World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: https://wjpsonline.com/
Research Article

Analytical Method Development and Validation of Macitentan and Tadalafil Using RP-HPLC in Bulk and Tablet Formulations.

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Received: 25-08-2025 / Revised Accepted: 27-08-2025 / Published: 29-08-2025

ABSTRACT:
the simultaneous evaluation of Macitentan and carbidoxopa. The chromatogram was performed using Agilent (250mm 4.6mm, 5µ). A mobile phase consisting of 0.1% OPA, acetonitrile, in a 45:55 ratio was injected across the column at a flow rate of 1.0 ml/min. The temperature was kept steady at 30°C. The optimal wavelength for Macitentan and carbidogopa was 220 nm. Macitentan and carbidogpa's percentage RSDs were 0.9 and 0.8, respectively. The retention durations for Macitentan and carbidogopa were found to be 2.443 and 2.990 minutes, respectively. The Recovery was found to be 99.89% and 99.58% Respectively. The regression equation for Macitentan is y = 142567x + 5088.2, whereas that for Tadalafil is y = 203509x + 9089.6.
Key Words: Macitentan, Tadalafil, Rp Hplc, Validation.

INTRODUCTION

The first type of pulmonary hypertension, known as pulmonary arterial hypertension (PAH), is a chronic, progressive condition marked by angioproliferative vasculopathy in the pulmonary arterioles, which causes inflammation, thrombosis, and the proliferation and dysfunction of smooth and endothelial muscles.[1] It is a dangerous illness that causes extremely high blood pressure in the arteries that supply your lungs. Your heart may be under stress from this pressure, which will make it more difficult for it to pump blood through your lungs. This illness requires cautious management because it can cause extreme exhaustion and dyspnoea.[2] The combination of macitentan and tadalafil is used to treat PAH, or pulmonary arterial hypertension.[3] Macitentan is an endothelin receptor antagonist used to manage pulmonary arterial hypertension to delay disease progression.[4] Tadalafil is a phosphodiesterase 5 inhibitor used to treat erectile dysfunction, benign prostatic hyperplasia, and pulmonary arterial hypertension.[5] Macitentan is chemically written as {[5-(4-bromophenyl)-6-{2-[(5-bromopyrimidin-2- yl)oxy] ethoxy}pyrimidin-4-yl]sulfamoyl}(propyl)amine and it is an antagonist that inhibits endothelin -1 and -2 signalling by binding to the endothelin A and B receptors (EA and EB). Tadalafil is chemically written as (2R,8R)-2-(2H-1,3-benzodioxol-5-yl)-6-methyl-3,6,17-triazatetracyclo [8.7.0.0^ {3,8}.0^{11,16}]heptadeca -1(10),11,13,15-tetraene-4,7-dione. It is a selective phosphodiesterase - 5 (PDE5) inhibitor produces several downstream effects with the most common therapeutic effect being smooth muscle relaxation.

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How to Cite this Article: Nandhimalla Sruchitha Reddy, Analytical Method Development and Validation of Macitentan and Tadalafil Using RP-HPLC in Bulk and Tablet Formulations. World J Pharm Sci 2025; 13(03): 127-137; https://doi.org/10.54037/WJPS.2022.100905

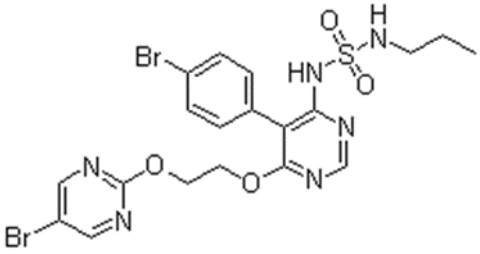


Figure 1: structure of Macitentan

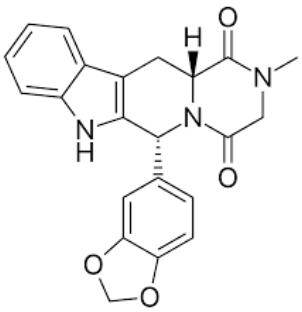


Figure 2: Structure of Tadalafil

Extensive literature research has unearthed a multitude of recorded analytical procedures, including the discovery of more economically efficient ways. Nevertheless, there is currently no documented approach for calculating stability studies. Hence, a reliable and cost-effective approach is suggested for assessing the stability of Macitentan, Tadalafil, and their medicinal dose form using RP-HPLC [6- 10] must be validated and developed as per ICH guidelines

Materials and Methods: Spectrum pharma Research Solution with Macitentan and Tadalafil pure drugs (API) gift samples and Combination Macitentan and Tadalafil tablets (**Opsynvi**). The chemicals and buffers utilized in this estimation were obtained from Rankem, an Indian supplier.

Instrumentation: The development and method validation were conducted using a WATERS HPLC, specifically the model 2695 SYSTEM, equipped with a Photo diode array detector. The system also included an automated sample injector and the Empower 2 software.

Objective: In order to fulfill ICH standards, we need to design and test an HPLC technique that can detect Tadalafil and Macitentan in pharmaceutical formulations at the same time.

Table 1: Chromatographic Conditions

Mobile phase	Acetonitrile and OPA (55:45 v/v)
Flow rate	1 ml/min
Column	Agilent C18 (4.6 x 150mm, 5µm)
Detector wave length	220 nm
Column temperature	30°C
Injection volume	10 µL
Run time	10.0 min
Buffer	Ortho Phosphoric Acid

Buffer Preparation: Accurately take 1.0 ml of OPA in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1ml of Triethylamine then PH adjusted to 4.8 with dil. Triethylamine.

API Preparation:

Preparation of Standard stock solutions: Accurately weighed 5 mg of Macitentan, 10 mg of Tadalafil and transferred to 50 ml volumetric flask separately. 3/4 th of diluents was added to both flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (100µg/ml of Macitentan and 200µg/ml of Tadalafil)

Preparation of Standard working solutions (100% solution): 1ml from stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (10µg/ml Macitentan and 20µg/ml of Tadalafil)Formulation Preparation:

Preparation of Sample stock solutions: 10 tablets were weighed and equivalent to 1 tablet is weighed and transferred to 100 ml volumetric flask, to this 50 ml of acetonitrile was added and sonicated. Volume was made upto 100ml with diluents and filtered through 0.45 µm or finer porosity membrane filter (100µg/ml of Macitentan and 200µg/ml of Tadalafil)

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (10µg/ml of Macitentan and 20µg/ml of Tadalafil)

System suitability parameters: Macitentan (10 ppm) and Tadalafil (20 ppm) standard solutions were prepared, injected six times, and metrics such as peak tailing, resolution, and USP plate count were measured in order to evaluate the system suitability parameters. The region of six standard injection results should have an RSD of no more than 2%.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific.

Table 2: System suitability results

S.no	Macitentan			Tadalafil			
Inj	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	2.430	7453	1.33	2.987	6290	1.78	4.2
2	2.431	7691	1.30	2.989	6641	1.81	4.2
3	2.432	7936	1.35	2.989	6232	1.86	4.2
4	2.435	8597	1.33	2.993	5666	1.92	4.0
5	2.440	7912	1.29	2.994	5973	1.88	4.2
6	2.445	7792	1.30	2.996	5716	1.85	4.1

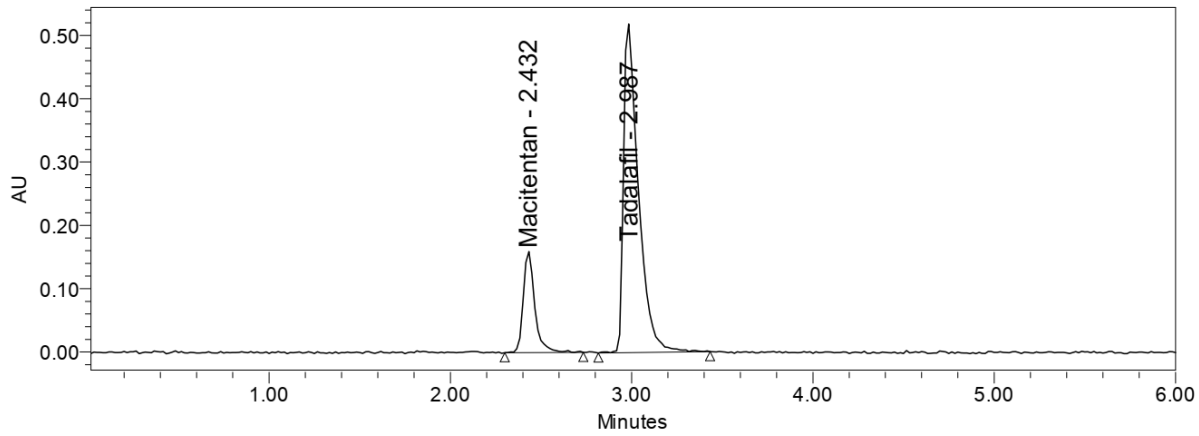


Figure 3: system suitability Chromatogram

Table 3: Specificity data

Sample name	Retention time	Area	Plate count	Tailing	Resolution
Macitentan	2.443	1429210	8467	1.1	
Tadalafil	2.990	4057732	8210	1.0	2.8

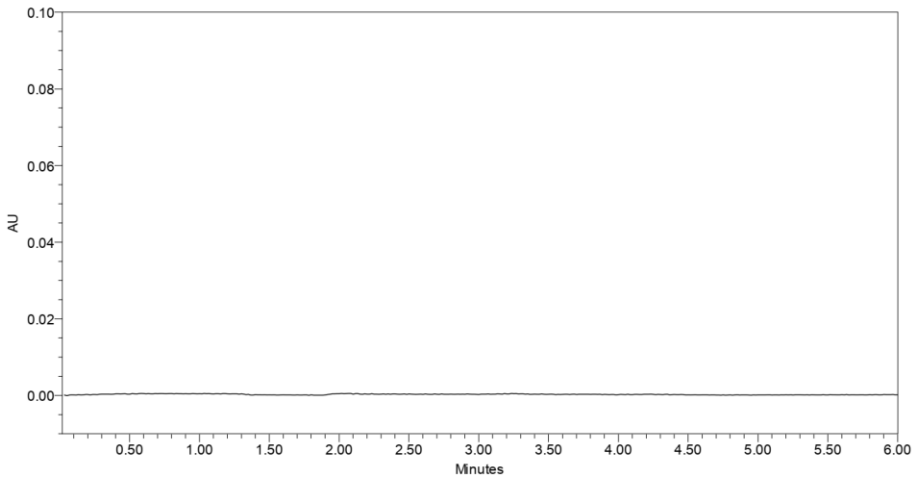


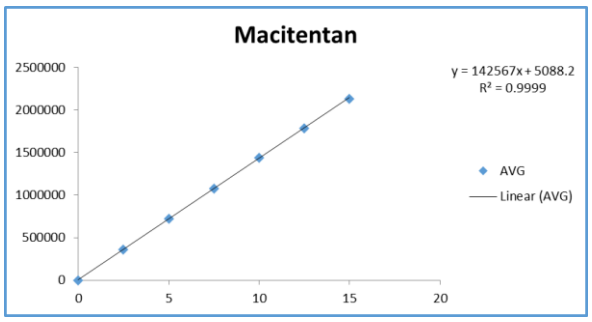
Figure.4 Specificity of Macitentan and Tadalafil

Linearity:

Calibration data is given in table 4 and regression data in table 5 and calibration curve in figure 6, 7

Table 4: Calibration data of Macitentan and Tadalafil

Macitentan		Tadalafil	
Conc (µg/mL)	**Peak area**	**Conc(µg/mL)**	**Peak area**
0	0	0	0
2.5	361192	5	988217
5	718498	10	2114444
7.5	1076947	15	3045618
10	1441543	20	4059694
12.5	1787587	25	5149819
15	2134619	30	6074259



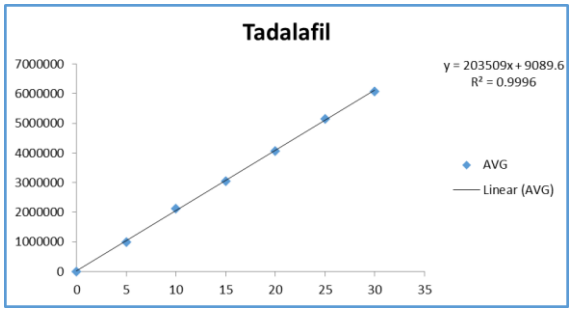


Figure 5 Calibration curve of Macitentan **Figure 6 Calibration curve of Tadalafil**

Table 5: regression data

Parameter	Macitentan	Tadalafil
Conc range (µg/mL)	2.5-15	5-30
Regression Equation	y = 142567x + 5088.2	y = 203509x + 9089.6
Co-relation	0.999	0.999

Accuracy:

Recovery data shown in table 6

Table 6: recovery data of Macitentan and Tadalafil

	Macitentan			Tadalafil		
% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**
50%	5	4.951	99.03	10	9.915	99.15
	5	4.954	99.08	10	9.969	99.69
	5	4.967	99.35	10	9.928	99.28
100%	10	9.934	99.34	20	20.035	100.18
	10	10.115	101.15	20	19.818	99.09
	10	10.006	100.06	20	19.808	99.04
150%	15	15.182	101.21	30	29.938	99.79
	15	14.988	99.92	30	30.134	100.45
	15	14.977	99.85	30	29.877	99.59
% recovery	**99.89**			**99.58**		

System precision was performed and the data was shown in table 8

Table 7: System precision of Macitentan and Tadalafil

S. No	Area of Macitentan	Area of Tadalafil
1.	1435907	4042716
2.	1438691	3997290
3.	1428726	3984344
4.	1465036	4065429
5.	1435574	4011325
6.	1436003	4006773
Mean	1439990	4017980
S.D	12711.7	30307.2
%RSD	0.9	0.8

The % RSD for the peak areas of Macitentan and Tadalafil obtained from six replicate injections of standard solution was within the limit.

Method Precision: The precision of the method was determined by analyzing a sample of Macitentan and Tadalafil and shown in table 8.

Table 8: method Precision

S. No	Area of Macitentan	Area of Tadalafil
1.	1437152	3994166
2.	1431131	4021032
3.	1434219	4018398
4.	1440345	3990315
5.	1435989	4014023
6.	1428801	3987269
Mean	1434606	4004201
S.D	4177.4	15241.5
%RSD	0.3	0.4

From the above results, the % RSD of method precision study was within the limit for Macitentan and Tadalafil.

Robustness: Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.0ml/min), mobile phase minus (40A:60B), mobile phase plus (50B:50A), temperature minus (27°C) and temperature plus(33°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Table 9: Robustness data for Macitentan and Tadalafil.

Condition	%RSD of Macitentan	%RSD of Tadalafil
Flow rate (-) 0.9ml/min	0.5	0.8
Flow rate (+) 1.1ml/min	0.3	0.7
Mobile phase (-) 40A:60B	0.4	0.2
Mobile phase (+) 50A:50B	0.2	1.2
Temperature (-) 27°C	0.6	1.0
Temperature (+) 33°C	0.6	0.3

Sensitivity:

Table 10: sensitivity of Macitentan and Tadalafil

Molecule	LOD	LOQ
Macitentan	0.02 µg/ml	0.08 µg/ml
Tadalafil	0.08 µg/ml	0.23 µg/ml

Force Degradation Studies: table 11 shows degradation conditions and table 10 shows the obtained degraded data and purity plot chromatogram in figure 8, 9.

Table 11: degradation conditions

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^{0}c	60 mins
Base	2N NAOH	60^{0}c	60 mins
Oxdation	20% H_2O_2	60^{0}c	60 mins
Thermal	Diluent	105^{0}c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^{0}c	60 mins

Table 12: degradation data

Type of degradation	Macitentan			Tadalafil		
	area	%recovered	% degraded	area	%recovered	% degraded
Acid	1482980	94.17	5.83	3851505	95.67	4.33
Base	1494952	93.91	6.09	3809255	94.62	5.38
Peroxide	1512114	94.1	5.94	3850029	95.63	4.37
Thermal	1527012	96.14	3.86	3914837	97.24	2.76
Uv	1530641	97.13	2.87	3947959	98.06	1.94
Water	1532456	99.12	0.88	3992087	99.16	0.84

Acid degradation chromatogram

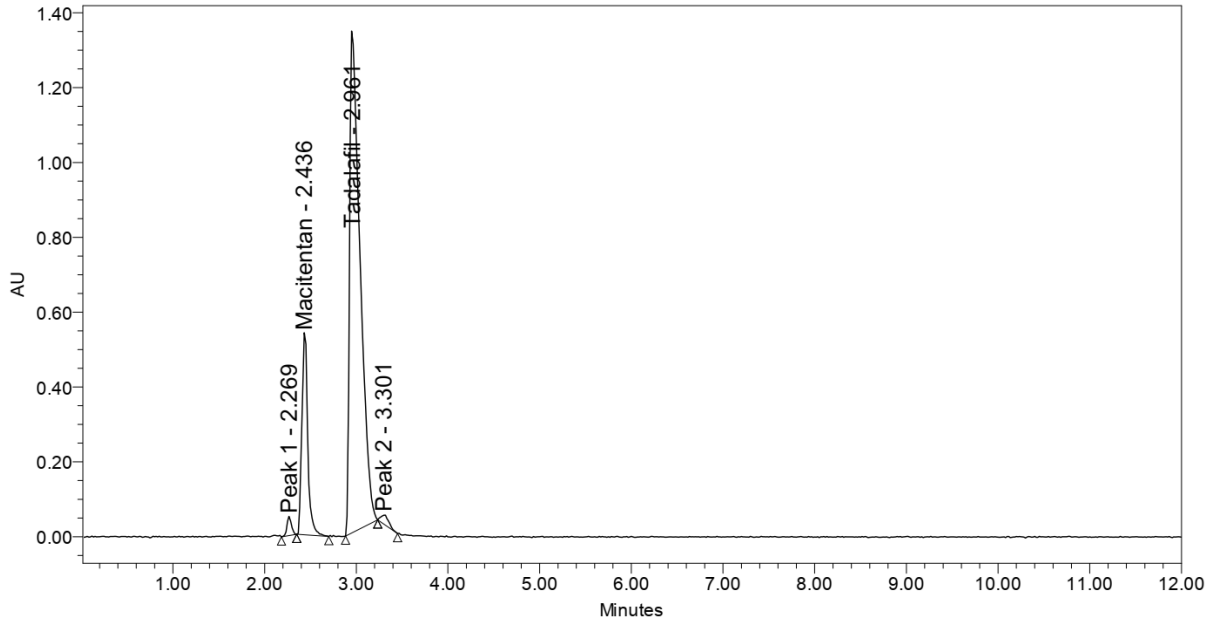


Figure 7 acid

Base degradation chromatogram

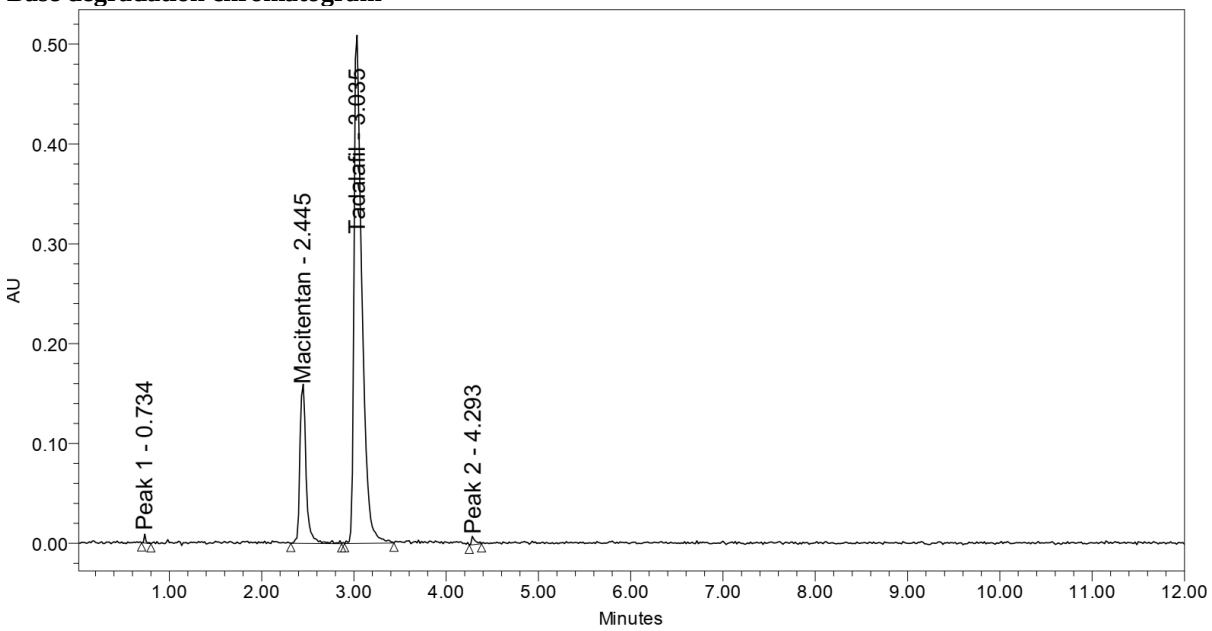


Figure 8 base

Peroxide degradation chromatogram

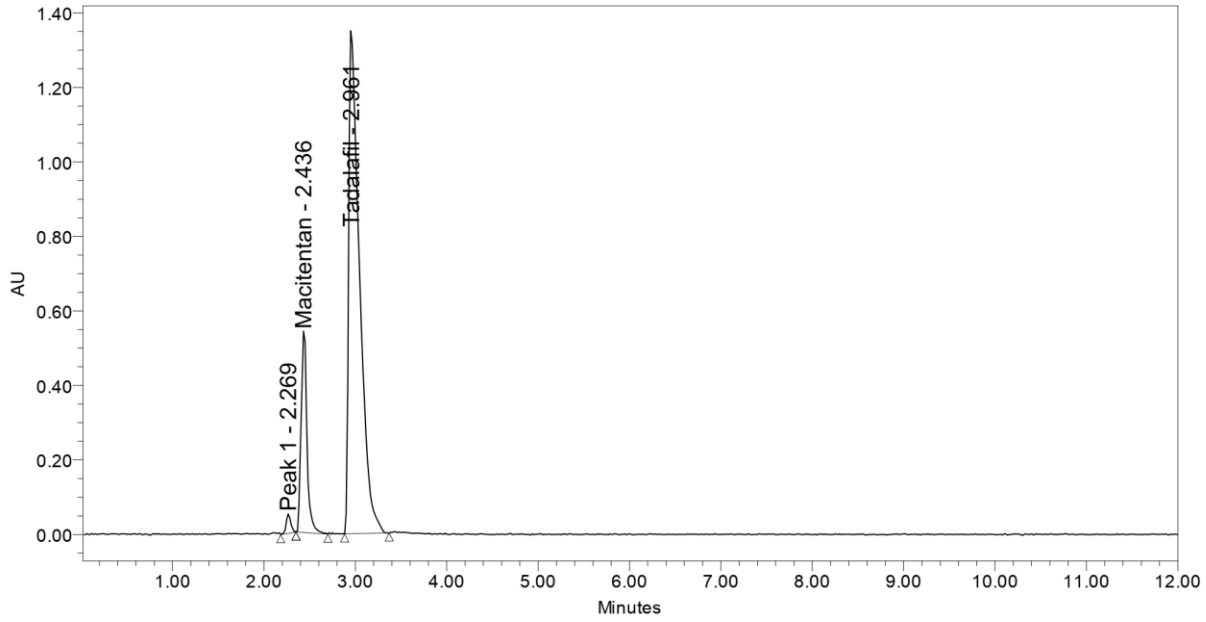


Figure 9 peroxide

Thermal degradation chromatogram

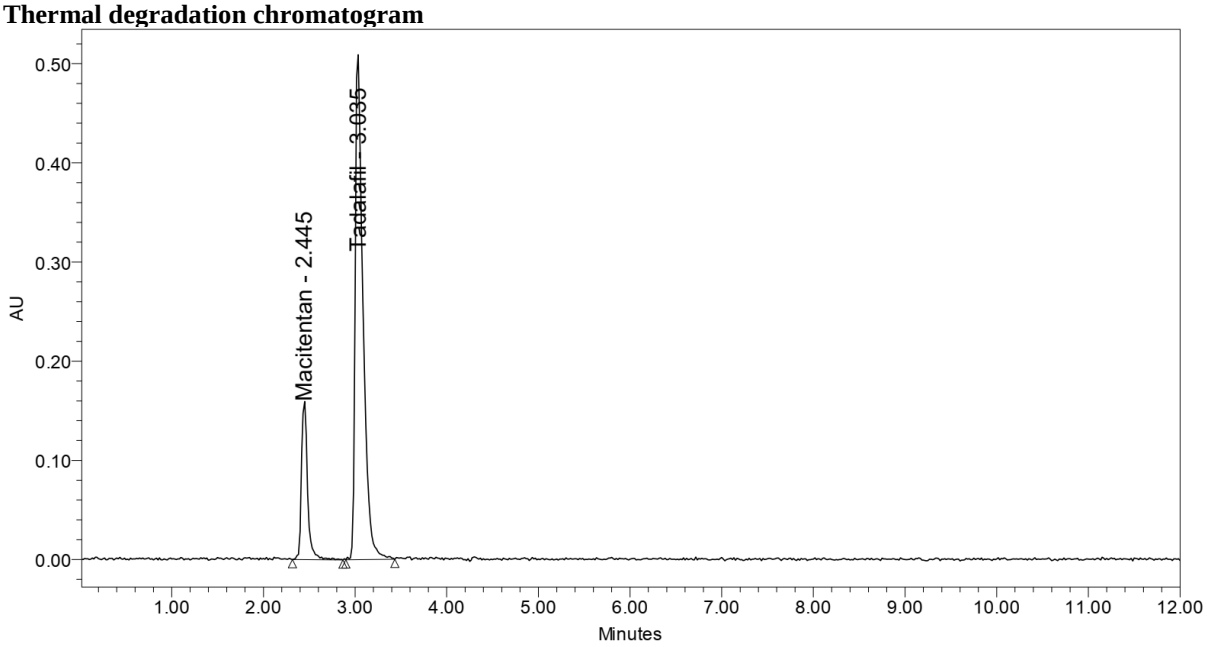


Figure 10 thermal

UV degradation chromatogram

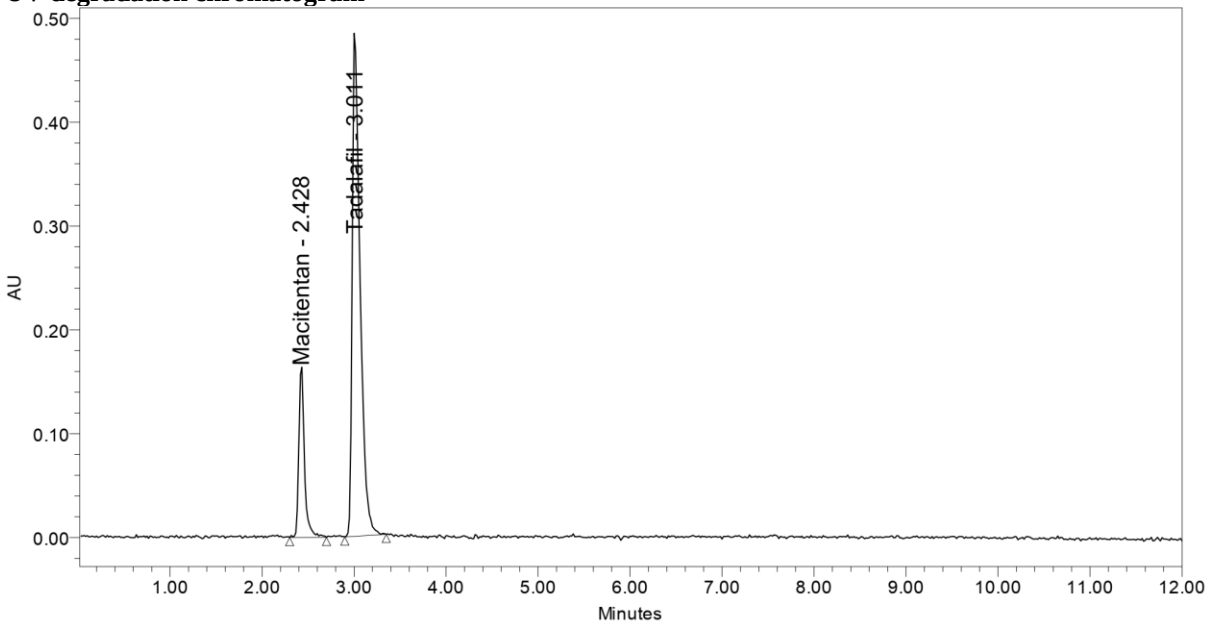


Figure 11 UV

Water degradation chromatogram

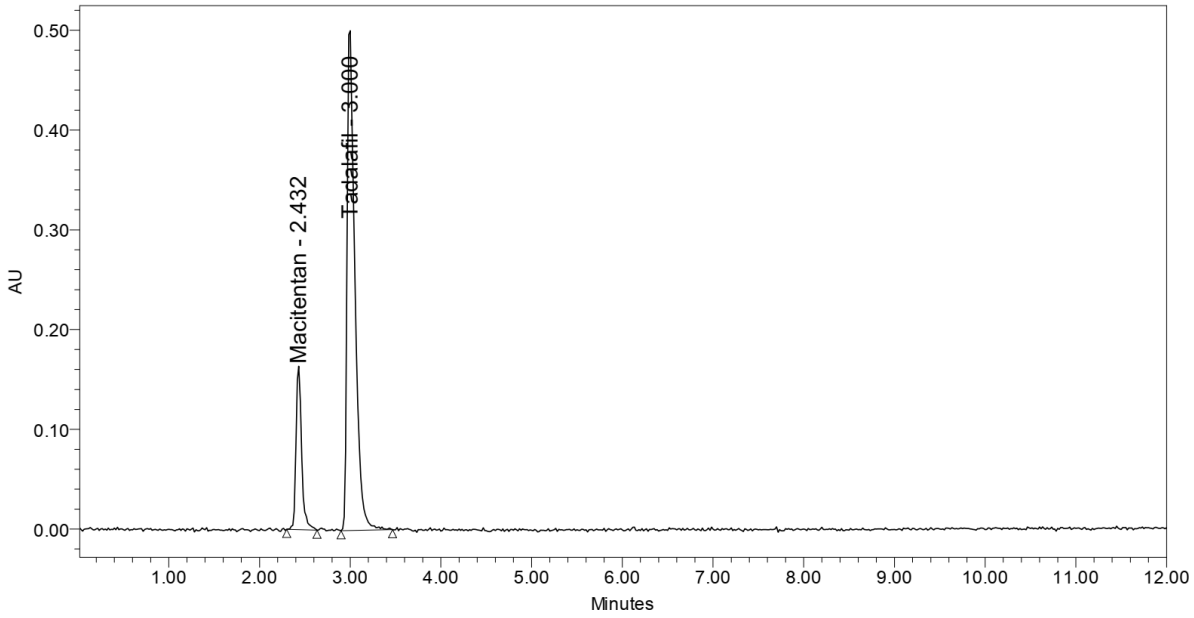


Figure 12 water

Assay: Opsynvi, bearing the label claim Macitentan 10mg, Tadalafil 40mg. Assay was performed with the above formulation. Average % Assay for Macitentan and Tadalafil obtained was 99.43% and 99.46% respectively.

Table 13: assay data

	Macitentan			Tadalafil		
S.no	**Std Area**	**Sample area**	**% Assay**	**Std Area**	**Sample area**	**% Assay**
1	1435907	1437152	**99.60**	4042716	3994166	**99.21**
2	1438691	1431131	**99.19**	3997290	4021032	**99.88**
3	1428726	1434219	**99.40**	3984344	4018398	**99.81**
4	1465036	1440345	**99.82**	4065429	3990315	**99.11**
5	1435574	1435989	**99.52**	4011325	4014023	**99.70**
6	1436003	1428801	**99.02**	4006773	3987269	**99.04**
Avg	1439990	1434606	**99.43**	4017980	4004201	**99.46**
Stdev	12711.7	4177.4	**0.29**	30307.2	15241.5	**0.38**
%RSD	0.9	0.3	**0.29**	0.8	0.4	**0.38**

Assay was calculated by the formula:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{1}{10} \times \frac{100}{1} \times \frac{10}{100} \times P \times \frac{FV}{L.C} \times 100$$

AT	Average Peak area of sample in test solution
AS	Mean peak area of sample in standard solution
WS	Weight of drug working standard taken in mg
P	Assay of drug working standard in % on dried basis
L.C	Label Claim

Figure 13 formula

Conclusion:

The results of the study will be very useful in assessing the quality of affordable medications that contain Macitentan and Tadalafil. This might be the consequence of the study's simple sample preparation procedure, which called for a short analysis time and minimal mobile phase. The evaluation of two drugs together in a single dosage showed that the newly developed analysis method was nearly full success.

ACKNOWLEDGEMENT:

The authors are thankful to, Department of Pharmaceutical Analysis, Pulla reddy college of Pharmacy, Affiliated to JNTUH India and Spectrum Pharma Research Solutions, Hyderabad, Telangana, India.

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