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METHOD DEVELOPMENT AND VALIDATION FOR IMPURITY PROFILING OF BOSENTAN MONOHYDRATE USING A NOVEL RP-HPLC METHOD

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ABSTRACT

A novel liquid chromatographic method has been developed and validated for the determination of Bosentan monohydrate (BOS), together with its four related substances (**Styrene, Hydroxy, Bosentan stage-3 and Dimer**) in a laboratory mixture as well as in marketed formulation. Efficient chromatographic separation was achieved on an Inertsil ODS-3V (450mm×4.6mm i.d., 5.0 µm particles), containing Mobile phase A – Buffer : Acetonitrile (50:50) and Mobile phase B – Buffer : Acetonitrile (20:80). Mobile phases were used in gradient combination for about 45 min at a flow rate 1.0ml/min and the eluant was monitored at 225nm. Regression analysis gave the correlation coefficient value greater than 0.999 for BOS and its four known impurities. The linearity of the method was determined over the concentration range of LOQ-150% of target concentration. The method has shown good and consistent recoveries for BOS (93.79-98.67%) and also for its four known impurities (97.2–101.3%). The proposed analytical method has been validated in accordance with ICH guidelines.

KEYWORDS: Gradient, Bosentan, Regression, Impurities, HPLC, ICH guidelines.

INTRODUCTION

Bosentan, 4-tert-butyl-N-[6-(2-hydroxy-ethoxy)-5-(2-methoxy- phenoxy)-[2, 2´] -Bipyrimidine-4-yl]-benzene sulfonamide monohydrate (Fig.1). It is a specific and competitive antagonist of endothelin receptor types ETA and ETB. Bosentan has a slightly

higher affinity for ETA receptors than for ETB receptors. The clinical impact of dual endothelin blockade is unknown.[1] This prompted a review of the literature, which showed that several HPLC methods have been developed over the years for bosentan content, but there is no limit applied to related impurities.[2-6] The work described in this paper is related to the development and validation of a gradient HPLC procedure capable of simultaneous determination of febuxostat content and related impurities. Thus, the aim of current study was to develop and validate an LC method for the determination of febuxostat and its known impurities (Imp-A, Imp-B, Imp-C & Imp-D) in bulk and tablet dosage forms, in accordance with the ICH guidlines.

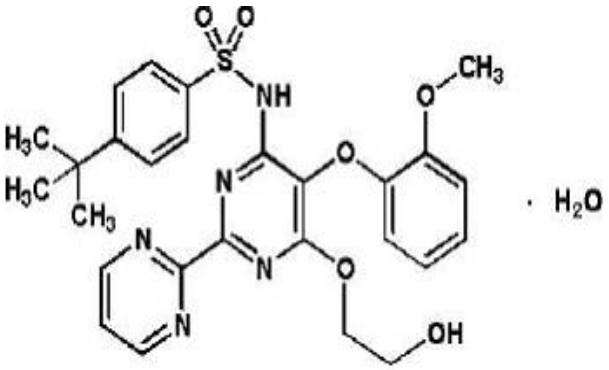


Fig. 1. Structure of the bosentan core molecule.

Table: 1 Chemical Names of Bosentan and its related impurities

Components	Name	Chemical Name
Bosentan	API	4-tert-butyl-N-[6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-[2,2´]- Bipyrimidine-4-yl]-benzene sulfonamide monohydrate
Impurity A	Styrene	N-[6-(ethene-1-oxy)-5-(2-methoxyphenoxy)-2-yl)-pyrimidin-4yl]-4-tert-butyl benzene sulphonamide
Impurity B	Hydroxy	N-[6-Hydroxy-5-(2-methoxyphenoxy)-2-yl)-pyrimidin-4yl]-4-tert-butyl benzene sulphonamide
Impurity C	Bosentan stage-3	4-ter-butyl-N-(6-chloro-5-(2-methoxyphenoxy)-2-2'-bipyrimidine-4-yl)benzene sulphonamide
Impurity D	Dimer	4-tert-butyl-N-{6-[2-({6-[4-tert-butyl benzene)sulphonamide]-5-(2-methoxyphenoxy)-2-(pyrimidin-2-yl)pyrimidin-4-yl}oxy)ethoxy]-5-(2methoxyphenoxy)-2-(pyrimidin-4yl}benzene-1-sulphonamide

2. EXPERIMENTAL

2.1. Materials and reagents

Qualified standards of Bosentan were gifted by NEULAND LABORATORIES Ltd

(Bothanpalli, Hyderabad, A.P, India.) Following authenticated impurity standards were obtained from Natco labs India: Imp-A, Imp-B, Imp-C & Imp-D. HPLC grade acetonitrile procured from Ranbaxy Fine Chemical Limited was used. Orthophosphoric acid of HPLC grade was obtained from Qualigens. HPLC grade water obtained from Ranbaxy Fine Chemical Limited was used throughout the analysis. TRACLEER 40mg, 80mg, 120mg tablets obtained from MNC Pharmaceuticals.

2.2. HPLC apparatus and operating conditions

The apparatus consisted of a gradient RP-HPLC (WATERS) 2996 series pump with PDA detector, with Empower 2 software and auto sampler. A column comprising of Inertsil ODS 3V, (450mm×4.6 mm i.d., 5.0 µm particles) with in-line pre-filter was used for the separation. The injection volume and flow rate were 10 µl and 1.0 ml/min, respectively. The composition of mobile phase A was *Buffer:Acetonitrile (50:50).* The composition of mobile phase B was *Buffer:Acetonitrile (20:80)* . The detection was carried out at 225nm.

2.3: Preparation of solution

2.3.1*: Preparation of Buffer*

1.36g of Potassium dihydrogen ortho phosphate in 1000ml of milli-Q water. Adjust the pH to 2.5 with dilute orthophosphoric acid.

2.3.2. *Preparation of Mobile phase A*

Mix equal volumes of buffer and acetonitrile (50:50).

2.3.3. *Preparation of Mobile phase B*

Mix buffer and acetonitrile in a ratio of 20:80.

2.3.4. *Diluent preparation*

Mobile phase A is used as diluent.

2.3.5. Standard Stock solution

Accurately 100mg of Bosentan monohydrate weighed and and transferred into 100ml volumetric flask. Dissolve in 40ml of acetonitrile and made upto volume with diluent, sonicated for 5minutes to dissolve completely and volume was made upto the mark with diluents and filtered through 0.45µ millipore nylon filter.

2.3.6. Standard Solution

5ml of standard stock solution was pipetted into a 10ml volumetric flask and diluted upto the mark with diluent. (0.5mg/ml)

2.3.7. Impurity Stock Solutions

Accurately 10 mg of each individual impurity (Impurity-A, Impurity-B, Impurity-C, Impurity-D) working standard was weighed and transferred into a 10 ml of clean dry volumetric flasks separately and 10ml of diluent was added, sonicated for 5minutes to dissolve completely and volume was made upto the mark with diluent and filtered through 0.45µ Millipore nylon filter.

2.3.8. Impurity solutions

5ml of each individual impurity stock solutions (Impurity-A, Impurity-B, Impurity-C, Impurity-D) was pipetted into a 10ml volumetric flask separately and diluted upto the mark with diluent.

2.3.9. Reference Solution

As per specification of each impurity (Impurity-A, Impurity-B, Impurity-C, Impurity-D) is NMT 0.15%. According to this, reference solution was prepared with 0.001mg/ml concentration.

Pipette out 2ml of the standard solution into a 100ml volumetric flask and make upto the volume with diluent and mix well further dilute 1ml of this solution to 10ml with the diluent.

2.3.10. Sample Stock Solution

20 tablets were weighed and average weight of tablet was determined. The tablets were equivalent to 50 mg of Tracleer into a 25ml clean dry volumetric flask added about 10ml of diluents and sonicated for 20minutes. Volume was made upto the mark with diluent.

2.3.11. Sample Solution

5 ml of supernatant sample stock solution was pipetted into a 10 ml volumetric flask and diluted upto the mark with diluents and filtered through 0.45µ Millipore nylon filter.

2.3.12. System suitability

System suitability parameters were evaluated to verify that the analytical system is working properly and can give accurate and precise results. Parameters such as peak asymmetry factor, tailing factor, resolution between Imp-A, Imp-B, Imp-C & Imp-D, resolution between

Imp-B and BOS, and %RSD of theoretical area obtained from two diluted standard solutions of BOS (in triplicate),were evaluated.

2.4. Analytical method validation

2.4.1. *Specificity*

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities, separation and resolution were observed between BOS standard solution and its four impurities, namely Bosentan, Imp-A, Imp-B, Imp-C and Imp-D (known impurities). Solutions of standard, each individual impurity solutions were prepared as per the test method and injected into the chromatographic system and the chromatograms were recorded.

2.4.2. *Linearity*

Linearity test for the method was performed according to the guidelines laid by ICH. Appropriate aliquots of BOS stock solution were spiked with appropriate volumes of stock solutions of known impurities (related substances) and diluted with the diluent to get solutions containing required concentrations. Linearity of BOS and its Impurities was determined over a range of obtained limit of quantification (range was inclusive of concentrations at LOQ, 20, 40, 80, 100, 120 and150%). Calibration curve was drawn by plotting the peak areas of BOS and Impurities versus its corresponding concentration. The process was repeated for three consecutive days (twice each day) in the same concentration range .Values of coefficient of regression; slope and Y-intercept of the calibration curve were calculated. There relative response factors were calculated and concentrations were adjusted accordingly.

2.4.3. Precision

The system precision was established by injecting six replicate injections of Reference solution in to the chromatographic system and the chromatograms were recorded. Six samples of drug product at 100% of the sample concentration (100% Spiked Solution) were prepared and injected into the chromatographic system and the chromatograms were recorded.

2.4.4. *Accuracy*

Recovery studies were performed in triplicate at concentration levels of 50,100 and 150% of BOS (0.5mg/mL) to evaluate the accuracy of the proposed method. Solutions for the purpose

were prepared by standard addition of BOS stock solution to laboratory mixture solution.

2.4.5. Limit of detection (LOD) and limit of quantification (LOQ)

Preparation of 0.01mg/ml solution

1 ml of standard stock solution and each individual impurity stock solution was diluted to 10ml with the diluents separately. From the above each individual solutions 1ml was diluted with 10ml diluents separately to prepare 0.01mg/ml solution.

Preparation of LOQ solution

0.178ml of IMP-A, 0.222ml of IMP-B, 0.215 ml of IMP-C, 0.248ml of IMP-D, 0.208ml of BOS-standard were taken from 0.01mg/ml above solutions respectively into 50ml clean dry volumetric flask and diluted upto the mark with the diluents. It was used as LOQ solution.

Preparation of LOD solution

3.3ml of LOQ solution was taken into clean dry 10 ml volumetric flask and make up the volume with diluents. The LOD and LOQ for BOS and all impurities were estimated by signal-to-noise ratio, 3:1and10:1, respectively, injecting a series of six diluted solutions with known concentrations.

2.4.6. *Stability of laboratory mixture solution*

The assessment of sample solution stability, the bosentan accuracy solutions were stored for 72 hr under both ambient and refrigerated conditions and injected versus fresh standard preparation. The difference in areas of respective peaks in the obtained chromatograms was calculated.

2.5.6. Robustness

For the robustness experiment related to specificity, two different composition of mobile phases comprises of mobile phase A was *Buffer: Acetonitrile (50:50)* and mobile phase B was *Buffer: Acetonitrile (20:80).*Reference solution and sample solution prepared as per the test method was injected into the chromatographic system maintaining flow rates, less flow (0.9 mL/min), more flow (1.1 mL/min) and actual flow (1.0 mL/min). A resolution mixture containing most of the peaks of interest was injected onto the chromatograph using each set of mobile phases.

3. RESULT AND DISCUSSION

3.1. Development of the chromatographic method

The method which was thought to be developed was envisaged to be capable of eluting wide range of compounds of different polarities, with excellent efficiency and sufficient band spacing. During development of chromatography, elution was performed using C18 columns. Mobile phase consisting of ACN, buffer was used preliminary in isocratic elution. Further, increasing the proportion of ACN in the mobile phase resulted in rapid elution of Bosentan. Therefore, a gradient mode of elution was tried for greater chance of success in the context. The gradient mobile phase consisted of two major components: Mobile Phase A containing Buffer: Acetonitrile (50:50) and Mobile Phase B was Buffer: Acetonitrile (20:80). The finally developed gradient method was consisting of % change in mobile phase B with respect to time (0.01-20 min: 100%A; 25-35min: 100% B; 40-45.00min: 100%A). The mobile phase was mixed and eluted at1.0mL/min by the system and column temperature was maintained at 25^0C.

3.2. System suitability

Chromatographic separation was performed with C18 column (Inertsil ODS-3V,450mm×4.6 mm i.d., 5.0 µm particles) with the above mentioned gradient mobile phase and a representative chromatogram is shown in the table, which display a tailing factor less than 1.5 for all the peaks, are solution of 5.31 and 3.72 for Imp-B and Imp-B with respect to BOS respectively. Tailing factor, a parameter that ICH guidelines consider as a factor to be controlled, was within the established limits. The resolution factor between two consecutive peaks approximately represents twice the minimum request to be considered.

3.3. Specificity

The HPLC chromatograms recorded separately for BOS alone and with its Impurities and blank preparations displayed a single, non-overlapped, peak for BOS. The resolution factor obtained between peak for BOS and other peaks was more than 2.1 and the tailing factor of peak for BOS and the Impurities was always in the range of 1.03–1.50. Thus, the HPLC method presented in this study is selective for BOS and also for the other four related compounds, which might co-exist as impurities.

3.4. Linearity

Calibration curves for BOS and its Impurities, examined in pure solutions as well as in the laboratory mixture solutions, were found to be linear; correlation coefficients ≥0.999 in all

the cases.UV-relative response factors (FR) were calculated for an each impurity using the following equation: FR¼S impurity/SFBX. Where, Impurity is slope of regression line for a given impurity and S_{FBX} is the slope of the regression line for BOS.

Concentrations of BOS and impurity were corrected. Statistical treatment of the linearity data of BOS shows a linear response between lower levels to highest level. In addition, the analysis of residuals shows values randomly scattered around zero, which fits well with in the linear model.

3.5. LOD and LOQ

LOD and LOQ, as a measure of method sensitivity, were provided for degradation products and impurity calculated by means of signal-to-noise ratio. From the results, it can be concluded that the proposed method can quantify small quantity of impurities in BOS samples. Acceptance Criteria are signal to noise ratio should be about 3:1, found to be between 2.94-3.49 which was within the acceptance criteria.

3.6. Precision and repeatability

Values of %RSD for system precision of BOS and total impurities were 2.39 and 1.29 respectively. Method precision has a % RSD below 1.9 for repeatability. Acceptance Criteria for precision at LOQ is the percentage Relative Standard Deviation (%RSD) for peak area of Bosentan and their known impurities at LOQ level should be less than or equal to 10. The % RSD values obtained for peak areas of bosentan and their known impurities at LOQ Level were in range of 9.98-10.69, which were within the acceptance criteria.

3.7. Accuracy

The results are expressed as percent recoveries of the particular components in the samples. The overall percent recoveries of BOS and its four Impurities at 50,100 and 150% of the test concentration. The method has shown good, consistent recoveries for BOS (97.2–101.3%).

3.8. Stability in analytical solution

The % area change in peaks of BOS and all impurities was less than 1.0% and5.0%, respectively. It was concluded that standard and sample solutions may be used up to72hr after preparation.

3.9. Robustness

Method robustness checked after deliberate alterations of mobile phase composition, flow

and temperature shows that the changes of the operational parameters do not lead to essential changes of the performance of the chromatographic system results are displayed in Tables. Tailing factor for BOS and its Impurities always ranged from 1to1.5 and the components were well separated. The percent recoveries of BOS and Impurities were good and did not show as significant change when the critical parameters were modified. Considering the results of modifications in the system suitability parameters and the specificity of the method, it would be concluded that the method conditions are robust.

Gradient Programme

Time(min)	Flow(ml/min)	M.P-A (%)	M.P-B (%)
0	1.0	100	0
10	1.0	100	0
35	1.0	0	100
40	1.0	0	100
42	1.0	100	0
45	1.0	100	0

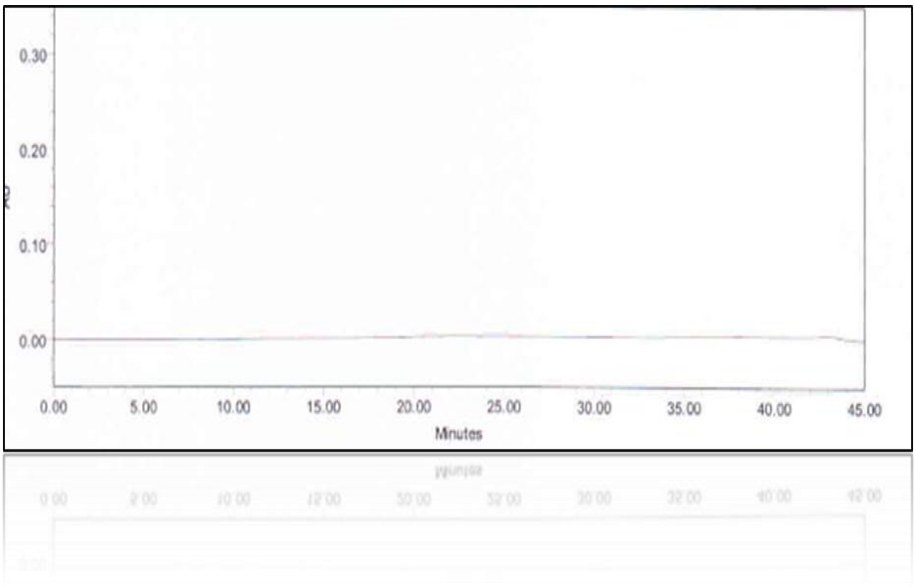
System suitability

	RETENTION TIME (min)		AREA		Resolution between bosentan monohydrate and hydroxy
	Bosentan	Hydroxy	Bosentan	Hydroxy	
Inj . 1	16.735	18.141	17421838	78356	2.99
Inj . 2	16.734	18.153	17945109	78687	2.97
Avg.	16.735	18.147	17683474	78522	2.98

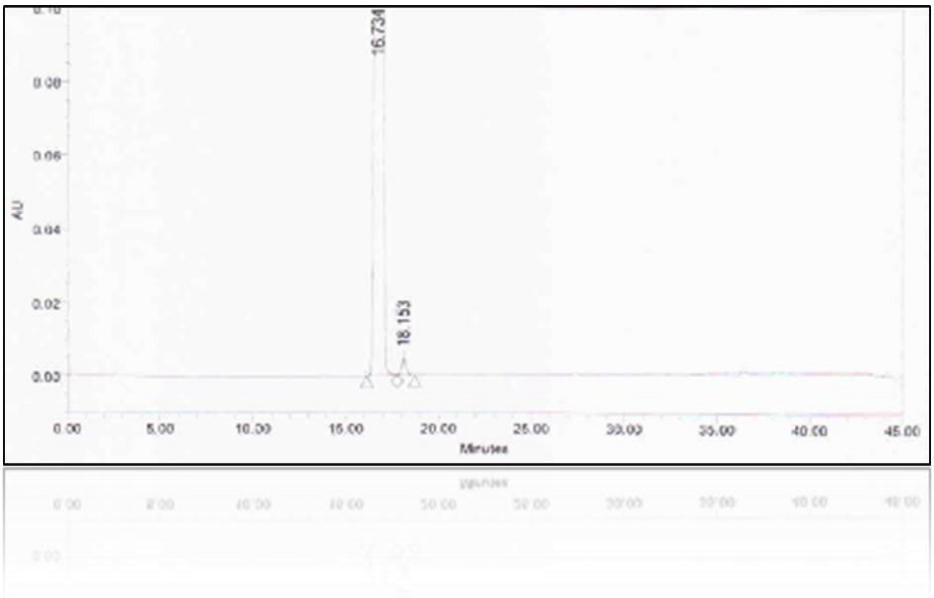
BLANK PREPARATION

COMPONENT	BOSENTAN MONOHYDRATE	
Conc. mg/ml	0.001	
INJECTIONS	RETENTION TIME (min)	AREA
1	16.747	36185
2	16.759	35717
3	16.748	36164
4	16.738	36121
5	16.739	36240
6	16.733	36253
Average	16.744	36113
% RSD	-------	0.55
Name of component	**Conc. (mg/ml)**	Retention time (min)
Bosentan	**0.5010**	16.699

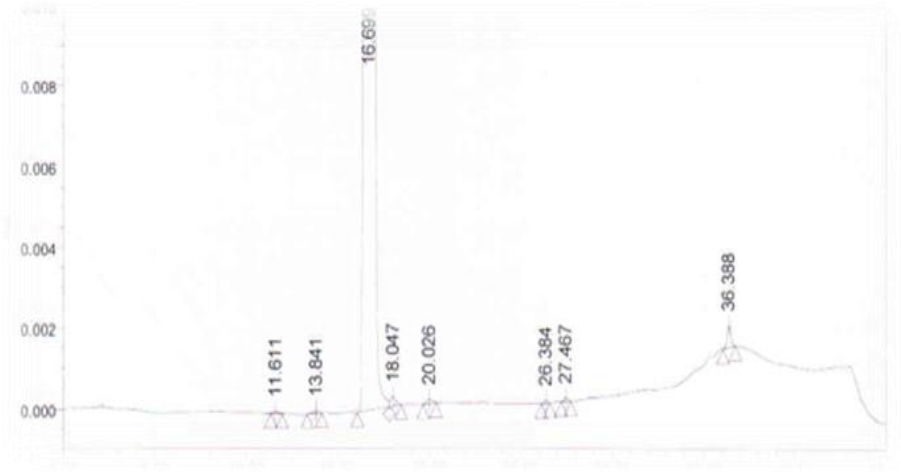
monohydrate Standard.		
Bosentan monohydrate sample.	**0.5006**	16.697
Bosentan stage-III.	**0.5008**	27.008
Dimer.	**0.1003**	36.424
Styrene.	**0.5012**	11.663
Hydroxy.	**0.5018**	18.147



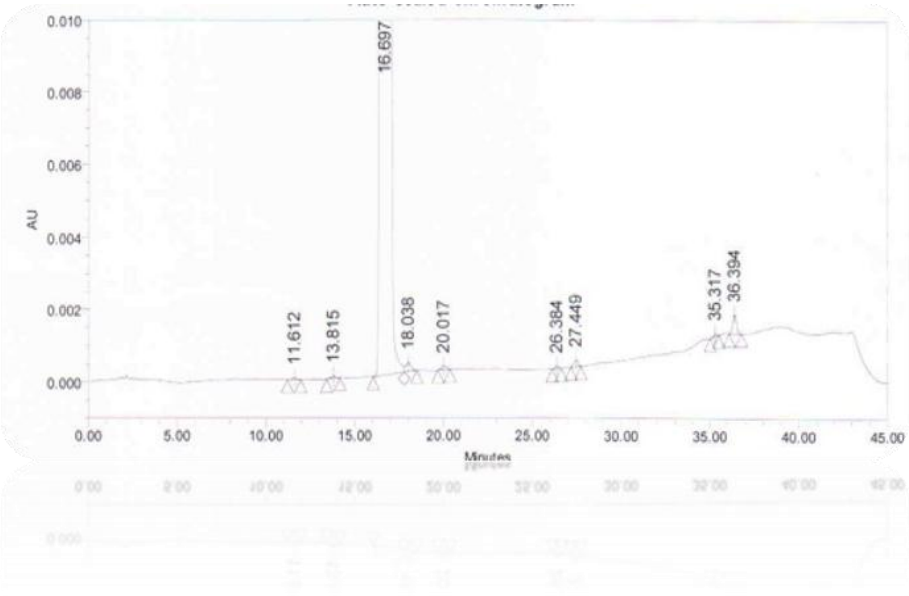
SYSTEM SUITABILITY



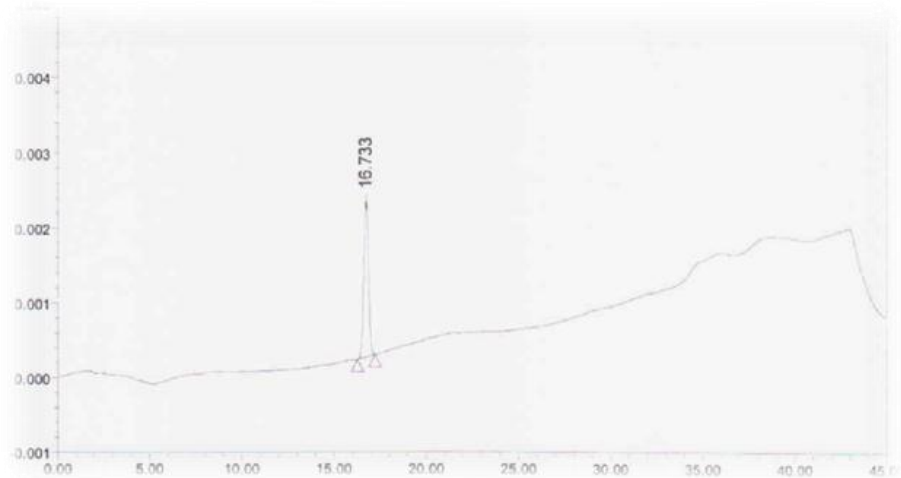
BOSENTAN MONOHYDRATE STANDARD



BOSENTAN MONOHYDRATE SAMPLE



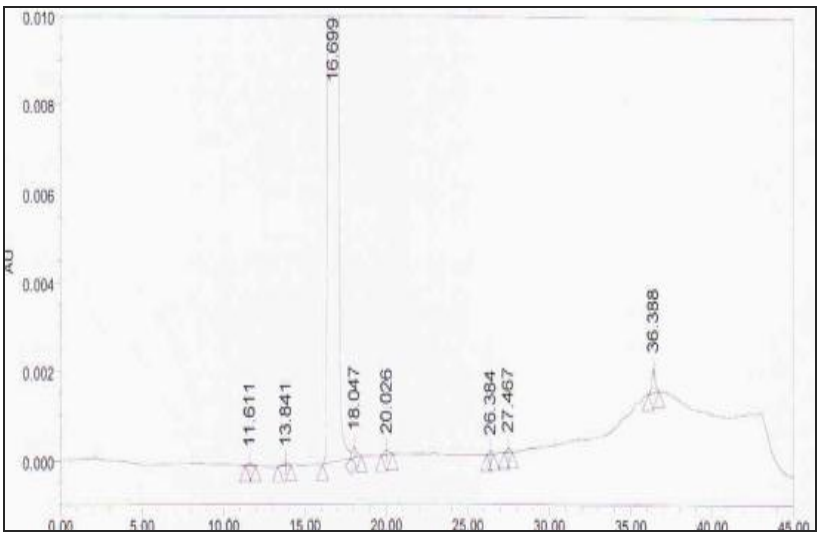
REFERENCE SOLUTION



VALIDATION DATA

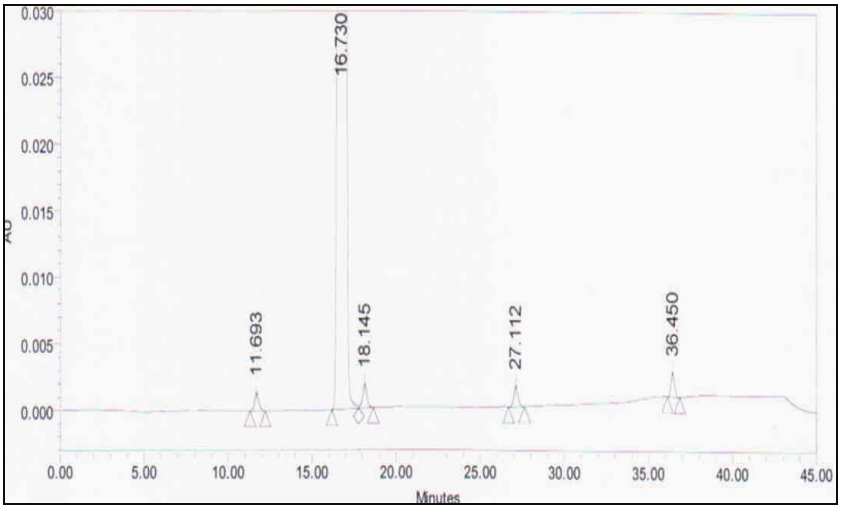
SPECIFICITY

Name of the component	Conc. (mg/ml)	Inj's	Retention time(min)
Bosentan Std	0.5010	Inj 1	16.699
		Inj 2	16.695
Bosentan sample	0.5006	Inj 1	16.708
		Inj 2	16.697
Bosentan stage-III	0.5008	Inj 1	27.008
		Inj 2	27.009
Dimer	0.1003	Inj 1	36.424
		Inj 2	36.415
Styrene	0.5012	Inj 1	11.663
		Inj 2	11.690
Hydroxy	0.5018	Inj 1	18.147
		Inj 2	18.140



SYSTEM PRECISION

	Content %						
	Styrene	Hydroxy	Bos Stg-III	Dimer	Single unknown	Total unknown	Total impurities
Inj's							
1	0.1465	0.1519	0.1547	0.1366	0.0131	0.0303	0.6200
2	0.1448	0.1506	0.1580	0.1376	0.0135	0.0322	0.6232
3	0.1459	0.1544	0.1602	0.1375	0.0135	0.0319	0.6299
4	0.1457	0.1530	0.1637	0.1380	0.0128	0.0324	0.6328
5	0.1459	0.1544	0.1672	0.1368	0.0130	0.0316	0.6359
6	0.1464	0.1529	0.1731	0.1383	0.0131	0.0314	0.6421
Avg	0.1459	0.1529	0.1628	0.1375	0.0132	0.0316	0.6307
% RSD	0.42	0.96	4.09	0.48	2.13	2.39	1.29

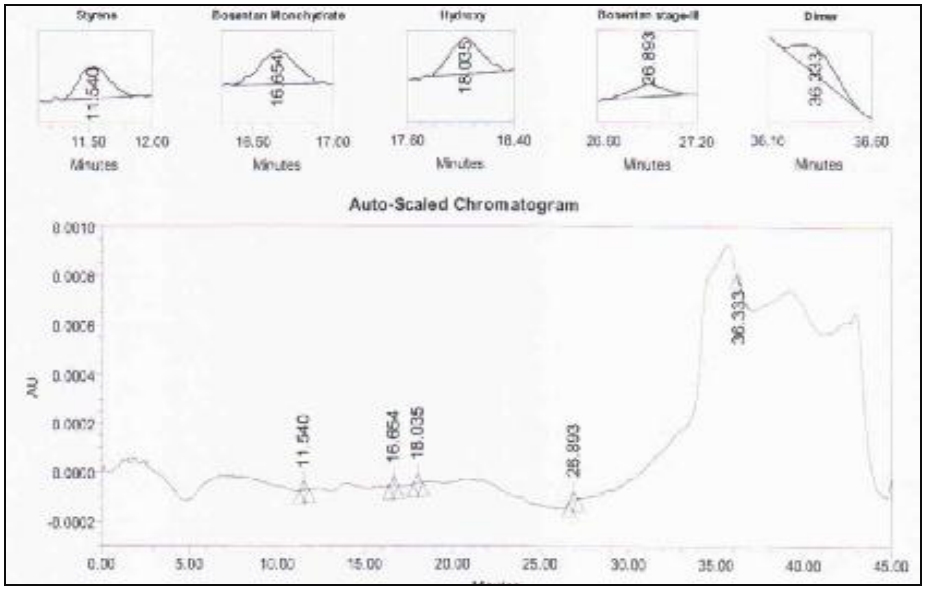


METHOD PRECISION

% Content							
S. No	**Styrene**	**Hydroxy**	**Bosentan stage 3**	**Dimer**	**Single Unknown**	**Total Unknown**	**Total impurities**
1	0.1471	0.1531	0.1563	0.1370	0.0137	0.0324	0.6259
2	0.1471	0.1553	0.1559	0.1393	0.0133	0.0314	0.6290
3	0.1464	0.1550	0.1538	0.1377	0.0133	0.0319	0.6248
4	0.1453	0.1563	0.1562	0.1378	0.0130	0.0320	0.6276
5	0.1452	0.1546	0.1552	0.1365	0.0127	0.0315	0.6230
6	0.1472	0.1556	0.1584	0.1398	0.0133	0.0322	0.6332
Avg	0.1464	0.1550	0.1560	0.1380	0.0132	0.0319	0.6273
% RSD	0.63	0.70	0.97	0.93	2.55	1.22	0.57

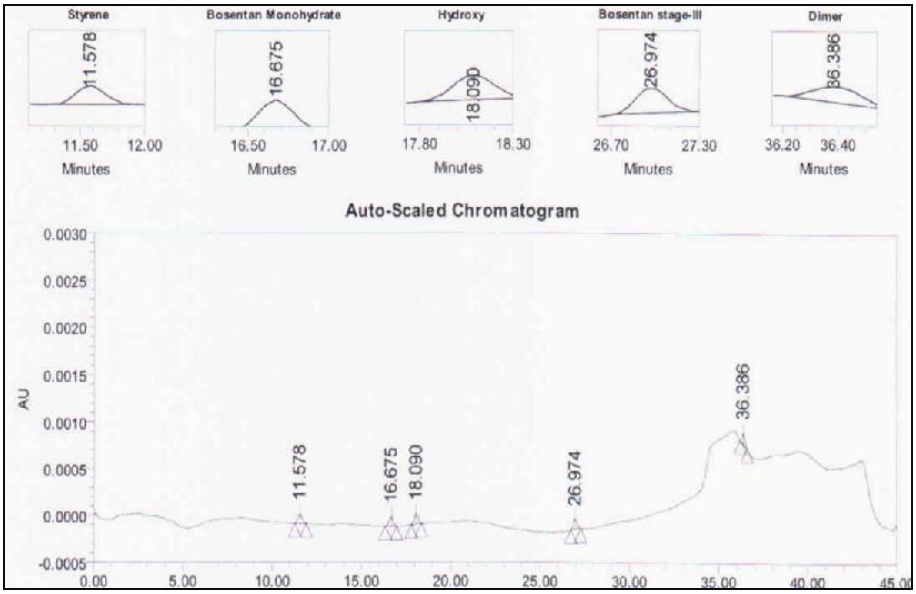
LIMIT OF DETECTION

Name of the component	**Signal to noise ratio**				
	Styrene	Bosentan monohydrate	Hydroxy	Bosentan Stage-3	Dimer
Conc. %	0.0033	0.0033	0.0033	0.0033	0.0033
Inj-1	3.02	3.26	3.58	3.56	3.30
Inj-2	3.23	3.24	3.27	3.33	3.15
Inj-3	2.56	3.39	3.63	3.44	3.01
Average	2.94	3.30	3.49	3.44	3.15



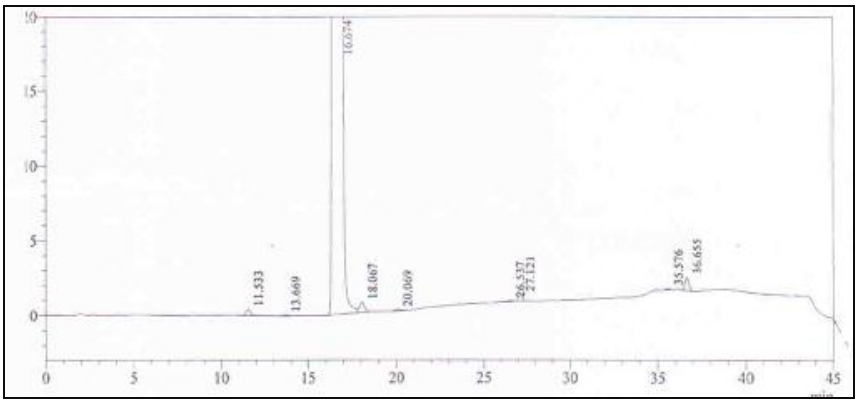
LIMIT OF QUANTITATION

Name of the component	Signal to noise ratio				
	styrene	Bosentan monohydrate	Hydroxy	Bosentan stg-3	Dimer
Conc. %	0.01	0.01	0.01	0.01	0.01
Inj-1	10.04	10.29	10.54	10.59	11.43
Inj-2	9.76	10.22	10.96	10.54	11.91
Inj-3	10.13	10.02	10.58	10.35	11.59
Average	9.98	10.18	10.69	10.49	11.64

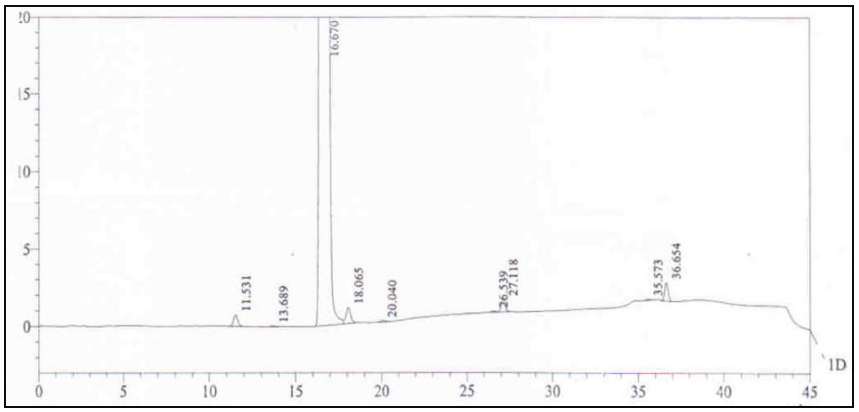


ACCURACY % RECOVERY

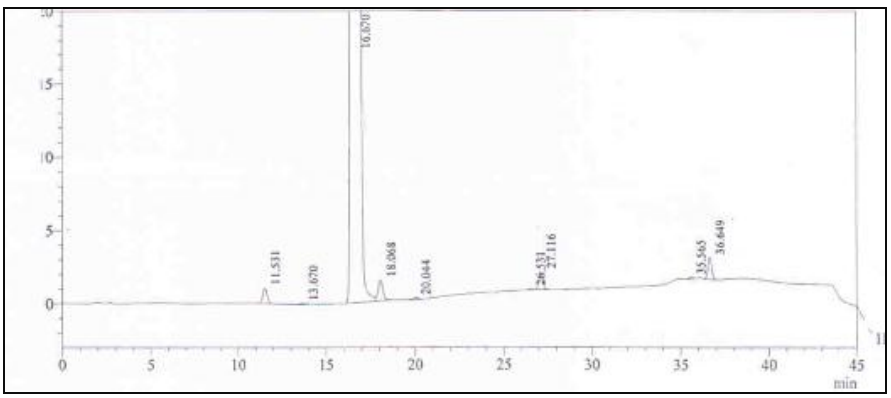
Name of the component	Average % Accuracy						
	At LOQ	25	50	75	100	125	150
Styrene	98.67	98.67	95.48	95.01	94.10	93.95	93.79
Hydroxy	113.83	111.97	105.62	103.37	100.45	101.19	100.69
Bosentan Stage– III	109.17	99.91	98.09	97.57	98.75	96.91	96.84
Dimer	96.83	98.32	98.09	91.46	94.29	89.49	89.93



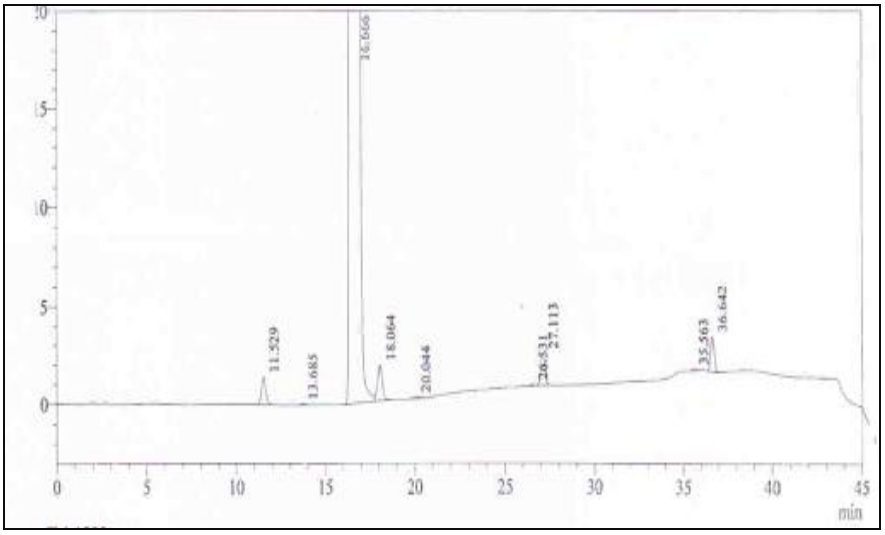
Sample + 25% level impurities spiked



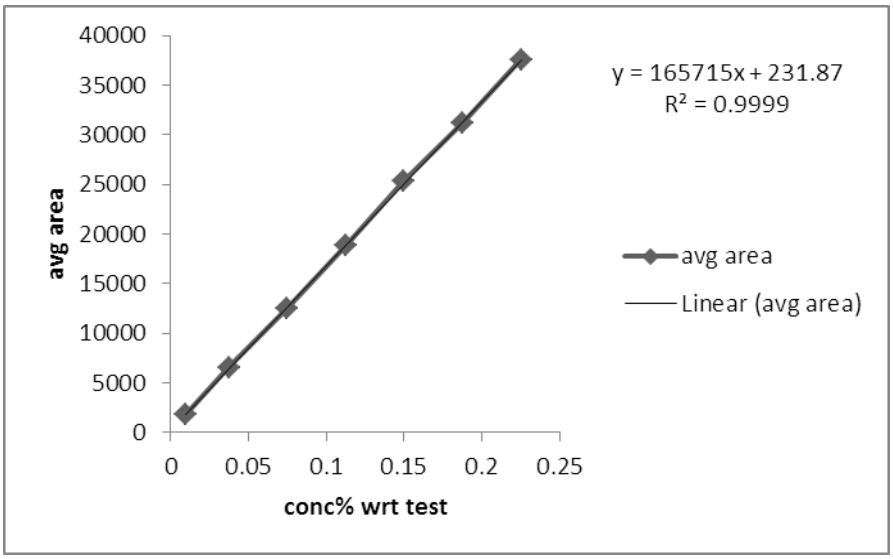
Sample + 50% level impurities spiked



Sample + 75% level impurities spiked:



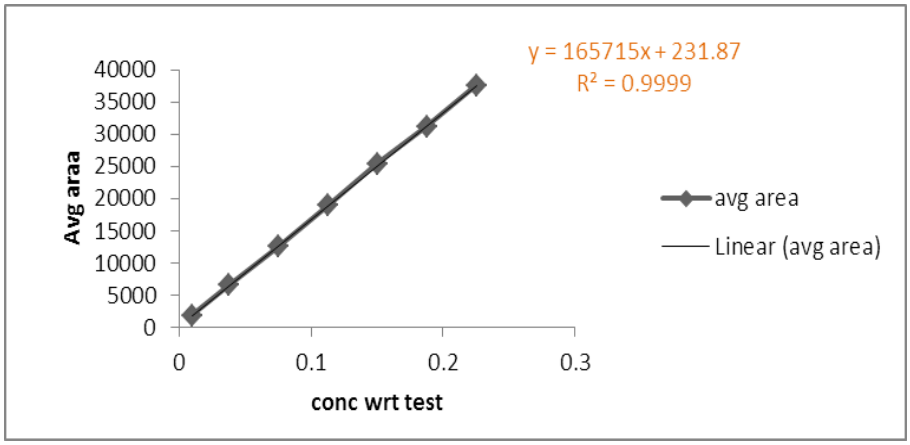
Sample + 100% level impurities spiked



LINEARITY

Hydroxy Impurity

Conc%	Avg area
0.01	2012
0.0376	7552
0.0753	14264
0.1129	21327
0.1505	27598
0.1882	33945
0.2258	40854



Bosentan stage III

CONC	avg area
0.01	1847
0.0376	6561
0.0751	12544
0.1127	18890
0.1502	25365
0.1878	31203
0.2254	37586

Ruggedness

	Content %						
	Styrene	**Hydroxy**	**Bosentan stg- III**	**Dimer**	**Single unknown**	**Total unknown**	**Total impurities**
Ruggedness prep 1	0.1461	0.1509	0.1547	0.1366	0.0127	0.0299	0.6182
Ruggedness prep 2	0.1457	0.1501	0.1542	0.1361	0.0116	0.0300	0.6161
Ruggedness prep 3	0.1457	0.1522	0.1557	0.1369	0.0126	0.0299	0.6204
Ruggedness prep 4	0.1472	0.1509	0.1553	0.1366	0.0118	0.0304	0.6204
Ruggedness prep 5	0.1458	0.1520	0.1555	0.1361	0.0129	0.0306	0.6200
Ruggedness prep 6	0.1465	0.1515	0.1556	0.1370	0.0116	0.0292	0.6198
M.Precision-1	0.1471	0.1531	0.1563	0.1370	0.0137	0.0324	0.6259
M.Precision-2	0.1471	0.1553	0.1559	0.1393	0.0133	0.0314	0.6290
M.Precision-3	0.1464	0.1550	0.1538	0.1377	0.0133	0.0319	0.6248
M.Precision-4	0.1453	0.1563	0.1562	0.1378	0.0130	0.0320	0.6276
M.Precision-	0.1452	0.1546	0.1552	0.1365	0.0127	0.0315	0.6230

5							
M.Precision-6	0.1472	0.1556	0.1584	0.1398	0.0133	0.0322	0.6332
Average	0.1463	0.1531	0.1556	0.1373	0.0127	0.0310	0.6232
% RSD	0.51	1.40	0.75	0.86	5.54	3.48	0.80

ROBUSTNESS:

	%RSD						
	styrene	**Hydroxy**	**Bosentan stg- III**	**Dimer**	**Single unknown**	**Total unknown**	**Total impurities**
Wavelength 227nm	0.97	1.20	1.82	0.82	5.49	1.81	0.63
Wavelength 223nm	3.42	2.09	0.89	0.85	7.39	3.27	1.67
10% increase flow	3.23	1.76	3.22	2.44	7.55	6.42	2.73
10% decrease flow	3.23	1.76	3.22	2.44	7.55	6.42	2.73
pH 2.7	1.64	0.82	3.33	1.15	3.55	4.62	1.71
pH 2.3	1.53	0.65	3.62	1.30	6.57	4.99	1.79

ROBUSTNESS CONT

	%RSD						
Temperature 30ºC	1.97	3.15	2.56	1.84	8.45	4.96	0.87
Temperature 20ºC	1.90	2.18	3.72	1.24	7.50	3.24	2.22
10% rise in buffer composition.	3.03	3.73	6.37	4.30	7.38	5.04	4.31
10% decrease in buffer composition.	5.63	4.29	6.53	3.86	5.89	1.39	4.83
Stability at 0 hr	0.51	1.40	0.75	0.86	5.54	3.48	0.80
Stability at 24 hr	0.59	0.76	1.23	0.48	4.63	3.31	0.73
Stability at 48 hr	8.38	8.65	6.90	11.64	8.97	5.19	8.54

VALIDATION SUMMARY REPORT

Parameter	**Observation**
Linearity range(ppm)	0.01-0.225
Correlation co-efficient(NLT-0.99)	0.9999
% Recovery	93.79-98.67%
System Precision % RSD (NMT-5)	0.82
Method Precision% RSD (NMT-5)	0.72
Range	LOQ- 150%

System Precision Ruggedness(NMT-5)	0.52
Method Precision Ruggedness(NMT-10)	1.40
Limit of detection	3
Limit of Quantitation	10

4. CONCLUSION

A simple, precise, rapid, accurate and economical RP-HPLC method was developed and validated for the determination of Related Substances of Bosentan in bulk and Pharmaceutical dosage form. This method yielded high Recoveries with good linearity and precision. It can be concluded that the proposed method is a good approach for obtaining reliable results and found to be suitable for routine analysis of related substances of Bosentan. On following the proposed method for analysis of related substances in TRACLEER tablets, it is concluded that impurities are within the limits (NMT 0.2%).

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