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ULTRAFAST LIQUID CHROMATOGRAPHIC ESTIMATION OF A NOVEL ANTI-HEPATITIS C AGENT PRESENT IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

An ultrafast liquid chromatographic method was developed and validated for estimation of a novel anti-hepatitis C agent, boceprevir (BOC) in bulk and tablet formulation. Chromatography was performed on a C-18 column using methanol: 10mM TBAHS (80:20, v/v) as mobile phase flowing at 1.2ml/min. PDA detection was carried out at 203 nm. Retention time for BOC was 2.451min.The method linearity was observed over a range of 2.5-40µg/ml. The developed method was validated as per ICH guidance for various validation parameters such as accuracy, precision, sensitivity, selectivity and specificity etc. The developed method was found suitable for estimating analyte in both bulk as well as in tablet dosage form. Overall the method was rapid, reliable and possesses the potential of application in routine and bio-analytical purposes.

KEYWORDS: Boceprevir, UFLC, validation, robustness.

INTRODUCTION

Liquid chromatography is a versatile analytical tool for drug analysis and testing, being capable of analyzing drugs in diverse samples. However, the recent development to liquid chromatography technique such as ultrafast liquid chromatography (UFLC) is finding its widespread applications in the field of pharmaceuticals. [1-3] The various merits like lower mobile phase usage, rapid analysis, and high sensitivity, thus advocates for utilization of

UFLC method over traditional HPLC for analysis of drugs in different samples during routine analysis.

Boceprevir (BOC), i.e. 3-{[(1R,2S,5S)-3-[(2S)-2-[(tert-butylcarbamoyl)amino]-3,3-dimethylbutanoyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexan-2-yl]formamido}-4-cyclobutyl-2-oxobutanamide is a novel anti-hepatitis C agent.[4] Literature reveals few chromatographic methods are reported for quantifying BOC in pharmaceuticals, which include, HPLC, HPTLC and LC-MS. [5-7] Most of these reported methods lack significantly in terms of method robustness effecting the reliability to the results produced. In order to overcome the above drawbacks and confirm method reliability and superiority than the reported methods a new liquid chromatographic method was developed for determination of BOC present in API as well as in the in-house prepared tablet formulation.

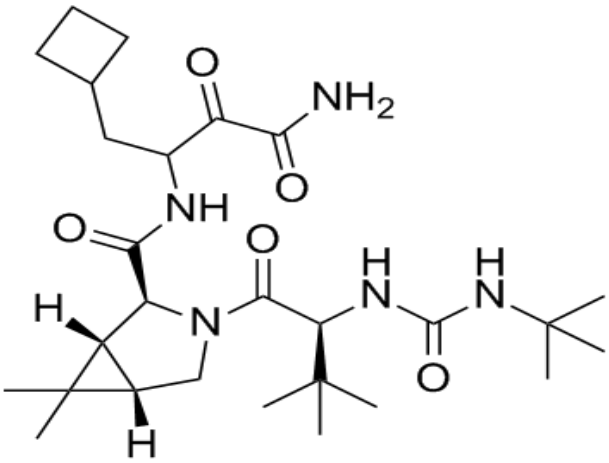

Figure 1: Chemical structure of boceprevir.

In this study, an accurate and precise UFLC method was developed for determining BOC in bulk drug and samples of in-house tablets. Validation study for the newly optimized chromatographic method was performed as ICH guidance.[8] Further, the amount of BOC present in the in-house tablet formulation was determined using UFLC.

MATERIALS AND METHODS

Materials

Boceprevir (purity > 98%) was obtained from Glenmark Generics Ltd., India. HPLC grade methanol and tetra butyl ammonium hydrogen sulfate (TBAHS) were procured from Merck Ltd. and HI-Media, Mumbai, India, respectively and were used. HPLC grade water prepared

by using TKA GenPure Ultra-Purification System, Germany was used for preparing TBAHS solution. The in-house tablet formulation containing 200mg of BOC was analyzed by the UFLC method.

Instrumentation

A SHIMADZU Prominence UFLC with binary pumps and a PDA detector with LC solution software were used to the chromatographic purpose. An Enable C-18 column, (250×4.6 mm, 5 µm) was used as stationary phase. A mobile phase of methanol: 10mM TBAHS (80:20, v/v) flowing at 1.2 mL/min was utilized for 15min. BOC was detected at 203nm. About 1.697g of TBAHS salt was dissolved in 500mL of water followed by ultrasonication and membrane filtration to produce 10mM solution.

Methods

Preparation of Calibration Curve

Around 50 mg of BOC was transferred into a 50 mL volumetric flask having 25 mL of mobile phase and dissolved in it. Finally, the volumes were made up to produce 1000µg/mL standard stock solution. From this solution, calibration concentrations of 2.5-40 µg/mL were prepared and chromatography was performed in triplicate (n=3). The calibration plot was generated taking concentration (µg/mL) on x-axis and peak area on the y-axis. Regression analysis (including ANOVA) of calibration data was performed to detect the regression statistics.

Method Validation Studies

Specificity

To evaluate specificity of the method analyte was added to known placebo and visual inspection was carried out for chromatographic interferences. The additives used in formulation were added to the standard solutions and analyzed.

Accuracy

Recovery studies were performed in triplicate by spiking the known excipients solutions with standard BOC at 80,100 and 120% of the test concentration (10 µg/mL). Further, the recovery of the spiked concentration of standard BOC was calculated.

Precision

The precision study in terms of system, interday and intraday was conducted. System precision was determined by six injections of a selected concentration (10 µg/mL) of analyte. The intraday (same day) and inter-day (different day) precision were calculated by injecting six solutions of a fixed concentration (10 µg/mL) of analyte. A % relative standard deviations (%RSD) lower than 2.0 was the acceptance value for all the precision studies.

Limit of detection (LOD) & Limit of quantitation(LOQ)

Signal to Noise (S/N) ratio of 3:1 and 10:1 were considered vital for visual detection of LOD and LOQ, respectively.

System Suitability Parameters

Various system suitability parameters such as retention time (Rt), theoretical plates (N), tailing factor (T) etc. were evaluated to monitor method performance.

Assay Procedure

In house tablet formulation powder equivalent to 25 mg of BOC was transferred into a 50 mL volumetric flask, containing 25 mL of mobile phase. The contents were vortexed for 5 min followed by 30 min of ultrasonication. Finally, volume was made up and filtration was done through a 0.45µm filter. Further, the filtered solution was diluted with mobile phase for UFLC analysis. These solutions were stored at 2-8ºC till further use.

RESULTS AND DISCUSSION

Method development studies

The early chromatographic method development studies were performed based on physicochemical properties of analyte. An Enable C-18 column was used for chromatography of the compound based on its suitability towards the analyte. Trials were performed using mobile phase composition of methanol: water, methanol: buffer, methanol: 10mM TBAHS at varying ratios (i.e., 50:50, 60:40, 70:30,80:20 v/v) and flow rates (1.0, 1.1 and 1.2 mL/min) at room conditions. Among these, methanol: 10mM TBAHS (80:20, v/v) flowing at 1.2 mL/min produced symmetrical peak shape with optimum system suitability conditions. Thereafter, the method validation studies were carried out as per the above chromatographic conditions. A typical chromatogram (Figure 2) of BOC in tablets revealed optimum peak shape in the optimized experimental conditions.

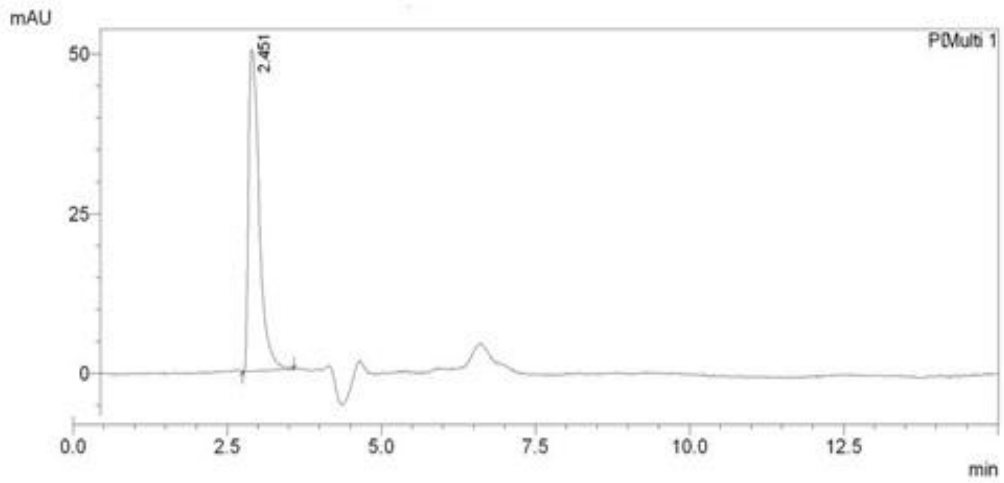

Figure 2: Typical chromatogram of BOC in in-house tablet formulation.

Method validation studies

Specificity

Visual assessment of chromatograms for both analyte and placebo revealed that the method is specific for determination of BOC, without any interference from placebo content.

Linearity

The method was found linear over concentration range of 2.5-40µg/mL (r^2=0.998). Further, satisfactory results obtained through regression analysis and ANOVA of linearity data indicated goodness of fit.

Accuracy

Satisfactory recoveries of BOC between 99.79-100.63%, advocated for optimum method accuracy and reliability.

Precision

The precision study revealed acceptable values of % RSD (<2%). The values were 0.41%, 1.11% and 0.39% for intraday, inter-day and system precision, respectively.

Limit of detection (LOD) & Limit of quantitation (LOQ)

The LOD and LOQ values were 1.0 and 2.5 µg/mL, respectively.

System Suitability

The observed system suitability results were found satisfactory (Table 1) as per the acceptance criteria.

Table 1: System Suitability of the UFLC Method.

Parameter	Criteria	Observed Values
Retention time (min)	NLT 2.0	2.451
Theoretical plates (N)	NLT 2000	3061
Tailing factor (T)	NMT 2.0	1.576

Assay of in-house formulation

The visual evaluation of chromatograms obtained for in-house tablet formulation indicated method selectivity due to non-interference of any of the formulation components. The mean (n= 3) content of BOC was found to be 99.14% (SD = ±0.28).

CONCLUSION

The present research explains optimization and development of an UFLC method for determining BOC in bulk and tablets. Overall, the chromatographic method was found suitable and trustworthy for determining BOC. Results of validation study were found compliant with ICH guidelines. Hence, this method is acceptable for estimating BOC in bulk and tablet formulation. Further, the above mentioned method has the potential for determining BOC in biological fluids.

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REFERENCES

1. Panda, S.S., Ravi Kumar, B.V.V., Patel, P.K., Panda, J. 2013. New RP-UFLC method for estimation of xanthinol nicotinate in pharmaceutical dosage form. *Chem Sci Trans*, 2(2): 656-662.
2. Panda, S.S., Ravi Kumar, B.V.V., Mohanta, G., Panda, J. 2013. Reverse phase ultra fast liquid chromatography method for simultaneous estimation of citicoline sodium and piracetam in tablets. *Int J Pharm Sci Nanotech*, 6(1): 1952-1957.
3. Panda, S.S., Ravi Kumar, B.V.V., Acharjya, S.K., Sahu, K. 2014. RP-UFLC method for estimation of propafenone in tablets. *Int J Pharm Sci Nanotech*, 7(4): 2671-2676.
4. Accessed online from: https://pubchem.ncbi.nlm.nih.gov/compound/Boceprevir.
5. Damle, M.C., Salunke, S.R. 2016. Development and validation of stability-indicating HPLC method for determination of boceprevir. *Int Res J Pharm*, 7(8): 47-53.

6. Damle, M.C., Salunke, S.R. 2016. Development and validation of stability-indicating HPTLC method for determination of boceprevir. *World J Pharm Res*, 5(8): 961-973.
7. Chandramowli, B., Rajkamal, B.B. 2016. Avalidated LC-MS/MS method for the estimation of boceprevir and boceprevir D6 (IS) in human plasma employing liquid-liquid extraction. *Int J PharmPharma Sci*, 8(7): 133-137.
8. Guideline of International Conference on Harmonization, Validation of Analytical Procedure: Text and Methodology- ICH, Q2 (R1). 2005.