Original Article

A New Stability Indicating Rp-Hplc Method Development And Validation For Estimation Of Baloxavir Marboxil In Pharmaceutical Dosage Form

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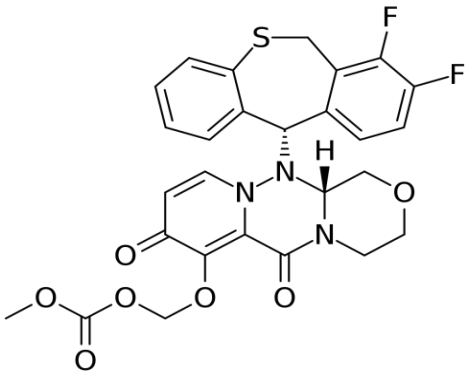
Abstract

A simple, precise, accurate method was developed for the estimation of Baloxavir marboxil by RP-HPLC technique. Chromatographic conditions used are stationary KromasilC18 (150mm x 4.6 mm) ,5µ , mobile phase 0.1%OPA: Methanol in the ratio of 50:50 and flow rate were maintained at 1.0ml/min, detection wave length was 220nm, column temperature was set to 30ºC and diluent was mobile phase conditions were finalized as optimized method. The retention time was found to be 2.199min. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to150 % levels, R^2 value was found to be as 0.999. Precision was found to be 0.9 for repeatability 0.8 and 0.2 for intermediate precision. LOD and LOQ are 0.08µg/ml and 0.25µg/ml respectively. By using above method , assay of marketed formulation was carried out and 99.69% was present. Degradation studies of Baloxavir marboxil were done in all conditions. Purity threshold was more than purity angle and within the acceptable range. Full length method was not performed; if it is done this method, it can be used for routine analysis of Baloxavir marboxil. The analytical procedure was specific and validated as per ICH guideline (Q2R1).

Keywords: RP- HPLC, Baloxavir, Method development , ICH Guidelines

INTRODUCTION

BLMX is an antiviral drug developed by Shionogi Co, a Japanese pharmaceutical company and Roche for the treatment of influenza A and influenza B infections. The drug was initially approved for use in Japan in February 2018 and approved by the FDA on October 24, 2018 for the treatment of acute uncomplicated influenza in patients 12 years of age and older who have been symptomatic for no more than 48 hours . BLMX, a cap-endonuclease inhibitor, has a unique mechanism of action when compared to the currently existing neuraminidase inhibitor drug class used to treat influenza infections. According to the literature study, no single method for quantifying BLMX has been described. According to the literature, a novel stability-indicating RP-HPLC technique is needed for the estimation of BLMX in pharmaceutical dosage form.



MATERIALS AND METHOD

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, Orthophosphoric acid and HPLC-grade milli Q water were bought from Rankem, avantor performance material India limited. Xofluza 40 mg tablets were obtained from a local pharmacy.

Instrument and Chromatographic Conditions

For the current study WATERS HPLC 2965 SYSTEM with auto injector and PDA(photodiode array detector), the separation was achieved by using Kromasil C18 (150mm length x 4.6 mm diameter with the particle size of 5µ) . 0.1% orthophosphoric acid and Methanol in the ratio of 50:50 (v/v) was used as a mobile phase with a flow rate of 1.0ml/min .The temperature of the column was maintained at 30^{o}C and detection wave length was 220nm . Integration of output signals was monitored and processed by waters Empower software-2.0.

Analytical Method Development

Preparation of buffer: 0.1% orthophosphoric acid

1mL of Orthophosphoric acid solution in a 1000 ml of volumetric flask was added and add about 100ml of milli-Q water and final volume was made up to 1000 ml with milli-Q water.

Diluent

Based up on the solubility of the drugs, diluent was selected, first dissolved in methanol then made up with acetonitrile and buffer taken in the ratio of 60:40.

Preparation of Standard stock solutions

Accurately weighed 20mg of BLMX was transferred to 50ml volumetric flasks, 3/4 th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as standard stock solution (400µg/ml of BLMX).

Preparation of Standard working solutions (100% solution)

1ml of BLMX from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (40µg/ml of BLMX).

Preparation of Sample stock solutions

20 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (400 µg/ml of BLMX).

Preparation of Sample working solutions (100% solution)

1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (40µg/ml of BLMX)

Optimized Chromatographic Condition

We tried with different mobile phase combinations (methanol, acetonitrile and buffer) and change in column (Agilent C18, BDS150, Ascentis C18, KromasilC18). At all the combinations, the resulting chromatograms has got poor resolution, theoretical plates and peak shape. Finally, excellent chromatographic efficiency parameters were obtained with the mobile phase composition of buffer 0.1% orthophosphoric acid and methanol in the ratio of 50:50 % (v/v) pumped through a KromasilC18 150mm x 4.6 mm, 5µ and flow rate was maintained at 1.0 ml/min, detection wave length was 220nm, column temperature was set to 30^{o}C. Based on the drug solubility and P^{ka} Value, all the dilutions were made with orthophosphoric acid and methanol in the ratio of 50:50%(v/v). Retention time of BLMX was found to be 2.199min.

ANALYTICAL METHOD OF VALIDATION

The developed method was validated according to standard ICH guideline for system suitability, linearity, robustness, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy (ICH guidelines).

SYSTEM SUITABILITY:

A standard solution of BLMX working standard was prepared as per procedure and was injected five times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms which is obtained by calculating the % RSD of retention time, tailing factor, theoretical plates and peak areas from five replicate injections that are within range and results were shown in Table:1 and figure 1

Table 1: SYSTEM SUITABILITY PARAMETERS

SL no	Baloxavir		
Inj	RT(min)	USP Plate Count	Tailing
1	2.185	2815	1.48
2	2.188	2696	1.46
3	2.193	2688	1.53
4	2.199	2785	1.45

5	2.200	2720	1.48
6	2.205	2535	1.46

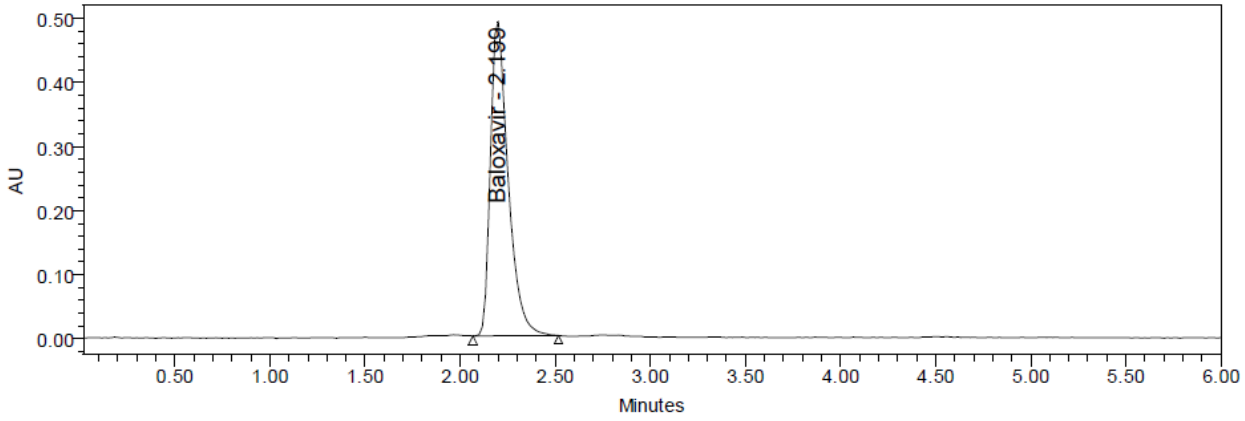
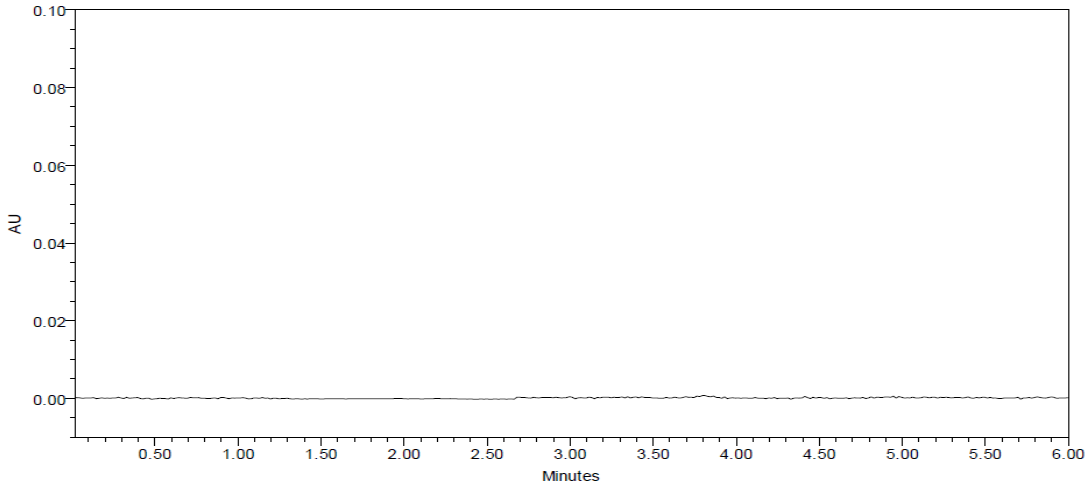


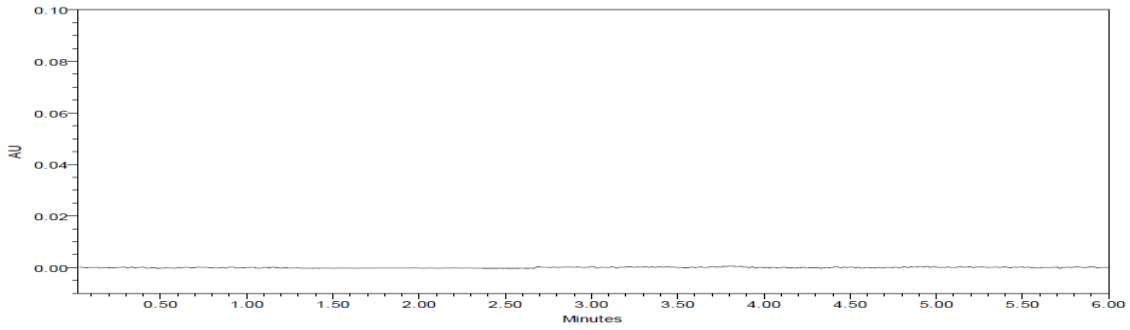
Fig:1 System suitability chromatograms

SPECIFICITY:

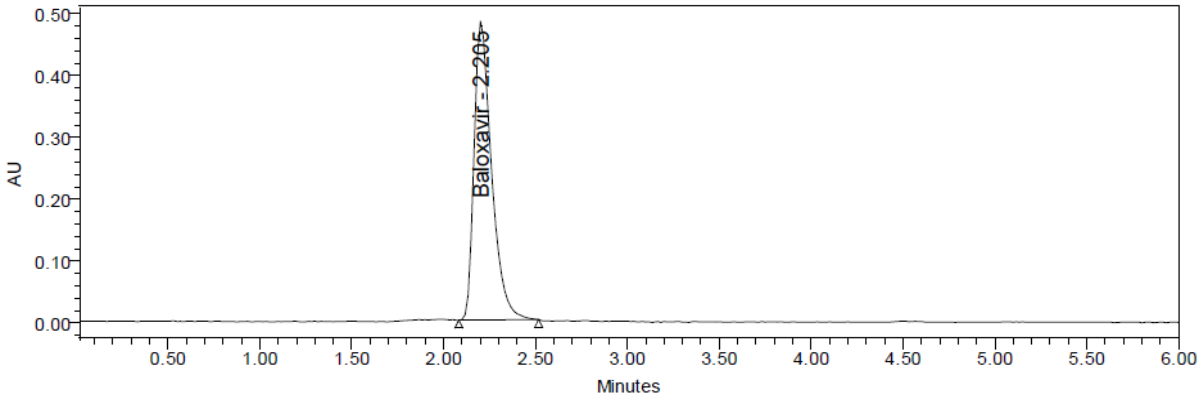
Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific. Blank, standard, formulation and placebo chromatograms were represented in figure 2,



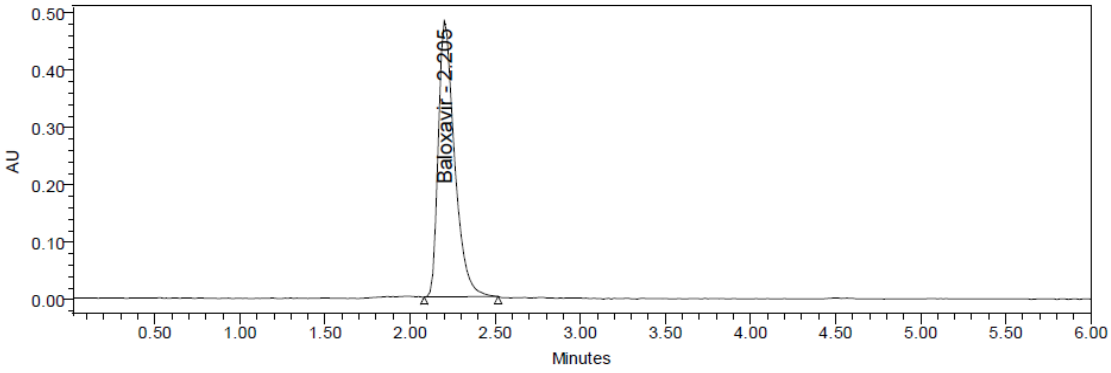
a) blank Chromatogram



b) **Placebo Chromatogram**



c) **standard**



d) **sample**

LINEARITY:

To demonstrate the linearity, inject 6 standard solutions with concentrations of about 0 ppm to 60 ppm of BLMX. The linearity graph created by plotting a graph of concentration versus peak area, it shows a good linearity over the range of 10 ppm to 60 ppm with a correlation co-efficient was found to be 0.999 and linearity plot was shown in Figure 3 and Table:2

Fig:3 Linearity Plot

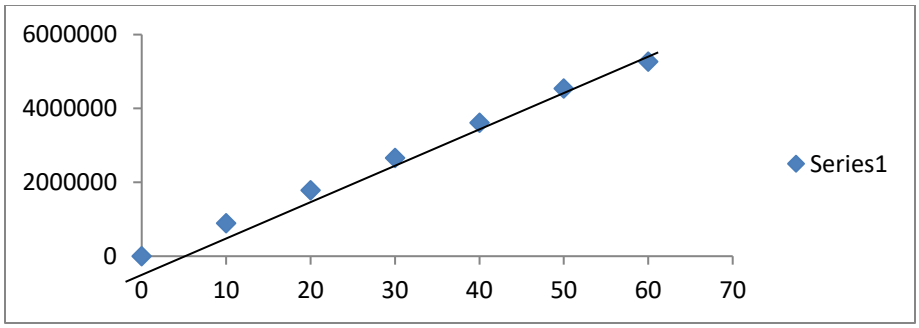


Table: 2 Linearity Concentration and Responses

Linearity Level (%)	Concentration (ppm)	Area
0	0	0
25	10	889803
50	20	1785161
75	30	2662417
100	40	3615367
125	50	4536681
150	60	5271935

PRECISION:

Precision of the method was evaluated in terms of method precision and intermediate precision. The method precision (repeatability) was estimated by injecting six working sample solutions of 40 ppm and the % amount found was calculated and %RSD was found to be 0.8 and the findings were represented in table: 3.

Table:3 Repeatability and Intermediate precision

S.No	Peak Area	
	Repeatability	**Intermediate precision**
1	3525516	3492786
2	3597707	3494624
3	3585581	3499739
4	3576895	3510147
5	3538296	3496776
6	3567338	3485645
AVG	3565222	3496620
STDEV	27971.7	8147.0
%RSD	0.8	0.2

Intermediate precision was evaluated by five working sample solutions(10ppm) that are injected on the next day of the preparation of samples and the % amount found was calculated and %RSD was found to be 0.2 (Table :3).

ACCURACY:

The accuracy of an analytical procedure is the similarity of the obtained value to the true value of the sample. To check the accuracy of the method, formulations were spiked with 50%, 100% and 150% of BLMX standard. The result was analyzed to find the % recovery of the BLMX (Table:4)

Tab: 4 Accuracy data

% Level	Amount Spiked (µg/mL)	Amount recovered(µg/mL)	% Recovery	Mean %Recovery
50%	20	20.36	101.82	99.99%
	20	19.75	98.73	
	20	20.14	100.72	
100%	40	40.01	100.03	
	40	39.30	98.25	
	40	39.62	99.04	
150%	60	59.90	99.83	
	60	60.17	100.29	
	60	60.70	101.17	

Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD and LOQ is the smallest concentration of the analyte that gives a response that can be detected and measured respectively. LOD and LOQ was calculated by using the following equation,

$$LOD = \frac{3.3 \times \text{Standard deviation (SD)}}{\text{Slope of calibration curve}}$$

$$LOQ = \frac{10 \times \text{Standard deviation (SD)}}{\text{Slope of calibration curve}}$$

The LOD and LOQ values were 0.08µg/ml and 0.25µg/ml respectively.

Robustness

It is the ability of a method to remain unimpaired when slight variations are applied. The robustness of the proposed techniques was checked by conditions like flow minus (0.9ml/min), flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. The findings were shown in the table 5.

Table 5 : Robustness Data

Parameter	%RSD
Flow Minus(0.9ml/min)	0.10
Flow Plus(1.1ml/min)	0.10
Mobile phase Minus(45B:55A)	0.20
Mobile phase Plus(55B:45A)	0.02
Temperature minus (25^0C)	0.10
Temperature plus (35^0C)	0.30

Assay Methodology

Assay of the marketed formulation was carried out by injecting sample corresponding to equivalent weight into HPLC system and percent purity was found by following formula. (Table:6,figure:4)

Table:6 : Assay Methodology

Sample No	Standard	Sample	%Assay
1	3525049	3525516	98.58
2	3579075	3597707	100.60
3.	3526521	3585581	100.26
4.	3585454	3576895	100.02
5.	3585317	3538296	98.94
6.	3592138	3567338	99.75
AVG	3565592	3565222	99.69
STDEV	31113.8	27971.7	0.782
%RSD	0.9	0.8	0.78

$$\text{Assay} = \frac{\text{Spl area}}{\text{Std area}} \times \frac{\text{Std.Dil.Fac}}{\text{Spl. Dil. Fac}} \times \frac{\text{Avg .Wt of Tab}}{\text{L.C}} \times \text{Potency of Std}$$

Spl area – Sample Peak area
Std area – Standard Peak area
Std. Dil. Fac- Standard dilution factor
Spl. Dil. Fac- Sample dilution factor
Avg. Wt of Tab- average weight of tablet
L.C – label claim
Potency of Std

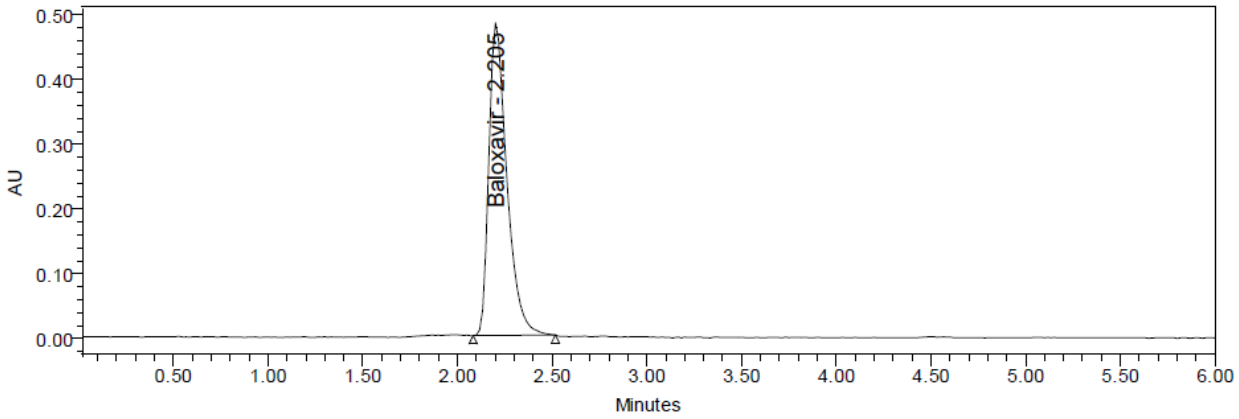
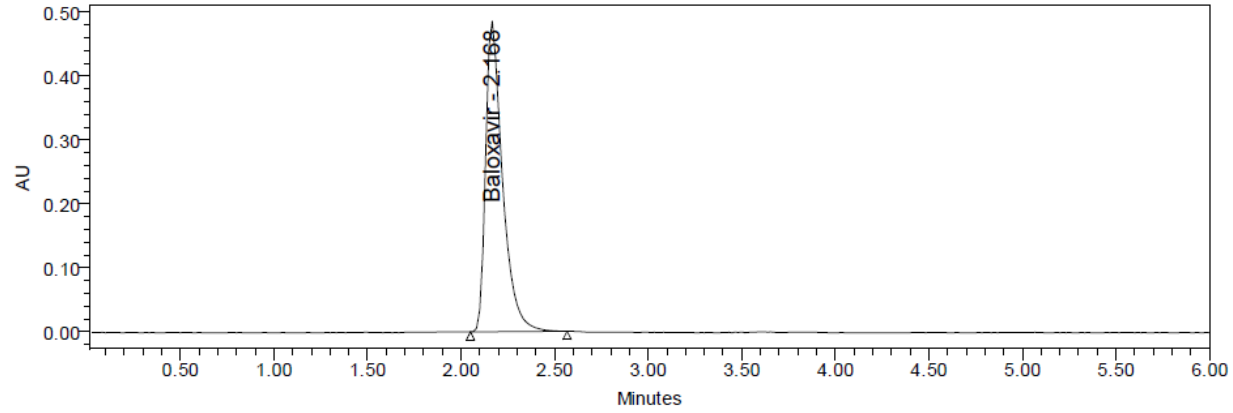


Fig 4: 1. Assay of Standard chromatogram



2. Assay of Sample chromatogram

Degradation studies

The degradation procedure was followed out according to ICH guidelines performed in Q1A (R2). Stability study of a new drug substance use a validated analytical method and results were shown in Table 7 with the following procedure.

Table:7 **Degradation Data of Baloxavir**

SL.NO	Degradation Condition	% Drug Undegraded	% Drug Degraded
1	Acid	94.61	5.39

2	Alkali	92.62	7.38
3	Oxidation	93.55	6.45
4	Thermal	97.46	2.54
5	UV	98.44	1.56
6	Water	99.10	0.90

Acid degradation study

1 mL of BLMX solution was transferred to a 10 ml volumetric flask, followed by 1 mL of standard solution of 2N hydrochloric acid was added and refluxed for a period of 30 minutes at 60ºC on a water bath. The resultant solution was diluted to obtain (40ppm) solution and 10µl solutions were injected into the system and the corresponding chromatogram is shown in the figure.

Basic Degradation Studies/alkali Degradation Studies

1 mL of BLMX solution was transferred to a 10 ml volumetric flask, followed by 1 mL of standard solution of 2 N sodium hydroxide was added and refluxed for a period of 30 minutes at 60ºC on a water bath. The resultant solution was diluted to obtain (40ppm) solution and 10µl solutions were injected into the system and the curresponding chromatogram is shown in the figure.

Oxidation

1 mL of BLMX solution was transferred to a 10 ml volumetric flask, followed by addition of 1 mL of 20% hydrogen peroxide (H2O2) separately. And refluxed for 30 minutes at a temperature of 60 °C on a water bath. The resultant solution was diluted to obtain (40ppm) solution and 10µl solutions were injected into the system and the curresponding chromatogram is shown in the figure.

Dry Heat Degradation Studies :

The standard drug solution was placed in oven at 105^0c for 6h to study dry heat degradation for HPLC study, the resultant solution was diluted to (40ppm) solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample. The resultant chromatograms were analysed for the stability of analyte. The findings were represented in the figure.

Photostability studies:

The photochemical stability of the drug was also studied by exposing the (400ppm) solution to UV Light by keeping the beaker in UV chamber for 7days or 200 Watt hours/m^2 in photostability chamber . For HPLC study, the resultant solution was diluted to obtain (40ppm) solutions and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample .

Neutral degradation Studies:

1 mL of BLMX solution was transferred to a 10 ml volumetric flask, followed by 5ml water was added in to a 10ml volumetric flask and kept for refluxing at 60 ºC for 1hr . The resultant solution was diluted to obtain (40ppm) solution and 10µl solutions were injected into the system and the corresponding chromatogram was shown in the figure

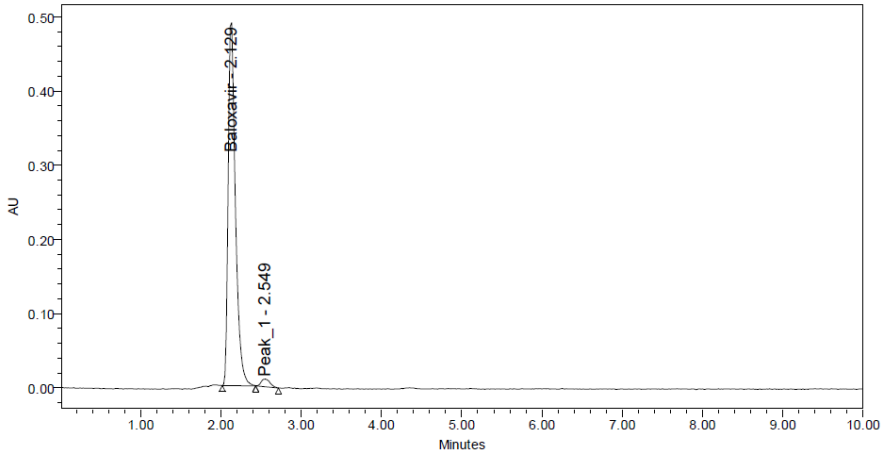


Fig 5. Acid degradation chromatogram

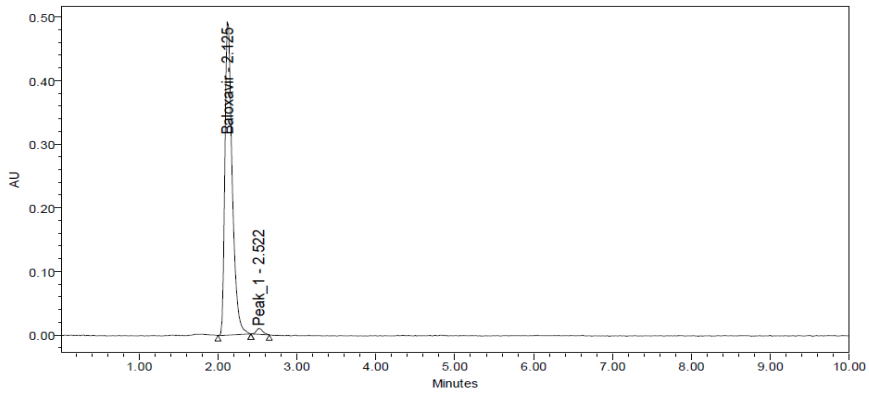


Fig6. Base degradation chromatogram

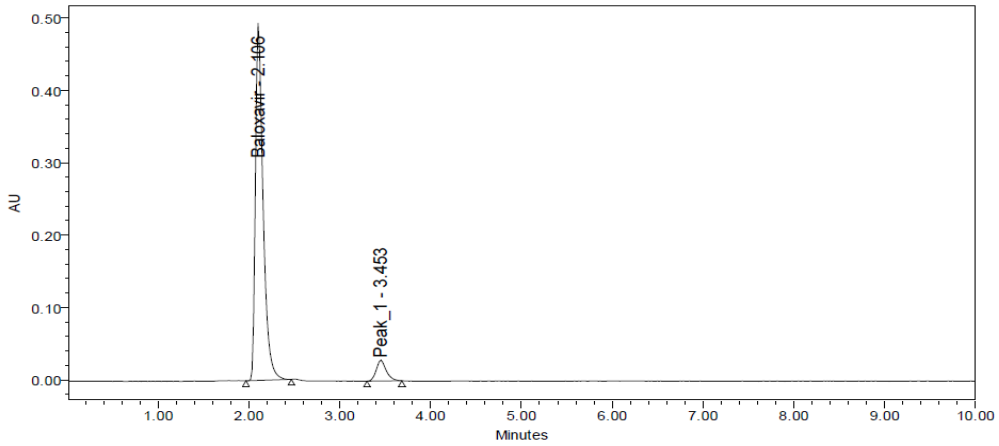


Fig 7 Peroxide degradation chromatogram

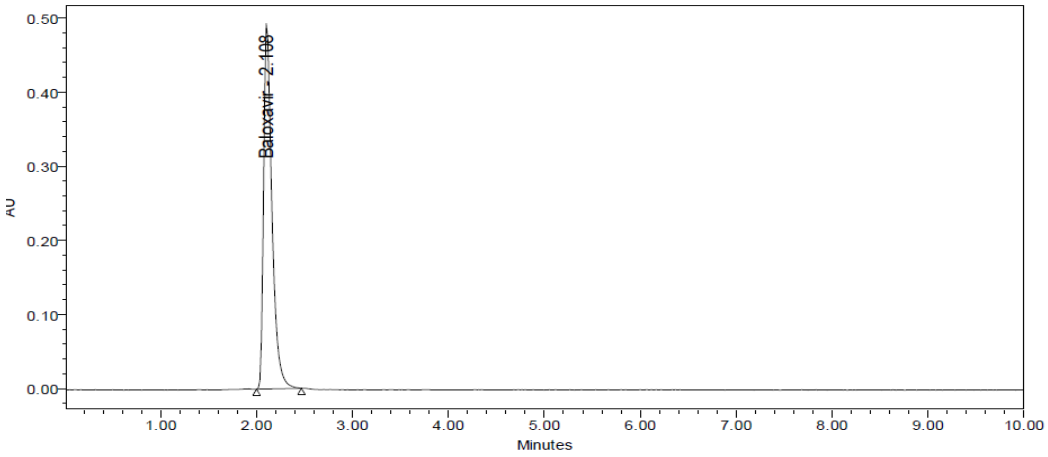


Fig 8. Thermal degradation chromatogram

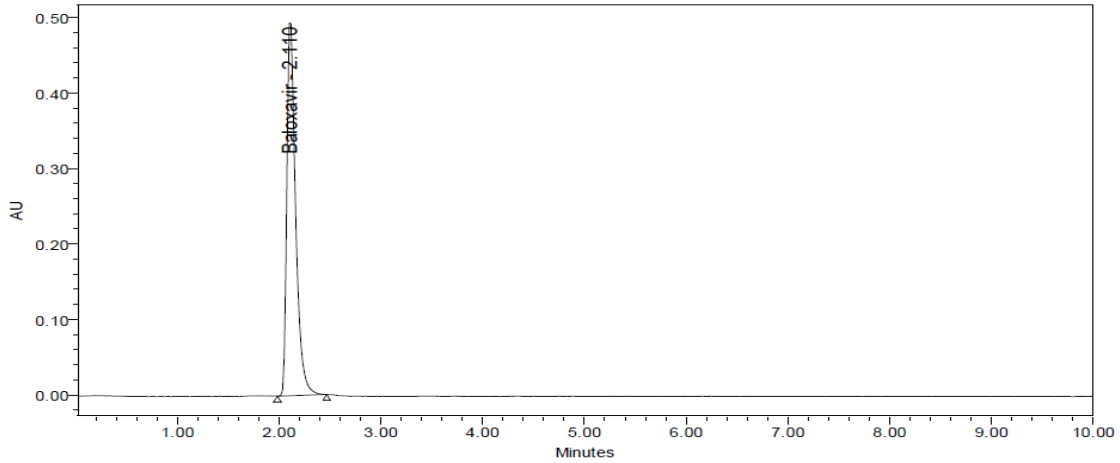


Fig 9. UV degradation chromatogram

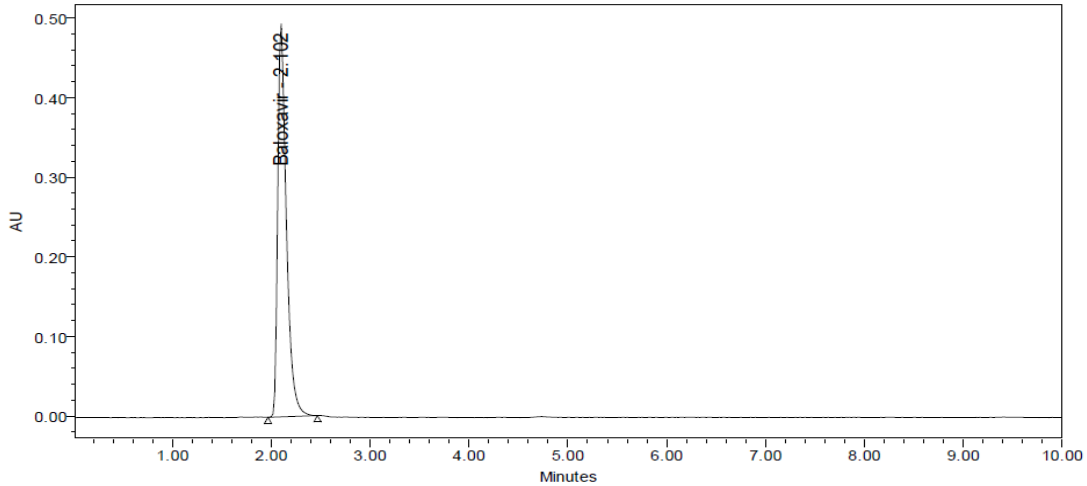


Fig 10. Water degradation chromatogram

CONCLUSION

The chromatographic developed method is quite simple, rapid for the estimation of Baloxavir marboxil , The method was validated according to standard ICH guidelines regarding accuracy, precision, linearity, the limit of detection, and LOQ and robustness . System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to150 % levels, R^2 value was found to be as 0.999. Precision was found to be 0.9 for repeatability 0.8 and 0.2 for intermediate precision. LOD and LOQ are 0.08µg/ml and 0.25µg/ml respectively. By using above method assay of marketed formulation was carried out and 99.69% was present. Degradation studies of Baloxavir marboxil were done in all conditions. Purity threshold was more than purity angle and within the acceptable range. Full length method was not performed; if it is done this method can be used for routine analysis of Baloxavir marboxil. It was concluded that the RP- HPLC method for BLMX, was found to precise, accurate, rapid, specific, and economical.

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