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ORIGINAL ARTICLE

Method Development and Validation for the Determination of Baloxavir Marboxil in Tablet Formulation by RP-UPLC; Stability Studies

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ABSTRACT
A simple, accurate and precise method was developed for the estimation of BLMX in Tablet dosage form by RP-UPLC technique. Retention time of BLMX was found to be 0.682min and an excellent chromatographic efficiency parameters were obtained with the mobile phase composition of buffer 0.1% orthophosphoric acid and acetonitrile in the ratio of 60:40%v/v pumped through an Zorbax C18 (100 x 2.1 mm, 1.8µ) reverse phase column, at a flow rate of 0.3 mL/min. Column oven temperature was maintained at 30℃ and the detection wavelength was processed at 240 nm.. Repeatability of the method was determined in the form of %RSD and the value was 0.2. The percentage mean recovery of the method was found to be 99.47%. Drug was subjected for acid, peroxide, photolytic, alkali, neutral and thermal degradation studies and the results shown that the percentage of degradation was found between 5.96% and 9.55%. Retention time and total run time of the drug was decreased and the developed method was simple and economical. So, the developed method can be adopted in industries as a regular quality control test for the quantification of ECRB and BMTB.
Keywords: Baloxavir marboxil, Antiviral, Robustness, Degradation Studies**,** RP-UPLC.

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INTRODUCTION

BLMX, sold under the brand name Xofluza, is an antiviral medication for the treatment of influenza A and influenza B flu [1]. Baloxavir marboxil was developed as a prodrug strategy, with its metabolism releasing the active agent, baloxavir acid (BXA). BXA then functions as inhibitor, targeting the influenza virus' dependent endonuclease activity, one of the activities of the virus polymerase complex [2]. In particular, it inhibits a process known as snatching, by which the virus derives short, capped primers cell RNA transcripts, which it then uses for the polymerase synthesis of its needed viral mRNAs [3]. A polymerase subunit binds to the host pre-mRNAs at their 5′-caps, then the polymerase's endonuclease activity catalyzes its cleavage "after 10 nucleotides". As such, its mechanism is distinct from inhibitors such as oseltamivir and zanamivir.

BLMX chemically designated as ({(12a*R*)-12-[(11*S* 11–dihydrodibenzo [*b*, *e*] thiepin-11-yl]-6, 8-dioxo hexahydro-1*H*-[1,4]oxazino[3,4-*c*]pyrido[2,1-*f*][1,2,4]triazin yl}oxy)methyl methyl carbonate with molecular weight of 571.55 g/mole and $C_{27}H_{23}F_2N_3O_7S$ respectively Fig.1. literature review unveils that no single method was reported for the quantification of BLMX. Based on the literature, there is a need to develop a stability-indicating RP-UPLC method for the quantification of BLMX in bulk and dosage forms [4].

MATERIAL AND METHODS

Chemicals and Reagents

API of BLMX was obtained from spectrum Pharma Research Solutions, Hyderabad. HPLC-grade methanol and acetonitrile were procured from Merck chemical division, Mumbai, India, Potassium dihydrogen ortho phosphate, orthophosphoric acid, sodium dihyrogen ortho phosphate and HPLC-grade water were bought from Rankem, avantor performance material india limited. Xofluza 40 mg tablets were obtained from local pharmacy.

Method development

During the method development various mobile phase compositions consisting of methanol, acetonitrile, water, phosphate buffers and different stationary phases were executed to get fine chromatographic conditions like theoretical plates, resolution, tailing and peak shape.

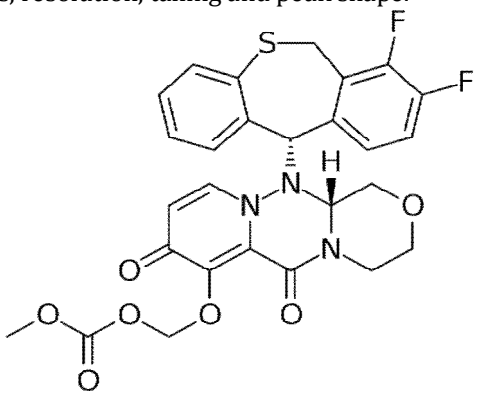

Fig. 1: Chemical structure of Baloxavir Marboxil.

Optimized conditions

Liquid chromatographic UPLC system of Waters equipped with PDA (photodiode array detector), auto-sampling unit and Zorbax C18 (100 x 2.1 mm, 1.8µ) reverse phase column. The mobile phase composition of buffer 0.1% orthophosphoric acid and acetonitrile in the ratio of 60:40 was pumped through a column at a flow rate of 0.3 mL/min. Column oven temperature was maintained at 30°C and the detection wavelength was processed at 240 nm. Integration of output signals were monitored and processed by waters Empower software-2.0.

Diluent

Depending up on the solubility of the drugs, diluent was optimized. Initially dissolved in methanol and diluted with acetonitrile and water (50:50).

Preparation of Standard Stock Solutions

Exactly weighed 20mg of ECRB and 9mg of BLMX poured in to a 50mL volumetric flask and 3/4th volume of diluent was added and vortexed for 20 min. Flasks were made up with water and acetinitrile (50:50) and marked as standard stock solution 1 (400µg/mL BLMX). 1mL from the resulting stock solution was pipetted out and taken into a 10mL volumetric flask and made up with diluent to get 40µg/mL BLMX.

Preparation of Sample Stock Solutions

5 tablets were weighed and the average weight of each tablet was calculated. The weight equivalent to 1 tablet was transferred into a 100mL volumetric flask and 25 mL of diluent was added and sonicated for 25 min. Further the volume was made up with diluent and filtered through 0.45 µ filter (400µg/mL BLMX). 1mL of the resultant solution was poured in to a 10mL volumetric flask and made up with diluent (40µg/mL BLMX).

Preparation of Buffer

0.1% orthophosphoric acid Buffer was prepared by diluting 1mL of concentrated orthophosphoric acid with water to up to 1000mL.

Method Validation

The developed method for BLMX was subjected for validation for the parameters like system suitability, linearity, robustness, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy as per the guidelines of ICH [5,6] .

RESULTS AND DISCUSSION

Method Development and Optimization

With different mobile phase compositions and stationary phases 4 different trials were executed and 5th trail was optimized. In all the 4 trials are not having the proper peak response and more noise was there. Optimized chromatographic peaks were shown in 5th trial.

Finally, excellent chromatographic efficiency parameters were obtained with the mobile phase composition of buffer 0.1% orthophosphoric acid and acetonitrile in the ratio of 60:40%v/v pumped through an Zorbax C18 (100 x 2.1 mm, 1.8µ) reverse phase column, at a flow rate of 0.3 mL/min. Column oven temperature was maintained at 30°C and the detection wavelength was processed at 240 nm. Based on the solubility, all the dilutions were made with acetonitrile and water in the ratio of 50:50%v/v. Retention time of BLMX was found to be 0.68min. An injection volume of 10 µL was infused through an UPLC system to get the better performance (Fig. 2).

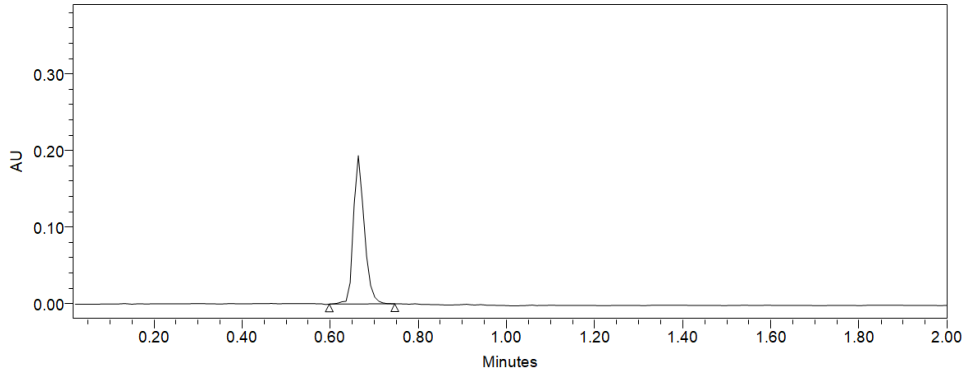

Fig. 2: Optimized chromatogram of BLMX

Method Validation

System Suitability

The system suitability variables were estimated by preparing standard solution of BLMX and the same were injected 6 times in to the chromatographic system[7,8]. The variables like peak tailing, and USP plate count were estimated. The results were shown in Fig. 3 and Table 1.

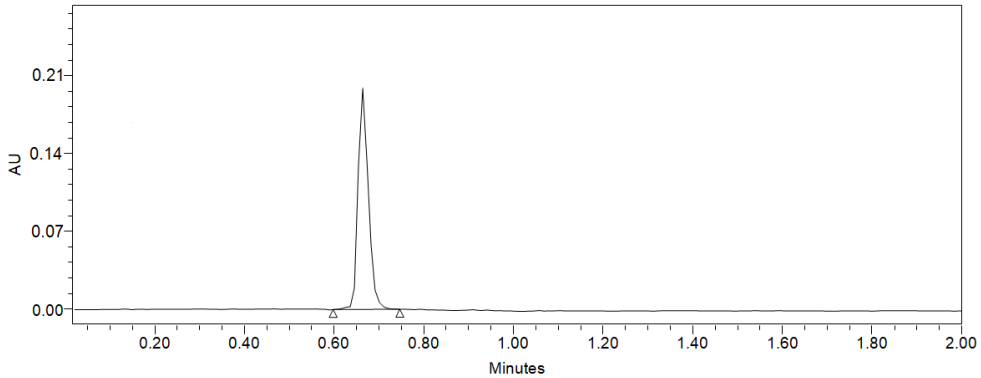

Fig. 3: Chromatogram showing standard injection.

Table. 1: System suitability parameters for BLMX

S.No	BLMX		
	RT(min)	USP Plate Count	Tailing
1	0.682	3701	1.18
2	0.682	3587	1.19
3	0.683	3658	1.16
4	0.681	3808	1.20
5	0.683	3659	1.24
6	0.682	3842	1.18

Specificity

Method specificity was determined by infusing the blank, placebo, standard and sample solutions in to a chromatographic system and the resulting chromatograms were evaluated for interference with the

excipients, degradants and other components may expected to be present[9,10]. Blank, standard, formulation and placebo chromatograms were represented in Fig. 4.

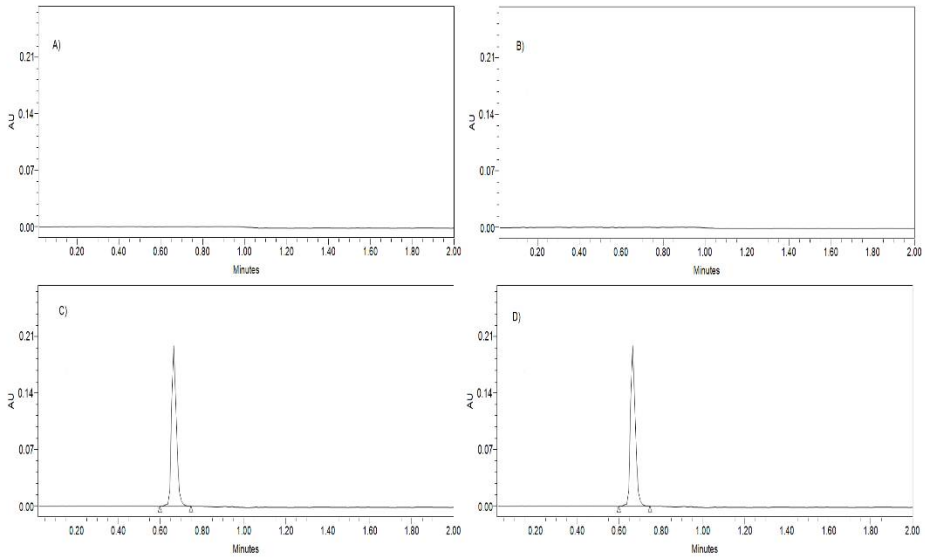

Fig. 4: Chromatograms of A) Blank, B) Placebo, C) Standard and D) Sample

Precision

Precision of the method was evaluated in terms of method precision and intermediate precision. The method precision (repeatability) was estimated by infusing 6 standard solutions and 6 sample solutions. Intermediate precision was evaluated by infusing 6 standard solutions and 6 sample solutions on different days by different employees on different chromatographic systems[11,12]. The peak responses of all the chromatograms were taken and standard deviation, % RSD (relative standard deviation) and percentage assay of sample solutions were calculated. The findings were represented in Table 2.

Table. 2: Repeatability and intermediate precision results of BLMX

S. No	Area of BLMX	
	Day-1	Day-2
1.	623135	615464
2.	625323	610345
3.	622733	613197
4.	625083	616754
5.	622016	611930
6.	625527	612846
Mean	623970	613423
SD	1519.2	2338.7
%RSD	0.2	0.4

Accuracy

Method accuracy was estimated at three variable concentrations of 50%, 100%, and 150% level by spiking the known amount of the analyte[13,14]. The % recovery at each level was calculated and the findings were represented in Table. 3 (Fig. 5 to 7).

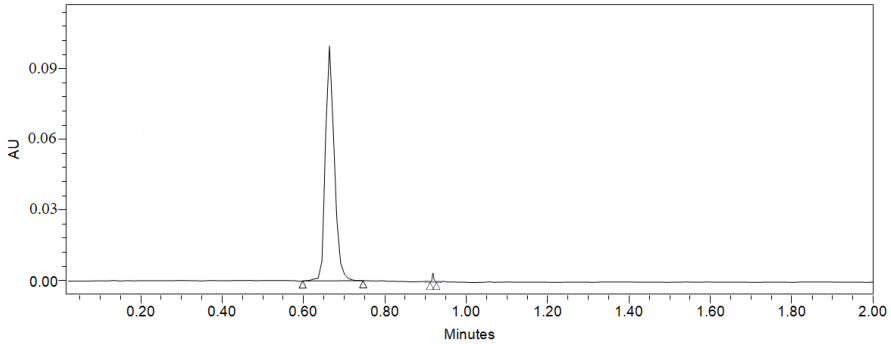

Fig. 5: Chromatogram showing accuracy 50%injection.

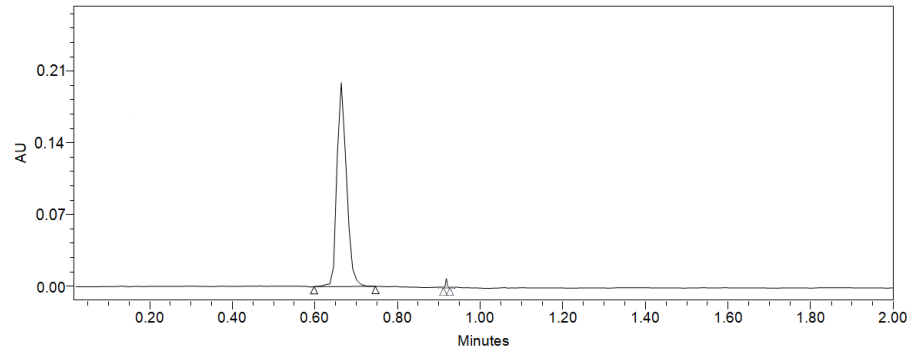

Fig. 6: Chromatogram showing accuracy 100%injection.

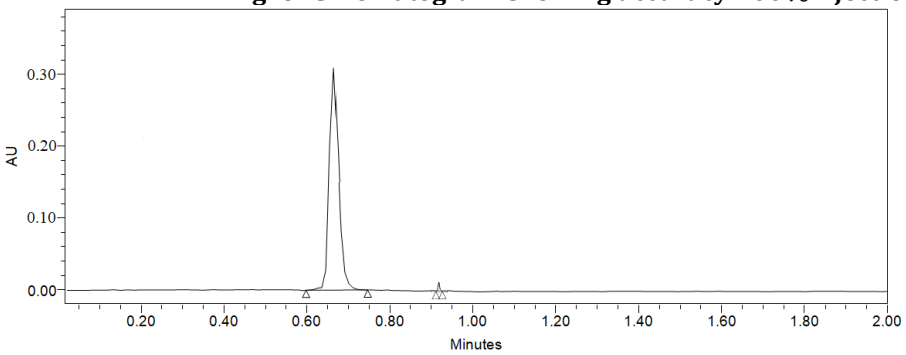

Fig. 7: Chromatogram showing accuracy 150%injection

Table. 3: Accuracy results of BLMX

% Level	Amount Spiked (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean %Recovery
50%	20	19.8932	99.47	99.47%
	20	19.81135	99.06	
	20	19.93662	99.68	
100%	40	39.84106	99.60	
	40	39.98523	99.96	
	40	39.91915	99.80	
150%	60	59.57467	99.29	
	60	59.5439	99.24	
	60	59.46752	99.11	

Linearity

Linearity of the developed method was evaluated by processing 6 different concentration levels of BLMX over the concentration of 10 to 60 µg/ml. Each concentration level was processed in triplicates[15,16]. The linearity plot was acquired by plotting peak response (on X-axis) versus concentration (on Y-axis). The results of the linearity were represented in Fig. 8 and Table. 4.

Table. 4: Linearity results of BLMX

S.No	Concentration (µg/ml)	Peak area
1	0	0
2	10	180652
3	20	351425
4	30	526977
5	40	677785
6	50	853069
7	60	1029005

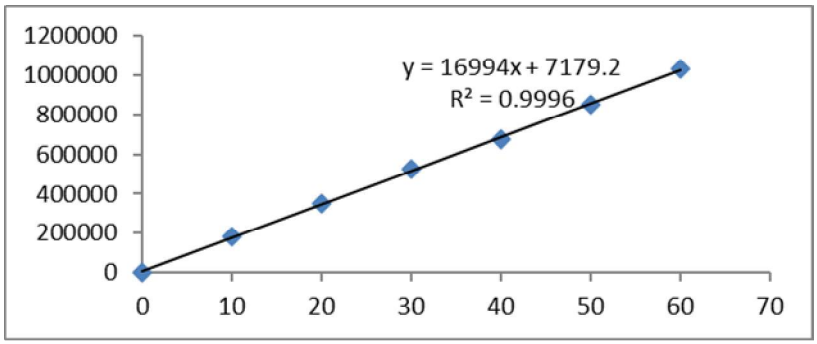

Fig. 8: Calibration curve of BLMX

LOD and LOQ

LOD is lowest quantity of drug in a sample that can be identified but cannot be quantify exactly. LOQ is the lowest quantity of a drug in an analyte which can be quantitatively estimated with a suitable accuracy and precision [17]. The LOD and LOQ values were calculated from the linearity data by utilizing standard deviation and slope of the curve and the values were 0.69 and 2.10 µg/ml respectively (Fig. 9 and10).

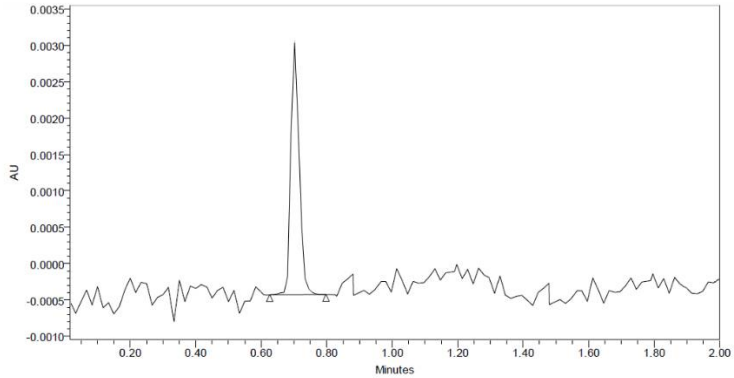

Fig. 9: LOD chromatogram of BLMX

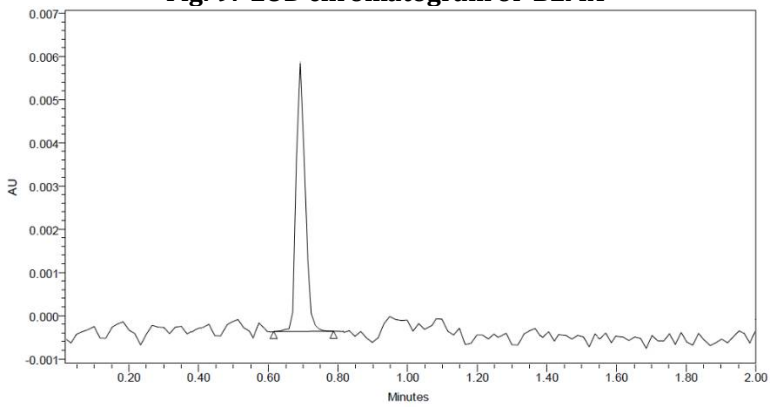

Fig. 10: LOQ chromatogram of BLMX

Robustness

The method robustness was processed by introducing small variation in the optimized LC conditions such as organic phase in mobile phase (±5%), flow rate (-0.1 and +0.1 mL/ min) and column temperature (±5°C). The findings were shown in the Table. 5.

Table. 5: Robustness data for BLMX

S.No.	Variation in LC conditions	BLMX % RSD
1	Flow rate -0.1ml/min	0.9
2	Flow rate +0.1ml/min	0.6
3	Organic phase -5%	0.8
4	Organic phase + 5%	1.0
5	Temperature at 25°C	0.5
6	Temperature at 35°C	0.9

Degradation Studies

Alkali Degradation Studies

To 1 ml of stock solution of BLMX, 1 ml of 2N NaOH was added in to a 10 ml volumetric flask and kept at 60°C for 30 min. Further, the resulting solution was made up to the mark to get 40 µg/ml BLMX. From that 10 µL of solution was infused in to an UPLC system and the resultant chromatograms were analysed for the stability of analyte[18]. The findings were represented in Table .6 and Fig.11.

Table. 6: Degradation data of BLMX

Type of degradation	BLMX		
	Area	%Recovered	% Degraded
Acid	573001	91.40	8.60
Alkali	567048	90.45	9.55
Peroxide	587048	93.64	6.36
Thermal	565739	90.24	9.76
UV light	589577	94.04	5.96
Neutral	577263	92.08	7.92

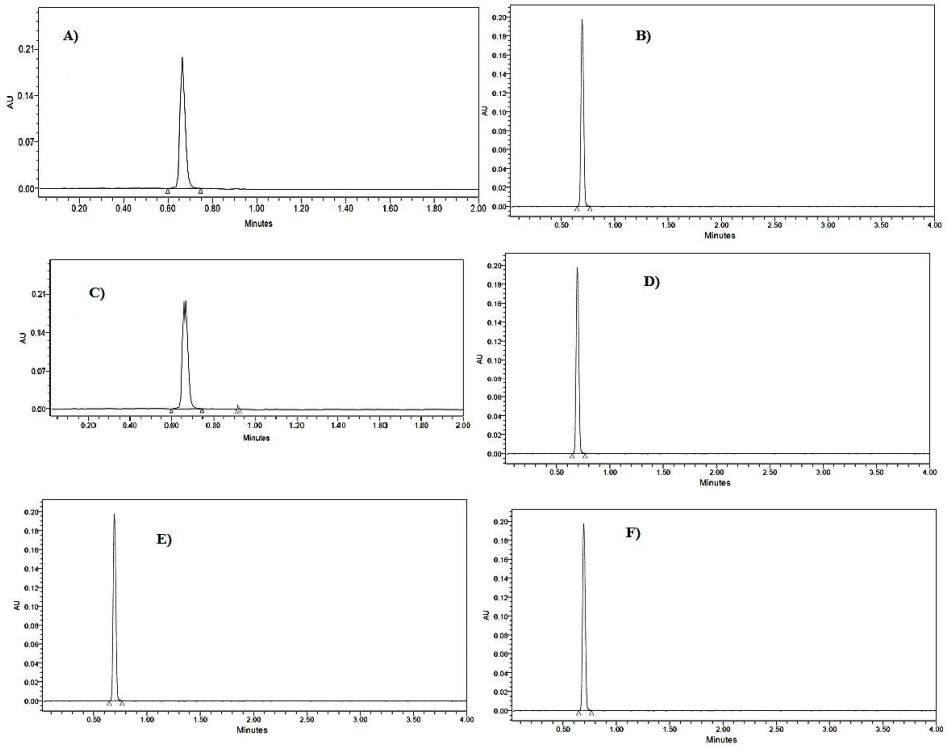

Fig. 11: Chromatogram for A) Alkali B) UV C) Acid D) Neutral E) Peroxide F) Thermal degradation study

Photolytic Stability Study

For the photolytic stability study, BLMX 400µg/mL solution was exposed to UV-light by placing the solutions in UV cabinet for 7 days or 200 Watt hours/m^2 in photo stability chamber. The resulting solution was transferred in to a 10 mL volumetric flask and made up to the mark with diluent to get 40µg/mL BLMX. From that 10 µL of solution was infused in to an UPLC system and the resultant chromatograms were analysed for the stability of analyte. The findings were represented in Table. 6 and Fig.11.

Acid Degradation Studies

To 1 mL of stock solution of BLMX, 1 mL of 2N Hydrochloric acid was added in to a 10 mL volumetric flask and refluxed at 60°C for 30 min. Further, the resulting solution was made up to the mark to get 40 µg/mL BLMX. From that 10 µL of solution was infused in to an UPLC system and the resultant chromatograms were analysed for the stability of analyte. The findings were represented in Table. 6 and Fig.11.

Neutral Degradation Studies

To 1 mL of stock solution of BLMX, 5 mL of water was added in to a 10 mL volumetric flask and kept for refluxing at 60°C for 1 h. Further, the resulting solution was made up to the mark to get 40 µg/mL BLMX. From that 10 µL of solution was infused in to an UPLC system and the resultant chromatograms were analysed for the stability of analyte. The findings were represented in Table. 6 and Fig.11.

Oxidation

To 1 mL of stock solution of BLMX, 1 mL of 20% hydrogen peroxide (H_2O_2) were added in to a 10 mL volumetric flask and kept at 60°C for 30 min. Further, the resulting solution was made up to the mark to get 40 µg/mL BLMX. From that 10 µL of solution was infused in to an UPLC system and the resultant chromatograms were analysed for the stability of analyte. The findings were represented in Table. 6 and Fig.11.

Dry Heat Degradation Studies

Standard stock solution of BLMX was monitored at 105°C for 6 h in a hot air oven to perform the dry heat stability study. Further, the resulting solution was subjected for dilution to get 40 µg/mL BLMX. From that 10 µL of solution was infused in to an UPLC system and the resultant chromatograms were analysed for the stability of analyte. The findings were represented in Table. 6 and Fig.11.

CONCLUSION

A simple, accurate and precise method was developed for the estimation of BLMX in Tablet dosage form by RP-UPLC technique. Retention time of BLMX was found to be 0.682min and an excellent chromatographic efficiency parameters were obtained with the mobile phase composition of buffer 0.1% orthophosphoric acid and acetonitrile in the ratio of 60:40%v/v pumped through an Zorbax C18 (100 x 2.1 mm, 1.8µ) reverse phase column, at a flow rate of 0.3 mL/min. Column oven temperature was maintained at 30°C and the detection wavelength was processed at 240 nm.. Repeatability of the method was determined in the form of %RSD and the value was 0.2. The percentage mean recovery of the method was found to be 99.47%. Drug was subjected for acid, peroxide, photolytic, alkali, neutral and thermal degradation studies and the results shown that the percentage of degradation was found between 5.96% and 9.55%. Retention time and total run time of the drug was decreased and the developed method was simple and economical. So, the developed method can be adopted in industries as a regular quality control test for the quantification of ECRB and BMTB.

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