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RP-HPLC Method Development And Validation For Simultaneous Estimation Of Ambrisentan And Tadalafil In Bulk And Tablet Dosage Forms.

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ABSTRACT

A simple, accurate, precise method was developed for the simultaneous estimation of the tadalafil and ambrisentan in bulk and tablet dosage form. Chromatogram was run through phenomex (250x4.6mm,5). Mobile phase containing phosphate buffer:acetonitrile taken in the ratio 70:30 v/v was pumped through column at a flow rate of 1ml/min. Buffer used in this method was 0.1% ortho phosphoric acid solution. Optimized wavelength selected was 262 nm. Retention time of tadalafil and ambrisentan were found to be 3.491min and 4.923 min.% RSD of the tadalafil and ambrisentan were and found to be 0.6 and 0.1 respectively. %Recovery was obtained as 99.48% and 99.52% for tadalafil and ambrisentan respectively. LOD, LOQ values obtained from regression equations of tadalafil and ambrisentan were 5.8µg/ml, 3.5µg/ml and 17.5µg/ml, 10.7µg/ml respectively. Regression equation of tadalafil is y=28466x+16260, and y=22874x+21411 of ambrisentan. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular quality control test in Industries.

Keywords: Tadalafil, Ambrisentan, Method Development, RP-HPLC.

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INTRODUCTION

Ambrisentan is a drug indicated for use in the treatment of pulmonary hypertension [1,2]

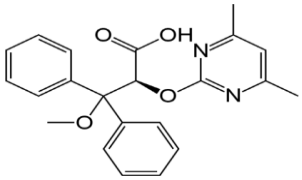

Figure 1: Ambrisentan structure

Molecular weight: 378.428

Chemical formula: $C_{22}H_{22}N_2O_4$

IUPAC name: (2S)-2-[(4,6-dimethylpyrimidin-2-yl)oxy]-3-methoxy-3,3-diphenylpropanoicacid.

Ambrisentan is a drug that blocks endothelin, an endogenous hormone found in higher quantities in patients with pulmonary arterial hypertension. Endothelin binds to two receptors, ET_A and ET_B. ET_A is responsible for cell growth in the vessels as well as vasoconstriction, while ET_B plays a role in vasodilation, endothelin 1 clearance, and antiproliferation of cells.

Tadalafil used to treat erectile dysfunction, benign prostatic hyperplasia, and pulmonary arterial hypertension.[3][4][5] It is taken by mouth.[5] Onset is typically within half an hour and the duration is up to 36 hours.[5]

Common side effects include headache, muscle pain, flushed skin, and nausea.[7] Caution is advised in those with cardiovascular disease.[5] Tadalafil is not recommended in people taking nitrovasodilators such as nitroglycerin, as this may result in a serious drop in blood pressure.[5] Tadalafil is a PDE5 inhibitor which increases blood flow to the penis.[5] It also dilates blood vessels in the lungs, which lowers the pulmonary artery pressure.[5]

Tadalafil inhibits PDE11 more than sildenafil or vardenafil.[6] PDE11 is expressed in skeletal muscle, the prostate, the liver, the kidney, the pituitary gland, and the testes.[6] The effects on the body of inhibiting PDE11 are not known.[6]

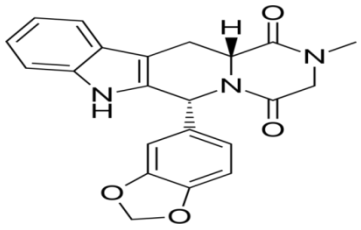

Figure 2: Tadalafil structure

Chemical formula: $C_{22}H_{19}N_3O_4$

Molecular weight: 389.404

IUPAC name: (6R,12aR)-2,3,6,7,12,12a-Hexahydro-2-methyl-6-(3,4-methylenedioxyphenyl)pyrazino [1',2':1,6]pyrido[3,4-b]indole-1,4-dione.

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EXPERIMENTAL

Method Development

UV method for wavelength detection

Preparation of tadalafil solutions: 200.2mg of tadalafil was weighed and transferred to100ml volumetric flask and 3/4 th of diluent was addedand sonicated for 10 minutes andvolume diluted with diluents and mixed well.From the above solution 1ml was pipetted out and transferred into a 50ml volumetric flask and volume diluted with diluent and mixed well and spectrum was taken in uv-visible spectrophotometer.

Preparation of ambrisentan solutions: 50.12mg of ambrisentan was weighed and transferred to 100ml volumetric flask and 3/4 th of diluent was added and sonicated for 10minutes and volume diluted with diluent and mixed well.From the above solution 1ml was pipetted out and transferred into a 50ml volumetric flask and volume diluted with diluent and mixed well and spectrum was taken in uv-visible spectrophotometer. The two spectrums was overlayed and isobestic point was taken as wavelength.i.e., 262nm

HPLC method development

0.1%OPAbuffer: 0.1ml of ortho phosphoric acid was diluted to 100ml with HPLC grade water and filtered through membrane filter.

Preparation of 0.02M potassium di hydrogen orthophosphate buffer pH 3.0:

Accurately weighed 3.48g of potassium dihydrogen ortho phosphate was dissolved in 1000ml of HPLC grade water.

Preparation of standard stock solutions: 200.2mg of tadalafil and 50.12mg of ambrisentan was weighed and transferred to 100ml volumetric flask and 3/4 th of diluent was added and sonicated for 10 minutes and volume diluted with diluent, mixed well and filtered through 0.45 µm pore nylon filter.

Preparation of working standard solution: From stock solution 4ml was pipetted out and transfer into a 50ml volumetric flask.

Preparation of sample stock solutions: 20 tablets were weighed and calculated average weight of tablets. Weighed 805.63 mg powder and transferred into a 100ml volumetric flask. 70ml of diluent was added, sonicated for 30 min with intermediate shaking and volume diluted with diluents and mixedwell up and filtered.

Preparation of working sample solutions: From stock solution 4ml was pipetted out and transferred into a 50ml volumetric flask.

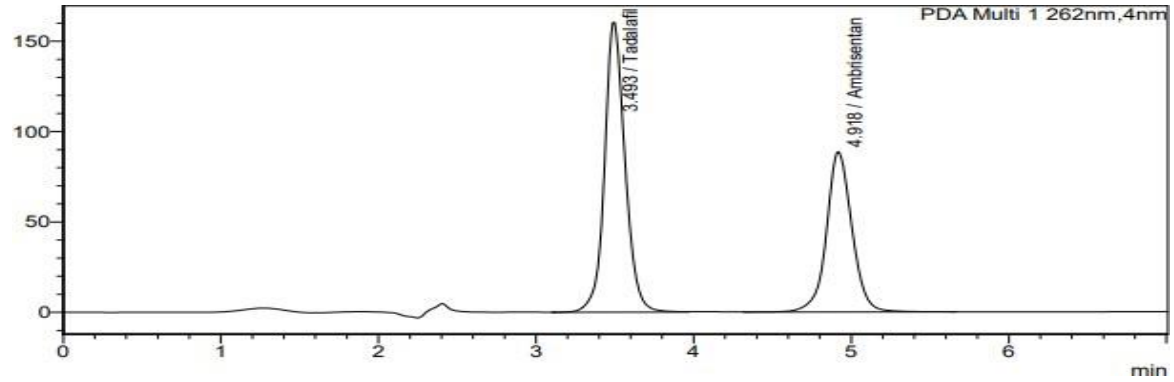

Figure 3: Optimized chromatogram

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System Suitability Procedure: 20µl of the standard solution of 100µg/ml was injected into the chromatographic system and chromatogram was recorded.

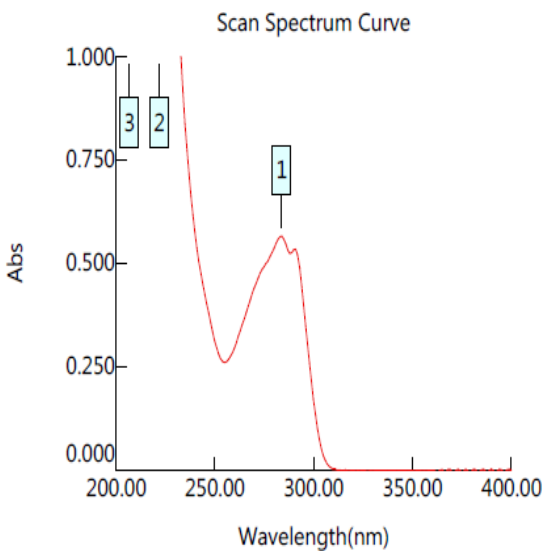

Figure 4: Uv spectrum of tadalafil

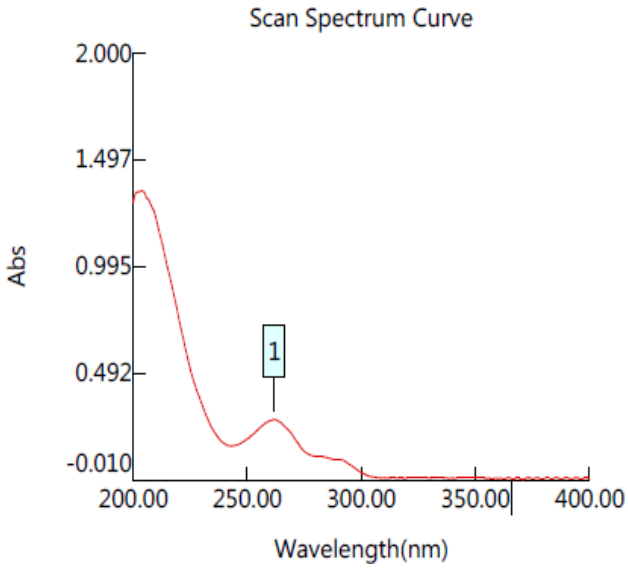

Figure 5: UV spectrum of ambrisentan

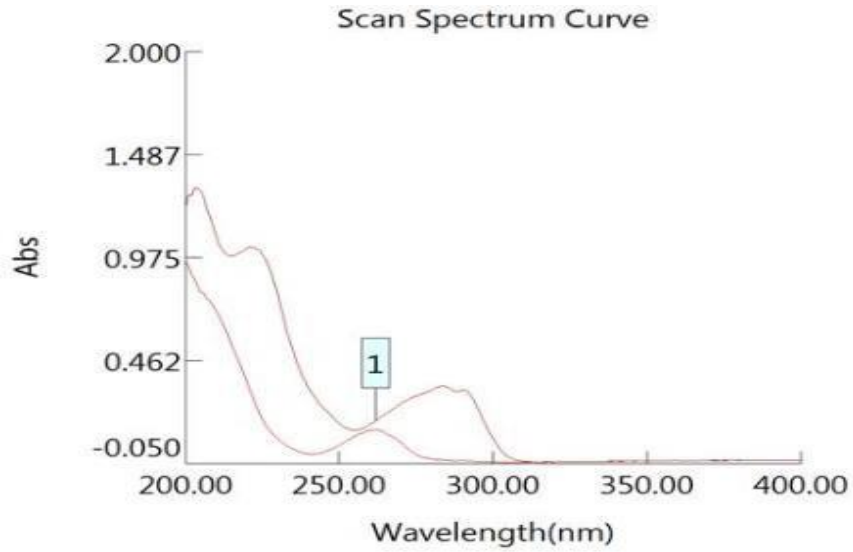

Figure 6: Overlay UVspectra of tadalafil and ambrisentan

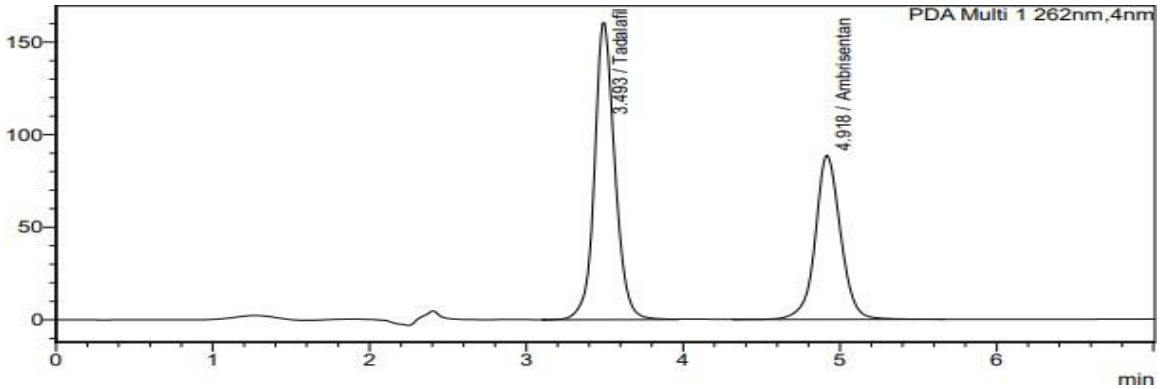

Figure 7: Optimized chromatogram

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System suitability

Table 1: System suitability parameters for tadalafil and ambrisentan

Tadalafil			Ambrisentan	
Injection No	**Retention time**	**Peak area**	**Retention time**	**Peak area**
1	3.483	2992720	4.890	1886023
2	3.481	2961889	4.890	1868021
3	3.480	2988078	4.890	1886012
4	3.477	2898891	4.889	1861899
5	3.481	2904594	4.895	1864808
6	3.483	2906035	4.889	1865216
Mean		2942035	1876997	
Standard deviation		43914.329	14967.04	
%RSD		1.5	0.8	

RESULTS AND DISCUSSION

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of tadalafil and ambrisentan was obtained and the isobestic point of tadalafil and ambrisentan showed absorbance's maxima at 262nm.

Systemsuitability:

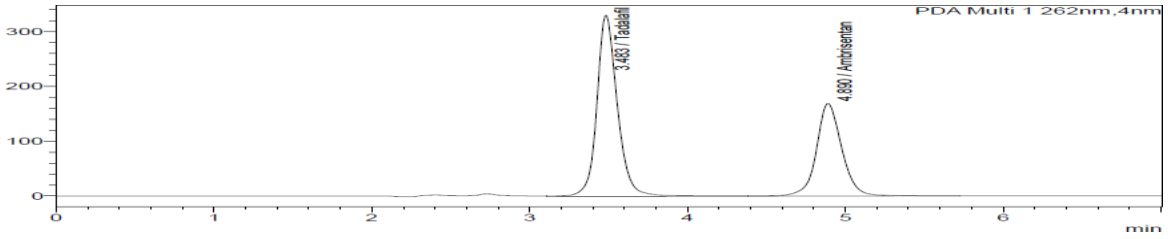

Figure 8: System suitability chromatogram

Table 2: Data of system suitability

Parameters	Tadalafil	Ambrisentan
Retentiontime	3.483	4.890
Tailing factor	1.153	1.070
Theoreticalplates(USP)	21282	29455
%RSD	1.5	0.8

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Specificity

Chromatogram of standard:

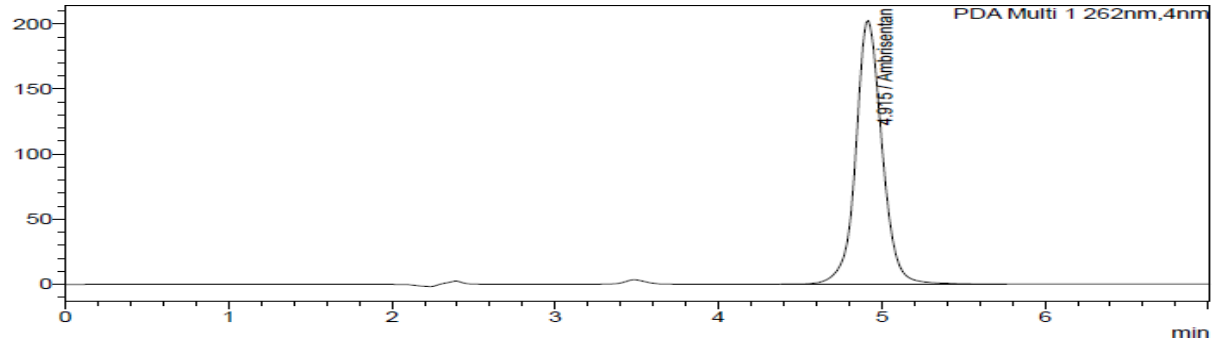

Figure 9: Chromatogram of ambrisentan

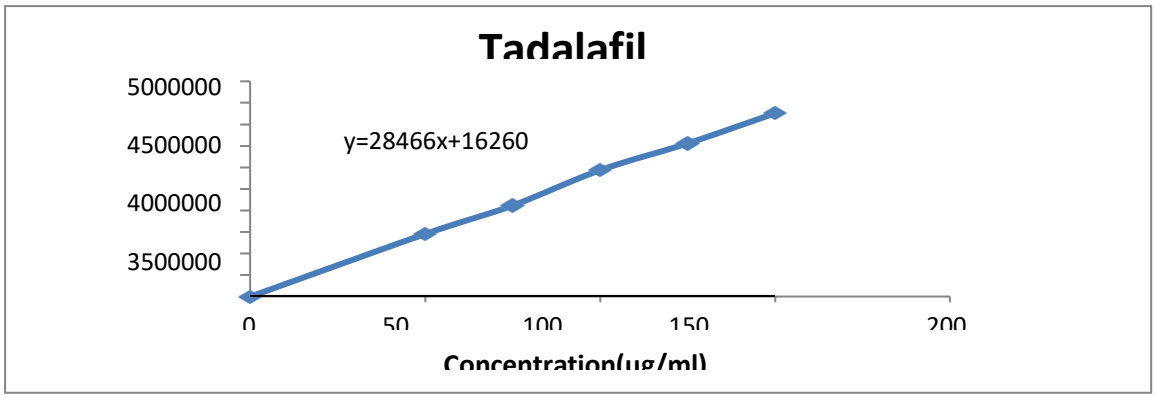

Figure 10: Calibration curve of tadalafil

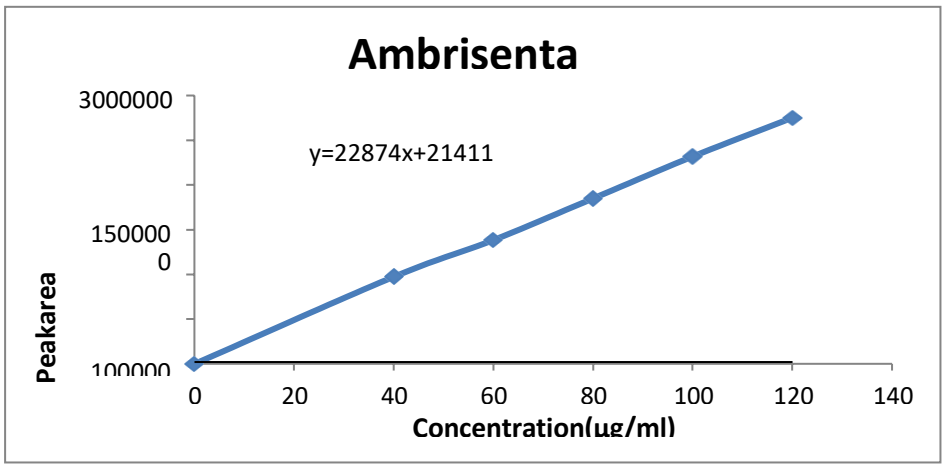

Figure 11: Calibration curve of ambrisentan

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Precision:

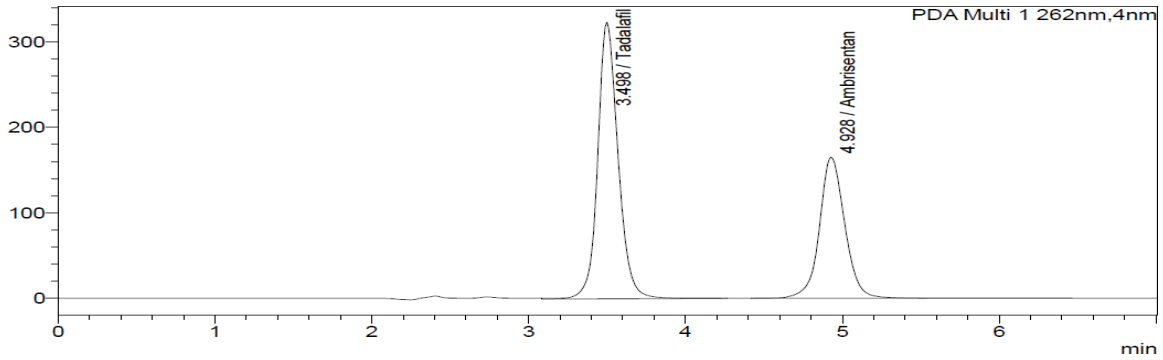

Figure 12: Chromatogram No-1

System precision

Table 3: System precision table of tadalafil and ambrisentan

Tadalafil			Ambrisentan	
Injection	**Peakarea**	**Assay**	**Peakarea**	**Assay**
1	2934840	99.7	1847734	98.4
2	2983341	101.5	1847840	98.5
3	2940851	99.9	1848675	98.4
4	2941349	100.0	1849700	98.5
5	2937747	99.9	1841826	98.4
6	2936081	99.8	1848139	98.4
Mean	**2945702**	**100.133**	**1847319**	**98.4**
Standard deviation	**18617.22**	**0.618241**	**2786.754**	**0.141421**
%RSD	**0.6**	**0.6**	**0.1**	**0.1**

Method precision

Table 4: Method precision table of tadalafil and ambrisentan

Tadalafil			Ambrisentan	
Injection	**Peak area**	**Assay**	**Peakarea**	**Assay**
1	2983441	101.4	1845754	98.3
2	2965421	100.9	1848675	98.5
3	2956321	100.5	1847954	98.4
4	2932154	99.7	1845632	98.3
5	2975412	101.1	1848587	98.4
6	2945230	100.1	1852100	98.6
Mean	**2959663**	**100.6167**	**1848117**	**98.41667**
SD	**19093.477**	**0.584285**	**2374.115**	**0.106719**
%RSD	**0.6**	**0.5**	**0.1**	**0.1**

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Accuracy:

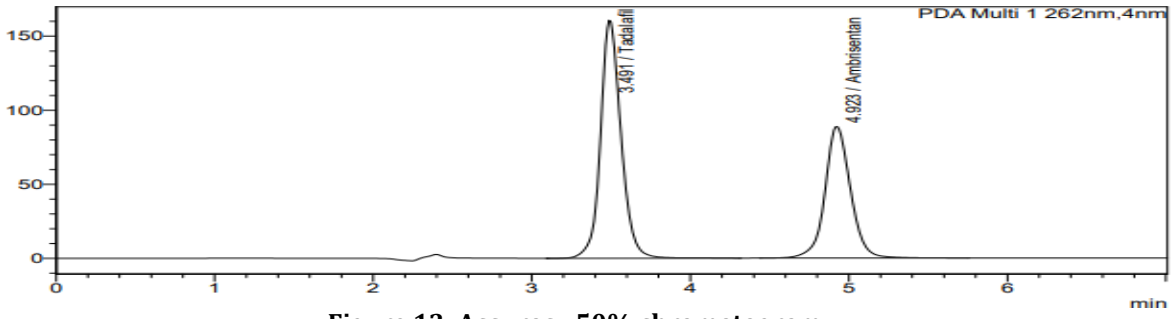


Figure 13: Accuracy 50% chromatogram

Figure 14: Accuracy100%chromatogram

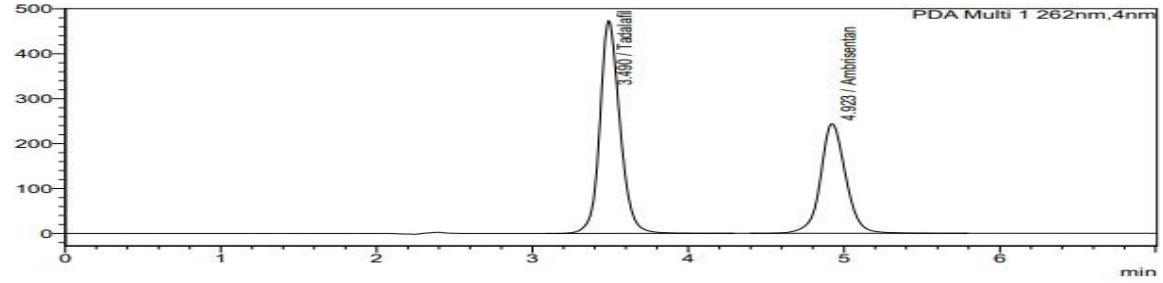
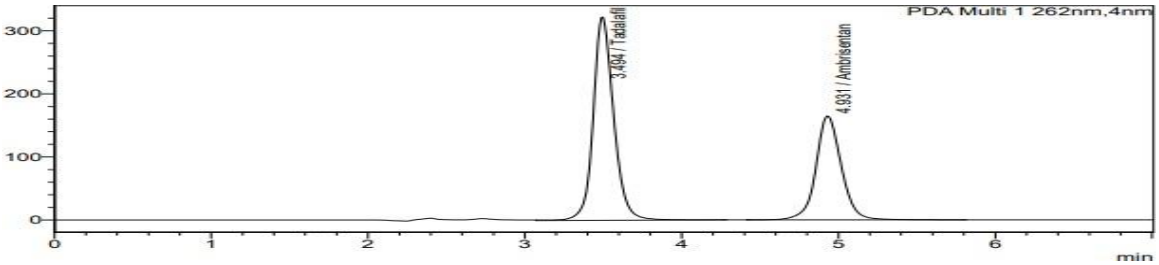
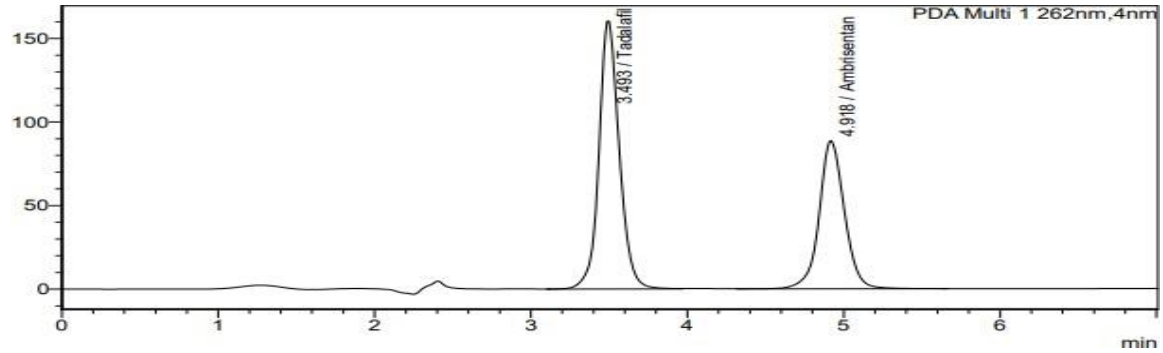


Figure 15: Accuracy 150 chromatogram



LOD and LOQ

Figure 16: LOD chromatogram



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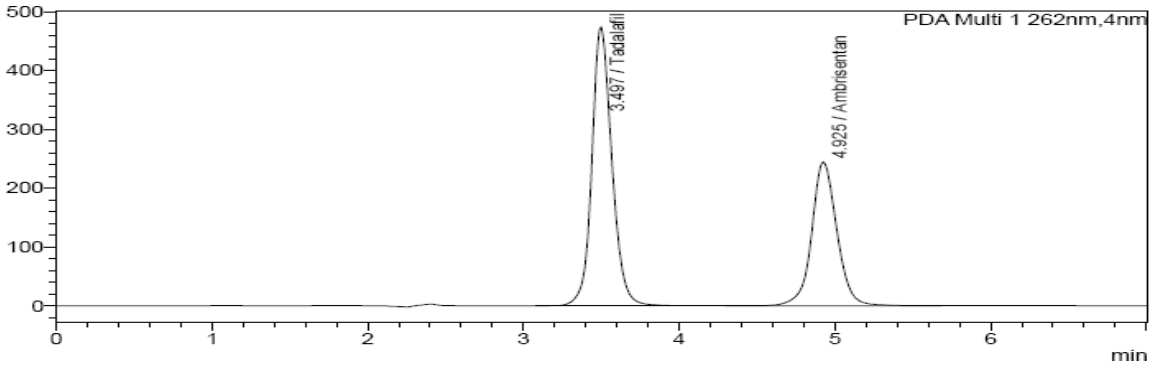

Figure 17: LOQ chromatogram

Robustness:

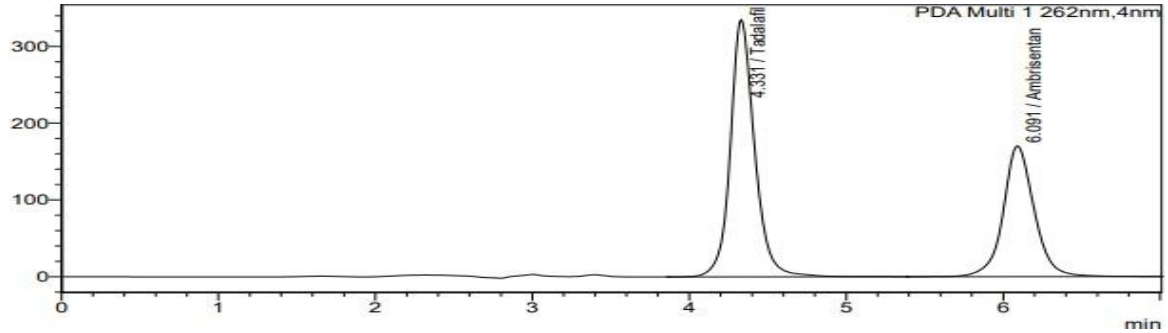

Figure 18: Flow minus chromatogram

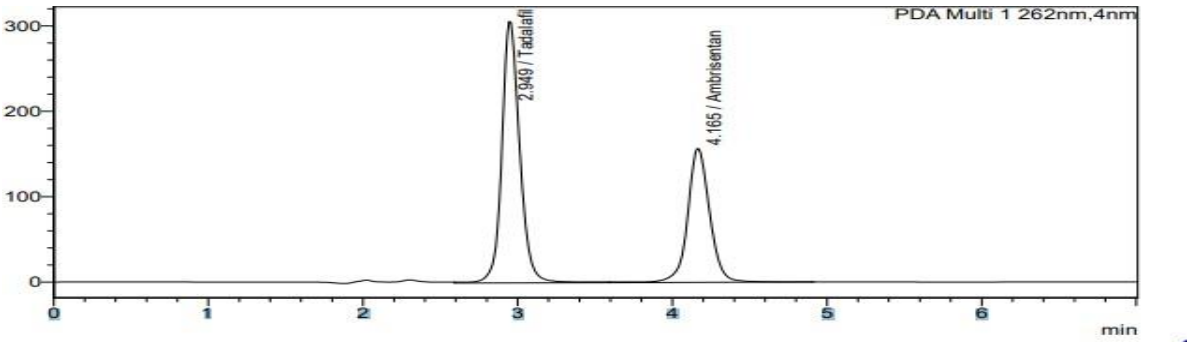

Figure 19: Flow pluschromatogram

CONCLUSION

The method demonstrated a linear response for tadalafil ranging from 50% to 150%, while for ambrisentan, the linear response was observed within the range of 40% to 125%. No interference was detected from the excipients used in the formulation of the capsule dosage form. The proposed method demonstrated results within the acceptance criteria of 98-102%, indicating good precision. The assay results exhibited a satisfactory agreement with the label claim. A novel and precise method was developed to simultaneously determine the quantities of tadalafil and ambrisentan in bulk and tablet formulations. The retention times for tadalafil and ambrisentan were identified as 3.491 and 4.923 minutes, respectively. The obtained %RSD values were 0.6% for tadalafil and 0.1% for ambrisentan, demonstrating excellent precision. The %recovery values achieved were 99.48% for tadalafil and 99.52% for ambrisentan, indicating accurate estimation. The LOD and LOQ values were established through regression equations, with tadalafil having LOD and LOQ values of 5.8 µg/ml and 17.5 µg/ml, respectively, while ambrisentan exhibited LOD and LOQ values of 3.5 µg/ml and 10.7 µg/ml, respectively. The regression equations for tadalafil and ambrisentan were determined as y = 28466x + 16260 and y = 22874x + 21411, respectively. Furthermore, the developed method demonstrated reduced retention

times and overall run time, resulting in a simplified and cost-effective approach.

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