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Research Article

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Simultaneous estimation of Tolterodine tartrate and Tamsulosin HCl by validated HPTLC assay method from combination capsule form

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ABSTRACT

A highly sensitive, simple and selective HPTLC method has been established for the assay determination as well In-process quality control of the Tolterodine and Tamsulosin in bulk as well as in capsule formulation. Separation of Tolterodine tartrate and Tamsulosin HCl was carried out on silica gel 60F254 backed on aluminum foil, 20 × 10 cm thin layer chromatography (TLC) plates with 6 mm band and 3 µl injection volume. Ascending chromatography TLC plate developed using mobile phase containing methanol: ethyl acetate: triethylamine in the ratio (5:5:0.3 v/v/v) with 30 min prior chamber saturation. The regression analysis was carried out of seven point calibration curve using 480-1920 ng/band and 48-192 ng/band concentration for Tolterodine tartrate and Tamsulosin HCl, respectively. The correlation coefficient found 0.999 for both the drugs. The limit of detection values of the compounds were 13.26 ng/band and 22.44 ng/band as well as limit of quantification values were found 44.34 ng/band and 74.85 ng/band for Tamsulosin HCl and Tolterodine tartrate. Densitometric signal was monitored at wavelength of 220 nm. Method was validated for solution stability, specificity, accuracy, precision, limit of detection and quantification, linearity, and robustness were statistical evaluation of the method proves that, method is highly robust accurate and precise for the intended use.

Keywords: High Performance Thin Layer Chromatography, Assay method, Combination capsule, Tolterodine, Tamsulosin.

INTRODUCTION

Management of lower urinary tract symptoms (LUTS) and benign prostatic hyperplasia (BPH) has been vital to urology for decades. In benign prostatic hyperplasia (BPH) there will be a sudden impact on overall quality of life of patient. [1-3]The quantity of prescriptions for α-blockers has been increasing gradually in the last 10 years. [4]

Tamsulosin hydrochloride, chemically (-)-(R)-5-[2-[[2-(o-ethoxyphenoxy) ethyl] amino] propyl]-2-methoxybenzene sulfonamide hydrochloride (fig. 1), was first developed by Yamanouchi Pharmaceuticals. Tamsulosin is a chiral molecule, currently marketed as a single enantiomer and its (R) enantiomer is used as a curative active substance.[5-6].

Tolterodine is chemically known as, - [(1S)-3-[bis(propan-2-yl)amino]-1-phenylpropyl]-4-methylphenol; (R)-N,N-diisopropyl-3-(2-hydroxy-5-methylphenyl)-3-phenylpropanamine L-hydrogen (fig. 2). Tolterodine comes under the pharmacological class of competitive muscarinic receptor antagonist; both urinary bladder tightening and salivation are mediated via cholinergic muscarinic receptors. [7-9].

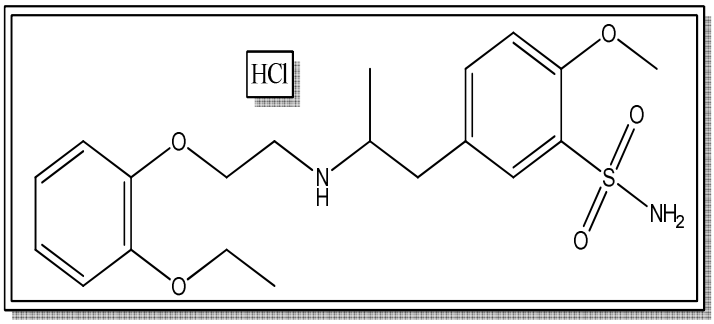

Figure 1: Chemical structure of Tamsulosin HCl

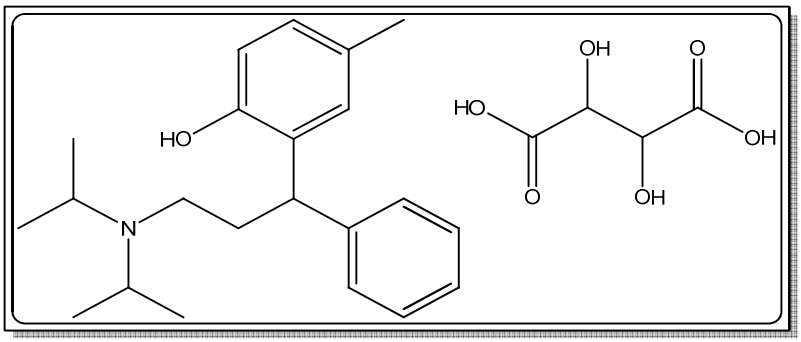

Figure 2: Chemical structure of Tolterodine tartrate

Various publications are available regarding determination method of Tolterodine tartrate and Tamsulosin HCl, but most of the methods are applicable for the analysis of Tolterodine tartrate and Tamsulosin HCl either alone or in combination with other drugs in pharmaceutical dosage form or in biological fluids. [10, 11] only few methods are reported for the simultaneous spectrometric estimation and HPLC method of Tolterodine tartrate and Tamsulosin HClin capsule dosage form. [12-14] According to our present knowledge, no validated HPTLC assay method for the simultaneous quantification of Tolterodine tartrate and Tamsulosin HCl in combine dosage forms has been published. Hence, the method is recommended for routine quality control analysis.

Consequently, the focus in the present study was to develop a simple, sensitive and low-cost, validated HPTLC method for the combination from its dosage form as per ICH [15].

EXPERIMENTAL SECTION

Chemicals and Reagents

Active pharmaceutical ingredient (API) working standards of Tolterodine Tartrate and Tamsulosin HCl were gifted by Symed Labs Limited, Hyderabad, India. A tablet containing 4 mg Tolterodine Tartrate and 0.4 mg Tamsulosin HCl (SYMED LABS LIMITED, Hyderabad, India) was purchased commercially.

HPLC grade methanol, ethyl acetate and triethylamine (TEA) were procured from Merck India Limited, Mumbai, India. High purity deionised water was prepared from a Millo-Q (Millipore, Milford, MA, USA) water purification system. Nylon syringe filters 0.22 µm were from Millex-Hn (Mumbai, India). TLC plates used were obtained from Merck India Limited, Mumbai, India.

Instrumentation

CAMAG HPTLC system used for quantitative analysis consisted of 100 µl sample syringe (Hamilton, Switzerland), CAMAG Linomat V applicator (Camag, Switzerland),CAMAG glass twin-trough chambers (20 × 10 × 4 cm^3); TLC plate visualizing chamber and CAMAG TLC scanner III. Chromatographic data acquisition and instrument control were doneusing WinCATS software (V 1.4.2, Camag). The source of radiation was a deuterium lamp emitting a continuous UV spectrum in the range 190–400 nm.

Standard preparation

Standard stock solution containing Tolterodine tartrate (4000 µg/ml) and Tamsulosin HCl (400 µg/ml) was prepared by transferring 100 mg Tolterodine tartrate and 10 mg Tamsulosin HCl working standard into a 25 ml volumetric

flask. A 5 ml of diluents (methanol) was added, sonicated and cooled to room temperature. The solution was diluted to the mark with diluents. Standard solution containing Tolterodine tartrate (400 ng/µl) and Tamsulosin HCl (40 ng/µl) was prepared by pipeting 1 ml stock solution into a 10 ml volumetric flask and diluted up to the mark with diluents.

Test preparation

Fifty capsules opened and granules of each were weighed; the average weight (96.0 mg) was calculated. The granules were mixed and crushed with a glass mortar and pestle for 5 min. Each capsule contains 4 mg Tolterodine tartrate and 0.4 mg Tamsulosin HCl. A portion of granule powder equivalent to the weight of 25 capsules was accurately weighed (2.4 gm) and transferred to a 25 ml volumetric flask. Approximately 5 ml diluent was poured and the mixture was sonicated for 10 min with intermittent shaking. The solution was restored to room temperature and make up to volume with diluents to prepare stock sample solution. The stock sample solution was filtered through 0.45 µm membrane filters and 1 ml of the filtered solution was transferred to a 10 ml volumetric flask and diluted up to 10 ml with diluents to furnish test solution containing 400 µg/ml Tolterodine tartrate and 40 µg/ml Tamsulosin HCl.

RESULTS AND DISCUSSION

Development and Optimization of the HPTLC Method

In the presence work, an analytical method based on TLC using densitometric detection was developed and validated for assay determination of Tolterodine tartrate and Tamsulosin HCl in capsule formulation. The analytical conditions were selected, keeping in mind the different chemical nature of Tolterodine tartrate and Tamsulosin HCl. The chemical and physical parameters e.g. solubility, pH, polar or nonpolar nature of the compound and λ max value. According to the literature review, the λ max values of Tamsolusin and Tolterodine were reported 220-225 nm and 280-285 nm, respectively. In such a case, if TLC plates were scanned at different λ max values of the multi-component analysis the purpose of high throughput analysis is lost. Hence, for the multi-component analysis of the drugs, most suitable wavelength was chosen; considering the ratio of both the drugs and the maximum response were observed at 220 nm for both the drugs.

The mobile phase selection has been done on the basis of resolution, Symmetry of peak, spot definition sensitivity and day-to-day reproducibility of the retardation factor and resolution between Tolterodine tartrate and Tamsulosin HCl peak. After evaluating all these factors, mid polar and polar solvent mixture was found to be giving satisfactory results. Initial trial was taken using methanol and chloroform as a mobile phase but we didn't get good peak shape as well as resolution of both the drug (Trial 1, fig. 4).

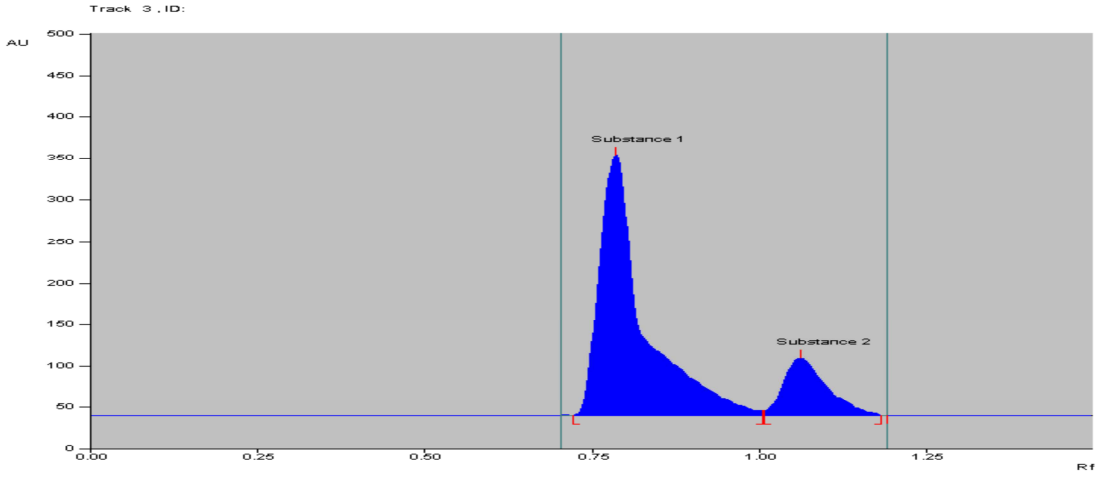

Figure 4: Chromatogram of method development (Trial 1)

When chloroform was replaced by ethyl acetate the peak shape and little separation of both the drugs were improved**.** (Trial 2, fig. 5)

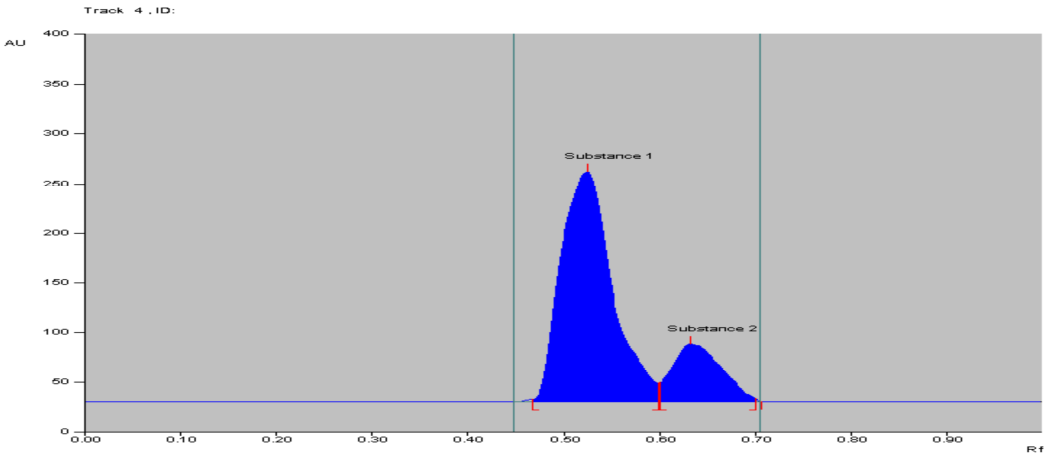

Figure 5: Chromatogram of method development (Trial 2)

The selection of basic modifier based on chemical structure of both the drugs to reduce the tailing of polar compounds. (Trial 3, fig. 6)

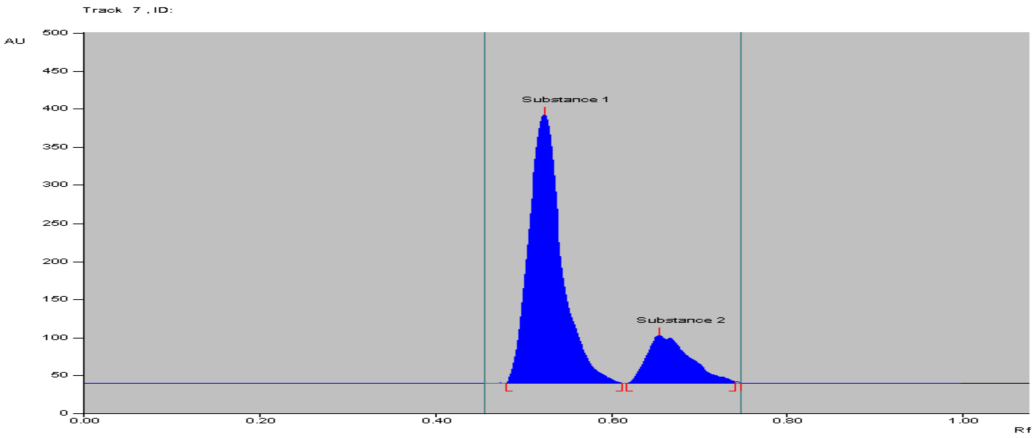

Figure 6: Chromatogram of method development (Trial 3)

After so many trial of method optimization, TEA was found suitable for faster development, resolution and peak shape of both components. Finally, the mobile phase composition consisted of a mixture of methanol: ethyl acetate: TEA (5.0: 5.0:0.3, v/v/v). Optimized mobile phase proportion was providing good resolution between Tolterodine tartrate and Tamsulosin HCl. For the selection of polar organic constituent of mobile phase, methanol was chosen to increase the retardation factor and to attain good peak shape with resolution. Figure 7 and 8 represent the chromatograms of standard and test preparation, respectively. Individual chromatographs of Tolterodine and Tamsulosin reference standards are also given in Figure9 and 10. The final optimized chromatographic conditions are given in the Table 1.

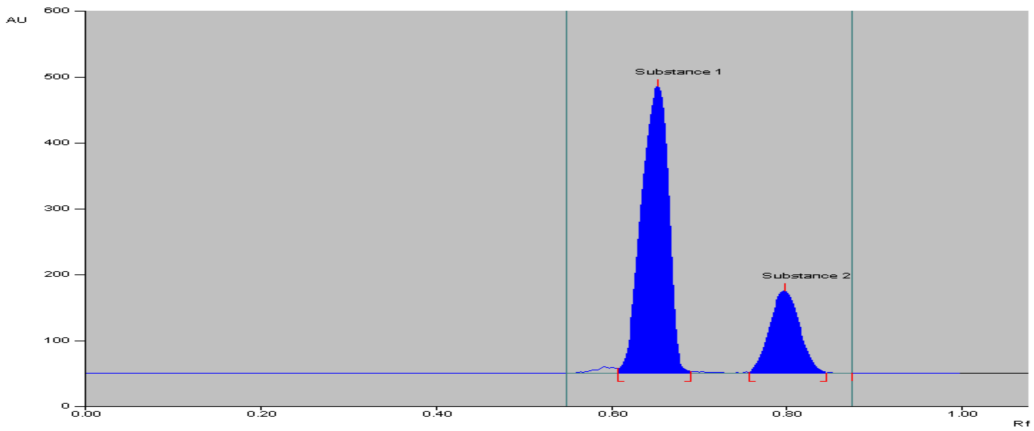

Figure 7: Chromatogram of Tolterodine tartrate and Tamsulosin HCl standard preparation

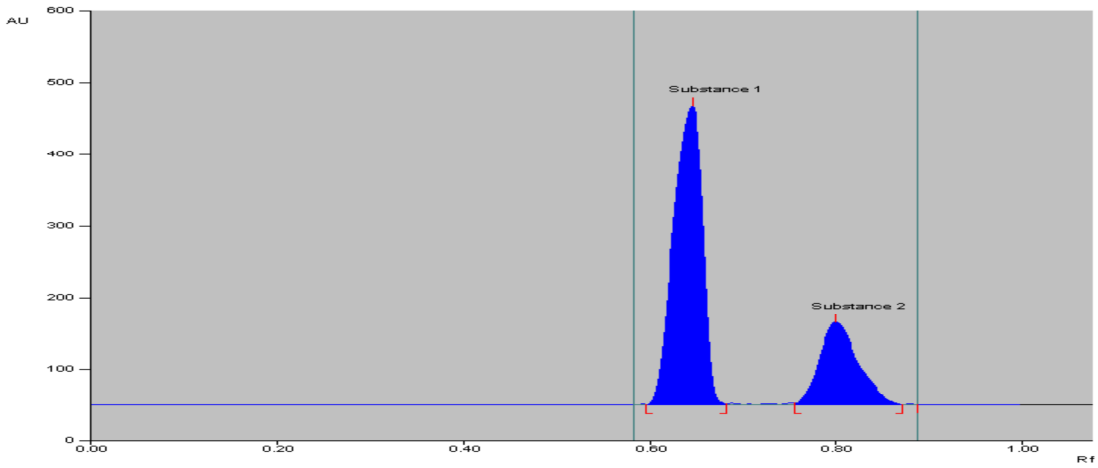

Figure 8: Chromatogram of Tolterodine tartrate and Tamsulosin HCl capsule

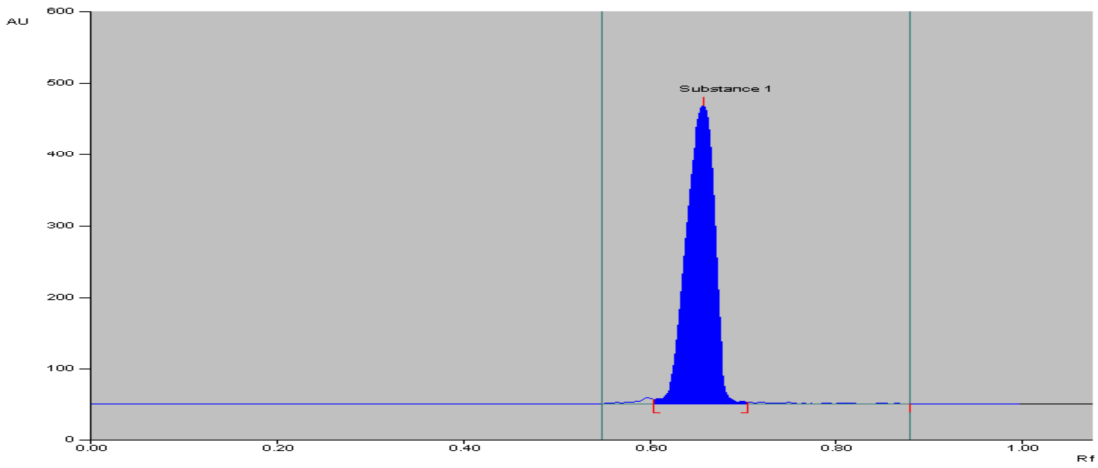

Figure 9: Chromatogram of Tolterodine tartrate standard preparation

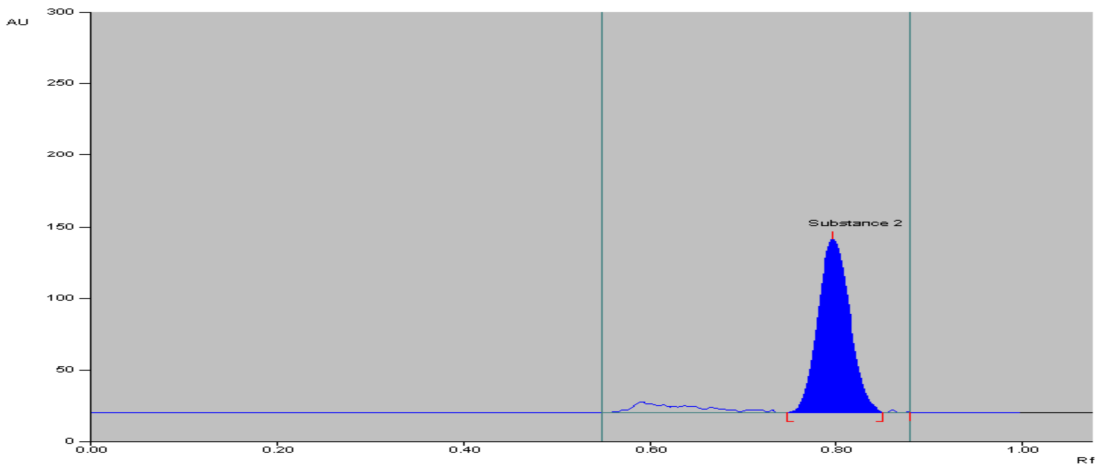

Figure 10: Chromatogram of Tamsulosin HCl standard preparation

Table 1: Optimized chromatographic conditions for HPTLC

Parameters	Chromatographic Conditions
Stationary phase	Silica gel GF254 pre-coated (aluminum sheet)
Diluents	Methanol
Sample Concentration (ng/band)	1200 (ng/band)TOL and 120 (ng/band)TAM
Sample applicator	CAMAG Linomate V
Sample application speed	150 nl/sec
Volume	3µl
Band	6mm
Space	9 mm
Development chamber	CAMAG Twin Trough Chamber
Mobile phase	Ethyl acetate: Methanol (5:5:0.3 v/v/v)
Chamber saturation	30 minutes
Sample preparation	Methanol as a diluent
Development distance	85 mm
Drying of plate	Room Temperature
Visualization	CAMAG UV Visualizing Chamber
Densitometric scanner	CAMAG TLC Scanner III
Scanning speed	20mm/sec
Detector	Deuterium Lamp
Wavelength	220 nm
Retardation Factor	0.65 TOL and 0.80 TAM

Solution Stability

Stability of solution was evaluated for standard solution and the test preparation. The solution were stored at ambient temperature without protection of light and tested after 12, 24, 36 and 48 hr. The response for the aged solution was evaluated by comparison with freshly prepared solution. According to results obtain from the study; solutions were stable up to 36 h after; results are given in the table 2.

Table 2: Summary of solution stability study of TOL and TAM capsule

Summary of solution stability study of Tolterodine tartrate and Tamsulosin HCl						
	Tolterodine tartrate			Tamsulosin HCl		
Duration	Mean Standard area	Mean area of test	%Assay	Mean Standard area	Mean area of test	%Assay
Initial	9897.88	9865.45	99.65	3189.99	3180.03	101.71
12 h	9851.23	9840.10	99.59	3170.76	3162.34	99.46
24 h	9866.68	9810.24	99.27	3180.59	3141.22	99.48
36 h	9901.09	9704.20	98.02	3186.88	3109.68	98.77
48 h	9899.96	9700.01	97.93	3145.52	3098.82	97.95

Specificity

Specificity is the measurement of the degree of interference from excipients that may be expected to be present in the sample matrix or pharmaceutical dosage form. Typically these might include placebo, impurities, diluents and other active ingredients. The peak purity of bands for Tolterodine tartrate and Tamsulosin HCl in the sample was confirmed by comparing the R_f values and spectra of the bands with standards. (Figure 11) It was assessed by comparing spectra at different levels, e. g. peak- start (S), peak- max (M) and peak-end (E) position of spots. There was no interference of any peak from these extraneous materials.

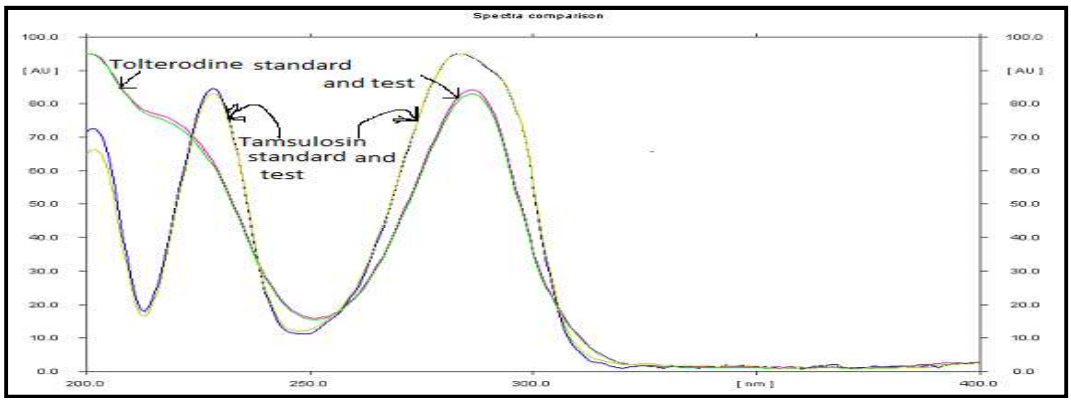

Figure 11: Overlay UV spectra of TOL and TAM, standard and sample

Linearity

In a chromatographic method, linearity study was carried out preparing seven point calibration curve of concentration range from 160-640 µg/ml (480-1920 ng/band) for Tolterodine tartrate and 16-64 µg/ml (48-192 ng/band) for Tamsulosin HCl. The linearity assessed by test results which are directly proportional to the concentration of analystes which are present in the sample. (Table 3) The linear regression equation for Tolterodine tartrate was y = 1973.0x+2063.0 with correlation co-efficient 0.999 and for Tamsulosin HCl was y = 580.2x+752.6 with correlation co-efficient 0.999. Where x is the concentration in µg/ml and y is the peak area in absorbance unit. The overlay chromatogram of all the levels of linearity is given in the figure 14.

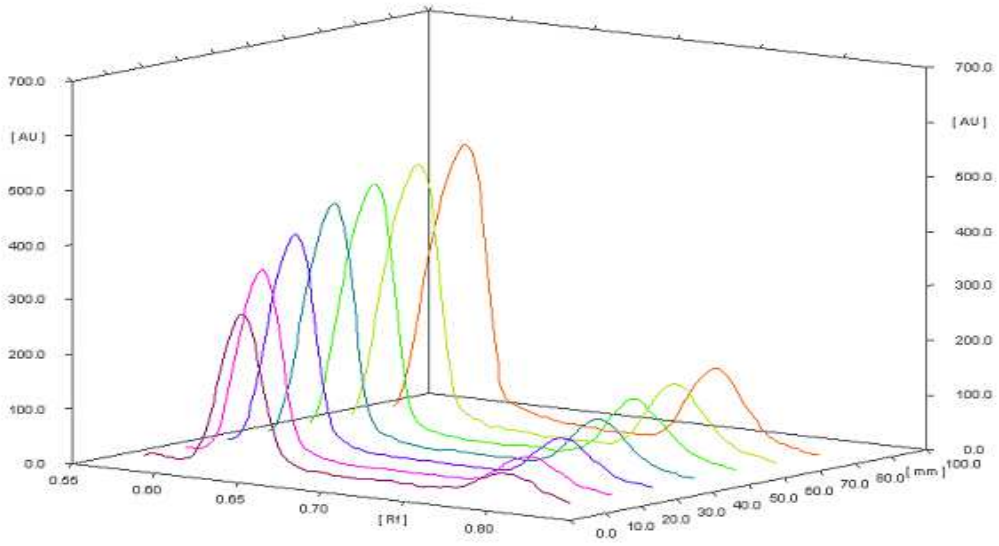

Figure 14: Overlay chromatogram of seven linearity levels

Table 3: Summary of concentration and evaluation of linearity curve

Linearity		Tolterodine tartrate			Tamsulosin HCl		
Level	**% of Level**	**Con. (µg/ml)**	**Con. (ng/band)**	**Mean Area**	**Con. (µg/ml)**	**Con. (ng/band)**	**Mean Area**
1	40	160	480	4122.94	16	48	1320.25
2	60	240	720	5988.08	24	72	1909.64
3	80	320	960	8049.55	32	96	2505.45
4	100	400	1200	9816.53	40	120	3098.38
5	120	480	1440	11779.8	48	144	3651.57
6	140	560	1680	13976.6	56	168	4211.60
7	160	640	1920	15976.6	64	192	4819.37
Correlation Co-efficient			0.999			0.999	
Slope			1973			580.2	
Intercept			2063			752.6	

LOD and LOQ

The limit of detection and limit of quantification were calculated using 3σ/S and 10σ/S equation for the developed method for LOD and LOQ, respectively. Where σ is the standard deviation of the y-intercept and S is the slope of the calibration curve. For the LOD and LOQ study, concentrations were selected in the range of 480-1920 ng/band and 48-192 ng/band for Tolterodine tartrate and Tamsulosin HCl, respectively. The LOD value for Tolerodine tartrate and Tamsulosin HCl were found to be 22.44 ng/band and 13.26 ng/band, respectively and the LOQ value 74.85 ng/band and 44.34 ng/band, respectively. The result shows the sensitivity of the methods.

Accuracy

The accuracy study of an analytical procedure states the degree of closeness of agreement between the conventional true value or accepted reference value and the practically found value. Moreover, for the combination drug product; percentage recovery of both the ingredients was determined at three different concentration levels. The mean recovery for Tolterodine tartrate was 99.19-99.39 % and 98.83-99.44 % for Tamsulosin HCl. The satisfactory results indicating that the method was accurate and % recovery found within acceptance criteria.

Precision

The precision of the method, as repeatability was evaluated by performing six independent assays of the test sample

preparation and calculating the SD, % RSD and mean % assay. The intermediate (inter-day) precision of the method was assessed by performing same procedure on different days by other analyst under the same experimental conditions with different make chemicals. The results obtain from the repeatability study was consider for the intra-day precision study also. The %RSD values for intermediate and method precision study was < 2.0% for Tolterodine tartrate and Tamsulosin HCl.

Robustness

The robustness study was evaluated with respect to the minute but a deliberate alteration in the chromatographic conditions, the result of the study delivers the reliability of the analysis. The change in chromatographic parameters e.g. composition of mobile phase, volume of mobile phase, chamber saturation time, detection wavelength and solvents from different company. The effects of such deliberate changes on peak area and retardation factor were evaluated in terms of % RSD for each parameter.

System suitability study

Before each measurement of validation parameter a system suitability test was performed by measurement of very important characteristics such as % RSD of peak area, % RSD of retardation factor observed and calculated for standard solutions. The experimental results obtained and calculated are suitable for the chromatographic method with in-house limits. (Table 4)

Table 4: Summary of system suitability data of TOL and TAM

System Suitability Parameter In-house Limits	%RSD[a] of Area NMT[b] 2.0	%RSD[a] of Rf[c] NMT[b] 2.0	%RSD[a] of Area NMT[b] 2.0	%RSD[a] of Rf[c] NMT[b] 2.0
Validation Parameter	**Tolterodine tartrate**		**TamsulosinHCl**	
Solution Stability	0.23	0.83	0.54	0.64
Specificity	0.16	0.62	0.62	0.79
Linearity	0.39	1.24	0.73	0.51
Method Precision	0.07	0.78	0.26	0.68
Intermediate Precision	0.07	1.16	0.12	0.94
Accuracy	0.28	1.15	1.02	0.94
Robustness	1.14	0.67	1.35	0.53

[a] Relative standard deviation
[b]Not more than
[c]Retardation Factor

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