Stability Indicating RP-UPLC Method for Quantification of Glycopyrrolate, Methylparaben and Propylparaben Assay in Liquid Oral Formulation

Sreenivas Pippalla[1]* , Arjuna Rao Nekkalapudi[2], Suresh Babu Jillellamudi[1]

[1]Sikkim Professional University, Gangtok, India
[2]Asphar Research Labs Pvt. Ltd., Hyderabad, India
Email: *sreenivas.pippala@gmail.com

Abstract

Stability indicating RP-UPLC technique was developed for the simultaneous quantification of glycopyrrolate, methylparaben, and propylparaben in glycopyrrolate oral solution. The method was established by using gradient UPLC and a Waters Acquity UPLC BEH C18, 100 mm 2.1 mm, i.d 1.7 µm particle size column with a gradient program of mobile phase A and mobile phase B with a flow rate of 0.25 mL/minute, UV wavelength detection at 222 nm, column temperature of 40°C, injection volume of 2 µL, mobile phase A contains 0.05% trifluoro acetic acid in water and Acetonitrile (90:10) v/v and mobile phase-B contains 0.05% trifluoro acetic acid in water and Acetonitrile (10:90) v/v. The current research describes a single UPLC method for developing an assay method for Glycopyrrolate Oral solution that includes Glycopyrrolate (Active), Methylparaben (Preservative), and Propylparaben (Preservative). The assay method was validated in accordance with ICH guidelines. The retention times of glycopyrrolate, methyl paraben and propylparaben were 6.051 min, 3.458 min and 8.095 min, respectively. Linearity range of glycopyrrolate, methyl paraben and propylparaben were in the range of 4 - 32 µg per mL, 35 - 290 µg per mL and 4 - 32 µg per mL, respectively. Recovery of glycopyrrolate, methylparaben and propylparaben ranged from 100.1% to 98.9%, 100.2% - 100.8%, and 100.2% - 100.8%. Validation of analytical method demonstrated that the method is suitable, specific, linear, accurate, precise, rugged and stability indicating for estimating three components in the pharmaceutical dosage form.

Keywords

Glycopyrrolate Oral Solution, UPLC, Assay, Methylparaben, Propylparaben

1. Introduction

Glycopyrrolate (Figure 1) is a white crystalline powder with a molecular weight of 393.3 that is soluble in water. Glycopyrrolate is a synthetic anticholinergic agent, and Glycopyrrolate oral solution is an anticholinergic used to treat long-term severe drooling in patients aged 3 to 16 years with neurologic conditions that cause drooling. Glycopyrrolate oral solution has a maximum daily dose of 9 mg (45 mL drug product) per day.

P-Hydroxybenzoic esters, Methylparaben (Figure 2) and Propylparaben (Figure 3) are synthetic chemicals that are regularly used as preservatives in the pharma industry, cosmetic and food products due to their effective antifungal and antimicrobial properties.

Glycopyrrolate oral solution is a clear to pale-yellow solution with a cherry flavor. Each solution contains 0.05 percent weight per milliliter of Methylparaben NF, 0.01 percent weight per milliliter of Propylparaben, NF, and 70% weight per volume of Sorbitol, 8% weight per volume of Glycerine, 5% weight per volume of Propylene glycol, and 0.3 percent weight per volume of Sodium citrate, 0.08 percent weight per volume saccharin sodium, 0.05 percent weight per volume flavor, and water up to 100% weight per volume.

Methylparaben and Propylparaben preservatives were present in the Glycopyrrolate oral solution. Before being released to the market, each drug product is subjected to a preservative assay to determine its initial and long-term stability. There have been no articles or methods published for the simultaneous estimation of active (glycopyrrolate) and preservatives (methylparaben and propylparaben) in Glycopyrrolate Liquid Oral Formulations. This method is quantifying three components simultaneously within a shorter run time, and it is a cost-effective and time-saving method for QC commercial testing. Liquid Chromatography-Tandem Mass Spectroscopy Method development and Validation of Pyrrolidinium, 3-hydroxy-1,1-dimethyl-, bromide (1:1) Impurity in Glycopyrrolate Oral Solution [1], the article was published with a runtime of 5 minutes, the articles explore only glycopyrrolate and its metabolite impurity, and not discussed about preservatives. Moreover, HPLC-MS/MS is not feasible at QC for routine testing. Utilizing various signal processing methods for ratio spectra, and simultaneous spectrophotometric detection of indacaterol and glycopyrronium in a newly approved pharmaceutical formulation [2], the spectroscopic method was published only for quantification of indacaterol and glycopyrrolate and the article, not explored preservatives. The development, validation, and forced degradation of an analytical method for the simultaneous evaluation of formoterol fumarate and glycopyrrolate in bulk drugs using the HPLC method [3], The
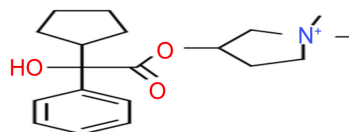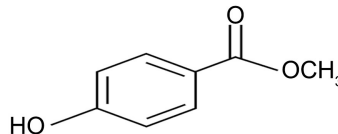
Figure 1. Chemical structure of Glycopyrrolate.
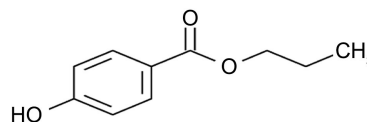

Figure 2. Methylparaben Chemical structure.

Figure 3. Propylparaben Chemical structure.

methylparaben and propylparaben components of the UHPLC technique were not separated from the analysis of glycopyrrolate and formoterol fumarate in bulk drugs and drug products. Benzyl alcohol and glycopyrrolate in glycopyrrolate injection estimation using reverse phase stability indicating HPLC method [4], Only benzyl alcohol and glycopyrrolate were explored by the HPLC method, which was published with a run duration of 12 minutes. Methylparaben and propylparaben were not separated using this approach. Formoterol Fumarate and Glycopyrrolate Quantification in Combination by Stability Indicating RP-HPLC Method with Photodiode Array Detector [5], The HPLC approach was solely described in relation to the measurement of formoterol fumarate and glycopyrrolate, not about parabens. Using ion-pair HPLC, (R, R)-glcopyrronium bromide (glycopyrrolate) and its associated impurities were determined [6], the article was published with a 60-minute run time, methylparaben and propylparaben were not examined in the article, it focused primarily on glycopyrrolate and its related contaminants. Development and validation of an RP-HPLC method for estimating glycopyrrolate in bulk and tablet dosage forms [7], the published method was with a 10-minute run duration and a 40% organic solvent, but the parabens (methylparaben and propylparaben) could not be separated in this method. RP-HPLC method for estimating glycopyrrolate and neostigmine in bulk and tablet dosage forms was created and validated [8], the method was explored in bulk drugs and tablet dosage forms and it was found to be ineffective for Glycopyrrolate Oral solution due to parabens. Analytical method validation of HPLC method for assay of the anticholinergic drug in the parenteral formulation [9], the method was published to estimate Glycopyrrolate and Neostigmine, not for preservative estimation. Simultaneous Estimation of Glycopyrrolate and Formoterol Fumarate in its Bulk and Pharmaceutical Rota Caps dosage form by using RP-UPLC [10], the article was published with 6 minutes runtime and it is unable to detect methylparaben and propylparaben. A novel stability-indicating

RP-HPLC method was created and validated for the simultaneous measurement of halometasone, flusidic acid, methylparaben, and propylparaben [11], the method has been published for simultaneous estimation of parabens, this article is unable to detect glycopyrrolate. For the coeval estimation of parabens (methylparaben and propylparaben), several research articles have been published [12]-[18], which are not disclosed or discussed Glycopyrrolate. In all of the articles, only the assay of methylparaben and propylparaben is proven to utilize the assay methods of HPLC, UV/PDA, and UPLC. In this research work, method development and validation were referred to [19] [20] [21]. In the quality control division, it is essential to measure the active compounds and their preservatives in pharmaceutical products. For the reasons mentioned above, a unique UPLC approach was developed and validated for the analysis of glycopyrrolate liquid oral solution.

2. Experimental

2.1. Reagents and Chemicals

Harman Finochem provided the reference standard for glycopyrrolate. On the market, a glycopyrrolate oral solution was purchased. Merck supplied the water, TFA, and acetonitrile, whereas Sigma supplied the methylparaben and propylparaben.

2.2. Instrumentation

The Ultra-High-Performance Liquid Chromatography, which consists of a pump, an injection module with an autosampler, and a UV detector, was utilised for method development and validation. Empower software was utilised to analyse the data.

2.3. Chromatographic Method Conditions

Using a chromatographic separation method, the medication and preservatives were separated using an Acquity UPLC BEH C18, 100 mm × 2.1 mm, 1.7 μm column. 0.05% TFA in water and Acetonitrile in a ratio of (90:10) v/v were used in mobile phase A, while 0.05% TFA in water and Acetonitrile in a ratio of (90:10) v/v were used in the mobile phase B. For active ingredients (glycopyrrolate) and preservatives (methylparaben and propylparaben), the gradient method (T/percent B) was finalised as 0.0/17, 2.0/17, 4.0/33, 9.0/33, 11.0/80, 12.0/17, and 15.0/17 with wavelength detection at 222 nm, injection volume of 2 μL, and column temperature of 40°C.

2.4. Standard and Samples Preparation

As a diluent, water, acetonitrile, and methanol are employed in a ratio of 60:30:10 v/v/v for the Standard, Placebo, and Sample solutions. The reference standard for glycopyrrolate, methylparaben, and propylparaben was used to create the standard solution at concentrations of 20 μg/mL for glycopyrrolate, 180 μg/mL for

methylparaben, and 20 µg/mL for propylparaben. A test sample and a placebo solution were produced by weighing around 5.8 g of glycopyrrolate oral solution and placebo solution into a separate 50 mL volumetric flask. Vertex with 35 mL of diluent for 5 minutes, then dilute to volume with diluent and completely combine both solutions.

2.5. Method Validation Procedure

The proposed RP-UPLC technique for the measurement of glycopyrrolate, methylparaben, and propylparaben was verified in accordance with the requirements of the International Conference on Harmonization (ICH).

2.5.1. System Suitability

As a part of the analytical test process, a standard solution was prepared and injected into the chromatographic device six times. System acceptability factors such as tailing factor, theoretical plates, and percent RSD were assessed in order to find the best strategy.

2.5.2. Specificity

Specificity tests are performed on the analytical solutions such as diluent solution, placebo solution, standard solution, and sample solutions to look for any interferences. The approach's specificity was validated because blank and placebo had no effect on the standard. The chromatograms (**Figure 4**) demonstrated that, under ideal chromatographic circumstances, Methylparaben, Propylparaben, and Glycopyrrolate were eluted at retention times of 3.45 min, 8.1 min, and 6.1 min, respectively in standard solution, with a complete runtime of 15 minutes.

(a)

(b)
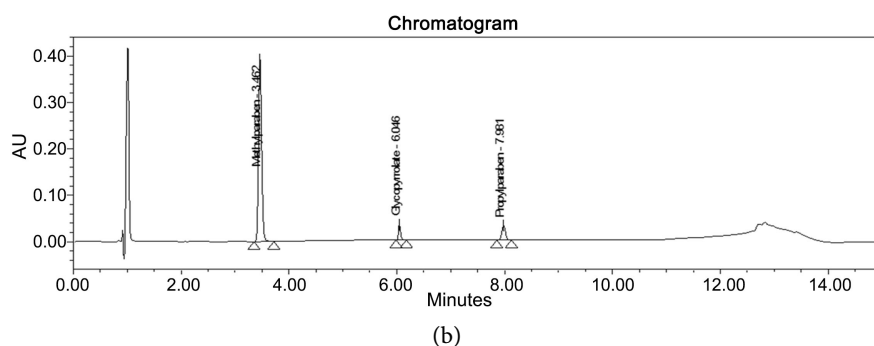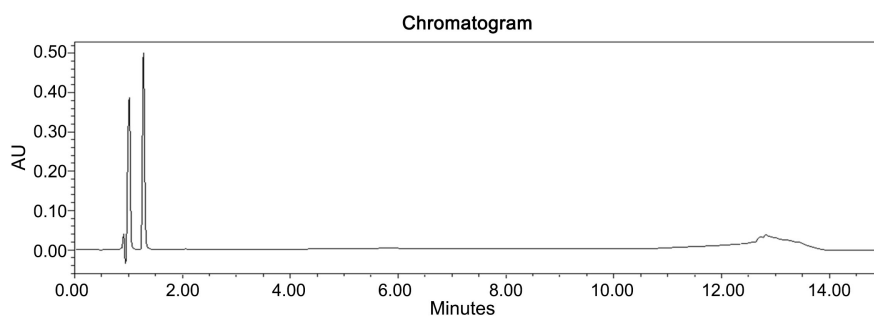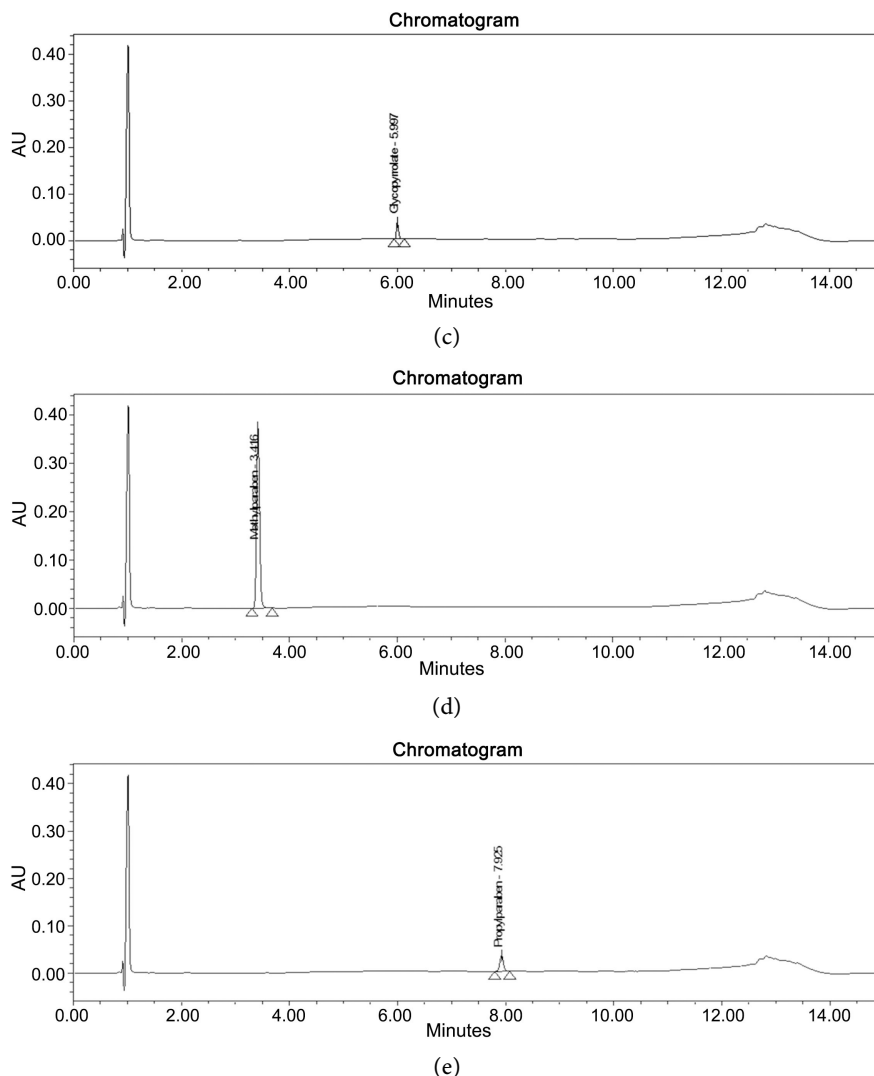
Figure 4. Testimonial chromatograms: (a) Chromatogram of Placebo, (b) Chromatogram of Standard, Individual Chromatograms (c) Glycopyrrolate, (d) Methylparaben, (e) Propylparaben.

2.5.3. Linearity

Glycopyrrolate, Methylparaben, and Propylparaben concentrations (X-axis) vs. peak areas (Y-axis) were plotted on a graph, and the correlation coefficient was calculated to determine the linearity. There were produced a number of solutions with concentrations between roughly 20% and 160% of the intended concentration. Glycopyrrolate linearity solutions of 4, 8, 16, 20, 24, and 32 μg/mL, methylparaben solutions of 35, 70, 140, 170, 210, and 290 μg/mL, and propylparaben solutions of 4, 8, 16, 20, 24 and 32 microgram/mL were prepared and injected into the UPLC in 2 μL portions.

2.5.4. Precision

Glycopyrrolate Oral Solution 1 mg/5mL was administered at aimed concentrations and evaluated using test methodologies to determine the accuracy of the test procedures. Six samples were processed in accordance with the analytical

test protocol, added to a chromatographic system, and assessed. The test chromatogram is presented in (**Figure 5**) together with the average assay percent and RSD for glycopyrrolate, methylparaben, and propylparaben.

2.5.5. Accuracy
Drug products were used to test the accuracy of glycopyrrolate recovery by changing sample quantities in response to the spike level. According to the test technique, test samples of glycopyrrolate, methylparaben, and propylparaben were created at the target assay test concentration in triplicate at each level. They were then preceded into a UPLC system and evaluated. % Recovery was calculated for each preparation, resulting in mean percent recoveries for each level of roughly 30%, 100%, and 150%.

2.5.6. Detection Limit (LOD) and Quantification Limit (LOQ)
The detection and quantification limits for glycopyrrolate, methylparaben, and propylparaben were investigated. The detection and quantification limits were determined using the signal-to-noise ratio. A series of solutions comprising glycopyrrolate, methylparaben, and propylparaben were created and injected into the chromatographic apparatus in accordance with the analytical test technique. The results in a signal-to-noise ratio of about 3 were used to calculate the limit of detection. The quantity that results in a signal-to-noise ratio of around 10 was used to define the quantification limit.

2.5.7. Ruggedness
Six samples of glycopyrrolate oral solution 1 mg/5mL were prepared in accordance with the test solution's specifications and evaluated in accordance with the analytical test technique for various UPLCs, various columns, various scientists, and various days. Differing days saw different evaluations of the system appropriateness traits for both the UPLC system and columns. Calculations were made to determine the %RSD of glycopyrrolate, methylparaben, and propylparaben.

2.5.8. Stability of Solutions
Solution Stability was established for standards and sample preparation. Solutions were made, and kept at 5 digress, and 25 degrees. These solutions were injected into the UPLC and evaluated initial and at three different intervals. Calculated the standard solution's similarity factor and the % assay for test preparation against freshly prepared standards. The difference was calculated using the initial results.

2.6. Stability Indicating Assessment

In order to determine the degradation of the active moiety, forced degradation studies were performed on a sample of glycopyrrolate oral solution. The amount of degradation depends on the concentration of the reactant, the time of the stress, and the exposure temperature. The percentage degradation range of 5 to
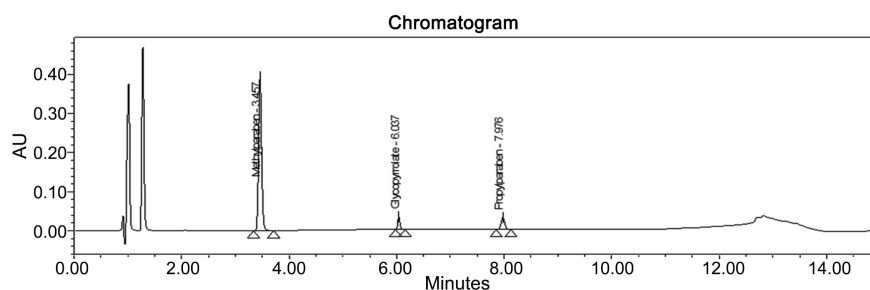
Figure 5. Sample chromatogram.

20 is meant to be connected with the stability of the therapeutic substance. In order to accomplish the desired degradation, the samples were therefore exposed to the ideal conditions. Experiments were conducted to test every conceivable scenario of degradation, and chromatograms were produced and compared to standards of undegraded samples. The peak purity of Glycopyrrolate was verified in all of the degradation sample chromatograms using the PDA detector and Empower 3. Refer to (Table 6) for information on how the Glycopyrrolate purity angle shows that all degradation samples' peak purity requirements were fulfilled.

2.6.1. Forced Degradation
Forced deterioration (FD) investigations were carried out to show the optimized method's capability to indicate stability under diverse stress circumstances. Details on the pathways for degradation and the generation of degradation impurities have been presented. According to stability testing of new drug substances and products, stress testing performed with the inclusion of API and drug product can help in detecting potential degradation products, resulting in the formation of the product's real-time stability (ICH guidelines). The conditions for quick stability testing are often easier than those for forced degradation. The Forced degradation assay on the material was run using the Glycopyrrolate reference standard. A PDA-UV detector verified the peak purity of the FD samples; refer to (Table 6) for findings.

2.6.2. Acid Degradation
Individually, 2 mL of 0.5 N hydrochloric acid were added to the sample solution and it was stressed for 30 minutes on the workbench. After a mentioned time, sample solutions were removed and kept on the benchtop to cool to room temperature. The solution was diluted to the mark of the volumetric flask with the diluent and then neutralized with 2 mL of 0.5N sodium hydroxide to obtain the final concentration.

2.6.3. Base Degradation
After being individually stressed with 2 mL of 0.1 N sodium hydroxide and kept on the benchtop for 15 minutes at 60˚C, the sample solution was examined. After the mentioned time, sample solutions were removed and kept on the benchtop to cool to room temperature. The solution was prepared to the mark of

the volumetric flask with the diluent and then neutralised with 2 mL of 0.1N hydrochloric acid to obtain the final concentration.

2.6.4. Peroxide Degradation

Two millilitres of 0.3 percent hydrogen peroxide were used to individually stress each sample solution, which was then kept at room temperature for an hour. Further made up to the volumetric flask up to mark with the diluent.

2.6.5. Thermal Degradation

For 24 hours, the test solution was heated at 80 degrees Celsius. A 50 mL volumetric flask was filled to the mark with the diluent after the sample had been heated in order to obtain the final concentration.

2.6.6. Photolytic Degradation

The sample's photolytic stability was examined by exposing the sample liquid to UV light directly for seven days. Samples were illuminated with 1.2 million lux hours and subjected to 200 -watt hours per square metre of exposure. Without any material loss, the exposed sample vial was quantitatively transferred to a 50 mL volumetric flask. The volume was then diluted with the diluent to get the final concentration.

2.6.7. Application of the Method

Using the optimised and validated method in combination with the in-process bulk solution of 0.2 mg/mL, the assay for glycopyrrolate, methylparaben, and propylparaben was tested. The method's use in estimating liquid oral formulations was demonstrated using the commercially available Glycopyrrolate Liquid Oral Solution 1 mg/5mL.

This UPLC application can be used to analyse pharmaceutical product formulations, drug substances, routine, and in-process samples, as well as the measurement of glycopyrrolate and preservatives. This technique is simple and cost effective for routine QC analysis.

This technique can be used to analyse pharmaceutical product formulations, drug substances, routine, and in-process samples, as well as the measurement of glycopyrrolate and preservatives.

Glycopyrrolate Oral Solution is carefully weighed at 5.8 g into a 50 mL volumetric flask. 30 mL of diluent should be shaken for one to two minutes before being diluted to volume. As final solutions, standard and sample solutions were added to the UPLC. Six injections of the test sample were made, and the average peak areas were calculated (refer to precision results).

3. Assessment Results

3.1. Modification of Chromatographic Conditions

According to the label, Methylparaben (preservative), Propylparaben (preservative), and Glycopyrrolate (active) are present in each 5 mL of Cuvposa Oral Solution 1 mg/5mL. The objective of this work is to extract glycopyrrolate more

quickly from a preservative using RP-UPLC technology.

The mobile phase was optimised using the resolution of the drug and preservative, tailing factor, and theoretical plates produced. The gradient mixture of mobile phases A and B as indicated in the chromatographic conditions (2.3) was discovered to be excellent at a flow rate of 0.25 mL/min, providing satisfactory separation and presenting symmetric peaks for glycopyrrolate and preservatives.

3.1.1. Wavelength Optimization

The detector wavelength was optimised using the maximum wavelength of methylparaben, propylparaben, and glycopyrrolate. The spectra showed that Methylparaben, Propylparaben, and Glycopyrrolate could be detected most effectively at a wavelength of 222 nm.

3.1.2. Column Optimization

Parabens (methylparaben, propylparaben), and Glycopyrrolate were separated using the waters Acquity BEH C18 150 mm × 2.1 mm, 1.7 µm column and it was selected for testing.

3.1.3. Optimisation of Sample Concentration and Injection Volume

For detection under the aforementioned chromatographic conditions, a sample concentration of 20 µg/mL glycopyrrolate, 180 µg/mL methylparaben, and 20 µg/mL propylparaben with a 2 µL injection volume. The data were deemed sufficient after the linearity of glycopyrrolate, methylparaben, and propylparaben in this sample concentration was verified.

3.1.4. Diluent Selection for Sample Preparation

According to the literature, Methylparaben and Propylparaben are highly soluble in organic solvents like acetonitrile and methanol but only minimally soluble in water. Glycopyrrolate is also water-soluble. Based on this knowledge, the solvents Methanol, Acetonitrile, and water were tested. Both water and a mixture of organic solvents don't affect the medication product's stability. Hence A blend of water, acetonitrile, and methanol (60:30:10 v/v/v) is used to prepare the diluent. It was determined that the chromatographic peak pattern and shape were satisfactory in this diluent.

3.1.5. Optimization of Column Oven Temperature

The temperature of the column was set to 40˚C based on the separation of glycopyrrolate, methylparaben, and propylparaben.

3.2. Method Validation

According to ICH recommendations, the procedure was validated. All the factors examined-specificity, linearity, precision, and accuracy were judged to be adequate.

3.2.1. System Suitability

Table 1 displays the findings of the parameters for system suitability which were

discovered to be within the acceptance criteria.

3.2.2. Specificity

No interference was seen at the retention time of any of the primary analytes in the analysis. Refer to Table 2 for further information.

3.2.3. Linearity

The detector response was verified to be within the proper range, according to Table 3.

3.2.4. Precision/Accuracy

According to Table 4, the six sample preparations' relative standard deviations and the mean recoveries percent from 30% to 150% of the specification are fell within the allowed range.

Table 1. System suitability results.

Parameter	Glycopyrrolate	Methylparaben	Propylparaben
Percent RSD (≤2.0) (n = 5)	1.3	1.1	1.3
Tailing factor (≤2.0) (n = 5)	1.2	1.3	1.4

Table 2. Specificity results.

Parameter	Glycopyrrolate	Methylparaben	Propylparaben
Retention Time (min)	6.51	4.01	8.83
Placebo Interference (Yes/No)	No	No	No

Table 3. Results of linearity study.

Name of the component	Linearity (μg/mL)	Intercept	Slope	Correlation coefficient
Glycopyrrolate	4 - 32	650	4670	1.000
Methylparaben	35 - 290	3460	8850	1.000
Propylparaben	4 - 32	205	7320	1.000

Table 4. Results of the precision study.

#	Glycopyrrolate	Methylparaben	Propylparaben
1	99.5	99.1	99.5
2	98.7	99.9	97.9
3	98.3	98.9	97.9
4	98.1	98.7	99.8
5	99.6	98.8	99.0
6	98.6	99.9	98.4
Mean	98.8	99.2	98.8
SD	0.6	0.5	0.8
%RSD	99.5	99.1	99.5

Results for Accuracy (Recovery)

Amount added	Glycopyrrolate	Methylparaben	Propylparaben
	% Recovery	% Recovery	% Recovery
30 %	100.1	100.2	100.2
100 %	99.8	101.0	100.0
150 %	98.9	100.8	100.8

3.2.5. Ruggedness

According to Table 5, the %RSD of glycopyrrolate, methylparaben, and propylparaben was discovered to be within the acceptance criteria.

Table 5. Results of Glycopyrrolate, Methylparaben, and Propylparaben between two different analysts.

Sample	Glycopyrrolate		Methylparaben		Propylparaben	
	Analyst-1	Analyst -2	Analyst -1	Analyst -2	Analyst -1	Analyst -2
01	99.5	99.6	99.1	99.5	99.5	98.0
02	98.7	99.4	99.9	98.9	97.9	99.8
03	98.3	99.0	98.9	98.9	97.9	97.8
04	98.1	99.0	98.7	99.0	99.8	97.9
05	99.6	99.3	98.8	97.4	99.0	99.8
06	98.6	99.0	99.9	99.0	98.4	98.9
Average	98.8	99.2	98.2	98.7	98.8	98.7
%RSD	0.6	0.2	0.5	0.7	0.8	0.9

%RSD-Percentage Relative standard deviation.

3.2.6. Forced Degradation

Table 6. The results from force degradation for the Acid, alkali, thermal, oxidative, and photolytic conditions are presented. All the conditions glycopyrrolate peak purity were passed. It indicates the validated method is stability indicating.

Sample Name	Purity Angle	Purity Threshold	% Assay	% Degradation	Mass Balance
Unstressed	0.591	0.653	100.1	Not Detected	99.8
Acid stress	0.678	0.790	88.4	8.20	96.6
Base stress	0.675	0.702	98.9	0.79	99.7
Peroxide stress	0.712	0.815	99.5	Not Detected	99.5
Thermal stress	0.675	0.693	99.8	Not Detected	99.8
Photolytic stress	0.868	1.025	99.5	Not Detected	99.5

3.2.7. Stability of Solutions

In order to compute the percent assay of parabens and active, a sample and standard solutions were studied for stability at 5°C and 25°C at various time in-

tervals. The medicine and preservatives were shown to be reasonably stable at room and refrigerator temperatures because the % assay was found to acceptable range at 5 and 25 degrees. The stability of the medication product was examined under several circumstances for quality control (QC) of samples.

4. Discussion

A method for measuring the concentration of glycopyrrolate and its preservatives in glycopyrrolate oral solution was developed using the RP-UPLC procedure. Obtaining optimal resolution between the Parabens and Glycopyrrolate peak with a shorter run time, choosing the right chromatographic conditions to obtain optimal separation between the parabens and active moiety, and optimizing the sample concentration to obtain the desired response levels for the Glycopyrrolate and its preservatives were the three main challenges faced by the current study. Glycopyrrolate Oral Solution was developed using a particular, precise, accurate, and trustworthy methodology. Although only the Glycopyrrolate Assay procedure has been established for measuring Glycopyrrolate API, Tablet, and Injections, Glycopyrrolate Liquid Oral formulation contains Glycopyrrolate (Active), Methylparaben (Preservative), and Propylparaben (Preservative). In order to quantify the three ingredients glycopyrrolate, methylparaben, and propylparaben, a single analytical method has been created and validated.

5. Conclusion

Glycopyrrolate, Methylparaben, and Propylparaben could all be identified and separated using the suggested method. Glycopyrrolate, Methylparaben, and Propylparaben in the Glycopyrrolate Oral Solution can be analysed using this technique. Glycopyrrolate and its preservatives in Glycopyrrolate Oral Solution have not yet been quantified using an assay method that has been developed. The Glycopyrrolate and its preservatives in a sample of an oral solution for Glycopyrrolate can be completely resolved using this RP-UPLC technology for the first time.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Chawla, R.K., Rao, G.S.N.K., Kulandaivelu, U., Panda, S.P. and Alavala, R.R. (2021) A Selective and Sensitive Method Development and Validation of 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide Impurity in Glycopyrrolate Oral Solution by Liquid Chromatography-Tandem Mass Spectroscopy. *Journal of Chromatographic Science*, **59**, 566-575. https://doi.org/10.1093/chromsci/bmab003

[2] Abdel Ghany, M.F., Hussein, L.A., Magdy, N. and Yamani, H.Z. (2016) Simultaneous Spectrophotometric Determination of Indacaterol and Glycopyrronium in a Newly Approved Pharmaceutical Formulation Using Different Signal Processing

Techniques of Ratio Spectra. *Spectrochimica Acta, Part A*: *Molecular and Biomolecular Spectroscopy*, **157**, 251-257. https://doi.org/10.1016/j.saa.2016.01.002

[3] Banoth, R.K. and Nethikoppula, R.L. (2021) A Study of Analytical Method Development, Validation and Forced Degradation for Simultaneous Estimation of Glycopyrrolate and Formoterol Fumarate Bulk Drug by UHPLC Method. *International Journal of Pharmaceutical Sciences and Research*, **12**, 3781-3790.

[4] Reddy, M.P., Reddy, O.S. and Subha, M.C.S. (2022) A Reverse Phase Stability Indicating HPLC Method Development for Estimation Assay of Benzyl Alcohol and Glycopyrrolate in Glycopyrrolate Injection. *International Journal of Pharmaceutical Investigation*, **12**, 51-55. https://doi.org/10.5530/ijpi.2022.1.9

[5] Rao, B.S., Reddy, M.V. and Rao, B.S. (2018) Combined Quantification of Formoterol Fumarate and Glycopyrrolate by Stability Indicating RP-HPLC Method with Photodiode Array Detector. *European Journal of Biomedical and Pharmaceutical Sciences*, **5**, 418-424.

[6] Nebiu, D., Walter, M., Lachmann, B., Kopelent, H. and Noe, C.R. (2007) Determination of (R,R)-Glycopyrronium Bromide and Its Related Impurities by Ion-Pair HPLC. *Pharmazie*, **62**, 406-410.

[7] Kumaraswamy, G., *et al.* (2016) RP-HPLC Method Development and Validation for Estimation of Glycopyrrolate in Bulk and Tablet Dosage Forms.
https://www.researchgate.net/publication/293247710

[8] Jogi, K., *et al.* (2016) Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Glycopyrrolate and Neostigmine in Bulk and Tablet Dosage Form. *International Journal of Scientific & Engineering Research*, **7**, 1236-1242.

[9] Mishra, S. and Arora, V. (2016) Analytical Method Validation of HPLC Method for Assay of the Anticholinergic Drug in Parental Formulation. *International Journal for Pharmaceutical Research Scholars*, **5**, 101-104.

[10] Venkateshwara Reddy, B., Anuradha, V., Ramachandran, D. and Naveen Reddy, S. (2020) Development and Validation of Simultaneous Estimation of Glycopyrrolate and Anol Fumarate in Its Bulk and Pharmaceutical Rota Caps Dosage Form by Using RP-UPLC. *SSRG International Journal of Applied Chemistry*, **7**, 49-56.
https://doi.org/10.14445/23939133/IJAC-V7I3P110

[11] Goswami, N., Gupta, V.R. and Jogia, H.A. (2013) Development and Validation of a Novel Stability-Indicating RP-HPLC Method for the Simultaneous Determination of Halometasone, Fusidic Acid, Methylparaben, and Propylparaben in Topical Pharmaceutical Formulation. *Scientia Pharmaceutica*, **81**, 505-518.
https://doi.org/10.3797/scipharm.1301-21

[12] Shabir, G.A. (2010) A New Validated HPLC Method for the Simultaneous Determination of 2-Phenoxyethanol, Methylparaben, Ethylparaben and Propylparaben in a Pharmaceutical Gel. *Indian Journal of Pharmaceutical Sciences*, **72**, 421-425.
https://doi.org/10.4103/0250-474X.73906

[13] Kumar, S., Mathkar, S., Romero, C. and Rustum, A.M. (2011) Development and Validation of a Single RP-HPLC Assay Method for Analysis of Bulk Raw Material Batches of Four Parabens That Are Widely Used as Preservatives in Pharmaceutical and Cosmetic Products. *Journal of Chromatographic Science*, **49**, 405-411.
https://doi.org/10.1093/chromsci/49.5.405

[14] Kokoletsi, M.X., Kafkala, S. and Tsiaganis, M. (2005) A Novel Gradient HPLC Method for Simultaneous Determination of Ranitidine, Methylparaben and Propylparaben in Oral Liquid Pharmaceutical Formulation. *Journal of Pharmaceutical and Biomedical Analysis*, **38**, 763-767. https://doi.org/10.1016/j.jpba.2005.02.022

[15] Qadir, M.A., Ahmed, M., Shafiq, M.I., Ali, A. and Sadiq, A. (2016) Analytical Method for the Identification and Assay of Kojic Acid, Methylparaben, and Propylparaben in Cosmetic Products Using UPLC: Application of ISO 12787:2011 Standard. *Journal of AOAC International*, **99**, 1191-1196.
https://doi.org/10.5740/jaoacint.16-0026

[16] Ahmed, M., *et al.* (2016) Sensitive and Selective UPLC Method for Simultaneous Determination of Methyl Paraben, Propyl Paraben and Loratadine in Antihistamine Suspension and Biological Samples: Stability Indicating Assay. *Latin American Journal of Pharmacy*, **35**, 869-876.
https://www.researchgate.net/publication/301794732

[17] Ahmed, M., Qadir, M.A., Shafiq, M.I. and Shahzad, S. (2012) Development and Validation of Novel Stability Indicating RP-UPLC Method for Simultaneous Determination of Nizatidine, Methylparaben, and Propylparaben in Oral Liquid Pharmaceutical Formulation.

[18] Soad, S., El-Hay, A. and Mohan, M.S. (2016) Development and Validation of New RP-HPLC Method for Simultaneous Determination of Methylparaben and Propylparaben with Levetiracetam in Pure Form and Pharmaceutical Formulation. *Chromatography Research International*, **2016**, Article ID: 4909547.
https://doi.org/10.1155/2016/4909547

[19] Nekkalapudi, A.R., Veldi, V.G. and Pippalla, S. (2022) A Novel RP-HPLC Method for Estimating Fulvestrant, Benzoyl Alcohol, and Benzyl Benzoate in Injection Formulation. *American Journal of Analytical Chemistry*, **13**, 229-240.
https://doi.org/10.4236/ajac.2022.137016

[20] Nekkalapudi, A.R., Gopal Veldi, V. and Bapatu, H.R. (2022) QBD Approach High-Performance Liquid Chromatographic Technique for Determination of Clofarabine Impurities in Clofarabine Api, Clofarabine Parenteral Dosage Form and Reconstitution Studies. *Natural Volatiles & Essential Oils Journal*, **9**, 82-98.

[21] Pippalla, S., Jillelamudi, S.B. and Nekkalapudi, A.R. (2022) Novel Stability-Indicating Method for Determination of Related Substances of Cyanocobalamin Injection Using Rp-HPLC. *European Journal of Biomedical and Pharmaceutical Sciences*, **9**, 351-358.