WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 6, Issue 1, 449-466. Research Article ISSN 2277– 7105

METHOD DEVELOPMENT AND VALIDATION OF NEW RP-HPLC METHOD FOR THE ESTIMATION OF BOSENTAN IN PHARMACEUTICAL DOSAGE FORM

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Article Received on
10 Nov. 2016,

Revised on 30 Nov. 2016,
Accepted on 20 Dec. 2016

DOI: 10.20959/wjpr20171-7207

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ABSTRACT

Present study aims to develop rapid, greater sensitivity and faster elution by RP-HPLC method for the estimation of Bosentan. The developed method will be validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines**.** The scope of developing and validating analytical methods is to ensure suitable methods for a particular analyte of more specific, accurate, precise and robust. The main objective for this is to improve the conditions and parameters, which should be followed in the development and validation. The existing physicochemical methods are inadequate to meet the requirements, hence it is proposed to improve the existing methods and to develop new methods for the assay of Bosentan.in pharmaceutical dosage forms adapting different available analytical techniques lik HPLC.

KEYWORDS: Bosentan, RP-HPLC, Method Development, Chromatographic Conditions, ICH guidelines.

1. INTRODUCTION

HPLC is a modern technique, it is a much more reliable and reproducible method for the standardization of both single and compound formulations. HPLC is a separation technique based on a stationary phase and a liquid mobile phase. Separations are achieved by partition, adsorption or ion exchange process, depending upon the size of stationary phase used.[1,2]

HPLC is one of the most versatile instruments used in the field of pharmaceutical analysis. It provides the following features[2,3]

- High resolving power
- Continuous monitoring of the column effluent
- Accurate quantitative measurement
- Repetitive and reproducible analysis using the same column
- Automation of the analytical procedure and data handling

TYPES OF MODES IN HPLC[3,4]

It includes.

BASED ON MODES OF SEPARATION

- Normal Phase Chromatography.
- Reverse Phase Chromatography.

BASED ON PRINCIPLE OF SEPARATION

- Adsorption Chromatography
- Ion exchange Chromatography
- Size exclusion Chromatography
- Affinity Chromatography
- Chiral phase Chromatography

BASED ON ELUTION TECHNIQUE

- Isocratic separation.
- Gradient separation.

NORMAL PHASE CHROMATOGRAPHY[5,6]

The term normal phase refers to a system where the stationary phase is a polar and mobile phase is a relatively non-polar liquid (Hexane, benzene, $CHCl_3$, etc). In this mode most probably used stationary phase is silica gel.

REVERSE-PHASE CHROMATOGRAPHY[6,7]

Reversed-phase chromatography refers to the use of a polar eluent with a non-polar stationary phase in contrast to normal-phase chromatography, where a polar stationary phase is employed with a non-polar mobile phase.

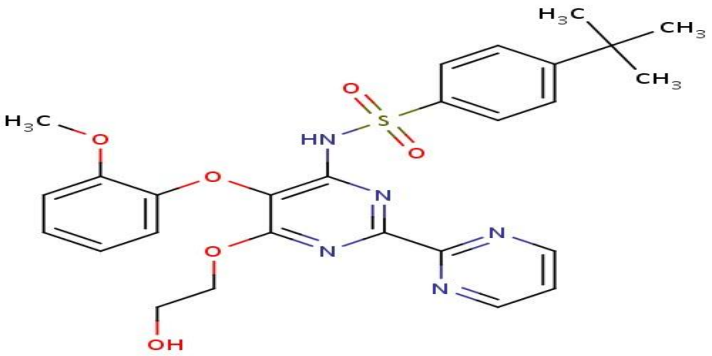
DEVELOPMENT OF RP- HPLC METHOD[7,8]

HPLC currently accounts for 35% of all instrument usage across the pharmaceutical and cosmetic industries and remains the fastest growing technique in both industries. HPLC provides reliable quantitative precision and accuracy, along with a linear dynamic range sufficient to allow for the determination of the Active Pharmaceutical Ingredient (API) and related substances in the same run using a variety of detectors along with excellent reproducibility and is applicable to a wide array of compound types by judicious choice of HPLC column chemistry. Major modes of HPLC include reverse phase and normal phase.

2. DRUG PROFILE

BOSENTAN

Structure



Chemical name : 4-tert-butyl-N-[6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)-2-(pyrimidin- 2-yl) pyrimidin-4-yl] benzene-1-sulfonamide

Molecular formula : $C_{27}H_{29}N_5O_6S$

Molecular weight : 551.614

Dose : 125mg

Description : Bosentan is a white to yellowish powder, In the solid state, Bosentan is very stable, is not hygroscopic and is not light Sensitive.

Solubility : Methanol

Melting point : 107-110°C

Category : Antihypertensive Agents, an endothelin receptor antagonist that belongs to a class of highly substituted pyrimidine derivatives.

Brand name : Tracellar

MECHANISM OF ACTION

Bosentan is a dual endothelin receptor antagonist. Endothelin-1 (ET-1) is a neurohormone, and a potent vasoconstrictor with the ability to promote fibrosis, cell proliferation and tissue remodeling. The effects of which are mediated by binding to ET_A and ET_B receptors in the endothelium and vascular smooth muscle. ET-1 concentrations are elevated in plasma and lung tissue of patients with pulmonary arterial hypertension, suggesting a pathogenic role for ET-1 in this disease. Bosentan exerts a specific and competitive antagonist at endothelin receptor types ET_A and ET_B, with a slightly higher affinity for ET_A than ET_B receptors. Bosentan decreases both pulmonary and systemic vascular resistance resulting in increased cardiac output without increasing heart rate.

3. MATERIALS AND METHODS

TABLE NO-1: SHOWS CHEMICALS AND REAGENTS

S. No.	Chemicals/standards and reagents	Grade	Make
1	Potassium dihydrogen phosphate	HPLC	Fisher
2	Ortho phosphoric acid	HPLC	Fisher
3	HPLC Grade Methanol	HPLC	Merck
4	HPLC Grade Acetonitrile	HPLC	Merck
5	Double Distilled Water	HPLC	Merck
6	Bosentan	N/A	Cipla

OPTIMIZED METHOD

Preparation of Phosphate buffer

Accurately weighed and placed 2.72gm of potassium dihydrogen phosphate in 1000 ml of volumetric flask. Add about 900 ml of water and sonicate and make up to the final volume with ml of water, adjust pH to 3 with dilute Orthophosphoric acid solution.

Preparation of mobile phase

A mixture of pH 3 phosphate buffer 500 ml (50%) and Acetonitrile 500 ml (50%) were mixed well, degassed in a Sonicator for about 10 minutes and filtered through 0.45 µ Millipore nylon filter.

Diluent Preparation

HPLC grade Methanol was used as diluent.

TABLE NO: 2: Optimized chromatographic conditions

PARAMETERS	CONDITIONS
Column (Stationary Phase)	Phenomenex C_{18} (250 x 4.6 mm, 5 µ)
Mobile Phase	Phosphate buffer pH 3: Acetonitrile (50:50)
Flow rate	0.8 ml/min
Run time	5min
Column temperature	Ambient
Volume of injection loop	10µl
Detection wavelength	223nm
Retention time (RT)	3.2

4. RESULTS AND DISCUSSION

TABLE NO-3: DATA OF TRAILS OF BOSENTAN

Trail No.	Mobile phase composition	Retention time Bosentan	Theoretical plates Bosentan	Tailing factor Bosentan	Inference
1.	Phosphate Buffer pH 3: Methanol (60:40) (V/V)	3.315	2282	1.3	low plate count and splitting of peaks
2.	Phosphate Buffer pH 3: Methanol (70:30) (V/V)	3.157	2156	1.3	low plate count and splitting of peaks
3.	Phosphate Buffer pH 3: Methanol (80:20) (V/V)	3.148	3013	1.2	Low R_t and high tailing factor
4.	Phosphate Buffer pH 3: Acetonitrile (70:30) (V/V)	2.807	4670	1.1	Low R_t and low tailing factor
5	Phosphate Buffer pH 3: Acetonitrile (50:50) (V/V)	3.240	2897	1.2	optimized

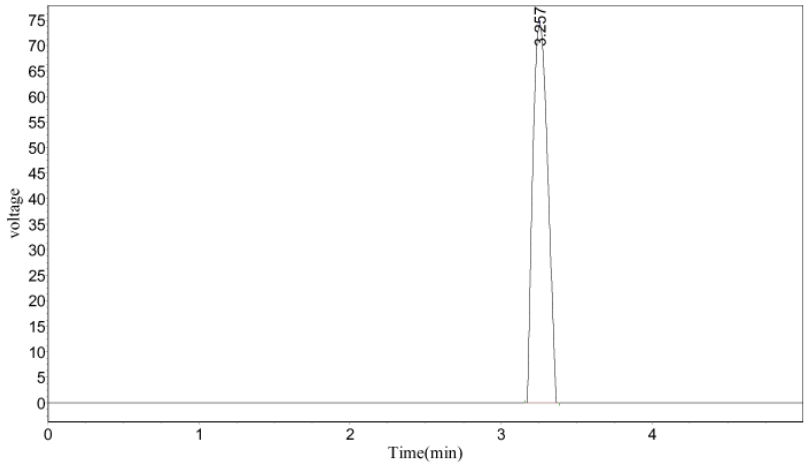


Fig-No-1: Standard Chromatogram – 1

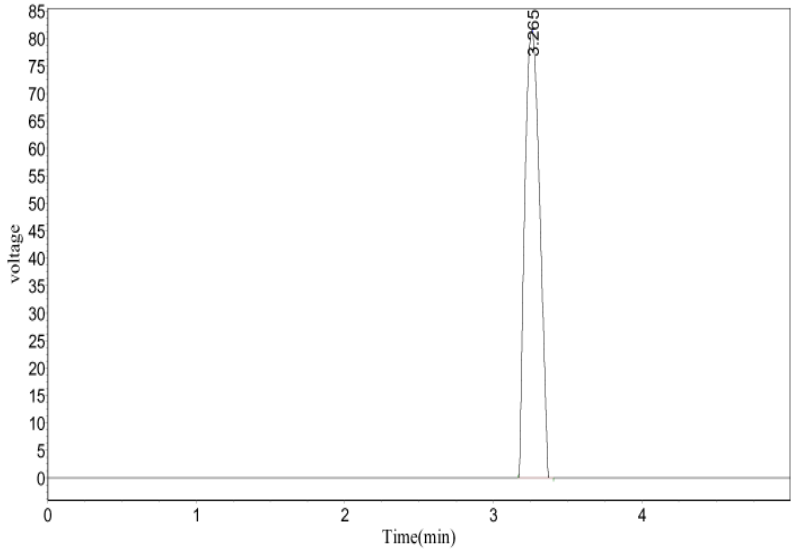


Fig-No-2: Standard Chromatogram -2

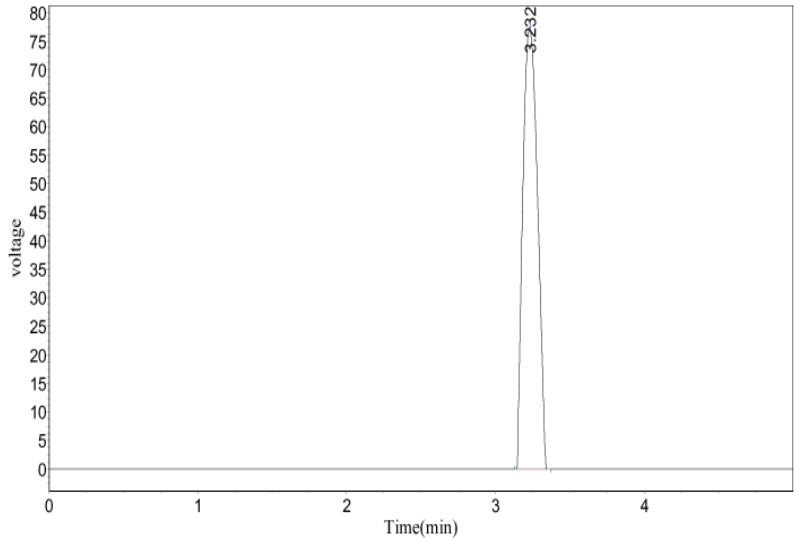


Fig-No-3: Standard Chromatogram – 3

TABLE NO-4: DATA OF ASSAY OF STANDARD CHROMATOGRAMS

Injection	Peak name	Retention time	Peak area	USP plate count	USP tailing
1.	Bosentan	3.25	509034	4196	1.17
2.	Bosentan	3.26	516539	4101	1.15
3.	Bosentan	3.23	511787	4131	1.17
Mean			512453		
Standard deviation			3796.61		
% RSD			0.740		

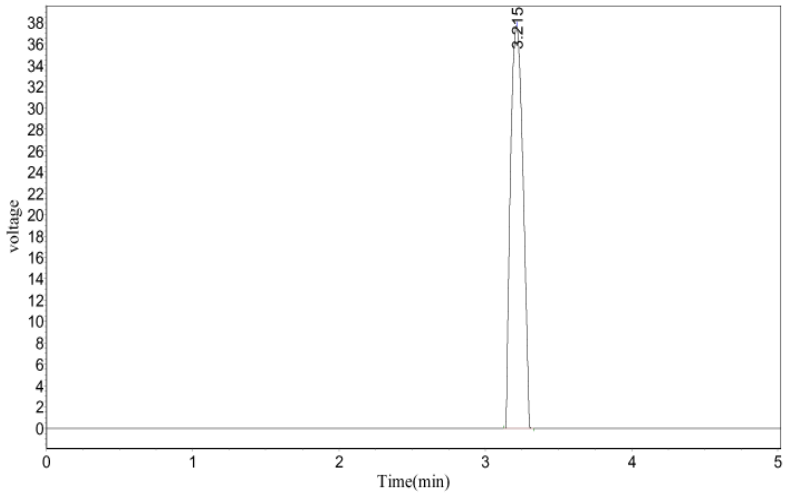


Fig-No-4: Sample chromatogram -1

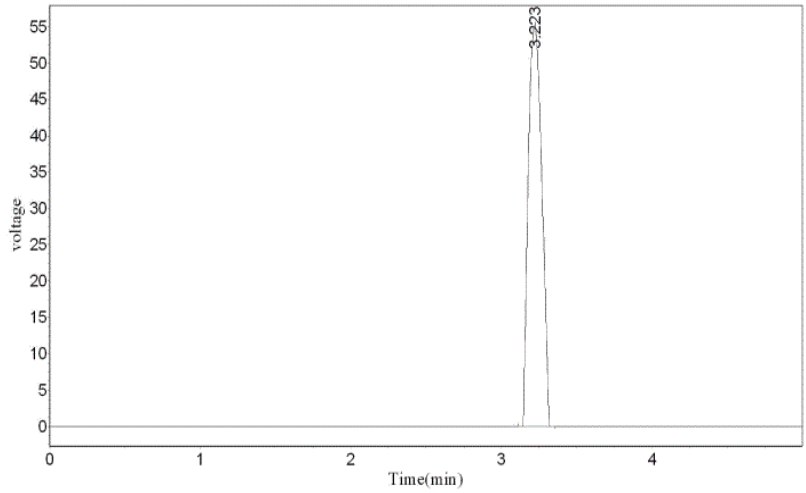


Fig-No-5: Sample Chromatogram-2

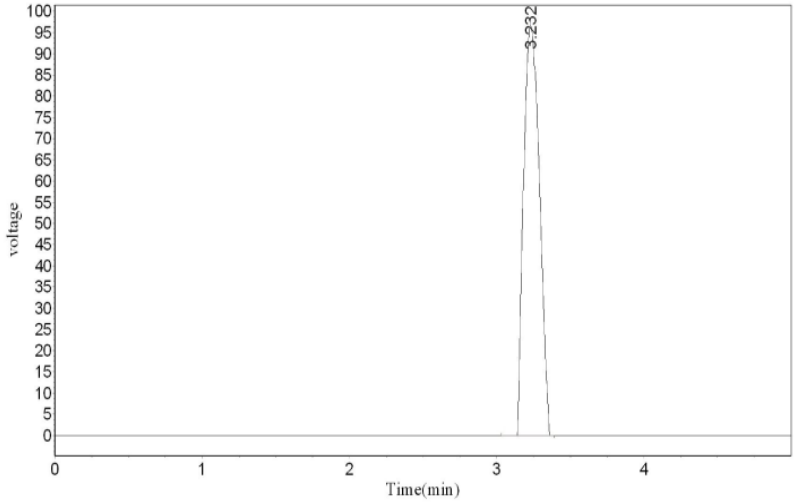


Fig-No-6: Sample Chromatogram-3

TABLE NO-5: DATA OF ASSAY OF SAMPLE CHROMATOGRAMS

Injection	Peak name	Retention time	Peak area	USP plate count	USP tailing
1.	Bosentan	3.21	515076	4396	1.17
2	Bosentan	3.223	528219	4201	1.16
3.	Bosentan	3.232	521794	4121	1.17
Mean			521696		
Standard deviation			6572.04		
% RSD			1.25		

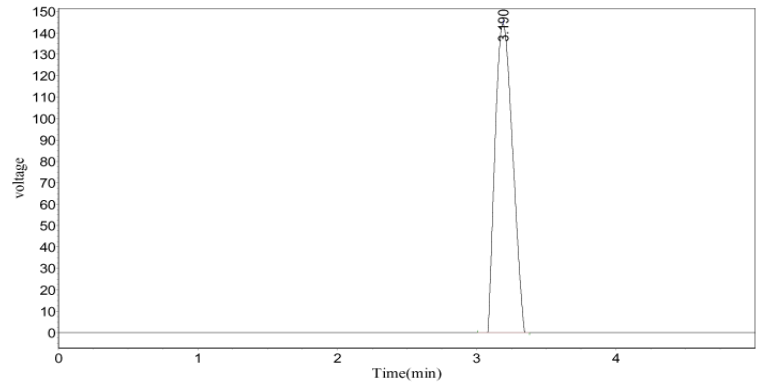


Fig-No-7: Accuracy 50% chomatogram -1

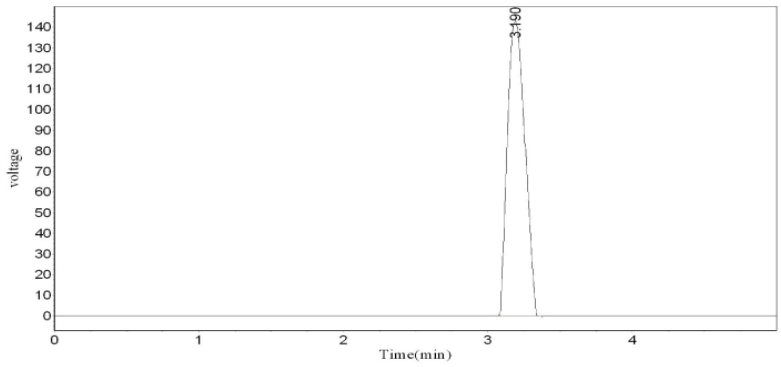


Fig-No-8: Accuracy 50% Chromatogram-2

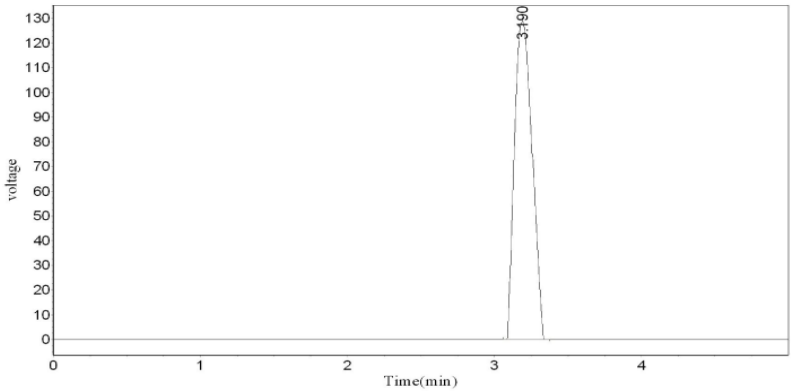


Fig-No-9: Accuracy 50 % Chromatogram -3

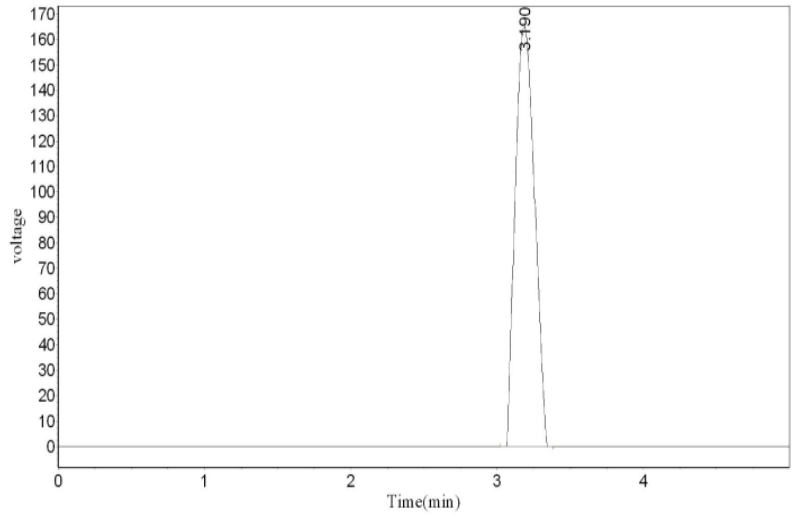


Fig-No-10: Accuracy 100 % Chromatogram -1

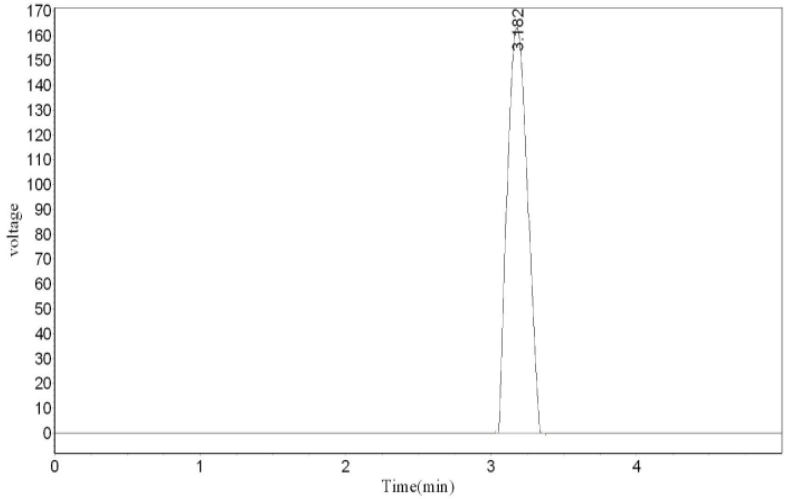


Fig-No-11: Accuracy 100 % Chromatogram -2

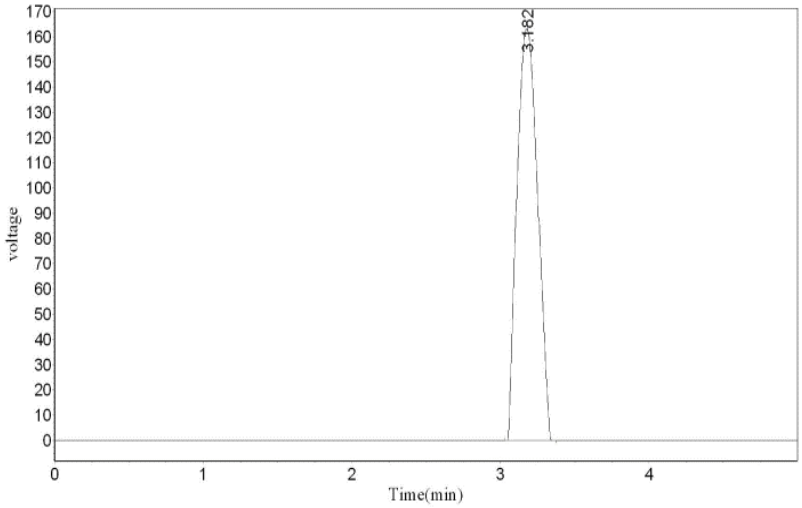


Fig-No-12: Accuracy 100 % Chromatogram -3

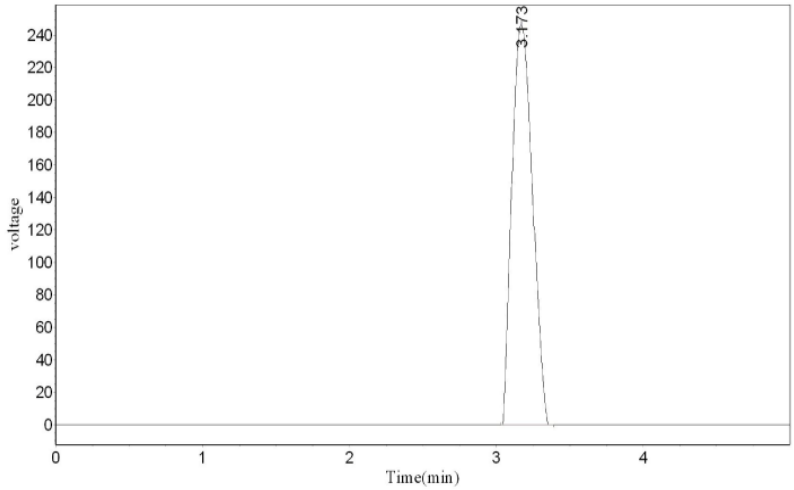


Fig-No-13: Accuracy 150% chromatogram -1

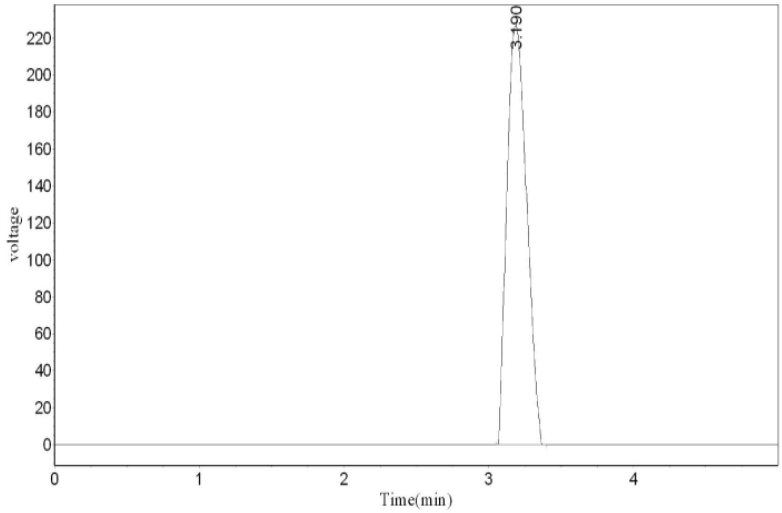


Fig-No-14: Accuracy 150% chromatogram -2

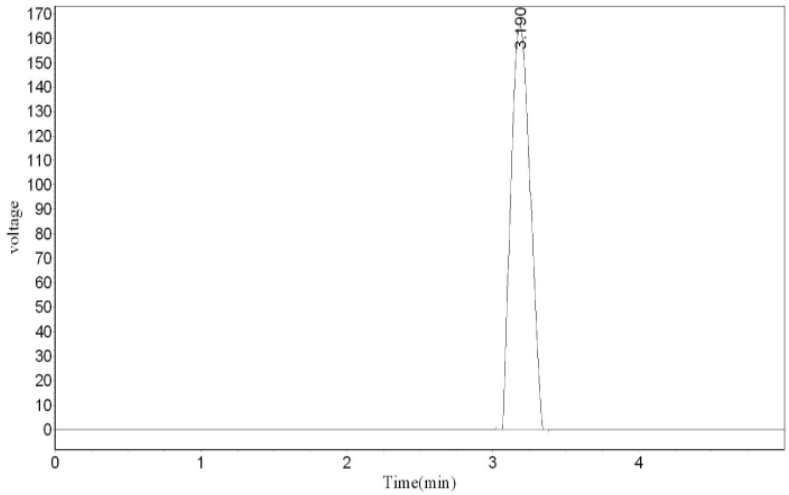


Fig-No-15: Accuracy 150% chromatogram -3

TABLE NO-6: ACCURACY OBSERVATION OF BOSENTAN

% Concentration (at specification Level)	Peak Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50 %	1255408	44.80	44.80	100.6	100.5
100 %	1669660	59.59	59.59	100.3	
150 %	2440788	76.98	76.98	100.8	

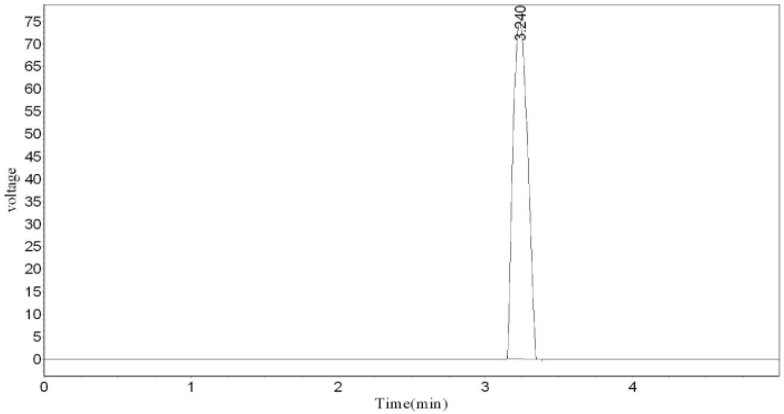


Fig-No-16: System precision Chromatogram -1

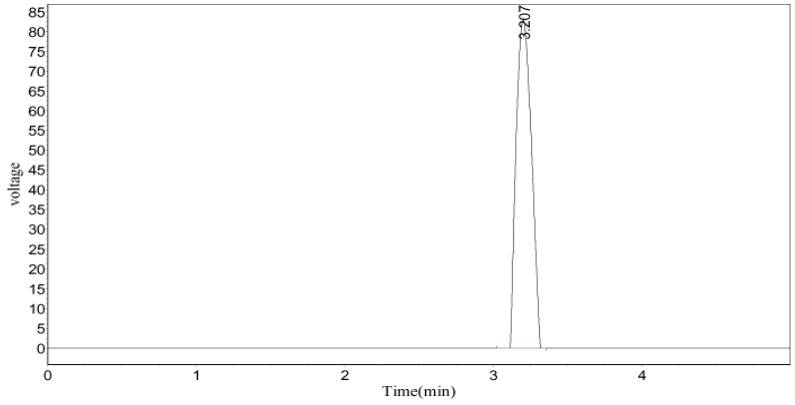


Fig-No-17: System Precision Chromatogram-2

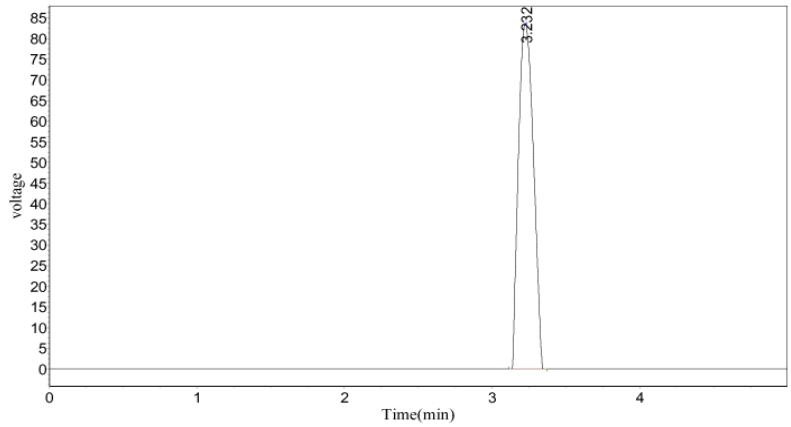


Fig-No-18: System Precision Chromatogram--3

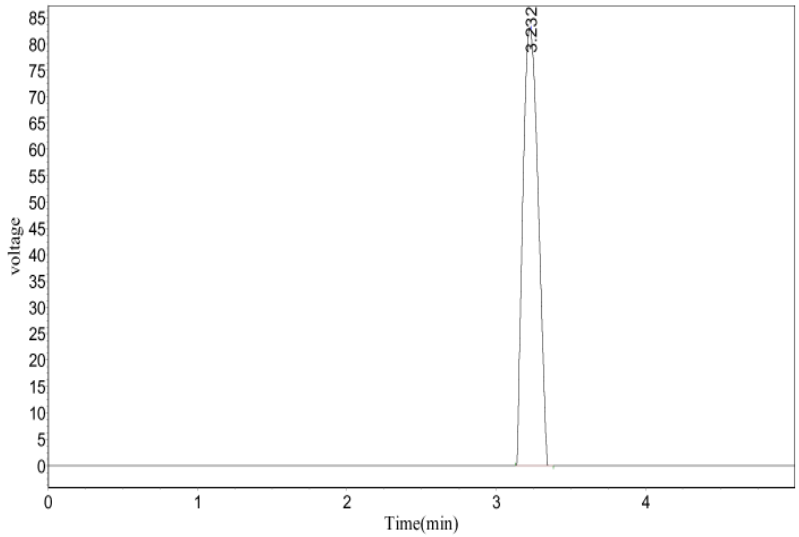


Fig-No-19: System Precision Chromatogram—4

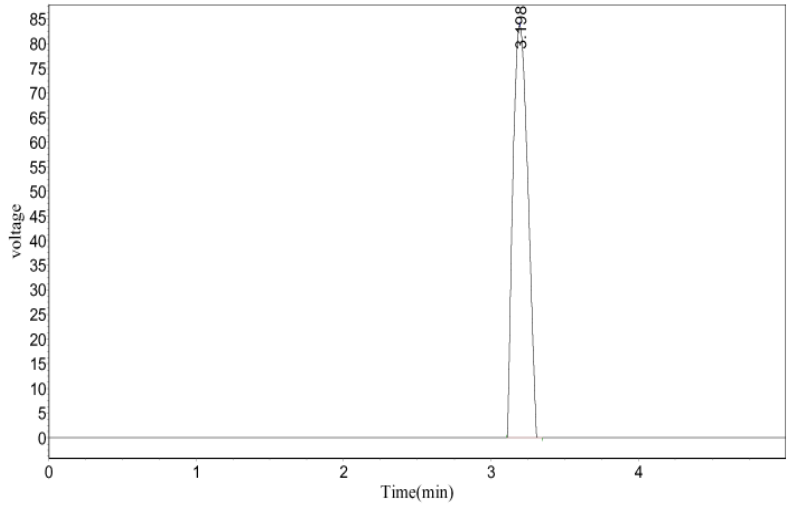


Fig-No-20: System Precision Chromatogram -5

TABLE NO-7: OBSERVATION OF SYSTEM PRECISION

INJECTION	BOSENTAN AREA
Injection1	590927
Injection2	587426
Injection3	589286
Injection4	587964
Injection5	584481
Average	588006
Standard Deviation	2417.9
% RSD	0.411

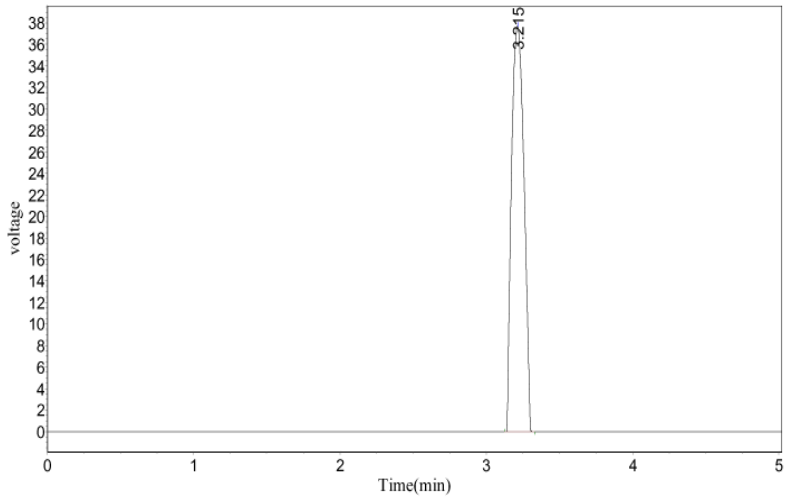


Fig-No-21: Linearity Chromatogram -1

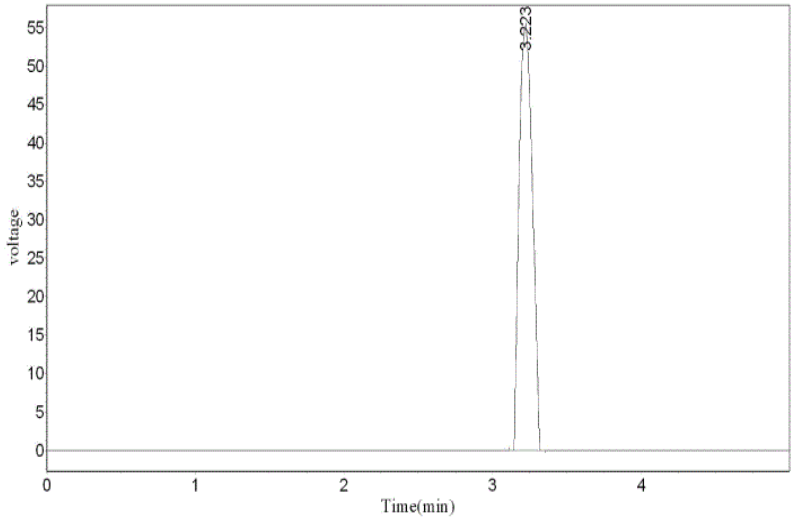


Fig-No-22: Linearity Chromatogram -2

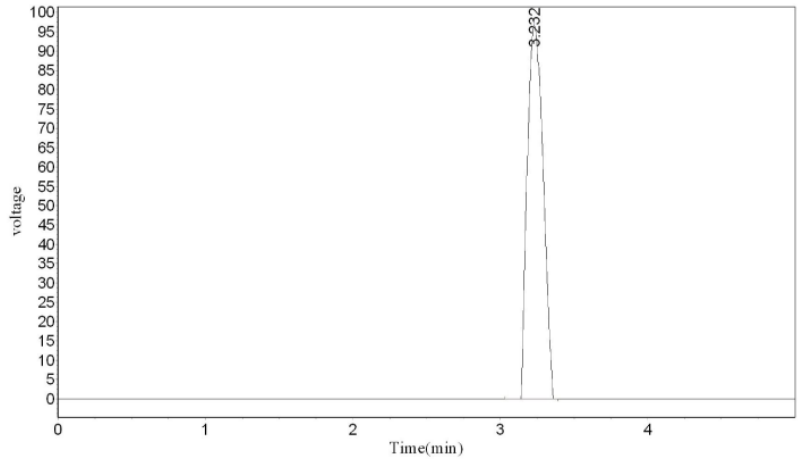


Fig-No-23: Linearity Chromatogram -3

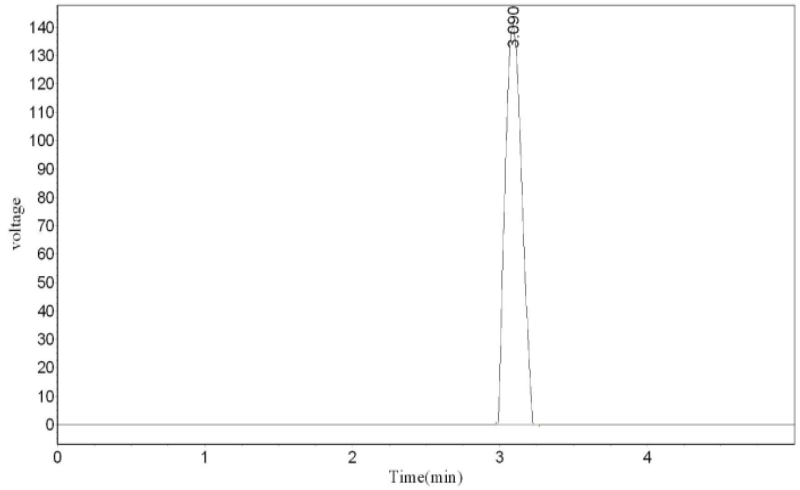


Fig-No-24: Linearity Chromatogram -4

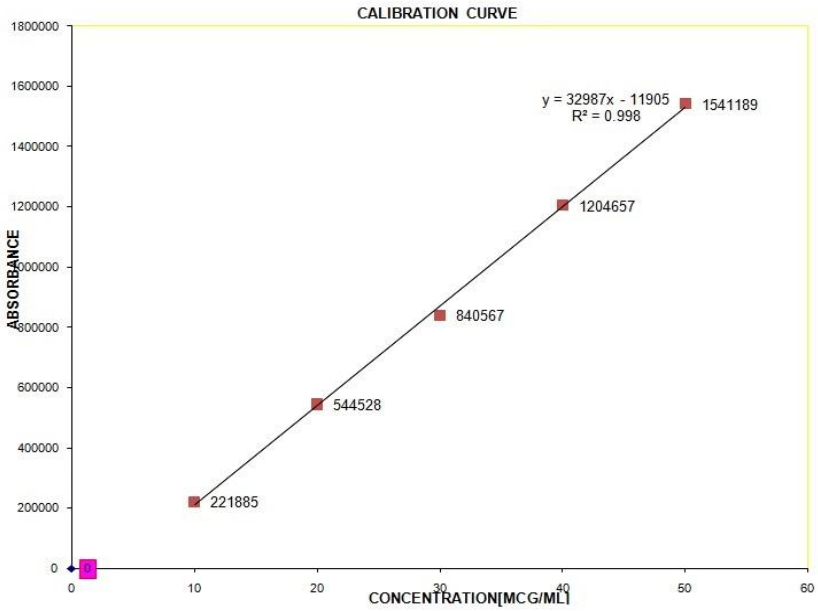


Fig-No-25: Calibration Curve for Bosentan

TABLE NO-8: LINEARITY OBSERVATION OF BOSENTAN

S.No	Level	Concentration	Retention time (min)	Peak Area
1	I	10 µg/ml	3.215	221885
2	II	20 µg/ml	3.223	544528
3	III	30 µg/ml	3.232	820567
4	IV	40 µg/ml	3.090	1204657
5	V	50 µg/ml	3.182	1541189
Slope	32987			
Intercept	11905			
Correlation Coefficient	0.999			

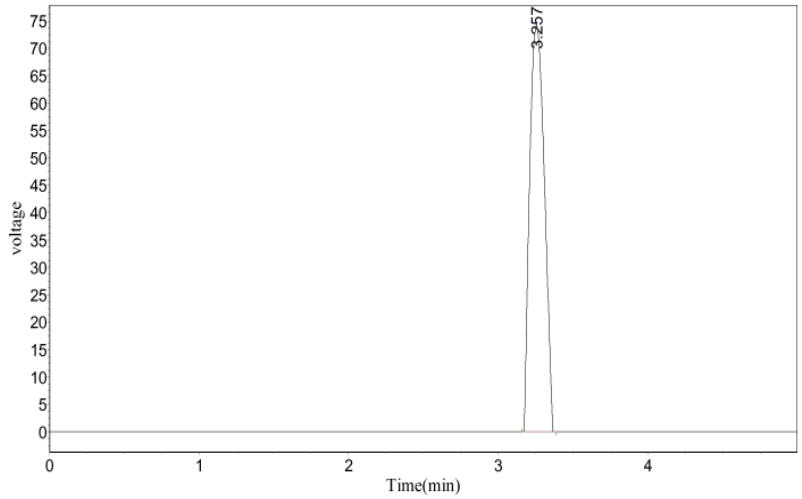


Fig-No-26: Actual flow rate Chromatogram-1(0.8 ml/Min)

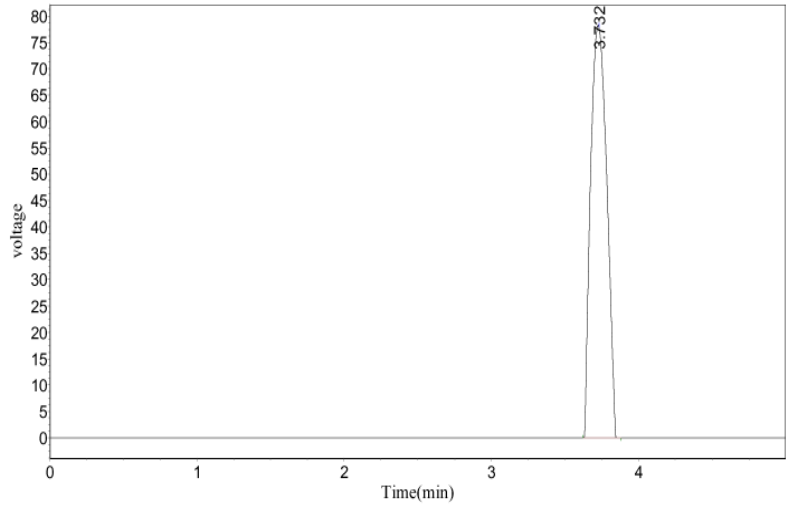


Fig-No-27: Less flow rate Chromatogram (0.7 ml/min)

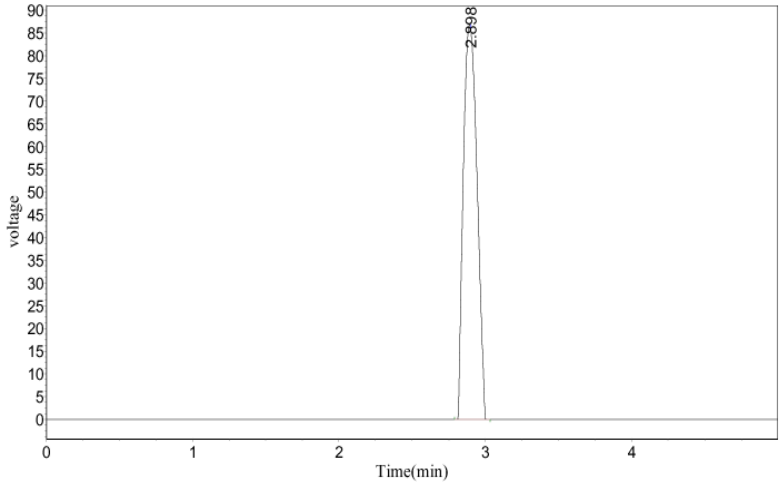


Fig-No-28: More flow rate Chromatogram (0.9 ml/min)

TABLE NO-9: FLOW RATE OBSERVATION OF BOSENTAN

S.No	Flow rate (in ml/min)	System suitability results		R_t (in min)	Peak area
		USP plate count	USP tailing		
1	0.7	4450	1.15	3.732	596509
2	0.8	4196	1.17	3.25	509034
3	0.9	3732	1.15	2.898	86493

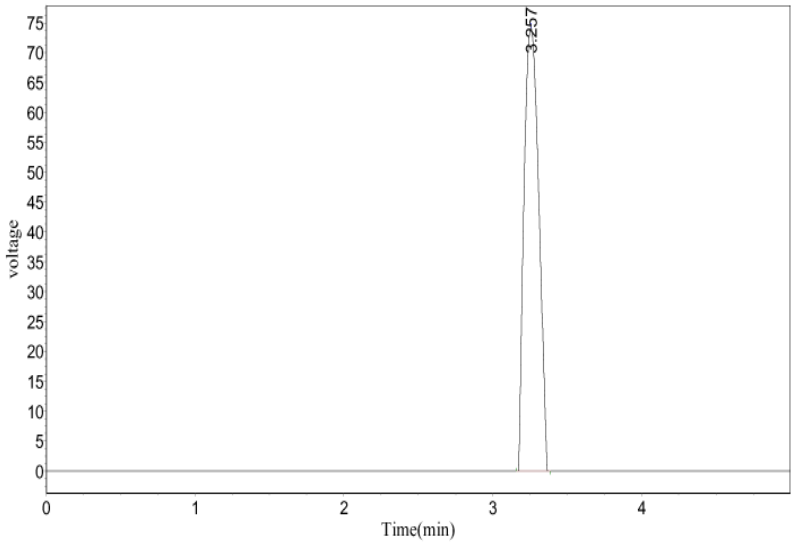


Fig-No-29: Actual Mobile Phase Chromatogram-1 (Buffer pH 3: Acetonitrile (60:40))

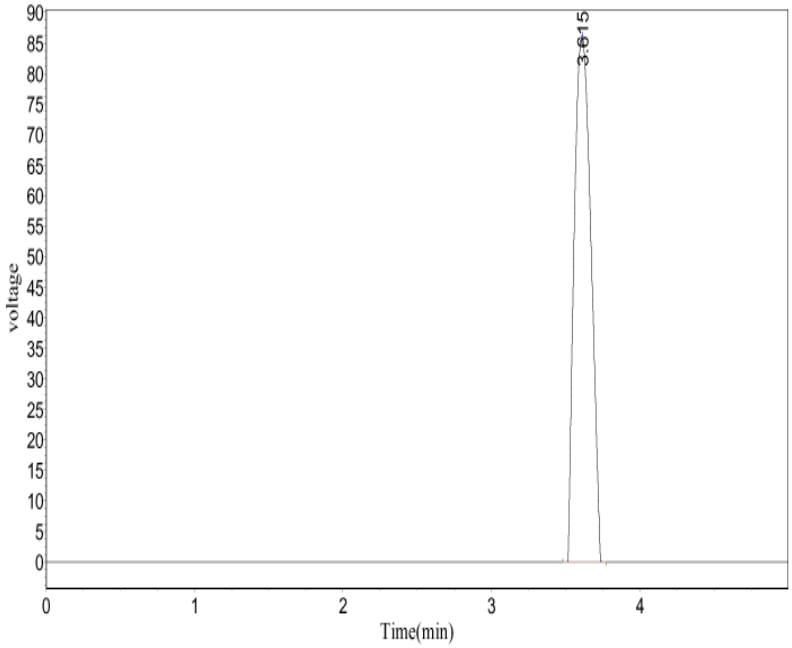


Fig-No-30: Less Organic Mobile Phase Chromatogram

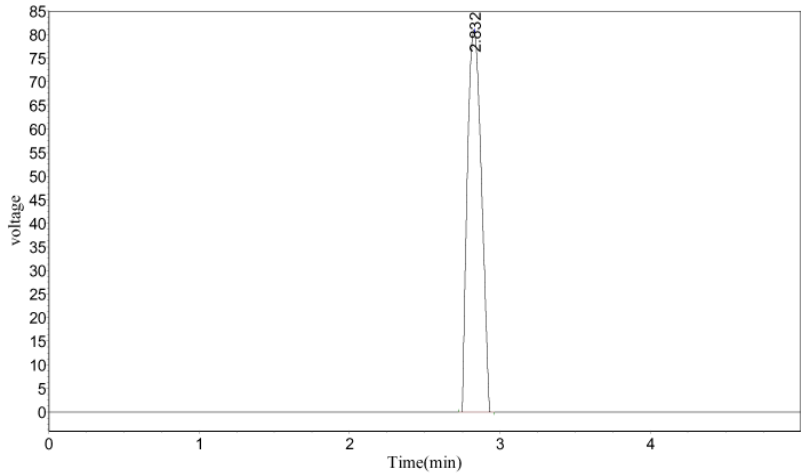


Fig-No-31: More Organic Mobile Phase Chromatogram

TABLE NO-10: MOBILE PHASE CHANGE OBSERVATION OF BOSENTAN

S.No	Flow rate (in ml/min)	System suitability results		R_t (in min)	Peak area
		USP plate count	USP tailing		
1	10% less	3972	1.16	3.615	671425
2	*Actual	4450	1.15	3.732	596509
3	10% more	3562	1.15	2.832	522305

TABLE NO-11: OBSERVATION OF SYSTEM SUITABILITY PARAMETERS

S. No	Parameter	Bosentan
1	Retention time	3.25
2	Theoretical plates	4196
3	Tailing factor	1.17
4	Area	509034
5	Resolution	3.23

TABLE NO-12: SUMMARY FOR RP-HPLC METHOD

S.NO	PARAMETER	ACCEPTANCECRITERIA	RESULTS OBTAINED
1	System suitability	Theoretical Plates-NLT2000	4196
		Tailing factor-NMT 2	1.17
		Resolution- NLT 2	3.23
2	Precision	% RSD of Bosentan	0.411%
3	ID Precision	% RSD of Bosentan	1.24%
6	Linearity	Correlation coefficient NLT 0.999	0.999
7	Accuracy	Percentage Recovery 98-102%	100.5

5. CONCLUSION

The proposed HPLC method was found to be specific, precise, accurate, rapid and economical for simultaneous estimation of Bosentan in Pharmaceutical dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness and results will be validated statistically according to ICH guidelines. The sample recoveries in all formulations were in good agreement with their respective Label Claims and this method can be used for routine Analysis.

6. REFERENCES

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