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Simultaneous Estimation of Finasteride and Tamsulosin Hydrochloride in Combined Dosage Forms by RP-HPLC-PDA Method

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ABSTRACT

The study describes development and subsequent validation of an RP-HPLC-PDA method for the simultaneous estimation of Tamsulosin (TAM) and Finasteride (FIN) in bulk and tablet dosage form. The chromatographic conditions comprised of a reversed-phase C₁₈ column (150 x 4.6 mm, 5 μ) with a mobile phase consisting of a mixture of methanol and formic acid (0.02% v/v in water) at a flow rate of 1 mL/min and ran in gradient mode. Detection was carried out at 230 nm. The retention times were 2.7 min and 10.08 min respectively for TAM and FIN. A good linear relationship in the concentration range 0.4-20 μg/mL with a correlation coefficient of 0.9981 for TAM and in the range of 5-50 μg/mL a correlation coefficient of 0.9987 was observed. Limit of detection (LOD) was found to be 0.16 μg/mL for TAM and 0.6 μg/mL for FIN. The method was validated for accuracy, precision, robustness and assay. The percent recovery values were in the ranges of 99.15-100.8 for TAM and 99.21-101.83 for FIN. The results of all the validation parameters were well within their acceptance values.

Keywords: Finasteride, Tamsulosin, HPLC, Gradient elution, Simultaneous estimation, excipients.

INTRODUCTION

FIN is a competitive inhibitor of the 5-alpha reductase, an enzyme that converts testosterone to dihydrotestosterone (DHT). Conversion of testosterone to DHT by 5-alpha reductase is essential for prostatic hyperplasia (Sweetman, 2005). Chemically, FIN is (5α,17β)-N-(1, 1-dimethylethyl)-3-oxo-4-aza-5azandrost-1-ene-17-carboxamide (Maryadele & Neil, 2001a). TAM is a selective alpha-1 adrenoceptor blocker, which blocks the adrenoceptors leading to the relaxation of smooth muscles in the bladder neck and prostate to relax, resulting in an improvement in urine flow rate and a reduction in symptoms of BPH (Tripathi, 2008). TAM chemically is (-)-(R)-5-[2-[[2-(o-ethoxy phenoxy) ethyl] amino] propyl] -2-methoxy benzene sulfonamide, monohydrochloride (Maryadele & Neil, 2001b). Combination of both drugs is indicated for the treatment of symptomatic benign prostatic hyperplasia in men with an enlarged prostate.

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Literature survey reveals that few spectrophotometric and chromatographic methods including UV-Visible determination (Agarwal, 2008; Ulu, 2007), ratio derivative (Vishnu, 2009), HPTLC (Rao, 2008; Sanjay, 2011) and HPLC methods including bio analytical have been reported for estimation of FIN and TAM either single or in combination (Basavaiah K, 2007, Manish KT et al., 2011, Patel DB 2010, Syed AA, 2001). An HPLC method developed by Patel DB (2010) even though used PDA detection, mobile phase composition comprises of methanol: ammonium acetate (0.02M) with 0.1% triethylamine (pH 9.2), which is not applicable with LC-MS detection and also there is a discrepancy with the LOD and LOQ values reported with the chromatogram shown. Hence, the present investigation was carried out in order to develop a reliable, economic and validated HPLC - PDA method with LC conditions suitable for MS detection for the simultaneous estimation of TAM and FIN in bulk and pharmaceutical dosage forms for routine and quality control tests.

EXPERIMENTAL

Materials and Methods

TAM and FIN were generous gifts from Dr. Reddy's Labs (Hyderabad, India). Tablets were purchased from local market, the labeled amount was 0.4mg TAM, and 5mg FIN each (FINAST-T, B.NO: D01210). Methanol, water and formic acid and other chemicals and reagents were of HPLC grade.

Apparatus

A Shimadzu Prominence HPLC system provided with DGU-20A3 degasser, LC-20AD binary pumps, SIL-20AHT auto sampler, and SPD-M20A PDA detector was used. Data acquisition was carried out using LC solutions software. The chromatographic analysis was performed on C₁₈ Phenomenex (150 × 4.6mm, 5μ, 100 Å) column.

Chromatographic conditions

Mobile phase is composed of 0.02% v/v formic acid in water (A) and methanol (B). Separation was carried out at gradient mode with 40% of B at 0 min and increased to 70% up to 3 min and increased linearly to 80% at 13 min and changed to 40% up to 15 min and maintained up to 25 min for equilibration. The flow rate was 1 mL/min and the injection volume was 20 μL. PDA detection was performed at 230 nm, because at this wavelength, sensitivity was higher than in other more characteristic wavelengths and it was necessary for the detection of minor responses.

Preparation of Standard Solutions

Stock solutions of TAM and FIN were prepared by dissolving 10 mg of the drugs in 5 mL of methanol in 10 mL of individual volumetric flasks. Drugs were solubilized by sonication and volume was made up to 10 mL to obtain a concentration of 1mg/mL. Standard solutions of TAM and FIN were prepared by

diluting the appropriate volumes of stock solution with 0.02% formic acid as diluent.

Preparation of sample solutions

In the present investigation a capsule containing a film coated tablet (FIN, 5 mg) and modified release pellets (TAM, 0.4 mg) was used for the assay procedure. The sample solutions were processed as follows:

Pellet form (TAM)

Pellets from ten capsules were weighed individually and finely powdered and the blend equivalent to 1 mg was transferred to a 10 mL volumetric flask (0.1mg/mL).

5 mL of methanol was added to the flask and the mixture was sonicated for 5 minutes and made up to the mark with the methanol. The mixture was centrifuged for 5 minutes at 4000 rpm; the supernatant was filtered through 0.22μm nylon syringe filter.

Tablet form (FIN)

Ten tablets from ten capsules were weighed individually and finely powdered. A powder blend equivalent to 10 mg of FIN was transferred to a 10 mL volumetric flask (1mg/mL). 5mL of methanol was added to the flask and the mixture was sonicated for 5 minutes and made up to the mark with the methanol. The mixture was centrifuged for 5 minutes at 4000 rpm; the supernatant was filtered through 0.22μm nylon syringe filter.

Appropriate volumes of TAM and FIN filtrates were diluted with diluent to get final concentrations of 1.6μg/mL of TAM and 20μg/mL of FIN for the analysis.

Method validation

This method described above had been validated as per the ICH guidelines (1996) and the results were summarized below.

Linearity

The linearity responses in the concentration range of 0.4-20μg/mL for TAM and 5-50μg/mL was determined and the data was given in Table-1.

Table. 1: Linearity data for TAM and FIN (n=3).

TAM		FIN	
Concentration (μg/mL)	Peak Area (± SD)	Concentration (μg/mL)	Peak Area (± SD)
0.4	9786.3 ± 50.8	5	36898.3 ± 31.75
0.8	18718.6 ± 235.8	10	82729 ± 38.105
2	36140.6 ± 712.8	20	236946.7 ± 26.55
6	203154.7 ± 5564.0	30	374789.7 ± 967.06
10	373415 ± 5027.4	40	486375.3 ± 5652.83
20	811877 ± 7311.5	50	654091.3 ± 1036.34
Regression equation	y = 41407x - 28343		y = 13676x - 41316
Correlation coefficient (R)	0.998		0.998
Regression coefficient (R²)	0.996		0.997

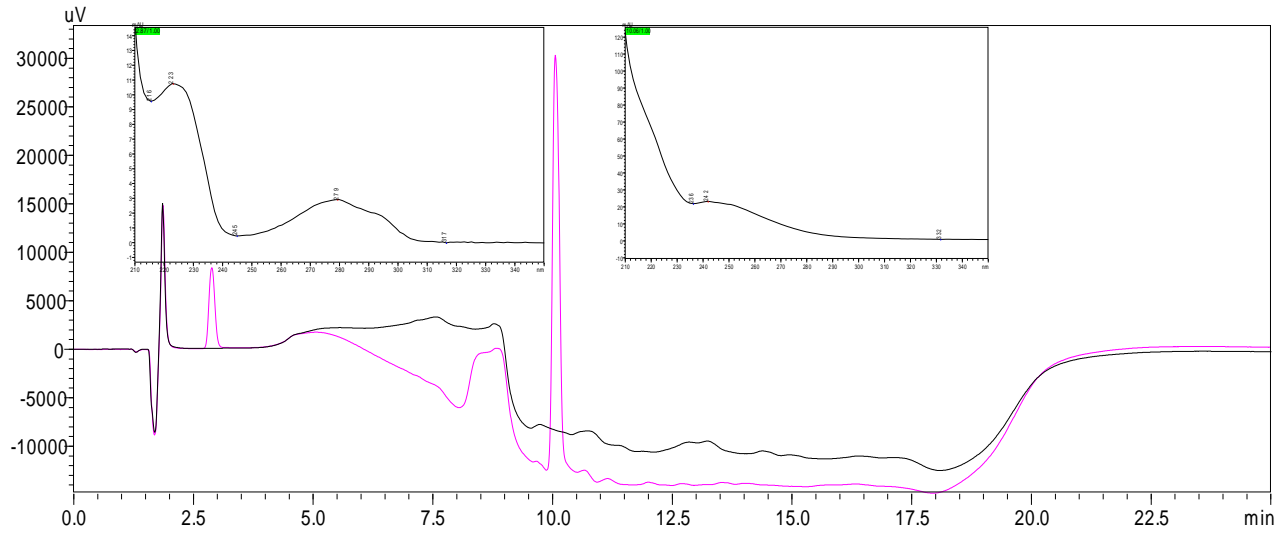


Fig. 1: Typical chromatograms of standard solution containing 0.8 µg/mL of TAM and 10 µg/mL of FIN and diluent with UV spectra.

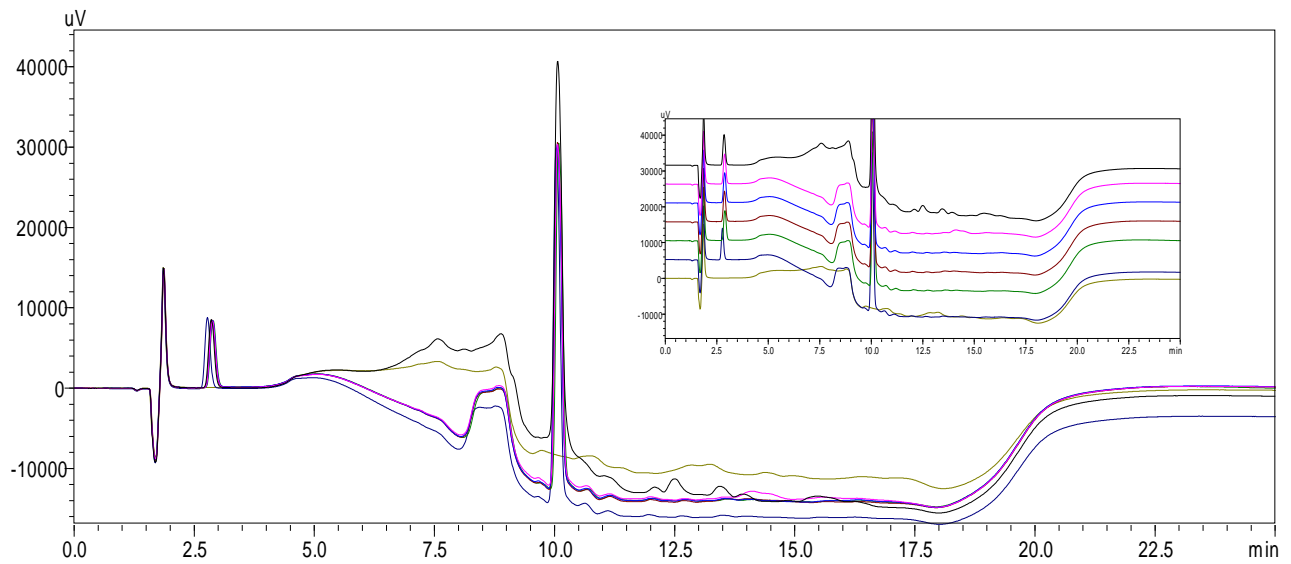


Fig. 2: Overlay of chromatograms of the TAM 2.4 µg/ml & FIN 30 µg/ml standard solutions.

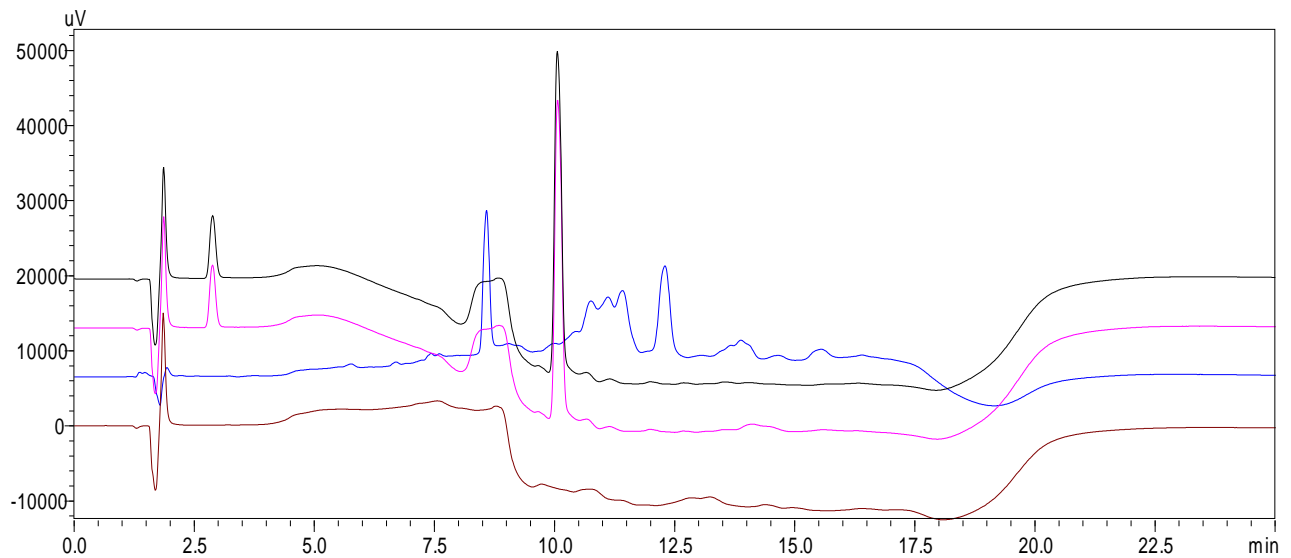


Fig. 3: Overlay of the chromatograms consisting of diluent (A), placebo (B), standard (C), and formulation (D).

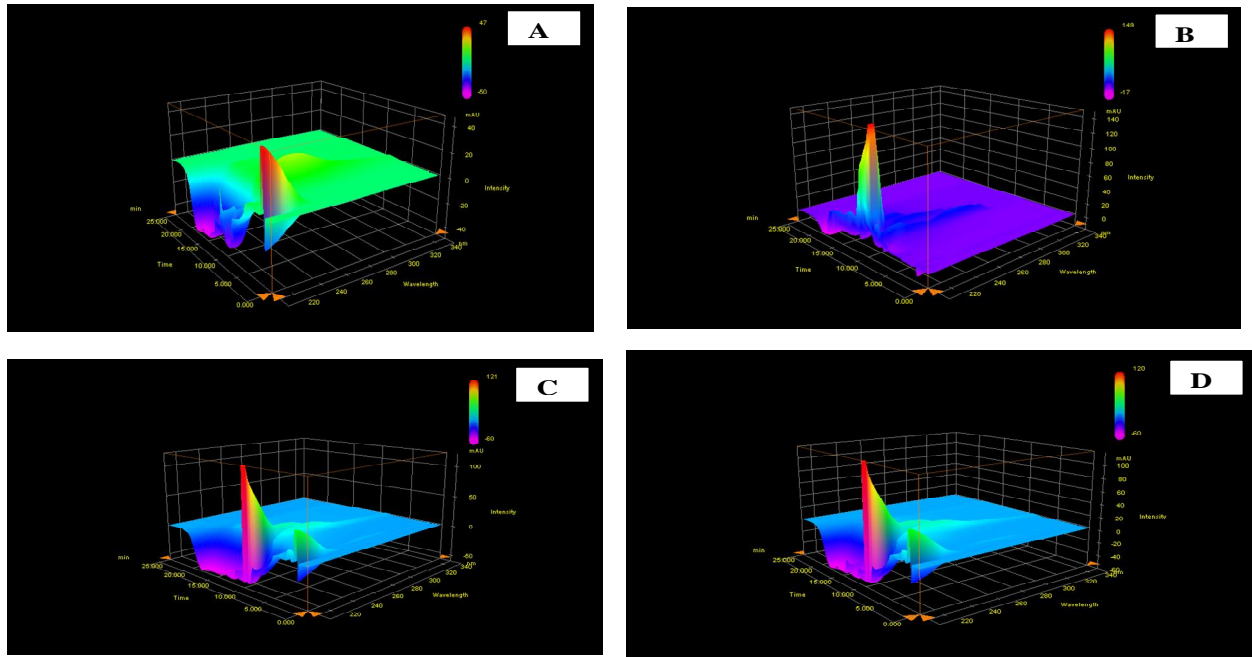


Fig. 4: 3D images of A-Mobile Phase, B-Placebo, C-Standard and D-Formulation.

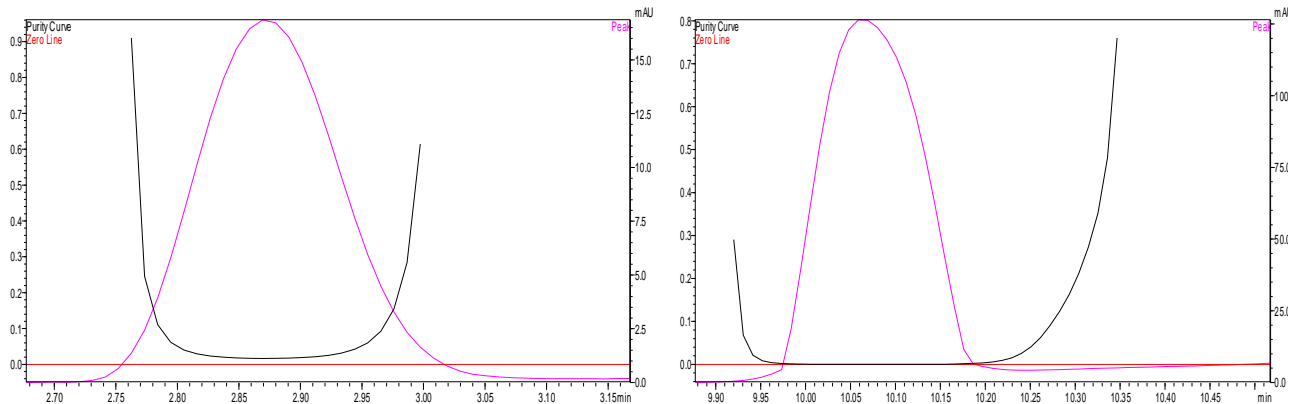


Fig. 5: Purity index profiles of (A) TAM; Peak purity index: 1.000000, single point threshold: 0.983256 and Minimum peak purity index: 16744 (B) Finasteride; peak purity index: 1.000000, single point threshold: 0.999793 and minimum peak purity indexes: 207

Precision

Precision was measured in terms of repeatability of application and measurement. Study was carried out by injecting six replicates of the standard at concentrations of 2.4 μ g/mL for TAM and 30 μ g/mL for FIN (Figure-2).

Specificity

Specificity studies were carried for both pure drug and drug product by comparing the 3D plots with diluent and placebo. Peak purity tests were also carried out to show that the analyte chromatographic peak is not attributable to more than one component as the impurities are not available by purity index data. The data was shown in (Figures-3 & 4).

Accuracy

Accuracy (Recovery) of the method was determined by spiking 80, 100 and 120% of working standard at a concentration

of 1.6 μ g/mL for TAM and 20 μ g/mL for FIN. Samples were injected in triplicate across its range according to the assay procedure and the data was given in Table-2 and shown in Figure-5.

LOD and LOQ

The LOD and LOQ values were determined by the formulae $LOD = 3.3 \sigma/m$ and $LOQ = 10 \sigma/m$ (Where, σ is the standard deviation of the responses and m is mean of the slopes of the calibration curves)

ASSAY

Sample solutions were prepared as mentioned above and were further diluted with diluents to get the required concentration 1.6MG/ML for tam & 20MG/ML for fin. The solution was injected three time into the column. From the peak area obtained, the drug content in the tablets was quantified. Results were given in table-3.

Table 2: Accuracy Data For TAM And FIN.

% Level of addition	Amount present ($\mu\text{g/mL}$)		Amount added ($\mu\text{g/mL}$)		Amount found in ($\mu\text{g/mL}$)		% Recovery		% Mean Recovery		% RSD	
	TAM	FIN	TAM	FIN	TAM	FIN	TAM	FIN	TAM	FIN	TAM	FIN
80%	1.6	20	1.28	16	2.86	35.1	98.75	98.65	99.15	99.21	1.72	1.8
80%	1.6	20	1.28	16	2.89	35.6	100.6	98				
80%	1.6	20	1.28	16	2.85	36.2	98.12	101				
100%	1.6	20	1.6	20	3.19	40.39	99.37	101.99	100	101.83	0.83	1.38
100%	1.6	20	1.6	20	3.22	40.14	100.01	100.7				
100%	1.6	20	1.6	20	3.21	40.56	100.62	102.8				
120%	1.6	20	1.92	24	3.54	44.62	100.56	101.25	100.8	101.23	1.2	1.75
120%	1.6	20	1.92	24	3.55	44.59	101.87	102.95				
120%	1.6	20	1.92	24	3.52	43.90	100	99.5				
							Mean		99.98	100.75		
							Limit		98-102%		NMT 2%	

Table 3: Assay results for TAM and FIN combination formulation.

Sample number	TAM		FIN	
	Area	% Assay	Area	% Assay
1	42277	96.9	235477	95.9
2	44122	101.1	237724	103
3	44460	101.9	237959	100.9
Mean	43619.6	99.96	237050.3	99.93

Table 4: Robustness data for TAM and FIN.

Robustness parameter	Retention Time (min)		Theoretical Plates (N)		Tailing factor		Capacity Factor		% Assay	
	TAM	FIN	TAM	FIN	TAM	FIN	TAM	FIN	TAM	FIN
<i>Flow Rate (mL/min)</i>										
0.95 (-5%)	2.79	10.01	3000.8	32372.1	1.19	1.02	2.918	5.879	98.76	99.32
1.0	2.76	10.01	2986.8	27646.7	1.09	1.06	2.972	5.139	99.16	99.44
1.05 (+5%)	2.65	10.16	2491.9	21223.4	1.15	1.16	2.765	5.749	99.25	99.23
<i>Wavelength (nm)</i>										
229 (-1)	2.88	10.08	2356.1	23773.8	1.15	1.24	2.753	5.925	99.23	100.4
230	2.88	10.08	2355.9	23721.3	1.15	1.25	2.782	5.980	100.2	99.98
231 (+1)	2.88	10.08	2353.7	23639.1	1.16	1.25	2.878	5.907	100.4	99.90

Table 5: System suitability data for TAM and FIN.

Injection volume (μL)	Retention time (min)		Tailing factor		Theoretical plates (N)	
	TAM	FIN	TAM	FIN	TAM	FIN
10	2.792	10.012	1.190	1.029	3000.84	32372.18
20	2.652	10.092	1.182	1.033	2728.98	14001.22
30	2.657	10.161	1.151	1.089	2991.91	22256.27
40	2.755	9.993	1.247	1.033	3244.56	42883.25
50	2.792	10.035	1.189	1.078	2963.23	32268.02
Mean	2.729	10.058	1.191	1.0524	2985.9	28756.19
%RSD	1.5	0.5	0.65	0.8	1.85	1.55

Robustness

Method robustness was determined by analyzing same sample at normal operating conditions and by changing, some operating analytical conditions such as flow rate and wavelength. The data was given in Table-4.

RESULTS AND DISCUSSION

Method development

The main of the present investigation is to develop a validated HPLC-PDA method for the simultaneous estimation of TAM and FIN. The method should be fast, specific, accurate, reproducible, robust, good peak symmetry and LC-MS compatible. Separation was achieved with Phenomenex C_{18} column (150 x 4.6mm, 5 μ , 100 Å). Mobile phase optimization was carried out by several compositions of 0.02% v/v formic acid (in water) and methanol. Initial trials were carried out in isocratic mode with

different proportions of formic acid (A): methanol (B) (60:40, 50:50, 30:70, and 20:80) at 1 mL/min flow rate and the eluents were monitored at 230 nm. With 60:40 composition the TAM was eluted at around 2.8 min where as, FIN was not eluted with in 20 min run time. Increasing the organic modifier to 50% TAM eluted along with solvent front, where as FIN was not eluted with in 20 min of runtime. Composition changed to 30:70 and with these conditions TAM eluted before solvent front and FIN was eluted at 8min. In another trial 20:80 composition was used where TAM was eluted again before solvent front and the FIN was eluted at 3.3min. Increasing the organic modifier in mobile phase composition the TAM was not retained on the column and eluted either along or with the solvent front in the isocratic mode. Further trials were carried out with gradient mode starting with 40% of B at 0 min and increased to 70% up to 3 min and increased linearly to 80% at 13 min and changed to 40% up to 15 min and maintained

for 25 min to achieve equilibration. Under these gradient conditions, TAM was eluted at 2.7min, whereas FIN was eluted at 10.8 min. A standard chromatogram of TAM and FIN under these conditions is shown in Figure-1 along with UV spectrum.

TAM exhibits two peaks at 223nm and 279nm whereas, FIN exhibits two peaks one at 236 nm and other at 242 nm. So, based on the UV cut off for the methanol, 230 nm was used in the studies for monitoring the eluents.

Method validation

The method described above had been validated as per the ICH guidelines (ICH–Guidelines Q2B, Switzerland, 1996) and the results were summarized below.

Linearity

Linearity was assessed in a concentration range of 0.4-20µg/mL for TAM and 5-50µg/mL for FIN. The range of concentrations was selected based on 80-120 % of the test concentration (for assay i.e. 1.6 and 20µg/mL). Samples were analysed in triplicate and the regression data was given in Table-1. Peak area and concentrations were subjected to least square regression analysis to calculate regression equation. The regression data indicates that the response is linear over range used. A good regression coefficient (R^2), correlation coefficient (R) values were obtained with regression equation from the calibration curve (Table-1).

Precision

Repeatability of standard application was carried out using six replicates of the same standard concentration (2.4µg/mL for TAM and 30µg/mL for FIN). Overlay of the chromatograms was shown in Figure-2. The % RSD was 0.2 for TAM and 0.16 for FIN peak areas and this low % RSD indicates the precision of the method.

Specificity

Diluent, placebo, standard and sample (formulation) solutions were run individually as per the method to examine any interference. From the base shifted overlay chromatograms as shown in (Figure-3) and the 3D plots of placebo and formulation in (Figure-4), it can be inferred that there were no co eluting or interfering peaks at the retention times of TAM and FIN. This shows that the peak of analyte was pure and excipients in the formulation did not interfere with the analysis and the peak purity indices of the standard and sample peaks were greater than 0.999 and confirms the specificity of the method (Figure-5).

Accuracy

Accuracy of the method was examined by performing recovery studies by standard addition method for drug product as the exact components are unknown and for drug substance the analyte peak is evaluated by 3D plot of the chromatogram in order to confirm the existence of single components at 2.7min for TAM and 10.08min for FIN as the impurities are not available. The

obtained recovery results were given in Table-2. The recovery of the added standard to the drug product sample was calculated and it was found to be 99.15-100.8% for TAM and 99.21-101.83% and the % RSD was less than 2 for both the drugs which indicates a good accuracy of the method to that of the label claim. The 3 D plots for standard chromatogram were shown in Figure 4. From the 3 D plot it is clear that the peaks eluted at 2.7 and 10.08 mins were of one component and free from impurities as confirmed by peak purity indices (Figure-5)

Limit of detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were determined based on statistical calculation using standard deviation of intercepts (s) and slopes (m) from calibration curves, where $LOD = 3.3 \times s/m$ and $LOQ = 10 \times s/m$. Compared with the results reported earlier by DB Patel et al (12), the present method conditions can be considered more sensitive by the fact that the intensity of the peaks was higher at the same injection volume and concentrations. A 10 µg/mL of TAM yielded a peak with an intensity of 100 mAU (Figure-6), while in the previously published method by DB Patel et al (2010) reported an intensity of 25 mAU with same injection volume and concentration. However, a 40 µg/mL of FIN showed an intensity of 70 mAU (Figure-6) approximately with the present method conditions while the previously reported method showed an intensity of 35 mAU. With these results, it is clear that the present method is superior in terms of LOD and LOQ. However, DB Patel et al calculated the LOD and LOQ based on visual inspection and reported LOD of 0.2 µg/mL and LOQ 0.5 µg/mL for TAM and 0.5 µg/mL and 1.0 µg/mL for FIN. In the present investigation, LOD and LOQ were calculated based on the slope, intercepts of the linear regression equation. The calculated LOD and LOQ for TAM were 0.16 µg/mL and 0.49 µg/mL whereas, for FIN 0.6 µg/mL and 1.9 µg/mL. Based on these results there is a discrepancy with the LOD and LOQ for FIN. However, based on their visual intensities observed from the both methods it can be conclude that the present method conditions were superior in terms of LOD and LOQ as supported by the higher peak intensities for both TAM and FIN.

Analysis of marketed formulation

The developed method was applied to the assay of commercial TAM and FIN combination formulation. The results of the assay were given in Table-3 and are in good agreement with the label amount and the error of the determination did not exceed the limits. The % assay of the formulation was calculated as an average of 3 determinations, which was about 99.96% for TAM and 99.93% for FIN.

Robustness

Method robustness was determined by analyzing same sample at normal operating conditions and by changing two operating analytical conditions such as wavelength of detection and flow rate. The results were given in Table-4 indicates that the changed conditions yielded similar results in terms of % assay. Hence, the method was sufficiently robust for normally expected variations in the chromatographic conditions.

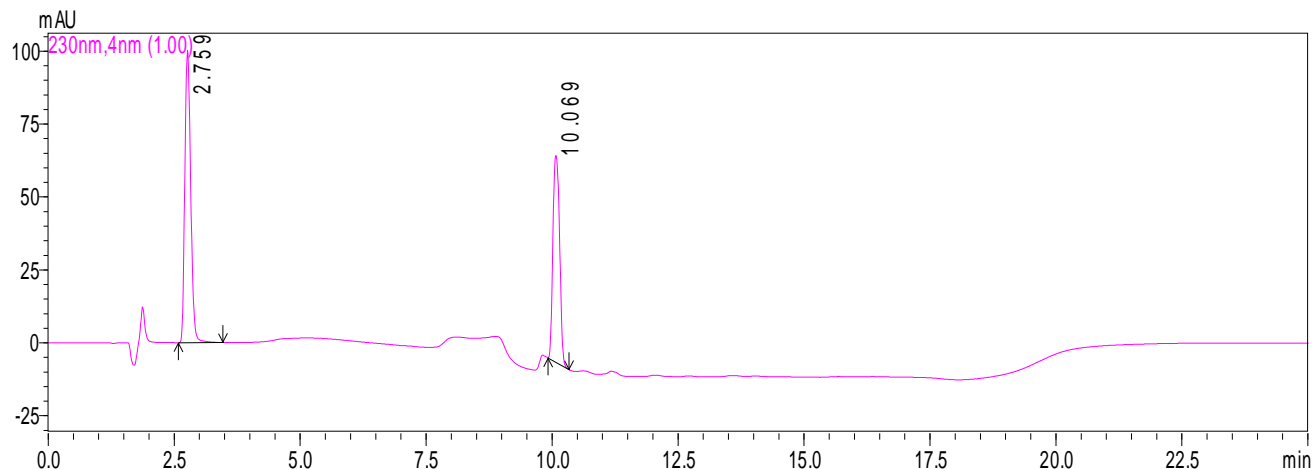


Fig. 6: Typical chromatogram of standard solutions containing of 10 µg/mL of TAM and 40µg/mL of FIN .

System suitability

Five injections of 2µg/mL (TAM) and 20µg/mL (FIN) standard solutions were given by increasing the injection volumes from 10 µL to 50 µL and the results were given in Table-5. The % RSD obtained for all the parameters was less than 2% and all these result indicates that the present method conditions were suitable for the analysis of TAM and FIN.

Stability of the analytical solution

The stability of the stock and standard solutions were assessed by analyzing the samples at different time intervals up to 7 days by storing at 4°C using a freshly prepared standard solution. The percentage variation was found to be less than 2% of the initial concentration and it was observed that the solution was stable for a period of 7 days when stored at 4°C.

CONCLUSION

The proposed RP-HPLC - PDA method was validated fully as per International Conference on Harmonisation (ICH) Guidelines, and found to be applicable for routine quality control analysis for the estimation of TAM and FIN in combination using gradient mode of elution. The results of linearity, precision, accuracy and specificity, proved to be with in the limits. The method provides selective quantification of TAM and FIN without interference from diluent and placebo. The proposed method is highly sensitive, reproducible, reliable, rapid and specific and also has the unique advantage of LC conditions being compatible with MS detection. Therefore, this method can be employed in quality control to estimate the amount of TAM and FIN in bulk and in combined dosage forms.

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