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Rapid determination of tamsulosin in human plasma by high-performance liquid chromatography using extraction with butyl acetate

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Abstract

A high-performance liquid chromatographic method with fluorescence detection for the determination of tamsulosin in human plasma is reported. The sample preparation involved liquid–liquid extraction of tamsulosin from alkalised plasma with butyl acetate and back-extraction of the drug to the phosphate buffer (pH 2). Butyl acetate is preferable to more commonly used ethyl acetate because of its much lower solubility in water. Liquid chromatography was performed on an octadecylsilica column (55 mm × 4 mm, 3 μm particles), the mobile phase consisted of acetonitrile–30 mM dihydrogenpotassium phosphate (25:75 v/v). The run time was 3.5 min. The fluorimetric detector was operated at 228/326 nm (excitation/emission wavelength). An analogue of tamsulosin, (*R*)-5-[2-[(3-(2-ethoxyphenoxy)propyl)amino]-2-methylethyl]-2-methoxybenzensulfonamide was used as the internal standard. The limit of quantitation was 0.4 ng/ml using 1 ml of plasma. Within-day and between-day precision expressed by relative standard deviation was less than 10% and inaccuracy did not exceed 5%. The assay was applied to the analysis of samples from several pharmacokinetic studies.

Keywords: Tamsulosin; Butyl acetate

1. Introduction

Tamsulosin is a subtype-selective (α_{1a} and α_{1d}) α_1 adrenoreceptor antagonist used for the treatment of patients with symptomatic benign prostatic hyperplasia. The result of its action is decrease of the smooth muscle tone in the prostate and prostatic urethra and thus the increase of an urinary flow. The irritation symptoms of benign prostatic hyperplasia are also affected by tamsulosin administration. The effects on symptoms during the collecting and emptying phase of the bladder are maintained during the long-term therapy [1].

Only few methods have been described for determination of tamsulosin in plasma. The first published assay used high-performance liquid chromatography (HPLC) with fluorescence detection [2]. The sample preparation involved three-step extraction procedure with ethyl acetate which was evaporated in the final step. The limit of quantitation was 0.5 ng/ml using 1.5 ml of plasma, run time was approximately 18 min.

Liquid chromatography coupled with mass spectrometry (LC–MS) [3] or tandem mass spectrometry (LC–MS–MS) [4,5] was used in the other methods. In the LC–MS–MS method published by Ding et al. [4], the limit of quantitation was 0.5 ng/ml using 0.2 ml of plasma, run time was 3 min. A single-step liquid–liquid extraction with ethyl acetate was required with subsequent evaporation of the organic solvent. The recently published LC–MS–MS assay for determination of tamsulosin in dog plasma [5] employed similar sample preparation (with hexane-ethyl acetate), the limit of quantitation was lower (0.1 ng/ml) and the run time was also 3 min.

The characteristics of the LC–MS assay [3] were similar, only the time of analysis was longer—7 min.

The aim of this study was to develop a rapid HPLC method for tamsulosin determination in plasma with elimination of tedious evaporation step and without requirements for costly

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mass spectrometric instrumentation. It was found that ethyl acetate is not suitable for back-extraction of tamsulosin into acidic aqueous phase because of its solubility in water. The direct injection of the aqueous phase with dissolved ethyl acetate causes peak distortion. We suggest butyl acetate as a replacement—it has similar extraction characteristics and much lower solubility in water.

2. Experimental

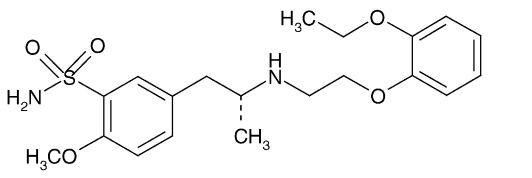
2.1. Chemicals

Tamsulosin hydrochloride and the internal standard, (*R*)-5-[2-[(3-(2-ethoxyphenoxy)-propyl)amino]-2-methylethyl]-2-methoxybenzenesulfonamide hydrochloride (Fig. 1) were obtained from Léčiva, Czech Republic. Acetonitrile (for liquid chromatography) was Riedel de Haën (Seelze, Germany) product. Potassium dihydrogenphosphate (analytical grade) and methanol (for liquid chromatography) were manufactured by Merck (Darmstadt, Germany), butyl acetate (puriss. p.a.) was purchased from Fluka (Buchs, Switzerland). All other reagents and chemicals were of analytical grade. Sodium carbonate and *o*-phosphoric acid were purchased from Lachema (Brno, Czech Republic).

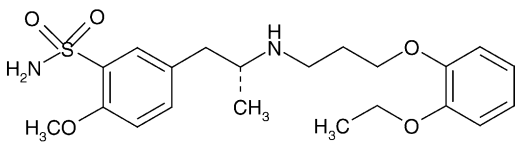
2.2. Apparatus and conditions

The HPLC system consisted of the P1000 pump, FL 2000 fluorimetric detector, data station with PC1000 software, version 2.5 (Thermo Separation Products, Riviera Beach, FL, USA) and the Midas automatic sample injector (Spark Holland BV, The Netherlands). The separation was performed on a Lichrocart 55 mm × 4 mm i.d. column filled with Purospher Star RP 18e, particle size 3 μm (Merck) protected with a C_{18} 4 mm × 3 mm i.d. precolumn (Phenomenex, Torrance, CA, USA).

The mobile phase consisted of acetonitrile–30 mM potassium dihydrogenphosphate (25:75 v/v). The flow-rate was 1.2 ml/min at 30 °C. The excitation and emission wavelengths were 228 and 326 nm, respectively and the time constant of the detector was set to 2 s.



Tamsulosin



Internal standard

Fig. 1. Chemical structures of tamsulosin and the internal standard.

2.3. Standards

Stock solutions of tamsulosin were made by dissolving of approximately 15 mg of the hydrochloride (factor 0.91798 for conversion to the free base) in 25 ml of methanol. Separate solutions were prepared for the calibration curve samples and quality control ones. Further solutions were obtained by serial dilutions of stock solutions with methanol. These solutions were added to drug-free plasma in volumes not exceeding 2% of the plasma volume.

The internal standard solution was prepared as follows: approximately 9 mg of the substance was dissolved in 10 ml of methanol and this solution was diluted with methanol to obtain the working solution of the internal standard (approximately 4 ng/μl). Ten microlitres of this solution was added to 1 ml of plasma as the internal standard.

The solutions were stored at −18 °C and were protected from light.

2.4. Preparation of the sample

The samples were stored in the freezer at −18 °C and allowed to thaw at room temperature before processing. Ten microlitres of the internal standard solution (40 ng) were added to 1 ml of plasma, the tube was briefly shaken. One hundred microlitres of 1 M sodium carbonate were added and the tube was shaken again. Then the mixture was vortex-mixed with 4 ml of butyl acetate for 2 min at 2000 rpm. The tube was centrifuged 2 min at 2000 × *g* and the upper organic phase was transferred to another tube. Tamsulosin was then back-extracted into 130 μl of 30 mM potassium dihydrogenphosphate (pH of this buffer was adjusted to 2 with concentrated *o*-phosphoric acid) by shaking the tube for 2 min at 2000 rpm. Then the tube was centrifuged 2 min at 2000 × *g* and 100 μl of the lower aqueous phase was transferred to the polypropylene autosampler vial. Ninety microlitres was injected into the chromatographic system.

2.5. Calibration curves

The calibration curve was constructed in the range 0.397–40.50 ng/ml to encompass the expected concentrations in measured samples. The calibration curves were obtained by weighted linear regression (weighing factor $1/x^2$): the ratio of tamsulosin peak height to internal standard peak height was plotted versus the ratio of tamsulosin concentration to that of internal standard in ng/ml. The suitability of the calibration model was confirmed by back-calculating the concentrations of the calibration standards.

3. Results and discussion

3.1. Sample preparation

In accordance with the published procedure [2] ethyl acetate had higher extraction efficiency compared to other solvents (diethyl ether, *t*-butyl methyl ether, hexane-isoamylalcohol). However, ethyl acetate has relatively high solubility in water (1 ml dissolves in 10 ml water at 25 °C [6] which precludes direct injection of the sample after back-extraction into acidic aqueous layer. The sample solvent has higher elution strength than the mobile phase which causes peak distortion and lowering of column efficiency. In the method with fluorimetric detection [2] it was necessary to re-extract tamsulosin from the aqueous layer again to ethyl acetate with subsequent evaporation. In the more selective procedures with mass spectrometric detection [3–5] single-step extraction was sufficient with evaporation of ethyl acetate or hexane–ethyl acetate mixture.

The back-extraction is more simple and rapid than evaporation. In order to overcome problems with solubility of extraction solvent in the aqueous layer butyl acetate was selected for extraction. This solvent has 12 times lower solubility in water than ethyl acetate [6]; the extraction efficiency was in the range 85–95% both for tamsulosin and the internal standard.

Several commercially available compounds were tested as internal standard. The selection is limited because the internal standard must have fluorescence spectra similar to tamsulosin and comparable extraction properties. Propranolol could be used, but the precision and accuracy was only moderate using this compound. In order to improve the performance of the assay, an analogue of tamsulosin (*R*)-5-[2-[(3-(2-ethoxyphenoxy)propyl)amino]-2-methylethyl]-2-methoxybenzensulfonamide was synthesized and it excellently compensated the losses of tamsulosin during extraction. Similar internal standards were used in all published procedures.

3.2. Chromatography

Typical chromatograms of drug-free plasma and plasma from a volunteer 16 h after the oral ingestion of 0.4 mg of tamsulosin hydrochloride are shown in Figs. 2 and 3, respectively. The method selectivity was demonstrated on six blank plasma samples obtained from healthy volunteers: the chromatograms were found to be free of interfering peaks.

We aimed at optimizing sensitivity and speed of the chromatography and therefore column dimensions 55 mm × 4 mm were selected. The benefit of this column is not only the increased sensitivity due to small peak volume, but also the possibility to obtain relatively large values of the capacity factor (4-6) in a short time. The retention times of tamsulosin and internal standard were 2.0 and 2.4 min, respectively, at a flow-rate 1.2 ml/min and the whole analysis was completed within 3.5 min. This is much faster than in the other method

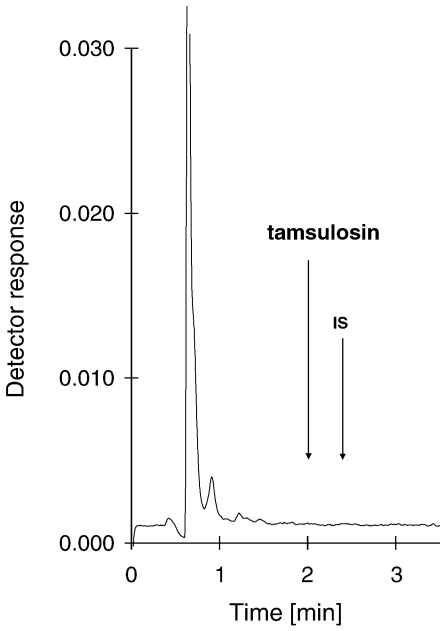


Fig. 2. Typical chromatogram of drug-free human plasma.

with fluorimetric detection [2] and comparable with assays using mass spectrometric detection [3–5]. The typical column efficiency expressed as the number of theoretical plates was about 3000 for both compounds.

3.3. Linearity and limit of quantitation

The calibration curves were linear in the studied range. The calibration curve equation is $y = bx + c$, where y represents the tamsulosin to internal standard peak height ratio and x represents the ratio of tamsulosin concentration to that of internal

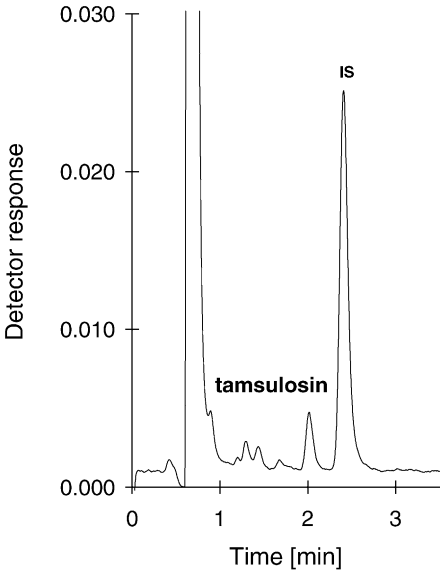


Fig. 3. Chromatogram of a plasma sample from a volunteer 16 h after administration of 0.4 mg of tamsulosin hydrochloride. The measured concentration of tamsulosin was 3.90 ng/ml.

Table 1
Intra-assay precision and accuracy

N	Concentration (ng/ml)			
	Added	Measured	Bias (%)	R.S.D. (%)
6	0.768	0.734	−4.5	2.5
6	4.035	3.960	−1.9	3.3
6	34.62	33.27	−4.1	0.8

standard. The mean equation (curve coefficients ± standard deviation) of the calibration curve ($N = 6$) obtained from six points was $y = 2.035(\pm 0.048)x + 0.00091(\pm 0.00072)$ (correlation coefficient $r = 0.9997$).

The limit of quantitation was 0.397 ng/ml ($N = 6$). At this concentration, the signal-to-noise ratio is approximately 5:1. The precision, characterised by the relative standard deviation, was 4.8% and accuracy, defined as the deviation between the true and the measured value expressed in percents, was 1.2% at this concentration ($N = 6$).

3.3.1. Intra-assay precision

Intra-assay precision of the method is illustrated in Table 1. It was estimated by assaying the quality control samples (low, medium and high concentration) six times in the same analytical run. The precision was better than 4% and the bias did not exceed 5% at all levels.

3.3.2. Inter-assay precision and accuracy

Inter-assay precision and accuracy was evaluated by processing a set of calibration and quality control samples (three levels analysed twice, results averaged for statistical evaluation) on six separate runs. The samples were prepared in advance and stored at −18 °C. The respective data are given in Table 2. The precision was 9.5% at the lowest concentration level and 3% at higher ones and the inaccuracy did not exceed 3% at all levels.

3.3.3. Stability study

3.3.3.1. Freeze and thaw stability. Stock solutions of a low and high concentration sample were prepared. The solutions were stored at −18 °C and subjected for three thaw and freeze cycles. During each cycle triplicate 1 ml aliquots were processed, analysed and the results averaged. The results are shown in Table 3. The concentration changes relatively to the nominal concentration are less than 6%, indicating no significant substance loss during repeated thawing and freezing.

Table 2
Inter-day precision and accuracy

N	Concentration (ng/ml)			
	Added	Measured	Bias (%)	R.S.D. (%)
6	0.768	0.754	−1.8	9.5
6	4.035	3.946	−2.2	2.7
6	34.62	33.89	−2.1	3.0

Table 3
Freeze and thaw stability

Concentration (ng/ml)	N	2.022		34.62	
		Measured	Bias (%)	Measured	Bias (%)
Cycle 1	3	2.022	0.0	35.52	2.6
Cycle 2	3	1.924	−4.9	32.69	−5.6
Cycle 3	3	1.930	−4.6	32.68	−5.6

Table 4
Stability of processed samples

Sample	Concentration (ng/ml)	N	Concentration found (ng/ml)	R.S.D. (%)	Bias (%)
New	0.768	5	0.734	2.5	−4.3
6 days old	0.768	5	0.764	4.0	−0.5
New	34.62	6	33.27	0.8	−3.9
6 days old	34.62	6	35.87	2.3	3.6

3.3.3.2. Processed sample stability. Two sets of samples with a low and a high concentration of tamsulosin were analysed and left in the autosampler at ambient temperature. The samples were analysed using a freshly prepared calibration samples six days later. The results are presented in Table 4. The processed samples are stable at room temperature for 6 days.

3.3.3.3. Stability of plasma samples. Two sets of plasma samples (with a low and high concentration) were stored in the freezer at −18 °C for five months. The samples were then analysed using freshly prepared calibration samples. The results are presented in Table 5. The samples are stable at −18 °C for five months.

The stability of thawed plasma samples (with a low and high concentration) was studied for 24 h period at room temperature. The samples are stable under studied conditions (see Table 5 for results).

3.4. Application to biological samples

The proposed method was applied to the determination of tamsulosin in plasma samples from a pharmacokinetic study, which was approved by the local ethics committee. The plasma samples were collected up to 36 h after a single oral dose of 0.4 mg tamsulosin hydrochloride in a controlled release capsule (Omnic® 0.4 mg cps. ret., Yamanouchi) to 26 healthy male volunteers: mean age of the group was 27

Table 5
Stability of plasma samples

Concentration (ng/ml)	Storage conditions	N	Concentration found (ng/ml)	R.S.D. (%)	Bias (%)
0.768	24 h/25 °C	3	0.803	5.9	4.7
34.62	24 h/25 °C	3	33.53	0.2	3.2
1.687	5 months/−18 °C	6	1.616	2.2	−4.2
33.69	5 months/−18 °C	6	31.18	1.0	−7.5

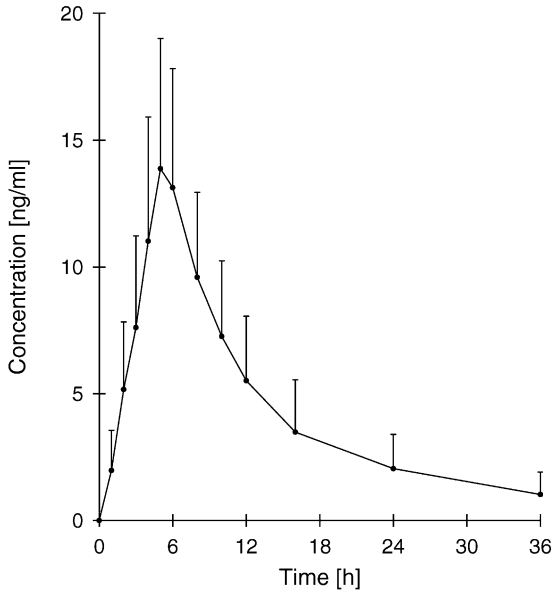


Fig. 4. Mean plasma concentrations (±S.D.) of tamsulosin after a single oral dose of 0.4 mg tamsulosin hydrochloride in a controlled release capsule (26 healthy volunteers).

years (range 18–41), mean weight was 77 kg (range 59–93). Fig. 4 shows the mean plasma concentrations of tamsulosin. The plasma levels reached their maximum 5 h after the administration and thereafter the plasma level declined with an elimination half-time of approximately 8 h. These values agree with previously published reports on the pharmacokinetics of tamsulosin, as a controlled release formulation [1]. In the majority of subjects the concentrations of tamsulosin were above the limit of quantitation at 36 h after drug administration which indicates a suitability of the analytical method for pharmacokinetic studies.

4. Conclusions

The validated method allows determination of tamsulosin in the 0.4–40 ng/ml range. The assay is rapid, the analysis time is only 3.5 min. About 150 samples can be prepared and analysed in one working day. The precision and accuracy of the method are well within the limits required for bioanalytical assays. The limit of quantification 0.4 ng/ml permits the use of the method for pharmacokinetic studies.

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