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QBD-Based Analytical Method Development and Validation for the Simultaneous Estimation of Voglibose and Metformin Using RP-HPLC

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Abstract
A scientifically rigorous and precise reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed using a Quality by Design (QbD) approach for the simultaneous estimation of Voglibose and Metformin in fixed-dose combination formulations. The method was systematically optimized through Design of Experiments (DoE), evaluating critical parameters such as flow rate, buffer pH, and organic solvent ratio for their impact on chromatographic responses like resolution and tailing factor. Statistical analysis via ANOVA confirmed the significance of the quadratic model in achieving optimal separation. The final method employed a Zorbax C18 column with phosphate buffer (pH 4.5) and acetonitrile (45:55 v/v) as the mobile phase, operating at a flow rate of 1.2 mL/min and detection at 225 nm. This setup yielded excellent peak separation with a resolution of 10.10 between the analytes. Validation was conducted in accordance with ICH Q2(R1) guidelines, demonstrating outstanding linearity for Metformin (25–125 µg/mL) and Voglibose (0.1–0.5µg/mL), with correlation coefficients of 0.9998 and 0.9994, respectively. Precision studies confirmed repeatability and intermediate precision (%RSD < 2%), while accuracy was validated through high recovery rates (99.4% for Voglibose and 99.6% for Metformin). Sensitivity was affirmed with acceptable LOD and LOQ values. This QbD-driven RP-HPLC method provides a robust, validated, and regulatory-compliant solution for simultaneous estimation of Voglibose and Metformin in pharmaceutical applications.

INTRODUCTION

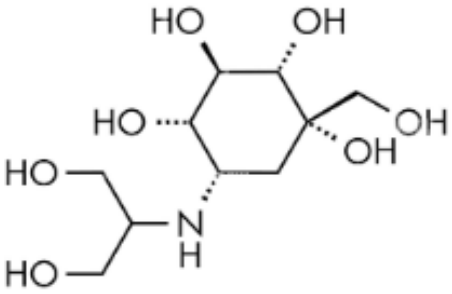

Fig.1: Voglibose

Basic Information
IUPAC Name: (1S,2S,3R,4S,5S)-5-(1,3-dihydroxypropan-2-ylamino)-1-(hydroxymethyl)cyclohexane-1,2,3,4-tetraol1
Molecular Formula: $C_{10}H_{21}NO_7$
Molecular Weight: 267.28 g/mol1
Category: Alpha-glucosidase inhibitor2
Physical Properties
Melting Point: Not readily available
pKa: Not readily available
Solubility: Soluble in water2
Description: Voglibose is an alpha-glucosidase inhibitor used to lower postprandial blood glucose levels in people with diabetes mellitus.

Mechanism of Action
Voglibose works by inhibiting alpha-glucosidase enzymes in the intestine, which delays the breakdown of complex carbohydrates into glucose, thereby reducing the postprandial rise in blood glucose levels21.
Pharmacodynamics
Voglibose helps manage postprandial hyperglycemia by delaying glucose absorption in the intestine2.
Pharmacokinetics
Absorption: Poorly absorbed from the gastrointestinal tract2
Metabolism: Metabolized in the liver
Route of Elimination: Primarily excreted in the feces
Protein Binding: Not readily available
Half-Life: Not readily available

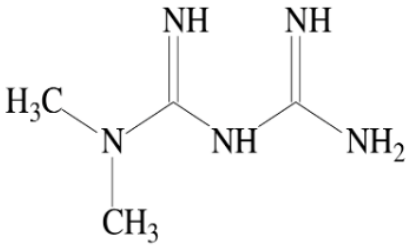

Fig.2: Metformin

Basic Information
IUPAC Name: N,N-Dimethylimidodicarbonimidic diamide

Molecular Formula: $C_4H_{11}N_5$
Molecular Weight: 129.16 g/mol
Melting Point: 223-226°C
pKa: 12.4
Category: Antidiabetic agent (Biguanides)
Solubility: Soluble in water, slightly soluble in ethanol, insoluble in acetone and ether

Description

Metformin is an FDA-approved medication primarily used to manage high blood sugar levels in individuals with type 2 diabetes. It works by reducing glucose absorption from the intestines, lowering liver glucose production, and improving insulin sensitivity1.

Mechanism of Action: Metformin activates AMP-activated protein kinase (AMPK), which helps to decrease hepatic glucose production and increase insulin sensitivity1.

Pharmacodynamics

Metformin decreases both basal and postprandial blood glucose levels. It does not stimulate insulin secretion; hence it does not cause hypoglycemia.

MATERIALS AND METHODS

Table 1: Instruments used

S.No	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

S.No	Chemical	Brand
1	Betamethasone	Supplied by KP LBAS
2	Calcipotriene	Supplied by KP LBAS
3	Ortho phosphoric acid	FINAR chemical LTD
4	Water and Methanol for HPLC	Standard solutions Ltd
5	Acetonitrile for HPLC	Standard solutions Ltd
6	HCl, H_2O_2, NaOH	MERCK

Optimization of Column:

Phenomenex Zorbax C18-SB, (150×4.6mm, 3□m) 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: Phenomenex Zorbax C18-SB, (150×4.6mm, 3µm)
Buffer : Phosphate buffer (pH-4.5)
Mobile phase: 45% KH2PO4: 55% ACN
Flow rate : 1.2 ml per min
Wavelength : 225 nm
Injection volume : 10µ
Run time : 8 min.

Preparation of buffer and mobile phase:

Preparation of KH2PO4 pH 4.5:
To prepare KH2PO4, by adding 6.8g of KH2PO4 in 1000ml water. Adjust this solution to pH 4.5 by using OPA.

Preparation of mobile phase:

Mix a mixture of above Acetonitrile 550ml (55%), 450 ml KH2PO4 (45%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Diluent Preparation:
Acetonitrile: KH2PO4 PH 4.5 (55:45 ml) ratio.

System Suitability:

Tailing factor for the peaks due to Metformin and Voglibose in Standard solution should not be more than 2.0.
Theoretical plates for the Metformin and Voglibose peaks in Standard solution should not be
Less than 2000.

Calculation: (For Metformin and Voglibose)

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

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.

Acceptance criteria of System Suitability

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

Method validation parameters:

Assay:

Standard Solution Preparation:

Accurately weigh and transfer 25mg of Metformin Working standard into 25ml clean dry volumetric flask and 1mg Voglibose Working standard into a 250ml 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 0.75ml of each of the above stock solutions into a 10ml volumetric flasks and dilute up to the mark with Diluents. (75ppm Metformin and 0.3ppm Voglibose)

Sample Solution Preparation:

Accurately weigh and transfer Equivalent weight of 25mg of Metformin into 25ml clean dry volumetric flask and 1mg Voglibose into a 250ml 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 0.75ml of each of the above stock solutions into a 10ml volumetric flasks and dilute up to the mark with Diluents. (75ppm Metformin and 0.3ppm Voglibose)

Procedure:

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

RESULTS AND DISCUSSION

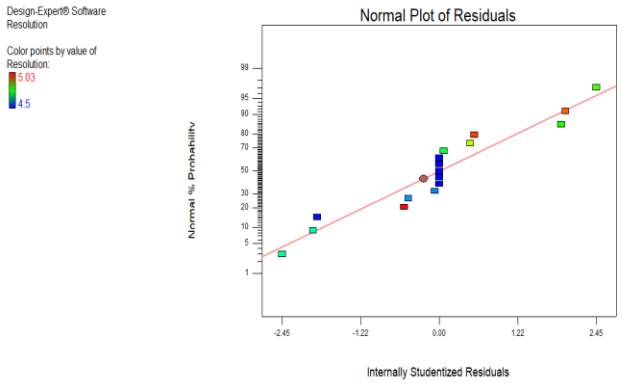

Fig.3: Normal plot of Residuals for Voglibose and Metformin

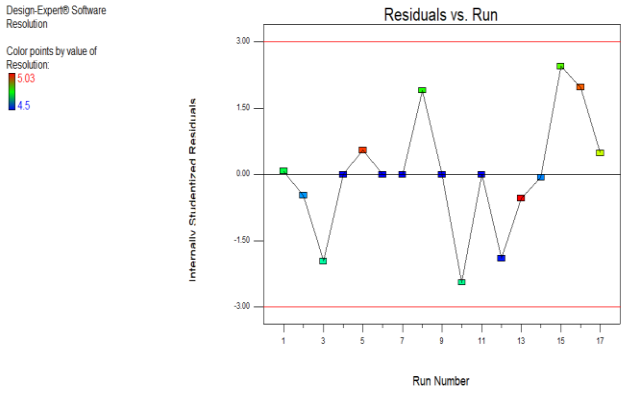

Fig.4: Residuals vs. Run for Voglibose and Metformin

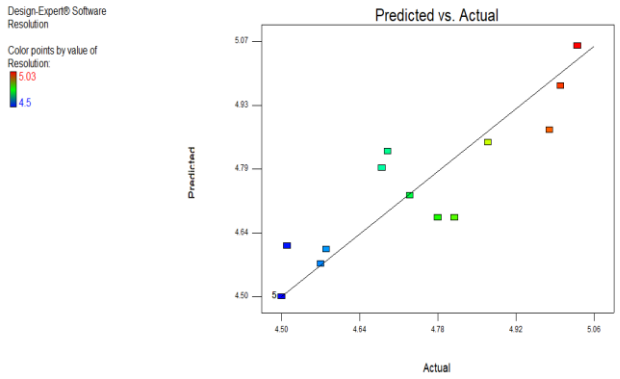

Fig.5: Predicted vs. Actual for Voglibose and Metformin

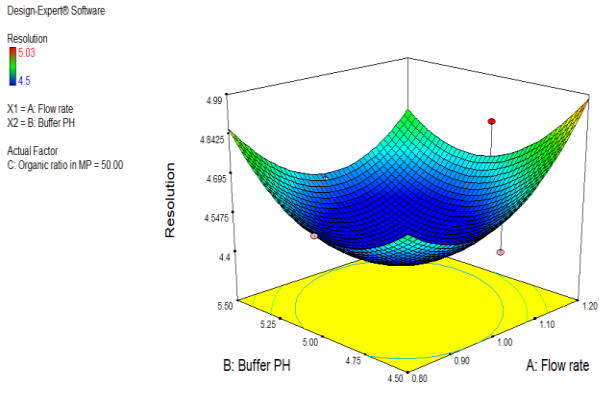

Fig.6: 3D Surface for Voglibose and Metformin

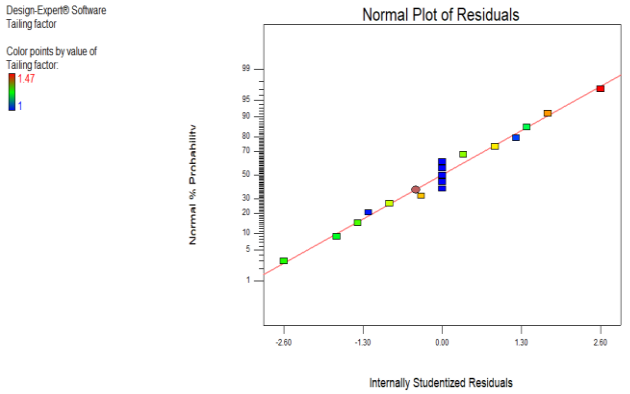

Fig.7: Normal plot of Residuals for Voglibose and Metformin

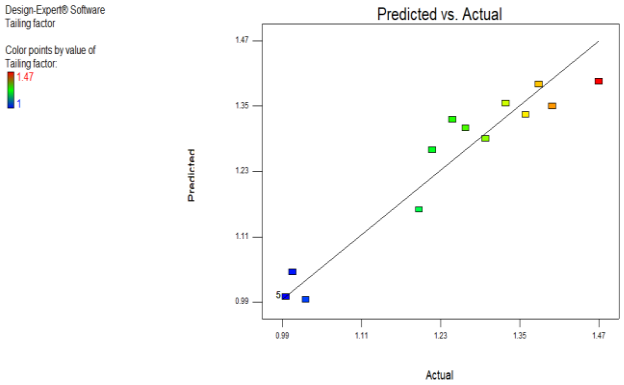

Fig.8: Predicted vs. Actual for Voglibose and Metformin

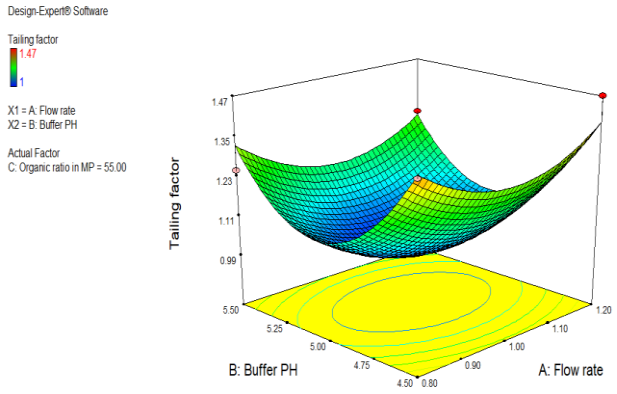

Fig.9: 3D Surface for Voglibose and Metformin

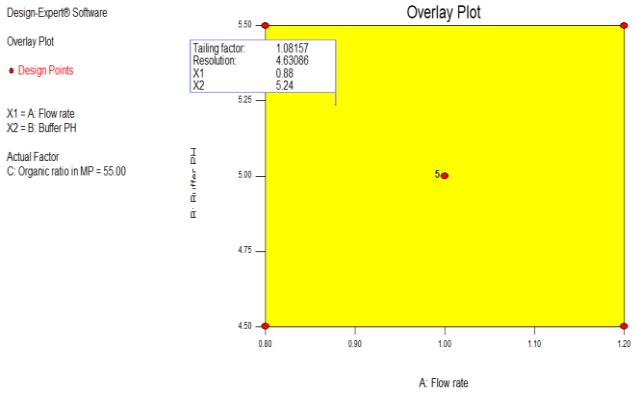

Fig.10: Overlay plot for Voglibose and Metformin

Table 3: (R_2) Tailing Factor of Voglibose and Metformin (FIT Summary)

Souces	Sum of square	df	Mean square	F value	P value Prob>F	
Mean vs Total	24.05	1	24.05			
Linear vs Mean	0.023	3	7.783E-003	0.23	0.8758	Suggested
2FI vs Linear	0.097	3	0.032	0.92	0.4645	Suggested
Quadratic vs 2FI	0.33	3	0.11	32.52	0.0002	Aliased
Cubic vs Quadratic	0.023	3	7.783E-003	6.366E+007	< 0.0001	Aliased
Residual	0.000	4	0.000			
Total	24.52	17	1.44			

Table 4: ANOVA for Response Surface Quadratic Model Tailing factor

Souces	Sum of square	df	Mean square	F value	P value Prob>F	
Model	0.45	9	0.049	14.83	0.0009	significant
A-Flow rate	4.500E-004	1	4.500E-004	0.13	0.7243	
B-Buffer PH	0.014	1	0.014	4.33	0.0759	
C-Organic ratio in MP	8.450E-003	1	8.450E-003	2.53	0.1555	
Residual	0.023	7	3.336E-003			
Lack of Fit	0.023	3	7.783E-003			
Pure Error	0.000	4	0.000			

Table 5: Results of system suitability parameters

NAME	RT	AREA	HEIGHT	RESOLUTION	TAILING FACTOR	PLATE COUNT
Voglibose	**2.722**	**4578**	**3741**	**10.10**	**1.02**	**2954**
Metformin	**5.510**	**36189**	**78254**		**1.12**	**9902**

Table 6: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio	Conc. In ppm
Voglibose	68	197	2.90	0.02
Metformin	68	196	2.88	0.30

Table 7: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained(µV)	S/N ratio	Conc. In ppm
Voglibose	68	662	9.74	0.05
Metformin	68	674	9.91	1.05

CONCLUSION

The developed method underwent rigorous validation in accordance with ICH Q2(R1) guidelines. It demonstrated excellent linearity for Metformin (25–125 µg/mL) and Voglibose (0.1–0.5 µg/mL) with correlation coefficients of 0.9998 and 0.9994, respectively. Precision studies showed %RSD values well below 2%, confirming the method's repeatability and intermediate precision. The assay results indicated high accuracy with mean recoveries of 99.4% for Voglibose and 99.6% for Metformin**,** while LOD and LOQ values were well within acceptable limits, indicating high sensitivity. Robustness testing confirmed the method's reliability under minor deliberate variations in analytical conditions. System suitability parameters, including theoretical plate count, tailing factor, and resolution, all complied with acceptance criteria, confirming the method's suitability for routine quality control. Thus, this QbD-based RP-HPLC method offers a scientifically sound, regulatory-compliant, and efficient tool for the simultaneous estimation of Voglibose and Metformin in pharmaceutical dosage forms.

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