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A Liquid Chromatography Tandem Mass Spectrometry Method for the Quantification of Tamsulosin: Application to a Pharmacokinetic Study in Healthy Human Subjects

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ABSTRACT

A simple, rapid and sensitive high throughput liquid chromatographic method coupled with tandem mass spectrometry (LC–MS/MS) has been developed and validated to quantify tamsulosin in human plasma using tamsulosin D4 as internal standard (I.S). The analyte and internal standard were extracted from 300 µL plasma via solid phase extraction and were separated on a Kromasil C18 column (100 × 4.6 mm, 5 µ) with isocratic elution using acetonitrile: 5 mM ammonium formate (70: 30, v/v) containing 0.05 % formic acid as mobile phase. Tamsulosin was quantified using a triple quadruple mass spectrometer operated in multiple-reaction-monitoring (MRM) mode using positive electrospray ionization. The mass transitions m/z 409.2→228.0 and m/z 413.2→228.0 were used to measure tamsulosin and tamsulosin D4 respectively. The calibration curves were linear ($r^2 > 0.99$) over the concentration of 0.1-89.4 ng/mL, where the regression model (1/x2) was best fitted. The intra- and inter-day batches precision (%CV) and accuracy values were found to be within the assay variability limit as per the FDA guidelines. The validated method was successfully applied to a pharmacokinetic bioequivalence study in human volunteers and found selective, sensitive and robust in quantitative measurement of tamsulosin in plasma samples.

Keywords: Tamsulosin, Solid-phase extraction, LC-MS/MS, Bioanalysis, Pharmacokinetic study

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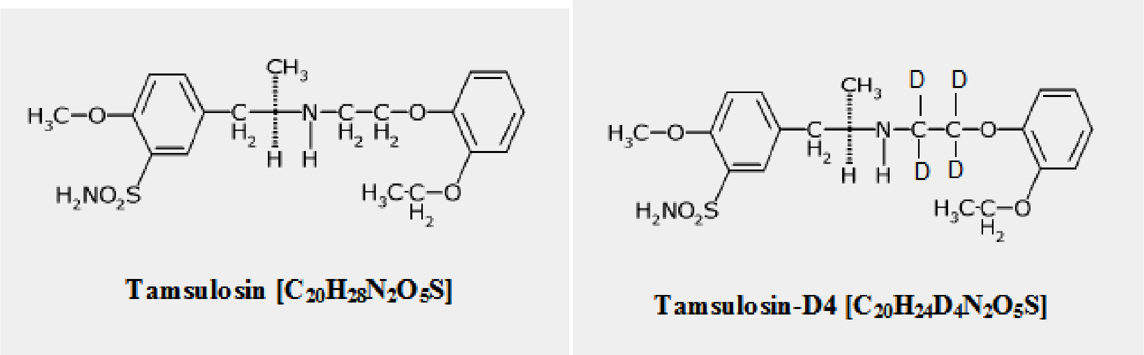
INTRODUCTION

Benign prostatic hyperplasia (BPH) is a progressive disease that is commonly associated with bothersome lower urinary tract symptoms (LUTS) such as urinary frequency, urgency, nocturia, decreased and intermittent force of stream and the sensation of incomplete bladder emptying[1]. The precise molecular etiology of this hyperplastic process is uncertain. The observed increase in cell number may be because of epithelial and stromal proliferation or to impaired programmed cell death or apoptosis leading to cellular accumulation. Androgens, estrogens, stromal–epithelial interactions, growth factors and neurotransmitters may play a role, either singly or in combination, in the etiology of the hyperplastic process[2]. Tamsulosin hydrochloride, (-)-(R)-5-[2-[[2-(o-ethoxyphenoxy) ethyl] amino] propyl]-2- methoxy benzene sulfonamide monohydrochloride [Figure 1], is indicated for the treatment of Lower Urinary Tract Symptoms (LUTS) associated with benign prostatic hyperplasis (BPH). After a single oral dose of 0.4 mg Tamsulosin hydrochloride in the fasted state, the plasma concentration of tamsulosin gradually increased reaching a C_{max} of approximately 6 ng/mL at a median time of 6 hours. The absolute bioavailability of tamsulosin ranged 55-59%[3].



Tamsulosin [$C_{20}H_{28}N_2O_5S$]

Tamsulosin-D4 [$C_{20}H_{24}D_4N_2O_5S$]

Figure 1: Chemical Structure of Tamsulosin and Tamsulosin-D4 (Internal Standard)

The current focus of pharmaceutical industry is on development and validation of bio-analytical methods which are more sensitive, precise, having less sample run time with wide range of linearity. Over the years, only few analytical methods were reported for the determination of tamsulosin in biological samples[4,5,6,7,8,9,10,11,12.] These published analytical methods were based on liquid-liquid extraction techniques for sample preparation and were performed using structural analogous of tamsulosin as internal standard. These reported methods either don’t have a high sensitivity to detect accurately and precisely the limit of quantification <0.1 ng/ml or having shorter validated linearity range for reliable estimation of tamsulosin in human plasma.

The currently proposed method has several merits over those reported earlier: (a) employing a single-step solid phase extraction procedure minimizes the chances of errors, saves considerable time and simplifies the sample preparation procedure (b) method has a wider analytical range (0.1-89.4 ng/mL) which make it suitable for pharmacokinetic and toxicokinetic studies (c) use of a stable isotopically labeled internal standard compensated for any possible matrix effect and improved robustness of the method (d) a greater sensitivity is achieved (0.1 ng/mL) even with low plasma volumes (e) low plasma volume requirements in sample processing significantly reduced the total blood withdrawn from pharmacokinetic studies and make it possible to introduced additional time point in sampling schedule and (f) rapid sample analysis turnaround time of 3.0 minutes makes it an attractive procedure in high-throughput bioanalysis of tamsulosin in human plasma. This method was fully validated and applied to a bioequivalence study in healthy volunteers after oral administration of tamsulosin 0.4 mg extended release capsules in healthy volunteers.

MATERIALS AND METHOD

Chemicals and materials

Working standards of tamsulosin (99.9%) and tamsulosin- D4 (98.12%) for use as an internal standard (ISTD) were procured from Vivan Life Sciences India and Clearsynth Labs (P) Ltd, India ,respectively. Ammonium formate of AR grade was obtained from FLUKA (Sigma-Aldrich, Steinheim, USA). Acetonitrile and methanol of HPLC grade was obtained from Spectrochem (Mumbai, India) and Fisher Scientific (Mumbai, India), respectively. Bond Elut Plexa 30 mg, 1 cc, solid phase extraction cartridges were obtained from Agilent Technologies India Pvt. Ltd. All aqueous solutions and buffers were prepared using water that was purified using Milli-Qs Gradient A10s (Millipore, MoscheimCedex France). Different individual lots of K_2EDTA (ethylene diamine tetraacetic acid dipotassium salt) human plasma, which were used to prepare calibration standards and quality control (QC) samples, were obtained from Mediplas Laboratories (Hyderabad, India).

Preparation of stock solution, calibration standards and quality control samples

The standard stock solutions of 1 mg/mL of tamsulosin and tamsulosin- D4 were prepared by dissolving requisite amount in methanol. Working solutions of tamsulosin (ranging from 5 to 4500 ng/mL) were prepared by serial dilution of the stock solution in methanol–water (50:50, v/v). Calibration standards and quality control (QC) samples were prepared by spiking (2 % total volume of blank plasma) blank plasma with stock solution. Calibration curve standards were made at 0.1, 0.2, 2.0, 14.9, 33.8, 56.3, 76.0 and 89.4 ng/mL respectively while quality control samples were prepared at four levels, viz. 70.2 ng/mL (HQC, high quality control), 35.1 (MQC, middle quality control), 0.3 ng/mL (LQC low quality control), 0.1 ng/mL (LOQQC limit of quantization quality control). The ISTD working solution (80 ng/mL) for routine use was prepared by diluting the tamsulosin-D4 stock solution in methanol-water (50:50, v/v). All the stock solutions were stored at 2-8°C and the spiked calibration curve standards along with quality control samples were stored at around -20 °C ± 10 °C until use.

Sample preparation

Plasma samples stored at around -20 °C ± 10 °C were thawed on the day of extraction at room temperature. To ensure homogeneity in content, samples were allowed to vortex. An eppendorf pipette was used to aliquot 300 µL of spiked plasma samples in to micro centrifuge tubes. Into each tube 50 µL of internal standard working solution (80 ng/mL) and 300 µL of 0.1 N hydrochloric acid solutions was added with the help of a multi stepper. After each addition tubes were allowed to vortex for 30 seconds. The contents of micro centrifuge tubes were then loaded on Bond Elut Plexa 30 mg, 1 mL extraction cartridge. Before loading of the samples, extraction cartridges were conditioned with 1 mL of methanol followed by 1 mL of water. The entire samples were allowed to pass through the extraction cartridge under a constant pressure using positive-pressure solid phase extraction unit. After the samples passed through, cartridges were washed with 1 mL of water twice and than were allowed to dry under a constant pressure and finally, the analyte and ISTD were eluted with 1 mL of methanol. The extracted samples were evaporated to dryness using a Zymark Turbo-Vap LV evaporator (Caliper, Hopkinton, MA, USA) and reconstituted with 500 µL of the mobile phase. The reconstituted samples were transferred to auto sampler vials and 15 µL was injected in to the LC–MS/MS system for analysis. The sample preparation was carried out under monochromatic light condition.

LC/ESI-MS/MS instrumentation and analytical conditions

The liquid chromatography separation was performed using a Shimadzu scientific instruments (Shimadzu Corporation; Kyoto, Japan) consisted of two LC-20AD delivery pumps, a non-line DGU-20A3 prominence solvent degasser, an SIL-HTc Shimadzu auto sampler and a CBM-20A prominence column oven. Liquid chromatographic separations were achieved using Kromasil C-18 column (100 × 4.6 mm, 5 µm) (Sigma-Aldrich, USA). An injection volume of 15 µL was used for each analysis. Mobile phase consisted of acetonitrile: 5 mM ammonium formate (70: 30, v/v) containing 0.05 % formic acid. The flow rate of the mobile phase was set at 1.0 mL/min. The column-oven and auto-sampler temperatures were maintained at 40 ± 2 °C and 10 ± 2 °C, respectively. Samples were analyzed with an API-3000 triple quadrupole mass spectrometer (MDS Sciexs; Toronto, Canada) equipped with an electrospray ionization source operating in positive ion mode. Nitrogen was used as the nebulizer, auxillary, collision and curtain gas. Analytes were detected by tandem mass spectrometry using multiple reaction monitoring (MRM) of precursor-product ion transitions with 200 ms dwell time, at m/z 409.2/228.0 for tamsulosin and m/z 413.2/228.0 for tamsulosin-D4. The instrument response was optimized by syringe pump infusion of tamsulosin and tamsulosin-D4 in the mobile phase by constant flow (10 µL/min) in to the stream of the mobile phase eluting from the LC column. The main working source/gas parameters of the mass spectrometer were optimized and maintained (Table 1). Calibration curves were constructed by calculating the analyte to ISTD peak area ratio (y) against analyte concentrations (x). Data acquisition and processing were performed using Analyst version 1.4.1 software (MDS Sciexs; Toronto, Canada).

Table 1 Mass spectrometry conditions for tamsulosin & tamsulosin-D4

Parameter	**Value**
Source temperature (◦C)	450
Dwell Time (msec)	200
Collision Activated Dissociation (CAD) gas	3
Curtain Gas (psi)	10
Nebulizer Gas (NEB) (psi)	13
Turboion Spray (IS) Voltage (V)	2000
Declustering Potential (DP) (V)	46
Entrance Potential (EP) (V)	10
Focusing Potential (FP) (V)	150
Collision Energy (CE) (V)	33
Collision Cell Exit Potential (CXP) (V)	14
Ion transition for (m/z)	409.2/228.0 (Tamsulosin)
Ion transition for (m/z)	413.2/228.0 (Tamsulosin-D4)

Method Validation

The method was validated to meet the acceptance criteria of industrial guidance for the bioanalytical method validation (FDA 2001). The method was validated for selectivity, sensitivity, linearity, accuracy, precision, recovery, matrix effect and stability of analyte during both short-term sample processing and long-term storage.

Selectivity

The selectivity of the method towards endogenous plasma matrix components and concomitant medications was assessed in eight lots of blank human plasma (six normal, one lipemic and one haemolyzed). They were processed and analyzed using the proposed extraction protocol and the set chromatographic conditions for tamsulosin at the lower limit of quantitation (LLOQ) level.

Linearity and LLOQ

The linearity of the method was determined by analysis of standard plots associated with an eight point standard calibration curve ranging 0.1-89 ng/mL. Calibration curves from three precision and accuracy batches were used to establish linearity. Peak area ratios of analyte/ISTD obtained from MRM were utilized for the construction of calibration curves using weighted (1/x2) linear least squares regression. Back calculations were made from these curves to determine the concentration of tamsulosin in each calibration standards and the resulting calculated parameters were used to determine concentrations of analyte in quality control or unknown samples. The correlation coefficient (r^2) >0.98 was desirable for all the calibration curves. The lowest standard on the calibration curve was to be accepted as the LLOQ, if the analyte response was at least five times more than that of drug free (blank) extracted plasma. In addition, the analyte peak of LLOQ sample should be identifiable, discrete, and reproducible with accuracy within ± 20.0% and a precision ≤ 20.0%. The deviation of standards other than LLOQ from the nominal concentration should not be more than ± 15.0%.

Accuracy and precision

Intra- and inter-day accuracies were expressed as a percentage of deviation from the respective nominal value and the precision of the assay was measured by the percent coefficient of variation (%CV) at concentrations. Intra-day precision and accuracy were assessed by analyzing six replicates of the quality control samples at four levels during a single analytical run. The inter-day precision and accuracy were assessed by analyzing 18 replicates of the quality control samples at each level through three precision and accuracy batches runs on 2 consecutive validation days. The deviation at each concentration level low, middle and high QC (0.3, 35.1 and 70.2 ng/mL) from the nominal concentration was expected to be within ± 15.0% except lower limit QC (0.1 ng/mL), for which it should not be more than 20.0%. Similarly, the mean accuracy should not deviate by ± 15.0% except for the LLOQC where it can be ± 20.0% of the nominal concentration.

Recovery

The relative extraction recoveries for the analyte and ISTD at low, middle and high QC (0.3, 35.1 and 70.2 ng/mL) concentration levels were determined by measuring the mean peak area response of six replicates of extracted quality control samples against the mean peak area response of six replicates each of three neat solutions containing analyte and a neat solution containing ISTD at concentrations equivalent to those obtained in the final extracted concentration for the analyte and ISTD in the quality control samples.

Matrix effect and matrix factor

Matrix effect (ME) is the suppression or enhancement of ionization of analyte by the presence of matrix components in the biological samples. The quantitative measurement of matrix effect can be termed as matrix factor (MF) and defined as a ratio of the analyte peak response in the presence of matrix ions to the analyte peak response in the absence of matrix ions.

The %ME were obtained from the mean of the MF as

$$\%ME = (1 - \text{Mean of the MF}) * 100$$

Preparation of matrix effect samples was done by processing and extracting two samples from each of six normal plasma batches along with one haemolysed and one lipemic plasma batches. The extracted samples were dried and one extracted sample of each plasma batch was reconstituted with 500 µL mobile phase solution pre-spiked with appropriate concentration of tamsulosin at low QC level and IS (Tamsulosin-D4) & other was reconstituted with 500 µL mobile phase solution pre-spiked with appropriate concentration of tamsulosin at high QC level and IS (Tamsulosin-D4). Two replicate injections of each sample were analyzed along with six replicate injections of aqueous sample representing the concentration of tamsulosin at the level of low QC and high QC & IS (Tamsulosin-D4). The variability of MF was measured as coefficient of variation and acceptance of results was predefined as %CV of MF that should be ≤15% and the %ME that should not be greater than 15% at the low & high QC concentrations.

Stability

The absolute stability of tamsulosin in plasma was assessed by analyzing six replicates of QC samples at low and high concentrations of 0.3 and 70.2 ng/mL during the sample storage and processing procedures. Bench top stability was assessed at room temperature for approximately 5 h, which exceeds the residence time of the sample processing procedures. The freeze-thaw stability was evaluated after undergoing three freeze (at -20 °C)-thaw cycles. Long-term stability was assessed after storage of the test samples at -20 °C for 54 days. The auto-sampler storage stability was determined by storing the reconstituted QC samples for approximately 77 h under auto-sampler condition (maintained at 10 °C) before being analyzed. All stability exercises were performed against freshly spiked calibration standards processed along with freshly spiked six replicates of QC samples at low and high concentrations which act as comparison samples for determining the percentage absolute stability of tamsulosin during the sample storage and processing procedures. The analyte was considered stable in plasma at each concentration if the mean calculated concentration of stability samples does not deviate by ± 15.0% of the mean calculated concentration of comparison quality control samples. The working solutions and stock solutions of tamsulosin and ISTD were also evaluated for their stability at room temperature for 8 h and at refrigerator temperature (2-8 °C) for 23 days, respectively.

Method application

A bioequivalence study between reference product (Flomax® capsules) and test product (0.4 mg extended release capsules, manufactured in India) was conducted on 14 healthy, adult, male, human subjects under fasting conditions. Each volunteer was judged to be in good health through medical history, physical examination and routine laboratory tests. Written consent was taken from all the volunteers after informing them about the objectives and possible risks involved in the study. An independent ethics committee constituted as per Indian council of Medical Research (ICMR) approved the study protocol. The study was conducted strictly in accordance with guidelines laid down by international conference on Harmonization and USFDA. Subjects were randomly assigned for this open label, balanced, randomized, two-treatment, two- period, two-sequence, single-dose, crossover study. A single oral dose of 0.4 mg extended release capsules was given to the volunteers with 240 mL of water in each period. Two periods were separated with a wash out period of 7 days. Blood samples were collected at pre-dose and at 1, 2, 2.5, 3, 3.5, 4, 4, 5, 5.5, 6, 6.5, 7, 8, 9, 10, 12, 16, 24, 36, 48 and 72 h post-dose in each period into K_2EDTA vacutainers. Plasma was separated by centrifugation (3200 × g, 10 ° C and 10 min) and kept frozen at – 20 ° c until analysis. During study, volunteers had a standard diet while water intake was free.

RESULTS AND DISCUSSION

Optimization of the mass spectrometric condition

Tamsulosin has amino functionality in its chemical structure; certainly the mass spectrometric detection in positive ion mode was evaluated using both electro spray ionization (ESI) as well as atmospheric pressure chemical ionization (APCI). The signal intensity obtained in the positive mode with ESI interface is much higher than that of APCI. The Q1 ESI spectra were dominated by the protonated parent ion $[M+H]^+$ at m/z 409.2 for tamsulosin and 413.2 for tamsulosin- D4. The MS/MS parameters, including the declustering potential (DP), focusing potential (FP), collision cell exit potential (CXP), gases (NEB, CAD) and collision energy (CE), were optimized to identify the most stable and intense product ion for the analyte and ISTD. The product ions at m/z 227.99 for tamsulosin and tamsulosin-D4 were observed with higher abundance and greater stability. Figure 2 shows the precursor and product ion spectra for tamsulosin. Use of ammonium formate solution of strength 5 mM in the mobile phase enhances the occurrence of $[M+H]^+$ and eventually results in an improvement in area response for both the analyte and ISTD.

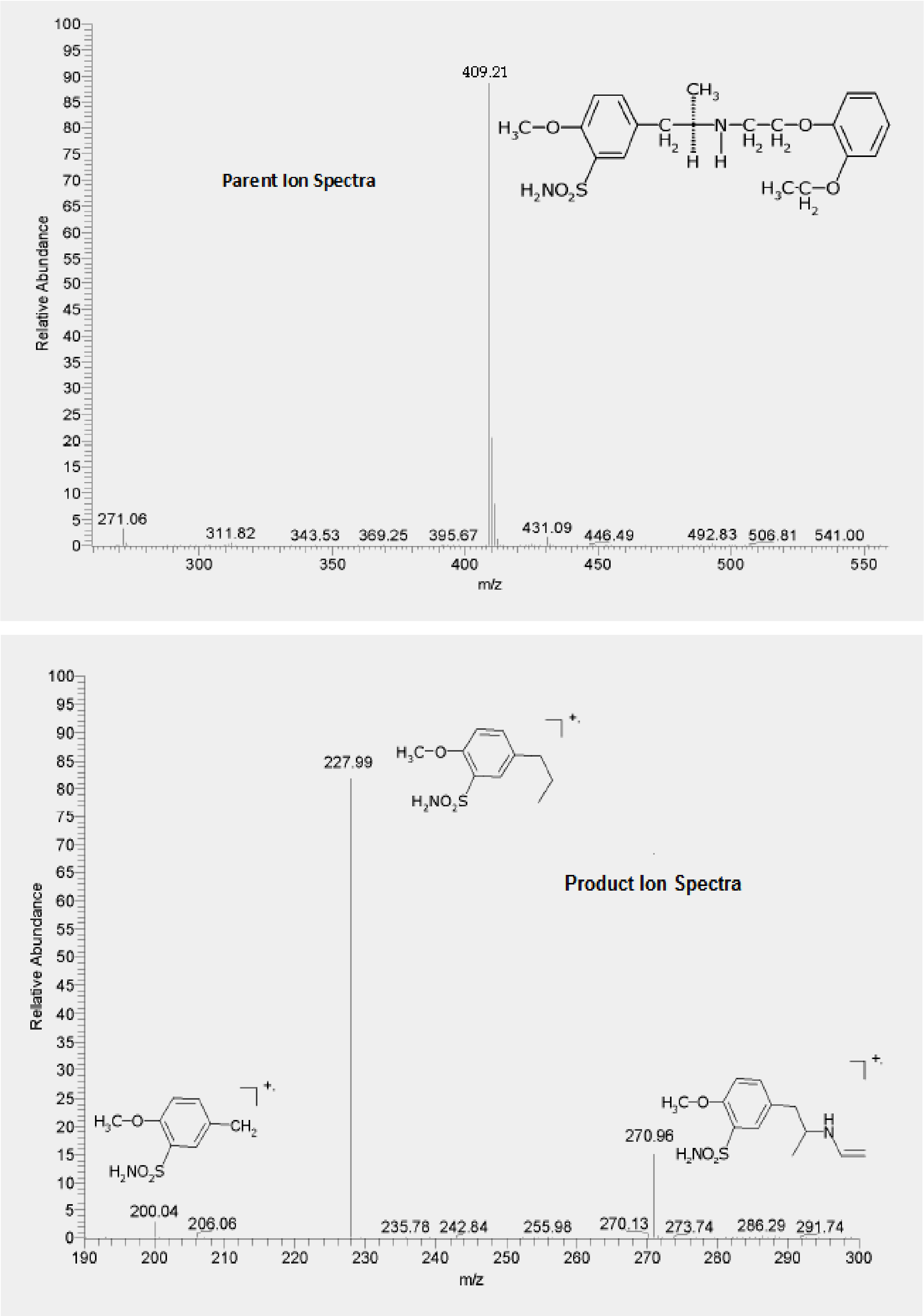


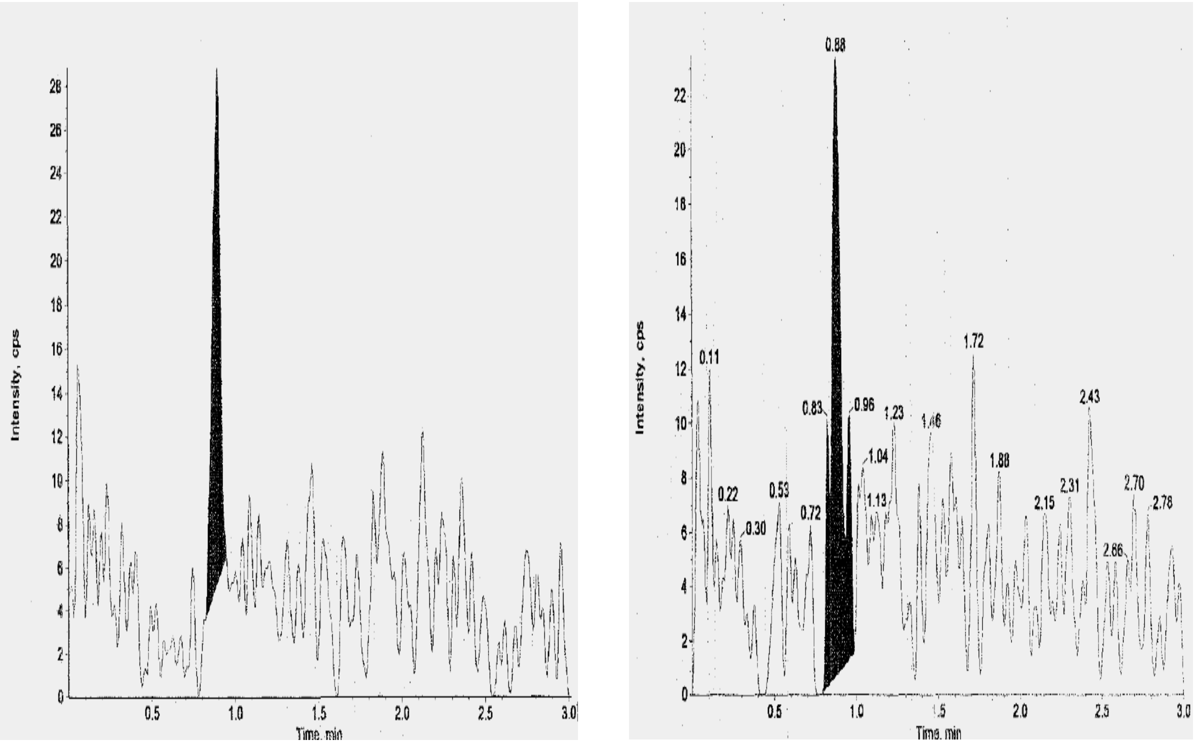
Figure 2: (a) Parent and (b) Product Ion Spectra of Tamsulosin

Optimization of chromatographic condition

A number of reversed-phase columns, such as Ascentis-C18, Hypersil-C18, ZorbaxSB-CN, Chromolith SpeedROD, Kromasil C18 and Phenomenex Luna, were tested to obtain optimal selectivity, efficiency, suitable retention time and symmetric peak shape for the analyte and ISTD. In industrial research cutting of the separation time is always in focus in order to attain the high throughput. The Kromasil C18 column (50×4.6 mm i.d.) was selected since it provided good peak shape and better sensitivity with greater signal to noise(S/N) ratio. The Kromasil C18 columns are composed of porous silica with 5µ particle size, which facilitates fast mass transfers and results in minimizing the total analytical run time to <3.0 min. Chromatographic separation was tried using various combination of methanol/acetonitrile, acidic buffers and additives like formic acid. The mobile phase consisting of acetonitrile, 5 mM ammonium formate solution and formic acid gave higher signal intensity. Acetonitrile was chosen as organic phase over methanol as it revealed a higher mass spectrometric response and with minimal background noise. Change in percentage of acetonitrile in the mobile phase was found to have profound influence on retention time and peak shape. Thus a mobile phase consisting of acetonitrile: 5 mM ammonium formate (70: 30, v/v) containing 0.05 % formic acid was used in the experiment. Under these conditions, tamsulosin and tamsulosin-D4 elute at retention time of approximately 0.90 min, Figure 3.

Tamsulosin **Tamsulosin-D4**

(a)



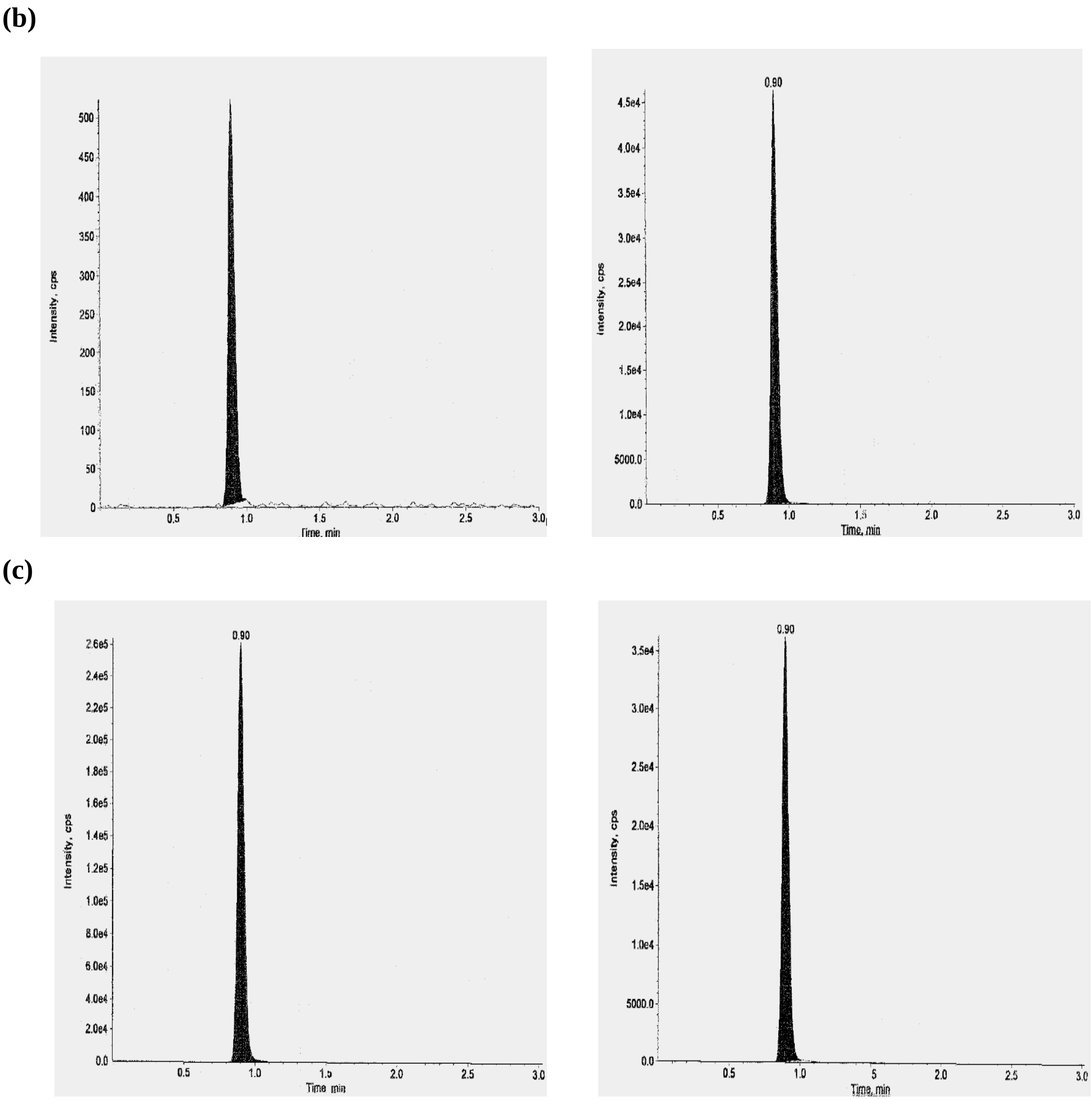


Figure 3: Representative Chromatograms of Tamsulosin and Tamsulosin-D4, (a) Blank Sample (b) Lower limit of quantification sample (LLOQ) and (c) Upper limit of quantification sample (ULOQ)

Sample preparation optimization

Sample clean-up was focused on solid phase extraction since this technique mostly provides the cleanest sample compared to protein precipitation or liquid–liquid extraction. During initial stages of the method development, three reversed-phase type solid phase polymeric supports (Waters Oasis HLB; Agilent Bond Elut Plexa; and Agelas Cleanert PEP-H) were tested for the extraction of analyte and ISTD from human plasma. All the three polymeric supports nearly produced comparable results. Owing to more economic, in this work we had utilized Agilent Bond Elut Plexa cartridges for isolation of analyte and ISTD from the plasma samples. Furthermore, the use of a stable isotopically labeled internal standard compensated for any possible matrix effect and improved robustness of this method. The extraction procedure described here offers a rapid way to isolate analyte and ISTD from plasma matrix.

Method validation

Selectivity

Selectivity of the method was ascertained in different batches of human plasma by comparing the chromatograms of blank plasma samples with the corresponding spiked LLOQ plasma samples. Figure 3 shows the typical chromatograms of a blank, blank spiked with ISTD, a spiked plasma sample with tamsulosin at low and upper limit of quantitation (LLOQ and ULOQ) level. No significant interference from endogenous components was observed at the retention time of the analyte and ISTD.

Linearity and sensitivity

The linearity of the method was determined by analysis of linear curves (n=5) containing eight non-zero concentration (Table 2). The ratio of area response for drug and IS was used for regression analysis. Each calibration curve was analyzed individually by using least square weighed (1/x2) linear regression. The developed and validated method was found linear over the concentration range of 0.1–89.4 ng/mL ($r^2 > 0.99$). The lower limit of quantification was 0.101 ng/mL and the lower limit of detection (based on a signal: noise ratio, S/N=3) was less than 0.07 .

Table 2. Precision and accuracy data of back-calculated concentration of calibration samples for tamsulosin from five representative calibration curves

Concentration added (ng/mL)	Concentration found (ng/mL) (mean ± SD)	Precision	Accuracy
0.101	0.099 ± 0.002	2.0	98.0
0.202	0.209 ± 0.009	4.1	103.5
2.020	2.171 ± 0.039	1.8	107.5
14.850	15.833 ± 0.314	2.0	106.6
33.751	32.140 ± 0.684	2.1	95.2
56.252	55.604 ± 0.406	0.7	98.8
76.016	74.087 ± 1.617	2.2	97.5
89.431	83.025 ± 1.273	1.5	92.8

Precision and accuracy

Precision and accuracy of the method were determined by analyzing quality control samples at four concentrations (0.1, 0.3, 35.1 and 70.2 ng/mL) within the calibration curve range to validate reproducibility. Table 3 summarizes inter-day (n=6) and intra-day (n=18) precision and accuracy values for quality control samples. The intra- and inter-day %CV ranged from 2.1-5.2% and 2.2-4.7%, respectively. Intra-and inter-day accuracies were less than ± 5.0% at all analyte concentrations. These results indicate that the purposed method has good accuracy, precision and reproducibility.

Table 3. Intra- and inter-day accuracy and precision of the method

Nominal Concentration (ng/mL)	Intra-day (n = 6)			Inter-day (n = 18)		
	Average ± SD (ng/mL)	Accuracy (%)	CV (%)	Average ± SD (ng/mL)	Accuracy (%)	CV (%)
0.102 (LOQQC)	0.100 ± 0.005	98.2	5.2	0.101 ± 0.005	99.2	4.7
0.295 (LQC)	0.302 ± 0.011	102.3	3.5	0.299 ± 0.012	101.6	3.9
35.108 (MQC)	34.148 ± 0.633	97.3	2.1	32.981 ± 0.748	96.8	2.2
70.216 (HQC)	66.989 ± 1.619	95.4	2.7	67.982 ± 1.904	96.8	2.8

Recovery

The extraction recovery (mean ± SD) for tamsulosin at three QC concentration levels 0.3, 35 and 70 ng/ mL were 63.4±4.5, 62.7±9.5 and 61.6±1.0, respectively. The mean recovery for the tamsulosin-D4 at the concentration employed was 67.1±2.7.The result indicates that the extraction efficiency for tamsulosin using solid phase extraction was satisfactory, consistent and was concentration independent.

Matrix effect and matrix factor

To ensure any ion suppression or enhancement, matrix effect as well as matrix factor on the presented method was evaluated using the procedure described above. Results of the exercise summarized in Table 4 and were well in predefined acceptance criteria. The mean absolute MF at the low and high QC concentrations from eight lots of plasma samples (six lots of normal, one lot of heamolysed and one lot of lipemic plasma) were 0.92 and 0.96 for tamsulosin and 0.94 and 0.97 for tamsulosin-D4, respectively. The % CVs of MF and %ME were <2.4% and <7.9%, respectively. These results showed that ion suppression or enhancement from the plasma matrix was negligible under the current conditions.

Stability studies

The absolute stability of tamsulosin was investigated at two concentration levels of QC samples (low and high). The stability studies include auto-sampler, bench-top, freeze/thaw and long-term stability. The stability results summarized in Table 5 showed that tamsulosin spiked in to human plasma was stable for at least 5.2 h on bench at room temperature, for at least 77 h in the mobile phase at 10 °C under auto-sampler storage condition, for 54 days at around -20 °C and during three freeze-thaw cycles. The stock solutions of tamsulosin and ISTD were found stable at refrigerator temperature (between 2 and 8 °C) for 23 days and the working solutions of tamsulosin and ISTD were found stable for about 8 h at room temperature (results not shown).

Table 4 Assessment of matrix factor (MF) in 8 different lots of human plasma at LQC and HQC levels (n = 2)

Lots of human Plasma	Tamsulosin		Tamsulosin-D4	
	LQC (0.295 ng/mL)	HQC (70.216 ng/mL)	LQC*	HQC*
Lot#1	0.92	0.98	0.96	0.99
Lot#2	0.89	0.97	0.90	0.97
Lot#3	0.94	0.97	0.96	0.98
Lot#4	0.91	0.95	0.93	0.95
Lot#5	0.91	0.98	0.94	0.97
Lot#6	0.96	0.93	0.96	0.95
Lot#7b	0.91	0.96	0.92	0.93
Lot#8c	0.93	0.97	0.94	0.98
Mean	0.92	0.96	0.94	0.97
SD (±)	0.022	0.017	0.022	0.020
CV (%)	2.4	1.7	2.3	2.1
Matrix Effect (%)	7.9	3.6	6.1	3.5

b- heamolysed plasma lot; c- lipemic plasma lot

*Tamsulosin-D4: 80.0 ng/mL

Table 5 Stability data for tamsulosin in human plasma under various conditions (n = 6)

QC Samples	Nominal Concentration (ng/mL)	Average ± SD	Accuracy (%)	CV (%)	Absolute stability (%)
Freeze/thaw stability (three freeze-thaw cycles at -20 °C)					
LQC [S]	0.295	0.301 ± 0.002	101.9	0.6	98.3
LQC [C]	0.296	0.307 ± 0.005	103.7	1.6	
MQC [S]	35.108	33.107 ± 0.510	94.1	1.9	97.8
MQC [C]	35.231	33.944 ± 0.477	96.3	1.4	
HQC [S]	70.216	66.032 ± 0.320	94.0	0.5	100.2
HQC [C]	70.461	66.153 ± 0.801	93.9	1.2	
Bench top stability (5.2 h at room temperature)					
LQC [S]	0.295	0.301 ± 0.008	101.9	2.7	96.4
LQC [C]	0.293	0.309 ± 0.006	105.7	1.9	
MQC [S]	35.108	33.231 ± 0.419	94.7	1.7	97.5
MQC [C]	34.853	33.848 ± 0.385	97.1	1.1	
HQC [S]	70.216	65.621 ± 1.231	93.5	1.9	97.8
HQC [C]	69.706	66.597 ± 0.391	95.5	0.6	
In-injector stability (77 h)					
LQC [S]	0.295	0.305 ± 0.007	10.34	2.3	102.6
LQC [C]	0.293	0.295 ± 0.005	100.8	1.6	
MQC [S]	35.108	33.387 ± 0.501	95.1	1.7	98.2
MQC [C]	34.853	33.740 ± 0.405	96.8	1.2	
HQC [S]	70.216	65.751 ± 0.244	93.6	0.4	98.8
HQC [C]	69.706	66.050 ± 0.655	94.8	1.0	

Long-term stability (54 days)					
LQC [S]	0.295	0.299 ± 0.005	101.2	1.6	101.1
LQC [C]	0.293	0.293 ± 0.007	100.1	2.3	
MQC [S]	35.108	32.988 ± 0.504	94.0	1.6	96.2
MQC [C]	34.936	34.118 ± 0.262	97.7	0.8	
HQC [S]	70.216	66.675 ± 0.375	95.0	0.6	99.2
HQC [C]	69.871	66.897 ± 0.326	95.7	0.5	
Working solutions stability at room temperature: 8 hours					
Stock solutions stability at refrigerator (2-8 ◦C): 23 days					

[S]- Stability Quality Control Samples; [C] - Comparison Quality Control Samples

Method application

The validated method was successfully applied for the assay of tamsulosin in healthy male Indian volunteers in the age group of 18-45 years. Figure 4 shows the plasma concentration vs. time profile of tamsulosin in human subjects under fating condition (n=14) and the mean estimated pharmacokinetic parameters derived from the plasma concentration profiles are summarized in Table 6. The method was sensitive enough to monitor the tamsulosin plasma concentration up to 72 h. Approximately 700 samples including the calibration and QC samples were within the acceptable limits. The pharmacokinetic parameters viz. Cmax, $AUC_{0\text{-}t}$, $AUC_{0\text{-}Inf}$, T_{max}, and $T_{1/2}$ for tamsulosin in a bioequivalence study were determined using the extra-vascular input non-compartmental analysis model of WinNonlin (version 5.0.1, Pharsight, Mountain View, CA, USA).

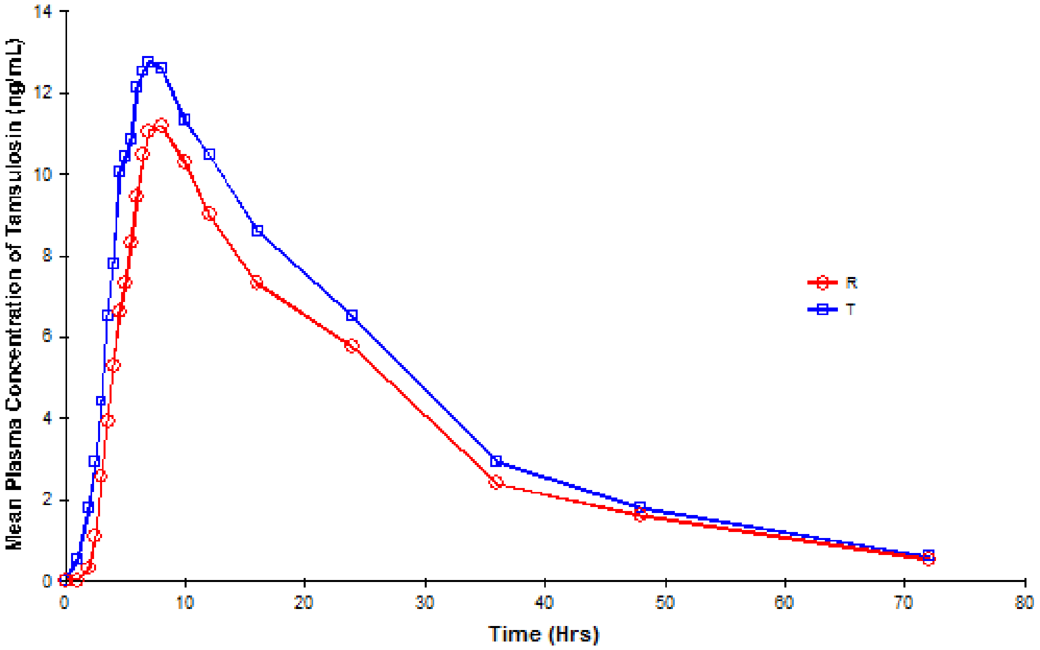


Figure 4: The mean concentration–time profile following oral administration of 0.4 mg of tamsulosin extended release (ER) capsules in healthy volunteers

Table 6 Pharmacokinetic parameters (mean ± SD) of tamsulosin, after the administration of Tamsulosin Hydrochloride 0.4 mg ER capsule (n = 14)

Parameters	**Reference**	**Test**
C_{max}	10.98 ± 3.97	12.52 ± 5.07
$AUC_{0\text{-}t}$	228.94 ± 83.96	240.48 ± 97.24
$AUC_{0\text{-}Inf}$	244.29 ± 90.57	256.93 ± 107.31
T_{max}^	7.00 (4.00 - 10.00)	7.00 (4.50 - 12.00)
Thalf ($T_{1/2}$)	11.66 ± 2.98	12.78 ± 3.19

^median (minima-maxima)

CONCLUSION

A rapid, sensitive and selective LC–MS/MS method for the quantification of tamsulosin in human plasma was developed and validated. The assay was linear from 0.1-89.4 ng/mL. Solid phase extraction methodology was adopted in plasma sample preparation, which provides consistent extraction recovery with minimal endogenous interference and matrix effect. Tamsulosin was found stable during storage, processing, and analysis in human plasma samples. The results indicate the method to be sensitive, selective, accurate and reproducible. The applicability of the method was demonstrated in a bioequivalence study of tamsulosin in healthy male volunteers.

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