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Short communication

Determination of silodosin in human plasma by liquid chromatography–tandem mass spectrometry

Xia Zhao [a], Yuwang Liu [a], Junyu Xu [a], Dan Zhang [b,c], Ying Zhou [a], Jingkai Gu [b,**], Yimin Cui [a,*]

[a] Department of Pharmacy, Peking University First Hospital, Beijing 100034, PR China
[b] Research Center for Drug Metabolism, Jilin University, Changchun 130023, PR China
[c] Clinical Pharmacology Department, Aerospace Clinical Medical College of Peking University, Beijing 100049, PR China

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ABSTRACT

A rapid, sensitive and specific liquid chromatography–tandem mass spectrometric (LC–MS/MS) method has been developed and validated for the determination of silodosin in human plasma. Silodosin and internal standard (IS) were extracted from human plasma by liquid–liquid extraction using methyl t-butyl ether and analyzed on an Agilent C_8 column with the mobile phase of acetonitrile–10 mM ammonium acetate (40:60, v/v) adjusted to pH 4.5 with acetic acid. Detection was carried out by MS/MS using TurboIonSpray (TIS) ionization and multiple reaction monitoring (MRM) in the positive-ion mode. The mass transitions monitored were m/z 496.3 → 261.4 and m/z 440.4 → 259.3 for silodosin and IS, respectively. The standard curve was linear in the range of 0.50–50.0 ng/ml with intra- and inter-day precision of 3.2–7.2% and 2.0–7.5%, respectively. The lower limit of quantification (LLOQ) for silodosin was 0.50 ng/ml using 500 μl plasma sample. This method was successfully applied to the pharmacokinetic study in healthy volunteers.

1. Introduction

Benign prostatic hyperplasia (BPH) is a common disorder of the male urogenital tract. It has reported that 78% of the men over 60 are struck by BPH [1]. As the ageing of the population, the amount of the relative involved patients will be still on the rise. Several α_1-adrenergic receptor (α_1-AR) antagonists, such as doxazosin and terazosin, are proven to relieve the symptoms of BPH, but these antagonists cause orthostatic hypotension in some cases. Pharmacological evidence and molecular cloning studies have demonstrated α_1-ARs are subdivided into the three subtypes α_{1A}, α_{1B} and α_{1D}-AR [2,3]. Studies have demonstrated α_{1A}-AR is the predominant receptor present in the prostate smooth muscle [4,5]. Selective α_{1A}-AR antagonists have been provided advanced in the treatment of BPH [6].

Silodosin ((-)-1-(3-Hydroxypropyl)-5-[(2R)-2-({2-(2,2,2-trifluoroethoxy) phenoxy] ethyl} amino) propyl-2, 3-dihydro-1H-indole-7-carboxamide), a selective α_{1A}-AR antagonist, has higher tissue selectivity for prostate. It has been proven to inhibit the contraction of prostate smooth muscle [7,8] and relieve the lower urinary tract symptoms associated with BPH [9]. A precise method for the determination of silodosin is very important for the investigation of its pharmacokinetics. To our knowledge, there is only one article mentioned the method for the determination of silodosin in human plasma. However, it has not reported any detail of the method [10]. This paper describes a rapid, sensitive, and specific LC–MS/MS method using liquid–liquid extraction for the quantification of silodosin in human plasma. The validated method has been successfully applied to the evaluation of silodosin pharmacokinetics in human.

2. Experimental

2.1. Materials and reagents

Silodosin (99.5%) and internal standard (IS) (99.8%) (Fig. 1) were provided by Daiichi Pharmaceutical Co. Ltd. (Beijing, China). Acetonitrile (HPLC grade) was purchased from Fisher Scientific (Fair Lawn, NJ, USA). All other chemicals (analytical grade) were used without further purification. Ultra pure distilled water was produced by a MUI-9000 Water Purification System (Nanjing, China).

2.2. Preparation of calibration standards and quality control samples

Stock solutions of silodosin at concentration of 0.1 mg/ml in acetonitrile were prepared from separate weighing for calibration

* Corresponding author. Tel.: +86 10 66110987; fax: +86 10 66110987.
** Corresponding author. Tel.: +86 431 85619955; fax: +86 431 5619955.
E-mail addresses: gujk@mail.jlu.edu.cn (J. Gu), cuiymzy@126.com (Y. Cui).

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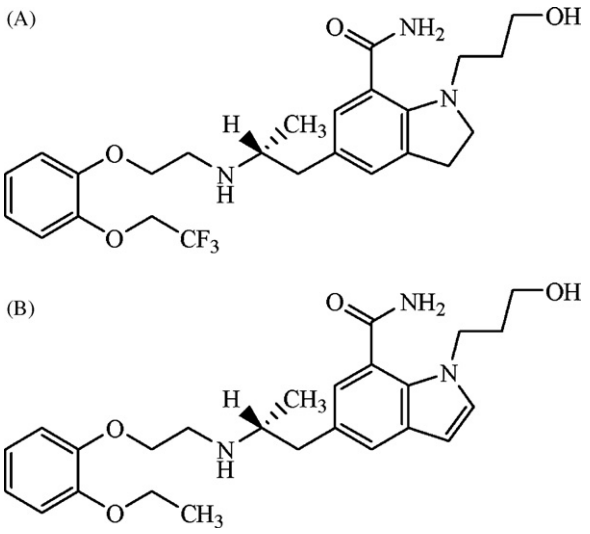

Fig. 1. Structures of silodosin (A) and IS (B).

standard samples and QC samples. Stock solution of IS (0.1 mg/ml) was prepared in 10 mM ammonium acetate buffer (pH 4.5). All the stock solutions were stored at 4 °C in the dark when not in use. Working solutions of silodosin at the desired concentrations for preparation of calibration standard samples and QC samples were made by serial dilution with acetonitrile. Working solution for IS at concentration of 250 ng/ml was prepared by diluting the stock solution with 10 mM ammonium acetate buffer (pH 4.5).

Both the calibration standard samples and QC samples were prepared by adding 50 μl of the appropriate working solutions to 4950 μl of blank human plasma. Seven calibration standard samples were prepared freshly before use at concentrations of 0.50, 1.00, 2.00, 5.00, 10.0, 20.0 and 50.0 ng/ml. Lower limit of quantification (LLOQ), low QC, medium QC, and high QC were prepared at concentrations of 0.50, 1.00, 10.0 and 50.0 ng/ml, respectively. The QC samples were prepared in the bulk, aliquoted into polypropylene tubes and stored at −20 °C.

2.3. Sample preparation

500 μl plasma samples were mixed with 50 μl of IS working solution and 100 μl of 0.5 M Na_2CO_3. The samples were extracted with 3 ml of methyl t-butyl ether in 5 ml glass tubes by vortex-mixing for 2 min at high speed and centrifuged at 2000 × *g* for 5 min at room temperature. The organic layer was removed and evaporated to dryness under nitrogen at 40 °C. The residues were dissolved in 500 μl mobile phase by vortex-mixing for 2 min and 30 μl were injected into LC–MS/MS system.

2.4. Instrumentation and conditions

The LC–MS/MS system consisted of an Agilent 1100 series HPLC (Agilent Technologies, Palo Alto, CA, USA) coupled to an API 3000 mass spectrometer (Applied Biosystems/MDS Sciex, Toronto, Canada). The separation was performed on a 150 mm × 4.6 mm, 5 μm Zorbax C_8 column using a mobile phase of acetonitrile–10 mM ammonium acetate (40:60, v/v) adjusted to pH 4.5 with acetic acid at a flow rate of 1.0 ml/min. The column effluent was split so that approximately 0.4 ml/min entered the mass spectrometer, which was equipped with a TIS source and operated on positive MRM mode. The source temperature was set at 425 °C. The ionspray voltage was 5000 V. Nitrogen was used as nebulizer gas, curtain gas and collision gas with flow rates at 12, 10 and 5 units, respectively. Declustering potential was 55 V for both silodosin and IS. Collision exit potential was 10 V. Collision energy was 36 V for silodosin and 30 V for IS. Singly charged precursor–product ion transitions were monitored at m/z 496.3 → 261.4 (silodosin) and m/z 440.4 → 259.3 (IS). The dwell time was 150 ms. The Q1 and Q3 resolutions were unit. Peak areas for all components were automatically integrated using Analyst 1.4 software (Applied Biosystems/MDS Sciex, Toronto, Canada).

2.5. Method validation

Specificity of the method was assessed by comparing the chromatograms for six different individuals of blank human plasma with those for the corresponding spiked plasma samples. Matrix effect was investigated by comparing the peak areas of silodosin in the blank plasma extracts spiked post-extraction with silodosin with those in blank water extracts spiked post-extraction with silodosin at the same concentration levels.

Calibration curves were constructed by plotting the peak area ratios of silodosin to the IS versus the nominal concentrations. The linear regression with weighting factor of $1/x$ was applied. Linearity was assessed by the correlation coefficient (r) and the relative error (R.E.) of calibration standard samples. Intra- and inter-day precision and accuracy were determined by analyzing six replicates of each QC level on 3 separate days, and on each day samples were analyzed together with an independently prepared calibration curve. The precision and accuracy were expressed as relative standard deviation (R.S.D.) and R.E., respectively. The LLOQ was defined as the lowest concentration at which both precision and accuracy were less than or equal to 15%.

The extraction recovery of silodosin was determined at three QC levels (n = 5) by comparing the peak areas of silodosin in the extracted QC samples with those obtained from the extracted blank plasma samples spiked post-extraction with corresponding solutions of silodosin.

Stability of silodosin was evaluated in QC samples stored at room temperature for 4 h, after three freeze–thaw cycles and stored at −20 °C for 38 days. Stability of silodosin in processed samples stored in autosampler vials at room temperature for 8 h was also assessed.

In order to evaluate the feasibility of the method to analyze samples whose concentrations extend beyond 50.0 ng/ml, three replicates of silodosin plasma samples at concentration of 100 ng/ml were diluted to be within the calibration range with blank human plasma before being analyzed.

2.6. Pharmacokinetic study

The validated assay was applied to evaluate the pharmacokinetics of silodosin in Chinese healthy volunteers. The study protocol was approved by the Ethical Committee of Peking University First Hospital (Beijing, China). Prior to the beginning of the study, volunteers provided written informed consent. Volunteers were administrated a single dose of an 8 mg capsule. Blood samples (3 ml) were collected before the dose and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 16, 24 and 36 h post-dose. Glass tubes used for transferring the whole blood had been mixed with sodium heparin. The whole blood samples were centrifuged at 3500 × *g* for 10 min at 4 °C immediately, and then the plasma samples were transferred into polypropylene tubes and stored at −20 °C prior to analysis. Noncompartmental pharmacokinetic parameters for silodosin were derived in WinNonLin Professional Version 5.0 (Pharsight Corporation, Mountain View, CA, USA) using actual sampling times.

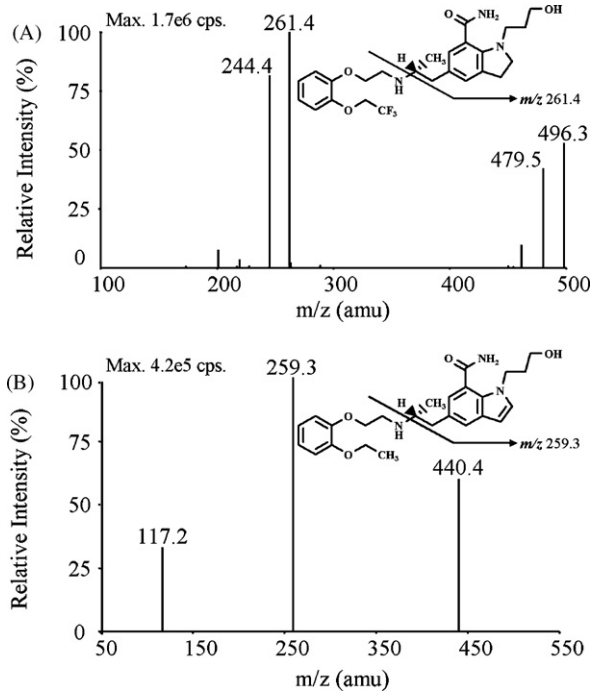

Fig. 2. Full-scan product ion spectra of $[M+H]^+$ for silodosin (A) and IS (B).

3. Results and discussion

3.1. Mass spectrometry/chromatography

Positive mode of detection was employed in the experiments. In this mode, the soft ionization process in the TIS source produces the precursor ions $[M+H]^+$. The precursor ions observed were m/z 496.3 for silodosin and m/z 440.4 for IS. The precursor ions were subjected to collision-induced dissociation in order to obtain product ions. The full product ion spectra of silodosin and IS are shown in Fig. 2. Ion transition of m/z 496.3 → 261.4 and m/z 440.4 → 259.3 were selected for the detection of silodosin and IS, respectively. The collision conditions were then optimized and it was found that the best detection was produced when the collision energy was at 36 V for silodosin and 30 V for IS.

To optimize peak shape with appropriate retention time, different analytical columns and various combinations mobile phases were investigated. The reversed phase C_8 column (150 mm × 4.6 mm, 5 μm, Zorbax) with a mobile phase of acetonitrile–10 mM ammonium acetate (40:60, v/v) adjusted to pH 4.5 with acetic acid was found to produce good peak shapes for silodosin and IS. Further improvement in peak shape with reduced cycle time was achieved by splitting the column effluent and increasing the flow rate. With a flow rate of 1.0 ml/min, the retention times of silodosin and IS were 2.3 and 1.9 min, the cycle time was 3.0 min. Representative chromatograms of silodosin and IS are shown in Fig. 3.

3.2. Assay validation

3.2.1. Specificity

Fig. 3 shows the representative LC–MS/MS MRM chromatogram obtained from the analysis of blank human plasma, human plasma spiked with silodosin at 0.50 ng/ml and a human plasma sample obtained 4 h after oral administration of silodosin capsule (8 mg). The analysis of blank plasma samples from six different sources did not show any interference at the retention times of silodosin (2.3 min) and IS (1.9 min) (Fig. 3), confirming the specificity of the present method.

Table 1
Back-calculated concentrations and correlation coefficient of silodosin.

Spiked concentration (ng/ml)	Mean concentration (ng/ml) ($n=5$)	R.S.D. (%)	R.E. (%)	Correlation coefficient ($n=5$)
0.50	0.51	5.1	1.4	
1.00	0.99	2.4	−0.5	
2.00	1.98	6.0	−1.1	
5.00	4.92	4.3	−1.7	0.9996 ± 0.0004
10.0	10.2	4.4	1.5	
20.0	20.2	3.0	0.8	
50.0	49.8	1.2	−0.4	

Three replicates each of three concentration levels (1.00, 10.0 and 50.0 ng/ml) were prepared from different sources of plasma to check the matrix effect. The results were 90.2 ± 2.5%, 92.4 ± 1.7% and 89.9 ± 2.1% for the low, middle and high concentration levels, respectively. It indicated that there was no obvious matrix effect of silodosin in this method.

3.2.2. Linearity, precision and accuracy

The assay was linear over the concentration range of 0.50–50.0 ng/ml of silodosin in plasma. The mean linear regression equation is listed below, where Y represents the ratios of silodosin peak area to that of IS and X represents the plasma concentrations of silodosin. Back-calculated concentration and R.E. are given in Table 1.

$$Y=(0.02148 \pm 0.0014)X+(0.00236 \pm 0.0021) \quad (n=5), \quad r=0.9996$$

Table 2 shows a summary of intra- and inter-day precision and accuracy data for LLOQ and QC samples containing silodosin. Both intra- and inter-day precisions ranged from 2.0% to 7.5% at three QC levels. The intra- and inter-day R.E. values for silodosin were −5.1% to 2.2% at three QC levels. These results indicated that the present method has an acceptable precision and accuracy.

The LLOQ was set at 0.50 ng/ml for silodosin using 500 μl of human plasma. Representative chromatogram of an LLOQ is shown in Fig. 3 and signal-to-noise ratio for silodosin is about 47 at 0.50 ng/ml. The intra-day R.S.D., inter-day R.S.D. and R.E. at the LLOQ level were 7.2, 7.3 and 2.6%, respectively.

3.2.3. Recovery

The one-step liquid–liquid extraction with methyl t-butyl ether adjusted pH by using 0.5 M Na_2CO_3 has been successfully applied to the extraction of silodosin from human plasma. The recoveries of silodosin from human plasma were 85.9 ± 2.5%, 84.6 ± 2.4%, 86.1 ± 3.1% at concentrations of 1.00, 10.0 and 50.0 ng/ml, respectively.

3.2.4. Stability

The results of the stability evaluation in human plasma are summarized in Table 3. Three freeze–thaw cycles or storage under

Table 2
Precision and accuracy for the determination of silodosin in human plasma (data are based on assay of six replicates samples of LLOQ and three QC concentrations on 3 different days).

Nominal concentration (ng/ml)	Measured concentration (mean ± S.D.) (ng/ml)	Intra-day R.S.D. (%)	Inter-day R.S.D. (%)	R.E. (%)
0.50	0.51 ± 0.04	7.2	7.3	2.6
1.00	0.95 ± 0.04	5.0	2.0	−5.1
10.0	9.99 ± 0.45	3.9	7.5	−0.1
50.0	51.1 ± 1.96	3.2	6.8	2.2

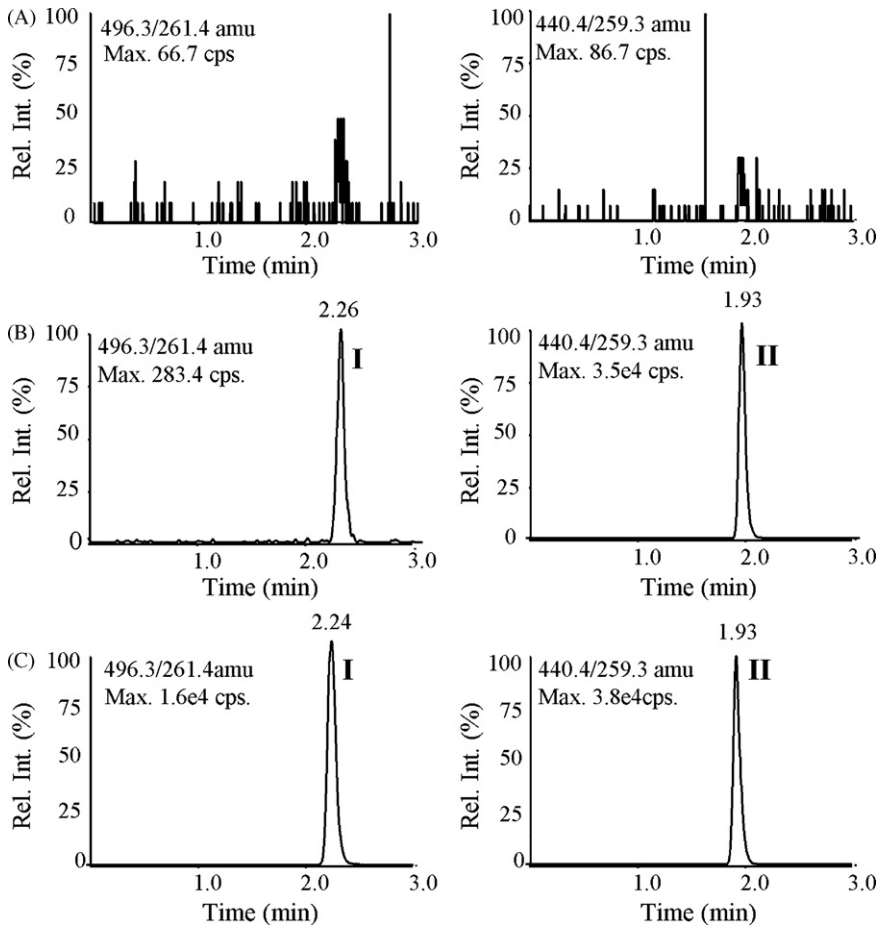

Fig. 3. Representative MRM chromatograms of (A) blank human plasma, (B) human plasma spiked with at the limit of quantification (0.50 ng/ml) and (C) a human plasma sample obtained 4 h after an oral administration of a capsule containing 8 mg silodosin. Peak I, silodosin; Peak II, IS.

autosampler condition for 8 h before analysis had little effect on the quantification. Plasma sample were allowed to store at room temperature for 4 h or −20 °C for 38 days before analysis without affecting the quantification.

3.2.5. Dilution

The precision (R.S.D.) and accuracy (R.E.) for the measured silodosin concentrations at 100 ng/ml following a 10-fold dilution with blank human plasma were 4.1% and −3.1%, respectively.

Table 3
Stability of silodosin in human plasma (data are means ± SD of three replicates).

Storage condition	Nominal concentration (ng/ml)	Calculated concentration (ng/ml)	R.E. (%)
In human plasma at room temperature for 4 h	1.00	0.98 ± 0.01	−2.2
	10.0	9.94 ± 0.26	−0.6
	50.0	50.9 ± 0.80	1.7
In human plasma after three freeze/thaw cycles	1.00	0.92 ± 0.03	−7.7
	10.0	9.75 ± 0.62	−2.5
	50.0	49.4 ± 1.3	−1.2
In human plasma at −20 °C for 38 days	1.00	0.91 ± 0.02	−8.5
	10.0	9.89 ± 1.13	−1.1
	50.0	51.7 ± 2.1	3.4
In processed samples at room temperature for 8 h	1.00	0.96 ± 0.04	−3.9
	10.0	10.3 ± 0.2	2.7
	50.0	52.0 ± 0.6	4.1

These results suggested that samples whose concentrations extend beyond the calibration range could be reanalyzed by dilution to obtain acceptable data.

3.3. Pharmacokinetic study

This method was successfully applied to the pharmacokinetic study of silodosin after administration of an 8 mg capsule to ten healthy male volunteers. This was the first evaluation of the pharmacokinetic properties of silodosin in Chinese subjects. Fig. 4 shows mean plasma concentration profiles of silodosin in

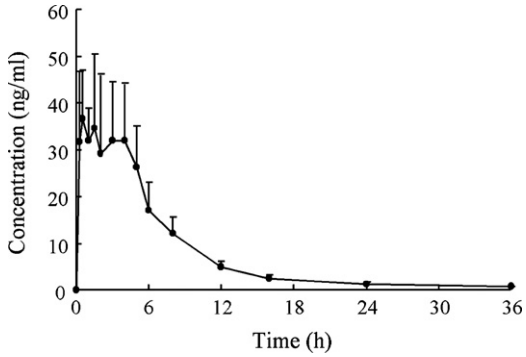

Fig. 4. Mean plasma concentration–time curve of silodosin after a single oral administration of 8 mg capsule to ten healthy male volunteers. Data are means + S.D.

10 healthy male volunteers. The pharmacokinetic parameters for silodosin are as follows: C_{max} 48.5 ± 12.4 ng/ml; T_{max} 1.9 ± 1.6 h; AUC_{0-t} 270.2 ± 54.7 ng h/ml; $t_{1/2}$ 6.7 ± 2.0 h. These values are comparable with silodosin exposure observed in Japanese volunteers administrated with the same dose. In the Japanese male volunteers, C_{max} was 59.3 ± 17.5 ng/ml; T_{max} was 2.3 ± 1.7 h; $AUC_{0-\infty}$ was 321.9 ± 75.9 ng h/ml; $t_{1/2}$ was 4.5 ± 0.4 h [11].

4. Conclusion

A LC–MS/MS method was successfully developed and validated for the quantitation of silodosin in human plasma. The LLOQ of silodosin was 0.50 ng/ml. One-step liquid–liquid extraction was used to extract silodosin from human plasma. The analysis time of each run was 3 min. The major advantages of the assay are simple sample preparation and short run time. The method was proved to be suitable for the clinical pharmacokinetic study of silodosin.

References

[1] J.E. Oesterling, N. Engl. J. Med. 332 (1995) 99.
[2] D.B. Bylund, D.C. Eikenberg, J.P. Hieble, S.Z. Langer, R.J. Lefkowitz, K.P. Minneman, P.B. Molinoff, R.R. Ruffolo Jr., U. Trendelenburg, J. Pharmacol. Rev. 46 (1994) 121.
[3] J.P. Hieble, D.B. Bylund, D.E. Clarke, D.C. Eikenburg, S.Z. Langer, R.J. Lefkowitz, K.P. Minneman, R.R. Ruffolo Jr., J. Pharmacol. Rev. 47 (1995) 267.
[4] C.R. Chapple, R.P. Burt, P.O. Andersson, P. Greengrass, M. Wyllie, I. Marshall, Br. J. Urol. 74 (1994) 585.
[5] I. Muramatsu, M. Oshita, T. Ohmura, S. Kigoshi, H. Akino, M. Gobara, K. Okada, Br. J. Urol. 74 (1994) 572.
[6] C. Chapple, K.E. Andersson, World J. Urol. 19 (2002) 397.
[7] M. Ishiguro, Y. Futabayashi, T. Ohnuki, M. Ahmed, I. Muramatsu, T. Nagatomo, J. Life Sci. 71 (2002) 2531.
[8] S. Murata, T. Taniguchi, M. Takahashi, K. Okada, K. Akiyama, I. Muramatsu, J. Urol. 164 (2000) 578.
[9] K. Kawabe, M. Yoshida, Y. Homma, Silodosin Clinical Study Group, Br. J. Urol. Int. 98 (2006) 1019.
[10] Y. Matsubara, T. kanazawa, Y. Kojima, Y. Abe, K. Kobayashi, H. Kanbe, H. Harada, Y. Momose, S. Terakado, Y. Adachi, I. Midgley, Yakugaku Zasshi 126 (2006) 237.
[11] T. Shimizu, I. Miyashita, Y. Matsubara, M. Ikeda, M. Yamaguchi, Yakugaku Zasshi 126 (2006) 257.