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Determination of tamsulosin in dog plasma by liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry

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Abstract

A rapid, sensitive and accurate liquid chromatographic–tandem mass spectrometric method is described for the determination of tamsulosin in dog plasma. Tamsulosin was extracted from plasma using a mixture of hexane–ethyl acetate (2:1, v/v) and separated on a C_{18} column interfaced with a triple quadrupole tandem mass spectrometer. The mobile phase consisting of a mixture of methanol, water and formic acid (80:20:1, v/v/v) was delivered at a flow rate of 0.5 ml/min. Atmospheric pressure chemical ionization (APCI) source was operated in positive ion mode. Selected reaction monitoring (SRM) mode using the transitions of *m/z* 409 → *m/z* 228 and *m/z* 256 → *m/z* 166.9 were used to quantify tamsulosin and the internal standard, respectively. The linearity was obtained over the concentration range of 0.1–50.0 ng/ml for tamsulosin and the lower limit of quantitation was 0.1 ng/ml. For each level of QC samples, inter- and intra-run precision was less than 5.0 and 4.0% (relative standard deviation (R.S.D.)), respectively, and accuracy was within ±0.3% (relative error (R.E.)). This method was successfully applied to pharmacokinetic study of a tamsulosin formulation product after oral administration to beagle dogs.

Keyword: Tamsulosin

1. Introduction

Tamsulosin hydrochloride (Fig. 1), (−)-(*R*)-5-[2-[[2-(*o*-ethoxyphenoxy)ethyl]amino]propyl]-2-methoxybenzenesulfonamide hydrochloride, is a structurally new type of sulfamoile derivative, possessing a highly selective α_1-adrenoceptor antagonistic property. It has been clinically used for urinary obstructed patients with benign prostatic hyperplasia [1,2].

For the pharmacokinetic study of a tamsulosin formulation product in dogs, an analytical method with simplicity and high sensitivity was required in our laboratory. A recent survey revealed that few methods were available for the determination of tamsulosin in biological samples, which involved radioreceptor assay [3], liquid chromatography with fluorescence detection [4,5] and liquid chromatography–tandem mass spectrometry (LC–MS–MS) [6,7]. Taking into consideration the low levels of tamsulosin in plasma, LC–MS–MS method is the first choice for our purpose. To our knowledge, atmospheric pressure chemical ionization (APCI) source offers some advantages over electrospray ionization source (ESI) in terms of less background noises and suitability for small molecular compounds [6,7]. Besides, a less sample mass onto the column is often preferred in ordinary laboratories for the benefits of both system maintenance and re-injections of the extracted sample when only a limited sample is available.

This paper describes a simple, specific and highly sensitive LC–MS–MS method with an APCI source in selected reaction monitoring (SRM) mode for the determination of tamsulosin in dog plasma. The described method was validated in terms of matrix effect, selectivity, sensitivity, linearity, accuracy, precision and stability of analyte in plasma and mobile phase, and successfully applied to the pharmacokinetic studies of tamsulosin hydrochloride sustained release tablets in beagle dogs.

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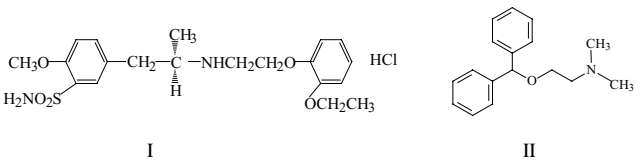

I II

Fig. 1. Chemical structures of tamsulosin hydrochloride (I) and diphenhydramine (II, internal standard).

2. Experimental

2.1. Chemicals and reagents

Tamsulosin hydrochloride (ca. 99.8% purity) and sustained release tablets (0.2 mg, batch no. 20011124) was supplied by Shenyang Pharmtech Institute of Pharmaceuticals (Shenyang, China). Harnal® capsules (0.2 mg, batch no. 0201126) from Yamanouchi Pharmaceutical Company Ltd. (Japan) were used as reference product. Diphenhydramine (ca. 99.6% purity, internal standard) was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC-grade methanol was purchased from Tianjin Concord Tech Reagent Company (Tianjin, China). Formic acid, *n*-hexane, ethyl acetate and sodium carbonate were all analytical-grade chemicals. Distilled water prepared from demineralized water was used throughout this study.

2.2. Instrumentation

A Shimadzu LC-10AD pump (Shimadzu, Kyoto, Japan) was used for solvent delivery. A Finnigan TSQ™ triple quadrupole tandem mass spectrometer with an atmosphere pressure chemical ionization source (Thermo Finnigan, San Jose, CA, USA) was used for quantitative determination of tamsulosin in dog plasma. Data collection was performed with Xcalibur 1.1 software (Thermo Finnigan). Peak integration and calibration were made with LCQuan software (Thermo Finnigan).

2.3. Chromatographic conditions

Chromatographic separation was performed on a Zorbax Extend-C_{18} column (150 mm × 4.6 mm i.d., 5 μm, Agilent, Palo Alto, CA, USA) with a SecurityGuard C_{18} guard column (4 mm × 3.0 mm i.d., Phenomenex, Torrance, CA, USA). The mobile phase consisting of a mixture of methanol, water and formic acid (80:20:1, v/v/v) was delivered at a flow rate of 0.5 ml/min. The column temperature was maintained at 20 °C. The injection volume was 20 μl.

2.4. Mass spectrometric conditions

APCI in positive ion detection mode was used as the ionization source with the corona discharge current set at 4 μA. The interface capillary temperature was maintained at 250 °C. The vaporizer temperature was 450 °C. High purity nitrogen served both as sheath gas with an operating pressure of 80 psi (1 psi = 6.895 kPa) and as auxiliary gas with a flow rate of 3 l/min. Argon (Ar) was used in the studies of collision induced dissociation (CID) with a collision gas pressure of 1.9 Pa and collision energy of 25 eV. Selected reaction monitoring mode was used for the quantitation. The transitions selected were m/z 409 → m/z 228 for tamsulosin and m/z 256 → m/z 166.9 for the internal standard, respectively, with a dwell time of 300 ms per transition.

2.5. Preparation of calibration standards and quality control samples

Stock solutions of tamsulosin and the diphenhydramine (internal standard, I.S.) were individually prepared at 400 μg/ml in methanol. The stock solution of tamsulosin was further diluted with mobile phase to give a series of standard solutions with concentration of 0.1, 0.25, 0.8, 2.0, 8.0, 20.0, 40.0 and 50.0 ng/ml. A solution containing 400 ng/ml I.S. was also prepared using mobile phase.

Calibration standards of tamsulosin (0.1, 0.25, 0.8, 2.0, 8.0, 20.0, 40.0 and 50.0 ng/ml) were prepared by spiking appropriate amount of the standard solutions in blank plasma obtained from healthy, non-smoking volunteers. Quality control (QC) samples were prepared using the pooled plasma at concentrations of 0.25, 2.0 and 40.0 ng/ml. The spiked samples were then treated following the sample preparation procedure as indicated in Section 2.6.

2.6. Sample preparation

A 200 μl aliquot of each plasma sample was transferred to a 5 ml polyethylene centrifuge tube. A 100 μl of mobile phase, a 100 μl of internal standard solution (400 ng/ml) and a 100 μl of sodium carbonate solution (1 mol/l) were added and the contents were briefly mixed by vortexing. And then after a 3 ml aliquot of a mixture of hexane and ethyl acetate (2:1, v/v) was added, the contents were vortexed for 1 min and centrifuged at 2000 × *g* for 5 min to separate the phases. The organic layer was removed into another tube and was evaporated to dryness under a stream of nitrogen in a 40 °C water bath. The residue of each sample was reconstituted in 200 μl of the mobile phase, and a 20 μl aliquot was injected onto the LC–MS–MS system.

2.7. Method validation

Validation runs were conducted on three separate days. Each validation run consisted of a set of the spiked standard samples at eight concentrations over the concentration range (each in triplicate), QC samples at three concentrations (n = 6, at each concentration), blank, stability and freeze–thaw samples. Standard samples were analyzed at the beginning of each validation run and other samples were distributed randomly throughout the run. The results from QC samples

in three runs were used to evaluate the accuracy and precision of the method developed. Concentrations of the analyte in plasma samples were determined by back-calculation of the observed peak-area ratios of the analyte and internal standard from the best-fit calibration curve using a weighted ($1/x^2$) linear regression. During routine analysis, each analytical run included a set of standard samples, a set of QC samples in duplicate and plasma samples to be determined.

The matrix effect was investigated by extracting blank plasma from six different sources, reconstituting the final extract in the mobile phase containing a known amount of the analyte, analyzing the reconstituted extracts and then comparing the peak areas of the analyte.

The extraction recovery of tamsulosin was determined at low, medium and high concentrations by comparing the responses from plasma samples spiked before extraction with those from plasma samples extracted and spiked after extraction. In both cases, the internal standard was added after extraction to eliminate bias introduced by sample processing.

Sample stability in terms of plasma freeze–thaw cycles, plasma short-term room temperature exposure and extracted sample stability was tested by analyzing QC samples at concentrations of 0.25, 2.0 and 40.0 ng/ml. The freeze–thaw stability was evaluated after three freeze (−20 °)–thaw (room temperature) cycles. The stability of tamsulosin in dog plasma at room temperature before being extracted and analyzed, and the stability of tamsulosin after reconstituted in mobile phase were determined.

2.8. Application of the LC–MS–MS method

The LC–MS–MS method developed was successfully applied to the pharmacokinetic studies of tamsulosin hydrochloride sustained release tablets in beagle dogs. Six male beagle dogs (9–10 kg) were purchased from the Laboratory Animal Center in Shenyang Pharmaceutical University. After an overnight fast (12 h), the dogs were given single dose of either three tamsulosin hydrochloride sustained release tablets (0.6 mg) or three Harnal® capsules (0.6 mg). No food was allowed until 8 h after oral administration of the doses while water intake was free. About 2 ml of blood samples were collected from the foreleg vein into heparinized tubes before (0 h) and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 h after dosing. Plasma was separated by centrifugation at $2000 \times g$ for 10 min and kept frozen at −20 °C until analysis.

3. Results and discussion

3.1. Method development

Liquid–liquid extraction (LLE) was used for the sample preparation in this work. LLE can be helpful in producing a spectroscopically clean sample and avoiding the introduction of non-volatile materials onto the column and MS system. Clean samples are essential for minimizing ion suppression and matrix effect in LC–MS–MS analyses. Three organic solvents, ethyl ether, ethyl acetate, hexane, and their mixtures in different combinations and ratios were evaluated. Finally, a mixture of hexane and ethyl acetate (2:1, v/v) was found to be optimal, which can produce a clean chromatogram for a blank plasma sample and yield the highest recovery for the analyte from the plasma.

The chromatographic conditions, especially the composition of mobile phase, were optimized through several trials to achieve good resolution and symmetric peak shapes of analytes as well as short run time. It was found that a mixture of methanol, water and formic acid (80:20:1, v/v/v) could achieve our purpose and was finally adopted as the mobile phase for the chromatographic separation. Moreover, it was necessary to reconstitute the residues with the mobile phase to produce the expected peak shapes of the analyte.

Internal standard is necessary for determination of analyte in biological samples. For an LC–MS–MS analysis, utilization of stable isotope-labeled drugs as internal standards proves to be helpful when significant matrix effect occurs. However, there are also many problems with the use of stable isotope-labeled internal standards. The major problems involve inadequate isotopic purity and stability, which often impose unfavorable impact on highly sensitive quantitative analyses. In initial stage of our work, several compounds were tried to find a suitable internal standard and finally diphenhydramine was found to be optimal for our work. Clean chromatograms were obtained and no significant matrix effect was found.

For the quantitation of tamsulosin in dog plasma, some parameters related with tandem mass spectrometry were investigated. Based on our experiences, APCI was preferred to ESI to quantify tamsulosin in dog plasma due to its lower levels of background noises. Parameters involving corona discharge, capillary temperature, vaporizer temperature, flow rate sheath and auxiliary gas were optimized to obtain the protonated molecules of tamsulosin and diphenhydramine. The collision energy was optimized to achieve maximum response of the fragment ion peak. Selected reaction monitoring was used for the detection of tamsulosin and diphenhydramine with a dwell time of 300 ms.

3.2. Specificity

The specificity of the method was investigated by comparing chromatograms of six different sources of dog plasma. The positive product ion mass spectra of the molecular ions of tamsulosin and the internal standard are shown in Fig. 2. The most intensive product ion was observed at *m*/*z* 228 for tamsulosin and *m*/*z* 166.9 for the internal standard. Representative chromatograms are shown in Fig. 3, indicating no interferences from endogenous substances in plasma with the analyte and internal standard. Tamsulosin and internal standard exhibited retention times of ca. 2.2 and 2.3 min, respectively. By monitoring the precursor-to-product ion

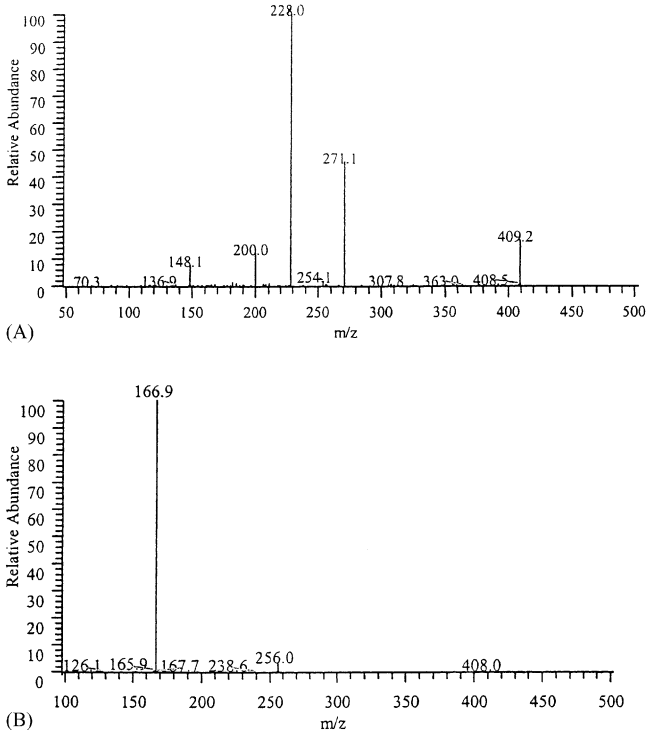

Fig. 2. Full-scan product ion spectra of $[M + H]^+$ of: (A) tamsulosin; (B) diphenhydramine.

transitions *m*/*z* 409 → *m*/*z* 228 for tamsulosin and *m*/*z* 256 → *m*/*z* 166.9 for the internal standard in the SRM mode, a highly sensitive assay for tamsulosin was developed.

3.3. Matrix effect

The ion suppression caused by the plasma matrix was evaluated. The matrix effect of the method was considerably reduced and suppressed by utilization of the APCI mode and by eliminating a number of endogenous components from plasma extracts during sample preparation. No matrix effect and interferences from endogenous compounds were detected for six different sources of dog plasma. The relative standard deviation (R.S.D.) value for the peak areas of the six reconstituted samples was 4.0%, indicating that the extracts were "clean" with no co-eluting "unseen" components interfering with the ionization of the analyte.

3.4. Linearity and sensitivity

The linearity of each calibration curve was determined by plotting the peak-area ratio (*y*) of tamsulosin to internal standard versus the nominal concentration (*x*) of tamsulosin. The calibration curves were obtained by weighted ($1/x^2$) linear regression analysis. To evaluate the linearity of the LC–APCI–MS–MS method, plasma calibration curves were determined in triplicate on three separate days. Representative regression equation for the calibration curve was $y = 4.167 \times 10^{-4} + 8.548 \times 10^{-3}x$ with a correlation coefficient of 0.9980. Good linearity was observed over the concentration range of 0.1–50.0 ng/ml for tamsulosin.

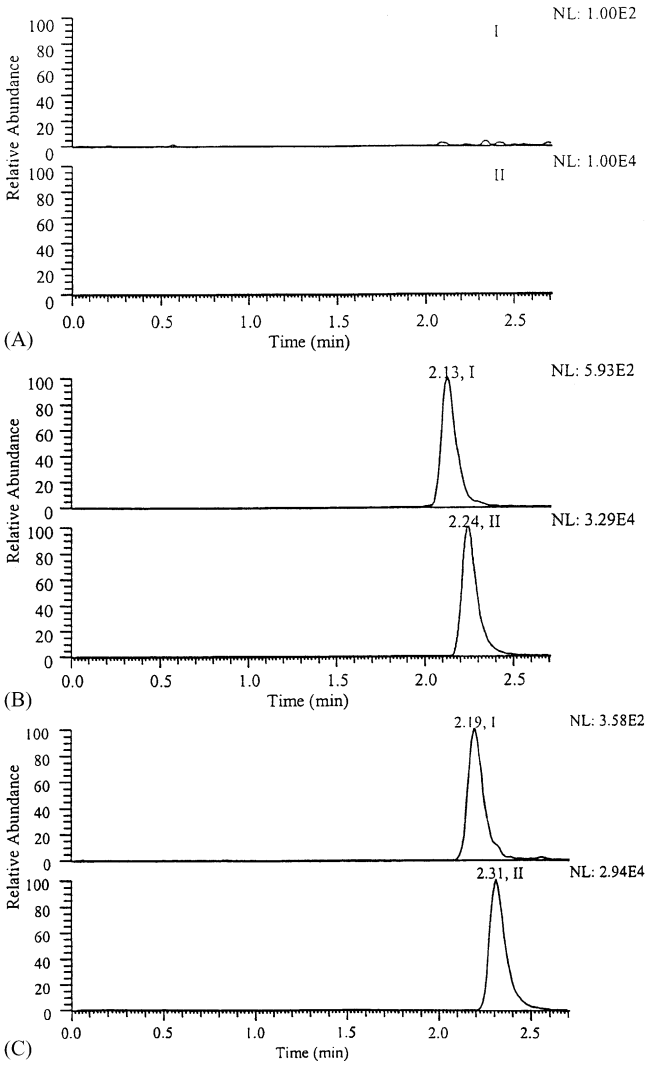

Fig. 3. Representative SRM chromatograms of: (A) blank dog plasma sample; (B) blank plasma sample spiked with tamsulosin (2.0 ng/ml) and diphenhydramine (200 ng/ml); (C) plasma sample from foreleg vein at 10 h after an oral dose of 0.6 mg tamsulosin hydrochloride sustained release tablets to a beagle dog. Peak I: tamsulosin; peak II: diphenhydramine (internal standard).

The lower limit of quantitation (LLOQ) for determination of tamsulosin in dog plasma, defined as the lowest concentration analyzed with an accuracy less than 15% and a precision less than 15%, was found to be 0.1 ng/ml, which is sufficient for pharmacokinetic study of tamsulosin in dogs.

3.5. Accuracy and precision

The accuracy and precision of the method were evaluated by a one-way analysis of variance (ANOVA) based on the data from QC plasma samples in three validation runs. The accuracy was determined by calculating the percentage deviation observed in the analysis of QC samples and

Table 1
Accuracy and precision for the determination of tamsulosin in dog plasma ($n = 3$ days, six replicates per day)

Added C (ng/ml)	Found C (ng/ml)	Intra-run R.S.D.[a] (%)	Inter-run R.S.D. (%)	Relative error (%)
0.25	0.25	4.4	3.9	0.05
2.0	2.01	3.4	1.8	0.07
40.0	39.9	4.7	1.2	−0.3

[a] Relative standard deviation.

expressed in the relative error. The intra- and inter-run precision was expressed as the relative standard deviation. As shown in Table 1, for each QC level of tamsulosin, the intra- and inter-run precision was less than 5.0 and 4.0%, respectively, and the accuracy was within ±0.3%, indicating the acceptable accuracy and precision of the method developed.

3.6. Extraction recovery

The extraction recovery of tamsulosin from dog plasma was determined by comparing peak areas from plasma samples spiked before extraction with those from plasma samples extracted and spiked after extraction. The results showed that the extraction recoveries of tamsulosin from dog plasma were 73.2 ± 2.2, 71.4 ± 2.6 and 75.9 ± 3.5% at concentrations of 0.25, 2.0 and 40.0 ng/ml, respectively.

3.7. Stability

The stability of tamsulosin in dog plasma and mobile phase were investigated. Tamsulosin was found to be stable after three freeze–thaw cycles in dog plasma with an accuracy within ±7.6% and in dog plasma at room temperature for at least 2 h (R.E. < 6.2%).

The stability of tamsulosin in the reconstitution mobile phase was also determined by analyzing QC samples at concentrations of 0.25, 2.0 and 40.0 ng/ml for a period of 24 h at room temperature. Tamsulosin was found to be stable for at least 24 h after sample preparation at room temperature with an accuracy ranging from −0.5 to −2.2% for three levels of QC samples.

3.8. Application

Th LC–APCI–MS–MS method developed yielded satisfactory results for the determination of tamsulosin in dog plasma and has been successfully used for the pharmacokinetic study of tamsulosin hydrochloride sustained release tablets and Harnal® capsules following oral administration to six beagle dogs. The mean plasma concentration–time profiles for tamsulosin hydrochloride sustained release tablets and Harnal® capsules are shown in Fig. 4.

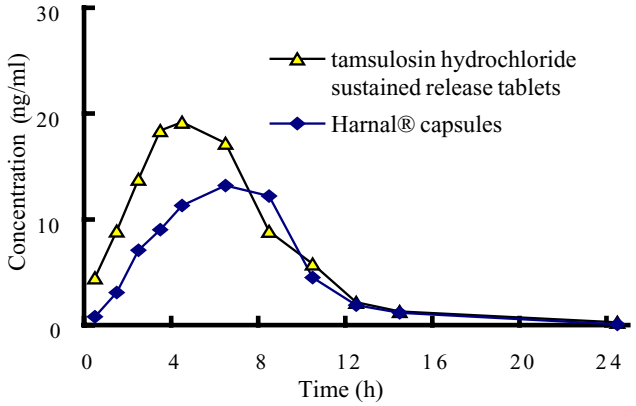

Fig. 4. Mean plasma concentration–time profiles of tamsulosin after oral administration of tamsulosin hydrochloride sustained release tablets (0.6 mg) and Harnal® capsules (0.6 mg) to six beagle dogs.

4. Conclusions

A rapid, sensitive and accurate liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry was developed for the determination of tamsulosin in dog plasma. The method offers high sensitivity with a low limit of quantitation of 0.1 ng/ml, wide linearity, specificity without interferences from endogenous substances and low sample volume. In addition, the simplicity of sample preparation facilitates its application in the pharmacokinetic studies of tamsulosin products.

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