Asian Journal of Chemical and Pharmaceutical Research
CODEN (USA):AJCPHP | ISSN: 2347-8322 | Publisher: Pharma Research Library
Home Page: https://pharmaresearchlibrary.org/journals/index.php/ajcpr
DOI: https://doi.org/10.30904/j.ajcpr.2025.4808
A. J. Chem. Pharm, Res., 2025, 13(1): 33-40

A Liquid Chromatography Method Development and Validation of Baloxavir in Pharmaceutical Dosage Form as Per ICH Guidelines

Anumalagundam Srikanth*[1], G Mallikarjuna[2], M. Pradeep Kumar[3]
[1]Associate Professor, Department of Pharmaceutical Analysis, Vasavi Institute of Pharmaceutical Sciences, Kadapa, A.P-516 247.
[2]Student, Department of Pharmaceutical analysis, Vasavi Institute of Pharmaceutical Sciences, Kadapa, A.P-516 247.
[3]Professor & Principal, Department of Pharmaceutics, Vasavi Institute of Pharmaceutical Sciences, Kadapa, A.P-516 247.

A B S T R A C T

A new method was established for the estimation of Baloxavir by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Baloxavir by using Platisil C18 (4.6x250mm, 5µm), mobile phase ratio was Methanol: KH2PO4 PH 2.5, Run time was found to be 10 min, and detection wavelength was 247nm. Phosphate buffer solution, by adding 0.1ml of formic acid in 1000ml water. Adjust this solution to pH 4.5 by using sodium hydroxide. The solutions were chromatographed at a constant flow rate of 1ml/min and Injection volume 20µl, the run time 10min, the linearity range was found to lie from 10µg/ml to 50µg/ml of Baloxavir. Results of %Assay for Baloxavir 99.63%, the % RSD values of Baloxavir are found to be 1.274372 indicating less than 2% precision of the method and Intermediate precision for Baloxavir found to be 1.261669. The LOD found to be 0.67 and LOQ found to be 2.25 were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements.

Keywords: Baloxavir, RP-HPLC, LOD, LOQ, Methanol, KH_2PO_4

A R T I C L E I N F O

***Corresponding Author**
Anumalagundam Srikanth
Associate Professor,
Department of Pharmaceutical Analysis,
Vasavi Institute of Pharmaceutical Sciences, Kadapa, A.P

Article History:
Received : 10 Mar 2025
Revised : 30 Mar 2025
Accepted : 12 April 2025
Published : 29 May 2025

Citation: *Anumalagundam Srikanth, et al. A Liquid Chromatography Method Development and Validation of Baloxavir in Pharmaceutical Dosage Form as Per ICH Guidelines. A. J. Chem. Pharm, Res., 2025, 13(1): 33-40*

C o n t e n t s

1. Introduction. 33
2. Methodology 34
3. Results and Discussion 34
4. Conclusion. 39
5. References. 39

1. Introduction

High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantify the compounds that are present in any sample that can be dissolved in a liquid. High performance liquid chromatography (HPLC) is the most accurate analytical methods widely used for the quantitative as well as qualitative analysis of drug product.[1]

Baloxavir marboxil is used to treat the flu (influenza) in patients who have had flu symptoms (eg, cough, fever, runny nose, headache, joint or muscle pain) for not more than 48 hours. It is used in patients 5 years of age and older who are healthy or who are at high risk of developing flu-related complications. Baloxavir marboxil is also used to prevent flu in patients 5 years of age and older who have been exposed with the virus (post-exposure prophylaxis).

Generic Name : Baloxavir marboxil
Brand Names : Xofluza
Drug Category : Miscellaneous antivirals
Indications : Treatment of influenza A and B
Pharmacology : Polymerase acidic endonuclease inhibitor used to treat uncomplicated influenza123
Availability: Available as an oral tablet (40 mg; 80mg)

Molecular Formula: C27H23F2N3O7S
Molecular Weight : 571.549 Da
IUPAC Name: ({(12aR)-12-[(11S)-7,8-difluoro-6,11-dihydrodibenzo[b,e]thiepin-11-yl]-6,8-dioxo-3,4,6,8,12,12a-hexahydro-1H-[1,4]oxazino[3,4-c]pyrido[2,1-f][1,2,4]triazin-7-yl}oxy)methylmethyl carbonate1.

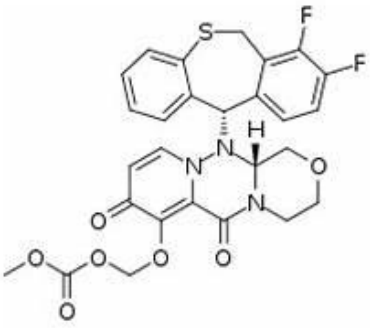

Fig.1. Chemical structure of boloxavir

2. Methodology

Table 1: Instruments used

SN	Instrument	Model
1	HPLC	WATERS, software: Empower, 2695 separation module.2487 UV detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000$^+$
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

Table 2: Chemicals used

SN	Chemical	Brand
1	Baloxavir	Supplied by MSN LAB
2	KH_2PO_4	FINAR chemical LTD
3	Water and Methanol for HPLC	Standard solutions Ltd
4	Acetonitrile for HPLC	Standard solutions Ltd
5	HCl, H_2O_2, NaOH	MERCK

Optimization of Column:
PLATISIL C18 (4.6 x 250mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1.0 ml/min flow.
OPTIMIZED CHROMATOGRAPHIC CONDITIONS:
Instrument used : High performance liquid chromatography Equipped with Auto Sampler and PDA detector
Temperature : Ambient
Column : PLATISIL C18 (4.6 x 250mm, 5µm)
Mobile phase : Methanol: KH2PO4 PH 2.5
Flow rate : 1ml/min
Wavelength : 247 nm
Injection volume : 20 µl
Run time : 10 min.

Preparation of Buffer and Mobile Phase:
Preparation of KH2PO4 pH 4.5:
To prepare phosphate buffer solution, by adding 0.1ml of formic acid in 1000ml water. Adjust this solution to pH 4.5 by using sodium hydroxide.
Preparation of mobile phase:
Mix a mixture of above buffer 600ml (60%), 400 ml Acetonitrile (40%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.
Diluent Preparation:
Methanol: KH2PO4 PH 2.5 (60:40) ratio.
System Suitability:
Tailing factor for the peaks due to Baloxavir in Standard solution should not be more than 2.0.
Theoretical plates for the Baloxavir peaks in Standard solution should not be less than 2000
Calculation: (For Baloxavir)

$$\%\,Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Where:
AT= average area counts of sample preparation.
AS= average area counts of standard preparation.
WS= Weight of working standard taken in mg.
P= Percentage purity of working standard
LC= Label Claim mg/ml.

3. Results and Discussion

HPLC Method Development:
Wave length selection:
UV spectrum of 10 µg / ml each drug of Baloxavir in diluent (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 247nm. At this wavelength both the drugs show good absorbance.
System Suitability Results:

1. Tailing factor Obtained from the standard injection is 1.16
2. Theoretical Plates Obtained from the standard injection is 333

Validation Parameters
ASSAY:
Standard Solution Preparation:
Accurately weigh and transfer 25 mg of Baloxavir working standard into a two 25 ml clean dry volumetric flasks add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3ml of each of the above stock solutions into a two 10ml volumetric flasks and dilute up to the mark with Diluents.
Sample Solution Preparation:
Accurately weigh and transfer equivalent to 25 mg of Baloxavir equivalent weight of the sample into a two 25 ml clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3ml of each of the above stock solution into a two 10ml volumetric flasks and dilute up to the mark with Diluents.

Procedure: Inject 10 µL of the standard, sample into the chromatographic system and measure the areas for the Baloxavir peaks and calculate the % Assay by using the formulae.

Linearity:

Preparation of stock solution:

Accurately weigh and transfer 25 mg of Baloxavir working standard into a two 25 ml clean dry volumetric flasks add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (10ppm of Baloxavir)

0.1ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – II (20ppm of Baloxavir):

0.2ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – III (30ppm of Baloxavir):

0.3ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – IV (40ppm of Baloxavir):

0.4ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – V (50ppm of Baloxavir):

0.5ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Procedure:

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

PRECISION:

Preparation of stock Solution:

Accurately weigh and transfer 25 mg of Baloxavir working standard into a 25 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh and transfer 12.5mg of Baloxavir working standard into a 25 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weigh and transfer 25 mg of Baloxavir working standard into a 25 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

For preparation of 150% solution (With respect to target Assay concentration):

Accurately weigh and transfer 37.5 mg of Baloxavir working standard into a 25ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure:

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Baloxavir and calculate the individual recovery and mean recovery values.

Limit of detection:

Preparation of Baloxavir solution:

Preparation of 0.6 µg/ml solution:

Accurately weigh and transfer 25 mg of Baloxavir working standard into a 25 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 0.6 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent. (0.6 ppm).

Limit of quantification:

Preparation of Baloxavir solution:

Preparation of 2.2µg/ml solution:

Accurately weigh and transfer 25 mg of Baloxavir working standard into a 25 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 2.2ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent. (2.2 ppm).

Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

The flow rate was varied at 0.81 ml/min to 0.99 ml/min.

Standard solution 30 µg/ml of Baloxavir prepared and analyzed using the varied flow rates along with method flow rate.

The Organic composition in the Mobile phase was varied from 40% to 60%:

Standard solution 30 µg/ml of Baloxavir was prepared and analyzed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

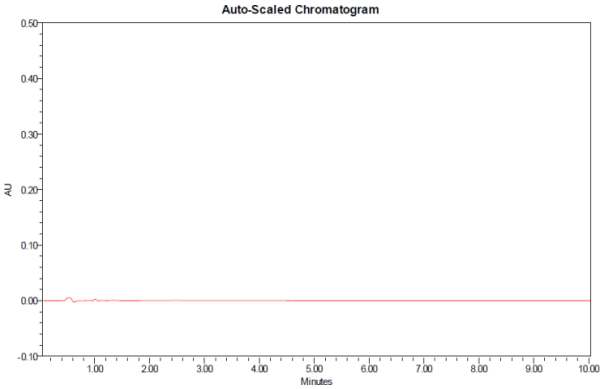

Figure 2: Chromatogram for system suitability

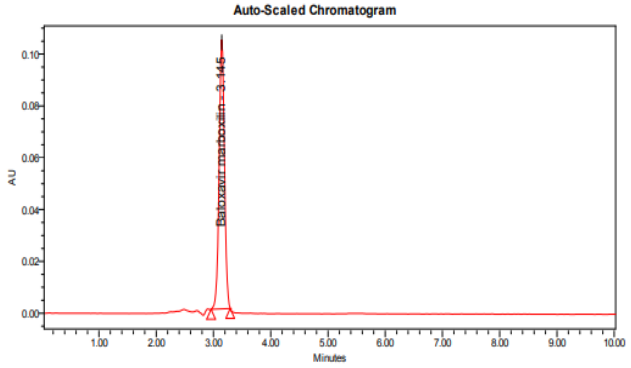

Figure 6: Chromatogram for Sample

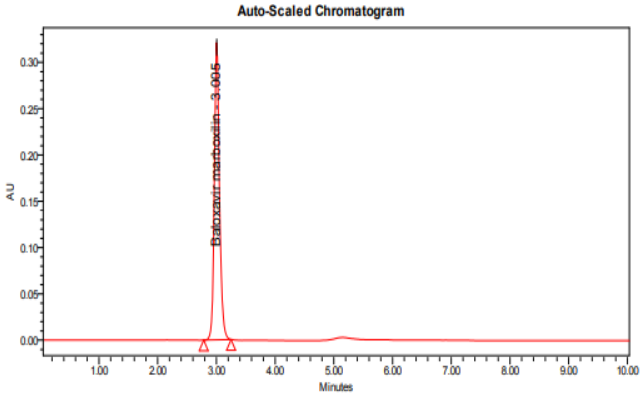

Figure 3: Chromatogram for system suitability of Standard solution

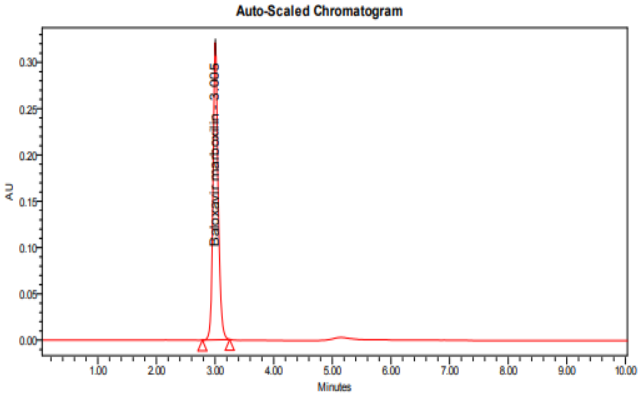

Figure 7: Chromatogram for linearity-5

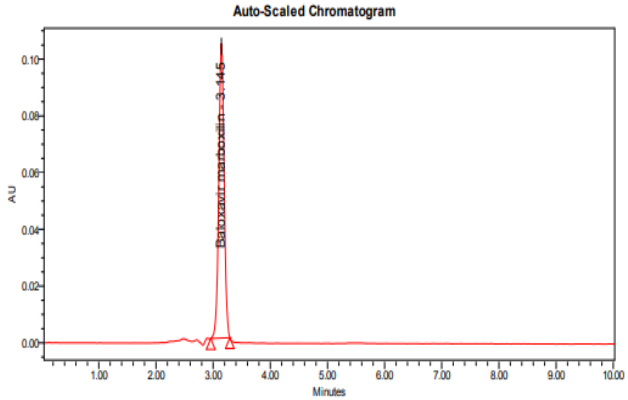

Figure 4: Chromatogram for system suitability of Sample solution

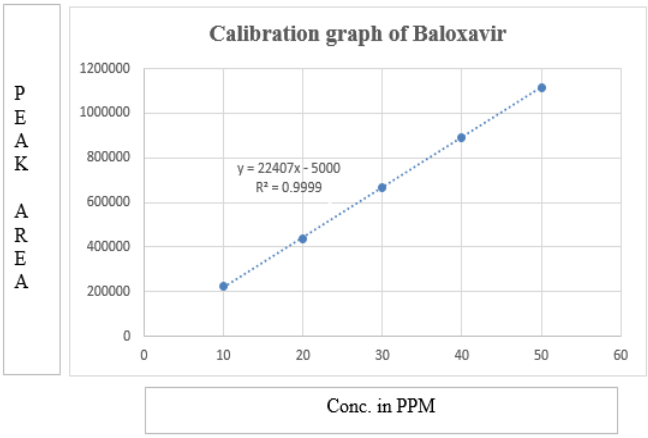

Figure 8: Calibration graph for Baloxavir

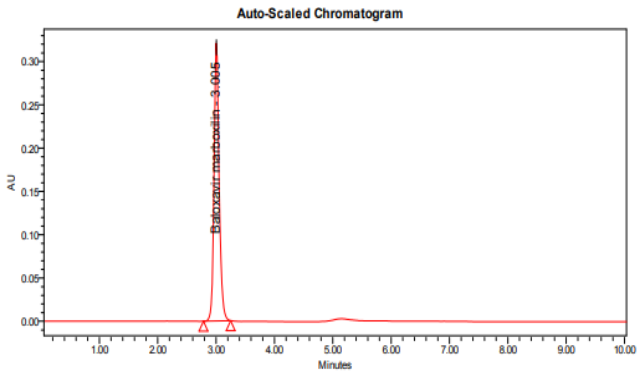

Figure 5: Chromatogram for Standard

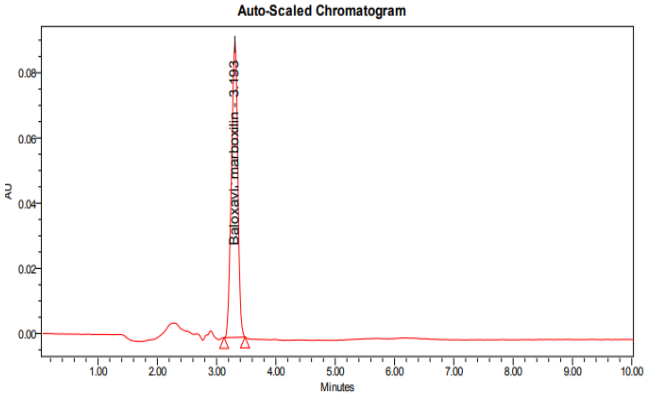

Figure 9: Chromatogram for Precision -6

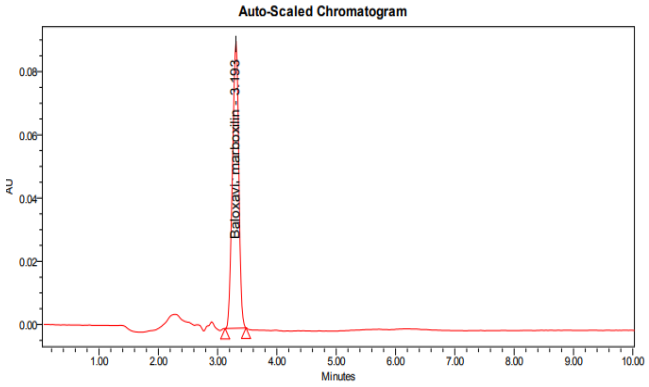

Figure 10: Chromatogram for ID Precision -6

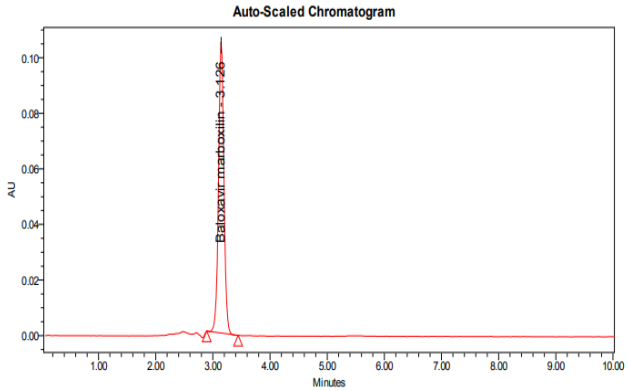

Figure 11: Chromatogram for Accuracy 50%-3

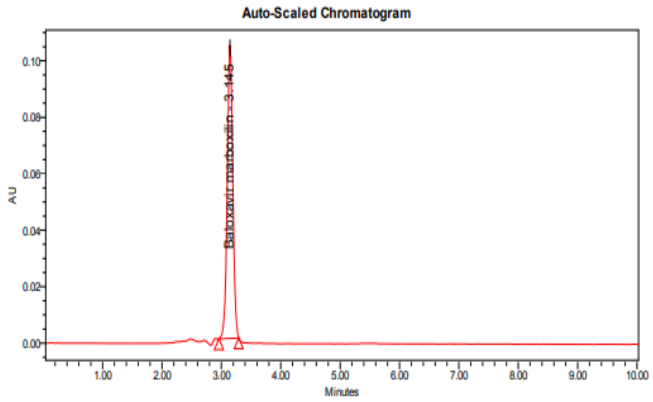

Figure 12: Chromatogram for Accuracy 100%-3

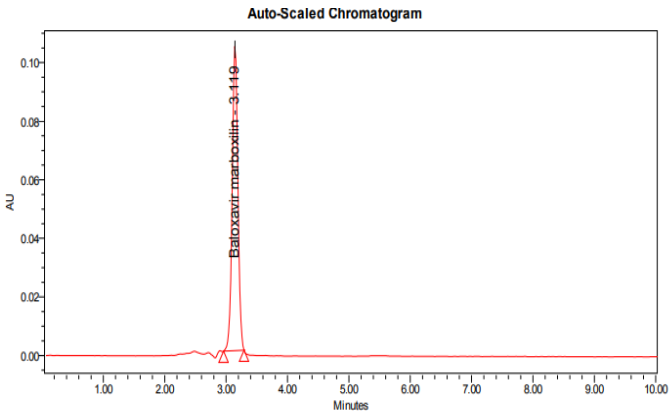

Figure 13: Chromatogram for Accuracy 150%-3

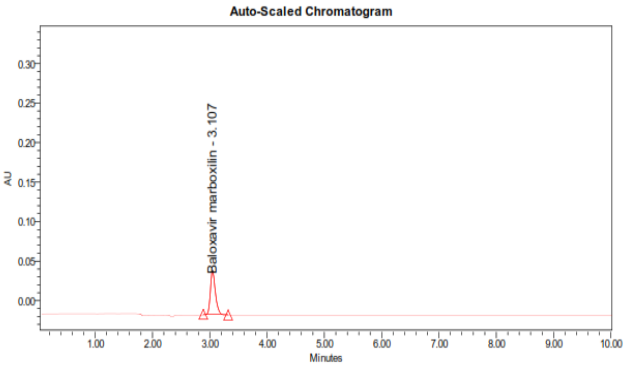

Figure 14: Chromatogram of Baloxavir showing LOD

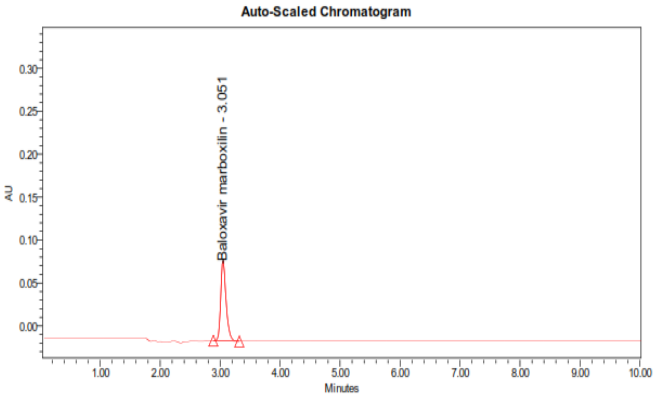

Figure 15: Chromatogram of Baloxavir showing LOQ

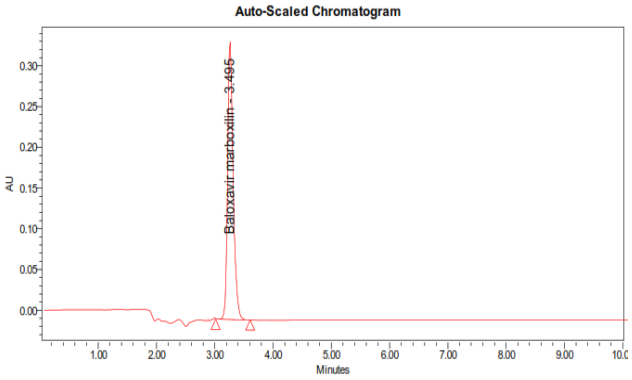

Figure 16: Robustness (less flow)

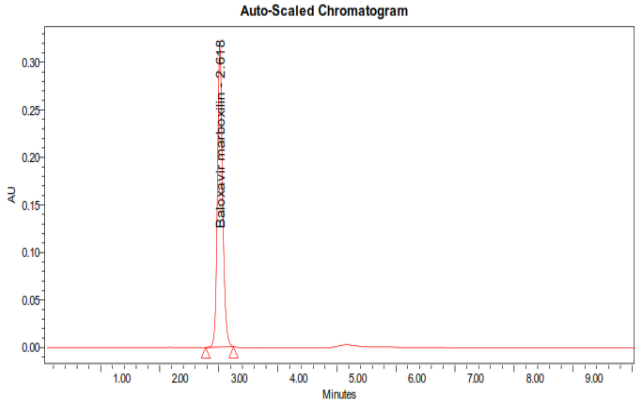

Figure 17: Robustness (more flow)

Table 3 : Results of system suitability parameters

S.No	Name	RT(min)	Area (µV sec)	Height (µV)	USP tailing	USP plate count
1	Baloxavir std	3.005	668116	12645	0.80	3262
2	Baloxavir sample	3.145	1955889	12749	0.85	3212

Table 4: Results of %Assay for Baloxavir

S.No	Label Claim(mg)	% Assay
Baloxavir	20 mg	99.63%

Table 5: Area of different concentration of Baloxavir

S.No	Concentration (µg/ml)	Areas of Baloxavir
1	10	223072
2	20	436144
3	30	669216
4	40	892288
5	50	1115360

Table 6: Analytical performance parameters of Baloxavir

Parameters	Baloxavir
Slope (m)	22407
Intercept (c)	5000
Correlation coefficient (R^2)	0.999

Table 7: Results of Precision for Baloxavir

Injection	Area
Injection-1	669216
Injection-2	648916
Injection-3	669216
Injection-4	669116
Injection-5	659206
Injection-6	669216
Average	664147.7
Standard Deviation	8463.712
%RSD	1.274372

Table 8: Results of Intermediate precision for Baloxavir

Injection	Area
Injection-1	668216
Injection-2	648916
Injection-3	669316
Injection-4	669116
Injection-5	659106
Injection-6	669216
Average	663981
Standard Deviation	8377.24
%RSD	1.261669

Table 9: Accuracy (recovery) data for Baloxavir

%Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mea Recovery
50%	334608	12.5	12.35	98.8	99.0
100%	669216	25	24.92	99.68	
150%	1003824	37.5	36.95	98.53	

Table 10: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained(µV)	S/N ratio	Conc. In ppm
Baloxavir	96	286	2.97	0.67

Table 11: Results of LOQ

Drug name	Baseline noise (µV)	Signal Obtained (µV)	S/N ratio	Conc. In ppm
Baloxavir	96	958	9.97	2.25

Table 12: Results for variation in flow for Baloxavir

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	3243	0.90
2	1	3212	0.85
3	1.2	3374	0.87

4. Conclusion

A new method was established for the estimation of Baloxavir by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Baloxavir by using Platisil C18 (4.6x250mm, 5µm), mobile phase ratio was Methanol: KH2PO4 PH 2.5, Run time was found to be 10 min, and detection wavelength was 247nm. phosphate buffer solution, by adding 0.1ml of formic acid in 1000ml water. Adjust this solution to pH 4.5 by using sodium hydroxide. 1. Tailing factor Obtained from the standard injection is 1. 16, Theoretical Plates Obtained from the standard injection is 333. The solutions were chromatographed at a constant flow rate of 1ml/min and Injection volume 20µl, the run time 10min, the linearity range was found to lie from 10µg/ml to 50µg/ml of Baloxavir. The correlation coefficient obtained was 0.999 which is in the acceptance limit. Results of %Assay for Baloxavir 99.63%, the % RSD values of Baloxavir are found to be 1.274372 indicating less than 2% precision of the method and Intermediate precision for Baloxavir found to be 1.261669. The percentage recovery varies from 98-102% of Baloxavir found to be 99. The LOD found to be 0.67 and LOQ found to be 2.25 were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

5. References

[1] Nagulancha BR, Lakka NS, Vandavasi KR. Stability-indicating method development and validation for quantitative estimation of assay and organic impurities of antiviral drug baloxavir marboxil in drug substance and pharmaceutical dosage form using HPLC and LC–MS methods. Biomedical Chromatography. 2023 Aug; 37(8): e5644.

[2] Divya M, Pawar AK. Development of RP-HPLC PDA method for concurrent quantification of Baloxavir marboxil and its impurities. Journal of Chemical Metrology. 2022 Jan 1;16(1):38-48.

[3] Gouda AS, Marzouk HM, Rezk MR, Abdel-Megied AM. Ecofriendly stability-indicating UHPLC-PDA method for determination of the new influenza antiviral prodrug Baloxavir Marboxil; application to degradation kinetic studies and structure elucidation of the major degradation products using LC-MS. Sustainable Chemistry and Pharmacy. 2023 Jun 1;33:101093.

[4] Gouda AS, Abdel-Megied AM, Rezk MR, Marzouk HM. LC-MS/MS-based metabolite quantitation of the antiviral prodrug baloxavir marboxil, a new therapy for acute uncomplicated influenza, in human plasma: Application to a human pharmacokinetic study. Journal of Pharmaceutical and Biomedical Analysis. 2023 Jan 20;223:115165.

[5] Hafez HM, Abdel-Halim M, Hemdan A, Hammam MA. Kinetic degradation study for the first licensed anti-influenza polymerase inhibitor, baloxavir marboxil, using high-performance liquid chromatography-mass spectrometry. Journal of Separation Science. 2022 Jul;45(14):2488-97.

[6] Ashira TV. A New Stability Indicating Rp-Hplc Method Development And Validation For Estimation Of Baloxavir Marboxil In Pharmaceutical Dosage Form. Journal of Pharmaceutical Negative Results. 2022 Nov 4:970-83.

[7] TV A, PC I. A New Stability Indicating Rp-Hplc Method Development And Validation For Estimation of Baloxavir Marboxil In Pharmaceutical Dosage Form. Journal of Pharmaceutical Negative Results. 2022 Oct 9;13.

[8] Lu H, Xu G, Kou J, Wu S, Zeng J, Liu Y, Lin J, Xu Y, Shang W, Li Y, Wang Z. Identification, Synthesis, Characterization, and Control Strategy Establishment for Process Impurities of Baloxavir Marboxil. Organic Process Research & Development. 2024, 28(4):1170-85.

[9] Mu H, Zhang W, Song Y, Liang R, Zhao H, Song C, Wen Q. Development and validation of an ultra

performance liquid chromatography–tandem mass spectrometry method for the determination of baloxavir in rat plasma and its application to pharmacokinetic studies. Biomedical Chromatography. 2023 Nov;37(11):e5729.