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Short communication

Determination of tamsulosin in human plasma by liquid chromatography/tandem mass spectrometry and its application to a pharmacokinetic study

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ABSTRACT

Tamsulosin, a selective α_1-adrenoceptor antagonist, is used for the treatment of benign prostatic hyperplasia (BPH). We developed and validated a rapid, sensitive, and simplified liquid chromatography analytical method utilizing tandem mass spectrometry (LC–MS/MS) for the determination of tamsulosin in human plasma. After liquid–liquid extraction with methyl *t*-butyl ether, chromatographic separation of tamsulosin was achieved using a reversed-phase Luna C_{18} column (2.0 mm × 50 mm, 5 μm particles) with a mobile phase of 10 mM ammonium formate buffer (pH 3.5)–methanol (25:75, v/v) and quantified by MS/MS detection in ESI positive ion mode. The flow rate of the mobile phase was 200 μL/min and the retention times of tamsulosin and the internal standard (IS, diphenhydramine) were 0.8 and 0.9 min, respectively. The calibration curves were linear over a range of 0.01–20 ng/mL ($r > 0.999$). The lower limit of quantification using 500 μL of human plasma was 0.01 ng/mL. The mean accuracy and precision for intra- and inter-day validation of tamsulosin were both within acceptable limits. The present LC–MS/MS method showed improved sensitivity for quantification of tamsulosin in human plasma compared with previously described analytical methods. The validated method was successfully applied to a pharmacokinetic study in humans.

1. Introduction

Tamsulosin (5-[(2R)-2-{[2-(2-ethoxyphenoxy)ethyl]amino}propyl]-2-methoxybenzene-1-sulfonamide) is a selective α_1-adrenoceptor antagonist, which has a relatively high affinity for the α_{1A}-subtype and, to a lesser extent, the α_{1D}-subtype [1,2]. Because the α_{1A}-adrenoceptor is the most abundant and functionally important subtype in the human prostate [3], tamsulosin is primarily used for the treatment of benign prostatic hyperplasia (BPH).

Tamsulosin is well absorbed in humans, with the absolute bioavailability of almost 100% when tamsulosin modified-release formulation is administered in fasted condition [4]. It undergoes extensive hepatic metabolism in humans, forming five primary metabolites, M-1 to M-4 and AM-1. Primary metabolites are further metabolized to glucuronide or sulfate conjugated form [5]. Within 168 h after tamsulosin administration, 97.8% of the total dose is eliminated in the urine (76.4%) and feces (21.4%) [5].

Several analytical methods for the determination of tamsulosin in human plasma have been described using high performance liquid chromatography (HPLC) coupled with either ultra-violet [5,6] or fluorescence detection [4,7–9]. However, the relatively large volume of plasma required for these methods, in addition to the complicated and time-consuming extraction procedures, long retention times, and insufficient sensitivity are obstacles for successful and reliable pharmacokinetic studies. Liquid chromatography–mass spectrometry (LC–MS) [10], and liquid chromatography–tandem mass spectrometry (LC–MS/MS) [11–19] analytical methods for the determination of plasma tamsulosin have also been described. Although several methods exhibit improved analytical conditions compared to previously described HPLC methods, several problems continue to persist including the need for a large plasma volume (1 mL), long total run time (>4 min) [14,18], and large injection volume (20 μL or more). Additionally, the majority of previously reported methods include an alkalinization step using saturated $NaHCO_3$ or Na_2CO_3 in order to improve extraction recovery of the analytes.

In the present study, we developed a rapid, sensitive, and simplified LC–MS/MS method employing a liquid–liquid sample extraction procedure without a sample-alkalinizing step for the

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determination of tamsulosin in human plasma. This method was validated using human plasma samples by evaluating pharmacokinetic parameters, including time-dependant concentration of tamsulosin.

2. Materials and methods

2.1. Reagents and chemicals

Tamsulosin hydrochloride (>99%) was purchased from Suven Life Science Ltd. (Monmouth Junction, NJ, USA). Diphenhydramine hydrochloride (>98%) was purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC grade methanol was purchased from J.T. Baker (Mallinckrodt Baker Inc., Phillipsburg, NJ, USA). Ammonium formate was purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade and used without further purification.

2.2. Chromatographic instruments and conditions

Chromatography was performed on an Agilent 1200 series HPLC system (Agilent Technologies Inc., Santa Clara, CA, USA). Separation was carried out on a 30 °C Luna C_{18} column (2.0 mm × 50 mm, 5 μm, Phenomenex Inc., Torrance, CA, USA). The mobile phase consisted of 10 mM ammonium formate buffer (pH 3.5 with formic acid) and methanol (25:75, v/v) at a flow rate of 200 μL/min. The autosampler was maintained at 4 °C and the total run time for each sample analysis was 2.0 min.

Mass spectrometric detection was performed using an API 3200 tandem mass spectrometer (Applied Biosystems/MDS SCIEX, Toronto, ON, Canada) equipped with an electrospray ionization (ESI) source. Ion source temperature and ion spray voltage were set to 600 °C and 5500 V, respectively. The mass spectrometer was operated in positive ion mode and the tandem mass spectrometry conditions for tamsulosin and the internal standard (IS, diphenhydramine) were optimized by carrying out full scans in positive ion detection mode. The detection and quantification of tamsulosin and the IS were performed in multiple reaction monitoring (MRM) mode. Quadrupoles Q1 and Q3 were set to unit resolution. Data acquisition and quantitation were carried out using Analyst® software version 1.4.2 (Applied Biosystems/MDS SCIEX).

2.3. Standard solution and quality control sample preparation

Stock solutions of 0.1 mg/mL tamsulosin and diphenhydramine (IS) were prepared by first dissolving the compounds in methanol. Next, intermediate solutions of each compound (10 μg/mL) were prepared by dilution in methanol; standard working solutions (0.5, 2.5, 12.5, 50, 250, 500, and 1000 ng/mL) were prepared by diluting intermediate solutions with the mobile phase. These working solutions were used for daily preparation of standard calibrators at concentrations of 0.01, 0.05, 0.25, 1, 5, 10, and 20 ng/mL in human plasma. The quality control (QC) working solution was prepared in the same way as the standard working solutions, and QC samples at concentrations of 0.01, 0.03, 0.9, and 18 ng/mL were prepared by diluting the working solution with blank human plasma. Medium (0.9 ng/mL) and high (18 ng/mL) concentrations were prepared by diluting the stock and working solutions with blank human plasma. The working IS solution (diphenhydramine 100 ng/mL) was prepared by diluting the intermediate solution with the mobile phase. All stock, standard working, and QC working solutions were stored at −20 °C.

2.4. Sample preparation

All samples were stored in a freezer at −70 °C until needed, and thereafter allowed to thaw at room temperature for 20 min before processing. For analyses, a total of 500 μL of each plasma sample was combined with 10 μL of IS solution (diphenhydramine, 100 ng/mL). After brief vortexing, 2 mL of methyl *t*-butyl ether (MTBE) was added and the mixture was vortexed for 30 s. After centrifugation at 3000 rpm for 10 min, the organic layer was transferred to a new glass tube and evaporated to dryness under a gentle stream of nitrogen gas at 50 °C. The resulting residue was reconstituted with 300 μL of the mobile phase, and a 10 μL aliquot was injected into the HPLC system.

2.5. Method validation

Validation was performed based on the US FDA guidelines.

Selectivity was assessed by comparing the chromatograms of six different batches of plasma obtained from six subjects. Plasma samples were spiked with tamsulosin and IS. The linearity was evaluated using five different calibration curves. The calibration curve was constructed from a blank sample (plasma sample processed without IS), a zero sample (plasma sample processed with IS), and seven non-zero calibration samples covering the range of 0.01–20 ng/mL. The blank and zero samples were not used for calibration curve regression. The LLOQ was defined as the lowest concentration yielding a signal to noise ratio of at least 10 with a coefficient of variation (CV) < 20% and accuracy of 80–120%. The LLOQ was analyzed five times for confirmation.

Matrix effect and recovery tests were performed in triplicate at three different QC sample concentrations (0.03, 0.9, and 18 ng/mL). The matrix effect was determined by extracting blank human plasma from six different sources and then reconstituting the final extract in the injection solvent, which contained known amounts of the analyte and IS. The extraction recovery was determined by comparing the mean peak areas of plasma QC samples spiked with analyte prior to extraction to those obtained from analysis of pure authentic samples.

The intra- and inter-day precisions were determined by replicate analysis of five sets of QC samples that were spiked with four different concentrations of tamsulosin within one day or on five consecutive days, respectively. Precision was determined as the CV and accuracy was defined as the relative standard error (RSE (%) = measured concentration/targeted concentration × 100).

2.6. Stability

The stability of tamsulosin in human plasma was evaluated in triplicate using three different concentrations of the QC sample. For short-term stability, frozen plasma samples (−70 °C) were kept at room temperature for 4 h before sample preparation. The freeze–thaw stability of the tamsulosin was determined over three freeze–thaw cycles within three days. In each freeze–thaw cycle, the spiked plasma samples were frozen for 24 h at −70 °C and thawed unassisted at room temperature. When completely thawed, the samples were refrozen for 12–24 h at −70 °C. Long-term stability was evaluated after storing the frozen plasma samples at −70 °C for 30 days. The stability of the prepared plasma samples was tested after keeping the samples in the autosampler at 4 °C for 24 h.

2.7. Pharmacokinetic application

Eleven male volunteers were enrolled in the present study. All subjects were healthy as defined by their medical histories, physical examinations, and routine laboratory tests (blood cell count,

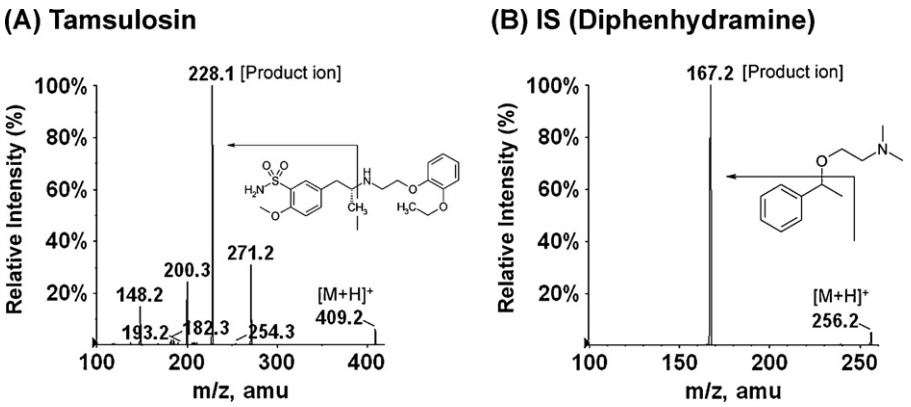

Fig. 1. Product ion mass spectra of (A) tamsulosin and (B) diphenhydramine (IS).

biochemical profile, and urinalysis). All subjects were restricted from ingesting any medications, caffeine, grapefruit products, or alcoholic beverages for at least 1 week before and during the study period. All subjects provided informed consent both verbally and in writing. The study was performed according to the Declaration of Helsinki and was approved by the Institutional Ethics Committee of the School of Pharmacy at Sungkyunkwan University (Suwon, Korea).

On the day of the study, following an overnight fast, each subject received a single 0.2 mg oral dose of tamsulosin (Harnal®-D, Astellas Pharma, Seoul, Korea) with 240 mL water. Subjects were maintained in the fasting state for 4 h after the drug administration. Venous blood samples (7 mL) were subsequently obtained into heparin sodium tubes up to 48 h after the administration of tamsulosin. Blood samples were centrifuged immediately and the plasma fractions were stored at −70 °C until needed for analysis.

The pharmacokinetic parameters of tamsulosin were estimated using non-compartmental methods and BA Calc 2007 software provided by the Korean Food and Drug Administration. The major pharmacokinetic parameters were obtained using the software.

3. Results and discussion

3.1. Method development

The tandem mass spectrometry ESI conditions for tamsulosin and IS were first optimized by carrying out full scans in positive ion detection mode. During a subsequent direct infusion experiment, the mass spectra for tamsulosin and IS produced protonated molecular ion $[M+H]^+$ peaks at 409.2 and 256.2 m/z, respectively. The major fragment ions observed in each product spectrum were at 228.1 m/z for tamsulosin and 167.2 m/z for IS, respectively (Fig. 1). The mass parameters were optimized by observing the maximum responses of the product ions. The adjusted values of the declustering potential (DP), entrance potential (EP), collision energy (CE), and collision cell exit potential (CXP) for tamsulosin were 40 V, 10 V, 31 V, and 5 V, and those for IS (diphenhydramine) were 26 V, 5 V, 20 V and 4 V, respectively. Optimized curtain gas (CUR), collision gas (CAD), nebulizer gas (GS1) and turbo gas (GS2) parameters were 10 psi, 7 psi, 70 psi, and 70 psi, respectively.

Chromatographic conditions, particularly the composition of the mobile phase, were optimized by several trials aimed at increasing the signal of the analyte and minimizing run times. The mobile phase consisted of a mixture of 10 mM ammonium formate buffer (pH 3.5 with formic acid) and methanol; ammonium formate is used primarily to improve peak shape and promote source ionization. Optimal ratios of ammonium formate buffer and methanol were tested from 10:90 to 30:70 (v/v), and a ratio of 25:75 (v/v) was selected as the final condition for the mobile phase. The flow rate of the mobile phase was set to 200 μL/min. The chromatographic sensitivity was sufficient using a Luna C_{18} column (2.0 mm × 50 mm, 5 μm). With Analyst® classic integration algorithms (Applied Biosystems/MDS SCIEX), default bunching factor, number of smoothes, and retention time window used for chromatography were 3, 3, and 30 s, respectively.

3.2. Method validation

3.2.1. Selectivity

No endogenous interference was observed at the retention times for tamsulosin and IS. Fig. 2 shows representative chromatograms for blank human plasma (Fig. 2A); human plasma spiked with tamsulosin (0.01 ng/mL) and IS (diphenhydramine 100 ng/mL; Fig. 2B); and a plasma sample obtained from a healthy volunteer 10 h after oral administration of 0.2 mg tamsulosin (Fig. 2C).

3.2.2. Accuracy and precision

Table 1 provides a summary of the accuracy and precision for four concentrations of tamsulosin. The intra- and inter-day accuracies for tamsulosin were 97.7–106.1 and 98.9–103.7%, respectively. The intra- and inter-day precisions for tamsulosin were 1.1–6.9 and 2.0–5.6%, respectively.

3.2.3. Recovery

Ethyl ether, MTBE, dichloromethane, and ethyl acetate were tested as solvents for extraction of tamsulosin from human plasma. Among these, MTBE was found to be the best solvent, producing a clean chromatogram for blank human plasma samples with the best recovery and least matrix effect. The recovery of analyte using the liquid–liquid extraction procedure with MTBE from 500 μL plasma samples was measured at three different concentrations of QC sample. With respect to sample concentration, the mean recoveries for tamsulosin and IS were 77.8 ± 2.1 and 91.3 ± 2.4%, respectively. These results are superior to those in several previous studies [7,10,15,17,18]. It indicates that our extraction procedures are more efficient, time-saving, and economic than other previously published methods.

3.2.4. Matrix effect

Matrix effects, which are a phenomenon of ion suppression or enhancement of the analyte of interest, should be evaluated during method development because the assay accuracy and precision of the LC–MS/MS method can be significantly affected [20,21]. Observed matrix effects for tamsulosin and IS ranged from 93.5 to 108.6%, and the respective CV values at each concentration from six

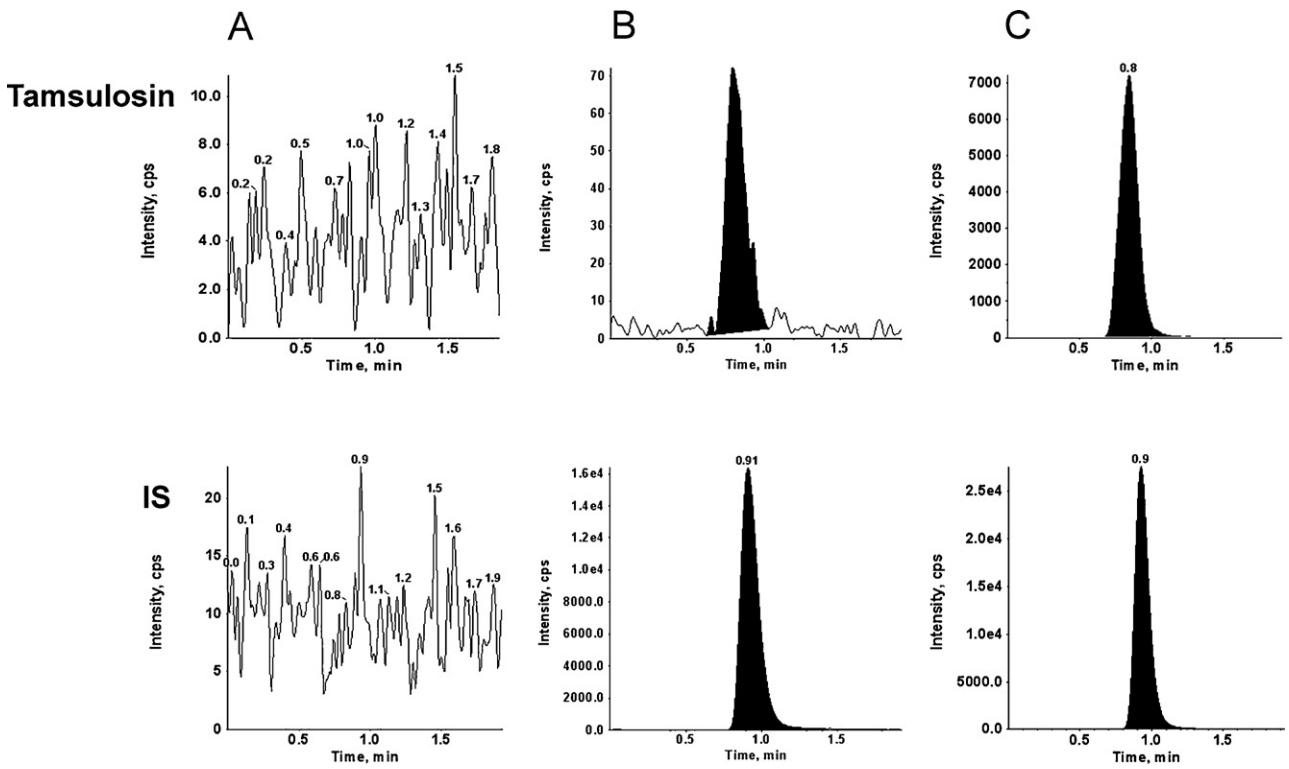

Fig. 2. Chromatograms of tamsulosin and IS (diphenhydramine) in human plasma. (A) Blank human plasma; (B) blank human plasma spiked with tamsulosin (0.01 ng/mL) and IS (100 ng/mL); (C) human plasma sample 10 h after administration of a single 0.2 mg oral dose of tamsulosin. MRM transitions for tamsulosin and IS are 409.2 → 228.1 m/z and 256.2 → 167.2 m/z, respectively.

Table 1
Precision and accuracy of the LC–MS/MS assay method for plasma tamsulosin.

Targeted concentration (ng/mL)	Intra-day ($n = 5$)			Inter-day ($n = 5$)		
	Measured concentration (ng/mL)	Accuracy (%)	Precision (CV%)	Measured concentration (ng/mL)	Accuracy (%)	Precision (CV%)
0.01	0.010 ± 0.001	100.1	6.9	0.010 ± 0.000	98.9	3.6
0.03	0.029 ± 0.002	97.7	5.4	0.031 ± 0.002	102.1	5.6
0.9	0.95 ± 0.02	106.1	1.7	0.93 ± 0.02	103.7	2.1
18	18.5 ± 0.2	103.0	1.1	18.2 ± 0.4	101.4	2.0

Table 2
Stability of tamsulosin in human plasma ($n = 3$).

Targeted concentration (ng/mL)	Measured concentration (ng/mL)	Accuracy (%)	Precision (CV%)
Post-preparative stability			
0.03	0.029 ± 0.001	95.0	2.1
0.9	0.95 ± 0.01	105.5	1.5
18	18.5 ± 0.2	102.6	0.8
Short-term stability			
0.03	0.031 ± 0.003	103.6	8.2
0.9	0.96 ± 0.01	106.6	1.2
18	18.8 ± 0.4	104.6	2.1
Freeze–thaw stability			
0.03	0.031 ± 0.002	104.7	7.6
0.9	0.99 ± 0.02	110.2	2.2
18	19.4 ± 0.4	108.0	2.1
Long-term stability			
0.03	0.028 ± 0.004	93.4	13.5
0.9	0.92 ± 0.01	101.9	1.2
18	18.2 ± 1.3	101.1	7.4

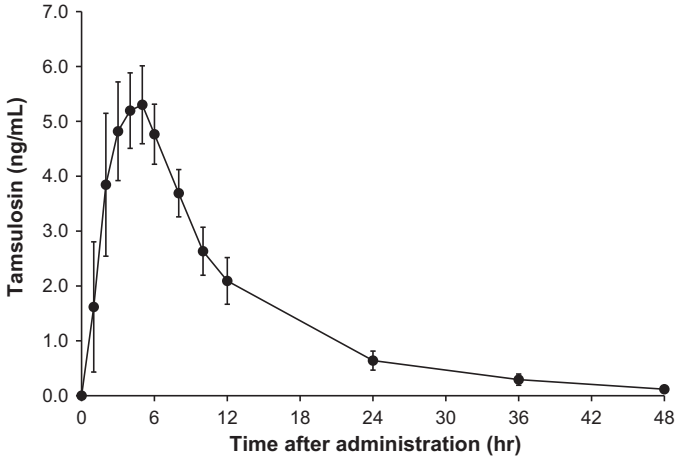

Fig. 3. Plasma concentration–time profile of tamsulosin after the administration of a single 0.2 mg oral dose of tamsulosin in healthy male subjects ($n = 11$).

lots of plasma were <5%, indicating the absence of co-eluting substances capable of influencing the ionization of either the analyte or IS [22]. This result indicates that the extraction efficiency for the analyte using liquid–liquid extraction was satisfactory, consistent, and concentration-independent. In addition, these results showed that ion suppression or enhancement from the plasma matrix was consistent utilizing the above protocol.

3.2.5. Linearity

The LLOQ was 0.01 ng/mL, and the signal-to-noise ratio of LLOQ was >10. The intra- and inter-day CV was no greater than 7% (Table 1). This value is at least 5-fold lower than that in other previously published reports [13,19]. The standard calibration curves were linear over tamsulosin concentration ranges in human plasma of 0.01–20 ng/mL, with a mean correlation coefficient (r) >0.999 ($n = 5$). The linear fit and least-squares residuals for the calibration curve were processed with a $1/x$ weighting factor.

3.2.6. Stability

Three freeze–thaw cycles of the QC samples did not appear to affect the quantification of tamsulosin. The QC samples were stored in a freezer at −70 °C and remained stable for at least 30 days. Thawing the frozen samples and maintaining them at room temperature for 4 h had no effect on quantification. The extracted samples were also analyzed after at least 24 h at 4 °C (Table 2). These results suggest that human plasma samples containing tamsulosin can be handled under normal laboratory conditions without any significant compound loss.

3.3. Pharmacokinetic application

Our method was applied successfully to a pharmacokinetic study of tamsulosin after the administration of a single oral dose of 0.2 mg tamsulosin. Fig. 3 shows the mean plasma concentration–time profile of tamsulosin. The mean C_{max}, T_{max}, $t_{1/2}$, and AUC_{inf} for tamsulosin in the 11 healthy male volunteers were 5.6 ± 0.6 ng/mL, 4.1 ± 1.1 h, 9.3 ± 2.8 h, and 66.6 ± 10.0 ng h/mL, respectively.

4. Conclusions

We have developed and validated a simple, rapid, and sensitive analytical LC–MS/MS method for the determination of tamsulosin levels in plasma samples with an LLOQ value of 0.01 ng/mL. This method was sufficiently sensitive for analyzing tamsulosin in human plasma for at least 48 h after the administration of a single oral dose of 0.2 mg of tamsulosin. Taken together, the lower LLOQ, shorter run time, and simplified sample extraction procedure makes our new method particularly suitable for use in routine assays compared with previous methods.

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