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QbD-Guided RP-HPLC Method Development and Validation for Bisoprolol and Perindopril in Pure and Formulated Dosage Forms

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Abstract:

A robust and optimized Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed for the simultaneous estimation of Perindopril and Bisoprolol in pharmaceutical dosage forms. Method optimization was performed using a Box-Behnken response surface design, with statistical analysis via ANOVA confirming the significance of resolution and tailing factor models ($p < 0.05$). Flow rate emerged as a critical factor influencing both responses. Diagnostic plots—including normal residuals, predicted vs. actual values, and 3D surface plots—validated the model's accuracy and predictive capability. System suitability parameters such as resolution (>5), tailing factor (<2), and theoretical plate count (>2000) met all acceptance criteria, ensuring reliable chromatographic performance. The method was validated according to ICH guidelines, demonstrating excellent linearity for Perindopril and Bisoprolol with correlation coefficients (R^2) exceeding 0.999. Precision studies showed %RSD values below 2%, confirming repeatability and intermediate precision. Accuracy was supported by recovery rates within 98–102% across multiple concentration levels. Sensitivity was established with low LOD and LOQ values for both analytes, and robustness was confirmed under deliberate variations in chromatographic conditions. Overall, the developed RP-HPLC method is simple, precise, accurate, and robust, making it highly suitable for routine quality control of Perindopril and Bisoprolol in bulk and formulated products.

Keywords: Reverse Phase High-Performance Liquid Chromatography (RP-HPLC), Perindopril, Bisoprolol, low rate, resolution, tailing factor, theoretical plate count, system suitability, ICH guidelines, linearity, precision, accuracy, %RSD, recovery, limit of detection (LOD), limit of quantification (LOQ)

Introduction

Bisoprolol

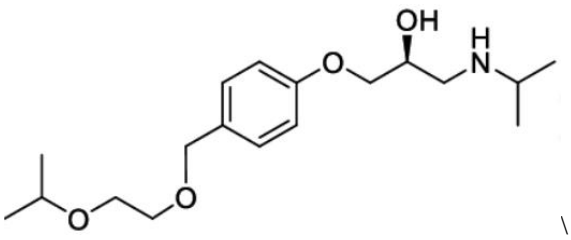

Fig.1: Bisoprolol

IUPAC: (±)-1-[4-[(2-Isopropoxyethoxy) methyl] phenoxy] -3-(isopropylamino)propan-2-ol
Molecular Formula: C[18]H[31]NO[4]
Molecular Weight: 325.45 g/mol
Melting Point: 100-102°C
pKa: 9.5
Category: Beta-1 selective adrenergic receptor blocker
Solubility: Slightly soluble in water; soluble in methanol and ethanol.
Description: Bisoprolol is a beta-blocker that affects the heart and circulation (blood flow through arteries and veins). It is used to treat hypertension (high blood pressure) and heart failure1.
Mechanism of Action: Bisoprolol selectively blocks beta-1 adrenergic receptors in the heart, reducing heart rate, cardiac output, and blood pressure2.
Pharmacodynamics: Bisoprolol reduces the workload on the heart and helps it to beat more regularly. This can help lower blood pressure and reduce the risk of heart attacks and strokes.

Perindopril

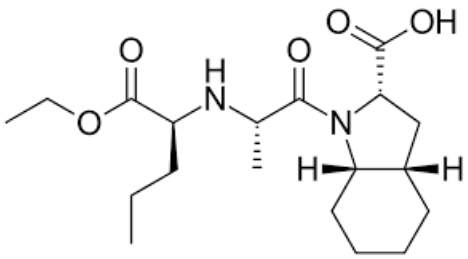

Fig.2: Perindopril

IUPAC: (2S,3aS,7aS)-1-[(2S)-2-[[(1S)-1-(ethoxycarbonyl) butyl] amino] propanoyl]octahydro-1H-indole-2-carboxylic acid
Molecular Formula: C[19]H[32]N[2]O[5]
Molecular Weight: 368.47 g/mol
Melting Point: 120-124°C
pKa: 3.8
Category: Angiotensin-converting enzyme (ACE) inhibitor
Solubility: Slightly soluble in water; soluble in methanol and ethanol.
Description: Perindopril is an ACE inhibitor used to treat high blood pressure, heart failure, or stable coronary artery disease. It works by relaxing blood vessels and decreasing blood volume.

Mechanism of Action: Perindopril inhibits the angiotensin-converting enzyme, which is responsible for the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor. This leads to vasodilation and reduced blood pressure[2].

Pharmacodynamics: Perindopril reduces blood pressure by decreasing peripheral vascular resistance without causing reflex tachycardia. It also has a beneficial effect on the heart by reducing preload and afterload[2].

Materials and Methods

Table 1: List of Proposed Materials

S.No.	Chemicals/standards and reagents	Make
1	Trifluoro acetic acid	Qualigens
2	Formic acid	Qualigens
3	Water	Qualigens
4	Acetonitrile	Qualigens
5	Methanol	Rankem

Table 2: List of **Equipment's**

S.No.	Equipment	Model/Type
1	Electronic Balance	SAB2032
2	Ultra-Sonicator	SE60US
3	Thermal Oven	i-THERM A17782
4	pH Meter	ORION STAR A111
5	Filter Paper	0.45 microns
6	HPLC System	Waters 2690 Separation Module

Optimization of Column

Spurcil C18, (250×4.6mm, 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 : RP-HPLC equipped with Auto Sampler and PDA detector
Temperature : Ambient
Column : Spurcil C18, (250×4.6mm, 5µm)
Buffer : 0.1% Formic Acid (pH-3.5)
Mobile phase : 40% 0.1% Formic Acid: 60% ACN
Flow rate : 1.0 ml per min
Wavelength :219 nm.
Injection volume: 20 µl
Run time : 10 min.

Preparation of buffer and mobile phase:

Preparation of 0.1% Formic Acid PH 3.5:

To prepare 0.1% Formic Acid buffer solution, by adding 1ml of formic acid in 1000ml water. Adjust this solution to pH 3.5 by using diluted NAOH.

Preparation of mobile phase:

Mix a mixture of above ACETONITRILE 600ml (60%) and 400 ml 0.1% OPA (40%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

Acetonitrile: 0.1% Formic Acid PH3.5 (60:40) ratio.

System Suitability:

Tailing factor for the peaks due to Bisoprolol and perindropil in Standard solution should not be more than 2.0. Theoretical plates for the Bisoprolol and perindropil peaks in Standard solution should not be less than 2000.

Method validation parameters:

Assay:

Standard Solution Preparation:

Accurately weigh and transfer 25mg Perindropil and 12.5mg of Bisoprolol 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 0.9ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents (90ppm of Perindropil and 45ppm of Bisoprolol).

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 25mg Perindropil and 12.5mg of Bisoprolol equivalent weight of the sample 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 0.9ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents (90ppm of Perindropil and 45ppm of Bisoprolol).

Procedure:

Inject 10 µL of the standard, sample into the chromatographic system and measure the areas for the Bisoprolol and Perindropil peaks.

Calculation: (For Bisoprolol and perindropil)

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

Acceptance criteria of System Suitability:

1. Tailing factor should be less than 2
2. Theoretical Plates should be above 2000

Results and Discussion

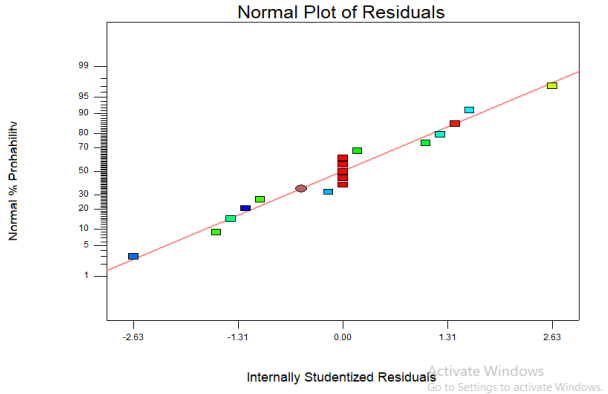

Fig.3: Normal plot of residuals for Perindropil and Bisoprolol

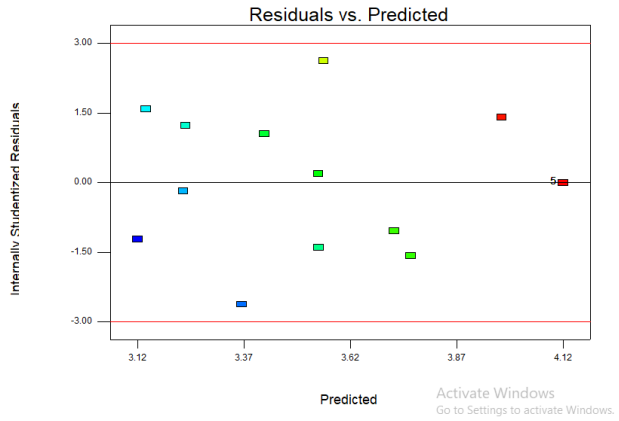

Fig.4: Residuals vs. Predicted for Perindropil and Bisoprolol

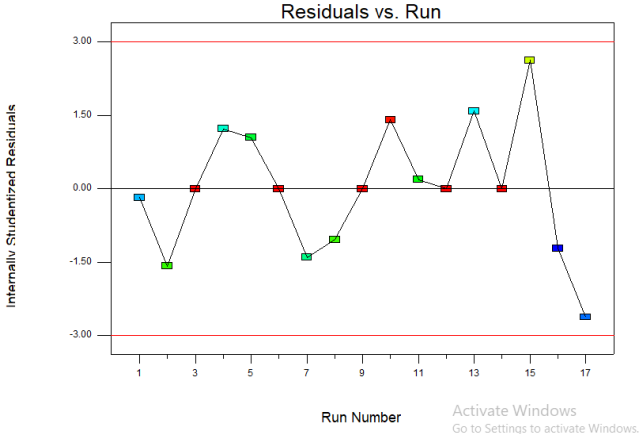

Fig.5: Residuals vs. Run for Perindropil and Bisoprolol

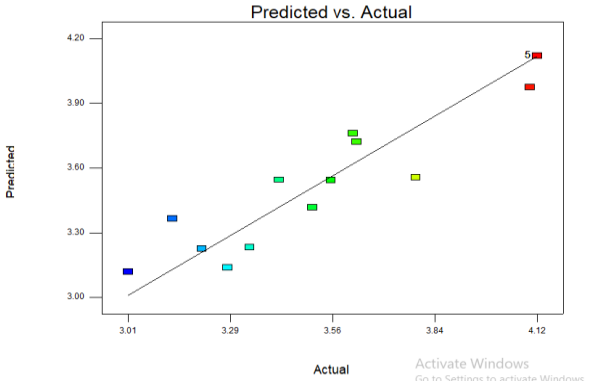

Fig.6: Predicted vs. Actual for Perindropil and Bisoprolol

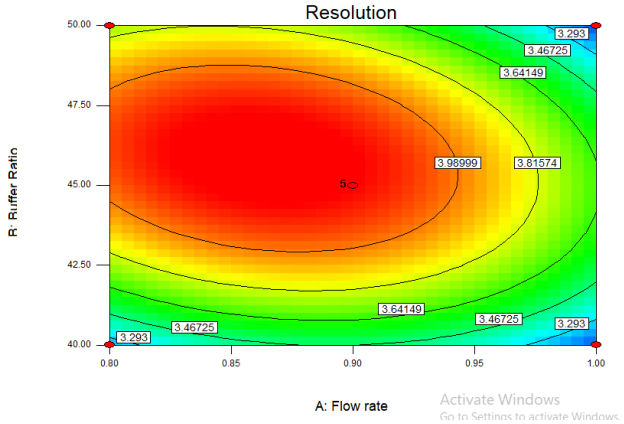

Fig.7: Resolution for Perindropil and Bisoprolol

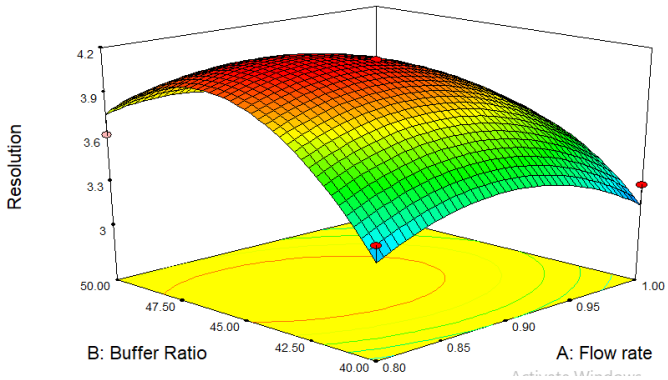

Fig.8: 3D Surface for Perindropil and Bisoprolol

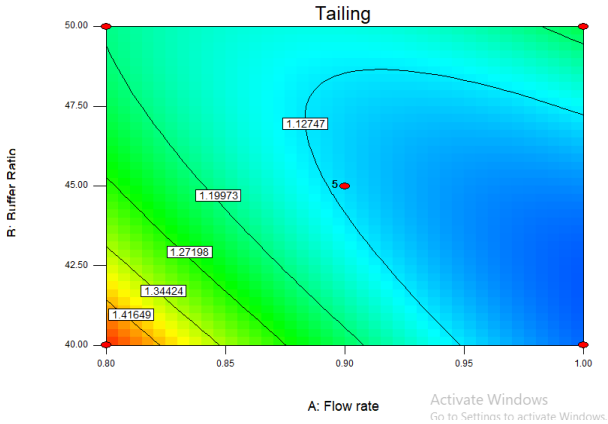

Fig.9: Tailing factor for Perindropil and Bisoprolol

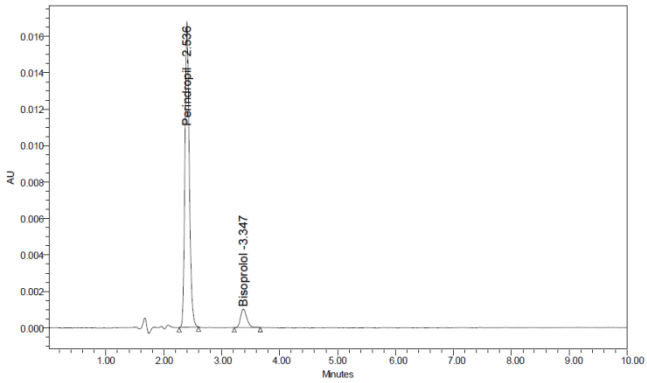

Fig.10: Chromatogram for system suitability

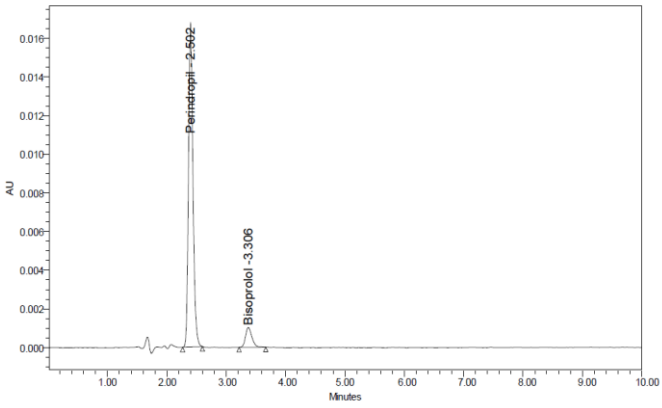

Fig.11: Chromatogram for Standard

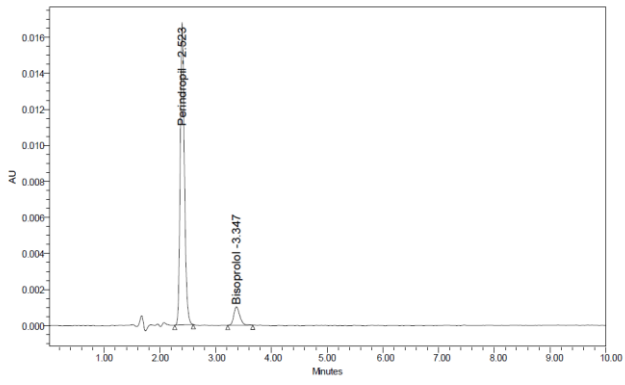

Fig.12: Chromatogram for Sample

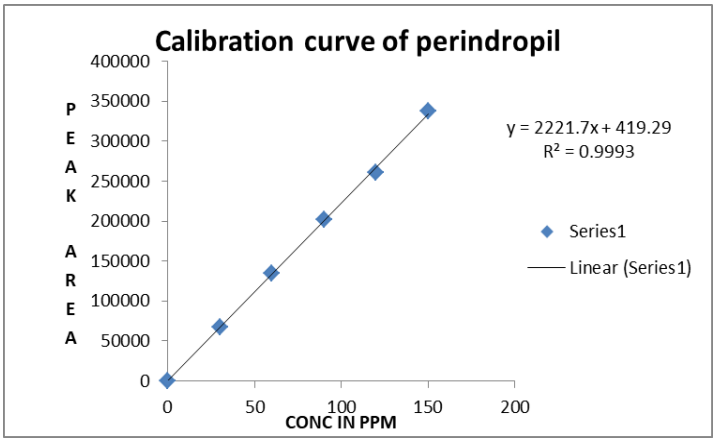

Fig.13: Calibration graph for perindropil

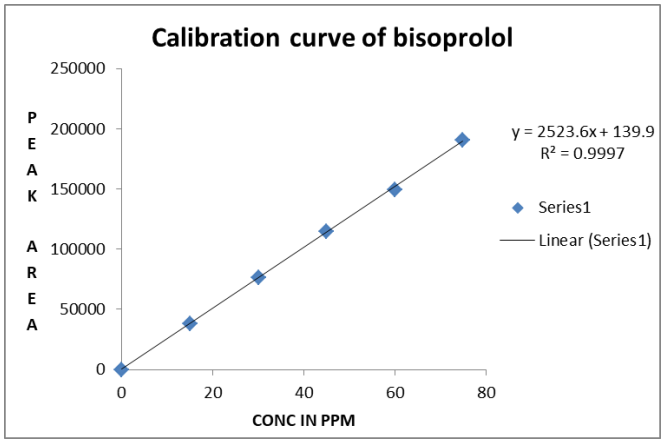

Fig.14: Calibration graph for Bisoprolol

Table 3: ANOVA for Response Surface Quadratic Model

Souces	Sum of square	df	Mean square	F value	P value Pro b>F	
Model	2.29	9	0.25	8.04	0.0059	significant
A-flow rate	0.27	1	0.27	8.53	0.0223	
B-buffer ratio	0.13	1	0.13	4.11	0.0824	
C-Organic ratio	7.812E-003	1	7.812E-003	0.25	0.6347	
Residual	0.22	7	0.032			
Lack of Fit	0.22	3	0.074			
Pure Error	0.000	4	0.000			

Table 4: Results of system suitability parameters

NAME	RT	AREA	RESOLUTION	TAILING FACTOR	PLATE COUNT
Perindropil	**2.563**	**201041**	**5.35**	**1.20**	**4821**
Bisoprolol	**3.347**	**114321**		**1.05**	**2823**

Table 5: Accuracy (recovery) data for Bisoprolol and Perindropil

%Concentration perindropil (at specification Level)	Area*	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	100521	12.5	12.39	99.12	99.2
100%	201324	25	24.8	99.26	
150%	301964	37.5	37.22	37.5	

%Concentration Bisoprolol (at specification Level)	Area*	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	56982	6.25	6.21	99.3	99.3
100%	113854	12.5	12.40	99.2	
150%	171089	18.75	18.64	99.4	

Table 6: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained(µV)	S/N ratio	Conc.
Perindropil	63	180	2.86	0.09 µg/ml
Bisoprolol	63	188	2.98	0.10 µg/ml

Table 7: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained(µV)	S/N ratio	CONC.
Perindropil	63	625	9.92	0.33µg/ml
Bisoprolol	63	601	9.54	0.32 µg/ml

Conclusion

The developed RP-HPLC method for the simultaneous estimation of Perindopril and Bisoprolol has been successfully optimized using a Box-Behnken response surface design. The statistical evaluation, including ANOVA, revealed that the resolution and tailing factor models were significant with acceptable p-values ($p < 0.05$), particularly highlighting the effect of flow rate on both responses. The normal plot of residuals, predicted vs. actual plots, and 3D surface plots confirm the suitability and accuracy of the developed models. System suitability parameters, such as resolution (>5), tailing factor (<2), and theoretical plate count (>2000), were all within the acceptable limits, indicating the system's performance and the method's reproducibility. The method was validated as per ICH guidelines and showed excellent linearity for both drugs with correlation coefficients (R^2) greater than 0.999. Precision studies demonstrated %RSD values well below 2%, confirming repeatability and intermediate precision. The accuracy studies

showed recovery values within the range of 98–102%, indicating the method's reliability. Sensitivity was proven with LOD values of 0.09 µg/ml and 0.10 µg/ml and LOQ values of 0.33 µg/ml and 0.32 µg/ml for Perindopril and Bisoprolol, respectively. Robustness testing under deliberate variations of chromatographic conditions confirmed the method's stability. Therefore, the developed method is simple, precise, accurate, robust, and suitable for routine analysis of Perindopril and Bisoprolol in bulk and pharmaceutical dosage forms.

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