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Research Article

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RP-HPLC METHOD DEVLOPMENT AND VALDATION OF BOSENTAN DRUG PRESENT IN TABLETS

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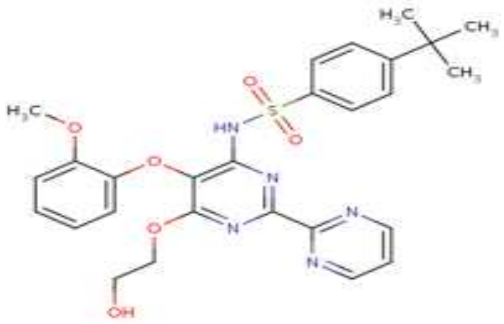
ABSTARCT:

A reverse phase isocratic high performance liquid chromatographic method was developed for the estimation of bosentan drug in tablet formulation. The separation was achieved by C-18 hypersil 250*4.6mm, 5µm and methanol: potassium dihydrogen orthophosphate buffer pH 7.8 (60:40 v/v) as mobile phase, at a flow rate of 0.8 ml/min. Detection was carried out at 220 nm. Retention time of Bosentan was found to be 8.26 +or-1. The method has been validated for linearity, accuracy and precision. Linearity of bosenatan was in the range 50-150 mcg/ml. The mean recovery obtained for was 99.1. Developed method was found to be accurate, precise, selective and rapid for estimation of bosentan in tablets.

Key words: RP-HPLC, Bosentan, method development and validation

INTRODUCTION: bosentan is competitive antagonist of endothelin-1 receptor acts on endothelin A and endothelin present smooth mussels of pulmonary blood vessels [1, 2, 3]. Bosentan used in the treatment pulmonary hypertension and digital ulcers in patients with systemic sclerosis .chemically it contain 4-tert-butyl-N-[6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)-2-(pyrimidin-2-yl) pyrimidin-4-yl] benzene-1-sulfonamide[2].

Molecular structure [1, 3]



Molecular weight 551.61. Molecular formula $C_{27}H_{29}N_5O_6S$.it is white to yellowish powder soluble in very low in water (1mg/100ml) and highly soluble in (43 mg/100ml) P^H 7.5[3, 4]. Bosentan available as tablets formulation as tracleer, Lupibose, Bosentas (125 mg, 62.5 mg) literature surveys reveals that bioanlytical methods are developed for analysis of metabolite of bosentan in blood plasma but there is no one RP-HPLC method available for analysis of drug present in formulation[5,6,7,8]. Present work explains that simple accurate and precise RP-HPLC method for estimation of drug present in the bosentan tablets.

MATERIAL AND METHOD:

Apparatus: HPLC (aglient 1200 series with PDA detector system) Ezchrome software,

PH meter (polmon), analytical balance (sartorius)

Chemicals: working standard of bosentan, methanol (standard chemicals), potassium dihydrogen ortho phosphates (standard chemicals), ortho phosphoric acid (standard chemicals), Millipore water (0.45µ filter)

Chromatographic condition [8, 9]:

Flow rate	**:**	1.0 ml/min
Column	**:**	hypersil C18, 250 x 4.6 mm, 5µ
Mobile phase	**:**	methanol: phosphate buffer P^H 7.5(60:40)
Detector wave length	**:**	220nm
Column temperature	**:**	Ambient
Injection volume	**:**	20 µl
Run time	**:**	15 mins

Standard Preparation: Accurately Weighed and transferred bosentan equivalent to 25 mg of bosentan Working Standard into a 100 ml clean dry volumetric flask, and 70 ml of mobile phase was added, sonicated for 5 minutes, and diluted to volume with mobile phase. Further diluted 5 ml to 50 ml with mobile phase.

Sample preparation: Weighed and powered 20 tablets. Transferred the powder equivalent to125mg of bosentan into 200 ml of clean, dry, volumetric flask and, to this added 140 ml of mobile phase and sonicated for about 15 minutes, further made up the volume with mobile phase and then filtered through 0.45 micron filter. Further diluted 2 ml of the filtrate to 50 ml with mobile phase.

% assay=samplearea/standardarea*standardweight/100*200/sample weight*50/2*average weight/label amount*standard potency /100*100

System suitability: The results obtained from five injections of standard solution shows that analytical method, equipment, chromatographic conditions and mobile phase suitable for analysis of drug present in the tablets (table 1,figure 2,3,4)

RESULTS AND DISCUSSION:

METHOD VALIDATION [10, 11]

The described method is validated as per ICH guidelines for the estimation bosentan drug present in the tablets of bosentan applying following parameter

LINEARITY

The linearity of chromatograph response is established by plotting a graph of concentration range 50 to 150% of standard preparation. The detector response found to be linear for 50% to 150% of concentration range. The correlation coefficient 0.9991(table 2, figure1)

PRECISION

The precision was demonstrated by repeatability and intermediate precision. The method was estimated by preparing by six samples for repeatability and six samples with different analyst for

intermediate precision .the repeatability and intermediate precision mean of assays and %RSD(relative standard deviation) was found to be 100.5%, 0.64and 101.1%, 1.17 respectively(table 3,table 4).

ACCURACY (RECOVERY):

Assay was performed in triplicate as per test method for various concentrations of bosentan equivalent to 50%, 75%, 100%, 125% and 150% of the labeled amount as per the test method. The average % recovery of Bosenatn was 99.1% and %RSD 0.70(table 5)

RUGGEDNESS:

Ruggedness of method is demonstrated by column to column variability as factors. Compare the both results of two different hplc systems and column variation. The mean assay was found to be 101.5 and %RSD 0.89(table6)

ROBUSTNESS:

Robustness of method demonstrated by changing of flow rate and temperature variations .the % RSD was calculated. the percentage %RSD should not be more than 2 (table7, 8)

Summary of validation parameter

Validation parameters	**results**
Linearity range	**50 -150 mcg/ml**
Correlation coefficient	**0.9991**
Precision	
a) Repeatability	**100.5 %(0.64%RSD)**
b) Intermediate precision	**101.1% (1.17%RSD)**
Accuracy (%recovery)	
50%	**100.05%**
75%	**98.91%**
100%	**99.24%**
125%	**98.91%**
150%	**98.14%**
Ruggeddeness	**101.50%, (0.89%RSD)**
Robustness	

A) Flow rate change

0.6 mL	**1.5(%RSD)**
0.8 mL	**0.05(%RSD)**
1.0 mL	**0.032(%RSD)**

B) Temperature change

20^{o}C	**0.15(%RSD)**
25^{o}C	**0.06(%RSD)**
30^{o}C	**0.83(%RSD)**

TABLES&FIGURES

Table-1: Summary of system suitability.

peak area	3893475
Theoretical plates	6773
Tailing factor	1.12
Mean retention time	8.26
%RSD	0.05

Table-2: Summary of linearity.

S.NO	% Test conc.	Average peak area
1	50	1818647
2	60	2437350
3	80	3104221
4	100	3867905
5	120	4589087
6	140	5306815
7	150	5911878

Figure-1: Linearity graph.

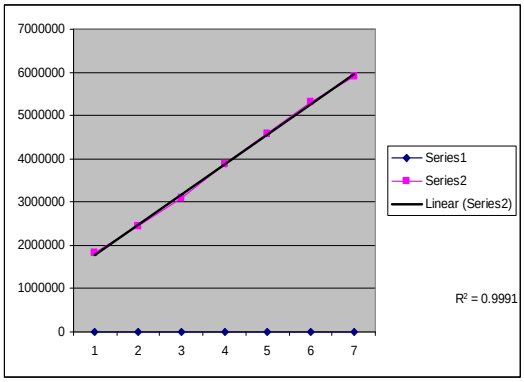


Table-3: Summary of repeatability.

Injection. No	Std Area	Sample area	%assay
1	3846748	3826768	99.26
2	3854717	3881412	100.68
3	3854748	3890480	100.93
4	3842345	3862787	100.20
5	3854751	3890149	100.90
6		3884587	100.76
Mean	3850662		100.5
SD:			0.64
%RSD			0.64

Table-4: Summery of intermediate precision.

S.No	Std Area	Sample area	%assay
1	3933354	3939201	100.06
2	3944563	4002197	101.66
3	3956713	4045632	102.76
4	3946282	3914862	99.44
5	3967323	3989201	101.33
6		3979221	101.07
Mean	394647		101.1
SD			1.18
%RSD			1.17

*** Std area=standard area, S.D=standard deviation,%RSD=percentage relative standard deviation**

Table-5: Summary of recovery studies.

s.no	Spiked levels	Mean recovery
1	50%	100.05
2	75%	98.91
3	100%	99.24
4	125%	98.91
5	150%	98.14
Mean		99.1
S.D		0.69
%RSD		0.70

Table-6: Summary of Ruggedness.

S.No	Std area	Sample area	%assay
1	3890567	3909021	100.21
2	3898245	4000151	102.55
3	3890921	3939245	100.99
4	3890618	3992206	102.35
5	3902430	3939405	100.99
6		3969201	101.76
Mean	3895936		101.5
S.D			0.90
%RSD			0.89

Table-7: Summary of temperature variation.

Temp 20°c	Std Area	Tailing factor	Temp25 °c (ambient)	Std Area	Tailing factor	Temp 30°c	Std Area	Tailing factor
	4000123	1.02		3943213	0.86		3829126	1.21
	4001834	1.04		3947418	0.87		3836715	1.20
	3990543	1.0		3943181	0.86		3824128	1.19
mean	3997500	1.02	mean	3944604	0.86	mean	3829990	1.2
SD	6085.37	0.02	SD	2437.04	0.005	SD	6337.79	0.01
%RSD	0.15	1.96	%RSD	0.06	0.66	%RSD	0.16	0.83

Table-8: Summary flow rate change.

Flow 0.6 ml	Std Area	Tailing factor	Flow 0.8 ml	Std Area	Tailing factor	Flow 1.0 ml	Std Area	Tailing factor
	3892560	0.97		3948568	0.73		3845212	1.02
	3889411	0.99		3950554	0.74		3843285	1.00
	3896024	0.99		3852342	0.74		3845028	1.03
Mean	3892665	0.996	Mean	3950438	0.74	Mean	3844708	1.02
SD	3307.75	0.005	SD	1887.87	0.005	SD	1250.07	0.015
%RSD	0.09	1.55	%RSD	0.05	0.78	%RSD	0.032	1.55

Figure-2: Chromatogram blank.

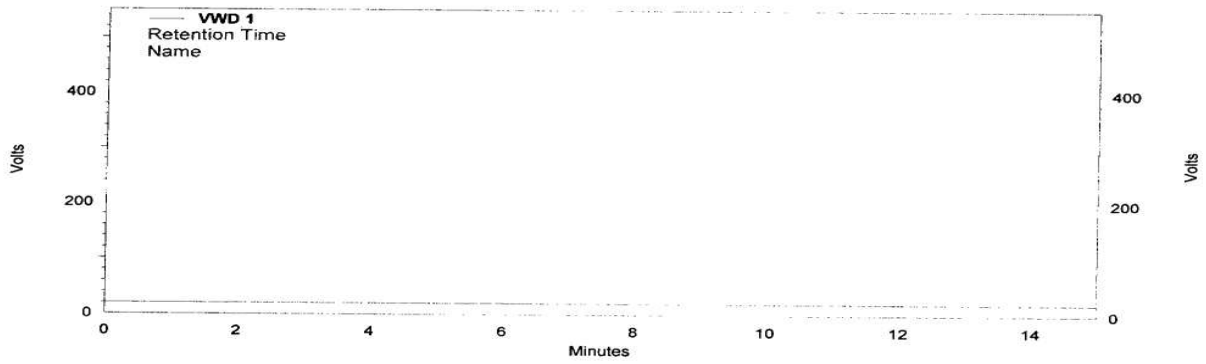


Figure-3: Chromatogram standard.

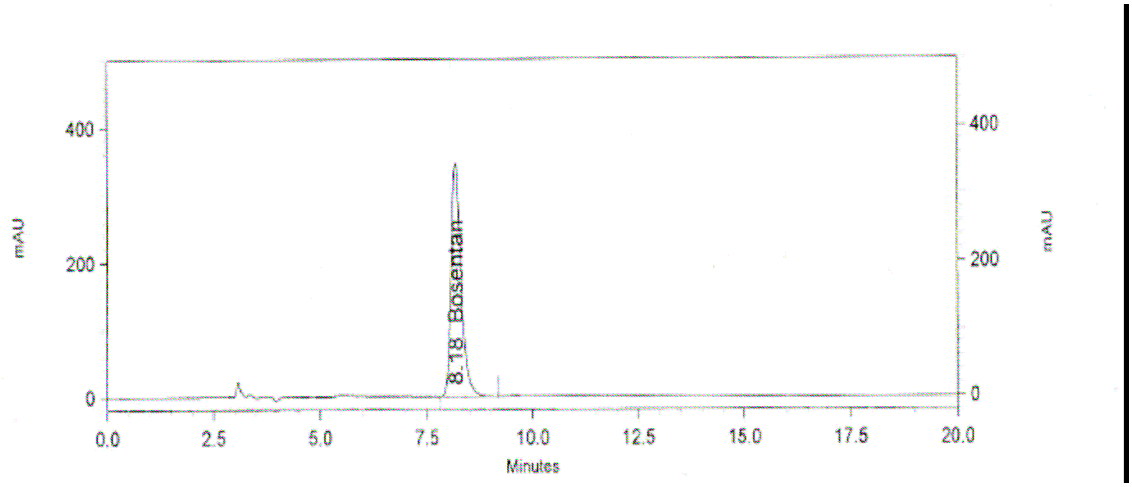
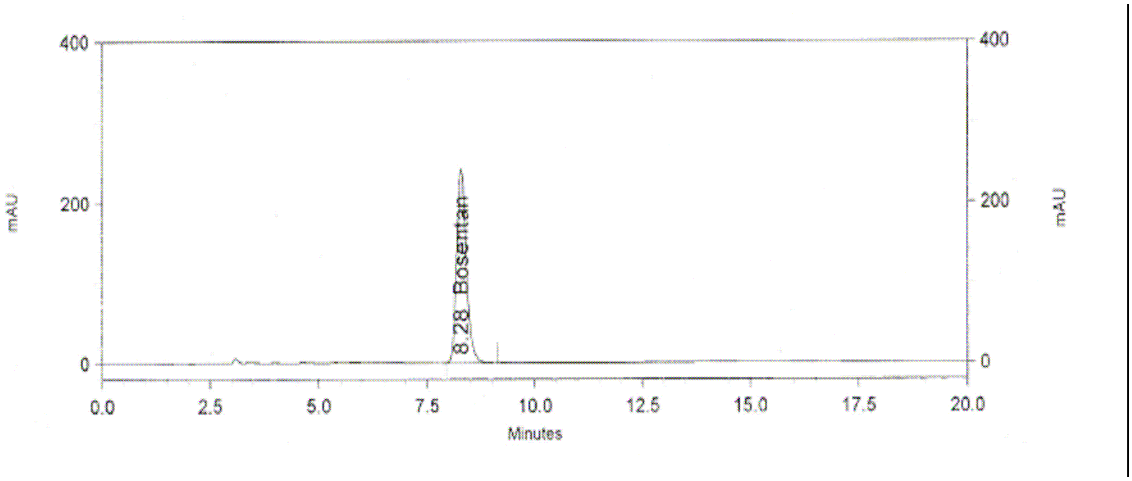


Figure-4: Chromatogram of sample.



CONCLUSION:

The newly developed and validated isocratic reverse phase high performance liquid chromatography with UV detection method for the estimation bosentan offers simplicity, linearity, precision, accuracy, ruggedness, robustness. The method is simple accurate, economical and rapid .the method easily adopted for routine analysis of laboratories and quality control test of raw material, finished products and dissolution studies of same formulation and also employed for impurity studies, stability studies, and toxicological, clinical studies of the same formulation.

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