# A LCMS-IT-TOF method for the quantification of tamsulosin in human plasma and application to a bioequivalence study in healthy Vietnamese volunteers

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# Abstract

Tamsulosin hydrochloride is an alpha1 adreno receptor blocking agent used for the treatment of lower urinary tract symptoms associated with benign prostatic hyperplasia. A highly sensitive, rapid and accurate LCMS-IT-TOF method was developed for determination of tamsulosin in human plasma. Analyte and internal standard were separated on Gemini  $C_{_{18}}$  (150 mm x 4.6 mm, 5  $\mu$ m particle size) column with a mobile phase of methanol and 5 % formic acid buffer in a ratio of 50:50 (v/v). The method had a chromatography run time of 4 min and was linear in the concentration range of 0.5-50 ng/mL; the intra-day and inter-day accuracy (bias  $\leq 9.8$  %) and precision (CV  $\leq 5.4$  %). The limit of quantitation was 0.5 ng/mL. This method was employed in bioequivalence study of tamsulosin. A bioequivalence study of two modified release formulations of tamsulosin hydrochloride 0.4 mg in healthy Vietnamese male populations was conducted (Tamsustad of STADA-VN Joint Venture Co., Ltd., Vietnam, as test product; FLOMAX relief®MR of Boehringer Ingelheim Limited, United Kingdom, as reference product). After dosing, serial blood samples were collected for a period of 96 hrs. No clinically significant or serious adverse events were observed during the study. The 90% confidence intervals for the ratios of  $C_{max}$ , AUC<sub>0-t</sub> and AUC<sub>0- $\infty$ </sub> suggested that a single dose of the test and reference modified-release formulations of tamsulosin hydrochloride met the FDA regulatory requirements of bioequivalence definition intervals of 80.00% - 125.00%.

Keyword: LCMS-IT-TOF, Tamsulosin, Bioeqivalence

## **1. INTRODUCTION**

Tamsulosin hydrochloride, (-)-(R)-5-[2-[[2-(*O*-ethoxyphenoxy)ethyl]amino] propyl]-2-methoxybenzenesulfonamide, monohydrochloride, isanalpha l adrenoreceptor (AR) blocking agent used for the treatment of lower urinary tract symptoms (LUTS) associated with benign prostatic hyperplasia (BPH)<sup>1,2</sup>. Tamsulosin hydrochloride is given as the 0.4 mg modified release formulation peak plasma concentrations occur about 6 hours and concentrations are between 11.8 ng/mL and 16.1 ng/mL after a single dose<sup>3-5</sup>. According to the literature, various articles have been reported for the determination of tamsulosin in human plasma by HPLC<sup>6,7</sup> and LC-MS <sup>8</sup> and LC–MS/MS <sup>3,9-13</sup> method. The aim of the present work is to establish a highly sensitive and accurate method for quantitation of tamsulosin using liquid chromatography mass spectrometry-ion trap-time of fight (LCMS-IT-TOF) which apply to pharmacokinetic studies and *in vivo* bioavailability of tamsulosin hydrochloride preparation. Method run time is only 4.0 min per sample, which helps to increase ESI-MS source life and reduce column

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back pressure during analysis of clinical samples. The application of this assay method to a bioequivalence study of two modified release formulations in healthy Vietnamese male volunteers is described.

## 2. MATERIALS AND METHODS

## 2.1. Chemicals and materials

Tamsulosin hydrochloride (99.60%) Diphenhydramine (99.74%; internal standard, Figure 2., IS.) were from Institute of Drug Quality Control Ho Chi Minh city, Vietnam. Methanol, dichloromethane and diethyl ether were of HPLC grade (J.T.Baker, Malaysia). Formic acid was PA (Merck, Germany). Distilled water was filtered through 0.22 µm filters (Millipore, USA). Blood samples were collected from HCMC Blood Transfusion and Hematology Hospital, Vietnam. Test product (T) was tamsulosin hydrochloride 0.4 mg capsules (Tamsustad) from STADA-VN Joint Venture Co., Ltd., Vietnam. Reference product (R) was FLOMAX relief<sup>®</sup> MR capsule (Tamsulosin hydrochloride 0.4 mg) from Boehringer Ingelheim Limited, United Kingdom.

## **2.2.** Analytical procedure

#### 2.2.1. Extraction protocol

One mL aliquot of human plasma and 50  $\mu$ L IS solution (5000 ng/mL) was added to glass centrifuge tube, then vortex-mixed for 30 s. The sample mixture was extracted twice by adding 2 mL of mixture of dichloromethane and diethyl ether in a ratio of 3:7, following vortex-mixed for 1 min and then centrifuged for 5 minutes at 1520 g-force. All the organic phase was transferred to another tube and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was reconstituted with 400  $\mu$ L water and 20  $\mu$ L aliquots were injected into the LCMS-IT-TOF system.



Figure 1. Structure and fragmentation pattern of tamsulosin



Figure 2. Structure and fragmentation pattern of diphenhydramine (IS.)

#### 2.2.2. Chromatographic system and conditions

The HPLC system was the Shimadzu LCMS-IT-TOF system (Shimadzu, Japan). Chromatogram was performed at ambient temperature on a steel,  $C_{18}$  (150 mm x 4.6 mm, 5 µm particle size) column (Gemini Phenomenex, USA). The mobile phase was a mixture of methanol and 5 % formic acid buffer in a ratio

of 50:50 (v/v). The flow rate was 1.0 mL min<sup>-1</sup>. An ion trap - time of fight mass spectrometer equipped with electrospray ionization source was used in positive ion, set with a drying gas ( $N_2$ ) flow of 1.5 L/min, nebulizer pressure at 200 kPa, temperature of CDL 250 °C, heat block of 300°C and capillary voltage of 4 kV.

Detector voltage was 1.6 kV. Ion accumulation of tamsulosin and diphenhydramine were 100.00 and 10.00 msec, respectively. Target ions were monitored at m/z 409.1792 for tamsulosin and m/z 256.1696 for IS. Full scan spectra of tamsulosin and diphenhydramine were illustrated in figure 3 and figure 4, respectively.



Figure 3. Full scan spectra for protonated tamsulosin in electronspray ionization mode, with m/z 409.1792 selected as qualifier ion



**Figure 4.** Full scan spectra for protonated diphenhydramine in electronspray ionization mode, with m/z 256.1696 selected as qualifier ion

## 2.3. Validation

The analytical method for tamsulosin quantitation in plasma samples was validated according to international guidelines<sup>14,15</sup>.

## 2.3.1. Specificity

Analyte specificity was ensured to avoid

potential interferences by other endogenous components in serum. Extracted drug-free serum was checked for interfering peaks at, or close to, the retention times of tamsulosin or diphenhydramine.

#### 2.3.2. Linearity

Calibration standards in plasma were

prepared at concentrations of 0.5; 1.5; 5; 15; 25; 40; 50 ng/mL. Linear was constructed by plotting peak area ratios of tamsulosin to the IS against concentrations in plasma. The correlation coefficient must be greater than 0.9900.

## 2.3.3. Precision and accuracy

The measure of precision and accuracy, were determined at 1.5, 25 and 40 ng/mL (LQC, MQC and HQC, respectively). Intra-day precision and accuracy was determined by repeated analysis of each QC sample on one day (n = 6), and inter-day was determined by repeated analysis on three consecutive days. The concentration of each sample was determined by linear on the same day. If they were within  $\pm 15\%$  of the actual value, except for the lower limit of quantification (LLOQ), for which  $\pm 20\%$  was regarded as satisfactory.

## 2.3.4. Recovery

Recovery was determined at low, medium and high levels of tamsulosin (1.5, 25 and 40 ng/mL). Recovery was evaluated by comparing the peak area of extracted samples to the peak area of unextracted samples.

#### 2.3.5. Stability

The stability of tamsulosin in human plasma was evaluated at two concentrations (1.5 and 40 ng/mL) under different conditions, simulating the same conditions that occurred during study sample analysis. The short-term stability study was established after 6 hours stored at room temperature, after 24 hours pretreatment stored at 10°C temperature in autosampler and freeze-thaw stability. The long-term was stored at -20 °C in 30, 60 and 90 days. Each level performed six replicates.

#### 2.4. Bioequivalence study

## 2.4.1. Study design

This was a single-center, randomized, open-label, single-dose, crossover, two-period, two-formulation study conducted to determining the bioequivalence study between the generic product and the reference product after oral administration of single dose of tamsulosin hydrochloride in healthy Vietnamese male volunteers. This study was designed and conducted at Institute of Drug Quality Control, Ho Chi Minh City, Ministry of Health - Vietnam, in December 2013.

The study was conducted according to the principles of the Declaration of Helsinki and its amendments for biomedical research involving human subjects and the principles of the Good Clinical Practice guidelines. The clinical trial followed the guidance provided in Regulations for Clinical Trials of the Ministry of Health of Vietnam, and the clinical protocol and the informed consent form were approved by the local Ethics Committee (approval No. [2013]97/QyĐ-HĐĐĐ). All eligible subjects were informed of the aim and risks of the study by the clinical investigators and provided written informed consent before participation.

#### 2.4.2. Study subjects

24 subjects were selected from healthy Vietnamese volunteers to participate in this study. Before study entry, subjects were interviewed and underwent a routine physical examination, including vital sign monitoring to ensure that they were healthy enough to participate in the study. Subjects met the following inclusion criteria: healthy; male; age between 18 and 50 years; body mass index (BMI) between 18 and  $27 \text{ kg/m}^2$ ; do not abuse tobacco product, drug, alcohol; no clinically significant medical history of hypertension, diabetes, respiratory illness, gastrointestinal illness, renal or liver impairment, hereditary disease, tuberculosis; no known allergy to study medication or similar drugs; no current infection; negative HIV/AIDS, HBsAg and anti HCV.

## 2.4.3. Blood sampling

A total of thirty-two 6 mL blood samples (total volume of 192 mL) were obtained by venipuncture from each subject during the course of the study for evaluation of tamsulosin. Blood samples were collected at 0 (pre-dose), 1, 2, 3, 4, 5, 6, 7, 9, 12, 16, 24, 36, 48, 72 and 96 hours after study drug administration. The blood samples for tamsulosin determinations were centrifuged within 20 minutes of blood collection at 5000 rpm for 5 minutes. Immediately following centrifugation, plasma was transferred to plastic 5 mL labeled storage tubes using clean pipettes. The plasma samples were stored at or below -20 °C until analysis.

#### 2.4.4. Pharmacokinetic and statistical analysis

The pharmacokinetic parameters were determined for all subjects using non-compartmental techniques. Excel software 2013 with an appropriate validated program was used for the calculation of pharmacokinetic parameters. To evaluate the bioequivalence of the test and reference formulations, ANOVA for the crossover design was conducted on In-transformed C<sub>max</sub>,  $AUC_{0-t}$ , and  $AUC_{0-t}$  by Equiv Test PK software (Ireland). Statistical test was also conducted on T<sub>max</sub> using Wilcoxon test at the significance level of 0.05. The two formulations were considered bioequivalent if the 90% confidence intervals for the ratios of the averages (population geometric means) of the measures of  $AUC_{0-t}$ ,  $AUC_{0-\infty}$ , C<sub>max</sub> of test and reference formulations were within a range of 80.00 % to 125.00 %<sup>16,17</sup>.

### **3. RESULTS AND DISCUSSION**

### 3.1. Assay validation

Under the LCMS-IT-TOF conditions previously described, and as shown in Figure 5a

and Figure 6 no endogenous peak was observed in the mass chromatogram of blank plasma. The mass chromatograms are shown in Figure 5b-c and Figure 6a, in which the retention time of tamsulosin and diphenhydramine were 1.8 and 2.8 min., respectively. A linear least-squares regression with a weighting index of 1/x was performed on peak area rations of tamsulosin and IS. vs. tamsulosin concentration of seven human plasma standards to generate a calibration curve. Calibration curve showed linear relationship between peak area ratios (tamsulosin/internal standard) and tamsulosin concentrations (y =ax, where v = peak area ratio and x = tamsulosinconcentration in ng/mL) A calibration curve was linear over the range 0.5 to 50 ng/mL. The mean coefficient of correlation (r) was greater than 0.999. The lower limit of quantification (LLOO) was 0.5 ng/mL. The recoveries observed were (means  $\pm$  SD) 82.63  $\pm$  3.29, 86.75  $\pm$  2.01 and  $86.39 \pm 1.58$  (1.5; 25 and 40 ng/mL, respectively) for tamsulosin and  $72.83 \pm 1.78$ for IS (5000 ng/mL). The precision and accuracy of quality control samples were shown in Table 1. No significant matrix effect was observed in all the six batches of human plasma for the analytes at LQC and HQC concentrations. Stability analysis was performed with plasma quality control samples (1.5 and 40 ng/mL) in Table 2. All samples showed no significance degradation under the conditions previously described in the experimental section.

| Table 1. P | recision and | accuracy | of method | for      | determining | tamsulosin | in | human | plasma    |
|------------|--------------|----------|-----------|----------|-------------|------------|----|-------|-----------|
|            |              |          |           | <b>.</b> |             |            |    |       | PIEROIIIE |

| Conc. (ng/mL) | Intra-day accuracy<br>and precision $(n = 6)$ |               | Inter-dayaccuracy<br>and precision $(n = 6)$ |               |
|---------------|---|---------------|--|---------------|
|               | Accuracy (%)                                  | Precision (%) | Accuracy (%)                                 | Precision (%) |
| 0.5           | 109.05  | 4.60          | 107.90                                       | 5.37          |
| 1.5           | 109.82  | 3.21          | 104.79                                       | 5.21          |
| 25            | 104.44  | 7.01          | 102.34                                       | 4.92          |
| 40            | 102.38  | 3.53          | 102.19                                       | 4.38          |



Figure 5. Chromatograms obtained from (a) blank plasma, (b) plasma spiked with 0.5 and 5000 ng/mL of tamsulosin and diphenhydramine, (c) plasma spiked with 40 and 5000 ng/mL of tamsulosin and diphenhydramine, respectively.

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**Figure 6.** Separate chromatograms obtained from blank plasma (a) and plasma spiked with 40 and 5000 ng/ml of tamsulosin and diphenhydramine (b), respectively

| <b>Table 2.</b> Stability samples result for tamsulosin in human pla |
|--|
|--|

| Stability test                               | QC (spiked conc. ng/mL) | Relative response (%) |
|--|-------------------------|-----------------------|
| Room temperature for 6 hours                 | 1.5                     | 95.14                 |
|  | 40                      | 107.97                |
| Freeze - thaw stability                      | 1.5                     | 95.07                 |
|  | 40                      | 111.23                |
| After 24 hours pretreatment (10 °C)          | 1.5                     | 101.41                |
|  | 40                      | 99.83                 |
| Long term stability (at - 20 °C for 30 days) | ) 1.5                   | 105.58                |
|  | 40                      | 105.31                |
| Long term stability (at - 20 °C for 60 days) | ) 1.5                   | 102.57                |
|  | 40                      | 100.56                |
| Long term stability (at - 20 °C for 90 days) | ) 1.5                   | 98.03                 |
|  | 40                      | 98.42                 |

## 3.2. Bioequivalence study results

A total of 24 male subjects (mean [SD] [range]) age, 22.0 [2.0][20-26] years; body mass index (BMI), 22.3 [2.3][19.2 - 26.7] kg/m<sup>2</sup> were enrolled in the study. All subjects completed both treatment periods. Table 3 summarizes the pharmacokinetic parameters (AUC<sub>0-t</sub>, AUC<sub>0-∞</sub>, C<sub>max</sub>) of tamsulosin. Table 4 and Table 5 show ratio AUC<sub>0-t</sub> / AUC<sub>0-∞</sub> results and the statistical comparison results of tamsulosin, respectively. The mean plasma tamsulosin concentrations are showed in Figure 7.

Table 3. Stability samples result for tamsulosin in human plasma

| Draduat            | Test                          | Reference           |  |
|--------------------|-------------------------------|---------------------|--|
| Product            | $(\text{mean} \pm \text{SD})$ | (mean ±SD)          |  |
| AUC <sub>0-t</sub> |                               |                     |  |
| (ng.h/mL)          | $239.05 \pm 148.62$           | $258.83 \pm 95.12$  |  |
| $AUC_{0-\infty}$   |                               |                     |  |
| (ng.h/mL)          | $264.26 \pm 165.90$           | $283.48 \pm 100.47$ |  |
| C <sub>max</sub>   |                               |                     |  |
| (ng/mL)            | $14.11 \pm 2.58$              | $14.44 \pm 3.17$    |  |
| T <sub>max</sub>   |                               |                     |  |
| (hour)             | $5.24 \pm 1.28$               | $5.23 \pm 1.17$     |  |
| $T_{1/2}$          |                               |                     |  |
| (hour)             | 20.64                         | 19.09               |  |
| K <sub>e</sub>     | 0.4391                        | 0.4695              |  |

| Table 4. AUC, /AUC, rat | tio of tamsulosin |
|-------------------------|-------------------|
|-------------------------|-------------------|

| Product | $AUC_{0-t}/AUC_{0-\infty}$ ratio (%) |
|---------|--------------------------------------|
| Т       | 90.40                                |
| R       | 91.51                                |

Table 5. The 90% confidence intervals of tamsulosin

| Parameters                | T/R ratio (%) | 90% Confidence Intervals (%) |
|---------------------------|---------------|------------------------------|
| AUC <sub>0-t</sub>        | 92.36         | 80.02 - 106.60               |
| $\mathrm{AUC}_{0-\infty}$ | 93.21         | 81.00 - 107.27               |
| C <sub>max</sub>          | 97.70         | 90.75 - 105.20               |



Figure 7. Mean plasma tamsulosin concentration-time curve

In our study, no period, treatment and sequence effects for any pharmacokinetic property were found using ANOVA in all subjects. These results indicate that a washout period of 7 days was adequate for total elimination of the drug between the 2 administration periods. Tamsulosin appeared to be well tolerated by all volunteers. No subject withdrew from our study, and no adverse events were found on analysis of vital signs or laboratory test results. No abnormalities were found in clinical or biochemical parameters when comparing baseline versus end-of-study assessments. This study assessed the bioequivalence of modified release formulation of tamsulosin hydrochloride. There were no significant differences between formulations in pharmacokinetic properties in the small, selected, fasting, healthy Vietnamese male population.

# **4. CONCLUSION**

The assay reported in this paper is rapid, specific and sensitive for quantification of tamsulosin in human plasma and was fully validated according to commonly acceptable FDA guidelines. The method showed suitability

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for pharmacokinetic and BA/BE studies in human. Our study found that the test and reference modified release formulations of tamsulosin hydrochloride met the regulatory criteria for bioequivalence in the fasting, healthy Vietnamese male volunteers.

## 5. ACKNOWLEDGMENTS

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