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A NEW RP-HPLC METHOD DEVELOPMENT & VALIDATION FOR SIMULTANEOUS ESTIMATION OF AMBRISENTAN AND TADALAFIL IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract:
A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Ambrisentan and Tadalafil in pharmaceutical dosage form. Chromatographic separation of Ambrisentan and Tadalafil was achieved on Shimadzu HPLC accomplished with cyber lab LC 100 software by using Cap cell pack C18 column and the mobile phase containing 1ml Triethyl amine is dissolved in 1lt water adjust pH-7.0 with OPA & ACN in the ratio of 60:40% v/v. The flow rate was 1.0 ml/min; detection was carried out by absorption at 249nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Ambrisentan and Tadalafil were NLT 2000 and should not more than 2 respectively. % Relative standard deviation of peak areas of all measurements always less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Ambrisentan and Tadalafil.

Key words: *HPLC, Ambrisentan and Tadalafil.*

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INTRODUCTION:

Administration of two or more drugs at a time becomes imperative for several therapeutic reasons. The multi component formulations have gained lot of importance now-a-days due to greater patient acceptability, increased potency, multiple action, fewer side effects and quicker relief. The combined dosage forms are complex in nature during the process of estimation, it is important to confirm that one component does not interfere with the estimation of the other. There is a plethora of analysis of such formulations without prior separation for the estimation of multi component formulation, the instrumental techniques, which are commonly employed are spectrophotometry, GLC, HP-TLC, HPLC etc. These methods are based up on the measurement of specific and nonspecific physical properties of the substances.

Ambrisentan is an orally active selective type an endothelin receptor antagonist indicated for the treatment of pulmonary arterial hypertension. (S)-2-(4,6-dimethylpyrimidin-2-yloxy)-3-methoxy-3,3-diphenylpropionic acid anti-hypertensive agent. Tadalafil is an orally administered drug used to treat male erectile dysfunction (impotence). pyrazino[1',2'1,6] pyrido[3,4-b]indole-1,4-dione, 6-(1,3-benzodioxol-5-yl)2,3,6,7,12,12a-hexahydro-2-methyl-, (6R,12aR).

EXPERIMENTAL WORK:

Determination of Working Wavelength (λ_{max})

The wavelength of maximum absorption of the solution of the drugs in mixture of Acetonitrile and 0.1% TEA (40:60) were scanned using PDA Detector within the wavelength region of 200–400 nm against Acetonitrile and 0.1% TEA (40:60) as blank. The absorption curve shows isobestic point at 249nm. Thus 249 nm was selected as detector wavelength for the HPLC chromatographic method.

Chromatographic conditions:

Preparation of standard stock solution

- Accurately weigh and transfer 20 mg of Ambrisentan, 80 mg of Tadalafil working standard into a 100 ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
- Further pipette 5 ml of the above stock solutions into a 50 ml volumetric flask and dilute up to the mark with diluent. (20ppm of Ambrisentan, 80ppm of Tadalafil)

Sample Solution Preparation:

- Accurately weighed and transfer equivalent to 20mg of Ambrisentan, 80mg of Tadalafil sample into a 100mL clean dry volumetric flask add Diluent and sonicate it up to 30 mins to dissolve, and centrifuge for 30min. to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.45-micron Injection filter. (Stock solution).
- Further pipette 5 ml of the above stock solutions into a 50ml volumetric flask and dilute up to the mark with diluent. (20ppm of Ambrisentan, 80ppm of Tadalafil)

RESULTS AND DISCUSSION:

RP-HPLC METHOD

Determination of Working Wavelength (λ_{max}):

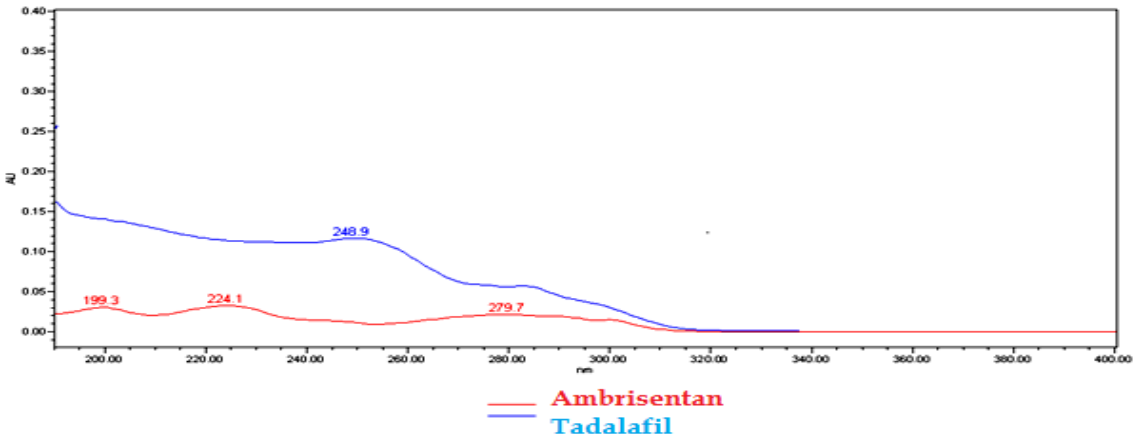

Fig: PDA - Spectrum of Ambrisentan and Tadalafil

Optimized chromatogram:

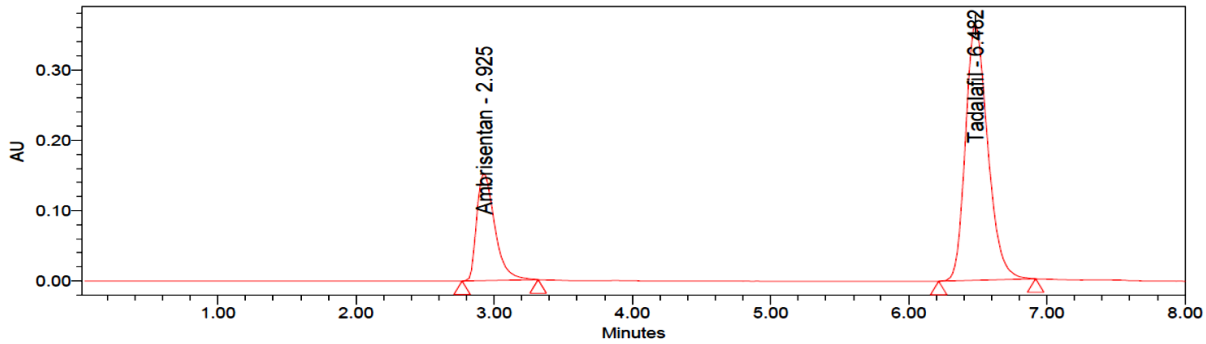

Fig: Optimized chromatogram

Table: Results for (Optimized trail)

S.No	Name	Retention Time	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
1	Ambrisentan	2.925	2068122	72.36	-	1.12	4852
2	Tadalafil	6.402	845623	27.64	13.21	1.10	5542

Table: Optimized chromatographic conditions

PARAMETERS	OBSERVATION
Instrument used	Shimadzu HPLC with PDA detector.
Injection volume	20µl
Mobile Phase	Acetonitrile and 0.1%triethylamine pH 7.0 with OPA 40:60
Column	Cap cell pack C18 (250×4.6nm, 5µ)
Detection Wave Length	249 nm
Flow Rate	1 mL/min
Runtime	7min
Temperature	Ambient(25° C)
Mode of separation	Isocratic mode

The Ambrisentan peak was observed at 2.521 min with peak area 2068122, tailing factor 1.12, Tadalafil peak was observed at 5.206 min, with peak area 845623, tailing factor 1.10 and resolution 13.21. This trial was optimized.

SYSTEM SUITABILITY:

Table: System suitability parameters for Ambrisentan and Tadalafil

S.no	Parameter	Ambrisentan	Tadalafil
1	Retention time	2.925	6.402
2	Plate count	2944	9315
3	Tailing factor	1.12	1.10
4	Resolution	----	13.21
5	%RSD	0.34	0.08

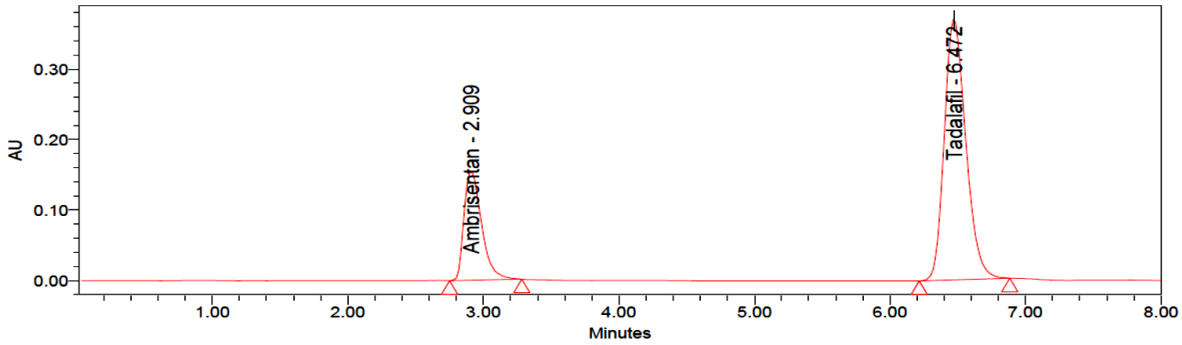

Fig: Chromatogram of standard

Acceptance Criteria: According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

Assay:

Table: Assay of Ambrisentan and Tadalafil

Brand	Drug	Avg sample area (n=5)	Std. wt (µg/ml)	Sample wt. (µg/ml)	Label amount (mg)	Std purity	Amount found (µg/ml)	% assay
-	A	2054326	20	228	5	99.9	20.2	99.45
	T	844234	80	228	20	99.9	80.1	99.69

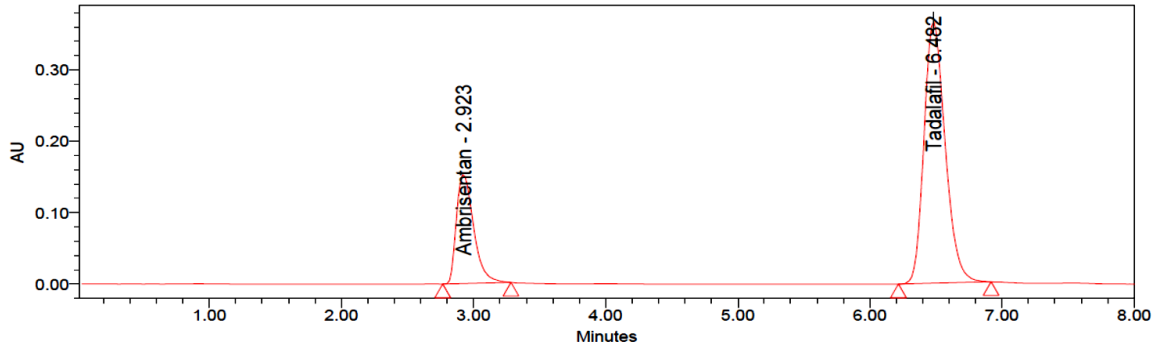

Fig: Chromatogram of Assay

ANALYTICAL METHOD VALIDATION (HPLC):

SPECIFICITY:

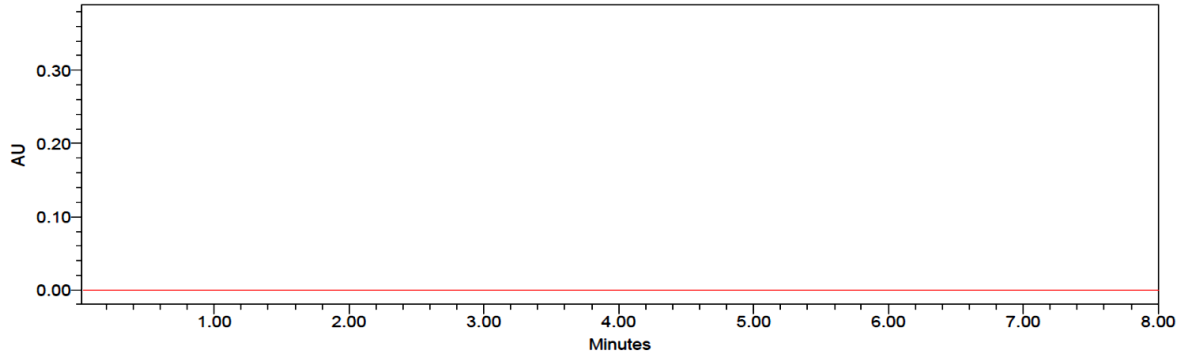

Fig: Chromatogram of blank

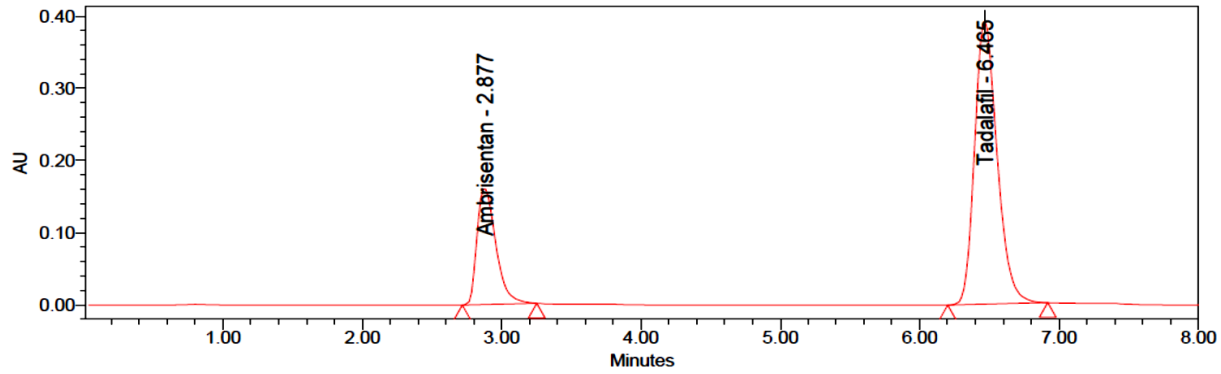

Fig: Optimized chromatogram

Discussion: Retention times of Ambrisentan and Tadalafil were 2.918 min and 6.480min respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

LINEARITY:

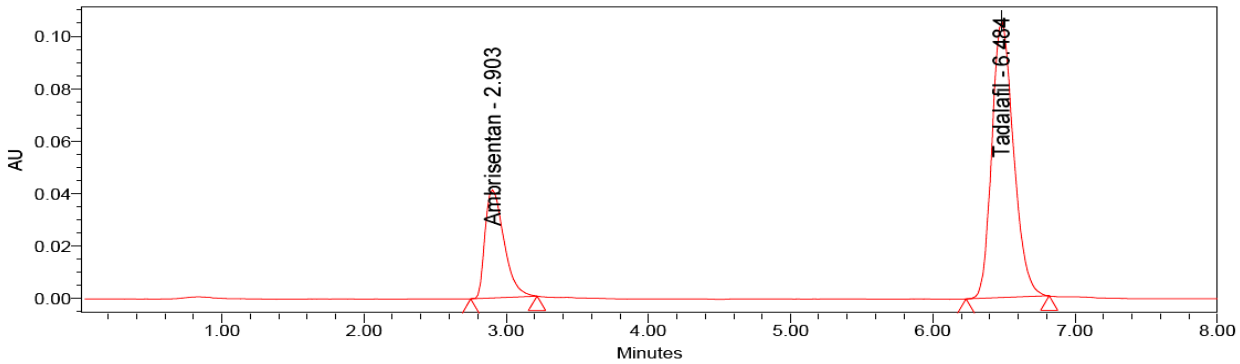

Fig: Chromatogram of Linearity-25%

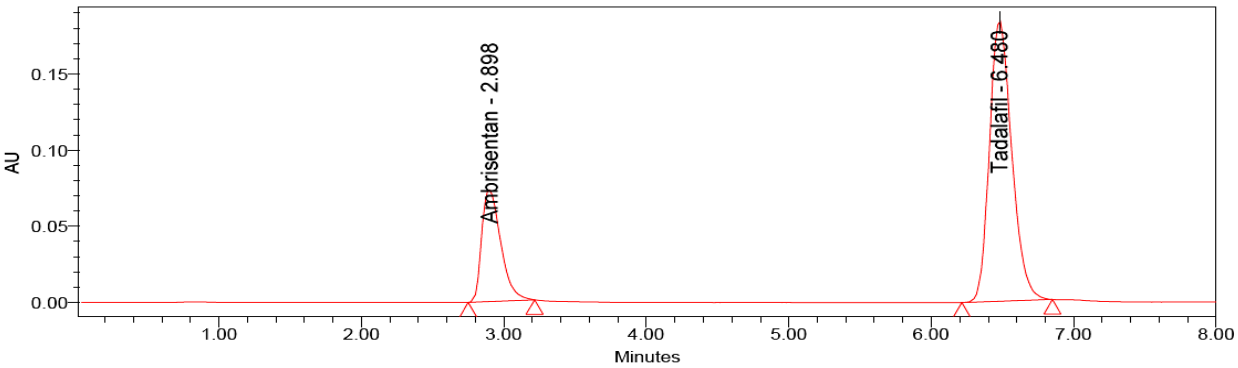

Fig: Chromatogram of Linearity-50%

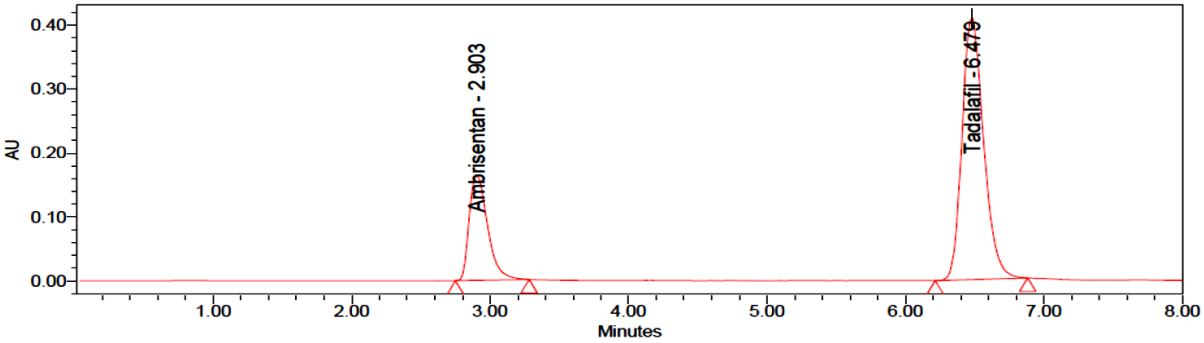

Fig: Chromatogram of Linearity-100%

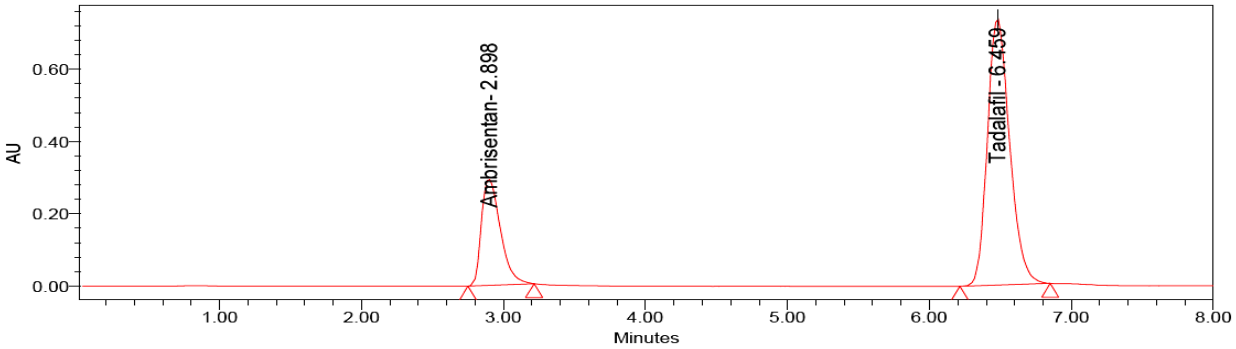

Fig: Chromatogram of Linearity-150%

Table: Results of linearity for Ambrisentan and Tadalafil

S.NO	Ambrisentan		Tadalafil	
	Conc.(µg/ml)	Peak area	Conc.(µg/ml)	Peak area
1	2	208924	8	134265
2	5	539862	20	252452
3	10	1081729	40	469575
4	15	1689432	60	683139
5	20	2163458	80	905367
6	25	2703340	100	1149622
7	30	3245828	120	1375856
Regression equation	y = 108409.5x +4093.6		y = 11239.79x +19955.64	
Slope	108409.5		11239.79	
Intercept	4093.63		19955.64	
R^2	0.9996		0.99958	

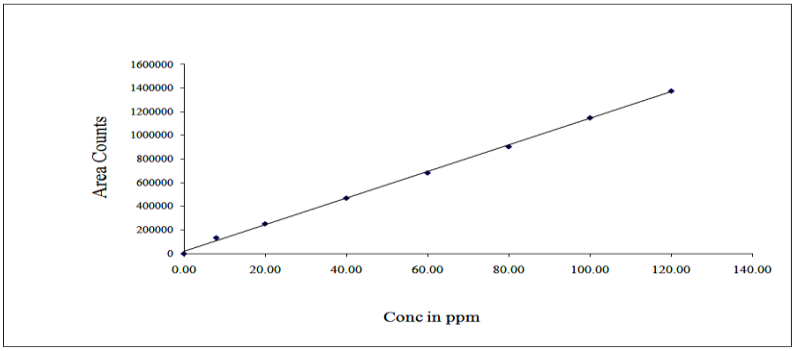

Fig: Calibration curve for Tadalafil at 249 nm

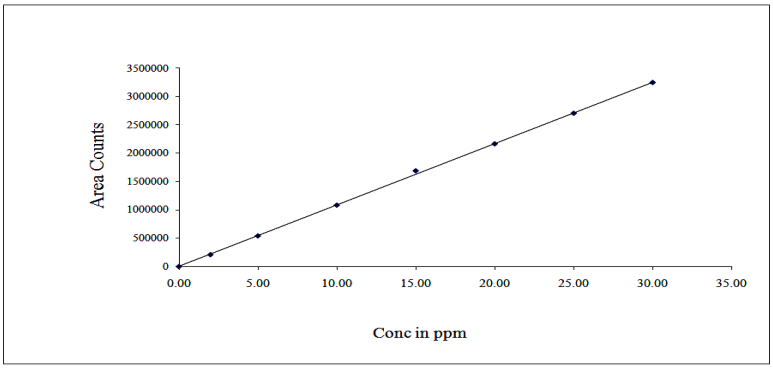

Fig: Calibration curve for Ambrisentan at 249 nm

PRECISION: System Precision:

Table: System precision table of Ambrisentan and Tadalafil

S. No	Concentration Ambrisentan (µg/ml)	Area of Ambrisentan	Concentration of Tadalafil (µg/ml)	Area of Tadalafil
1.	20	2064257	80	844562
2.	20	2058964	80	846572
3.	20	2045268	80	845962
4.	20	2054268	80	845721
5.	20	2047895	80	845936
6.	20	2056421	80	846257
Mean		2054512		845835
S.D		7038.826		690.088
%RSD		0.34		0.08

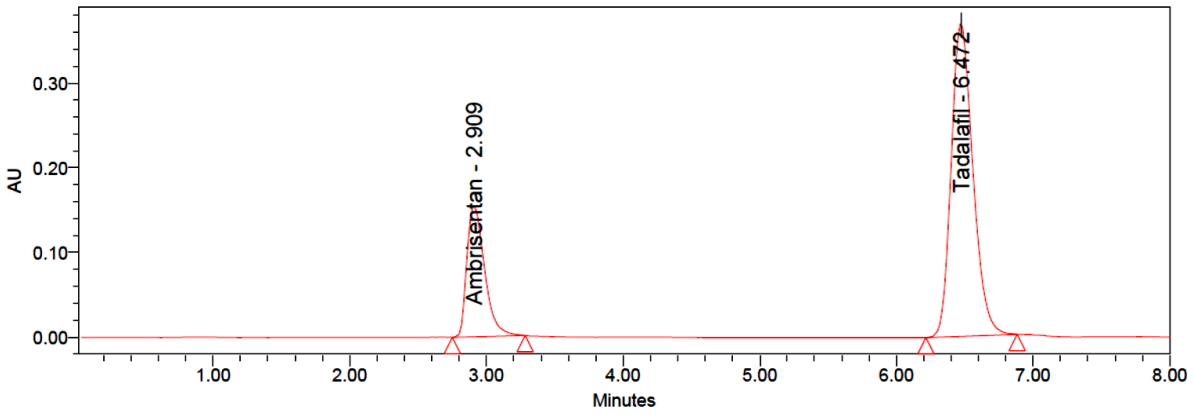

Fig: System precision chromatogram-1

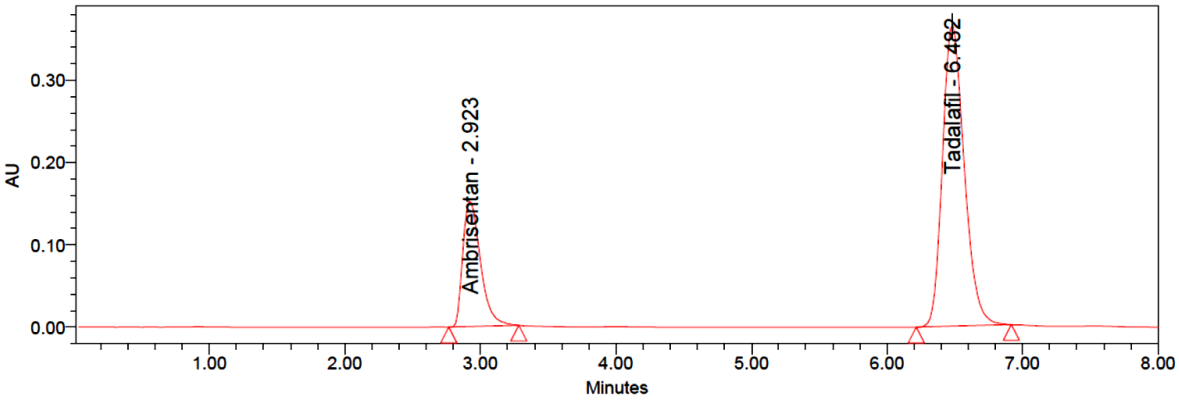

Fig: System precision chromatogram-2

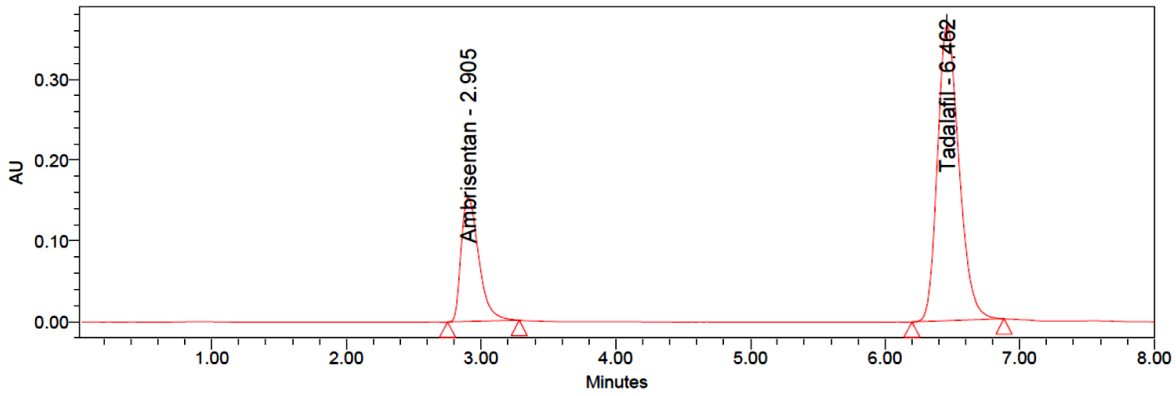

Fig: System precision chromatogram-3

Discussion: From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.34% and 0.08% respectively for Ambrisentan and Tadalafil. As the limit of Precision was less than "2" the system precision was passed in this method.

REPEATABILITY:

Table: Method Precision for Ambrisentan and Tadalafil by RP-HPLC method

S. No.	Area for Ambrisentan	Area for Tadalafil
1	2046315	845316
2	2048761	845796
3	2048697	842678
4	2043679	842368
5	2013675	842679
6	2016795	845796
Average	2036320	844106
Standard Deviation	16468.425	1689.524
%RSD	0.81	0.20

Acceptance Criteria: The % RSD for the area of six standard injections results should not be more than 2%**.**

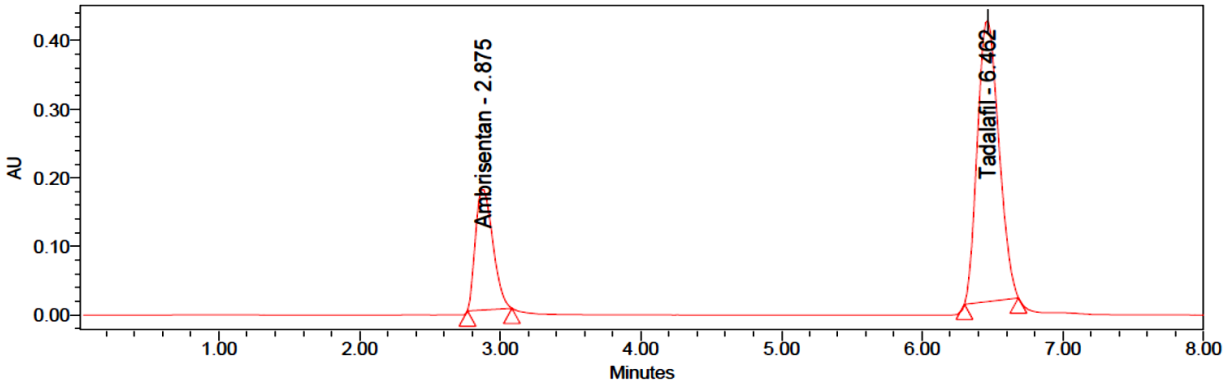

Fig: Repeatability chromatogram

INTERMEDIATE PRECISION (DAY-DAY PRECISION):

Table: Intermediate Precision (Day variation)

S. No.	Area for Ambrisentan		Area for Tadalafil	
	Day-1	Day-2	Day-1	Day-2
1	2016375	2045632	847658	846532
2	2046972	2048965	841675	845293
3	2046966	2051493	842597	847632
4	2043675	2065871	842365	846325
5	2045325	2045236	845794	842638
6	2069586	2058912	845316	843795
Average	2044817	2052685	844234	845369
Standard Deviation	16930.89	8162.571	2368.523	1861.683
%RSD	0.83	0.40	0.28	0.22

Acceptance Criteria: The % RSD for the area of six standard injections results should not be more than 2%.

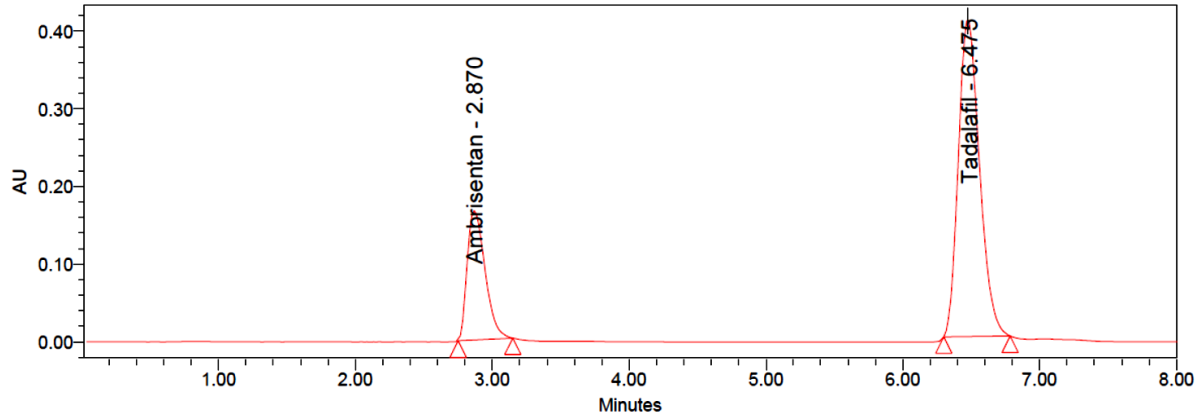

Fig: Inter day precision chromatogram

ACCURACY:

Table: Accuracy results of Ambrisentan by RP-HPLC method

%Concentration(at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1118353	10	10.2	100.26	100.29
100%	2036487	20	20.12	99.75	
150%	3255143	30	30.15	101.49	

Table: The Accuracy results for Tadalafil by RP-HPLC method

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	432691	40	40.24	100.25	100.35
100%	845362	80	80.17	99.95	
150%	1377946	120	120.16	100.86	

Discussion: Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 100.25% and 100.86% for Ambrisentan and Tadalafil respectively.

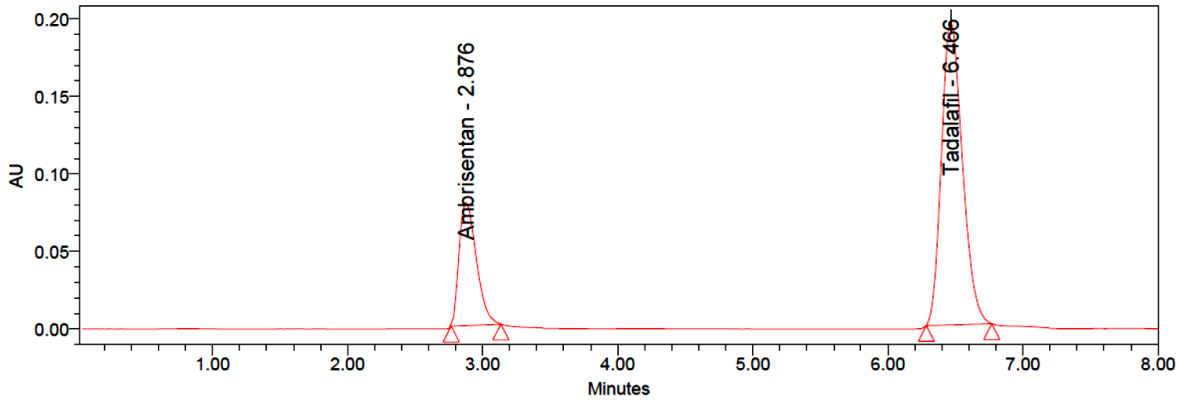

Fig: Chromatogram of Accuracy 50%

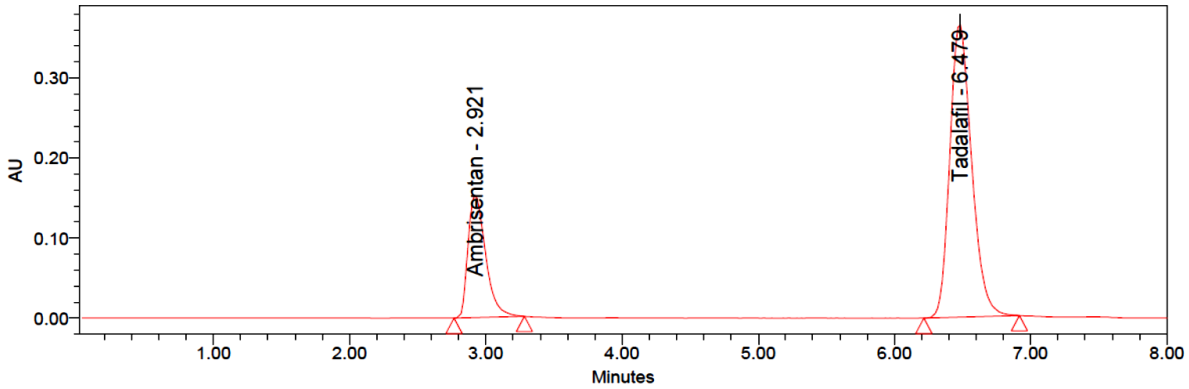

Fig: Chromatogram of Accuracy 100%

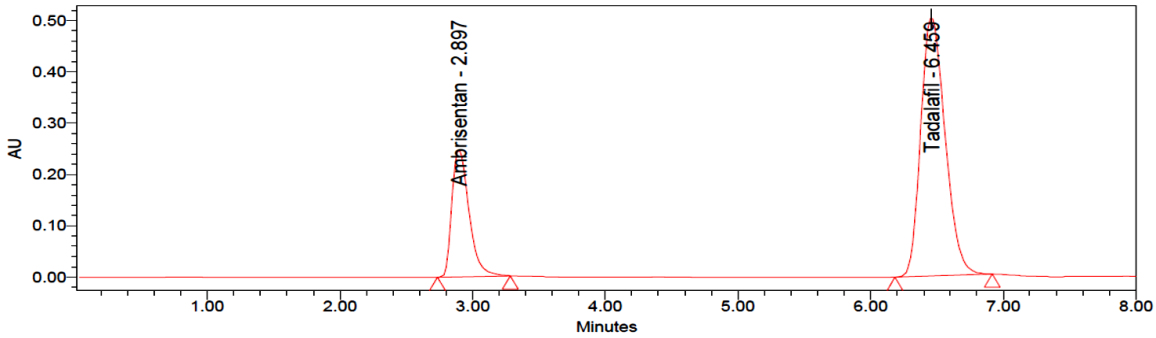

Fig: Chromatogram of Accuracy 150%

ROBUSTNESS:

Table: Robustness results of Ambrisentan by RP-HPLC

Parameter	Ambrisentan					
	Condition	Retention time(min)	Peak area	Resolution	Tailing	Plate count
Flow rate Change(mL/min)	Less flow(0.8ml)	3.811	2146875		1.12	4679
	Actual(1ml)	2.824	2046252		1.11	4645
	More(1.2ml)	2.308	1946759		1.12	4792
Organic Phase change	Less Org (36:64)	2.944	2147682		1.13	4893
	Actual(40:60)	2.842	2046972		1.15	4728
	More Org(44:56)	2.601	1967925		1.14	4896

Table: Robustness results of Tadalafil by RP-HPLC

Parameter	Tadalafil					
	Condition	Retention time(min)	Peak area	Resolution	Tailing	Plate count
Flow rate Change(mL/min)	Less flow(0.8ml)	8.665	856478	14.26	1.11	5579
	Actual(1ml)	6.415	845623	13.21	1.10	5542
	More flow(1.2ml)	5.213	826248	11.28	1.15	5679
Organic Phase change	Less Org (36:64)	8.546	856972	15.24	1.16	5816
	Actual(40:60)	6.462	845931	14.28	1.13	5684
	More Org(44:56)	5.469	821679	10.39	1.12	5795

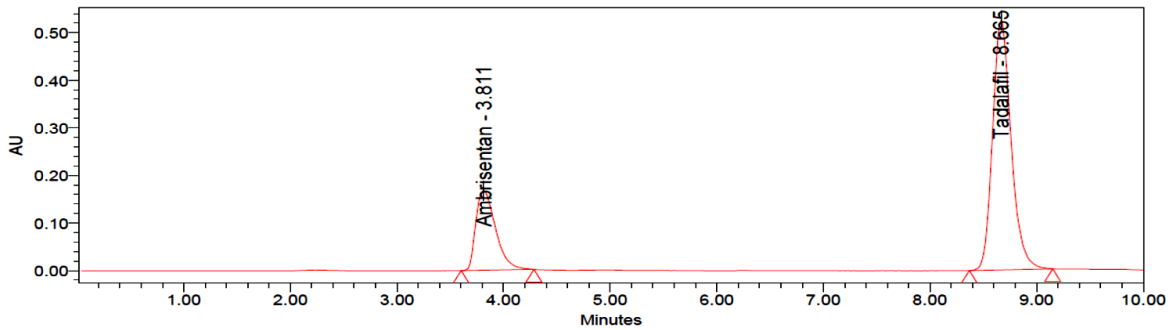

Fig: Chromatogram for less flow rate (0.8 ml)

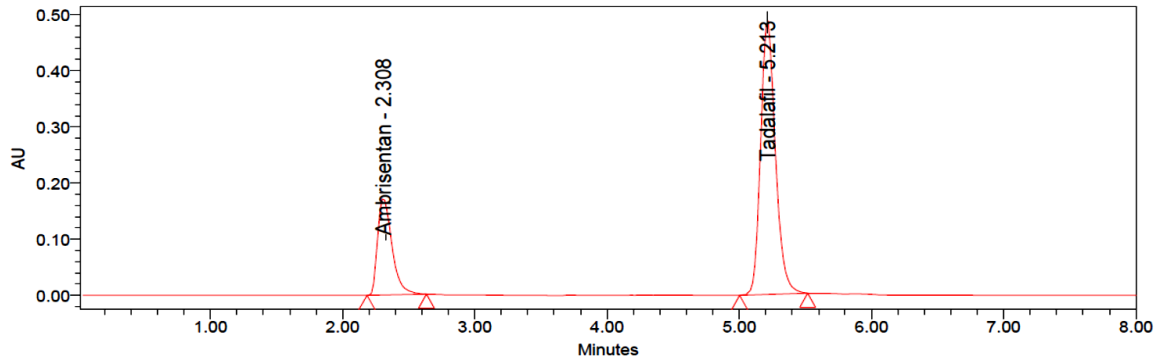

Fig: Chromatogram for more flow rate (1.2mL)

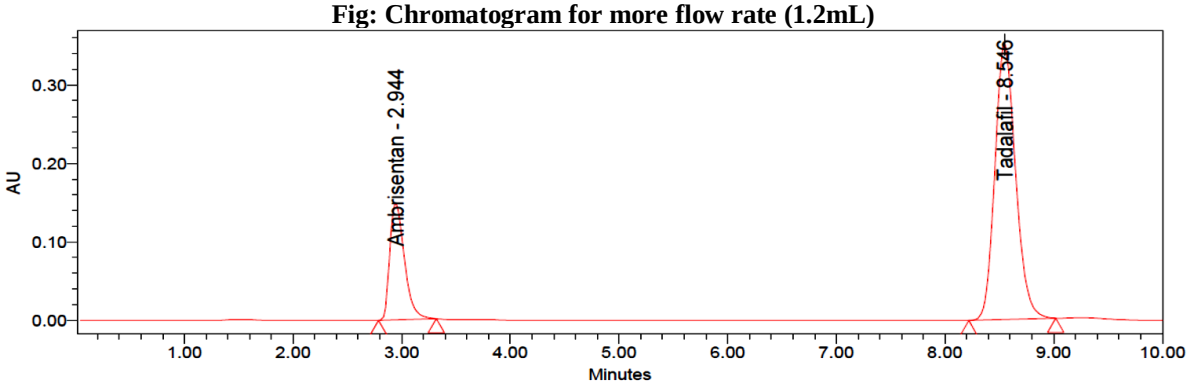

Fig: Chromatogram for less Organic Phase (36:64)

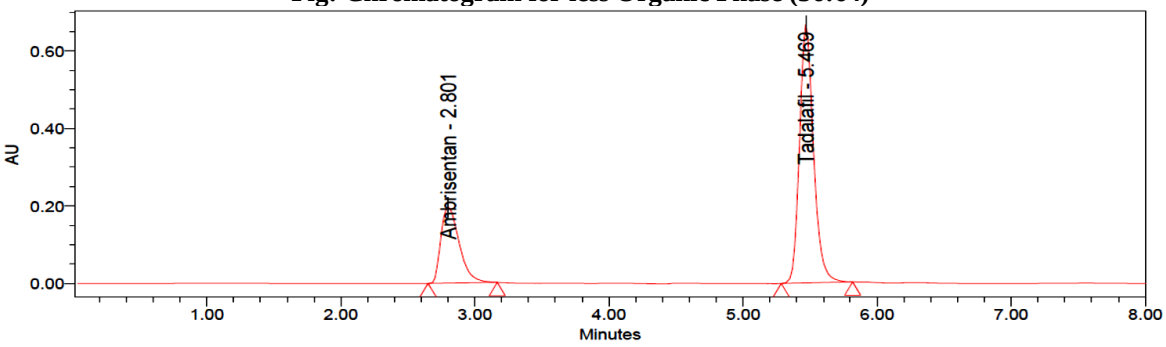

Fig: Chromatogram for more Organic Phase (44:56)

LOD and LOQ (µg/ml):

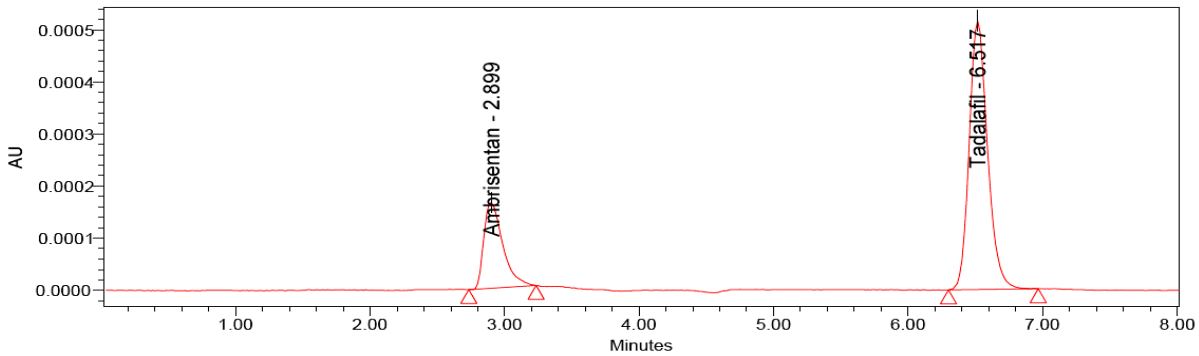

Fig: Chromatogram for LOD

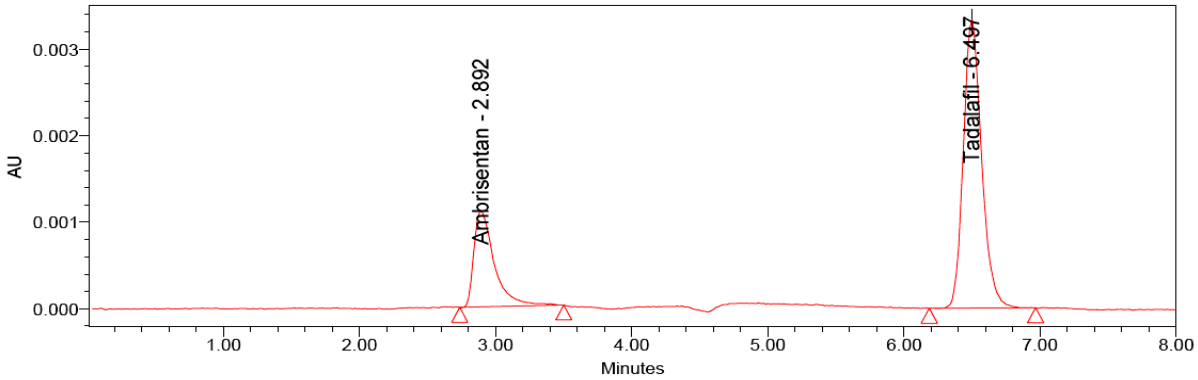

Fig: Chromatogram for LOQ

Table: Sensitivity parameters (LOD & LOQ) by RP-HPLC

Name of drug	LOD(µg/ml)	LOQ(µg/ml)
Ambrisentan	0.02	0.2
Tadalafil	0.08	0.8

SUMMARY

- Gives the general information on RP-HPLC and method development.
- Discusses about drug profiles and official status of selected drugs i.e., Ambrisentan and Tadalafil
- Explains in detail the previous literature available for drugs used for developed research work.
- Gives in detail about the aim, objective and plan of the proposed work by using selected drugs.
- Include RP-HPLC Method Development and Validation for Simultaneous Estimation of Ambrisentan and Tadalafil in Bulk and their Pharmaceutical dosage form. Using Shimadzu HPLC accomplished with Cap cell pack C18 column with 20µl is injected eluted with the mobile phase containing 0.1% Triethyl amine pH=7.0 adjusted with Ortho phosphoric acid and Acetonitrile in the ratio of 60:40 v/v which is pumped at a flow rate of 1ml/min and detected by UV detector at 249nm. The peak of Ambrisentan and Tadalafil were eluted at retention times of 2.921 min and 6.428 min respectively.
- In this proposed HPLC method for the selected drugs showed good linearity. Results for the recoveries of selected drugs were found to be within limits (98 – 102 %). These indicate that the proposed method was accurate for the analysis.

CONCLUSION:

The developed HPLC method for the estimation of selected drugs is simple, rapid, accurate, precise, robust and economical. The mobile phase and solvents are simple to prepare and economical, reliable, sensitive and less time consuming. The sample recoveries were in good agreement with their respective label claims and they suggested non-interference of formulation recipients in the estimation and can be used in laboratories for the routine analysis of selected drugs.

Since the system validation parameters of HPLC method used for estimation of selected drugs in pure and have shown satisfactory, accurate and reproducible results (without any interference of recipients) as well, it is deduced that the simple and short proposed methods be most useful for analysis purpose. The present work concluded that assay method by RP-HPLC was simple, accurate, precise, and specific and has no interference with the placebo. Hence these can be used for routine analysis of Ambrisentan and Tadalafil.

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