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Original Article

METHOD DEVELOPMENT AND VALIDATION OF BAXDROSTAT BY USING INTEGRATED ANALYTICAL QUALITY BY DESIGN APPROACH IN RP-HPLC

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ABSTRACT

An optimized RP-HPLC method for quantifying Baxdrostat was developed and validated using a Platisil ODS C18 column with a mobile phase of acetonitrile and sodium dihydrogen phosphate (70:30, pH 4), achieving a retention time of 2.504 minutes, a tailing factor of 1.38, and a plate count of 2969. The method, which showed excellent linearity (10-50 µg/ml, R^2 = 0.999), high precision (%RSD 1.08 for standard injections, 0.81 for intermediate precision), and accuracy (96%-99.84% recovery), was robust against slight variations in flow rate and mobile phase composition. The study employed an integrated Quality by Design (QbD) approach, utilizing systematic experimentation and Design of Experiments (DOE) to optimize critical method parameters, ensuring robustness and reliability. Validation followed ICH guidelines, confirming the method's accuracy, precision, linearity, specificity, and robustness. This QbD-driven RP-HPLC method is reliable and efficient for the quantitative analysis of Baxdrostat, supporting quality assurance and regulatory compliance in pharmaceutical development and manufacturing.

Keywords *– Baxdrostat, QBD – quality by design, RP-HPLC – Reverse phase – High performance liquid chromatography, DOE - Design of Experiments, International Conference on Harmonization (ICH)*

INTRODUCTION

The introduction sets the stage for the method development and validation of Baxdrostat employing an integrated analytical Quality by Design (QbD) approach in RP-HPLC. Baxdrostat, a pharmaceutical compound with potential therapeutic significance, necessitates precise and reliable analytical methods for its quantification in formulations. RP-HPLC stands as a widely utilized technique for such purposes due to its versatility and sensitivity. However, the traditional trial-and-error approach in method development often lacks efficiency and may result in suboptimal methods. In contrast, QbD offers a systematic and scientific framework for method development, focusing on understanding the relationship between critical method parameters and the desired analytical outcomes. By integrating QbD principles into RP-HPLC method development, various factors affecting method performance can be systematically explored and optimized. This approach enhances method robustness, ensuring consistent results across different conditions and laboratories. Furthermore, regulatory authorities emphasize the application of QbD in pharmaceutical analysis to ensure product quality and patient safety. The current study aims to bridge the gap between traditional method development approaches and modern QbD principles in RP-HPLC analysis of Baxdrostat. Through a comprehensive exploration of critical method parameters and rigorous validation, the developed method is expected to offer accurate and reliable quantification of Baxdrostat, contributing to the pharmaceutical industry's quality assurance and regulatory compliance efforts.

Quality by Design (QbD)

Quality by Design (QbD) is a systematic approach to pharmaceutical development that begins with predefined objectives and emphasizes understanding of product and

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process, and utilizes quality risk management. The concept of QbD was introduced by the International Conference on Harmonization (ICH) in the pharmaceutical industry to ensure the quality of pharmaceuticals by designing quality into the product rather than relying solely on end-product testing.

At its core, QbD aims to enhance the quality and performance of pharmaceutical products by systematically identifying and controlling all factors that could affect product quality. This proactive approach involves the integration of scientific principles, risk assessment, and process understanding throughout the product lifecycle.

DRUG PROFILE OF BAXDROSTAT

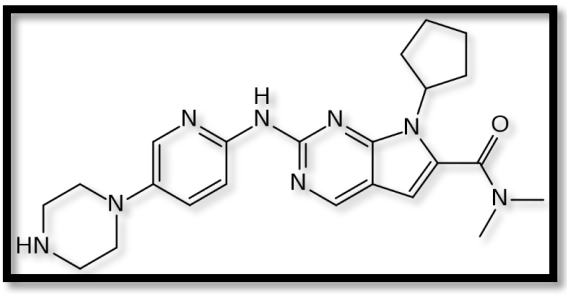


Fig 1: Molecular structure of Baxdrostat

Table No 1: Drug Information of Baxdrostat

Property	Information
Molecular Formula	C23H30N8O
Molecular Weight	Average: 434.55 g/mol, Monoisotopic: 434.25 g/mol
IUPAC Name	Baxdrostat
ChemSpider ID	DB11730
Generic Name	Baxdrostat
Brand Names	Kisqali (200 mg daily dose carton), Kisqali Femara Co-pack
Drug Category	Selective cyclin-dependent kinase inhibitor
Indications	Treatment of HR+, HER2- advanced or metastatic breast cancer in combination with letrozole.
Pharmacology	Inhibits cyclin-dependent kinase 4 and 6 (CDK4/6) to slow cancer progression.
Potency	Highly selective inhibitor of CDK4/6 kinases with low nanomolar IC50 concentrations.

MATERIALS AND METHODS

Equipment used:

The laboratory was equipped with a WATERS2695 separation module, HPLC system, which featured Empower3 software, PAD photodiode array detector water 996 – USA . It also included a LABINDIA UV 3000+ UV/VIS spectrophotometer, an Adwa AD 1020 pH meter, an Afcoset ER-200A weighing machine, and Borosil pipettes, burettes, and beakers.

Chemicals and reagents:

The chemicals used in the study were Baxdrostat, supplied by MSN LAB, and KH2PO4 from FINAR Chemical LTD. Water and methanol for HPLC, as well as acetonitrile for HPLC, were provided by Standard Solutions Ltd. Additionally, HCl, H2O2, and NaOH were sourced from MERCK.

Chromatographic Conditions:

When several mobile phases were tried, the optimized chromatographic conditions were established using high performance liquid chromatography equipped with an auto sampler and PDA detector. The analysis was conducted at ambient temperature. A Platisil C18-EP column (4.6 x 250mm, 5µm) was utilized. The mobile phase consisted of ACN and NaH2PO4 at pH 4 in a 70:30 ratio. The flow rate was set at 1 ml/min, and the detection wavelength was 275 nm. An injection volume of 20 µl was used, and the total run time for the analysis was 10 minutes.

Standard Solution Preparation:

25 mg of Baxdrostat working standard was accurately weighed and transferred into 25 ml clean, dry volumetric flasks. Diluent was added, and the mixture was sonicated to dissolve completely. The volume was then made up to the mark with the same solvent to prepare the stock solution, which had a concentration of 1000 ppm. Subsequently, 0.3 ml of each stock solution was pipetted into 10 ml volumetric flasks and diluted up to the mark with diluent, resulting in a concentration of 30 ppm.

Sample Solution Preparation:

The equivalent of 25 mg of Baxdrostat was accurately weighed and transferred into 25 ml clean, dry volumetric flasks. About 7 ml of diluent was added, and the mixture was sonicated to dissolve completely. The volume was then made up to the mark with the same solvent to prepare the stock solution,which had a concentration of 1000 ppm. Subsequently, 0.3 ml of each stock solution was pipetted into 10 ml volumetric flasks and diluted up to the mark with diluent, resulting in a concentration of 30 ppm.

Assay:

10 µL of the standard and sample were injected into the chromatographic system. The areas for the Baxdrostat peaks were measured, and the % Assay was calculated using the formulae.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used: High performance liquid chromatography equipped auto sampler and PDA detector.

Column: Platisil ODS C18 (250× 4.6mm 5µm)

Mobile phase: CAN (acetonitrile): NaH2PO4 (Sodium dihydrogen phosphate) PH- 4 (70:30)

Flow rate: 1ml/min

Injection volume: 20 µl

Temperature: 25°c

Wavelength: 275nm

Run Time: 10mins

OBSERVATION

Retention time: 2.504 min

Tailing: 1.38

Plate count: 2969

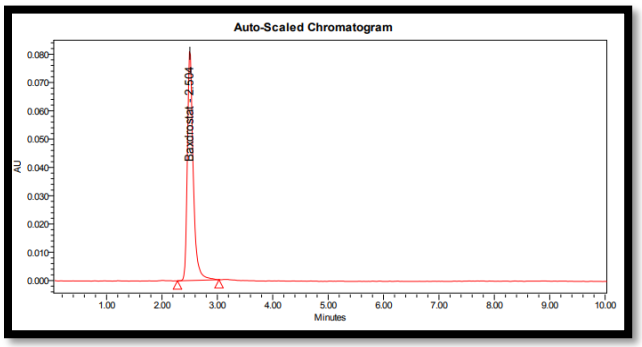


Fig 2: Optimized Chromatogram of Baxdrostat

RESULTS AND DISCUSSION

Response-1 retention time of baxdrostat:-

Table No-2: Fit summary of Baxdrostat

File Version	23.1.0.0		
Study Type	Response Surface	Subtype	Randomized
Design Type	Box-Behnken	Runs	17
Design Model	Quadratic	Blocks	No Blocks
Build Time (ms)	18.00		

ANOVA FOR QUADRATIC MODEL:

Table No 3: Response 1: Retention time

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Mean vs Total	119.66	1	119.66			
Linear vs Mean	0.5443	3	0.1814	137.29	< 0.0001	
2FI vs Linear	0.0005	3	0.0002	0.0996	0.9584	
Quadratic	0.0166	3	0.0055	371.01	<	Suggested
vs 2FI					0.0001	
Cubic vs Quadratic	0.0001	3	0.0000			Aliased
Residual	0.0000	4	0.0000			
Total	120.22	17	7.07			

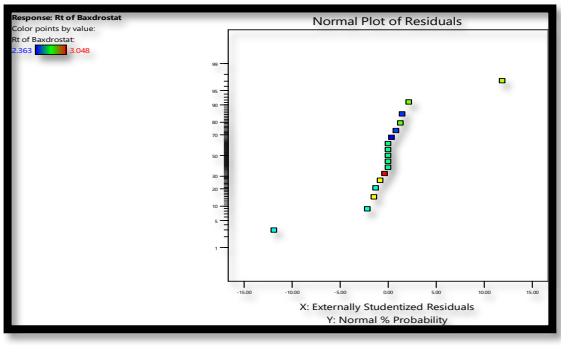


Fig 3: Normal plot of Residuals for Baxdrostat

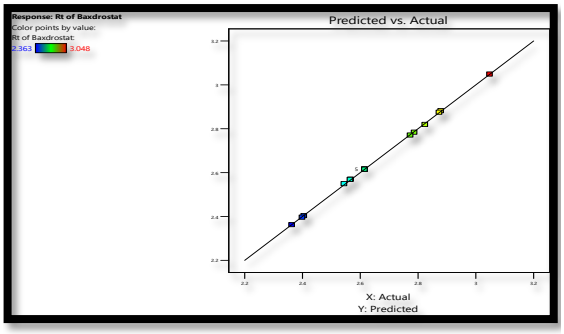


Fig 4: Predicted vs. Actual for Baxdrostat

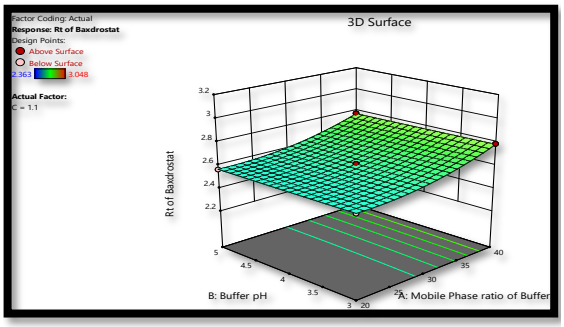


Fig 5: 3D Surface for Baxdrostat

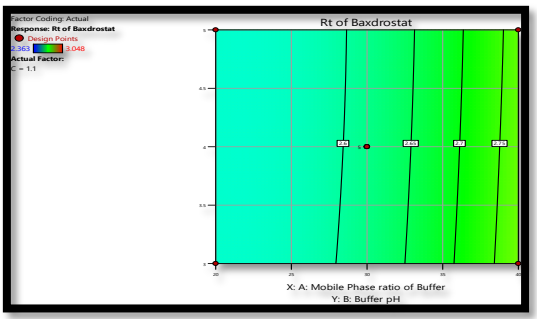


Fig 6: Retention time of Baxdrostat

TAILING FACTOR OF BAXDROSTAT:-

ANOVA for Quadratic model

Table No 4: Response 2: tailing factor

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.2548		0.0897	-0.5082	
2FI	0.4463		0.0825	-1.7798	
Quadratic	0.0185		0.6596	-1.3825	Suggested
Cubic			1.0000		Aliased

Table No 5: Sequential Model sum of squares

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Mean vs Total	23.48	1	23.48			
Linear vs Mean	0.0068	3	0.0023	1.53	0.2548	
2FI vs Linear	0.0043	3	0.0014	0.9660	0.4463	
Quadratic vs 2FI	0.0110	3	0.0037	6.65	0.0185	Suggested
Cubic vs Quadratic	0.0039	3	0.0013			Aliased
Residual	0.0000	4	0.0000			
Total	23.51	17	1.38			

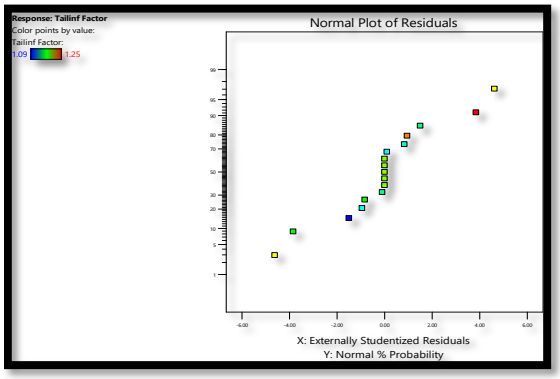


Fig 7: Normal plot of Residuals for Baxdrostat

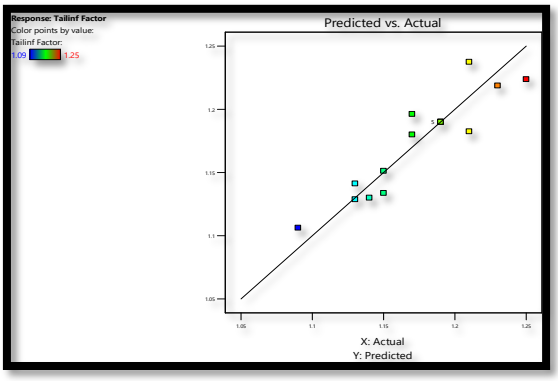


Fig 8: Predicted vs Actual for Baxdrostat

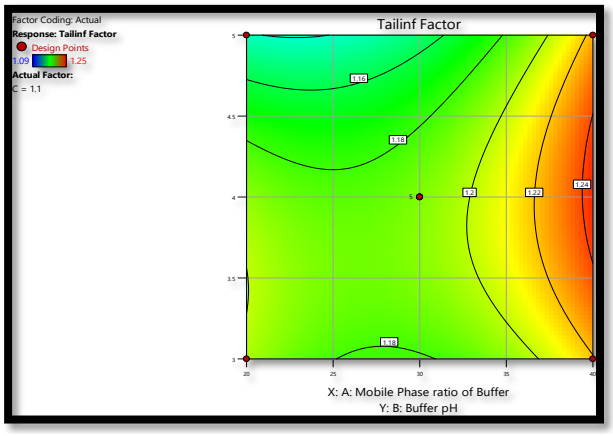


Figure 9: R tailing factor for Baxdrostat

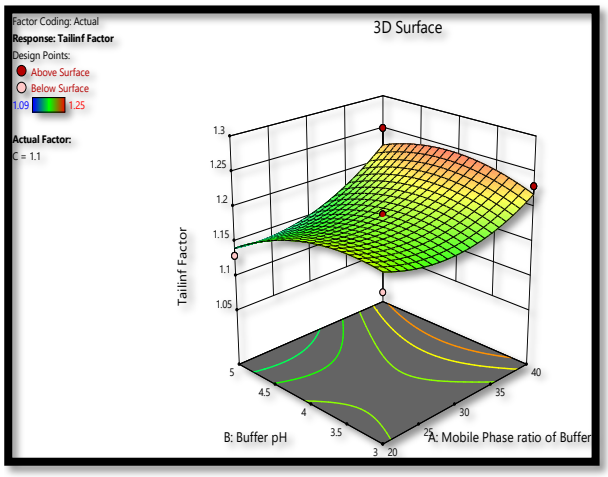


Figure 10: 3D Surface for Baxdrostat

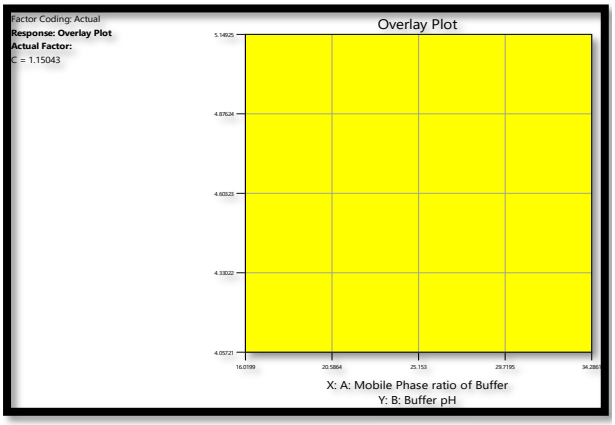


Figure11: Overlay plot for Baxdrostat

ASSAY:

Standard and sample solution injected as described under experimental work. The corresponding chromatograms and results are shown below.

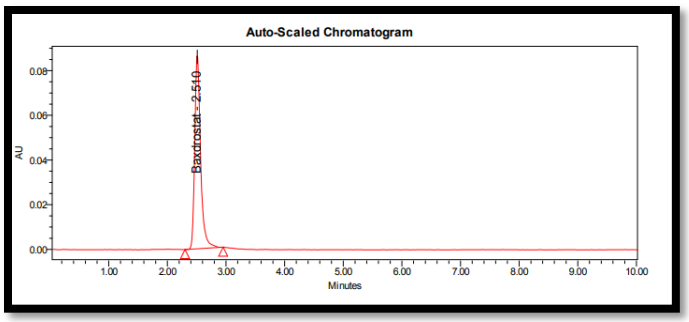


Fig 12: Chromatogram for Standard

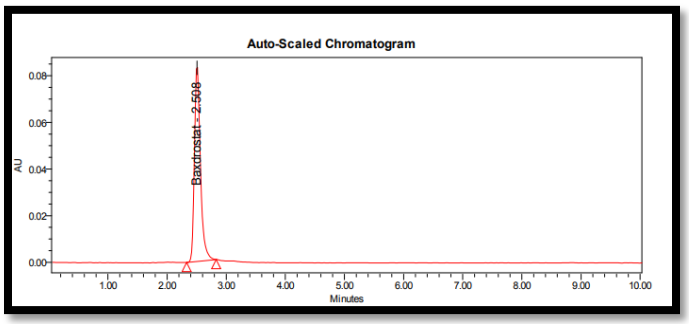


Fig 13: Chromatogram for Sample

System suitability

System suitability tests were carried out on freshly prepared standard solution of Baxdrostat to check the various parameters such as efficiency, retention time, and peak tailing which was found to comply with ICH requirements. The instrumental precisions as determined by six successive injections of the standard solutions give RSD below 2% of Retention Time and Area. The data is given in Table no:1 as shown in figure-2.

Table No 6: System suitability parameters of Baxdrostat.

Parameters	Baxdrostat
Tailing factor	**1.38**
Retention time	**2.504**
Theoretical plates per unit	**2969**

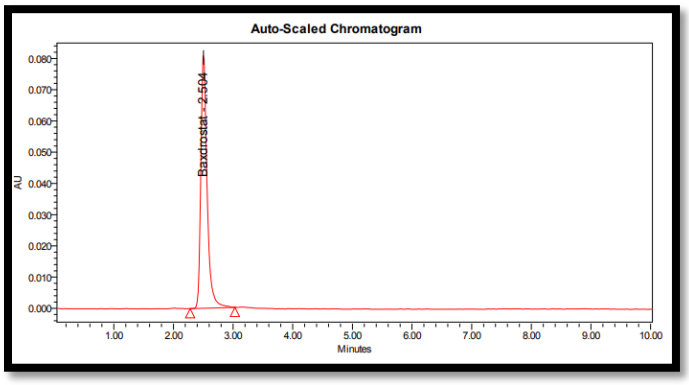


Fig 14: Chromatogram showing system suitability testing of Standard Solution of Baxdrostat

LINEARITY:

Linearity is the ability of the method to obtain test results that are directly proportional to the analyte concentration within a given range.

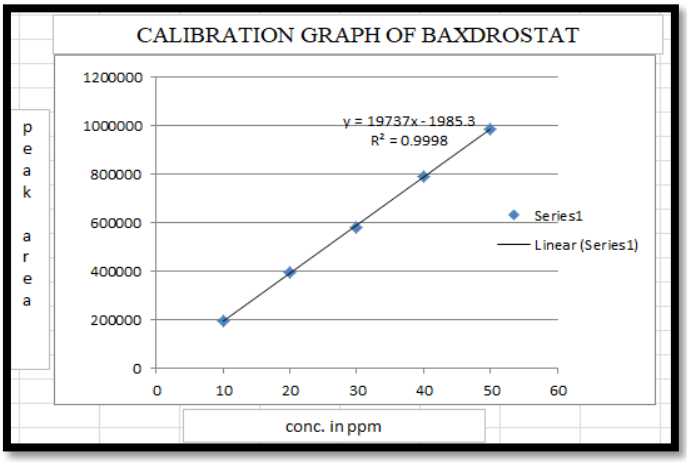
Procedure:

From the above standard stock solution pipette out a series of dilution i.,e0.1 ml ,0.2 ml ,0.3 ml, 0.4 ml, and 0.5 ml, to five 10 ml volumetric Flasks and make up with diluent in. Each level was injected into the chromatographic system, and the peak areas were measured. A graph of peak area versus concentration was plotted, and the correlation coefficient was calculated.

Table No 7: Linearity Results for Baxdrostat:

Parameters	Baxdrostat
Linear Dynamic Range	10 to 50µg/ml
Correlation Co-efficient	0.999
Slope (m)	19737

LINEARITY GRAPH



PRECISION

The precision study was conducted by preparing a stock solution where 25 mg of Baxdrostat working standard was accurately weighed and transferred into a 25 ml clean, dry volumetric flask. Diluent was added, and the mixture was sonicated to dissolve completely, then made up to the mark with the same solvent. Subsequently, 0.3 ml of the stock solution was pipetted into a 10 ml volumetric flask and diluted to the mark with diluent. The standard solution was injected six times, and the area for all six injections was measured using HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Table No 8: Results of Precision for Baxdrostat

Injection	Area
Injection-1	602744
Injection-2	585088
Injection-3	600178
Injection-4	598776
Injection-5	596473
Injection-6	601692
Average	597491.8
Standard Deviation	6465.05
%RSD	1.08

Intermediate Precision:

To evaluate the intermediate precision (ruggedness) of the method, precision was assessed on different days within the laboratory. A stock solution was prepared by accurately weighing and transferring 25 mg of Baxdrostat working standard into a 25 ml clean, dry volumetric flask. Diluent was added, and the mixture was sonicated to dissolve completely, then made up to the mark with the same solvent. Subsequently, 0.3 ml of the stock solution was pipetted into a 10 ml volumetric flask and diluted to the mark with diluent. The standard solution was injected five times, and the area for all six injections was measured using HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Table No 9: Results of Intermediate precision for Baxdrostat

Injection	Area
Injection-1	602744
Injection-2	595088
Injection-3	600178
Injection-4	608776
Injection-5	596473
Injection-6	601692
Average	600825.2
Standard Deviation	4897.435
%RSD	0.81

Accuracy (recovery study) for Baxdrostat

The validation of the proposed method was further verified by recovery studies. Acceptance criteria are 98 - 102 % w/v. The results are reported in below

Table No 10: Accuracy data for Baxdrostat

Parameters	Baxdrostat
% recovery	
50 %	96 % w/v
100 %	99.84 % w/v
150 %	98.29 % w/v

This serves as a good index of the accuracy and reproducibility of the proposed method.

A known amount of Baxdrostat was applied to 50%, 100% and 150% of specification in triplicate and analyzed determine the accuracy of the method.

LIMIT OF DETECTION (LOD)

The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value. LOD can be calculated as: LOD = 3.3 σ / S Where, σ =Standard deviation of the response (y-intercept) S= Slope of the calibration curve. The limit of detection (LOD) was found to be 0.06µg/ml for RAB respectively.

Table No 11: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio	Conc. In ppm
Baxdrostat	64	189	2.95	0.06

LIMIT OF QUANTIFICATION (LOQ)

The limit of quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined with suitable precision and accuracy. LOQ can be calculated as: LOQ= 10 σ / S Where, σ = Standard deviation of the response (y- intercept) S= Slope of the calibration curve whereas the limit of quantification (LOQ) was found to be 0.23µg/ml.

Table No 12: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio	Conc. In ppm
Baxdrostat	64	635	9.92	0.23

ROBUSTNESS

Robustness was determined by carrying out the assay during which flow rate and organic were altered slightly. The results are reported.

Table No 13: Results for variation in flow for Baxdrostat

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	2394.93	1.28
2	1	2969.06	1.38
3	1.2	2457.91	1.32

***Results for actual flow (0.8 ml/min) have been considered from Assay standard.**

Table No 14: Results for variation in mobile phase composition for Baxdrostat

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2782.31	1
2	*Actual	2969.06	2
3	10% more	2885.61	3

*Results for actual Mobile phase composition have been considered from Accuracy standard.

Table No15: Robustness data for Baxdrostat

Parameters	Baxdrostat
Flow rate	0.8
Detection wave length	220nm

The above value of robustness indicated that the method is robust and does not show variations in the results on slight variations in flow rate and detection wavelength.

CONCLUSION

In conclusion, the development and validation of Baxdrostat using an integrated analytical Quality by Design (QbD) approach in RP-HPLC have proven to be highly effective and beneficial. By employing QbD principles, we have systematically optimized the method parameters to ensure robustness, reliability, and efficiency in the analysis of Baxdrostat.

The Baxdrostat HPLC method was developed using a quality by design (QbD) approach. Critical process parameters (CPPs) were identified, including phosphate buffer, flow rate, and temperature. The critical quality attributes (CQAs) considered were retention time (1.196) and tailing factor (1.41). The method was optimized through a Design of Experiment (DOE) study, with the following condition like Column: DIKMA SPURSIL C18-EP (3.0 x 150 mm, 3 µm)Mobile phase: 50% KH2PO4:50% acetonitrile, flow rate: 0.9 mL/min .UV wavelength (λmax): 220 nm The Baxdrostat retention time was 2.44, and system suitability parameters included a tailing factor of -1.16 and a plate count of 3338. The assay showed 99.55% recovery, indicating suitability for routine analysis. Linearity was confirmed (correlation coefficient = 0.999), and precision met acceptance criteria (RSD ≤ 2.0%). Intermediate precision was also demonstrated. Accuracy (98.8%) and LOD/LOQ (2.97/9.97) were within limits. The method exhibited robustness under varying mobile phase and flow rate conditions.

Through a thorough understanding of critical method attributes and their impact on the performance of the method, we have achieved a method that not only meets regulatory requirements but also delivers consistent and accurate results. The use of a risk-based approach allowed us to identify and mitigate potential sources of variability, thus enhancing the method's robustness and ensuring its suitability for routine analysis.

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