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Quantitation of tamsulosin in human plasma by liquid chromatography–electrospray ionization mass spectrometry

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Abstract

A highly sensitive method for quantitation of tamsulosin in human plasma using 1-(2,6-dimethyl-3-hydroxylphenoxy)-2-(3,4-methoxyphenylethylamino)-propane hydrochloride as the internal standard (I.S.) was established using liquid chromatography–electrospray ionization-mass spectrometry (LC–ESI-MS). After alkalization with saturated sodium bicarbonate, plasma were extracted by ethyl acetate and separated by HPLC on a C_{18} reversed-phase column using a mobile phase of methanol–water–acetic acid–triethylamine (620:380:1.5:1.5, v/v). Analytes were quantitated using positive electrospray ionization in a quadrupole spectrometer. LC–ESI-MS was performed in the selected ion monitoring (SIM) mode using target ions at *m/z* 228 for tamsulosin and *m/z* 222 for the I.S. Calibration curves, which were linear over the range 0.2–30 ng/ml, were analyzed contemporaneously with each batch of samples, along with low (0.5 ng/ml), medium (3 ng/ml) and high (30 ng/ml) quality control samples. The intra- and inter-assay variability ranged from 2.14 to 8.87% for the low, medium and high quality control samples. The extraction recovery of tamsulosin from plasma was in the range of 84.2–94.5%. The method has been used successfully to study tamsulosin pharmacokinetics in adult humans.

Keywords: Tamsulosin

1. Introduction

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 [1,2]. The drug has been used clinically for urinary obstructed patients with benign prostatic hyperplasia. The α_1-adrenoceptor antagonist activity of tamsulosin hydrochloride has been found to be more potent than other drugs such as prozosin [3]. Because of low oral dose of 0.1–0.2 mg, the human plasma concentration of tamsulosin is very low. After an oral dose of 0.2 mg the maximum plasma concentration of tamsulosin in adult humans was about 7 ng/ml [4]. Few articles reported quantitation of tamsulosin in human plasma. Soeishi et al. [5]reported a method for determination of tamsulosin by HPLC 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. More-

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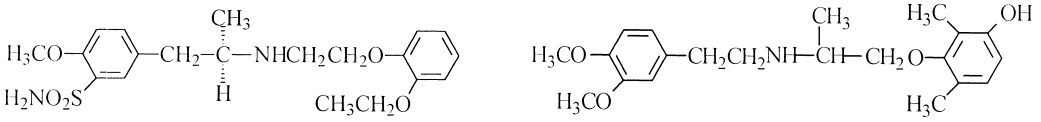

Fig. 1. Chemical structures of tamsulosin (I) and the I.S. (II).

over, the detection limit (0.5 ng/ml) reported in the paper was not adequate for pharmacokinetic studies. Recent progress in the field of mass spectrometry (MS) has resulted in development of new techniques such as electrospray ionization (ESI) and tandem MS (MS–MS) which have significantly improved detection sensitivity for 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. Hiroshi et al. [6] has reported a high-performance liquid chromatography-electrospray tandem MS (LC–MS–MS) method for the 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. In this study, we established a highly sensitive and rapid assay method using LC–ESI-MS instead of using LC–MS–MS. The assay is validated over the range of 0.2–30 ng/ml and the method has been used successfully to study tamsulosin pharmacokinetics in adult humans.

2. Experimental

2.1. Materials and reagents

Tamsulosin was supplied by Zhejiang Hailisheng Pharmaceutical Company. The internal standard (I.S.), 1-(2,6-dimethyl-3-hydroxylphenoxy)-2-(3,4-methoxyphenylethylamino)-propane (Fig. 1) hydrochloride, was a gift from Organic Chemistry Laboratories of China Pharmaceutical University. Sodium bicarbonate and ethyl acetate were analytical grade; methanol, acetic acid, triethylamine were HPLC grade, all the chemicals were purchase from Nanjing Chemical Reagent Co. (Nanjing, China).

2.2. Instrument and conditions

HPLC analyses were performed using a Hewlett-Packard HP1100 LC/DAD/MSD system (Hewlett-Packard, USA) with a Hypersil ODS-2 C_{18} column (4.6×250 mm, 5 μm, Dalian Elite Science Co., China). The mobile phase was methanol–water–acetic acid–triethylamine (620:380:1.5:1.5, v/v), the column temperature was maintained at 25°C. A constant flow-rate of 1.0 ml/min was employed throughout the analyses. LC–ESI-MS was carried out using nitrogen to assist nebulization. A quadrupole mass spectrometer equipped with an electrospray ionization source was used in positive ion selected ion monitoring (SIM) mode, set with a drying gas (N_2) flow of 10 l/min, nebulizer pressure of 40 pisg, drying gas temperature of 350°C and capillary voltage of 4 kV. The fragmentor voltage was 150 V. Target ions were monitored at m/z 228 for tamsulosin and m/z 222 for I.S. in the SIM mode.

2.3. Preparation of standard solutions, calibration standards, limit of quantitation (LOQ) and quality control samples

Stock solutions of tamsulosin and the I.S. were prepared at 1 mg/ml in methanol, respectively and stored at −20°C. These solutions were stable for 9 months at least. Standard solutions containing 0.01, 0.1 and 1 μg/ml tamsulosin were prepared by diluting the stock solution with methanol. A solution containing 1 μg/ml I.S. was also prepared using methanol.

Calibration standards of tamsulosin (0.2, 0.5, 1, 3, 10, 20, 30 ng/ml) and a LOQ sample at 0.1 ng/ml were prepared by spiking appropriate amount of the standard solutions in control plasma obtained from healthy, non-smoking volunteers. Quality control

(QC) samples were prepared in blank control plasma at concentrations of 0.5, 3 and 30 ng/ml.

2.4. Sample preparation

To a 1-ml aliquot of plasma in a 10-ml centrifuge tube, 40 μl I.S. solution (1 μg/ml), 1 ml of saturated sodium bicarbonate solution and 5 ml of ethyl acetate were added. The centrifuge tube was vortexed for 5 min, and then was evaporated to dryness under a stream of nitrogen in a 45°C water bath. The residue was reconstituted in 100 μl of the mobile phase, and a 40-μl aliquot was injected onto the LC–ESI-MS system.

2.5. Assay validation

2.5.1. Linearity and LOQ

Calibration standards of seven concentrations of tamsulosin ranged 0.2–30 ng/ml and LOQ plasma sample of tamsulosin were extracted and assayed. A calibration curve was constructed by plotting the area ratios of tamsulosin to the I.S. against tamsulosin concentrations in plasma. LOQ for tamsulosin was established based on a S/N ratio of 10.

2.5.2. Precision and accuracy

The precision of the assay was determined from the low, medium and high QC plasma samples by replicate analyses of the three different concentrations (0.5, 3, 30 ng/ml). Intra-day precision was determined by repeated analysis of each QC sample on one day ($n=5$), and inter-day precision and accuracy was determined by repeated analysis on five consecutive days ($n=1$ series per day). The concentration of each sample was determined using calibration standards prepared on the same day. Accuracy is defined as the relative deviation in the computed value (E) of a standard from that of its true value (T) expressed as a percentage (RE%). It was calculated using the formula $RE\% = (E-T)/T \times 100$. Assay precision was defined as the relative standard deviation (SD) from the mean (M), calculated using the equation $RSD\% = SD/M \times 100\%$.

2.5.3. Extraction recovery

The absolute recovery (extraction efficiency) of tamsulosin through the extraction procedures was

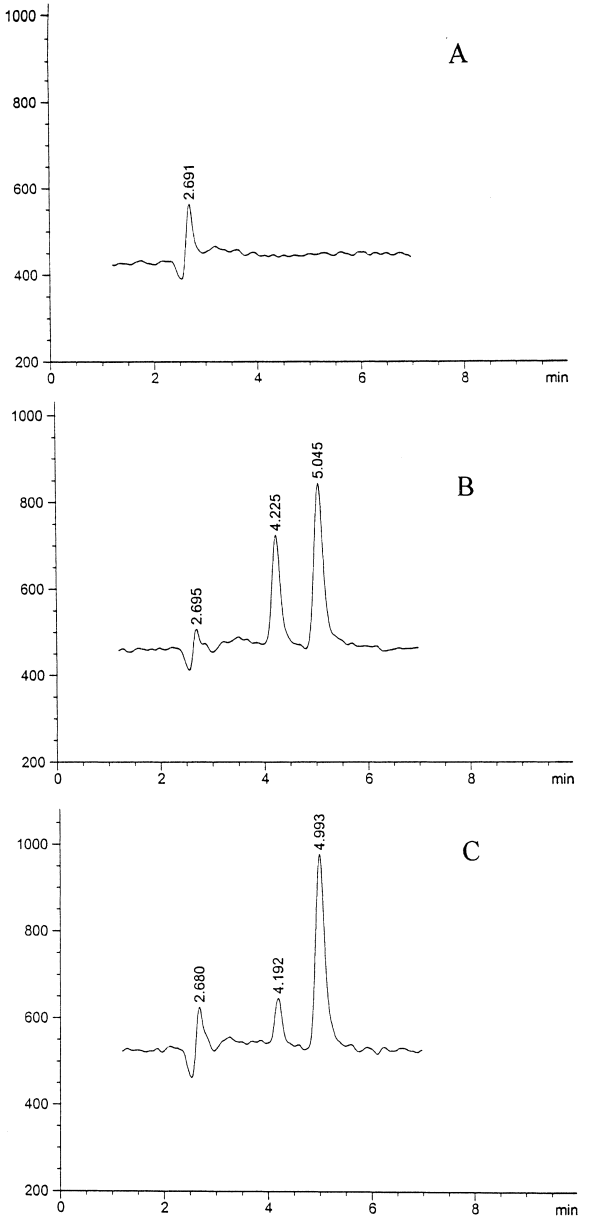

Fig. 2. Typical chromatograms of: (A) blank plasma, (B) plasma spiked with tamsulosin (3.11 ng/ml) and the I.S. and (C) plasma obtained from a volunteer 15 h after tamsulosin administration. The tamsulosin concentration of the plasma sample shown in (C) was estimated to be 1.05 ng/ml. Each plasma sample was extracted as described in the text and a 40-μl aliquot (of 100 μl total) was analyzed by LC–ESI-MS.

determined at low, medium and high concentrations by the external standard method. A known amount of tamsulosin was added to human plasma prior to extraction as described in Section 2.4. The internal standard was added after extraction to eliminate bias introduced by sample processing. The concentration of tamsulosin following extraction was calculated using the calibration curves prepared on the same day, and was compared to the nominal concentration to estimate extraction recovery.

2.6. Pharmacokinetics and study

Each of 20 healthy male volunteers received a controlled release capsule containing 0.2 mg tamsulosin, respectively after overnight fasting. Blood samples were drawn at appropriate intervals and centrifuged to obtain plasma samples.

3. Result and discussion

3.1. Conditions of chromatography

I.S. was selected as an internal standard because its chemical properties and mass spectral fragmentation were similar to those of tamsulosin. The selection of mobile phase components was a critical factor in achieving good chromatographic peak shape and resolution. A solvent system of acetic acid and triethylamine was selected as a buffer for its good volatility. Moreover, acetic acid could improve the ionization efficiency and triethylamine could inhibit the tailing problem of chromatographic peaks of tamsulosin and I.S. Good separation of target compounds and short run time were obtained using an elution system of methanol–water–triethylamine–acetic acid (620:380:1.5:1.5, v/v). Representative chromatograms are shown in Fig. 2 in which the retention times were 4.2 min for tamsulosin and 5.0 min for I.S.

3.2. Conditions for ESI-MS

The ESI mass spectrum at a fragmentor voltage of 70 V showed that the protonated molecular ion $[M+H]^+$ of tamsulosin was at m/z 409. By increasing fragmentor voltage, the fragmentation pattern of this protonated molecular ion was observed. The product ion mass spectrum of this protonated molecular ion is shown in Fig. 3 in which the most intensive product ion was observed at m/z 228. By monitoring this product ion, a highly sensitive assay for tamsulosin was developed. The intensity of product ion of tamsolusin at m/z 228 was compared at fragmentor voltages of 100, 120, 150, 160, 180 and 200 V in order to determine the optimal collision energy. The result showed that the highest sensitivity was ob-

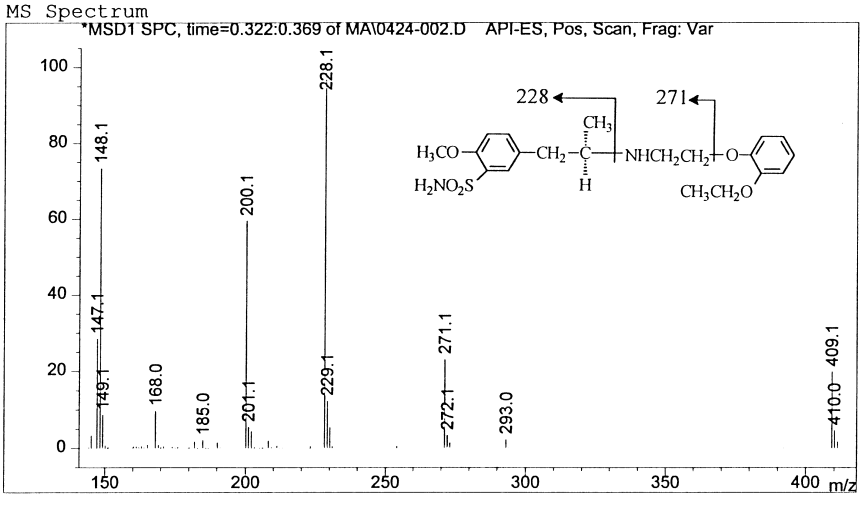

Fig. 3. Positive production mass spectra of the protonated molecular ions (MH^+) of tamsulosin at 150 V.

tained using a fragmentor voltage of 150 V. Therefore, a fragmentor voltage of 150 V was used to carry out LC–ESI-MS in the SIM mode. At this collision energy the most intensive product ion of I.S. protonated molecular ion was at m/z 165 (Fig. 4). However, when this ion was selected as the target ion of I.S. in the SIM, typical plasma extracts showed a little interference in the retention time range of tamsolusin. Therefore, another prominent product ion (m/z 222) of I.S. was selected as the target ion of I.S. in the SIM.

3.3. Method validation

3.3.1. Calibration curve and sensitivity

The calibration curves which relate the concentrations of tamsulosin to the area ratio of tamsulosin to I.S. were linear over the range of 0.2–30 ng/ml. A typical calibration curve for tamsulosin had a slope of 0.2081, an intercept of 0.00539 and $R = 0.9998$. A calibration curve was prepared contemporaneously with each batch of samples. LOQ for tamsulosin in plasma, defined at a N/S = 10:1, was 0.1 ng/ml (Fig. 5).

3.3.2. Precision and accuracy

The intra- and inter-day ($n = 5$) precision and accuracy, shown in Table 1, were satisfactory for our purpose. The intra-day precision expressed as relative standard deviation (RSD) for each QC concentration (0.5, 3, 30 ng/ml) was 2.14–3.26%, and the inter-day RSD for the same QC samples was 2.67–8.87%.

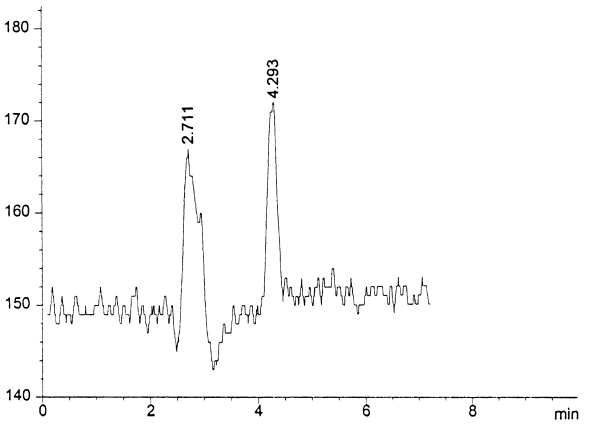

Fig. 5. Chromatogram of tamsulosin at LOQ (0.1 ng/ml in plasma).

3.3.3. Extraction recovery

Ethyl acetate and a mixture of ethyl acetate–cyclohexane (7:3, v/v) were evaluated as extraction solvents. Ethyl acetate was chosen as the extraction solvent for its higher extraction efficiency to the two target compounds. The mean recovery of tamsulosin from human plasma with ethyl acetate was 90.47±5.51% (range: 84.17–94.45%). The recovery

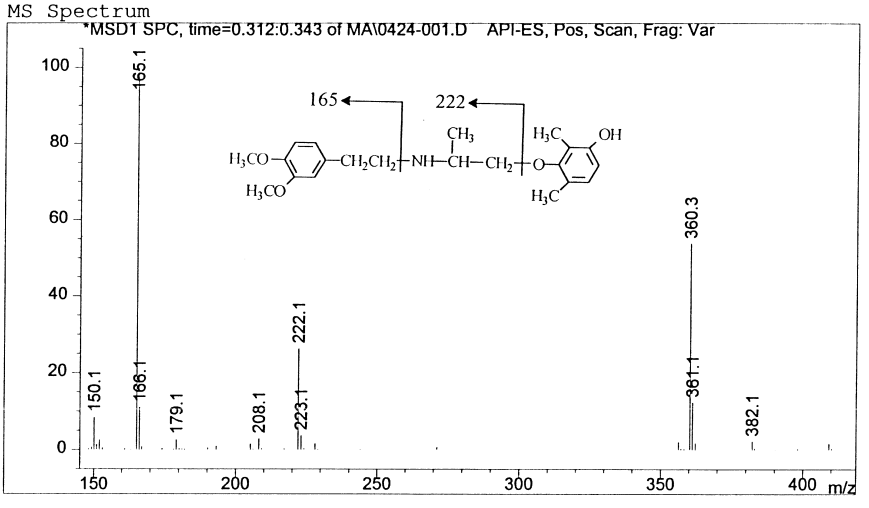

Fig. 4. Positive production mass spectra of the protonated molecular ions (MH^+) of the I.S. at 150 V.

Table 1
Precision and accuracy of the assay for determination of tamsulosin in plasma ($n=5$)

Added to plasma (ng/ml)	Intra-assay measured concentration (mean±SD) (ng/ml)	RE (%)	RSD (%)	Inter-assay measured concentration (mean±SD) (ng/ml)	RE (%)	RSD (%)
0.50	0.55±0.017	10.88	3.14	0.53±0.047	5.96	8.87
3.01	2.98±0.097	−0. 92	3.26	2.99±0.14	−0.77	4.68
30.15	30.88±0.656	2.43	2.14	30.01±0.80	−0.47	2.67

Table 2
The extraction recovery of tamsulosin from human plasma ($n=5$)

Added concentration (ng/ml)	Measured concentration (ng/ml; mean±SD)	Extraction recovery (%)	RSD (%)
0.50	0.42±0.016	84.17	3.92
3.01	2.79±0.099	92.78	3.54
30.15	28.43±0.813	94.45	2.86

data reported here is the average for the three QC standards shown in Table 2.

3.4. Application

The method described above was successfully applied to the pharmacokinetic study in which plasma concentrations of tamsulosin in 20 healthy male volunteers were determined up to 36 h after administration of 0.2 mg of tamsulosin hydrochloride as a controlled release capsule. The mean plasma concentration–time curve is shown in Fig. 6. The pharmacokinetic parameter values are calculated. The maximum plasma concentration of 6.0±1.6 ng/ml was achieved 5.5±1.1 h after administration. The elimination half-life and mean residence time of tamsulosin in healthy human body were 8.1±3.8 and 11.5±1.4 h, respectively.

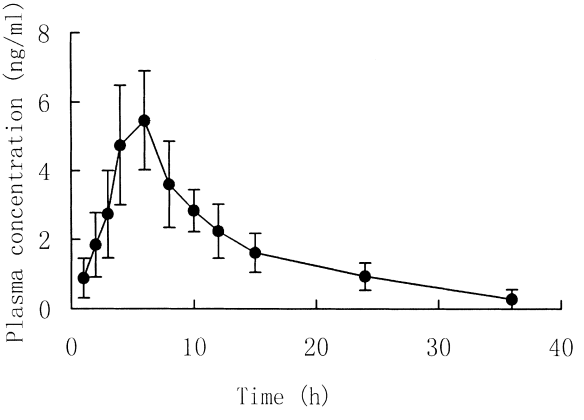

Fig. 6. Mean tamsulosin plasma concentration–time profile in 20 healthy volunteers after a 0.2-mg oral dose.

4. Conclusion

This assay achieved higher sensitivity and better specificity for analysis of tamsulosin in human plasma. The limit of quantitation of 0.1 ng/ml for tamsulosin was better than attainable by HPLC–FL (fluorescence detection). The I.S. proved to be a good internal standard for this assay. No significant interference caused by endogenous compounds was observed. This simple and rapid assay can be successfully used in pharmacokinetic studies of tamsulosin in human.

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