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Quantification of Tamsulosin in Human Plasma by High-Performance Liquid Chromatography Coupled with Electrospray Tandem Mass Spectrometry

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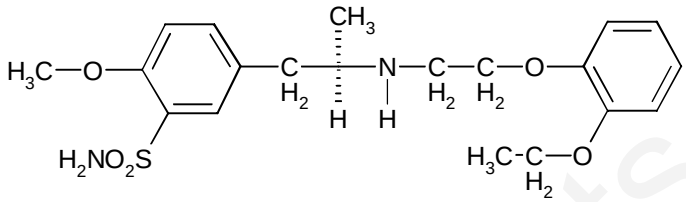
Summary. A sensitive and specific high-performance liquid chromatographic–electrospray ionization tandem mass spectrometric (HPLC–ESI–MS–MS) method for quantification of tamsulosin in human plasma, using propranolol as internal standard (IS), has been developed, validated successfully, then used in a clinical study. Plasma (0.5 mL) was mixed with 50 μL 1 M sodium carbonate solution. Tamsulosin and propranolol were isolated from the mixture by liquid–liquid extraction with 7:3 (*v*/*v*) hexane–ethyl acetate. Reversed-phase chromatography was performed on a C_8 column at 25°C with 70:30:0.1 (*v*/*v*) methanol–water–formic acid as mobile phase at a flow-rate of 1.0 mL min^{-1}. Quantification was achieved in positive-ion mode by monitoring the product ions at *m*/*z* 409.1 → 270.9, 228.0, and 200.0 (tamsulosin) and *m*/*z* 260.1 → 183.0 (IS). The lowest limit of quantification was 0.25 ng mL^{-1}, and the calibration range was 0.25–50 ng mL^{-1}. Within and between batch precision (expressed as coefficient of variation, CV) did not exceed 10.8% and accuracy was within 5.0% deviation of the nominal concentration. Recovery of tamsulosin from plasma was >83.0%. The validated method was used for clinical study of tamsulosin in human volunteers.

Key Words: tamsulosin, LC–ESI–MS–MS (ion trap), validation, bioequivalence application, clinical study

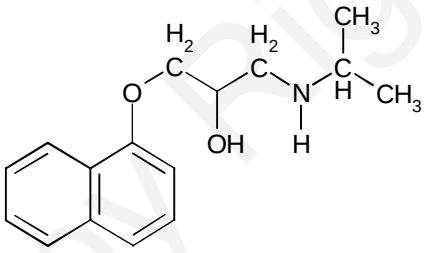
Introduction

Tamsulosin hydrochloride, (–)-(*R*)-5-[2-[[2-(*o*-ethoxyphenoxy)ethyl]amino] propyl]-2-methoxybenzene sulfonamide hydrochloride (*Fig. 1*), is a structurally new type of sulfamoyl derivative with highly selective α_1-adrenoceptor antagonistic properties. It has been used clinically for urinary obstructed patients with benign prostatic hyperplasia [1–4]. Tamsulosin, an $_1$-adrenoceptor blocking agent, has selectivity for $_{1}$-receptors in the human

prostrate. At least three discrete $_1$-adrenoceptor subtypes, α_{1A}, $_{1B}$, and α_{1D}, have been identified, and their distribution differs between human organs and tissue. Approximately 70% of the α_1-receptors in the human prostrate are the α_{1A} subtype [5]. An in-vitro study has revealed that the selectivity of this drug for the prostrate α_1-adrenoceptor was approximately ten times higher than that for the aorta $_1$-adrenoceptor [6]. The α_1-adrenoceptor antagonist activity of tamsulosin hydrochloride has been found to be more potent than that of other drugs such as prazosin [7].



a)



b)

Fig. 1. The structures of (a) tamsulosin and (b) propranolol (IS)

For pharmacokinetic study of tamsulosin, sensitive and simple methods are required. A recent survey revealed a few chromatographic methods for analysis of tamsulosin in biological samples; these involved HPLC with fluorescence detection [8, 9] and liquid chromatography–tandem mass spectrometry (LC–MS–MS) [10–15].

Soeishi et al. [8] reported an LC method with fluorescence detection for analysis of tamsulosin, but the method was affected by interference from endogenous substances and potential loss of the drug in the re-extraction procedure. The sample-preparation process was also tedious and time-consuming. The limit of quantitation was 0.5 ng mL^{-1}, using 1.5 mL plasma, and the run time was 18 min. Macek et al. [9] have reported an HPLC method with fluorescence detection after liquid–liquid extraction. The limit of quantitation was 0.4 ng mL ^{1} using 1 mL plasma.

Recent progress in mass spectrometry (MS) has resulted in the development of new techniques, for example electrospray ionization (ESI) and tandem MS (MS–MS) which have significantly improved detection sensitivity for drugs, and quantification at picogram levels is becoming possible. Matsushima et al. [10] have reported a high-performance liquid chromatography–electrospray-tandem MS (LC–MS–MS) method for analysis of tamsulosin in plasma dialysate, plasma, and urine, in which response to the plasma concentration was linear over the range 0.5–50 ng mL ^{1}. Ding et al. [11] reported a highly sensitive assay method using LC–ESI–MS rather than LC–MS–MS. The assay was validated over the range 0.2–30 ng mL ^{1} using 1 mL of plasma. Qi et al. [12] reported a simple, specific and highly sensitive LC–APCI–MS–MS method for analysis of tamsulosin in dog plasma. Ramakrishna et al. [13] reported a rapid, simple, and highly sensitive LC–MS–MS method for quantification of tamsulosin in plasma. Recently, Rahkonen et al. [14] reported the analysis of tamsulosin in human aqueous humor and serum by gradient LC–ESI–MS with a linear ion-trap mass detector. The method was validated utilizing 1 mL serum; the concentration range was 0.1 to 19.3 ng mL^{-1} and the total run time 8 min. Simultaneous LC–MS–MS analysis of tamsulosin and dutasteride in human plasma has been reported [15]. The lower limit of quantification for both tamsulosin and dutasteride was 1 ng mL ^{1} using 0.9 mL plasma.

It is well known that HPLC–tandem MS (MS–MS) further enhances specificity, with an improved signal-to-noise ratio compared with single-stage MS [16]. The ion-trap mass spectrometer also enables MS–MS at an affordable cost compared with triple-stage quadrupole MS. The purpose of this work was to exploit the high sensitivity and selectivity of an ion-trap detector operated in MS–MS mode with an ESI interface to develop and validate a robust, cost-effective reversed-phase LC–MS–MS method for analysis of tamsulosin in human plasma utilizing a commercially available internal standard. The C_{max} of tamsulosin ranges from 5.17–13.74 ng mL ^{1} after oral administration of a 0.4 mg OCAS (oral controlled absorption system) tablet or modified release formulation. For a 1.2 mg OCAS tablet, the C_{max} of tamsulosin reached 31.6 ng mL^{-1} [17]. It was essential to establish an assay capable of quantifying tamsulosin at concentrations down to 0.25 ng mL ^{1} (i.e. 5% of C_{max}). At the same time, it was expected the method would enable efficient analysis of large numbers of plasma samples obtained in pharmacokinetic, bioequivalence, or bioavailability studies after administration of a therapeutic dose of tamsulosin.

This paper describes a simple and sensitive HPLC–ESI–MS–MS method, with a commercially available internal standard, for analysis of tamsulosin in plasma. The lower limit of quantitation of this validated method is 250 pg mL ^{1} (using 0.5 mL human plasma). The advantages of the

method presented in this paper, in comparison with that of Rahkonen et al. [14] and other methods [8–11, 15] are:

- less sample is used – 0.5 mL plasma instead of 1 mL – so the volume of sample collected per time point from an individual during a study is reduced substantially, enabling inclusion of additional points and making it suitable for pharmacokinetic and/or bioequivalence studies;
- rapidity – sample turnaround time of ~4 min compared with 8 min makes it an attractive procedure in high-throughput bioanalysis of tamsulosin; and
- isocratic rather than gradient elution is used.

Experimental

Ethics

The bioequivalence study was conducted in accordance with the Declaration of Helsinki and ICH guidelines on good clinical practice. The report was written in accordance with ICH Topic E3 Structure and Content of Clinical Study Reports, Step 4, Consensus Guideline, 30.11.95. Archiving of essential documents was performed according to European Commission Directive 91/597/EEC of 19.07.1991. The protocol was approved by the IEC of Al-Mowasah Hospital. All subjects were screened within 14 days before entering the study. Before screening, a specific screening informed consent form was signed. The non-clinical part of the study was conducted at JCPR, Amman, Jordan (data on file).

Chemicals, Reagents, and Solutions

Tamsulosin drug substance (purity 99.5%) and propranolol (99.4%) (internal standard, IS) were obtained from Hetero Drug, Medak, Andhra Pradesh (India). The chemical structures are presented in *Fig. 1*. LC–MS quality LiChrosolv deionized water (conductance <1 μS cm $^{-1}$), hexane, and ethyl acetate were purchased from Merck (Darmstadt, Germany). All other chemicals were of analytical grade. Blank plasma was collected from the Blood Bank, Islamic Hospital, Amman, Jordan. Plasma was obtained by centrifugation of blood plasma treated with EDTA–heparin.

Stock solutions (1.0 mg mL $^{-1}$) of tamsulosin and propranolol were prepared in acetonitrile and methanol, respectively. These solutions were

stored at 4°C. Working solutions were prepared by appropriate dilution with methanol–water (50:50, *v*/*v*) as required.

Separate calibration (5, 10, 20, 50, 100, 200, 600 and 1000 ng mL^{-1}) and control (5, 15, 500 and 800 ng mL^{-1}) working solutions were prepared from the stock solution by appropriate dilution with 50:50 (*v*/*v*) acetonitrile–water. Propranolol working solution (2 μg mL^{-1}) was also prepared by appropriate dilution of the stock solution. Tamsulosin working solutions (50 μL) were added to 950 μL drug-free plasma to furnish tamsulosin concentrations of 0.25, 0.5, 1, 2.5, 5, 10, 30 and 50 ng mL^{-1}. Quality-control samples were prepared in pooled plasma at concentrations of 0.25 ng mL^{-1} (LLOQ), 0.75 ng mL^{-1} (low), 25 ng mL^{-1} (medium), and 40 ng mL^{-1} (high), as a single batch of each concentration, and then divided into aliquots that were stored in the freezer at −70°C until required for analysis.

Sample Processing

Plasma (0.5 mL) was placed in a 15-mL polypropylene tube and 25 μL propranolol working solution (2 μg mL^{-1}) was added. After addition of 50 μL 1 M sodium carbonate buffer solution, 6 mL extraction solvent (hexane–ethyl acetate, 7:3, *v*/*v*), was added by use of a Dispensette Organic (Brand, Postfach, Germany). The sample was vortex mixed for 1 min using a Vibrax type VX-Z, VXR basic vortex mixer (IKA-Werke, Staufen, Germany) and centrifuged for 5 min at 800*g* by use of Multitude 3S-R (Sorvall-Heraeus, Germany). The organic layer was transferred by freeze-decanting (−70°C; Platinum 500 V; Angelantoni Industrie, Italy) to an 8-mL glass test tube and evaporated to dryness at 40°C under a stream of nitrogen. The dried extract was reconstituted by addition of 200 L methanol–water (70:30, *v*/*v*) containing 1% formic acid. It was then vortex mixed for 10 s and 50 μL of this sample was injected for analysis.

LC–MS–MS Instrumentation and Conditions

Chromatographic analysis was performed with a Thermo Finnigan Spectra-System equipped with a binary solvent-delivery pump (P 2000), on-line vacuum degasser (SCM 1000), and injector (Rheodyne 7125, with 100-μL loop) linked to an autosampler (AS 3000), and supported by a tray-cooling system and column oven. Remote control and data treatment were performed by Windows XP-based Finnigan Xcalibur Software (Version 1.4).

Compounds were separated at room temperature (25 ± 1°C) on a 10 cm × 4.6 mm i.d., 5- m particle, LiChrospher 60, RP-Select B C_8 analytical

column protected by a 4 mm × 2.0 mm i.d., 5-μm particle, Phenomenex C_{18} guard column. The mobile phase was 70:30:0.1 (*v*/*v*) methanol–water–formic acid at a constant flow rate of 1.0 mL min^{-1}.

Mass spectrometric analysis was performed with a Finnigan LCQ-AdvantageMax, ion-trap mass spectrometer (Finnigan Thermo Electron, USA) equipped with an electrospray ionization (ESI) source operating in positive-ion mode. The built-in waste/detector switcher valve was programmed to allow mobile phase flow into the mass spectrometer from 1.8–3.8 min of each run. Data acquisition, instrument control, and quantification were performed by Finnigan Xcalibur data-management software.

The optimum settings were obtained by automatic tuning while a built-in infusion pump continuously supplied the ESI source with 5 μg mL^{-1} tamsulosin in acetonitrile, aided by normal HPLC flow via a T-connecter, in the infusion mode. The optimum settings were: sheath gas flow 62 units (units refer to arbitrary values set by the LCQ software), capillary temperature 350°C, and spray potential 4.0 kV. Collection time for the ion trap was set at 200 ms and no cross talk was found between transitions. Positive-ion scan-mode spectra contained strong signals at *m*/*z* 409.06 and 260.07 for the monoprotonated molecules $[MH]^+$ of tamsulosin and propranolol, respectively; these masses were detected in selected ion monitoring (SIM) mode, and these parent ion molecules were subsequently fragmented by a helium collision gas in the ion trap at 34.0 V collision energy to produce significant daughter-ion fragments. The mass spectra resulting from these fragmentation processes were acquired in SRM mode at *m*/*z* 270.96, 227.99, and 200.04 for tamsulosin and *m*/*z* 183.02 for propranolol. These product ions were monitored and selected for quantification of tamsulosin.

Bioanalytical Method Validation

Calibration

A calibration plot was constructed after analysis of a blank sample (i.e. a plasma sample processed without addition of the IS), a zero sample (i.e. a plasma sample processed with IS), and eight non-zero samples covering the total range (0.25–50 ng mL^{-1}), including the lower limit of quantification (LLOQ).

Validation runs were conducted on three separate days. Each validation run consisted of the spiked calibration standards at eight concentration over the range of interest ($n = 5$ at each concentration), QC samples at three concentrations ($n = 10$ at each concentration), blank, and zero samples. Calibration samples were analyzed from low to high concentration at the

beginning of each validation run and the other samples were distributed randomly through the run, except for the blank plasma samples which were placed after the high calibration sample. Carryover was evaluated to ensure that the rinsing solution used to clean the injection needle prevented injected sample contaminating subsequent runs. Stability and the freeze–thaw samples were analyzed on day three, with other validation samples. Linearity was assessed by weighted ($1/x^2$) least-squares regression analysis. The calibration plot had to have a correlation coefficient (r) of 0.998 or better. The acceptance criterion for each back-calculated standard concentration was 15% deviation from the nominal value except at the LLOQ, at which 20% was acceptable. Results from at least 67% of non-zero standards should meet these criteria, including an acceptable LLOQ and upper limit of quantitation [18].

Recovery and Matrix Effect

Recovery of tamsulosin by the extraction procedure was determined by comparison of peak area of tamsulosin in spiked plasma samples (five low, medium, and high quality controls) with the peak area of tamsulosin from samples prepared by spiking extracted drug-free plasma with the same amount of tamsulosin at the step immediately before chromatography. Similarly, recovery of the IS was determined by comparing mean peak areas of the IS in samples prepared by spiking extracted drug-free plasma with the same amount of IS at the step immediately before chromatography.

Endogenous matrix components may change the efficiency of droplet formation or droplet evaporation, which in turns affects the amount of charged ion in the gas phase which ultimately reaches the detector. Five sets were prepared by direct addition of the analytes to reconstitution solution with or without the presence of residue extracted from the control plasma; ion suppression was assessed at the three QC sample concentrations by comparing the mean analyte peak area obtained from these sets of testing samples. Matrix effect was calculated [19, 20] by use of the equation:

Matrix effect = [(analyte peak area of extracted plasma residue / analyte peak area of neat solution) × 100] 100

Accuracy and Precision

Within-batch accuracy and precision were evaluated by analyzing ten sets of quality-control samples in a batch. Between-batch precision and accuracy were determined by analyzing ten sets of quality control samples in three

different batches. The quality control samples were randomized daily, processed, and analyzed in the positions:

(a) immediately after the standard curve;
(b) in the middle of batch; or
(c) at the end of the batch.

The acceptance criteria for within and between-batch precision were 20% or better for the LLOQ and 15% or better for the other concentrations; that for accuracy was 100 ± 20% or better for the LLOQ and 100 ± 15% or better for the other concentrations [18].

Stability

Bench-top stability was examined by storing replicates of the low and high-concentration plasma quality-control samples at room temperature for approximately 12 h. Freeze–thaw stability of the samples was measured over three freeze–thaw cycles of thawing at room temperature for 2–3 h then re-freezing for 12–24 h. Autosampler stability of tamsulosin was tested by analysis of processed and reconstituted low and high-concentration plasma QC samples stored in the autosampler tray for 24 h. Stability of tamsulosin in human plasma was tested after storage at approximately 70°C for 30 days. For each concentration and each storage condition, six replicates were analyzed in one analytical batch. The concentrations of tamsulosin after each storage period were related to the initial concentration as determined for the samples. The stability of the IS in human plasma under different temperature and time conditions was also evaluated. The stability of stock solutions of tamsulosin and propranolol was tested, and established, at room temperature for 2 and 24 h and under refrigeration (~4°C) for 30 days.

Results and Discussion

Separation and Specificity

MS–MS detection is highly selective, and no interferences were observed. Tamsulosin and the IS gave protonated molecules $[M + H]^+$ in positive MS mode. The major ions observed were *m*/*z* 409.06 for tamsulosin and *m*/*z* 260.07 for propranolol. The most intense product ions observed in the MS–MS spectra were *m*/*z* 270.96 for tamsulosin and *m*/*z* 183.02 for propranolol. The corresponding positive product-ion spectra of tamsulosin and propranolol (IS) are shown in *Figs 2* and *3*, respectively.

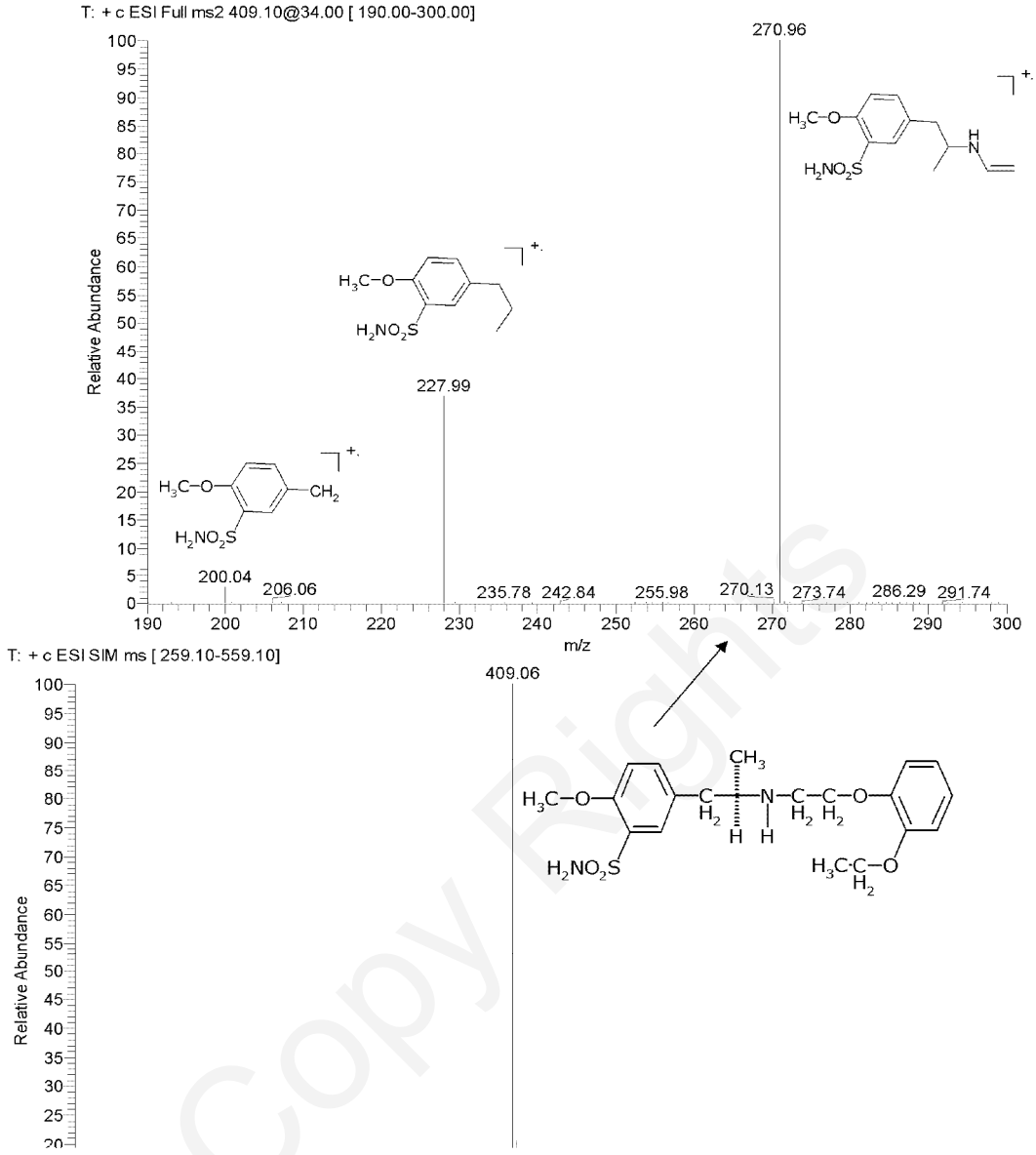


Fig. 2. Positive-ion electrospray mass spectrum (bottom) and product ion mass spectrum (top) used in SRM for analysis of tamsulosin

The chromatographic conditions, especially the composition of mobile phase, were optimized by means of several trials to achieve good resolution and symmetric peak shapes for the analyte and the IS, and short run time. This was achieved by use of methanol–water–formic acid 70:30:0.1 (*v*/*v*) as mobile phase. Addition of 0.1% formic acid resulted in good peak shape, ionization, and fragmentation in the mass spectrometer.

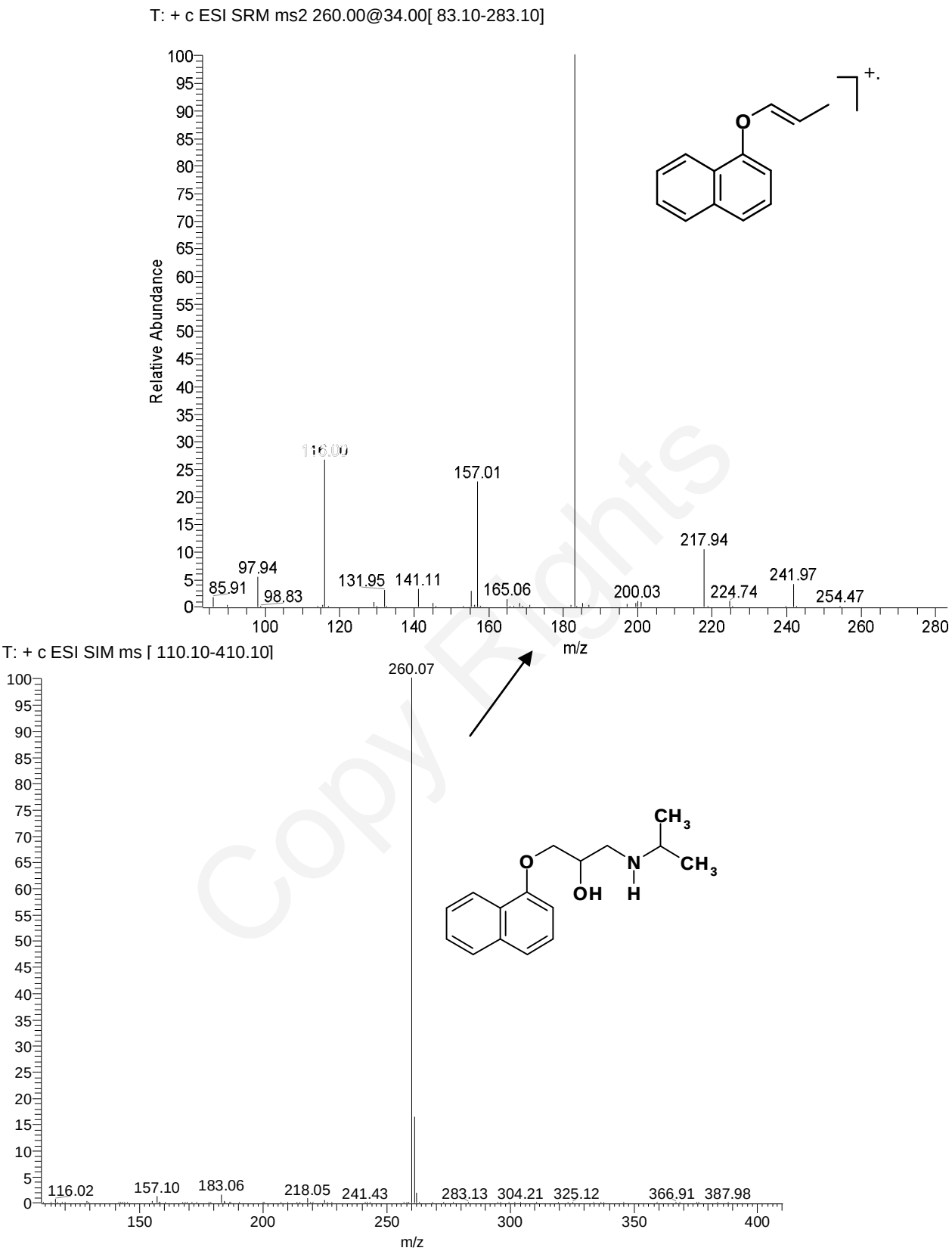


Fig. 3. Positive-ion electrospray mass spectrum (bottom) and product ion mass spectrum (top) used in SRM for analysis of propranolol

Tandem mass spectrometry enables selective detection of substances of different masses and with different fragmentation without chromatographic separation. Development of this method was focused on short run time to ensure high throughput, paying attention to matrix effects and good peak shapes. The high concentration of methanol eluted tamsulosin and the IS with retention times of ~2.4 and 2.9 min, respectively. Total HPLC–MS–MS analysis time was 4 min per sample. No interference of the analytes was observed, because of the high selectivity of MS–MS.

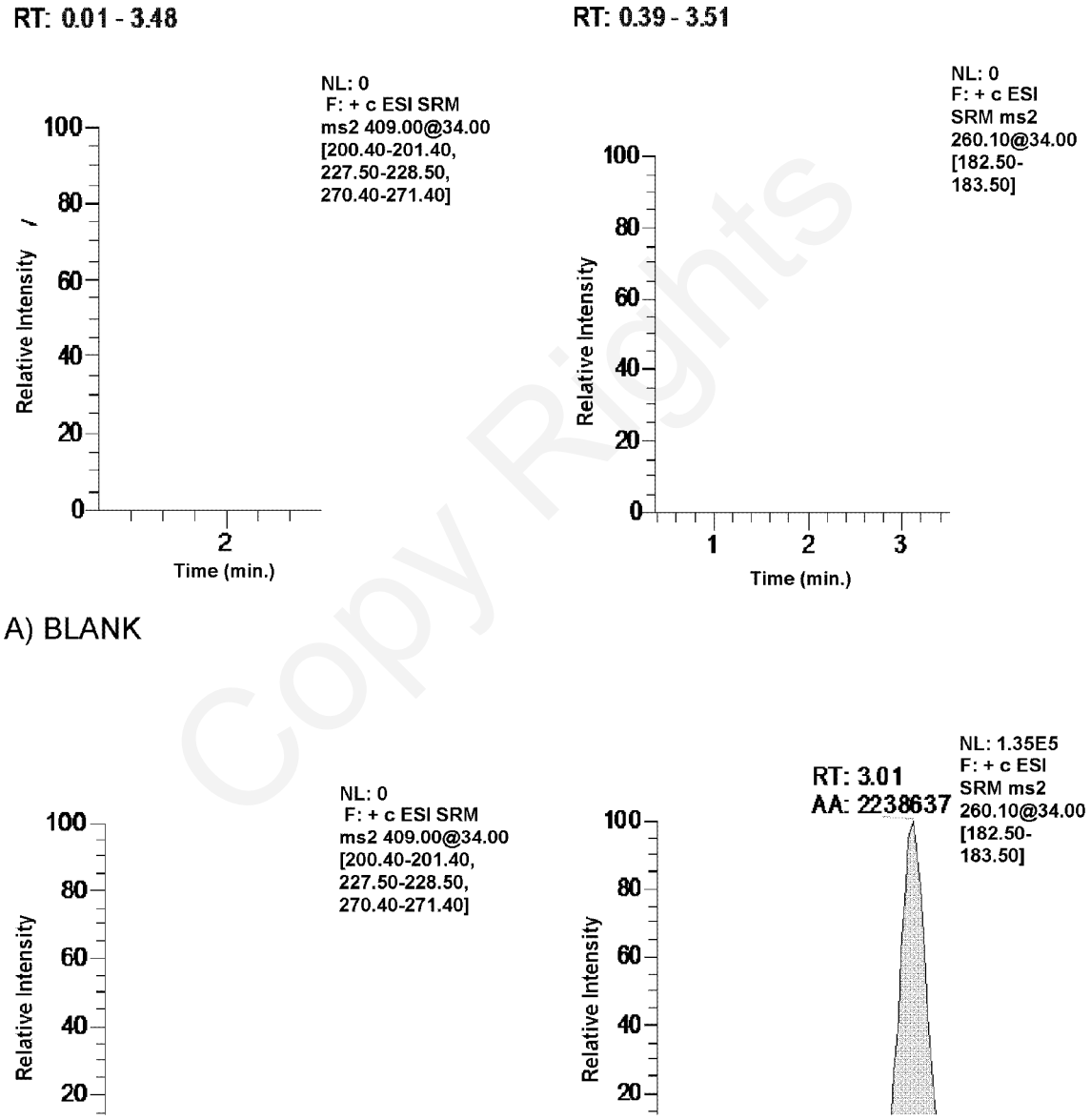


Fig. 4A,B

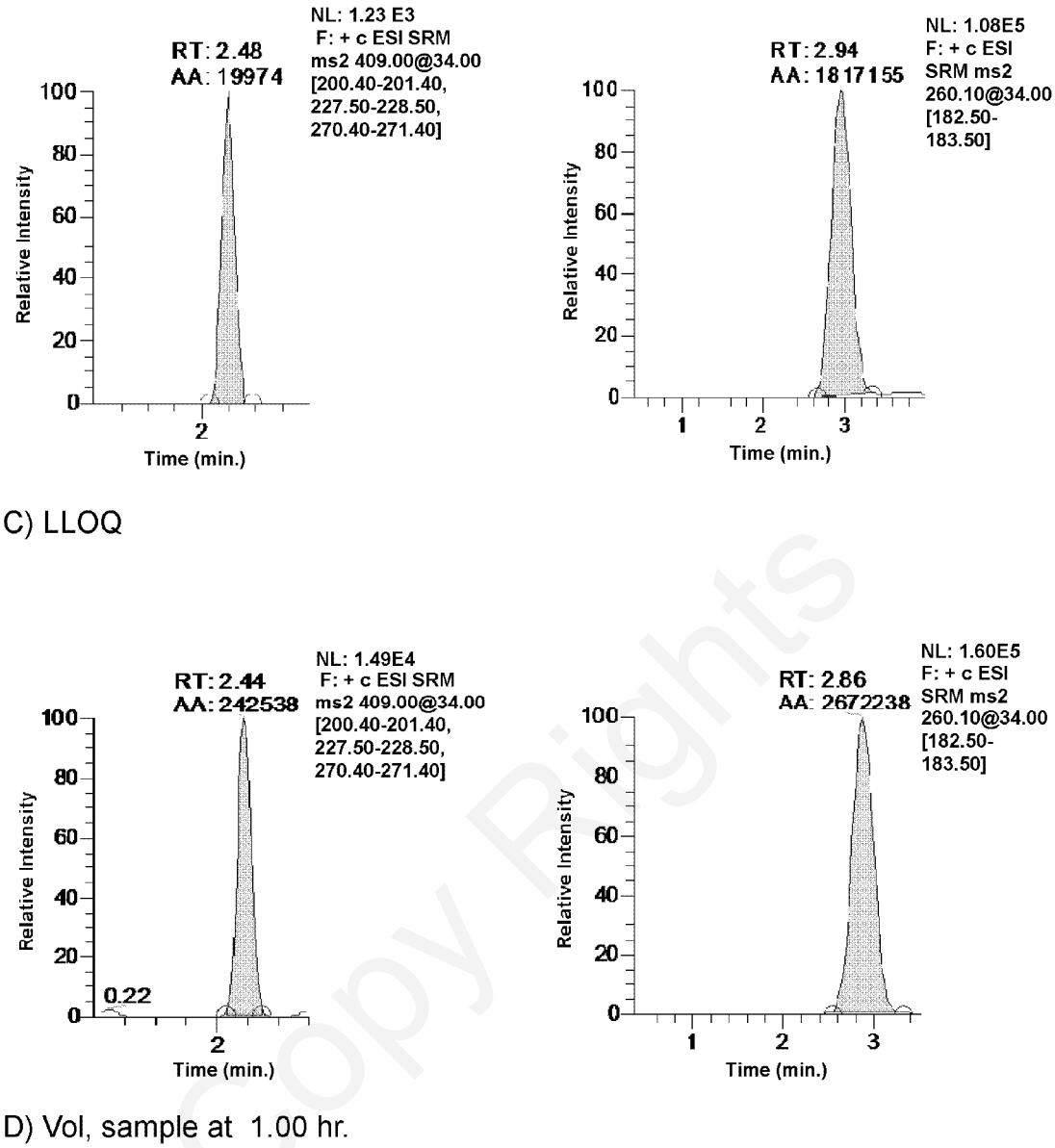


Fig. 4. LC–MS–MS chromatograms obtained from: (A) blank human plasma; (B) human plasma spiked with 100 ng mL^{-1} IS; (C) human plasma spiked with 0.25 ng mL^{-1} tamsulosin and 100 ng mL^{-1} IS; (D) plasma from a volunteer after oral administration of a 0.4 mg tablet of tamsulosin (the concentration was 4.029 ng mL^{-1})

The liquid–liquid extraction procedure described here enables rapid isolation of the analytes from the plasma matrix. Several extraction solvents of different composition were tested, and 7:3 (*v*/*v*) hexane–ethyl acetate was found to meet the criteria of clean injection extracts, and high and constant recovery. Recovery of the analyte and IS was good, consistent, precise, and reproducible, and the assay proved to be robust in high-throughput bioanalysis.

Choosing the appropriate internal standard is an important aspect of achieving acceptable method performance, especially with LC–MS–MS, with which matrix effects can lead to poor analytical results. Ideally, isotopically labeled internal standards for all analytes should be used, but these are not economical. Several compounds were investigated to find a suitable IS, and finally propranolol was found to be suitable.

The specificity of the method was examined by analyzing six different blank human plasma extracts ($n = 6$) and an extract spiked with the internal standard only. Product ion chromatograms extracted from plasma and volunteer samples are depicted in *Fig. 4*. Because of the high specificity of MS–MS detection, no interfering peaks were found when chromatographing blank extract (*Fig. 4A*). *Fig. 4* shows representative product ion chromatograms obtained from (B) human plasma spiked with IS, (C) human plasma spiked with tamsulosin at the LLOQ (0.25 ng mL ^{1}) and the IS, and (D) an extracted volunteer plasma 1.0 h after an oral dose of 0.4 mg tamsulosin. Tamsulosin was unambiguously identified and was quantified at 4.029 ng mL ^{1}. There was no response from blank plasma after injection of high-concentration calibration sample, which indicates there was no carryover of the analyte in subsequent runs.

Validation

Linearity and Limit of Quantitation

The tamsulosin-to-IS peak-area ratio for human plasma was a linear function of analyte concentration over the range 0.25–50 ng mL^{-1}. The mean linear regression equation ($y = mx + c$) of the calibration plot was $y = 0.02382\ (\pm\ 00338)x - 0.00164\ (\pm\ 0.00588)$, where y is the analyte-to-IS peak-area ratio and x is analyte concentration. The correlation coefficient (r) for tamsulosin was >0.998 over the concentration range used. *Table I* summarizes the calibration results for the analyte. The limit of quantification was 0.25 ng mL^{-1} ($n = 5$). At this concentration the signal-to-noise ratio is approximately 12:1. The precision, as relative standard deviation, was 7.4% and accuracy, defined as the deviation between the nominal and measured values was 3.4% at this concentration (*Table II*).

Table I. Representative calibration data for HPLC assay of tamsulosin in plasma[a]

Calibration plot no.	Slope	*y* intercept	*r*
1	0.02926	−0.00527	0.99999
2	0.02659	−0.00462	0.99997
3	0.02287	−0.00459	0.99994
4	0.02103	−0.00653	0.99994
5	0.02084	0.00824	0.99972
6	0.02232	0.00291	0.99998
Mean	0.02382	−0.00164	0.99996
SD	0.00338	0.00588	0.00010

[a]Eight calibration standards were used to construct each calibration plot

Table II. Results from statistical analysis of the calibration data for tamsulosin

Concentration added (ng mL ^{1})	Concentration found (mean ± SD, n = 5) (ng mL ^{1})	Precision (%)	Bias (%)
0.25	0.24 ± 0.02	7.4	−3.4
0.50	0.49 ± 0.01	2.8	−2.0
1.00	0.99 ± 0.02	2.0	−0.6
2.50	2.48 ± 0.05	2.1	−0.8
5.00	5.12 ± 0.09	1.8	2.5
10.00	10.15 ± 0.19	1.9	1.5
30.00	29.92 ± 0.34	1.1	−0.3
50.00	49.84 ± 0.24	0.5	−0.3

Extraction Recovery and Matrix Effect

Average recovery of tamsulosin and propranolol were 81.8 ± 2.2 and 84.2 ± 3.2%, respectively, at the concentrations used in the assay (*Table III*). Extraction recovery was satisfactory because it was consistent, precise, and reproducible. Thus the liquid–liquid extraction procedure used in this method proved to be efficient and simple enough to extract the drug and the internal standard simultaneously from human plasma.

Table III. Extraction recovery of tamsulosin and propranolol from plasma, and matrix effect

Analyte	Concentration (ng mL ^{1})	Concentration found (ng mL^{-1}, mean ± SD)	Recovery (%, mean ± SD)	Mean recovery	Matrix effect (%)
Tamsulosin (*n* = 5)	0.75	0.62 ± 0.05	81.5 ± 6.8	81.8 ± 2.2	–3.6
	25	20.04 ± 1.25	80.2 ± 5.0		–2.0
	40	33.53 ± 0.96	83.8 ± 2.4		2.3
Propranolol (*n* = 5)	100	84.2 ± 3.2	–	84.2 ± 3.2	1.3

Endogenous components are the main cause of ion-suppression effects during electrospray ionization. The extent of this effect mainly depends on the sample-extraction procedure and is also compound-dependent [21]. The matrix effects on drug and IS were −3.6 to 2.3% (*Table III*), which indicated there was no marked endogenous matrix effect.

Precision and Accuracy

Accuracy was expressed as percentage error (relative error, or bias, %), calculated as:

[(measured concentration − spiked concentration)/spiked concentration] × 100 (%)

whereas precision was quantified by calculating within and between-day CV (%).

The precision and accuracy at the LLOQ and for low, medium and high concentrations of tamsulosin in plasma were within acceptable limits (*Table IV*). Within and between-day precision at the LLOQ was <9.3 and 10.8%, respectively. Within and between-day relative error was less than −0.1 and 5.0%, respectively.

Within and between-day precision was less than 2.8 and 5.5% for quality-control samples other than at the LLOQ. Within and between-day relative error was less than 1.3 and 3.4%, respectively.

Table IV. Accuracy and precision of the HPLC method for analysis of tamsulosin in plasma

Concentration added (ng mL ^{1})	Concentration found (mean ± SD)(ng mL^{-1})	Precision (%)	Bias (%)
Within-batch precision (n = 10)			
0.25 (LLOQ)	0.25 ± 0.02	9.3	−0.1
0.75 (Low)	0.76 ± 0.02	2.8	1.3
25 (Medium)	25.18 ± 0.58	2.3	0.7
40 (High)	40.06 ± 0.67	1.7	0.1
Between-batch precision (n = 30)			
0.25 (LLOQ)	0.26 + 0.03	10.8	5.0
0.75 (Low)	0.73 ± 0.08	5.5	−3.4
25 (Medium)	24.59 ± 1.01	4.0	−1.6
40 (High)	38.83 ± 1.57	4.1	−2.9

Stability

Stock solutions of tamsulosin and propranolol in acetonitrile and methanol, respectively, were stable for at least two months when stored at 4°C. The compounds were also stable in plasma samples through three freeze–thaw cycles and when low and high-concentration quality-control samples were stored for 12 h at room temperature. QC samples were stable for at least 30 days if stored frozen at approximately 70°C in the deep freezer (*Table V*). Samples were also stable when kept in an autoinjector for up to 24 h. The stability of the IS in human plasma under different temperature and time conditions was also verified (data not shown).

Results from this study of the stability of tamsulosin in plasma were indicative of reliable behavior, because mean results for the samples were within acceptance criteria of ±15% of the initial values for control samples. The findings also indicated that storage at −70°C is suitable for samples of tamsulosin in plasma, and no stability-related problems would be expected during routine analysis of samples for pharmacokinetic, bioavailability, or bioequivalence studies.

The validated method has been successfully used to quantify tamsulosin concentrations in human plasma after oral administration of a single dose of 0.4 mg tamsulosin. The analyses were accomplished in accordance with bio-analytical method validation guidance [18].

Table V. Results from stability testing

Sample concentration (ng mL $^{-1}$)	Concentration found (ng mL $^{-1}$)	Precision (%)	Bias (%)
Short-term stability for 12 h (n = 6) in plasma			
0.75	0.68 ± 0.08	12.0	−8.8
40	40.19 ± 0.90	2.3	0.5
Three freeze and thaw cycles (n = 6)			
0.75	0.73 ± 0.04	5.4	−3.3
40	38.43 ± 3.11	8.1	−3.9
Autosampler stability for 24 h at 5 ± 1°C (n = 6)			
0.75	0.77 ± 0.05	6.5	−3.4
40	39.33 ± 2.69	6.8	−1.7
30-day stability at −70°C (n = 6)			
0.75	0.78 ± 0.60	8.6	3.5
40	39.93 ± 0.26	0.7	−0.2

Conclusion

This HPLC–ESI–MS–MS (ion-trap) method employing simple liquid–liquid extraction for sample preparation enables rapid, economical, very simple, and convenient quantification of tamsulosin in human plasma in the range 0.25–50 ng mL $^{-1}$. The method is simple, sensitive, and fully validated in accordance with recognized guidelines. The precision, accuracy, and sensitivity of the method are acceptable, and adequate for use in clinical studies. The method has been used for analysis of tamsulosin in healthy volunteers. The method also has a short turnover time (less than ~4.0 min) and is suitable for clinical pharmacokinetic / bioequivalence studies. In conclusion, this paper describes a very simple, rapid, and sensitive HPLC method for quantification of tamsulosin in human plasma.

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