RESEARCH PAPER

Analytical Method Development and Validation of Glycopyrronium Bromide using RP-HPLC

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ABSTRACT

A simple, sensitive, accurate, and cost-effective RP-HPLC method was developed and validated for the quantitative estimation of Glycopyrronium Bromide in bulk drug and in its pharmaceutical dosage form. Chromatographic separation was carried out on a Nucleosil C18 column (250 × 4.6 mm, 5 µm) using an isocratic mobile phase of Acetonitrile:Methanol (70:30, v/v) at a flow rate of 1.0 mL/min, with detection at 220 nm and an injection volume of 20 µL. The method was validated as per ICH guidelines for system suitability, specificity, linearity, accuracy, precision, LOD, LOQ, and robustness. Glycopyrronium Bromide eluted at a retention time of 2.84 min with an asymmetry factor of 1.27 and a theoretical plate count of 2536.81. The method was linear over 5–30 µg/mL (R^2 = 0.994), with mean recoveries at 50%, 100%, and 150% concentration levels within acceptable limits and %RSD values below 1.0% for precision. LOD and LOQ were found to be 1.221 µg/mL and 3.700 µg/mL, respectively, and assay of Glycolate 1 tablets gave a mean recovery of 99.746%. The proposed method is reliable, precise, and suitable for routine quality control analysis of Glycopyrronium Bromide in bulk and pharmaceutical dosage forms.

Keywords: Glycopyrronium Bromide, RP-HPLC, Method Development, Method Validation, ICH Q2 (R1), Anticholinergic, Antimuscarinic, Quality Control, Pharmaceutical Analysis.

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1. INTRODUCTION:

Gastrointestinal and respiratory disorders linked to excessive cholinergic activity, such as peptic ulcer, irritable bowel syndrome, and chronic obstructive pulmonary disease, affect several hundred million people worldwide [1]. Excessive cholinergic stimulation causes discomfort and can impair a patient's quality of life, and patients with these disorders often show higher rates of related secretory and motility complications [2]. A range of anticholinergic agents acting through different mechanisms has been developed, but achieving selectivity for peripheral over central muscarinic receptors remains a major challenge. This need to minimise central side effects led to the development of a newer class of antimuscarinic agents with higher specificity, potency, and better tolerability.

Glycopyrronium bromide (GBr) was developed to address these limitations. It was approved as an antimuscarinic agent for the management of peptic ulcer disease and as a premedication to reduce salivary, tracheobronchial, and pharyngeal secretions during anaesthesia [3]. Decades of clinical use have confirmed its tolerability and efficacy in patients requiring reduction of gastric and respiratory secretions [4, 5].

Glycopyrronium Bromide is a synthetic quaternary ammonium anticholinergic agent and a derivative of mandelic acid; its IUPAC name is 3-[(2-cyclopentyl-2-hydroxy-2-phenylacetyl)oxy]-1,1-dimethylpyrrolidin-1-ium bromide. The molecule contains a chiral centre arising from the cyclopentylmandelic acid moiety, which gives rise to diastereomers; these diastereomeric impurities are analytically important in drug development [6,7]. Glycopyrronium Bromide is marketed under several brand names, including Glycolate, and is produced by various pharmaceutical manufacturers [3].

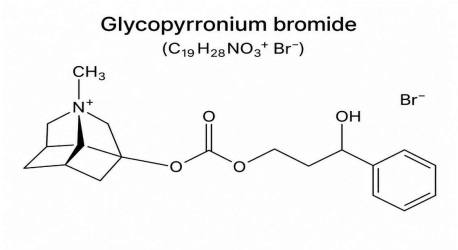

Figure 1: Chemical Structure of Glycopyrronium Bromide

Glycopyrronium Bromide acts as an anticholinergic, binding with high affinity and selectivity to muscarinic acetylcholine receptors (M1, M2, and M3 subtypes). These receptors are widely distributed across smooth muscle, glandular, and cardiac tissue, where they regulate secretory and motor responses following parasympathetic nerve activation. By blocking acetylcholine at these sites, the drug reduces gastric, salivary, and bronchial secretions and can also help control smooth muscle spasm [8, 9]. The quaternary ammonium structure also limits the drug's passage across the blood-brain barrier compared to tertiary amine anticholinergics, which accounts for its predominantly peripheral action and lower incidence of central side effects [8]. Glycopyrronium Bromide binds competitively to muscarinic receptors, blocking acetylcholine and the resulting parasympathetic responses, which reduces glandular secretions, smooth muscle tone, and gastrointestinal motility [9]. It may also reduce gastric acid secretion, which could contribute to its therapeutic effect in peptic ulcer disease, though the extent of this pathway's contribution is still being investigated [8].

Pharmacokinetically, Glycopyrronium Bromide shows poor and variable oral bioavailability owing to its quaternary ammonium structure, with absolute bioavailability typically below 10% [10,14]. Its volume of distribution is relatively small, with high plasma protein binding in some compartments, suggesting limited tissue distribution [10, 11]. The elimination half-life is around 1.5–3 hours, which means some formulations need multiple daily doses. The drug is mainly eliminated unchanged via renal excretion, with a minor contribution from hepatic metabolism, and is cleared primarily through the kidneys and bile [10].

Because closely related diastereomeric impurities can compromise drug safety, precise testing methods are needed to quantify Glycopyrronium Bromide in both the bulk substance and the finished product. HPLC is used throughout drug discovery, development, and manufacture for tasks such as peak purity studies, reaction progress monitoring, formulation analysis, and quality control of the final product [12,13,17]. RP-HPLC, the most common form of HPLC, uses a non-polar C18-bonded stationary phase with a polar aqueous-organic mobile phase, and accounts for roughly 65–80% of pharmaceutical HPLC separations due to its versatility, ease of development, and compatibility with most drug substances [12, 13]. As Glycopyrronium Bromide is a water-soluble, polar quaternary ammonium molecule, it is well suited to RP-HPLC for routine quality control analysis.[15]

2. MATERIALS AND METHOD:

2.1. Chemicals:

Glycopyrronium Bromide API was procured from Harman Finocchem Ltd. Acetonitrile (HPLC grade) and methanol (HPLC grade) were obtained from Merck Ltd., Mumbai, and HPLC-grade water was generated in-house. Glycolate 1 tablets (Intas Pharmaceuticals Ltd., label claim 1 mg per tablet) were procured from a local pharmacy.

2.2. Instrumentation:

All instruments were calibrated and performance-qualified before use. The instruments and equipment used in this study are listed in Table 1.

Table 1: Instruments and Equipment Used in the Study

Instrument / Equipment	Make and Model
HPLC Pump	JASCO PU-2080 Plus Intelligent HPLC Pump
UV-VIS Detector	JASCO UV-2075 UV-VIS Detector
Analytical Column	Nucleosil C18 Column (250 × 4.6 mm, 5 µm particle size)
Sample Injector	Rheodyne injector with 20 µL loop
HPLC Software	Borwin Chromatography Software Version 1.50
UV-Visible Spectrophotometer	Shimadzu Double-beam UV-1780
Analytical Balance	Shimadzu AY-120
Sonicator / Ultrasonic Bath	Prama Solutions
Water Purification System	ELGA Purelab UHQ-II (Conductivity < 0.05 µS/cm)
DSC Instrument	Mettler Toledo STAR SW 19.00
FTIR Spectrometer	JASCO FTIR-4600 A

3. EXPERIMENTAL WORK:

3.1. Preformulation Studies:

Preformulation studies were carried out to characterize the drug substance in terms of physical appearance, solubility, melting point (thermal

behaviour), and spectral properties before method development.

3.1.1. Physical Appearance:

The Glycopyrronium Bromide sample was examined visually for its organoleptic characteristics and found to be a white to off-white, odourless, crystalline powder, free from extraneous matter, consistent with the pharmacopoeial description of the drug substance.

3.1.2. Solubility:

The solubility of Glycopyrronium Bromide was determined by visual solubility testing at room temperature. The drug was freely soluble in water and methanol, soluble in ethanol, sparingly soluble in acetone, and practically insoluble in non-polar organic solvents such as diethyl ether and n-hexane. Given its good solubility in methanol, this solvent was chosen as the diluent for preparing standard and sample solutions.

3.1.3. Melting Point (Differential Scanning Calorimetry):

The melting point of Glycopyrronium Bromide was determined by Differential Scanning Calorimetry (DSC) using a STAR System (Mettler Toledo, SW 19.00). A 5.0000 mg sample was heated from 25°C to 350°C at 10.00°C/min under an air atmosphere. The DSC thermogram showed a sharp endothermic peak with onset, peak, and endset temperatures of 193.10°C, 194.33°C, and 196.98°C, respectively, consistent with reported literature values and confirming the identity and purity of the drug sample.

3.1.4. UV Spectroscopy – Selection of Analytical Wavelength (λmax):

A standard solution of Glycopyrronium Bromide (20 µg/mL) was prepared by diluting the standard stock solution (1000 µg/mL in methanol) with methanol. This solution was scanned over 200–400 nm on a Shimadzu UV-1900 double-beam UV-visible spectrophotometer, using methanol as the blank. The wavelength of maximum absorbance (λmax) was found to be 220 nm.

3.1.5. Fourier Transform Infrared (FTIR) Spectroscopy:

The FTIR spectrum of Glycopyrronium Bromide was recorded on a JASCO FT/IR-4600 A spectrophotometer fitted with an ATR PRO ONE accessory over 4000–650 cm^{-1}. The spectrum showed characteristic absorption bands at 3329.5 cm^{-1} (O–H stretching), 2961.16 cm^{-1} (C–H stretching), 1736.58 cm^{-1} (C=O stretching), 1371.14 cm^{-1} (C–N stretching), and 1227.47–1032.69 cm^{-1} (C–O stretching), confirming the presence of the expected functional groups and establishing the identity of the drug sample.

3.2. Preparation of Standard Solutions:

3.2.1. Mobile Phase Preparation:

The mobile phase was prepared by mixing **Acetonitrile and Methanol in the ratio of 70:30 v/v**. Both solvents were of HPLC grade and were filtered separately through a **0.45 µm membrane filter** under vacuum to remove particulate matter. The filtered solvents were then mixed in the required proportion and degassed by sonication for **15 minutes** prior to use. The prepared mobile phase was used throughout the chromatographic analysis.

3.2.2. Preparation of Standard Stock Solution:

An accurately weighed **10 mg of Glycopyrronium Bromide** was transferred into a **10 mL volumetric flask** and dissolved in methanol. The volume was then made up to the mark with methanol to obtain a **standard stock solution containing 1000 µg/mL of Glycopyrronium Bromide**. From this standard stock solution, a **working standard solution (100 µg/mL)** was prepared by transferring **2.5 mL** into a **25 mL volumetric flask** and diluting to volume with methanol. Further dilutions were prepared from the working standard solution using methanol as diluent to obtain concentrations in the range of **5–30 µg/mL** for method development and validation studies.

3.2.3. Preparation of Working Standard Solution:

An aliquot of **2.5 mL** was withdrawn from the standard stock solution of **Glycopyrronium Bromide (1000 µg/mL)** and transferred into a **25 mL volumetric flask**. The volume was made up to the mark with **methanol** to obtain a working standard solution containing **100 µg/mL** of Glycopyrronium Bromide. Further dilutions were prepared to obtain concentrations of **5–30 µg/mL**. The **10 µg/mL solution** was used for system suitability testing, assay, precision, and other validation parameters.

3.2.4. Preparation of Sample Solution (Tablet Assay):

Twenty tablets of **Glycolate 1 Tablet** were accurately weighed and the average tablet weight was calculated. The tablets were finely powdered, and a quantity of powder equivalent to **10 mg of Glycopyrronium Bromide** was transferred into a **10 mL volumetric flask**. The drug was dissolved in methanol with sonication, and the volume was made up to the mark with methanol to obtain a sample stock solution containing **1000 µg/mL** of Glycopyrronium Bromide. The resulting solution was filtered through **Whatman filter paper No. 41**. An appropriate aliquot of the filtrate was further diluted with methanol to obtain a final concentration of **10 µg/mL**. The prepared sample solution was used for assay determination of Glycopyrronium Bromide in the marketed formulation.

3.3. Chromatographic Method Development and Optimization:

Chromatographic conditions for Glycopyrronium Bromide were optimized by testing various mobile phase compositions on a Nucleosil C18 column (250 mm × 4.6 mm, 5 µm). The optimized conditions,

Acetonitrile:Methanol (70:30, v/v) at a flow rate of 1.0 mL/min with detection at 220 nm, gave a sharp, symmetrical peak at a retention time of approximately 2.84 min.

3.4. Analytical Method Validation:

The developed RP-HPLC method was validated as per ICH Harmonized Guideline Q2 (R1) Validation of Analytical Procedures. The following validation parameters were examined: system suitability, specificity, linearity and range, accuracy, precision (intraday and interday), limit of detection (LOD), limit of quantification (LOQ), and robustness.[14]

4. RESULTS AND DISCUSSION:

4.1. Physical Appearance:

The Glycopyrronium Bromide bulk drug was visually inspected and was found to be a **white crystalline powder**. The observed appearance was consistent with the description reported in the literature and confirms the identity of the procured drug substance.

4.2. Solubility Profile:

The solubility of Glycopyrronium Bromide was assessed in several solvents during method development. The drug was **freely soluble in methanol**, and was therefore selected as the solvent for preparing standard and sample solutions throughout the study. This solubility behaviour supported development of a simple, reproducible RP-HPLC method.

Table 2. Solubility Profile of Glycopyrronium Bromide

Solvent	Solubility Observed
Methanol	Freely soluble
Water	Freely soluble
Ethanol	Soluble
Acetonitrile	Sparingly soluble
Acetone	Sparingly soluble
Diethyl ether	Practically insoluble

4.3. Differential Scanning Calorimetry (DSC):

The DSC thermogram of Glycopyrronium Bromide showed a sharp endothermic peak with an onset temperature of 193.10°C and a peak temperature of 194.33°C, corresponding to its melting point. This single, well-defined melting peak confirmed the crystalline nature and purity of the drug sample, with an enthalpy of fusion of −41.88 J/g. The DSC thermogram is shown in Figure 2.

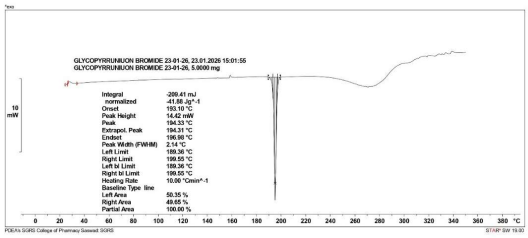

Figure 2: DSC Thermogram of Glycopyrronium Bromide

4.4. FTIR Spectroscopy:

The FTIR spectrum of Glycopyrronium Bromide was recorded on a JASCO FTIR-4600 spectrophotometer in ATR mode over 4000–650 cm^{-1}, showing characteristic absorption bands corresponding to the functional groups present in the drug molecule. These peaks matched the reported structural features of Glycopyrronium Bromide, confirming the identity and purity of the drug substance. The bands are summarized in Table 3, and the spectrum is shown in Figure 3.

Table 3. FTIR Spectral Interpretation of Glycopyrronium Bromide

Sr. No.	Functional Group	Standard IR Range (cm^{-1})	Observed IR Peak (cm^{-1})
1	O–H Stretching (Alcohol group)	3600–3200	3329.50
2	C–H Stretching (Aliphatic CH_2/CH_3)	3000–2850	2961.16
3	C=O Stretching (Ester Carbonyl)	1750–1700	1736.58
4	CH_2 Bending / CH_3 Deformation	1500–1400	1480.10, 1455.99
5	C–N Stretching (Quaternary ammonium)	1380–1200	1371.14, 1227.47
6	C–O Stretching (Ester group)	1250–1000	1181.19, 1167.69, 1118.51, 1093.44, 1067.41, 1032.69

7	Fingerprint Region Vibrations	1000–650	997.02, 955.56, 941.09, 861.06, 741.50, 700.03, 667.25

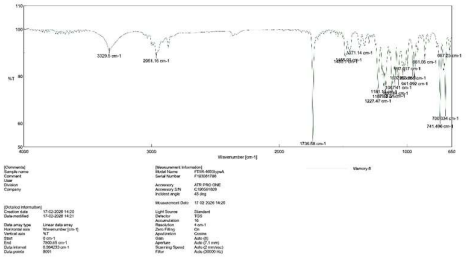

Figure 3: FTIR Spectrum of Glycopyrronium Bromide

4.5. UV Spectroscopic Analysis:

A standard solution of **Glycopyrronium Bromide (20 µg/mL)** was prepared in methanol and scanned in the wavelength range of **200–400 nm** using a **Shimadzu UV-1780 double-beam UV-Visible spectrophotometer**. The UV absorption spectrum exhibited a characteristic maximum absorbance (λmax) at **220 nm** [16]. Therefore, **220 nm** was selected as the analytical wavelength for the RP-HPLC method development and validation studies. The UV spectrum of Glycopyrronium Bromide is shown in Figure 4.

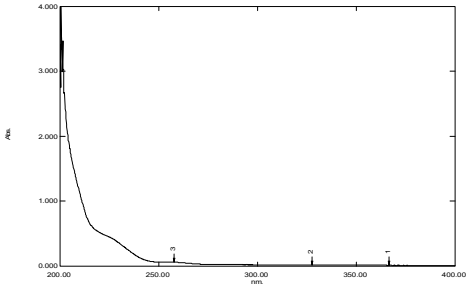

Figure 4: UV Absorption Spectrum of Glycopyrronium Bromide (λmax = 220 nm)

4.6. Optimized Chromatographic Conditions for Glycopyrronium Bromide:

The final optimized chromatographic conditions for the determination of Glycopyrronium Bromide are presented in Table 4.

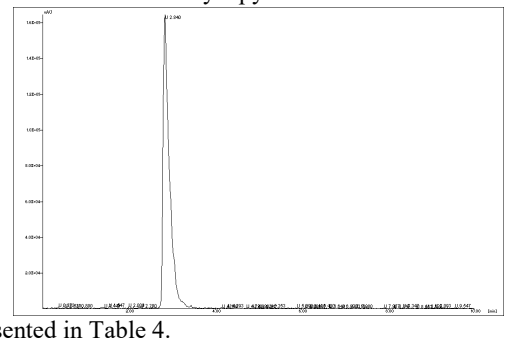

Table 4. Optimized Chromatographic Conditions for Glycopyrronium Bromide

Chromatographic Parameter	Optimized Conditi
Column	Nucleosil C18 Colu
Mobile Phase	Acetonitrile : Metha
Flow Rate	1.0 mL/min
Detection Wavelength	220 nm
Injection Volume	20 µL
Column Temperature	Ambient (25 °C)
Retention Time (Glycopyrronium Bromide)	2.84 min
Run Time	10 min

4.7. System Suitability:

System suitability testing was performed by injecting the standard solution of Glycopyrronium Bromide (10 µg/mL) under the optimized chromatographic conditions. The chromatogram showed a sharp, symmetrical peak at a retention time of 2.84 min, with a theoretical plate count of 2536.81 and an asymmetry factor of 1.27. The obtained values were within the acceptable limits prescribed by ICH guidelines (theoretical plates > 2000 and asymmetry factor between 0.8 and 2.0), confirming that the chromatographic system was suitable for the intended analysis.

Table 5: System Suitability Parameters of the Developed RP-HPLC Method

Parameter	Obtained values
Concentration (µg/ml)	10 (µg/ml)
RT (min)	2.84
Asymmetry	1.27
Theoretical Plates (N)	2536.81

4.8. Specificity:

Specificity is the ability of an analytical method to unequivocally assess the analyte in the presence of components that could reasonably be present, such as impurities, degradation products, excipients, or other matrix components. To evaluate specificity, the blank solution (methanol) was checked for interference at the retention time of Glycopyrronium Bromide. The blank chromatogram showed no interfering peaks at the retention time of the analyte (2.84 min), confirming that the method is specific for Glycopyrronium Bromide.

Figure 5: Chromatogram of Glycopyrronium Bromide (10 µg/mL; RT: 2.84 min)

4.9. Linearity and Range:

Linearity is the ability of an analytical procedure to give results that are directly proportional to the concentration of analyte over a given range. Range is the interval between the upper and lower concentration levels over which the method has been shown to give acceptable precision, accuracy, and linearity.

The linearity of the developed RP-HPLC method for Glycopyrronium Bromide was established by preparing six concentrations (5, 10, 15, 20, 25, and 30 µg/mL) from the working standard solution and analyzing in six replicates. The peak area for each concentration was recorded and a calibration curve was plotted. The regression equation and correlation coefficient (R^2) were calculated. The results are summarized in Table 6.

Table 6: Linearity of Glycopyrronium Bromide

Sr. No.	5	10	15	20	25	30
1	621835.56	708270.81	827673.96	997150.48	1103835.64	1211721.92
2	636783.95	705445.84	852692.78	974551.80	1109895.22	1210556.11
3	636408.65	706583.86	831569.17	979246.42	1087592.02	1187652.96
4	622589.02	719060.43	812626.20	969868.87	1101150.81	1225411.70
5	627540.52	707164.00	821290.91	945593.18	1115726.22	1197310.22
6	636553.87	698217.60	820731.18	981061.62	1085466.00	1216833.17
AVG	630285.260	707457.088	827764.033	974578.725	1100610.982	1208247.676
SD	7171.994	6716.570	13830.732	16944.878	12035.125	13634.399
%RSD	1.138	0.949	1.671	1.739	1.093	1.128

The calibration curve for Glycopyrronium Bromide exhibited good linearity over the concentration range of **5–30 µg/mL**. The regression equation obtained was: **y = 24091.93x + 486548.45** with a correlation coefficient (R^2) of **0.9940**. The %RSD values for all concentration levels were below 2%, indicating acceptable repeatability of the analytical response. These results demonstrate that the developed RP-HPLC method provides a linear response over the studied concentration range and is suitable for quantitative analysis of Glycopyrronium Bromide.

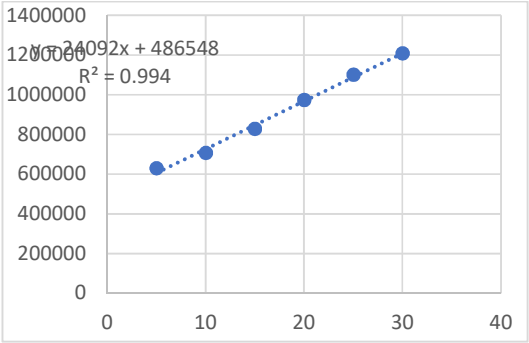

Figure 6: Calibration Curve of Glycopyrronium Bromide (5–30 µg/mL)

4.10. Accuracy:

Accuracy expresses the closeness of agreement between the value accepted as a true value and the value found using the analytical method. The accuracy of the developed RP-HPLC method for Glycopyrronium Bromide was assessed by the standard addition method at three spiking levels — 50%, 100%, and 150% of the target concentration. The study was performed in triplicate, and the results were expressed as percentage recovery and percentage relative standard deviation (%RSD). The results are summarized in Table 7.

Table 7: Results of Accuracy Studies

Spiked Level (%)	Conc. of Drug added (µg/ml)	Amount of Drug added (µg/ml)	Area	Amount recovered (µg/ml)	% Recovery	Mean ± %RSD
50	10	5	849859.51	15.080	100.534	100.233 ± 0.405
	10	5	849348.88	15.059	100.393	
	10	5	847104.53	14.966	99.772	
100	10	10	966721.98	19.931	99.654	100.098 ± 0.393
	10	10	970339.11	20.081	100.405	
	10	10	969514.36	20.047	100.234	
150	10	15	1090355.21	25.063	100.250	100.169 ± 0.396
	10	15	1087269.73	24.934	99.738	
	10	15	1091978.80	25.130	100.520	

The percentage recovery values obtained at 50%, 100%, and 150% spiking levels were 100.233%, 100.098%, and 100.169%, respectively. The %RSD

values at all concentration levels were less than 2%, indicating excellent accuracy and reproducibility of the developed method. The recovery results were within the acceptable limits of 98–102%, demonstrating that the method is accurate and suitable for the quantitative determination of Glycopyrronium Bromide in pharmaceutical formulations.

4.11. Precision:

Precision expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. The precision of the developed RP-HPLC method for Glycopyrronium Bromide was evaluated in terms of intraday and interday precision at three concentration levels (10, 20, and 30 µg/mL). The results were expressed as percentage recovery and percentage relative standard deviation (%RSD). The acceptance criterion for precision was %RSD not more than 2%. The results obtained are summarized in Table 8 and Table 9.

Table 9: Interday Precision Studies

Theoretical Conc (µg/ml)	Peak Area	Amount Recovered (µg/ml)	%Recovery	Mean	SD	%RSD
10	727160.82	9.987	99.872			
10	728287.31	10.034	100.340	99.895	0.435	0.435
10	726195.05	9.947	99.472			
20	967195.59	19.951	99.753			
20	967039.48	19.944	99.720	99.968	0.402	0.402
20	970470.57	20.086	100.432			
30	1212684.40	30.140	100.467			
30	1211623.44	30.096	100.320	100.257	0.249	0.248
30	1209179.00	29.995	99.982			

The developed RP-HPLC method demonstrated excellent precision for the determination of Glycopyrronium Bromide. The %RSD values obtained for intraday precision were 0.348%, 0.618%, and 0.462% at concentrations of 10, 20, and 30 µg/mL, respectively. Similarly, the %RSD values for interday precision were 0.435%, 0.402%, and 0.248% at the corresponding concentration levels. Since all %RSD values were well below the acceptance limit of 2%, the method was found to be precise and reproducible for routine quantitative analysis of Glycopyrronium Bromide.

4.12. Limit of Detection (LOD) and Limit of Quantification (LOQ):

The limit of detection (LOD) is the lowest concentration detectable by the analytical method, but not necessarily quantifiable with accuracy. The limit of quantification (LOQ) is the lowest concentration that can be quantified with acceptable accuracy and precision. LOD and LOQ were calculated using the following formulae based on the calibration curve data:

Table 8: Intraday Precision Studies

Theoretical Conc (µg/ml)	Peak Area	Amount Recovered (µg/ml)	%Recovery	Mean	SD	%RSD
10	726887.16	9.976	99.759			
10	726425.35	9.957	99.567	99.856	0.347	0.348
10	728049.20	10.024	100.241			
20	966721.98	19.931	99.654			
20	972268.21	20.161	100.805	100.099	0.618	0.618
20	967605.86	19.968	99.838			
30	1211721.92	30.100	100.334			
30	1212977.22	30.152	100.508	100.158	0.463	0.462
30	1206655.40	29.890	99.633			

LOD = 3.3 × σ/S **LOQ = 10 × σ/S**

Where σ = standard deviation of the y-intercept and S = slope of the calibration curve. The results are summarized in Table 10.

Table 10: Results of LOD and LOQ Studies of Glycopyrronium Bromide

		S.D of Y-intercept (*σ*)	Slope (m)	Concentration (µg/ml)
LOD	3.3 (*σ*/*S*)	8913.806	24091.934	1.221
LOQ	10 (*σ*/*S*)	8913.806	24091.934	3.700

The LOD and LOQ were found to be 1.221 µg/mL and 3.700 µg/mL, respectively, indicating adequate sensitivity of the developed RP-HPLC method for the determination of Glycopyrronium Bromide.

4.13. Assay:

Assay of Glycopyrronium Bromide was performed using the marketed formulation Glycolate 1 tablets (Intas Pharmaceuticals Ltd., label claim 1 mg per tablet). Tablet powder equivalent to 10 mg of Glycopyrronium Bromide was dissolved in methanol, filtered through Whatman filter paper No. 41, and diluted appropriately to obtain a working concentration of 10 µg/mL. Six replicates were evaluated. The results are summarized in Table 11.

Table 11: Assay Results of Glycopyrronium Bromide Commercial Tablet

Sr. No	Peak Area	Amount Recovered	%Recovery
1	727610.43	10.006	100.059
2	727145.30	9.987	99.866
3	728049.20	10.024	100.241
4	722110.82	9.778	97.776
5	727941.31	10.020	100.196
6	728272.64	10.033	100.334
AVG	726854.950	9.975	99.746
SD	2357.034	0.098	0.978
% RSD	0.324	0.981	0.981

(Glycolate 1)

The mean assay value of Glycopyrronium Bromide in Glycolate 1 tablets was found to be **99.746 ± 0.981%**, which is within the acceptance limit of 98–102%. This confirms that the method is accurate and suitable for quantitative analysis of Glycopyrronium Bromide in its pharmaceutical dosage form without interference from excipients.

4.14. Robustness:

Robustness is a measure of the capacity of the method to remain unaffected by small but deliberate variations in method parameters. The acceptance criterion is no significant change in results. Robustness was evaluated by deliberately varying the mobile phase ratio (±2 mL: ACN:MeOH 72:28 and 68:32 v/v), flow rate (±0.1 mL/min: 0.9 and 1.1 mL/min), and detection wavelength (±1 nm: 219 and 221 nm), while keeping all other parameters constant. The results are summarized in Table 12.

Table 12: Results of Robustness Study

	FLOW RATE VARIATION (± 0.1 ml/min)		
	0.9	1	1.1
	705053.06	718186.60	714830.67
	701957.38	728323.87	719892.95
	714696.29	732700.03	707992.64
AVG	707235.574	726403.498	714238.752
SD	6643.980	7444.847	5972.193
%RSD	0.939	1.025	0.836
	WAVELENGTH VARIATION (± 1 nm)		
	219	220	221
	704546.06	733264.78	714629.89
	707556.38	718987.79	709712.95
	689346.29	726748.86	728272.64
AVG	700482.907	726333.810	717538.494
SD	9761.340	7147.541	9615.640
%RSD	1.394	0.984	1.340
	MOBILE PHASE VARIATION (± 2 ml)		
	72:28 v/v	70:30 v/v	68:32 v/v
	709565.36	732751.70	714830.67
	701669.80	713053.87	713816.50
	704556.29	716679.34	697919.57
AVG	705263.812	720828.299	708855.579
SD	3995.048	10483.872	9484.431
%RSD	0.566	1.454	1.338

All %RSD values obtained during robustness testing were below 2.0%, demonstrating that small deliberate variations in flow rate, detection wavelength, and mobile phase composition did not significantly affect the analytical performance of the method. These results confirm the robustness of the developed RP-HPLC method.

5. CONCLUSION:

A simple, rapid, accurate, and economical RP-HPLC method was successfully developed and validated for the quantitative estimation of Glycopyrronium Bromide in bulk drug and tablet dosage form using a Nucleosil C18 column (250 × 4.6 mm, 5.0 µm) with an isocratic mobile phase consisting of Acetonitrile:Methanol (70:30 v/v) at a flow rate of 1.0 mL/min and UV detection at 220 nm. Preformulation studies including DSC and FTIR confirmed the identity and purity of the drug substance. The DSC thermogram exhibited a sharp endothermic peak at 194.33°C, indicating the crystalline nature of Glycopyrronium Bromide, while FTIR analysis confirmed the presence of characteristic functional groups corresponding to the drug molecule.

The optimized chromatographic method produced a sharp and symmetrical peak of Glycopyrronium Bromide at a retention time of 2.84 min with an asymmetry factor of 1.27 and a theoretical plate count of 2536.81. Validation of the method was carried out in accordance with ICH Q2(R1) guidelines. The method exhibited good linearity over the concentration range of 5–30 µg/mL with a regression equation of $y = 24092x + 486548$ and a correlation coefficient (R^2) of 0.994. Accuracy studies demonstrated excellent recovery in the range of 100.098–100.233%, while precision studies showed %RSD values below 2% for both intraday and interday analyses, confirming satisfactory repeatability and reproducibility of the method.

The limits of detection (LOD) and quantitation (LOQ) were found to be 1.221 µg/mL and 3.700 µg/mL, respectively, indicating adequate sensitivity of the developed method. The robustness study demonstrated that small deliberate variations in chromatographic parameters such as flow rate, mobile phase composition, and detection wavelength did not significantly affect the analytical performance of the method.

Assay of the marketed formulation, Glycolate 1 Tablet, yielded a mean assay value of 99.746 ± 0.981%, confirming the suitability of the method for routine quantitative analysis without interference from formulation excipients.

Therefore, the developed RP-HPLC method is simple, precise, accurate, robust, and suitable for routine quality control analysis of Glycopyrronium Bromide in both bulk drug and pharmaceutical dosage forms.

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