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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF TAMSULOSIN AND TOLTERODINE IN CAPSULE DOSAGE FORM BY RP-HPLC

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ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the simultaneous determination Tamsulosin hydrochloride and Tolterodine tartrate in capsule dosage form. The column used was Inertsil ODS C18 column, 150mm x 4.6 mm, 5µm in isocratic mode, with mobile phase containing phosphate buffer and acetonitrile (50:50 v/v) adjusted to pH 3 with dilute ortho phosphoric acid solution. The flow rate was 1.0 ml/ min and effluents were monitored at 221 nm by PDA detector. The retention times of Tamsulosin and Tolterodine were found to be 2.537 min and 4.660 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 98.5 to 100.25% and 99.95 to 100% Tamsulosin hydrochloride and Tolterodine tartrate respectively. The proposed method was validated and successfully applied to the estimation of Tamsulosin hydrochloride and Tolterodine tartrate in combined capsule dosage forms.

KEYWORDS: Tamsulosin, Tolterodine, 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.

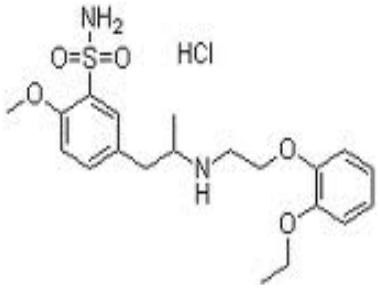

Fig. 1: Chemical Structure of Tamsulosin HCl.

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.

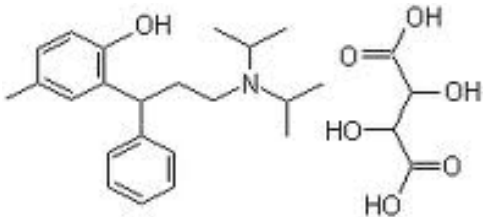

Fig. 2: Chemical Structure of Tolterodine Tartrate.

Different analytical methods have been reported in the literature for the assay of Tamsulosin[1-7] and Tolterodine[8-12] in pharmaceuticals and include spectrophotometry, TLC, HPLC, HPTLC, LC-MS. Literature survey reveals that few spectrophotometric and HPLC methods have been reported for their individual analysis, along with other combinations[13-16] in pharmaceutical formulations.

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. Later the method developed was been compared with the reported methods.

EXPERIMENTAL

Reagents

Reference standard of Tamsulosin and Tolterodine were gifted by Bioleo laboratories Kukatpally. ROLIFLO OD was procured from the local pharmacy. Acetonitrile, water (HPLC grade, Merck). Potassium di hydrogen orthophosphate and di potassium hydrogen orthophosphate used were of E. Merck (India) Ltd.

Instrumentation

Waters HPLC 2 2695 series consisting four pumps, Auto sampler with five racks, each has 24 vials holding capacity to inject 5µl to 500µl. Thermostat column compartment connected having capacity to maintain 5˚ to 60˚ column temperature was utilized for study. 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 phosphate buffer(pH 3) and acetonitrile (50:50 v/v). The mobile phase was filtered through a 0.45-µm membrane filter prior to use. A Inertsil ODS C18 column (150 x 4.6mm x 5 µm) was used for determination. The flow rate was 1.0 ml/min and the column was operated at ambient temperature (~25˚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 detection wavelength was set at 221nm.

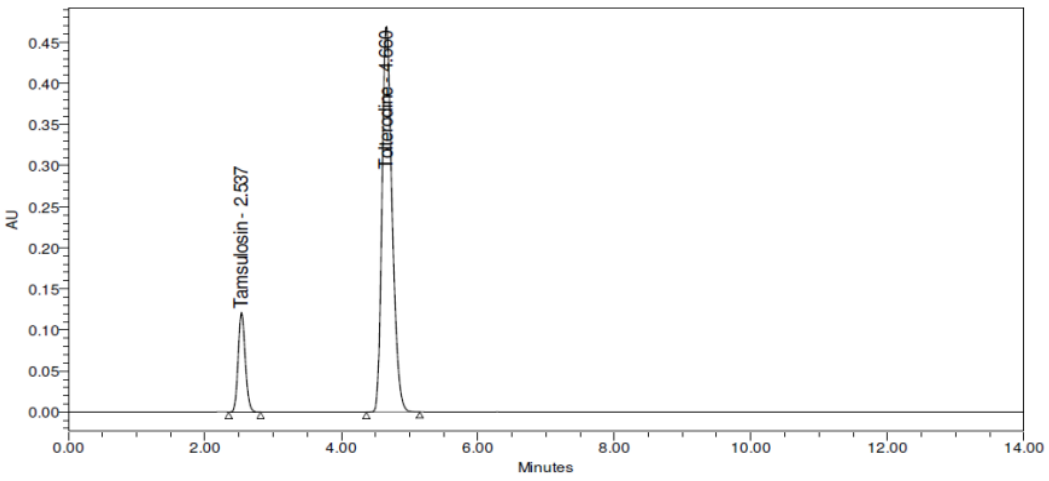

Fig. 3: Optimized chromatogram of Tamsulosin and Tolterodine.

Standard Preparation

Accurately weighed and transferred 2mg of Tamsulosin and 20mg of Tolterodine working Standards into a 10 ml clean dry volumetric flask, added 7ml of diluent, sonicated for 10 minutes and made up to the final volume with diluent. From the above stock solution, 1ml was pipetted out in to a 10ml volumetric flask and then made up to the final volume with diluent.

Diluent: Phosphate buffer and Acetonitrile (50:50 v/v).

Sample Preparation

Twenty capsules were weighed and average weight was calculated. The capsule contents were powdered and a quantity of powder 465.3mg (equivalent to 2mg Tamsulosin hydrochloride and 20mg Tolterodine tartrate) was transferred into 50mL volumetric flask, and diluent added was added about 25ml. The solution was sonicated for 10 minutes and diluted to volume with diluent. Further solution was filtered through filter paper.

From the above solution 1mL was transferred into 10mL volumetric flask and diluted to volume with diluent.

RESULTS AND DISCUSSION

The developed method was validated as per ICH guidelines for its accuracy, linearity, precision, specificity, robustness and 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. The data was given in **table 1**.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradation products, matrix components, etc. The data was given in **table 2**.

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. 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 3**.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and value found. Accuracy was performed in triplicate for various concentrations of Tamsulosin and Tolterodine equivalent to 80%, 100% and 120% 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 4**.

Precision

A) Method Precision

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) System Precision

Standard solution was prepared and injected same solution six times into the HPLC system as per test procedure. The data was given in **table 5**.

Limit of detection

Limit of detection is the lowest concentration of the substance that can be detected, not necessarily quantified by the method. The minimum concentration at which the analyte can be detected is determined from the linearity curve by applying the following formula.

Limit of detection (LOD) = $\sigma / S \times 3.3$

Where S – slope of the calibration curve

σ – Residual standard deviation

Limit of Quantification

It is defined as lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy and reliability by a given method under stated experimental conditions. LOQ is expressed as a concentration at a specified signal to noise ratio. It can be determined from linearity curve by applying the following formula.

Limit of Quantification (LOQ) = σ / *S*×10

Where S – slope of the calibration curve

σ – Residual standard deviation

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 0.7µg/ml and 1.2µg/ml respectively. The LOD and LOQ of Tolterodine were found to be 2.1µg/ml and 3.9µg/ml respectively.

Robustness

Robustness was done by small deliberate changes in the chromatographic conditions and retention time of Tamsulosin and Tolterodine were noted. The factors selected were flow rate and variation in the column temperature. The results remained unaffected by small variations in these parameters as shown in **table 6**.

Table 1: Results for system suitability parameters for the drugs Tamsulosin and Tolterodine.

S.NO	Peak Name	R_t	Area	Height	% Area	USP Resolution	USP Tailing	USP Plate Count
1	Tamsulosin	2.537	873425	121322	15.266		1.14	2814.32
2	Tolterodine	4.660	4848044	470449	84.734	8.96	1.29	4596.48

Table 2: Specificity data of Tamsulosin and Tolterodine.

S.NO	Name	Tamsulosin		Tolterodine	
		Rt	Area	Rt	Area
1	Blank	-	-	-	-
2	Tamsulosin and Tolterodine standard	2.538	874427	4.662	4993174
3	Placebo	-	-	-	-
4	Sample	2.537	878271	4.666	4978241

Table 3: Linearity data of Tamsulosin and Tolterodine.

S.No	Tamsulosin			Tolterodine		
	Conc.(µg/ml)	Rt(mins)	Area	Conc.(µg/ml)	Rt(mins)	Area
1	1	2.528	241659	10	4.666	1338276
2	2	2.528	424675	20	4.652	2356096
3	3	2.527	641305	30	4.645	3576201
4	4	2.528	811194	40	4.646	4506710
5	5	2. 528	1016933	50	4.638	5664898
6	6	2. 533	1238844	60	4.640	6812492
$r^2 = 0.998$				$r^2 = 0.999$		

Table 4: Accuracy data of Tamsulosin and Tolterodine.

S.No	Spiked level	Tamsulosin			Tolterodine		
		Amount added (µg/ml)	Amount present (µg/ml)	Average %Recovery	Amount added (µg/ml)	Amount present (µg/ml)	Average %Recovery
1	80%	0.32	0.3154	98.59	3.2	3.1828	99.46
2	100%	0.4	0.4	100	4	3.9978	99.95
3	120%	0.48	0.481	100.25	4.8	4.8	100

Table 5: System Precision data of Tamsulosin and Tolterodine.

S.No	Tamsulosin		Tolterodine	
	RT	Area	RT	Area
1	2.533	828174	4.654	4590330
2	2.534	834710	4.657	4633377
3	2.535	835370	4.657	4695188
4	2.537	839258	4.652	4694235
5	2.536	835962	4.656	4689325
6	2.539	839965	4.655	4682589
Average	2.536	835573	4.655	4664174
Std Dev	0.0022	4208.36	0.0019	42985.13
%RSD	**0.085**	**0.504**	**0.042**	**0.922**

Table 6: Method Precision data of Tamsulosin and Tolterodine.

S.NO	Tamsulosin		Tolterodine	
	R_t	Area	R_t	Area
1	2.537	873925	4.66	4998044
2	2.537	876263	4.658	4983145
3	2.538	879265	4.661	4972563
4	2.536	878523	4.678	4945623
5	2.539	879236	4.662	4982362
6	2.535	885145	4.677	4973015
Average	2.537	878726	4.666	4975792
Std Dev	0.0014	3765.94	0.0090	17439.55
%RSD	0.056	0.429	0.193	0.350

Table 7: Robustness data relating to change in flow rate (1.0ml/min).

S.No		Tamsulosin			Tolterodine		
	Flow rate (ml/min)	Average Peak Area	Std.dev	%RSD	Average Peak Area	Std.dev	%RSD
1	0.9ml/min	795688	50.05	0.006	4420342	104.42	0.002
2	1ml/min	835573	4208.36	0.504	4664174	42985.13	0.922
3	1.1ml/min	1040516	18.08	0.002	5784964	393.96	0.007

Table 8: Robustness data relating to change in column temperature.

		Tamsulosin			Tolterodine		
S.No	**Column temperature**	**Average Peak Area**	**Std.dev**	**%RSD**	**Average Peak Area**	**Std.dev**	**%RSD**
1	25˚C	927088	7.58	0.001	5133350	34.40	0.001
3	30˚C	931154	51.37	0.006	5177047	449.59	0.009

Table 9: System suitability parameters.

S.no	Characteristics	Acceptance criteria	Tamsulosin	Tolterodine
1	Linearity	r2> 0.999	0.998	0.999
2	Limit of Detection	S/N > 3	0.7	1.2
3	Limit of Quantitation	S/N> 10	2.1	3.9
4	Accuracy	98-102%	98.5-100.2%	99.46- 100%
5	Precision	%RSD< 2	0.52	0.66
6	Robustness Flow rate: (0.9ml/min & 1.1ml/min)	%RSD< 2	0.006 0.002	0.002 0.007
7	Colum temperature 25˚C & 30˚C	%RSD< 2	0.001 0.006	0.001 0.009

Table 10: Comparison chart of available methods and proposed method.

A)

Cromatographic Conditions	Method Developed	REF-15(2012)	REF-9(2013)	REF-13 (2009)
HPLC SYSTEM	WATERS E 2695	Agilent 1100 series	WATERS E 2695	ALachrome HPLC
Detector	PDA	UV	PDA	UV
column	INERTSIL ODS C 18 (150x4.6mmx 5µm)	INERTSIL ODS C 18 (250x5.4mmx 5µm)	C 8 (250x4.6mmx 5µm)	Kromosil c18(250x4.6mmx 5µm)
Temperature	25◦C	Room Temp.	30◦C	
Elution	Isocratic	-	Isocratic	Isocratic
Mobile Phase	Phosphate buffer :ACN(50:50v/v)	Ammonium Acetate buffer :ACN(40:60v/v)	Phosphate buffer :MEOH(50:50v/v)	Phosphate buffer :ACN(57:43v/v)
Flow Rate	1ml/min	1ml/min	1ml/min	1.5ml/min
Injection volume	20µl		20µl	20µl
Detection Wavelength	221nm	220nm	247nm	211nm
sample	RolifloOD	RolifloOD, Tamlet 4, Bapter	RolifloOD	

B)

	Method Developed		REF -15(2012)		REF-9(2013)		REF-13(2009)	
System suitability parameters	TAM	TOL	TAM	TOL	TAM	TOL	TAM	TOL
RT	2.537	4.660	4.189	5.876	2.753	4.678	2.36	4.12
Sensitivity								
LOD(µg/ml)	0.7	2.1	0.0044	0.0030	-	-	-	-
LOQ µg/ml)	1.2	3.9	0.013	0.011	-	-	-	-
Linearity(µg/ml)	1-6	10-60	0.04-0.8	0.4-8	0.02-0.06	0.2-0.6	2-10	10-50
Accuracy(n=3)								
80%	98.59	99.46	104.40	97.15			98.12	98.66
100%	100.00	99.95	103.74	95.04	100	100	98.16	100.36
120%	100.25	100	102.52	92.79			100.36	100.95
Precision	0.42	0.35	1.48	1.09	0.18	0.21	0.29	0.62

CONCLUSION

It was concluded that the proposed new RP-HPLC method developed for the quantitative determination of Tamsulosin and Tolterodine in capsule dosage form was simple, selective, sensitive, accurate, precise and rapid. The method was proved to be equally good when compared to most of the reported methods. The mobile phases were simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence the method can be easily adopted as an alternative method to report routine determination of Tamsulosin and Tolterodine.

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