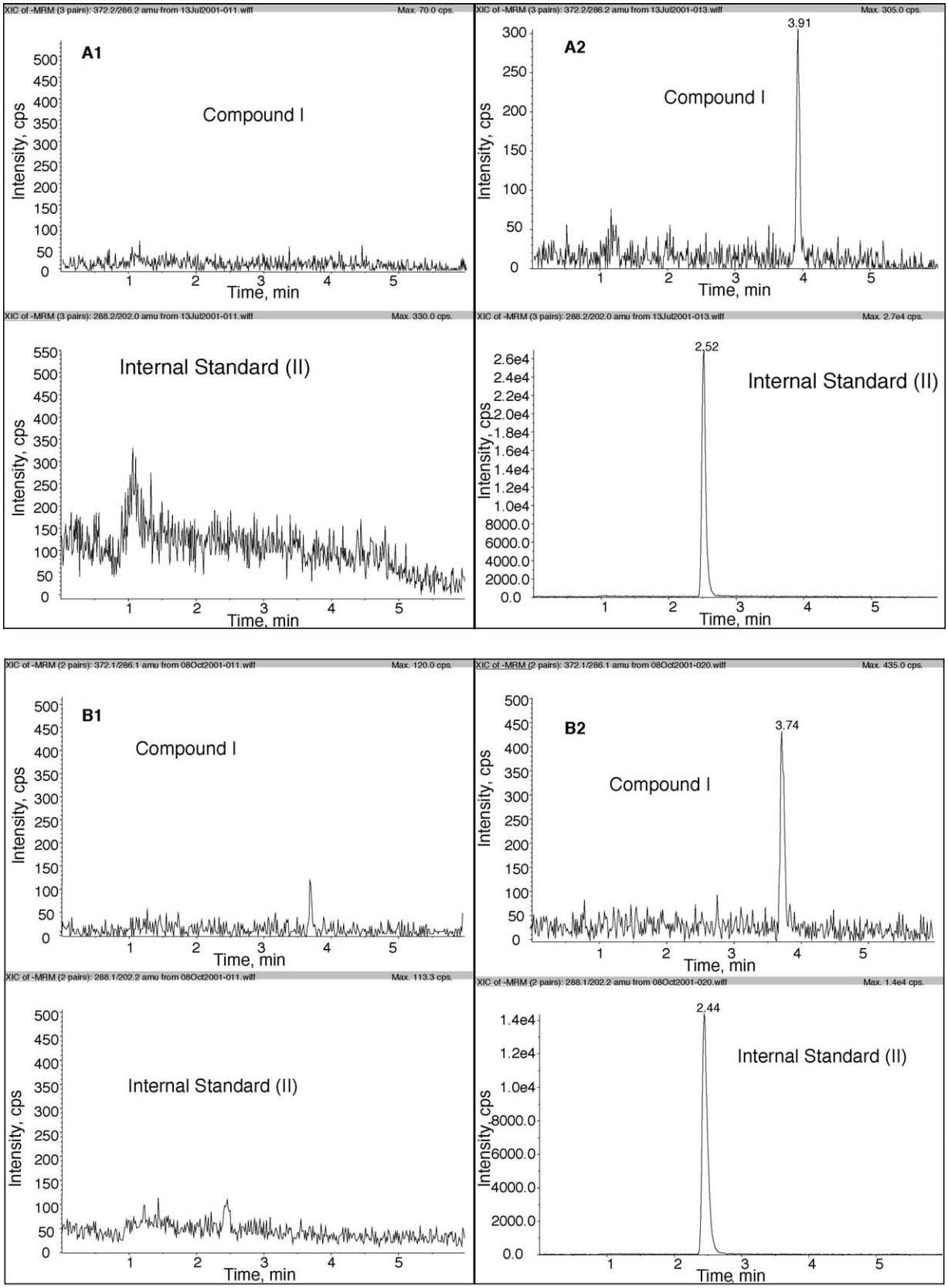

Fig. 3. Representative chromatograms from plasma samples analyzed using Methods A and B. Ions monitored were *m*/*z* 372.2 → *m*/*z* 286.1 for **I** and *m*/*z* 288.1 → *m*/*z* 202.2 for **II**. A1, Control plasma, double blank; A2, plasma standard at LOQ: **I**, 0.05 ng/mL; **II**, 5 ng/mL using Method A, B1, Control plasma, double blank; B2, plasma standard at LOQ: **I**, 0.2 ng/mL; **II**, 10 ng/mL analyzed using Method B.

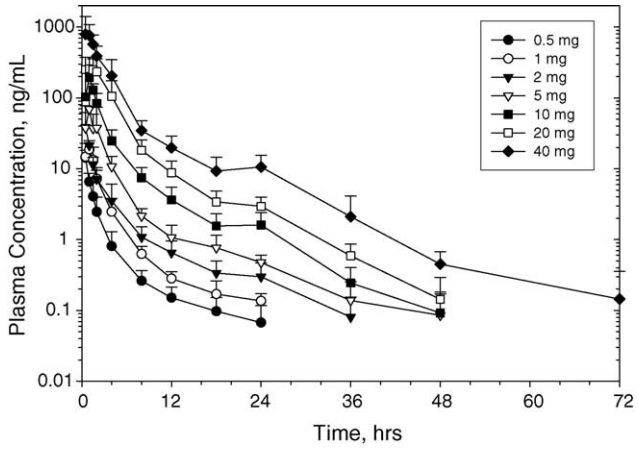

Fig. 4. Mean concentration plots for **I** following single oral doses ranging from 0.5 to 40-mg ($n = 6$ subjects per dose). Plasma samples were analyzed using Method A. Error bars represent one standard deviation.

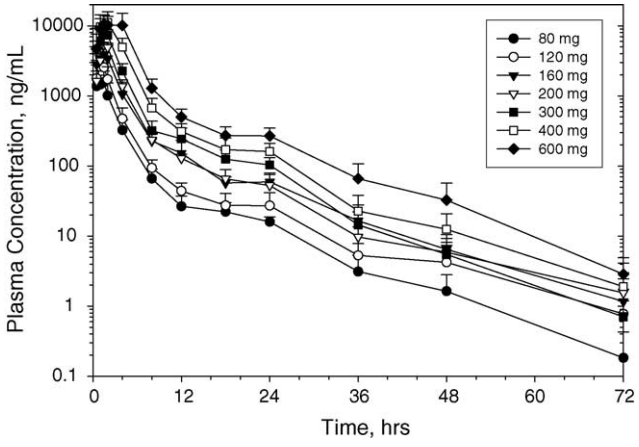

Fig. 5. Mean concentration plots for **I** following single oral doses ranging from 80 to 600-mg ($n = 6$ subjects per dose). Plasma samples were analyzed using Method B. Error bars represent one standard deviation.

mean daily concentrations of QC samples ($n = 18$) illustrate the precision and accuracy of the method. Precision (CV%) was $\leq$11.7% and accuracy ranged from 94.8 to 97.5% for the three QC concentrations.

Fig. 5 shows mean concentration plots for **I** following single oral doses ranging from 80 to 600 mg analyzed using Method B. Results from mean daily concentrations of QC samples ($n = 17$) illustrate the precision and accuracy of the method. For all QC concentrations, precision (CV%) was $\leq$10.7% and accuracy ranged from 92.7 to 104.0%.

4. Conclusions

A fully automated liquid–liquid extraction procedure has been described for the extraction of **I** in human plasma samples. The automated process reduces the possibility of systematic error due to manual sample transfer. Other advantages of the automated process are the limited contact with clinical samples by the analyst and improved throughput and efficiency.

Reproducibility, specificity and sample stability were assessed under various conditions for the analysis of **I** in human plasma. The two methods described here highlight the advantages of using either a turbo ionspray or heated nebulizer source on a PE Sciex API 3000 mass spectrometer. The sensitivity of Method A illustrates the sensitivity that can be achieved using the turbo ionspray source. Method B illustrates the wide dynamic range that is achievable using the heated nebulizer interface, at the expense of sensitivity. Samples from a clinical study were successfully analyzed using both methods. The results from both methods show that each is accurate, reproducible and selective. The methods provide good sensitivity and selectivity for **I** and its internal standard while eliminating interference from the acylglucuronide metabolite not quantitated using these methods.

References

[1] N. Zhang, K.L. Hoffman, W. Li, D.T. Rossi, J. Pharm. Biomed. Anal. 22 (2000) 131.
[2] S. Steinborner, J. Henion, Anal. Chem. 71 (1999) 2340.
[3] L. Ramos, R. Bakhtiar, F.S.L. Tse, Rapid Commun. Mass. Spectrom. 14 (2000) 740.
[4] M. Jemal, D. Teitz, Z. Ouyang, S. Khan, J. Chromatogr. B 732 (1999) 501.
[5] G. Basileo, M. Breda, G. Fonte, R. Pisano, C.A. James, J. Pharm. Biomed. Anal. 32 (2003) 591.
[6] M. Shipkova, V.W. Armstrong, M. Oellerich, E. Wieland, Ther. Drug Monit. 25 (2003) 1.
[7] C. Li, L.Z. Benet, M.P. Grillo, Chem. Res. Toxicol. 15 (2002) 1309.
[8] T. Ebner, G. Heinzel, A. Prox, K. Beschke, H. Wachsmuth, Drug Metab. Disp. 27 (1999) 1143.
[9] T. Mizuma, L.Z. Benet, E.T. Lin, J. Chromatogr. B 718 (1998) 153.
[10] K. Jemnitz, L. Vereczkey, J. Chromatogr. B 681 (1996) 385.
[11] L.H. Spahn-Langguth, L.Z. Benet, Drug Metab. Rev. 24 (1992) 5.
[12] M.L. Hyneck, A. Munafo, L.Z. Benet, Drug Metab. Disp. 16 (1988) 322.
[13] P.C. Smith, J. Hasewaga, P.N.J. Langendijk, L.Z. Benet, Drug Metab. Disp. 13 (1985) 110.
[14] E.M. Faed, Drug Metab. Rev. 14 (1984) 1213.
[15] P.N.J. Langendijk, P.C. Smith, J. Hasewaga, L.Z. Benet, J. Chromatogr. 307 (1984) 371.
[16] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 70 (1998) 882.
[17] I. Fu, E.J. Woolf, B.K. Matuszewski, J. Pharm. Biomed. Anal. 18 (1998) 347.
[18] D.L. Buhrman, P.I. Price, P.J. Rudewicz, J. Am. Soc. Mass Spectrom. 7 (1996) 1099.