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A New Quantitative Analytical Method Development and Validation for the Analysis of Boceprevir in Bulk and Marketed Formulation

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ABSTRACT

A simple, precise and accurate RP-HPLC technique was developed and the developed method was validated for the regular analysis of Boceprevir. Chromatographic analysis was performed by selecting X-Terra ODS (C18) column (4.6 mm i.d. × 250 mm, 5µ), Acetonitrile : Phosphate buffer pH -3 (90 : 10% v/v) as mobile phase, 1.0 ml/min as flow rate and 20µl injection volume. The LC chromatographic peak was eluted at 3.6 min at 235 nm as UV detection wavelength. The developed method was validated as per the ICH guidelines and the Validation parameters were specificity, accuracy, linearity, precision, LOD and LOQ. Linear relationship for Boceprevir established in the concentration range of 50 to 150µg/mL. Accuracy in terms of percentage recovery found in the range between 98 to 101%. LOD and LOQ values were found to be 2.3 and 7.123µg/mL respectively. The results of the method established that the new RP-HPLC method is convenient and simple in regular analysis of Boceprevir in bulk and capsule formulation.

Keywords: Boceprevir, Protease-inhibitor, Hepatitis-C, RP-HPLC, Accuracy, ICH guidelines.

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INTRODUCTION

Chromatography is a simple and economical tool for the analysis of different kinds of drugs. RP-HPLC a one of the chromatographic method, used in present research work. Chemically Boceprevir denoted as (1*R*,5*S*)-*N*-[3-Amino-1-(cyclobutylmethyl)-2,3-dioxopropyl]-3-[2(*S*)- [[[(1,1-dimethylethyl)amino] carbonyl] amino] -3,3-dimethyl-1-oxobutyl]- 6,6-dimethyl -3-azabicyclo[3.1.0]hexane-2(*S*)-carboxamide. Boceprevir drug is a protease-inhibitor used in the treatment of hepatitis caused by Hepatitis-C virus (HVC) genotype-1.[1-2] Mechanism of drug is a NS3/4a

protease inhibitor which inhibits viral-HCV replication. NS3/4a protease is an important part of viral replication and facilitates the cleavage of virally encoded poly-protein to mature proteins (NS5A, NS5B, NS4A and NS4B). It covalently and reversibly binds through (α)-keto-amide group to serine (S-139) residue in the active site. It inhibits the HCV 1a and 1b encoded enzyme proteolytic activity.

Boceprevir belongs to DAAs (Direct Acting Antivirals) medication and useful in combination therapy for the treatment of chronic Hepatitis-C, liver disease affected by Hepatitis-C Virus (HCV). HCV comes under the single stranded RNA virus which is classified into nine different genotypes. The genotype-1 is the most common type affecting 72% of all chronic Hepatitis-C virus patients in USA. [3] Treatment choices for chronic Hepatitis-C have advanced pointedly since 2011, with the improvement of Direct Acting Antivirals such as Boceprevir. The development in resistance to NS3/4A inhibitors is poorer than that of NS5B inhibitors (another class of DAAs). [4] Literature review for the estimation of Boceprevir revealed that the drug was estimated by Spectroscopic [5], High Performance Liquid Chromatography (HPLC) [6-7] and LC-MS/MS [8-9] methods. The objective of present research work is to simplify the estimation of Boceprevir with good precision and accuracy. The recommended method was validated according to the guidelines given by International Conference on Harmonization (ICH). [10]

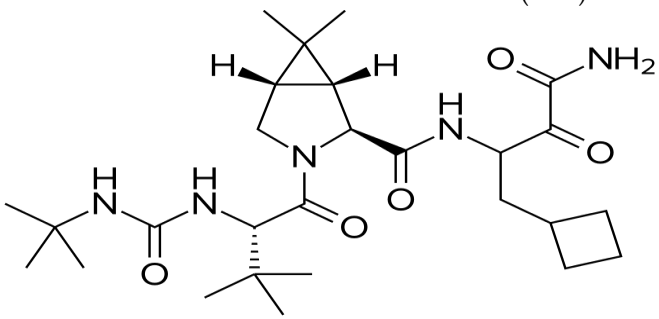

Fig. 1: Structure of Boceprevir

MATERIALS AND METHODS

Materials and reagents

Pharmaceutical reference grade Boceprevir drug was gifted by Hetero drugs, one of the leading company in Hyderabad, Telangana. Victrelis (containing 200 mg Boceprevir) Capsules bought from the local Pharmacy store. Other reagents and chemicals like KH_2PO_4, HPLC grade Acetonitrile and orthophosphoric acid were Purchased from MERCK, Mumbai, India.

Instruments

Shimadzu LC system with Isocratic (single) pump, model prominence LC-20AD, with prominence UV/Visible detector (SPD-20A) and rheodyne injector with 20µL capacity fixed loop was used. All samples weighed by using LC-GC AGN-204PO balance.

Chromatographic conditions

Chromatographic analysis was performed on X-Terra ODS (C18) column (4.6 mm i.d. × 250 mm, 5µ) using Acetonitrile : Phosphate buffer pH -3 (90 : 10% v/v) as mobile phase composition. Shimadzu LC system flow rate maintained at 1.0 ml/min and 20µL was the injection volume through fixed loop. The HPLC chromatographic peak was eluted at 3.6 min at 235 nm as UV detection wavelength. In this method mobile phase (as diluent) only used for all the standard and sample dilutions.

Preparation of phosphate buffer pH 3.0

1.36 g of potassium dihydrogen orthophosphate and 2 ml of water added to 800 ml of water. Contents were dissolved in water and the pH was adjusted to 3.0 with orthophosphoric acid and sufficient water added to make up to 1000 mL.

Preparation of mobile phase

Mobile phase was prepared by mixing Acetonitrile: Phosphate buffer pH -3 (90: 10% v/v) and the mixture was degasified by sonication and vacuum filtration using 0.45µ filter. Freshly prepared mobile phase used as diluent for the preparation of standard and sample stock and working solutions.

Preparation of Stock and working standard solutions

An accurately weighed sample of 100 mg of Boceprevir (working standard) was transferred into a 100 mL volumetric flask. The solvent mobile phase was added and sonication to dissolve it completely and made up to the mark with the same solvent. 10 mL of the above stock solution was pipetted into a 100 mL volumetric flask and diluted up to the mark with mobile phase. The contents were mixed well and filtered through 0.45µ filter paper.

Preparation of working Sample solutions

Twenty tablets of Boceprevir (INVOKANA) were crushed to give finely powdered material. Powder equivalent to 100 mg of Boceprevir was taken in 100 mL of volumetric flask containing few ml of mobile phase and was shaken to dissolve the drug and then diluted to the mark with mobile phase. Above solution filtered through 0.45µ filter paper and from the filtrate 10 mL transferred into a 100 mL volumetric flask and diluted the mark with the same solvent to obtain concentration of 100 ppm.

Validation of Analytical Method

The objective of the method validation is to make evident that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for specificity, linearity, precision, accuracy, robustness, limit of detection, limit of quantification and system suitability.

Specificity

Method specificity determined by observing and comparing the test results obtained for sample solution (containing excipients) with that of standard results those obtained for pure drug.

System Suitability Parameters

System suitability parameter was carried out by preparing standard stock solutions of Boceprevir and by calculating the standard deviation of it by injecting

standard solution in six replicates and the values were recorded.

Linearity

Boceprevir linearity performed by taking five different concentrations of working standard i.e., 50, 80, 100, 120 and 150 ppm were injected into HPLC system. Linearity graph was plotted by taking peak area on Y - axis and Concentration on X – axis and the r^2 value and Y- intercept values were calculated from the calibration curve.

Accuracy

Method accuracy was performed in terms of percentage recovery of added standard Boceprevir at 50%, 100% and 150% spiked levels. For each triplicate spiked concentration, mean, standard deviation and %RSD values were calculated and the values will be within the limit as per the ICH guidelines.

Precision

The precision of the method expresses the closeness between a series of determinations obtained from multiple sampling of the same sample under the same analytical conditions. Precision may be performed in three different levels: repeatability, reproducibility and intermediate precision. Precision is stated by mean, standard deviation and percentage relative standard deviation.

Limit of Detection (LOD) & Limit of Quantification (LOQ)

The detection limit defined as the lowest amount of analyte which can be identified but cannot be quantified exactly. The quantitation limit defined as the lowest amount of analyte which can be quantified with suitable accuracy and precision. LOD and LOQ can be estimated by following formulas:

$$LOD=3.3\times\sigma/S$$

$$LOQ=10\times\sigma/S$$

σ = Standard deviation estimated based on the calibration curve, S = Slope of the calibration curve.

Robustness

The robustness of method expresses the resistance of chromatographic conditions by small change in the analytical conditions. To estimate robustness of analytical method chromatographic conditions like temperature, pH of mobile phase, flow rate and mobile phase composition were varied.

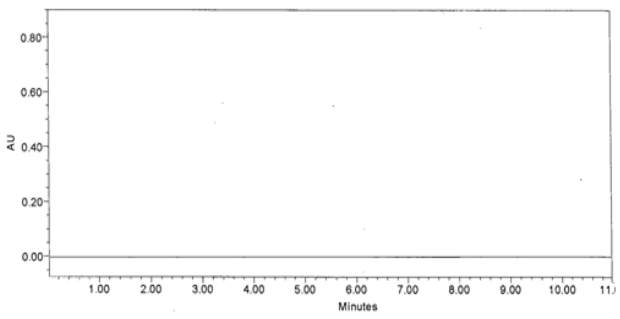

Fig. 2: Blank chromatogram Boceprevir

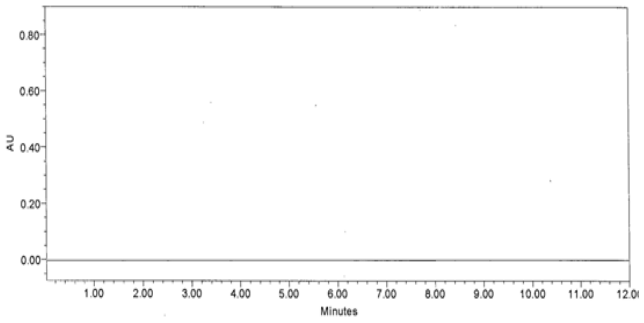

Fig. 3: Placebo Chromatogram of Boceprevir

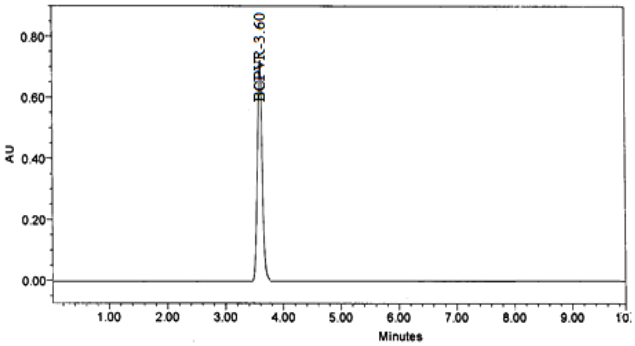

Fig. 4: Standard chromatogram of Boceprevir

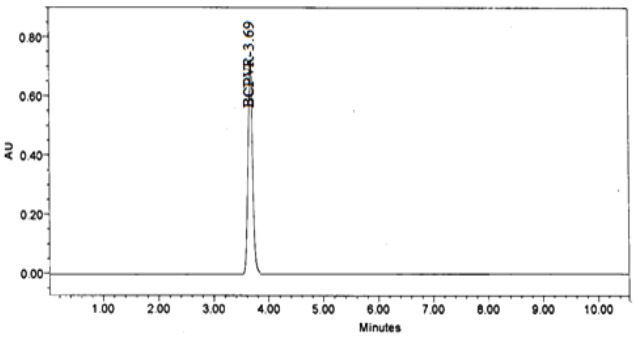

Fig. 5: Sample chromatogram of Boceprevir

RESULTS AND DISCUSSION

System Suitability Parameters

System suitability parameters were studied by injecting Boceprevir standard in six replicates and the values were shown in the Table 1.

Accuracy

The accuracy of the projected method was estimated on three different concentration levels by recovery experiment and the method was reported by addition of known quantity of standard in the placebo. The three triplicate concentration levels were 50%, 100%, 150% by the addition known quantity of drug. Results for percentage recovery values were found to be between 98% and 102%. These recovery values specify that method was accurate and values were indicated in the Table 2.

Linearity

From the standard stock solution 50, 80, 100, 120 and 150 ppm solutions were prepared and injected into HPLC system and corresponding peak areas were measured. From the concentration and peak area values calibration curve was plotted and the r^2 and Y-intercept values were found to be 0.999 and 78201

respectively. The linearity curve was shown in Figure-6. Results were shown in the Table 3.

Table 1: System Suitability Parameters of Boceprevir

S. No	Parameters	Boceprevir
1	Retention time (min)	3.6
2	Theoretical plates	9542
3	Tailing factor	1.02
4	%RSD	0.54

Table 2: Accuracy of Boceprevir

Sample name	Amount added (µg/ml)	Peak area found	% recovery	% RSD
50% solution	50	1318858	98.3	
50% solution	50	1319880	99.2	0.82
50% solution	50	1320820	100.3	
100% solution	100	2616641	98.8	
100% solution	100	2656656	100.2	0.59
100% solution	100	2616749	99.8	
150% solution	150	3969449	99.3	
150% solution	150	3976534	99.5	0.34
150% solution	150	3986399	100.1	

Table 3: Linearity of Boceprevir

S. No	Concentration (ppm)	Peak area
1	0	0
2	50	1318820
3	80	2104079
4	100	2616641
5	120	3145310
6	150	3986456

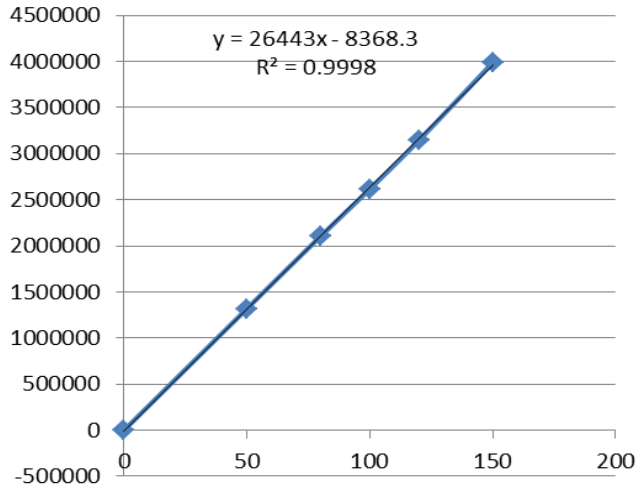

Fig. 6: Linearity of Boceprevir

Precision

Intra-day precision: It was performed at three different concentration levels low (80µg/mL), mid (100µg/mL) and high (120µg/mL) within the same day at three different times (session 1, 2, 3).

Inter-day precision: It was carried out at same concentration levels on three consecutive days, using same homogeneous sample. The % RSD values for both intra-day and inter-day precision were found within acceptable limit. Results are presented in Tables 4 and 5 respectively.

LOD AND LOQ

Values of LOD and LOQ were calculated using slope of calibration curve. LOD and LOQ values of Boceprevir for HPLC method are tabulated in Table 6. Determined based on the standard deviation of the response and slope of the calibration curve.

Table 4: Intra-day precision results

Level		Low	Mid	High
Concentration (ppm)		80	100	120
Peak area	Session 1	2094080	2617625	3141150
	Session 2	2102056	2625990	3205421
	Session 3	2102346	2599896	3204496
Average peak area		2099494	2614504	3183689
Standard Deviation		3830.106	10879.07	30081.99
%RSD		0.18243	0.416105	0.944878

Table 5: Inter-day precision results

Level		Low	Mid	High
Concentration (ppm)		80	100	120
Peak area	Session 1	2084085	2616628	3141856
	Session 2	2103059	2627994	3204429
	Session 3	2101346	2598891	3204599
Average peak area		2096163	2614504	3183628
Standard Deviation		8569.255	11975.77	29537.35
%RSD		0.408807	0.458051	0.927789

Table 6: Results of LOD and LOQ

Parameters	Result
LOD	2.3 ppm
LOQ	7.123 ppm

Table 7: Robustness of Boceprevir

Chromatographic condition	Variation	% RSD
Mobile phase	A) Potassium dihydrogen phosphate buffer pH- 3.0 adjusted with orthophosphoric acid and acetonitrile in 11:89 ratio	0.76
	B) Potassium dihydrogen phosphate buffer pH- 3.0 adjusted with orthophosphoric acid and acetonitrile in 9:91 ratio	0.89
Flow rate	A) 0.9 mL/min	0.69
	B) 1.1 mL/min	0.85

Robustness

Robustness of method was studied by making slight but deliberate changes in chromatographic conditions such as proportion of organic phase in mobile phase composition and flow rate. Effects of these changes on both the retention time (RT) and peak area were evaluated by calculating the relative standard deviations (%RSD). The results obtained are tabulated in Table 7.

A new economical, simple and precise RP-HPLC method has been developed for the analysis of Boceprevir in capsule formulation. Chromatographic conditions for Boceprevir estimation includes X-Terra ODS (C18) column (4.6 mm i.d. × 250 mm, 5µ) and with mobile phase combination consists of potassium dihydrogen phosphate buffer pH- 3.0 adjusted with orthophosphoric acid and acetonitrile in 10:90 ratio. The drug peak eluted at 3.6 min by operating the instrument at the flow rate1 ml/min and detected at 235 nm by using ultraviolet detector. Accuracy in terms of percentage recovery found in the range between 98 to 101%. LOD and LOQ values for the drug were 2.3

ppm and 7.123 ppm respectively. Linear relationship for Boceprevir established in the concentration range of 50 to 150µg/mL. The %RSD values of method validation parameters reveals that the developed method is simple, sensitive, robust, rugged and accurate and the method also useful for regular quantitative analysis of Boceprevir.

REFERENCES

1. Degertekin B, Lok AS. Update on viral hepatitis: 2007. Curr. Opin. Gastroenterol. 2008; 24 (3): 306–11.
2. Njoroge FG, Chen KX, Shih NY, Piwinski JJ. Challenges in modern drug discovery: a case study of boceprevir, an HCV protease inhibitor for the treatment of hepatitis C virus infection. Acc. Chem. Res. 2008; 41 (1): 50–9.
3. American Association for the study of Liver Diseases; Infectious Diseases Society of America. HCV Guidance. http://hcvuidelines.org. Accessed June 12, 2017.
4. Bagaglio S, Uberti-Foppa C, Morsica G. Resistance Mechanisms in Hepatitis C Virus: implications for Direct-Acting Antiviral Use. Viruses. 2017; 77(10):1043-1055.
5. Govardhan Reddy P, Kiran Kumar V, Appala Raju, VVSS, Raghu Ram J, Appala Rraju N. Novel Spectrophotometric Method Development for the Estimation of Boceprevir in Bulk and in Pharmaceutical Formulations. Research J. Pharm. and Tech 2017; 10(12): 4313-4316.
6. Ganji S, Satyavati D. Development and Validation of RP – HPLC Method for the Analysis of Boceprevir and Related Impurities in Bulk and Pharmaceutical Dosage Forms. Indo American Journal of Pharmaceutical Research. 2015; 5(6): 2391-2401.l
7. Damle MC, Salunke SR. Development and Validation of Stability Indicating HPLC Method for Determination of Boceprevir. International Research Journal of Pharmacy. 2016; 7(8):47-53.
8. Chandramowli B, Rajkamal BB. A Validated LC-MS/MS Method for the Estimation of Boceprevir and Boceprevir D6 (IS) in Human Plasma Employing Liquid-Liquid Extraction. International Journal of Pharmacy and Pharmaceutical Sciences. 2016; 8(7): 133-137.
9. Harald Farnik H, El-Duweika J, Welsch C, *et al*. Highly sensitive determination of HCV protease inhibitors boceprevir (SCH 503034) and telaprevir (VX 950) in human plasma by LC–MS/MS. Journal of Chromatography B. 2009; 877(31):4001-4006.
10. International conference on harmonization (ICH), Validation of Analytical Procedures: text and methodology Q2 (R1), Geneva, 2005.

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