ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 7, Issue 2, 2014

ISSN - 0974-2441

Research Article

# ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF TAMSULOSIN AND TOLTERODINE IN BULK & PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

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*Received: 2 February 2014, Revised and Accepted: 6 March 2014*

**ABSTRACT**

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the simultaneous determination tamsulosin and tolterodine in pharmaceutical dosage form. The column used was Hypersil BDS $C_{18}$, 100 x 4.6 mm, 5µ in isocratic mode, with mobile phase containing phosphate buffer and acetonitrile (65:35 v/v) adjusted to pH 3.8 with dilute ortho phosphoric acid solution. The flow rate was 1.0 ml/ min and effluents were monitored at 220 nm. The retention times of tamsulosin and tolterodine were found to be 2.285 min and 4.334 min, respectively. The linearity for tamsulosin and tolterodine were in the range of 1-6 µg/ml and 10-60 µg/ml respectively. The recoveries of tamsulosin and tolterodine were found to be 98.40 to 100.42% and 98.16 to 99.76%, respectively. The proposed method was validated and successfully applied to the estimation of tamsulosin and tolterodine in combined tablet dosage forms.

**Keywords:** tamsulosin, tolteraotine, hplc, rsd, dosage.

## INTRODUCTION

Chemically, Tamsulosin is 5-[(2R)-2-{[2-(2-ethoxyphenoxy) ethyl] amino} propyl]-2-methoxybenzene-1-sulfonamide. It is used in the treatment of signs and symptoms of benign prostatic hyperplasia. Tamsulosin is a selective antagonist at alpha-1A and alpha-1B-adrenoceptors in the prostate, prostatic capsule, prostatic urethra, and bladder neck. At least three discrete alpha1-adrenoceptor subtypes have been identified: alpha-1A, alpha-1B and alpha-1D; their distribution differs between human organs and tissue. Blockage of these receptors causes relaxation of smooth muscles in the bladder neck and prostate, and thus decreases urinary outflow resistance in men[1].

Tolterodine is chemically, 2-[(1R)-3-[bis(propan-2-yl)amino]-1-phenylpropyl]-4-methylphenol. It is an antimuscarinic drug that is used to treat urinary incontinence. Tolterodine acts on M2 and M3 subtypes of muscarinic receptors. Tolterodine and its active metabolite, 5-hydroxymethyltolterodine, act as competitive antagonists at muscarinic receptors. This antagonism results in inhibition of bladder contraction, decrease in detrusor pressure, and an incomplete emptying of the bladder[2].

Different analytical methods have been reported in the literature for the assay of tamsulosin and tolterodine in pharmaceuticals and include spectrophotometry, TLC, HPLC, HPTLC, LC-MS[3–14]. The present study was to establish a simple, sensitive and low cost RP-HPLC method for simultaneous estimation of tamsulosin and tolterodine in bulk as well as in other dosage forms. The developed method was validated as per ICH guidelines[15, 16].

## EXPERIMENTAL

### Reagents

Tamsulosin and Tolterodine were kindly supplied by Dr. Reddy Labs (Hyderabad, A.P., and India). Acetonitrile, water (HPLC grade, Merck) and all the other reagents of AR grade were purchased from M R Enterprisers. A tablet Bapter(Dr. Reddy's) and Roliflo OD(Ranbaxy) containing 0.4mg of tamsulosin and 4mg of tolterodine were used.

### Instrumentation

The LC system consisted of a Waters model 515, PDA detector 2998 with 20 µL sample loop. The output signals were monitored and integrated using Empower 2 software.

### Chromatographic conditions

The elution was isocratic and the mobile phase consisted of a mixture of buffer (accurately weighed and transferred 2.72gm of Potassium dihydrogen Orthophosphate in a 1000ml of volumetric flask add about 900ml of milli-Q water, add 1ml of triethylamine and degass to sonicate and finally make up the volume with water, then pH adjusted to 3.8 with dil. Ortho phosphoric acid solution) and acetonitrile (65 : 35 v/v). The mobile phase was filtered through a 0.45-µm (HVLP, Germany) membrane filter prior to use. A Thermohypersil BDS $C_{18}$ column (250 x 4.6mm x 5 µ) was used for determination. The flow rate was 1.0 ml/min and the column was operated at ambient temperature (~25 $^{o}$C). The volume of sample injected was 20 µL. Prior to injection of the solutions, column was equilibrated for at least 30min with mobile phase flowing through the system. The UV detector was set at wavelength of 220nm. A typical RP-HPLC chromatogram of tamsulosin and tolterodine is shown in (Fig. 1).

**Diluent:** Methanol and Water (70:30) v/v

### Standard Preparation

Accurately weighed and transferred 0.4mg of tamsulosin and 4mg of tolterodine working Standards into a 10 ml clean dry volumetric flask, add 7ml of diluent, sonicated for 30 minutes and make up to the final volume with diluent. From the above stock solution, 1ml was pipetted out in to a 10ml volumetric flask and then make up to the final volume with diluent.

### Sample Preparation

About20 tablets were taken and their average weight was calculated. The tablets were crushed to a fine powder and drug equivalent to 0.4 and 4mg were transferred to a 10ml volumetric flask,

dissolved in diluent. Transfer 1ml from the above solution into 10ml volumetric flask and filtered through 0.45µ membrane filter to get concentration of 4 µg/ml and 40µg/ml.

**METHOD VALIDATION**

The developed method was validated as per ICH guidelines [13-14] for its accuracy, linearity, precision, specificity, robustness, ruggedness, limit of detection and limit of quantification by using the following procedures. The parameters are validated as shown in table 9.

**System suitability**

System suitability and chromatographic parameters were validated such as asymmetry factor, tailing factor and number of theoretical plates were calculated.

**Linearity**

Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solutions of tamsulosin and tolterodine at different concentration levels. Absorbance of resulting solutions was measured and the calibration curve was plotted between absorbance vs concentration of the drug (Figure: 2 & 3). The response was found to be linear in the range 1-6 µg/ml & 10-60 µg/ml for tamsulosin and tolterodine. The data was given in table 1.

**Accuracy**

Accuracy was performed in triplicate for various concentrations of tamsulosin and tolterodine equivalent to 50%, 100% and 150% of the standard amount were injected into the HPLC system per the test procedure. The average % recovery was calculated. The data was given in table 2.

**Precision**

**A) Method Repeatability**

Six sample solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure. The results were given in table 3.

**B) Intermediate Precision (Day to Day variability)**

Two days as per test method conducted the study. For Day-1 and Day-2, six sample solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure. The results were given in table 6.

**Limit of detection and Limit of Quantification**

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve as per ICH guidelines. The LOD and LOQ of tamsulosin were found to be 0.105µg/ml and 0.318µg/ml respectively. The LOD and LOQ of tolterodine were found to be 0.859µg/ml and 2.603µg/ml respectively.

**Robustness and Ruggedness**

Robustness was done by small deliberate changes in the chromatographic conditions and retention time of tamsulosin and tolteodine were noted. The factors selected were flow rate and variation in the mobile phase composition. The results remained unaffected by small variations in these parameters as shown in table 4 and 5.

Ruggedness of the method was checked by using different days and instruments. The relative standard deviation of the results obtained from different days and instruments was <2.0%. The results were given in table 6 and 7.

**Assay**

The assay and % purity were calculated for two brands Bapter(Dr. Reddy's) and Roliflo OD(Ranbaxy) with label claim 0.4mg and 4mg. The observed value was compared with that of standard value without interference from the excipients used in the tablet dosage form. The results were given in table 8.

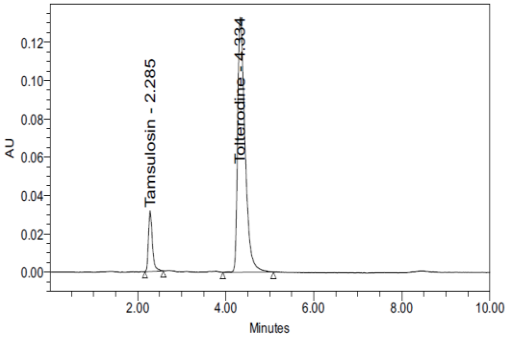


**Fig - 1: HPLC chromatogram of Tamsulosin and Tolterodine in optimized chromatographic conditions**

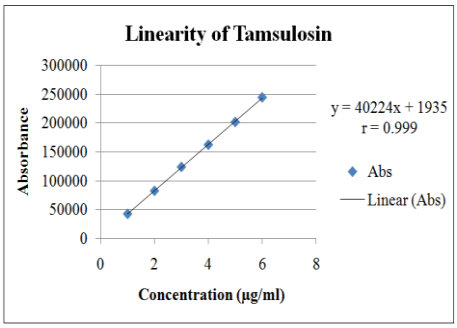


**Fig .2: Linearity of Tamsulosin in the range 1 to 6 µg/ml.**

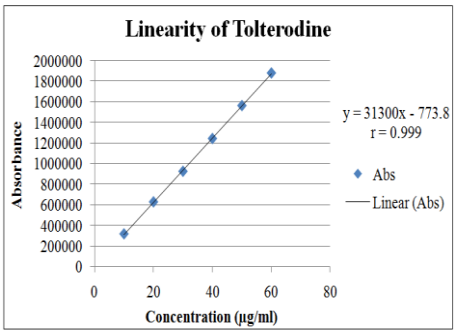


**Fig .3: Linearity of Tolterodine in the range 10 to 60 µg/ml.**

**Table 1: Linearity data of Tamsulosin and Tolterodine**

| | Tamsulosin | | | Tolterodine | | |
|---|---|---|---|---|---|---|
| **S.No** | **Conc(µg/ml)** | **Rt(mins)** | **Area** | **Conc(µg/ml)** | **Rt(mins)** | **Area** |
| 1 | 1 | 2.307 | 42163 | 10 | 4.399 | 317640 |
| 2 | 2 | 2.266 | 82167 | 20 | 4.238 | 629389 |
| 3 | 3 | 2.287 | 123735 | 30 | 4.351 | 926685 |
| 4 | 4 | 2.28 | 162261 | 40 | 4.296 | 1245904 |
| 5 | 5 | 2.301 | 201660 | 50 | 4.432 | 1565787 |
| 6 | 6 | 2.321 | 244330 | 60 | 4.49 | 1882960 |
| | r = 0.99992 | | | r = 0.99993 | | |
| | y = 40224x + 1935 | | | y = 31300x - 774 | | |

**Table 2: Accuracy data**

| | | Tamsulosin | | | Tolterodine | | |
|---|---|---|---|---|---|---|---|
| **S.No** | **Spiked level** | **Amount added (µg/ml)** | **Amount present (µg/ml)** | **Average %Recovery* + %RSD** | **Amount added (µg/ml)** | **Amount present (µg/ml)** | **Average %Recovery* + %RSD** |
| 1(n=6) | 50% | 1.99 | 1.96 | 98.40 + 0.05 | 19.92 | 19.68 | 98.80 + 0.28 |
| 2(n=6) | 100% | 3.98 | 4.00 | 100.42+ 0.65 | 39.84 | 39.75 | 99.76 + 0.19 |
| 3(n=6) | 150% | 5.98 | 5.89 | 98.49 + 0.03 | 59.76 | 58.65 | 98.16 + 0.14 |

***n=6 (Average of 6 determinations)**

**Table 3: Precision data of Nebivolol and Valsartan**

| | Tamsulosin | | | Tolterodine | | |
|---|---|---|---|---|---|---|
| **S.No** | **Conc(µg/ml)** | **Rt(mins)** | **Area** | **Conc(µg/ml)** | **Rt(mins)** | **Area** |
| 1 | 4 | 2.307 | 166792 | 40 | 4.399 | 1286105 |
| 2 | 4 | 2.266 | 166812 | 40 | 4.238 | 1290671 |
| 3 | 4 | 2.227 | 167962 | 40 | 4.351 | 1290176 |
| 4 | 4 | 2.281 | 168945 | 40 | 4.296 | 1285868 |
| 5 | 4 | 2.301 | 166973 | 40 | 4.432 | 1306381 |
| 6 | 4 | 2.322 | 167619 | 40 | 4.491 | 1297918 |
| **Mean** | | | 167517 | | | 1292853 |
| **Std.dev** | | | 845 | | | 7936 |
| **%RSD** | | | 0.50 | | | 0.61 |

**Table 4: Robustness data relating to change in flow rate (1.0ml/min)**

| | | Tamsulosin | | | Tolterodine | | |
|---|---|---|---|---|---|---|---|
| **S.No** | **Flow rate (ml/min)** | **Average Peak Area*** | **Std.dev** | **%RSD** | **Average Peak Area*** | **Std.dev** | **%RSD** |
| 1 | 0.9ml/min | 164423 | 469 | 0.28 | 1264278 | 1874 | 0.15 |
| 2 | 1.0ml/min | 162491 | 500 | 0.31 | 1247818 | 2254 | 0.18 |
| 3 | 1.1ml/min | 157248 | 1522 | 0.97 | 1271579 | 2402 | 0.19 |

***n=3 (Average of 3 determinations)**

**Table 5: Robustness data relating to change in mobile phase composition**

| | | Tamsulosin | | | Tolterodine | | |
|---|---|---|---|---|---|---|---|
| **S.No** | **Mobile phase variation (%)** | **Average peak area*** | **Std.dev** | **%RSD** | **Average peak area*** | **Std.dev** | **%RSD** |
| 1 | M.P-1 (Buffer:ACN:: 66:34) | 164743 | 1066 | 0.65 | 1139759 | 5014 | 0.44 |
| 2 | M.P-2 (Buffer:ACN:: 65:35) | 162818 | 608 | 0.37 | 1251151 | 5719 | 0.46 |
| 3 | M.P-3 (Buffer:ACN:: 64:36) | 156559 | 764 | 0.49 | 1289912 | 3831 | 0.30 |

***n=3 (Average of 3 determinations)**

**Table 6: Ruggedness data relating to change of day**

| | | | Inter-day precision | | | |
|---|---|---|---|---|---|---|
| | Tamsulosin | | | Tolterodine | | |
| | Peak area | | | Peak area | | |
| **S.No** | **Conc (µg/ml)** | **Day-1** | **Day-2** | **Conc (µg/ml)** | **Day-1** | **Day-2** |
| 1 | 4 | 165261 | 166812 | 40 | 1275708 | 1243567 |
| 2 | 4 | 166786 | 164972 | 40 | 1265975 | 1239854 |
| 3 | 4 | 167397 | 165893 | 40 | 1278675 | 1259873 |
| 4 | 4 | 166939 | 165939 | 40 | 1285632 | 1249821 |
| 5 | 4 | 167937 | 165038 | 40 | 1273649 | 1250932 |
| 6 | 4 | 166837 | 166692 | 40 | 1287462 | 1249845 |
| **Mean** | | 166860 | 165891 | | 1277850 | 1248982 |
| **SD** | | 896 | 783 | | 7961 | 6878 |
| **%RSD** | | 0.54 | 0.47 | | 0.62 | 0.55 |

**Table 7: Ruggedness data relating to change of instrument**

| Instrument to Instrument | | | | | | |
|---|---|---|---|---|---|---|
| | Tamsulosin | | | Tolterodine | | |
| S.No | Conc (µg/ml) | Peak area Day-1 | Day-2 | Conc (µg/ml) | Peak area Day-1 | Day-2 |
| 1 | 4 | 168743 | 166753 | 40 | 1278473 | 1243857 |
| 2 | 4 | 167594 | 165749 | 40 | 1267922 | 1239854 |
| 3 | 4 | 168744 | 165629 | 40 | 1278675 | 1258621 |
| 4 | 4 | 166539 | 165921 | 40 | 1285632 | 1249947 |
| 5 | 4 | 167733 | 165238 | 40 | 1273649 | 1250932 |
| 6 | 4 | 166831 | 166692 | 40 | 1287462 | 1249231 |
| **Mean** | | 167697 | 165997 | | 1278636 | 1248740 |
| **Std.dev** | | 927 | 606 | | 7301 | 6433 |
| **%RSD** | | 0.55 | 0.36 | | 0.57 | 0.52 |

**Table-8: Results of analysis of laboratory samples (Assay)**

| | | | Tamsulosin | | Tolterodine | |
|---|---|---|---|---|---|---|
| S.No | Sample | Label | Amount found | %Purity + RSD* | Amount found | %Purity + RSD* |
| 1 | Brand-1 (BAPTER) | 0.4mg/4mg | 0.402 | 99.99 + 0.51 | 3.992 | 99.40 + 0.17 |
| 2 | Brand-2 (ROLIFLO OD) | 0.4mg/4mg | 0.401 | 99.97 + 0.48 | 3.998 | 99.54 + 0.56 |

***n=3 (Average of 3 determinations)**

**Table 9: System suitability parameters**

| Validation parameter | Results | |
|---|---|---|
| | Tamsulosin | Tolterodine |
| Linearity range (µg/ml) | 1 – 6 | 10 - 60 |
| Regression equation | y = 40224x + 1935 | y = 31300x – 773.8 |
| Correlation Coefficient(r) | 0.999917 | 0.99993 |
| Accuracy | 98.40% to 100.42% | 98.16% to 99.76% |
| Precision (%RSD) | 0.50 | 0.61 |
| Robustness (%RSD) | | |
| Flow rate: (0.9ml/min & 1.1ml/min) | NMT 0.28 | NMT 0.15 |
| Mobile phase: Buffer : ACN(66:34 & 64:36) | NMT 0.97 | NMT 0.19 |
| Ruggedness (%RSD) | | |
| Interday – (Day 1 & Day 2) | NMT 0.65 | NMT 0.49 |
| Instrument to Instrument (Inst-1 & Inst-2) | NMT 0.44 | NMT 0.30 |

## RESULTS

A reverse-phase column procedure was proposed as a suitable method for the simultaneous estimation of tamsulosin and tolterodine dosage form. The chromatographic conditions were optimized by changing the mobile phase composition. Different ratios were experimented to optimize the mobile phase. Finally, buffer and acetonitrile in the ratio 65:35v/v was used as mobile phase, which showed good resolution of tamsulosin and tolterodine peak. The wavelength of detection selected was 220nm, as the drug showed optimized absorbance at this wavelength. By our proposed method the retention time of tamsulosin and tolterodine were about 2.285mins and 4.334mins and none of the impurities were interfering in its assay.

## DISCUSSION

The statistical analysis of data and the drug recovery data showed that the method was simple, rapid, economical, sensitive, precise and accurate. It can thereby easily adopt for routine quality control analysis. The results of this analysis confirmed that the proposed method was suitable for determination of drug in pharmaceutical formulation with virtually no interference of additives. Hence the proposed method can be successfully applied in simultaneous estimation of tamsulosin and tolterodine in marketed formulation.

## CONCLUSION

The proposed method is rapid, accurate and sensitive. It makes use of fewer amounts of solvents and change of set of conditions requires a short time. This method can be suitably analyzed for the routine analysis of tamsulosin and tolterodine in bulk and its pharmaceutical dosage forms. It does not suffer from any interference due to common excipients present in pharmaceutical preparation and can be conveniently adopted for quality control analysis.

## ACKNOWLEDGEMENTS

The authors are thankful to Mallareddy College of pharmacy, Secunderabad for providing all facilities to complete the work and Dr. Reddy Labs for providing the tamsulosin and tolterodine as gift sample.

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