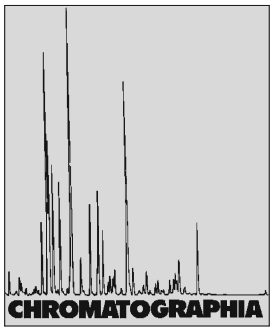
Simultaneous Determination of Tamsulosin and Dutasteride in Human Plasma by LC–MS–MS



2008, *67*, 893–903

Sangita Agarwal[1], K. Veeran Gowda[1], Amlan Kanti Sarkar[1], Debotri Ghosh[1], Uttam Bhaumik[1], Tapas Kumar Chattaraj[2], Tapan Kumar Pal[1,✉]

[1] Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700 032, West Bengal, India; E-Mail: sangita_agarwal2000@yahoo.com
[2] N.R.S. Medical College and Hospital, Kolkata, India

Received: 16 November 2007 / Revised: 8 January 2008 / Accepted: 22 February 2008
Online publication: 20 May 2008

Abstract

A sensitive and selective liquid chromatographic tandem mass spectrometric (LC–MS–MS) method was developed for simultaneous identification and quantification of tamsulosin and dutasteride in human plasma, which was well applied to clinical study. The method was based on liquid–liquid extraction, followed by an LC procedure with a Gemini C-18, 50 mm × 2.0 mm (3 μm) column and using methanol:ammonium formate (97:3, *v/v*) as the mobile phase. Protonated ions formed by a turbo ionspray in positive mode were used to detect analytes and internal standard. MS–MS detection was by monitoring the fragmentation of 409.1 → 228.1 (*m/z*) for tamsulosin, 529.3 → 461.3 (*m/z*) for dutasteride and 373.2 → 305.3 (*m/z*) for finasteride (IS) on a triple quadrupole mass spectrometer. The lower limit of quantification for both tamsulosin and dutasteride was 1 ng mL^{-1}. The proposed method enables the unambiguous identification and quantification of tamsulosin and dutasteride for clinical drug monitoring.

Keywords

Column liquid chromatography–mass spectrometry
Pharmacokinetics
Validation
Tamsulosin and dutasteride

Introduction

Benign prostatic hyperplasia (BPH) is the most prevalent urological disorder in men and is caused due to the enlargement of the prostate gland. The available epidemiological data are somewhat variable and most observers report that its prevalence ranges from 30 to 60% of men over 65 years. However, it is perhaps more noteworthy that virtually all men who reach their average life expectancy will at some point be affected by prostate disease [1].

The management of BPH has changed radically over the past 30 years [2, 3]. The surgical techniques have been refined and equipment improved, the spectrum of treatment has progressed from simple vaporization of tissue to the complete removal, or enucleation, of intact lobes of prostatic adenoma. Holmium laser enucleation of the prostate (HoLEP) combined with mechanical morcellation represents the latest refinement of holmium:YAG surgical treatment for BPH [4, 5]. Over this period there has been a major shift from surgical intervention [e.g., transurethral resection of the prostate (TURP) and transurethral incision of the prostate (TUIP), holmium:YAG laser, holmium laser enucleation of the prostate (HoLEP)] to medical management, which is now the recommended first-line management choice in uncomplicated BPH.

Medical treatment focuses on two distinct areas—reducing the size of an enlarged prostate and improving urinary flow by relaxation of the smooth muscle

DOI: 10.1365/s10337-008-0613-7
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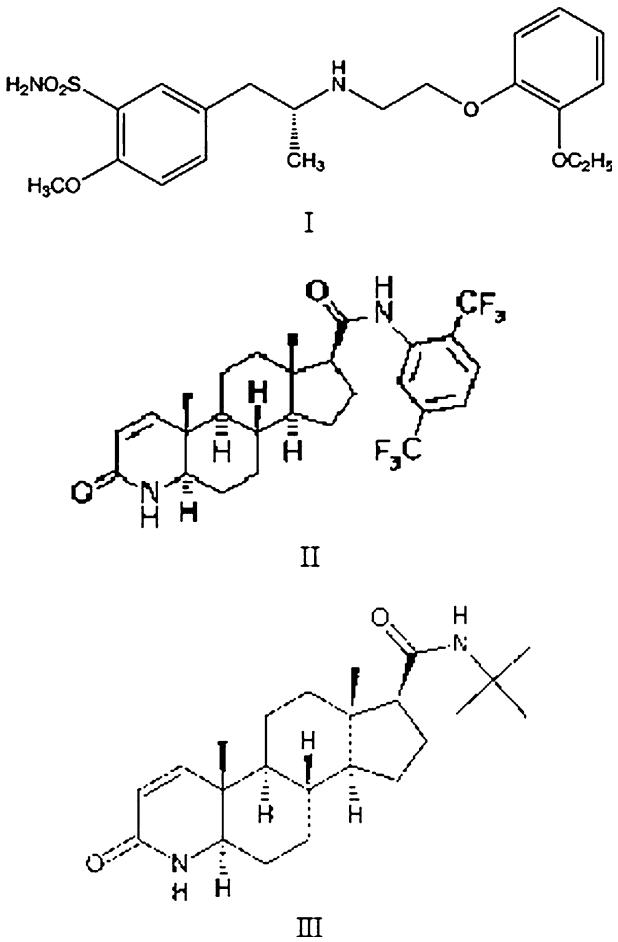

Fig. 1. Chemical structures of tamsulosin (**I**), dutasteride (**II**) and the IS (**III**)

in the prostate. Two distinct classes of agents are available to achieve these objectives, 5α-reductase inhibitors and α-blockers. Whilst α-blockers provide rapid onset of action and rapid symptomatic relief, they do not reduce the size of an enlarged prostate or alter its composition. Moreover, 5α-reductase inhibitors retard the progression of the disorder, and further reduce the likelihood of surgery. As is the case with so many other disease areas, there is growing evidence indicating a potentially valuable role for combination therapy in managing BPH [6].

Tamsulosin hydrochloride, (−)-(*R*)-5-[2-[[2-(*O*-ethoxyphenoxy)ethyl]amino] propyl]-2-methoxybenzensulfonamide (Fig. 1) hydrochloride, is a structurally new type of highly selective α_1-adrenoceptor antagonist, having a molecular weight of 408.51 [7, 8]. The drug has been used clinically for urinary obstructed patients with BPH. The α_1-adrenoceptor antagonist activity of tamsulosin hydrochloride has been found to be more potent than other drugs such as prozosin.

Soeishi et al. [9] reported a method for determination of tamsulosin by LC with fluorescence detection, but the method was influenced by interference of endogenous substances and potential loss of drug in the re-extraction procedure, and the overall plasma preparation process was tedious and time-consuming. Matsushima et al. [10] had reported an LC–MS–MS method for determination of tamsulosin in plasma dialysate, plasma and urine, in which the plasma concentration was linear over the range of 0.5–50 ng mL^{-1}. Dinga et al. [11] reported a rapid assay method for determination of tamsulosin using LC–ESI–MS.

Dutasteride is a synthetic 4-azasteroid compound (Fig. 1) that is a selective inhibitor of both type 1 and type 2 isoforms of steroid 5α-reductase (5AR), an intracellular enzyme that converts testosterone to 5α-dihydrotestosterone (DHT). Dutasteride is chemically designated as (5α,17β)-*N*-{2,5 bis(trifluoromethyl) phenyl}-3-oxo-4-azaandrost-l-ene-17-carboxamide. The empirical formula of dutasteride is $C_{27}H_{30}F_6N_2O_2$, representing a molecular weight of 528.5.

Dutasteride inhibits the conversion of testosterone to DHT. DHT is the androgen primarily responsible for the initial development and subsequent enlargement of the prostate gland. Testosterone is converted to DHT by the enzyme 5α-reductase, which exists as two isoforms, type 1 and type 2. The type 2 isoenzyme is primarily active in the reproductive tissues while the type 1 isoenzyme is also responsible for testosterone conversion in the skin and liver.

Dutasteride is a competitive and specific inhibitor of both type 1 and type 2 5α-reductase isoenzymes, with which it forms a stable enzyme complex. Dissociation from this complex has been evaluated under in vitro and in vivo conditions and is extremely slow.

Recent progress in the field of mass spectrometry (MS) has resulted in development of new techniques such as electrospray (ESI) and tandem MS (MS–MS) which has significantly improved the detection sensitivity of drugs, and quantification in the order of picograms is becoming possible. Therefore, LC–MS–MS has attracted attention as a highly sensitive and specific first-choice method for assaying clinical samples. Ramakrishna et al. [12] reported an LC–MS–MS method developed and validated for the quantitative determination of dutasteride in human plasma. In recent years, a number of laboratories have reported the use of high-throughput bioanalytical procedures using LC–MS–MS [12–15].

The purpose of this study was the identification and quantification of tamsulosin hydrochloride and dutasteride by LC–MS–MS and its subsequent utilization in bioequivalence study on healthy human volunteers. Basic information about BPH, tamsulosin hydrochloride and dutasteride, combination therapy and the individual methods of analysis of

both the drugs, sample preparation, detection mode and validation data have been extensively studied, however, very little literature is available on the application of a validated and robust LC–MS–MS method for simultaneous determination of tamsulosin and dutasteride to a pharmacokinetic study in humans.

In order to quantify plasma concentrations of tamsulosin and dutasteride simultaneously in clinical trials, it was necessary to develop and validate an assay with appropriate sensitivity, selectivity, accuracy and precision. The most attractive approach for an assay which would be robust, sensitive, selective and would allow high throughput is a liquid chromatographic tandem mass spectrometric (LC–MS–MS) method [13]. Our method for simultaneous determination of both the drugs is simple, rapid, robust, specific and sensitive that makes it an attractive procedure in high-throughput bioanalysis [12–14]. Moreover, this type of study would be beneficial in our local population. Over 50% of men above the age of 60 and around 80% beyond the age of 80, suffer from BPH in India alone. If left untreated, BPH can lead to serious health problems, including urinary tract infections, bladder and kidney damage, bladder stones and inability to hold urine.

Experimental

Materials and Reagents

The pure substances of tamsulosin, dutasteride and finasteride (internal standard, IS) were obtained from Central Drugs Laboratory, Kolkata, India. Chemical structure of the analytes and the internal standard are presented in Fig. 1. Stock solutions of tamsulosin (0.5 mg mL^{-1}) were prepared in MeOH:H_2O (1:1, *v*/*v*). Stock solutions of dutasteride (0.5 mg mL^{-1}), and finasteride (1 mg mL^{-1}) were separately prepared in a 10 mL volumetric flask with methanol. LC-grade ammonium formate, methanol, diethylether and methylene chloride were from Merck India, Mumbai, India. Water (resistivity of 18 MΩ) was collected from a Milli-Q gradient system of Millipore (Elix 3, Milli-Q A10 Academic). All other chemicals were of analytical grade. The blank human plasma with EDTA-K_3 anticoagulant was collected from the Clinical Pharmacological Unit (CPU) of Bioequivalence Study Centre, Jadavpur University, Kolkata, India.

Table 1. Tandem mass spectrometric parameters of dutasteride, tamsulosin and finasteride

Parameter	Value
Source temperature (°C)	550
Dwell time per transition (ms)	200
Ion source gas (gas1) (psi)	70
Ion source gas (gas2) (psi)	20
Curtain gas (psi)	10
Collision gas (psi)	12
Ion spray voltage (V)	5,000

	Tamsulosin	Dutasteride	Finasteride (IS)
Entrance potential (V)	11.5	11.8	11.8
Declustering potential, DP (V)	24	105	95
Focussing potential (V)	398	395	390
Entrance potential (V)	11.5	11.8	11.8
Collision energy (V)	33.4	49	43.5
Collision cell entrance potential (V)	31.05	33.81	30.22
Collision cell exit potential (V)	5.29	5	8
Ion transition (*m*/*z*)	409.1/228.1	529.3/461.3	373.2/305.3

LC–MS–MS

The LC system consisting of solvent delivery LC 10ADVP, controller LC10ADVP and column oven CTO10ASVP was from Shimadzu, Kyoto, Japan. Sample injection was by using SIL HTC autosampler from Shimadzu, Kyoto, Japan. The analytical column used was a Gemini C-18, 50 mm × 2.0 mm (3 µm) from Phenomenex, USA. Elution was performed at room temperature using the mobile phase (MeOH:ammonium formate, 97:3, *v*/*v*) The LC system was operated isocratically at 1 mL min^{-1}. The column eluent was split and approximately 400 µL were introduced in the mass spectrometer. The total run time for each sample analysis was 1 min only.

The LC system was coupled with a turbo ionspray ionization–triple quadrupole mass spectrometer API 2000 made by AB Sciex Instruments (Toronto, Canada) for detection. Sciex Analyst software version 1.4.1 was used for data acquisition and processing. The turbo ionspray ionization source was operated in positive mode for mass spectrometric detection. The main working parameters of the mass spectrometer are summarized in Table 1. In this method, both Q_1 and Q_3 quadrupoles were operated at unit resolution. For each injection, the total acquisition time was 1 min.

Standard Solutions and Quality Control (QC) Samples

Working solutions for calibration and controls were prepared from the stock solution by dilution using water/methanol. Dilutions of 100 and 10 ng mL^{-1} were made from the stock solutions, which were used to prepare the calibration curve and QC samples. An eight-point standard curve was prepared by spiking the blank plasma with appropriate amounts of working solution to obtain final concentrations of 2.0, 2.5, 5.0, 7.5, 10.0, 15.0, 20.0 and 25 ng mL^{-1} for tamsulosin and 2.0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 20.0 ng mL^{-1} for dutasteride. The concentration of IS in plasma the sample was 8.0 ng mL^{-1}. All stock solutions and working standard solutions were stored in polypropylene vials in a −20 °C freezer.

The linear regression of the peak area ratio of analyte/IS versus concentration using a weighed $1/x^2$ was used to obtain a calibration curve from the calibrators. The regression equation of the calibra-

Table 2. Precision and accuracy data of back-calculated concentrations of calibration samples for tamsulosin in human plasma

Concentration added (ng mL^{-1})	Concentration found (mean ± SD, $n = 6$) (ng mL^{-1})	Precision (%)	Accuracy (%)
2	1.82 ± 0.03	1.63	90.83
2.5	2.28 ± 0.15	6.75	91.07
5	4.58 ± 0.13	2.74	91.57
7.5	6.78 ± 0.05	0.76	90.44
10	8.13 ± 0.20	2.20	91.33
15	14.04 ± 0.35	2.46	93.60
20	19.21 ± 0.51	2.65	96.05
25	23.77 ± 0.71	2.97	95.09

Table 3. Precision and accuracy data of back-calculated concentrations of calibration samples for dutasteride in human plasma

Concentration added (ng mL^{-1})	Concentration found (mean ± SD, $n = 6$) ng mL^{-1}	Precision (%)	Accuracy (%)
2	1.81 ± 0.05	2.73	90.56
2.5	2.31 ± 0.09	4.10	92.39
5	4.53 ± 0.25	5.59	90.63
7.5	6.81 ± 0.18	2.68	90.82
10	9.20 ± 0.14	1.52	91.97
12.5	11.88 ± 0.34	2.86	95.00
15	14.26 ± 0.31	2.14	95.09
20	19.06 ± 0.23	1.22	95.29

tion curve was then used to calculate the plasma concentration. The back calculated values of the concentrations (Tables 2, 3) were statistically evaluated.

QC samples were prepared as a bulk, and at concentrations of 2 ng mL^{-1} (lower limit of quantitation, i.e., LLOQ), 6 (low-), 12 (medium-), and 22 ng mL^{-1} (high-) for tamsulosin and at concentrations of 2 ng mL^{-1} (lower limit of quantitation, i.e., LLOQ), 6 (low-), 12 (medium-), and 18 ng mL^{-1} (high-) for dutasteride. QC samples were prepared in a 50-mL pool then aliquoted into prelabeled 2 mL polypropylene vials and stored at −20 °C.

Sample Preparation

For calibration standards, an aliquot of 0.1 mL for each spiking solution was spiked into 0.9 mL of control blank plasma in a polypropylene tube. Then 0.1 mL of IS working solution was added to each tube and all the samples were vortex-mixed for 30 s. Afterwards, 0.2 mL of NaOH (1 M) were added and mixed for 30 s followed by adding 6 mL diethyl ether:methylene chloride::70:30(*v*/*v*). The solution was then hand mixed for 15 min. All the samples were centrifuged for 15 min at 5,000 rpm. The upper organic layer was separated and evaporated at 50 °C under N_2 atmosphere and reconstituted with 300 µL of methanol and filtered through a 0.45 µm membrane filter. The resulting samples were transferred into a 1.0 mL glass vial which was loaded into the autosampler cabinet and 20 µL aliquot of each extracted sample were injected into the LC–MS–MS system.

Method Validation

The within-batch precision and accuracy were determined by analyzing six sets of QC samples in a batch. The between-batch precision and accuracy was determined by analyzing six sets of QC samples on three different batches. The QC samples were randomized daily, processed and analyzed in position either (a) immediately following the standard curve (b) in the middle of the batch or (c) at the end of the batch. The acceptance criteria of within- and between-batch precision were 20% or better for LLOQ and 15% or better for the rest of the concentration and the accuracy was 100 ± 20% or better for LLOQ and 100 ± 15% or better for the rest of concentrations. The precision of the method was expressed in terms of bias (percentage deviation from the bias).

Recovery of analyte was evaluated by comparing the mean peak areas of six extracted low and high QC samples to mean peak areas of six reference solutions at the same concentration level. Recovery of IS was evaluated by comparing the mean peak areas of ten extracted QC samples to mean peak areas of ten references solutions at the same concentration level.

Application

The above-mentioned validated method was successfully applied to the bioequivalence study of fixed dose combination of tamsulosin and dutasteride. The study was approved by the ethical committee of Jadavpur University, Kolkata, India. It was an open, randomized crossover study to determine relative bioavailability of tamsulosin and dutasteride in 12 healthy male volunteers following single dose administration of a tablet containing tamsulosin 0.4 mg MR and dutasteride 0.5 mg. After the screening visit, each eligible volunteer entered into a randomized schedule to either receive reference formulation or test formulation after overnight fast. All subjects were admitted to the Clinical Pharmacological Unit (CPU) of Bioequivalence Study Centre, Jadavpur University, Kolkata, India, in the evening prior to the dosing day and remained in the ward until 24 h post dosing. Test preparation was a tamsulosin 0.4 mg MR and dutasteride 0.5 mg tablet manufactured by Burgeon Pharmaceutical Pvt., Chennai, India and tablet Veltam plus containing tamsulosin 0.4 mg MR and dutasteride 0.5 mg (Batch No. DH0138) manufactured by Intas Pharmaceuticals, Ahmedabad, India was used as reference preparation.

Approximately, 5 mL blood samples were drawn into Vacutainer tubes containing EDTA from a forearm vein using an indwelling catheter or by direct venipuncture before dosing (0 h) and then at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 6.0, 8.0, 10.0, 12.0 and 24.0 h after dosing. Collected blood samples were centrifuged immediately, plasma was separated and stored frozen at −20 °C with appropriate labeling of volunteer code number, study date and collection time, till the date of analysis.

Abnormal signs/symptoms were monitored, during the study period and for 1 week after the study period and if noticed, their details were entered in the case report sheets and tabulated at the end of the study. On the study days, volunteers were permitted normal activities, excluding strenuous exercise.

The instrumentation and chromatographic conditions employed for analysis are described earlier. The pharmacokinetic parameters like area under the plasma concentration–time curve from zero to the last measurable tamsulosin and dutasteride sample time and to infinity (AUC_{0-t} and AUC_{0-inf}), maximum concentration (C_{max}), time to maximum concentration (t_{max}), elimination rate constant (K_{el}) and elimination half-life ($t_{1/2}$) were directly determined or calculated by the standard non-compartmental method using WinNolin 4.1 Pro software package (Pharsight Inc.). Both maximum plasma concentration (C_{max}) and time to peak plasma concentration (t_{max}) were directly obtained from the data. The elimination half-life ($t_{1/2}$) was calculated as $0.693/K_{el}$, where K_{el} is the apparent elimination rate constant. K_{el} was in turn, calculated as the slope of the linear regression line of natural log-transformed plasma concentrations. The last seven quantifiable levels were used to determine K_{el}. The area under the plasma concentration–time curve (AUC_{0-t},) was calculated from the measured levels, from time zero to the time of last quantifiable level, by the linear trapezoidal rule. ($AUC_{0-\infty}$) was calculated according to the following formula: $AUC_{0-\infty} = AUC_{0-t} + C_{last}/K_{el}$, where C_{last} is the last quantifiable plasma level.

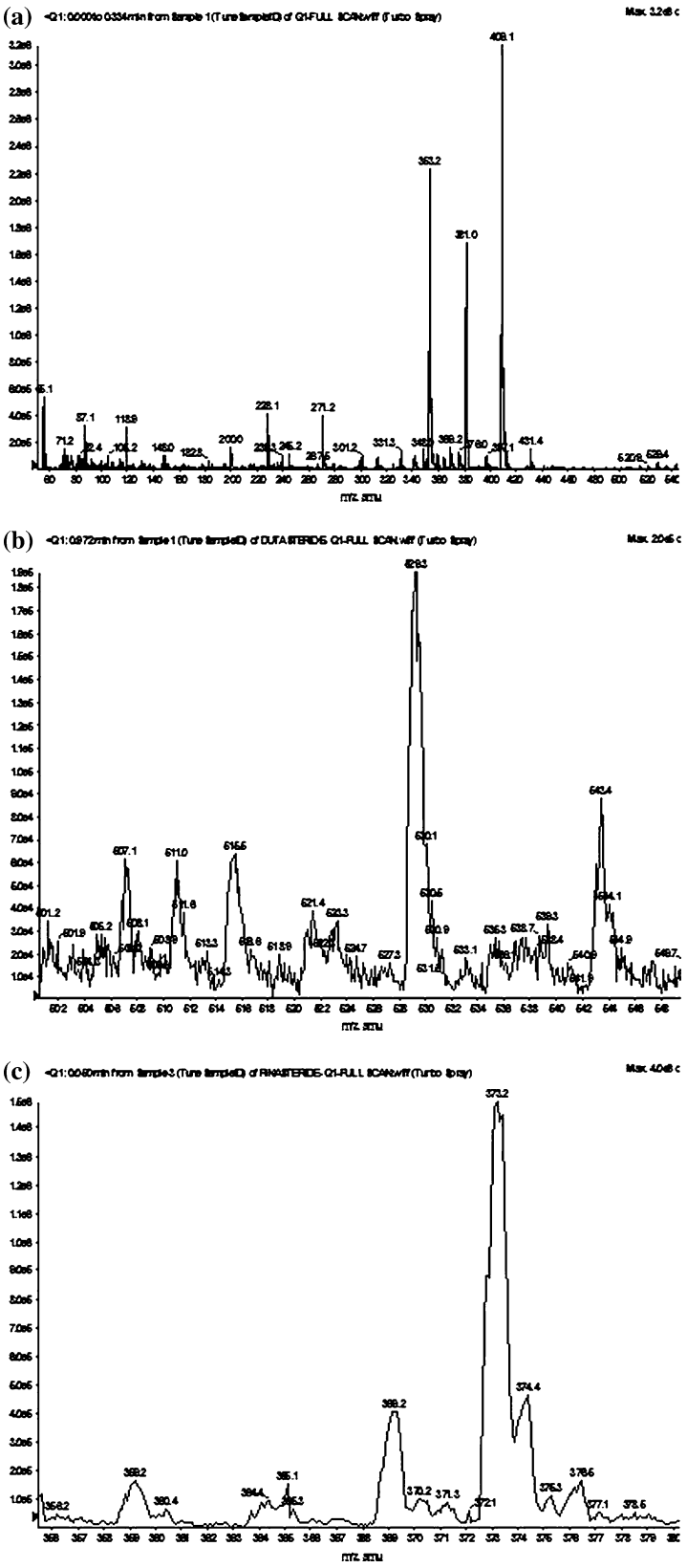

Fig. 2. Parent ion mass spectra: **a** tamsulsoin (m/z 409), **b** dutasteride (m/z 529) and **c** finasteride (m/z 373)

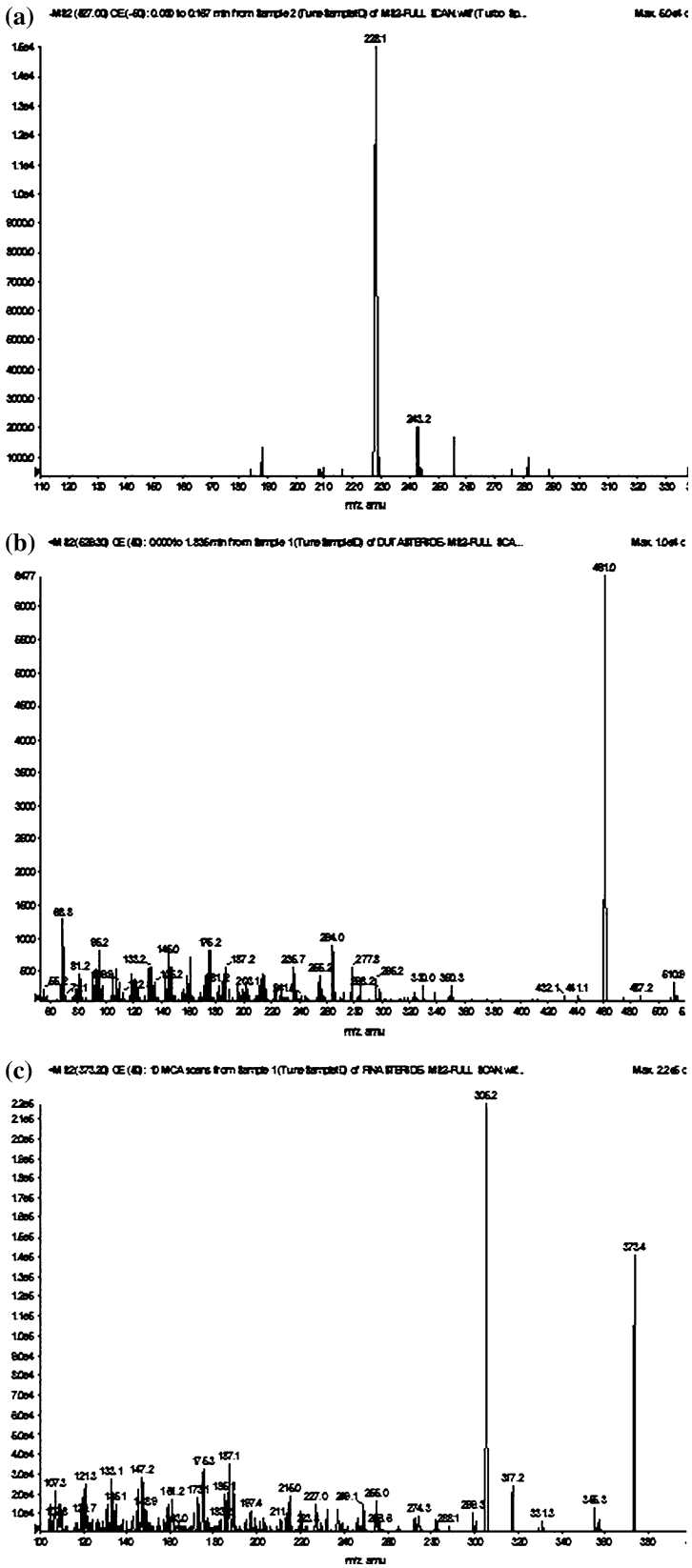

Fig. 3. Product ion mass spectra: **a** tamsulsoin (m/z 228), **b** dutasteride (m/z 461) and **c** finasteride (m/z 305)

Results and Discussion

Mass Spectrometry

Electrospray MS–MS was used to analyze both the analytes as it is beneficial in developing a selective and sensitive method. The positive ion turbo ionspray Q1 mass spectrum and product ion mass spectrum of tamsulosin, dutasteride and the IS are shown in Figs. 2a–c and 3a–c, respectively. [M + H]+ was the predominant ion in the Q1 spectrum and was used as the precursor ion to obtain product ion spectra. The most sensitive mass transition was from m/z 409.1 to 228.1 for tamsulosin, m/z 529.3–461.3 for dutasteride and from m/z 373.2–305.3 for the IS. LC-MRM is a powerful technique for pharmacokinetic studies since it provides sensitivity, selectivity and specificity requirements for analytical methods. Thus, the MRM technique was chosen for the assay development. The MRM state file parameters were optimized to maximize the response for the particular analyte. The parameters presented in Table 1 are the result of optimization.

Separation and Specificity

Typical MRM chromatograms from the study of tamsulosin, dutasteride and IS in human plasma are shown in Fig. 4b, c and d, respectively. Retention time of tamsulosin, dutasteride and IS are at 0.80, 0.39 and 0.39 min, respectively. The specificity of the method was examined by analyzing blank human plasma extract (Fig. 4a) and an extract spiked only with the internal standard (Fig. 4d). As shown in Fig. 4a no significant interference in the blank plasma traces was seen from endogenous substances in drug-free human plasma at the retention time of the analyte. Figure 4b and c show the absence of interference from the internal standard to the MRM channels of both the analytes.

Limit of Quantitation, Linearity, Precision and Accuracy

The lower limit of quantitation of tamsulosin in human plasma assay was

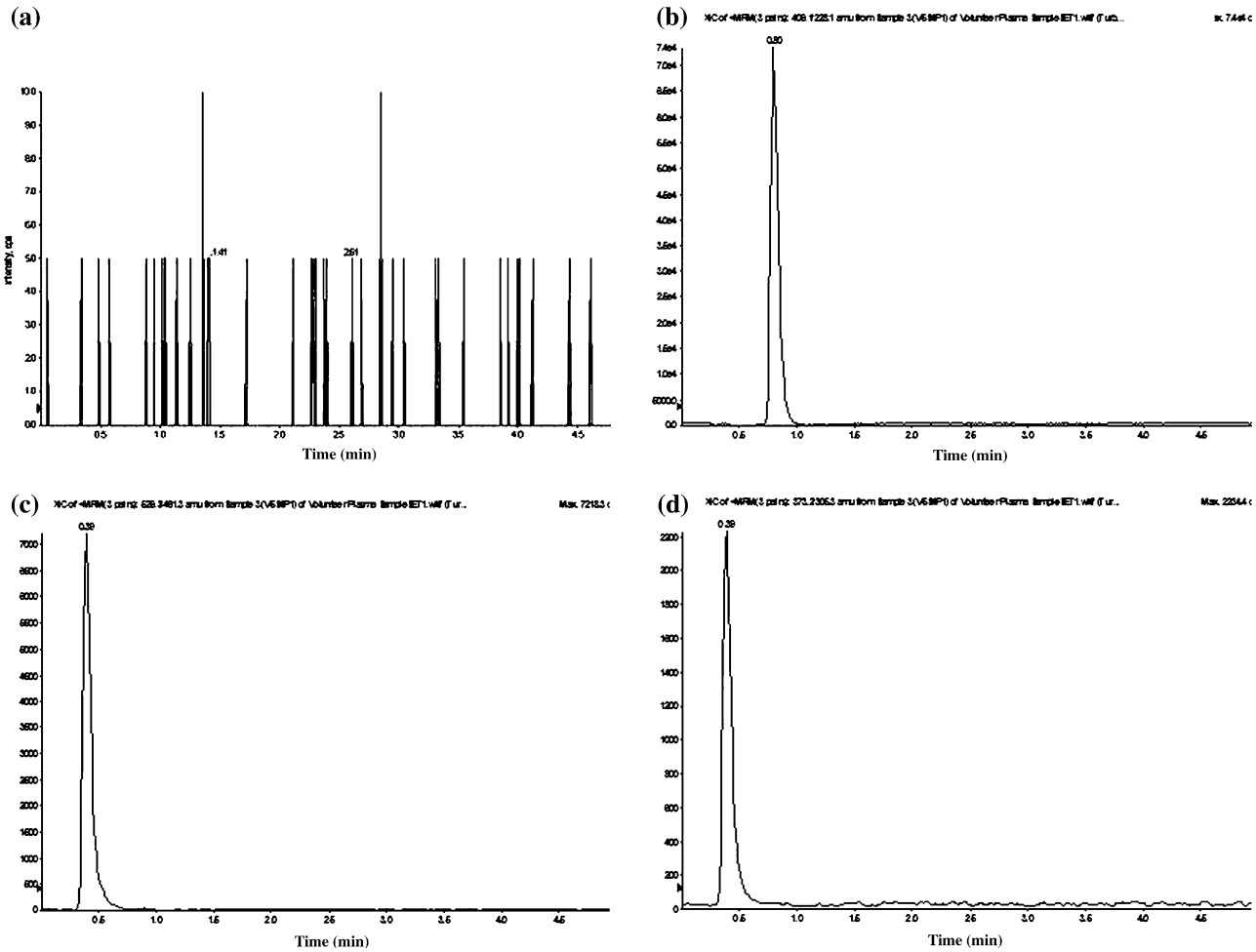

Fig. 4. Representative MRM chromatogram of tamsulosin, dutasteride and finasteride (IS): **a** blank human plasma; plasma sample of a volunteer showing separation of **b** tamsulosin (0.80 min) **c** dutasteride (0.39 min) and **d** finasteride (IS) (0.39 min) after oral administration of tablet containing tamsulosin 0.4 mg MR and dutasteride 0.5 mg

Table 4. Precision and accuracy of the LC–MS–MS method for determining tamsulosin concentrations in plasma samples

Concentration added (ng mL^{-1})	Within-batch precision			Between-batch precision		
	Concentration found (mean ± SD, $n = 6$) (ng mL^{-1})	Precision (%)	Accuracy (%)	Concentration found (mean ± SD, $n = 6$) (ng mL^{-1})	Precision (%)	Accuracy (%)
2	1.63 ± 0.19	11.84	81.61	1.66 ± 0.13	7.93	82.85
6	5.08 ± 0.13	2.48	84.72	5.06 ± 0.33	6.55	84.41
12	10.58 ± 0.36	3.35	88.19	10.56 ± 0.57	5.34	88.0
22	19.91 ± 0.37	1.88	90.48	19.41 ± 1.28	6.60	88.23

2 ng mL^{-1}. The between batch precision of tamsulosin at the LLOQ was 7.93%. The between batch accuracy of tamsulosin was 82.85% (Table 4). The within-batch precision was 11.84% and the accuracy was 81.61% of tamsulosin. The lower limit of quantitation of dutasteride in human plasma assay was 2 ng mL^{-1}. The between batch precision of dutasteride at the LLOQ was 6.0%. The between batch accuracy of dutasteride was 84.57% (Table 5). The within-batch precision was 14.03% and the accuracy was 87.23% of dutasteride.

The middle and upper quantitation levels of tamsulosin ranged from 6 to 22 ng mL^{-1} in human plasma (Fig. 5). The middle and upper quantitation levels of dutasteride ranged from 6 to 18 ng mL^{-1} in human plasma (Fig. 6). For the between batch experiment, the precision ranged from 5.34 to 6.60% and the accuracy ranged from 84.41 to 88.23% for tamsulosin (Table 4). Similarly, for dutasteride the precision ranged from 4.94 to 7.47% and the accuracy ranged from 87.15 to 93.25%

Table 5. Precision and accuracy of the LC–MS–MS method for determining dutasteride concentrations in plasma samples

Concentration added (ng mL^{-1})	Within-batch precision			Between-batch precision		
	Concentration found (mean ± SD, $n = 6$) (ng mL^{-1})	Precision (%)	Accuracy (%)	Concentration found (mean ± SD, $n = 6$) (ng $mL^{-1)}$	Precision (%)	Accuracy (%)
2	1.75 ± 0.245	14.03	87.23	1.69 ± 0.10	6.0	84.57
6	5.37 ± 0.26	4.85	89.50	5.23 ± 0.39	7.47	87.15
12	10.81 ± 0.23	2.16	90.09	10.85 ± 0.73	6.72	90.45
18	17.22 ± 0.34	2.00	95.64	16.79 ± 0.83	4.94	93.25

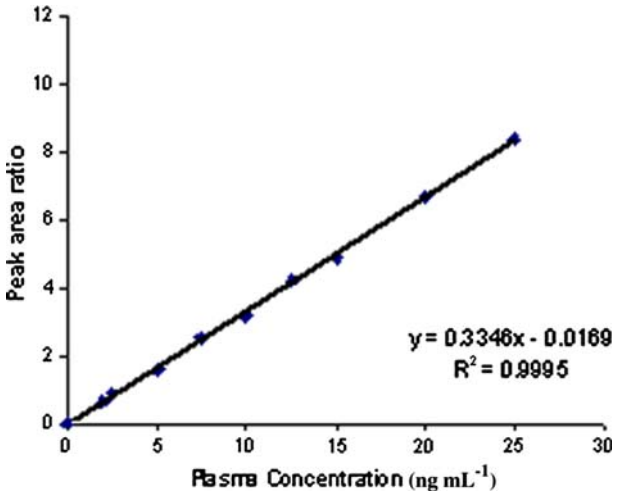

Fig. 5. Calibration curve of tamsulosin in plasma

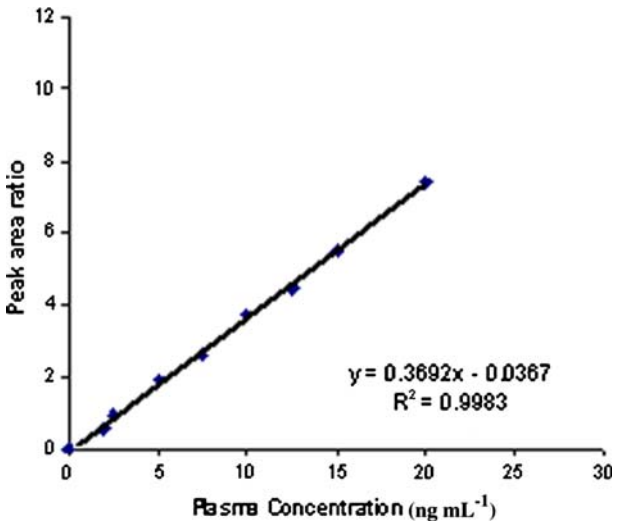

Fig. 6. Calibration curve of dutasteride in plasma

for the between batch experiment (Table 5). For the within-batch experiment, the precision and accuracy for tamsulosin and dutasteride met the acceptance criteria ($< \pm 15\%$) and precision was below 15% at all concentrations tested.

Stability

Each stability test included six replicates of three levels of QC samples (for tamsulosin at concentrations of 6, 12 and 22 ng mL^{-1} and for dutasteride at concentrations of 6, 12 and 18 ng mL^{-1}). The freeze–thaw stability of both the analytes were determined by measuring the assay precision and accuracy for the samples, which underwent three freeze–thaw cycles. The stability data were used to support repeating analysis. In each freeze–thaw cycle, the frozen plasma samples were thawed at room temperature for 2–3 h and refrozen for 12–24 h. After completion of each cycle the samples were analyzed and results was compared with that of zero cycle. The results showed that both the analytes were stable in human plasma through three freeze–thaw cycles. The results demonstrated that human plasma samples could be thawed and refrozen without comprising the integrity of the samples.

The storage time in long-term stability evaluation brackets the time between the first sample collection and the last sample analysis. The sample long-term storage stability at −20 °C was evaluated to establish acceptable storage conditions for subject samples.

All stability results for tamsulosin and dutasteride are presented in Tables 6 and 7. There were no significant differences between the responses of spiked standard at time zero and after 24 h both for tamsulosin and dutasteride, indicating the stability of both analytes at room temperature over 24 h. Moreover, both the analytes were found to be stable after reconstitution in diluent for at least 12 h at 4 °C.

Extraction Recovery

The extraction recovery of tamsulosin was 52.61% on average and dutasteride was 55.24% on average and the dependence on concentration is negligible. The recovery of IS was 68.78% at the concentration used in the assay (100 ng mL^{-1}). Recovery of both the analytes and IS were low, but it was consistent, precise and reproducible. With the consistency in the recovery of tamsulosin, dutasteride and IS, the assay has proved to be robust in high throughput bio-analysis.

Application to Pharmacokinetic Study

The validated method has been successfully used to quantitate the plasma concentrations of tamsulosin and dutasteride in an open, balanced, randomized, pharmacokinetic study of 12 healthy volunteers and to assess the bioavailability of tamsulosin 0.4 mg MR and dutasteride 0.5 mg after administration of a single fixed dose combination tablet. The limit of quantitation of tamsulosin and dutasteride allowed the plasma concentration to be followed for up to 24 h after drug administration. The calculated pharmacokinetic parameters are given in Tables 8 and 9. The concentration versus time profiles of a subject receiving a single dose of tamsulosin and dutasteride are presented in Figs. 7 and 8.

Conclusions

All calculated pharmacokinetic parameters summarized in Tables 7 and 8 agree with the previously reported values [14, 15]. C_{max} levels for tamsulosin were observed after 5.2 ± 0.283 h (Test) and 5.17 ± 0.342 h (Reference) and for dutasteride C_{max} levels were observed after 1.71 ± 0.40 h (Test) and 1.83 ± 0.49 h (Reference). The C_{max} values for tamsulosin of Test and Reference were 13.46 ± 1.51 and 14.19 ± 1.77 ng mL^{-1}, respectively. The C_{max} values for dutasteride of Test and Reference were 8.91 ± 1.51 and 9.66 ± 1.75 ng mL^{-1}, respectively. The mean $t_{1/2}$ for tamsulosin of Test and Reference were 8.41 ± 0.96 and 7.41 ± 1.58 h, respectively. The mean $t_{1/2}$ for dutasteride of Test and Reference were 5.71 ± 0.34 and 5.09 ± 0.45, respectively. The relative bioavailability between Test and Reference of tamusolin and dutasteride were 98.26 and 97.09%, respectively.

In summary, the described method of simultaneous analysis of tamsulosin and dutasteride from human plasma by LC–MS–MS in positive ionization mode using multiple reaction monitoring has shown acceptable precision and adequate sensitivity and was successfully

Table 6. Stability of human plasma samples of tamsulosin

Sample concentration (ng mL^{-1}) (n = 6)	Concentration found (mean ± SD, n = 6) (ng mL^{-1})	Precision (%)	Accuracy (%)
Short-term stability (24 h)			
6	5.48 ± 0.25	4.68	89.93
12	10.39 ± 0.18	1.77	86.59
22	19.21 ± 0.18	0.95	87.34
Long-term stability (10 days at −20 °C)			
6	4.37 ± 0.26	4.85	89.50
12	10.58 ± 0.36	3.35	88.19
22	19.91 ± 0.37	1.88	90.48
Freeze–thaw stability			
6	5.24 ± 0.19	3.62	87.32
12	10.64 ± 0.19	1.77	88.67
22	19.53 ± 0.19	0.95	88.77

Table 7. Stability of human plasma samples of dutasteride

Sample concentration (ng mL^{-1}) (n = 6)	Concentration found (mean ± SD, n = 6) (ng mL^{-1})	Precision (%)	Accuracy (%)
Short-term stability (24 h)			
6	5.20 ± 0.09	1.77	86.68
12	11.08 ± 0.17	1.55	92.31
18	17.22 ± 0.56	3.23	95.67
Long-term stability (10 days at −20 °C)			
6	5.16 ± 0.09	1.77	86.01
12	11.05 ± 0.19	1.71	92.08
18	17.08 ± 0.55	3.23	94.86
Freeze–thaw stability			
6	5.24 ± 0.18	3.46	87.39
12	11.17 ± 0.35	3.13	93.09
18	16.93 ± 0.55	3.23	94.06

Table 8. Pharmacokinetic parameters of tamsulosin after oral administration of Test and Reference fixed dose combination tablet

Pharmacokinetic parameters	Reference preparation	Test preparation
C_{max} (ng mL^{-1})	13.46 ± 1.51	14.19 ± 1.77
t_{max} (h)	5.20 ± 0.28	5.17 ± 0.34
AUC_{0-24} (ng h mL^{-1})	83.88 ± 10.33	78.94 ± 6.91
$AUC_{0-\infty}$ (ng h mL^{-1})	105.59 ± 13.89	104.89 ± 12.41
K_{el} (h^{-1})	0.17 ± 0.04	0.14 ± 0.03
$t_{1/2}$ (h)	8.41 ± 0.96	7.41 ± 1.58
Relative bioavailability (%)	100	98.26

Table 9. Pharmacokinetic parameters of dutasteride after oral administration of Test and Reference fixed dose combination tablet

Pharmacokinetic parameters	Reference preparation	Test preparation
C_{max} (ng mL^{-1})	8.91 ± 1.51	9.66 ± 1.75
t_{max} (h)	1.71 ± 0.40	1.83 ± 0.49
AUC_{0-24} (ng h mL^{-1})	56.85 ± 8.98	55.20 ± 10.46
$AUC_{0-\infty}$ (ng h mL^{-1})	71.80 ± 10.31	66.56 ± 12.89
K_{el} (h^{-1})	0.12 ± 0.01	0.14 ± 0.01
$t_{1/2}$ (h)	5.71 ± 0.34	5.09 ± 0.45
Relative bioavailability (%)	100	97.09

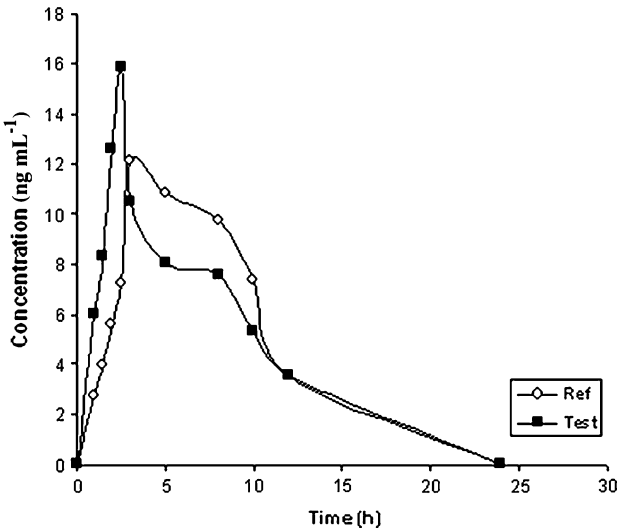

Fig. 7. Plasma concentration–time curves of tamsulosin after administration of Test and Reference formulations of tamsulosin 0.4 mg MR and dutasteride 0.5 mg

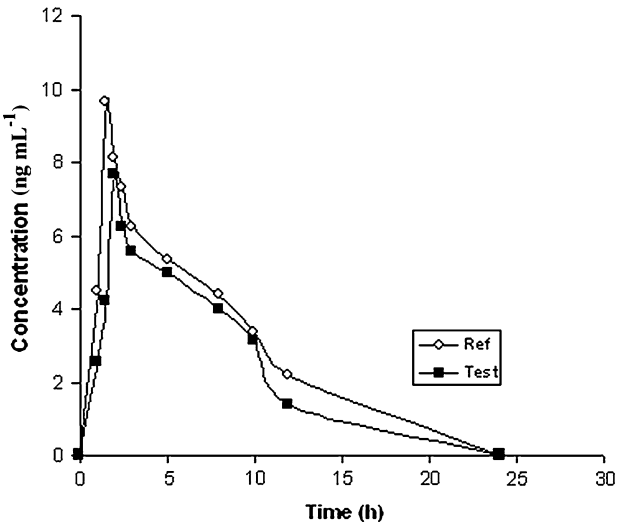

Fig. 8. Plasma concentration–time curves of dutasteride after administration of Test and Reference formulations of tamsulosin 0.4 mg MR and dutasteride 0.5 mg

applied to pharmacokinetic and bioequivalence studies. The assay method is specific due to the inherent selectivity of tandem mass spectrometry. The method described is simple, rapid, sensitive, specific and fully validated as per FDA guidelines [16]. Our method for simultaneous determination of both the analytes is simple, rapid, robust, specific and sensitive which makes it an attractive procedure in high-throughput bioanalysis [12–14]. As mentioned before, this study would be beneficial in India as more than 50% of men above the age of 60 and around 80% beyond the age of 80, suffer from BPH. If left untreated, BPH can lead to serious health problems, including urinary tract infections, bladder and kidney damage, bladder stones and inability to hold urine.

Acknowledgments

The authors are thankful to DPRP/PRDSF of Department of Science and Technology (DST), New Delhi (Grant No.VII-PRDSF/56/05-06/TDT) for providing the instrumental facilities and financial support for carrying out this study and also to Burgeon Pharmaceutical Pvt. Ltd., Chennai, India for supplying the samples.

References

1. Wei JT, Calhoun E, Jacobsen SJ (2005) J Urol 173:1256
2. Sarma AV, Jacobson DJ, McGree ME et al (2005) J Urol 173:2048
3. Sung JC, Curtis LH, Schulman KA, Albala DM (2006) J Urol 175:1023
4. Gilling PJ, Cass CB, Cresswell MD, Malcolm AR, Fraundorfer MR (1996) J Endourol 10:459–461
5. Kuo RL, Paterson RF, Kim SC, Siqueira TM Jr, Elhilali MM, Lingeman JE (2003) World J Surg Oncol 1:6
6. McVary KT (2007) Clin Ther 29:387
7. Honda K, Nakagawa C (1986) J Pharmacol Exp Ther 239:512
8. Honda K, Nakagawa C, Terai M, Naunyn S (1987) Arch Pharmacol 336:295
9. Soeishi Y, Kobori M, Kobayashi SL, Higuchi S (1990) J Chromatogr B 533:290
10. Matsushima H, Takanuki KI, Kamimura H, Watanabe T, Higuchi S (1997) J Chromatogr B 695:317

11. Dinga L, Limin L, Taob P, Yanga J, Zhengxing Z (2002) J Chromatogr B 767:75–81
12. Ramakrishna NVS, Vishwottam KN, Puran S, Manoj S, Santosh M, Wishu S, Koteshwara M, Chidambara J, Gopinadh B, Sumatha B (2004) J Chromatogr B 805:13–20
13. Lee MS, Kerns EH (1999) Mass Spectrom Rev 18:187
14. Niessen WMA (2003) J Chromatogr A 1000:413
15. Macek J, Klima J, Ptacek P (2004) J Chromatogr B 809:307–311
16. Guidance for Industry: Bioanalytical Method Validation (2001) US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Rockville, MD