

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF TAMSULOSIN HCl AND TOLTERODINE TARTRATE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, rapid, and accurate reversed phase high-performance liquid chromatographic (RP-HPLC) method has been developed and subsequently validated for the simultaneous determination of Tamsulosin HCl (TAM) and Tolterodine Tartrate (TOL) in combination. The separation was carried out using a mobile phase consisting of Acetonitrile and 20mM Ammonium Acetate buffer in the ratio of 60: 40. The pH of the mobile phase was adjusted to 7.8 with Triethylamine. The column used was Inertsil ODS-3V (250 × 4.6 mm, 5 μm) with flow rate of 1.0 mL/min using UV detection at 220nm. The total run time was 8 min and the retention time of TAM and TOL was 4.1 min and 5.8 min, respectively. The described method was linear for the assay of TAM and TOL over a concentration range of 0.2-0.6 μg/mL and 2-6 μg/mL respectively. Results of the analysis have been validated statistically and by recovery studies. The limit of quantitation for TAM and TOL was found to be 0.013μg/mL and 0.011μg/mL respectively. The results of the studies showed that the proposed RP-HPLC method is simple, rapid, precise, and accurate, which is useful for the routine determination of TAM and TOL in bulk drug and its pharmaceutical dosage form.

Keyword: Tamsulosin HCl, Tolterodine Tartrate, HPLC,

INTRODUCTION

TAM (Fig 1) is a selective, potent and competitive α₁ – adrenoreceptor antagonist and has a greater affinity for these receptors, predominantly present in the human prostate. Chemically, TAM is 5 – [(2R) – 2- [[2-(2-ethoxy phenoxy) ethyl] amino] propyl] – 2- methoxy benzene sulfonamide hydrochloride. TAM is used in men to treat the symptoms of an enlarged prostate which includes difficulty in urination, painful urination, and urinary frequency and urgency [1].



Fig. 1: Chemical Structure of Tamsulosin HCl

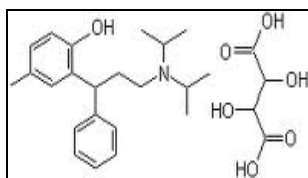


Fig. 2: Chemical Structure of Tolterodine Tartrate

TOL (Fig 2), chemically designated as (R)-N,N-di-isopropyl- 3-(2-hydroxy-5-methylphenyl)-3-phenylpropanamine L-hydrogen Tartrate is a new muscarinic receptor antagonist intended for the treatment of urinary urge incontinence and other symptoms related to an unstable bladder [2,3]. It is used to treat an overactive bladder

with symptoms of urinary frequency, urgency, and incontinence. Both, urinary bladder contraction and salivation are mediated via cholinergic muscarinic receptors [4]. Both of these drugs i.e. TAM and TOL are marketed as combined dose tablet formulation in the ratio (0.4:4mg) respectively.

A literature survey revealed that, TAM was developed as a controlled release delivery system, pellets and an oral controlled delivery system [5-9]. Only TAM was estimated in pharmaceuticals and biological fluids by HPLC, HPLC – MS, LC – MS/MS methods [10-13]. In Development and optimization of a novel oral controlled delivery system for TAM using response surface methodology, chiral separation of Tamsulosin by capillary electrophoresis and methods for Voltammetric investigation have been reported [14-16]. Similarly, for TOL there are stability indicating HPLC methods and also for enantiomeric separation and for routine quality control samples [17-20]. A simultaneous determination method for pellets of these two molecules using the gradient mode has been described [21]. This study aimed at developing and validating an RP-HPLC method, being simple, accurate and selective and the proposed method can be used for the estimation of these drugs in combined dosage forms.

MATERIAL AND METHOD

Reagents and Chemicals

Standards of TAM and TOL were received as gift samples from 'Corporate Analytical Development Lab'. Methanol, Acetonitrile (of HPLC grade) and Ammonium acetate (of AR grade) were obtained from E. Merck (India) Ltd. Triethylamine of AR Grade was used of Qualigens. Sodium Chloride, Monobasic Potassium Phosphate were of Qualigens and Hydrochloric Acid, Polysorbate 80, Sodium Hydroxide used were of E. Merck (India) Ltd. in preparation of acidic solution of pH 1.2 and basic solution of pH 8.6 [22]. The details of various brands used in the study are explained in Table 1.

Table 1: Details of brand used in the study

S. No.	Brands	Content (mg)		Manufacturer
		Tamsulosin HCl	Tolterodine Tartrate	
1	Roliflo-OD	0.4	4.0	Ranbaxy
2	Tamlet 4	0.4	4.0	Sun Pharma
3	Bapter	0.4	4.0	Dr. Reddy's

Preparation of Standard Stock Solution and Linearity Solutions

The standard stock solutions of TAM and TOL (1 mg/mL) were prepared separately by dissolving 50mg of each drug in 50mL of methanol. Several aliquots of these standard stock solutions were taken in different 10 mL volumetric flasks and diluted upto the mark with the mobile phase such that the final linearity concentrations of TAM and TOL were 0.2-0.6 µg/mL and 2.0-6.0 µg/mL, respectively.

Preparation of Sample Solution

Twenty Capsules of all three brands each containing 0.4 mg of TAM and 4.0 mg of TOL were weighed and powdered equivalent to dose, transferred to a 100 mL volumetric flask. In this flask 40 mL of acidic solution of pH 1.2 was added and sonicated for 30 min. After which 60 mL of a basic solution of pH 8.6-8.7 was added, to give a final pH

of 6.8. The solution was filtered through Whatman filter paper 42, as per procedure and diluted to the target concentration. The solution was injected into HPLC and recovery was calculated, the results are given in Table 2.

Instruments and Method

A surveyor HPLC system (Agilent 1100 series) consisting of G1379A degasser, a G1311A quaternary pump, a G1330B autosampler, a G1330B autosampler thermostat-ALS Therm, a G1316A column oven (COL COM) and a G1314A UV - Visible detector were used for the experiment. The HPLC column used was Inertsil ODS-3V C18 (250 × 4.6 mm) 5 µm. The mobile phase consisted of Acetonitrile-20mM Ammonium Acetate buffer (60:40), pH adjusted to 7.8 with Triethylamine, at a flow rate of 1.0 mL/min through the analytical column.

Table 2: Analysis of Formulation

Brands	Drug	Labelled Amount (mg)	Amount Found (mg)	% Label Claim	% RSD (n=6)
Roliflo-OD	TAM	0.4	0.41	102.5	1.48
	TOL	4.0	3.8	95.0	1.09
Tamlet 4	TAM	0.4	0.409	102.25	1.40
	TOL	4.0	3.86	96.5	1.09
Bapter	TAM	0.4	0.412	103.0	1.40
	TOL	4.0	3.82	95.5	1.09

RESULTS AND DISCUSSION

Method Development and Optimization

A literature survey revealed the pKa values of TAM and TOL to be 8.4, 10.7 and 9.87 respectively [23, 24]. The buffer was chosen considering the fact that the pKa values were on the basic side. 20 mM ammonium acetate buffer was chosen so as to avoid precipitation when used with an organic modifier. The mobile phase used was acetonitrile and buffer 60:40 pH adjusted to 3.0 with

Glacial Acetic acid. Since both these drugs were not ionized in the acidic mobile phase, it made them more hydrophilic and thus enabled longer retention. Hence rather than keeping the mobile phase acidic, attempts were made to develop methods using basic mobile phases. Among the different mobile phases investigated, the optimized mobile phase comprising acetonitrile and ammonium acetate in 60:40, pH being adjusted to 7.8 with Triethylamine. The typical chromatogram obtained under optimized chromatographic condition of the sample is shown in Fig 3.

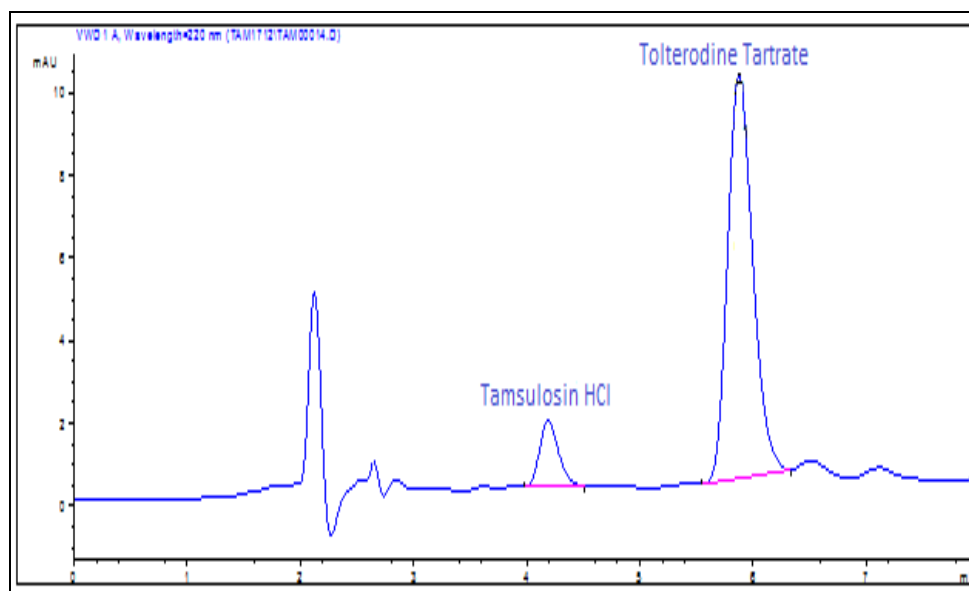


Fig. 3: A Chromatogram of mixture containing Tamsulosin HCl (4.1min, 0.4µg/mL) and Tolterodine Tartrate (5.8min, 4.0µg/mL)

Method Validation

System Suitability studies

The column efficiency, resolution, and peak symmetry were calculated for the standard solutions (n=6). The values obtained (Table 3) demonstrated the suitability of the system for the analysis of this drug combination.

Solution Stability

In order to demonstrate the stability of both the analyte during analysis, sample solutions were analyzed over a period of 48 h at room temperature. The results indicated that both the analyte did not show much variation in retention time and peak response (% RSD less than 2.0). There was no degradation within the indicated

period. Hence, it was concluded that both the solutions were stable for 48 h at room temperature.

Limit of Detection and Limit of Quantitation

The linearity for TAM was determined from 0.2-0.6 µg/mL and that for TOL from 2.0-6.0 µg/mL. A linearity graph was plotted and the correlation coefficient (r) determined. The limit of detection (LOD) was calculated from the linearity curve using the formula

$$\text{LOD} = 3.3 \times \text{SD}/\text{Slope}^{[25]}$$

The LOD for TAM was confirmed to be 0.00445 µg/mL and TOL it was confirmed to be 0.0036 µg/mL. The limit of quantitation (LOQ) was calculated from the linearity curve using the formula.

$$\text{LOQ} = 10 \times \text{SD}/\text{Slope}^{[25]}$$

The LOQ for TAM was confirmed to be 0.0134 µg/mL and TOL was confirmed to be 0.011 µg/mL.

Linearity Study

The peak areas of TAM and TOL were linear with respect to the concentrations over the range of 0.04-0.8 µg/mL and 0.4-8.0 µg/mL. The slope and intercept values for the calibration curves for TAM and TOL are given in Table 3. The results showed that an excellent correlation exists between peak area and concentration of the drugs within the concentration range indicated previously. The data were analyzed by "Linear Regression Least Squares Fit".

Accuracy

Accuracy was determined by spiking the solution containing 0.4 µg/mL of TAM and 4.0 µg/mL of TOL with three different concentrations of the drug i.e. 80%, 100% and 120%. In effect, this mean that, the three mixed solutions calculated were 0.32, 0.4 and 0.48 µg/mL of TAM and 3.2, 4.0 and 4.8 µg/mL of TOL respectively. These solutions were then diluted with the mobile phase in tenfold stages so as to yield solutions; having concentrations in the calibrations range. Results are given in Table 3.

Precision

Precision was calculated in terms of method precision and intermediate precision [26]. In method precision SST solutions are injected to check precision while in intermediate precision these SST solutions are injected on two different days and precision is calculated. This precision is expressed in terms of RSD i.e. relative standard deviation. The % recovery was well within 85 to 115 % as per guideline, indicating that the developed method can accurately quantify TAM and TOL simultaneously in pharmaceutical formulation.

Ruggedness and Robustness

Ruggedness of the method was determined by carrying out the experiment on different instruments JASCO - 1500 series by different operators using the same Chromatographic conditions.

Robustness of the method was determined by subjecting the method to slight changes in the chromatographic conditions. No significant changes in the chromatograms were observed, proving that the developed method is rugged and robust. The obtained robustness results are presented in Table 4.

Table 3: Summary of Validation

S. No.	Validation Parameter		TAM	TOL
1	System Suitability Parameter (n=6)	Retention Time	4.189	5.876
		Area	18.3	150.2
		Height	1.6	9.7
		Symmetry Factor	0.808	0.804
		RSD	1.62	0.94
		NTP	7596	8332
		Resolution	7.5	
2	Sensitivity	LOD (µg/mL)	0.0044	0.0030
		LOQ (µg/mL)	0.013	0.011
3	Linearity (µg/mL)	Range	0.04 - 0.8	0.4 - 8.0
		Slope (m)	34.63	37.28
		Intercept (b)	-2.01	-2.36
		Coefficient Correlation(r)	0.99	0.99
4	Accuracy (n=3)	80 %	104.40	97.15
		100 %	103.74	95.04
		120 %	102.52	92.79
5	Precision	Method Precision	1.48	1.09
		Intermediate Precision	1.48	1.09

Table 4: Robustness Study

Changing Parameter	Actual Parameter	Modification	Tamsulosin HCl (n=3)	Tolterodine Tartrate (n=3)
MP	40 : 60	45 : 55	101.29	91.87
		35 : 65	105.41	91.83
pH	7.8	7.6	97.58	92.47
		8.0	88.15	89.62
Flow rate	1.0 mL/min	1.2	100.89	89.95
		0.8	96.29	89.62
Wavelength	220 nm	225	95.18	93.51
		215	92.76	92.00
Temperature	24.0°C	+5.0° C	90.80	94.25
		-5.0° C	94.01	100.82

CONCLUSION

The proposed method gave a good resolution between TAM and TOL within a short analysis time (< 8.0 min). Solution stability studies showed that the active pharmaceutical ingredients remained stable

for 48 h at room temperature. The changes in flow rate, pH of mobile phase, composition of mobile phase, and temperature of column did not affect the percentage assay of the drug, confirming the robustness of the method. Ruggedness of the method was confirmed as no significant changes were observed on analysis using different

instruments. High percentage recovery of drug indicated that the method is free from interference of excipients present in the formulation. Thus the proposed RP-HPLC method for the simultaneous estimation of TAM and TOL in combined dosage forms is accurate, precise, linear, rugged, robust, simple and rapid. Hence the present RP-HPLC method is suitable for the quality control of the raw materials, formulations and dissolution studies.

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