https://doi.org/10.48047/AFJBS.6.Si3.2024.2917-2928

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Development of a Stable Indicative Approach for the Simultaneous Quantification of Voglibose and Nateglinide for Combined Dosage form

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ArticleInfo

Volume6, Issue Si3, July2024

Received: 13 May 2024

Accepted: 20 June 2024

Published: 09 July2024
doi:

10.48047/AFJBS.6.Si3.2024.2917-2928

ABSTRACT:

The aim of this study is to develop a comprehensive understanding of Nateglinide (NGL) and Voglibose (VGB), focusing on their pharmaceutical formulation, analytical methods, and therapeutic applications in diabetes management with their different mode of action of the human body. The primary objective is to address critical gaps in current knowledge by exploring the solubility profiles, stability characteristics, and compatibility of NGL and VGB to develop the combined dosage forms. This research aims to develop robust stability-indicating methods for the simultaneous estimation of NGL and VGB, ensuring accuracy and specificity in pharmaceutical formulations. In this paper, mainly focus on the estimation of both of the drug by High Performance Liquid Chromatography technology (mainly RP-HPLC) with estimating the stability of drugs by performing the food degradation analysis like acid hydrolysis, alkaline hydrolysis, oxidative degradation and Thermal degradation. Also performing the recovery studies of the both of the drug with their different percentage level like 80%, 100%, and 120%. By achieving these objectives, the research aims to contribute to advancements in diabetes treatment, pharmaceutical sciences, and patient care outcomes through evidence-based innovations in drug development and formulation technology with formulating the combined drug that are ensuring the stability of the components present in the formulated drug.

Keywords: Voglibose, Nateglinide, Diabetes management, Stability-indicating methods, HPLC.

1. Introduction:

Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose levels due to insulin deficiency, insulin resistance, or both. Effective management of diabetes is crucial to prevent complications such as cardiovascular diseases, neuropathy, retinopathy, and nephropathy. Pharmacological interventions play a significant role in managing blood glucose levels, and among these interventions, Nateglinide and Voglibose are noteworthy for their unique mechanisms of action and therapeutic benefits [(1-5)]. In the realm of pharmaceutical analysis, the development of stability-indicating methods is crucial for ensuring the efficacy, safety, and quality of pharmaceutical products. Stability indicating methods are analytical procedures that accurately and precisely measure active pharmaceutical ingredients (APIs) without interference from degradation products, impurities, or excipients. This is particularly important for combination therapies where multiple drugs are present. Nateglinide and Voglibose are commonly combined in a dosage form to manage postprandial hyperglycemia in type 2 diabetes mellitus. This article details the development of a stability-indicating method for the simultaneous estimation of Nateglinide and Voglibose in a combined dosage form. Nateglinide is a D-phenylalanine derivative and a member of the meglitinide class of oral hypoglycemic agents. It primarily stimulates the pancreatic beta cells to release insulin, thereby reducing postprandial blood glucose levels. Nateglinide acts rapidly and is particularly effective in managing mealtime glucose spikes due to its fast onset of action. This characteristic makes it an ideal choice for controlling postprandial hyperglycemia, a significant contributor to overall glycemic control in diabetes management. Voglibose is an alpha-glucosidase inhibitor, a class of drugs that delay carbohydrate digestion and absorption in the intestine. By inhibiting alpha-glucosidase enzymes in the brush border of the small intestine, voglibose slows down the breakdown of complex carbohydrates into glucose, thereby attenuating postprandial hyperglycemia. This mechanism provides a unique approach to managing blood glucose levels, complementing other oral hypoglycemic agents and insulin.[(2-5)]

a) **Mechanism of Action:** Nateglinide binds to the sulfonylurea receptor on the pancreatic beta cells, triggering the closing of ATP-sensitive potassium channels. This closure leads to cell depolarization, opening of voltage-dependent calcium channels, and subsequent influx of calcium ions. The increased intracellular calcium concentration stimulates the exocytosis of insulin-containing granules, thereby enhancing insulin secretion. Unlike traditional sulfonylureas, nateglinide exhibits a more glucose-dependent insulinotropic effect, reducing the risk of hypoglycemia.[(3-10)]

The primary action of voglibose involves the competitive and reversible inhibition of alpha-glucosidase enzymes, including sucrase, maltase, and isomaltose. By blocking these enzymes, voglibose prevents the rapid conversion of disaccharides and oligosaccharides into monosaccharides, leading to a slower and more gradual increase in postprandial blood glucose levels. This reduction in the rate of glucose absorption helps in mitigating the postprandial glycemic spikes that are common in diabetes patients.[(4-8)]

b) **Clinical Benefits[(25-30)]**:The rapid action and short half-life of nateglinide offer several clinical advantages. It helps achieve better postprandial glucose control without significantly increasing the risk of prolonged hypoglycemia. Nateglinide's effectiveness in combination therapy with other oral hypoglycemic agents or insulin further enhances its utility in comprehensive diabetes management. Additionally, its ability to preserve beta-cell function over time makes it a valuable addition to the therapeutic arsenal against type 2 diabetes mellitus. Voglibose offers several clinical benefits, particularly in the management of postprandial hyperglycemia. Its ability to reduce post-meal glucose excursions is beneficial in achieving overall glycemic control. Voglibose is well-tolerated, with gastrointestinal side

effects being the most common but generally mild and transient. Additionally, voglibose does not cause hypoglycemia when used as monotherapy, making it a safe option for patients with a tendency towards hypoglycemia. Furthermore, voglibose has shown potential in reducing cardiovascular risk factors associated with diabetes, adding to its therapeutic value.[5-8]

c) Comparative Analysis: While both nateglinide and voglibose are effective in managing postprandial blood glucose levels, they differ significantly in their mechanisms of action. Nateglinide works by enhancing insulin secretion, whereas voglibose slows down carbohydrate absorption. These complementary mechanisms allow for their concurrent use in combination therapy, providing a synergistic effect in controlling blood glucose levels. This combination approach can be particularly beneficial for patients who struggle with achieving optimal glycemic control through monotherapy.[6-14]

2. Material and Methods:

The goal of this method development is to establish a robust, accurate, and precise analytical procedure that can simultaneously estimate Nateglinide and Voglibose. High-Performance Liquid Chromatography (HPLC) is the preferred technique due to its sensitivity, specificity, and suitability for stability studies.[7-13]

Chromatographic Conditions[8-12]

1. **Column**: C18 column (250 mm × 4.6 mm, 5 µm particle size) is selected for the separation of the two drugs.
2. **Mobile Phase**: A gradient or isocratic elution using a suitable buffer (e.g., phosphate buffer, pH adjusted to around 3.0-3.5) and an organic solvent (e.g., acetonitrile or methanol) in a ratio that provides optimal separation.
3. **Flow Rate**: Typically set at 1.0 mL/min.
4. **Detection Wavelength**: UV detection at an appropriate wavelength where both drugs exhibit sufficient absorbance (e.g., around 210-220 nm for Nateglinide and 250-260 nm for Voglibose).
5. **Injection Volume**: 20 µL.

Preparation of Standard and Sample Solutions[9-15]

1. **Standard Solution**: Prepare standard solutions of Nateglinide and Voglibose in the mobile phase at appropriate concentrations based on their therapeutic dosage.
2. **Sample Solution**: Accurately weigh and dissolve the combined dosage form containing both drugs in the mobile phase, followed by suitable dilution to achieve the desired concentration within the linearity range of the method.

Method Validation[10-17]: The developed method must be validated according to ICH guidelines for various parameters to ensure its reliability and reproducibility.

1. **Specificity**: Demonstrate that the method can unequivocally assess the analytes in the presence of components such as impurities, degradation products, and excipients.
2. **Linearity**: Evaluate the method's ability to obtain test results proportional to the concentration of the analyte within a given range. Prepare calibration curves for both Nateglinide and Voglibose and determine the correlation coefficient (R^2).
3. **Accuracy**: Assess the closeness of the test results to the true value. Perform recovery studies by spiking known quantities of Nateglinide and Voglibose into the matrix and calculate the percentage recovery.

4. **Precision**: Determine the method's repeatability and reproducibility. Analyze multiple replicates of standard solutions and assess the relative standard deviation (RSD) for intra-day and inter-day precision.
5. **Robustness**: Evaluate the effect of slight deliberate variations in method parameters (e.g., pH, flow rate, column temperature) on the method's performance.
6. **Limit of Detection (LOD) and Limit of Quantitation (LOQ)**: Establish the smallest amount of analyte that can be detected and quantified with acceptable precision and accuracy.
7. **Forced Degradation Studies**: Subject the sample solutions to stress conditions (e.g., acidic, basic, oxidative, thermal, and photolytic) to generate potential degradation products and ensure that the method can separate and quantify the analytes from these degradation products.[12-15]

Application to Stability Studies[17-26]: Apply the validated method to conduct stability studies on the combined dosage form of Nateglinide and Voglibose. Store samples under different conditions (e.g., room temperature, refrigerated, accelerated stability conditions) and analyze them at predetermined intervals to assess any changes in the content and purity of the drugs over time.

Characterization and Identification of Nateglinide (NGL) and Voglibose (VGB)[16-25]

a) Solubility: The solubility of Nateglinide and Voglibose was determined using the I.P. method. The results are presented in Table2.

b) FTIR Spectrum: The IR absorption spectra of Nateglinide and Voglibose were obtained using the KBr pellet method. The spectra are shown in Figure 1.

c) UV-Visible Absorption Maxima: Preparation: 10 mg each of Nateglinide and Voglibose were dissolved in methanol and diluted with 0.1 N HCl to 1000 µg/ml.

Dilution: 1 ml of this stock was diluted to 10 ml with 0.1 N HCl to 100 µg/ml.

Aliquots: Prepared concentrations of 5, 10, 15, 20, and 25 µg/ml.

Measurement: Absorbance was measured at 260 nm using a UV-Visible Spectrophotometer, and a concentration versus absorbance graph was plotted. Chemicals and solvents

Table 1: Chemicals and Solvents Used

S. No.	Chemicals	Manufacturer
1	Nateglinide and Voglibose	Gift sample, Aurobindo Pharma Limited
2	Methanol (AR Grade)	Solvent Merck Ltd., India
3	Acetonitrile (HPLC)	Merck Ltd., India
4	Methanol (HPLC)	Merck Ltd., India
5	Water (HPLC)	Merck Ltd., India

3. Result and Discussion

a) Results of system suitability parameters

The mobile phase saturated the column at 1.0 ml/min. Six replicates of the NGL standard and 2 µg/ml VGB were injected. Peak and column performance reports were recorded for all chromatograms.

Table 2:System Suitability Parameters of NGL

System suitability Parameter	RT	AUC	No. of theoretical plates	Tailing Factor

RC1	3.455	15745.658	2655	1.15
RC2	3.452	15635.658	2568	1.18
RC3	3.453	15669.985	2474	1.15
RC4	3.452	15660.321	2565	1.14
RC5	3.456	15798.854	2498	1.13
RC6	3.453	15674.854	2513	1.14
Mean	3.4535	15697.56	2545.50	1.15
S.D.	0.0016	61.76	65.27	0.02

Table 3: System Suitability Parameters of VGB

System suitabilityParameter	**RT**	**AUC**	**No. of theoretical plates**	**Tailingfactor**
RC1	5.618	5036.647	2585	1.12
RC2	5.617	5040.365	2545	1.15
RC3	5.615	5074.658	2624	1.19
RC4	5.614	5033.145	2574	1.17
RC5	5.619	5074.665	2598	1.14
RC6	5.619	5032.445	2578	1.16
Mean	5.617	5048.65	2584.00	1.16
S.D.	0.0021	20.34	26.28	0.02

b) Results of Validation of developed Method : Response ratio data for Linearity

Table 4: Response Ratio Data for Linearity of NGL

Concentration (□g/ml)	**Mean AUC**	**Response Ratio**
2	3469.761	1734.880
4	6685.493	1671.373
6	9653.683	1608.947
8	12654.429	1581.804
10	15697.555	1569.756
Mean		1633.352
SD		69.013
%RSD		4.225

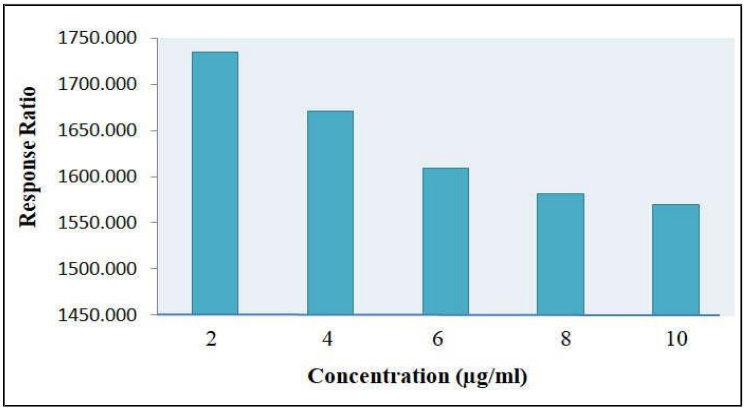

Figure 1:Response ratio curve of NGL

Table 5: Response ration data for linearity of VGB

Concentration (□g/ml)	**Mean AUC**	**Response Ratio**
2	2569.8817	1284.941
4	5048.6542	1262.164
6	7551.1945	1258.532
8	10164.2623	1270.533
10	12575.4695	1257.547
Mean		1266.74331
SD		11.385
%RSD		0.899

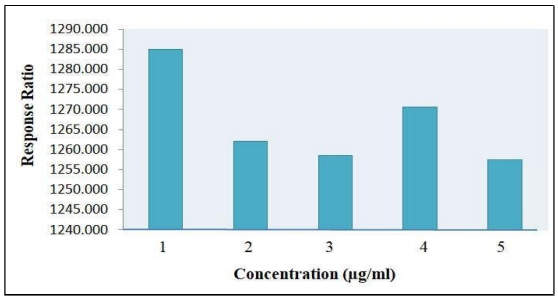

Figure 2: Response Ratio Curve of VGB

c) Results of Specificity

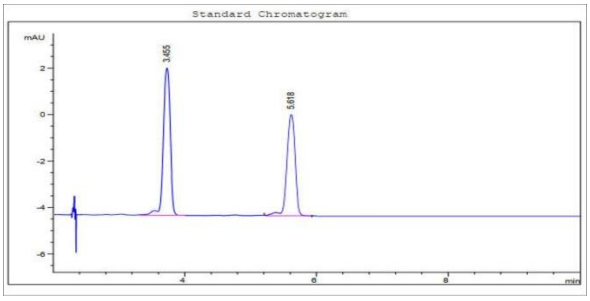

Figure 4: Chromatogram of Both the drug

d) Results of Accuracy

Table 6: Recovery Study of NGL (80% Level)

Conc. of sample (mg)	Amt. Added (mg)	Conc. Found (mg)			% conc. Found			Mean % conc.
		RC1	**RC2**	**RC3**	**RC1**	**RC2**	**RC3**	
2	1.6	1.58	1.62	1.55	98.75	101.25	96.88	98.96
4	3.2	3.18	3.17	3.19	99.38	99.06	99.69	99.38
6	4.8	4.78	4.75	4.79	99.58	98.96	99.79	99.44
							MEAN	99.26
							SD	0.263
							% RSD	0.265

Table 7:Recovery study of NGL (100% Level)

Conc. Of sample(mg)	**Amt.Added(mg)**	**Conc. Found (mg)**			**% conc. Found**			**Mean conc.**
		RC1	**RC2**	**RC3**	**RC1**	**RC2**	**RC3**	
2	2	1.98	1.95	1.87	99.00	97.50	93.50	96.67
4	4	3.74	3.88	3.89	93.50	97.00	97.25	95.92
6	6	5.85	5.95	5.87	97.50	99.17	97.83	98.17
							MEAN	96.92
							SD	1.146
							% RSD	1.182

Table 8: Recovery study of NGL (120% Level)

Conc.of sample(mg)	Amt.Added (mg)	Conc. Found (mg)			% conc. Found			Mean conc.
		RC1	**RC2**	**RC3**	**RC1**	**RC2**	**RC3**	
2	2.4	2.38	2.39	2.38	99.17	99.58	99.17	99.31
4	4.8	4.75	4.65	4.75	98.96	96.88	98.96	98.26
6	7.2	7.15	7.18	7.19	99.31	99.72	99.86	99.63
							MEAN	99.07
							SD	0.714
							% RSL)	0.720

Table 9: Recovery Study of VGB (80% Level)

Conc. Of sample(mg)	**Amt.Added(mg)**	**Conc. Found (mg)**			**% conc. Found**			**Mean conc.**
		RC1	**RC2**	**RC3**	**RC1**	**RC2**	**RC3**	
1	0.8	0.79	0.78	0.81	98.75	97.50	101.25	99.17
2	1.6	1.58	1.59	1.57	98.75	99.38	98.13	98.75
3	2.4	2.39	2.38	2.37	99.58	99.17	98.75	99.17
							Mean	99.03
							SD	0.241
							% RSD	0.243

Table 10: Recovery study of VGB (100% Level)

Conc. Of sample(mg)	**Amt.Added (mg)**	**Conc. Found (mg)**			**% conc. Found**			**Mean conc.**
		RC1	**RC2**	**RC3**	**RC1**	**RC2**	**RC3**	
1	1	0.98	0.99	0.97	98.00	99.00	97.00	98.00
2	2	1.98	1.99	1.95	99.00	99.50	97.50	98.67
3	3	2.98	2.95	2.96	99.33	98.33	98.67	98.78
							MEAN	98.48
							SD	0.421
							% RSD	0.427

Table11:Recovery study of VGB (120% Level)

Conc. Of sample(mg)	**Amt.Added (mg)**	**Conc. Found (mg)**			**% conc. Found**			**Mean conc.**
		RC1	**RC2**	**RC3**	**RC1**	**RC2**	**RC3**	
1	1.2	1.18	1.15	1.19	98.33	95.83	99.17	97.78
2	2.4	2.39	2.38	2.39	99.58	99.17	99.58	99.44

3	3.6	3.58	3.59	3.57	99.44	99.72	99.17	99.44
							MEAN	98.89
							SD	0.962
							% RSD	0.973

e) Results of Precision: Repeatability

Table 12: Repeatability of NGL

Replicate	**Concentration Found (ug/ml)**					
	2	4	6	8	10	
RC1	1.98	3.95	5.88	7.88	9.85	
RC2	1.96	3.85	5.98	7.96	9.65	
RC3	1.98	3.96	5.75	7.85	9.78	
RC4	I .95	3.78	5.65	7.98	9.74	
RC5	1.98	3.93	5.99	7.88	9.65	
MEAN	1.97	3.894	5.85	7.91	9.88	
% MEAN	98.5	97.35	97.50	98.875	98.80	98.205
SD	0.014	0.077	0.148	0.057	0.086	0 076
% RSD	0.014	0.079	o. 152	0.057	0.087	0.078

Table 13: Repeatability of VGB

Replicate	**Concentration Found (ug/ml)**					
	1	2	3	4	5	
RC1	0.95	1.98	2.98	3.98	4.78	
RC2	0.98	1.95	2.85	3.65	4.65	
RC3	0.99	1.78	2.74	3.95	4.78	
RC4	0.96	1.96	2.96	3.92	4.65	
RC5	0.97	1.85	2.87	3.78	4.65	
MEAN	0.97	1.904	2.88	3.856	4.88	
% MEAN	97	95.2	96.00	96.4	97.60	96.440
SD	0.016	0.086	0.096	o. 138	0.071	0.081
% RSD	0.016	0.090	0.100	0.143	0.073	0.085

Intermediate precision: Day to day precision

Table 14:Day-to-Day variation of NGL

Replicate	**Concentration Found (ug/ml)**					
	2	4	6	8	10	
Day 1	1.95	3.85	5.87	7.98	9.98	
Day 2	1.99	3.74	5.65	7.65	9.85	
Day 3	1.85	3.65	5.85	7.95	9.65	
MEAN		3.98	5.96	7.84	9.78	
% MEAN	98.00	99.50	99.33	98.00	97.80	98.527
SD	0.072	0.100	0. 122	0.182	0. 166	0.129
% RSD	0.074	0.101	0.122	0.186	0. 170	0. 131

Table 15: Day-to-day variation of VGB

Replicate	Concentration Found (ug/ml)					
	1	2	3	4	5	
Day I	0.98	1.98	2.85	3.65	4.78	
Day 2	0.96	I .95	2.96	3.85	4.95	
Day 3	0.99	1.85	2.85	3.95	4.65	
MEAN	0.98	1.93	2.89	3.82	4.79	
% MEAN	97.67	96.33	96.22	95.42	95.87	96.301
SD	0.015	0.068	0.064	0.153	0.150	0.090
% RSD	0.016	0.071	0.066	0.160	0.157	0.094

Analyst to Analyst

Table 16: Analyst to analyst variation of NGL

Replicate	Concentration Found (ug/ml)					
	2	4	6	8	10	
Analyst 1	1.95	3.85	5.98	7.96	9.99	
Analyst 2	1.88	3.65	5.78	7.95	9.78	
MEAN	1.92	3.75	5.88	7.96	9.885	
% MEAN	95.75	93.75	98.00	99.44	98.85	97.158
SD	0.049	0.141	0.141	0.007	0.148	0.098
% RSD	0.052	0.151		0.007	0. 150	0. 101

Table 17: Analyst to analyst of VGB

Replicate	Concentration Found (ug/ml)					
	1	2	3	4	5	
Analyst I	0.98	1.98	2.69	3.99	4.78	
Analyst 2	0.96	1.87	2.85	3.87	4.65	
MEAN	0.97		2.77	3.93	4.715	
% MEAN	97.00	96.25	92.33	98.25	94.3	95.627
SD	0.014	0.078	0.113	0.085	0.092	0.076
% RSD	0.015	0.081	o. 123	0.086	0.097	0.080

f) Results of Robustness

Table 18: Robustness of NGL

Replicate	Concentration Found (pg/ml)					MEAN
	2	4	6	8	10	
RC1	1.98	3.85	5.98	7.85	9.85	
RC2	1.85	3.96	5.87	7.96	9.65	
RC3	1.96	3.78	5.65	7.85	9.78	
RC4	1.85	3.65	5.88	7.65	9.65	
RC5	I .93	1.85	5.77	7.78	9.89	
MEAN	1.914	3.418	5.83	7.818	9.97	
% MEAN	95.7	85.45	97.17	97.725	99.70	95.148
SD	0.061	0.884	0.125	14	0.111	0.259
% RSD	0.064	1.034	0.129	0.117	0.112	0.291

Table 19: Robustness of VGB

Replicate	Concentration Found (gg/ml)					MEAN
	1	2	3	4	5	
RC1	0.95	1.85	2.98	3.96	4.85	
RC2	1.02	1.96	2.78	3.85	4.96	
RC3	0.96	1.99	2.96	3.74	4.92	
RC4	0.93	1.87	2.95	3.65	4.88	
RC5	0.99	I .65	2.96	3.99	4.98	
MEAN	0.97	1.864	2.926	3.838	4.918	
% MEAN	97	93.2	97.53	95.95	98.36	96.409
SD	0.035	o. 133	0.082	0.144	0.054	0.090
% RSD	0.036	0.143	0.084	0.150	0.055	0.094

g) Results of Detection Limit and Quantitation Limit

Table 20: LOD and LOQ of NGL and VGB

Name	LOD (□g/ml)	LOQ (□g/ml)
NGL	0.10	0.35
VGB	0.15	0.40

h) Results of Analysis of both the drug in physical mixture

Table21: Result of assay of physical mixture

	NGL	**VGB**
Label Claim (mg)	60mg	0.2mg
% Found (mg)	0.1	0.25
% Assay	0.098	0.24
% RSD	98.00	96.00

i) Results of forced degradation studies

Table22: Results of Forced degradation studies of NGL

Stress conditions	**Drug recovered (%)**	**Drug decomposed (%)**
Standard drug	99.95	0
Acidic hydrolysis	89.85	10.1
Alkaline hydrolysis	84.65	15.3
Oxidative degradation	92.12	7.83
Thermal degradation	96.65	3.3

Table 23: Results of Forced degradation studies of VGB

Stress conditions	**Drug recovered (%)**	**Drug decomposed (%)**
Standard drug	99.5	0
Acidic hydrolysis	85.65	13.85
Alkaline hydrolysis	91.15	8.35
Oxidative degradation	88.87	10.63
Thermal degradation	90.25	9.25

4. Conclusion:

Developing a stability-indicating method for simultaneous estimation of nateglinide (NGL) and voglibose (VGB) in a physical mixture is crucial for quality and stability assessment. Forced degradation studies showed NGL and VGB are susceptible to acidic, alkaline, oxidative, and thermal stress. The method must detect and quantify both drugs amidst degradation products. Appropriate chromatographic conditions (column type, mobile phase, detection wavelength) are selected to ensure resolution, sensitivity, and selectivity. Method validation ensures reliability and specificity. This method is essential for monitoring the stability of NGL and VGB during formulation, storage, and use, ensuring the efficacy and safety of the pharmaceutical products.

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