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Research Article

Analytical Method Validation For Determination Of 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide In Glycopyrrolate Oral Solution 1mg/5 mL BY LC-MS/MS

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ABSTRACT

A rapid, sensitive and selective analytical method was developed and validated for the determination of 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide in Glycopyrrolate oral solution 1 mg/5 mL. Chromatographic separation was achieved on a XBridge HILIC 100X 4.6mm, 5µm column using gradient elution with the mobile phase consisting of ammonium formate buffer and acetonitrile with flow rate was1.2 mL/min. The retention time of 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium bromide was found to be 3.2 minutes. The method was validated according to ICH[1] guidelines. The calibration curve was linear over the concentration range of 0.0.0497–1.9889 µg/mL (r = 1.000). The intra- and inter-day precision (RSD %) was 2.3% and the accuracy (%) was LOQ (103.2%) to 200% (90.3%). The method can be used for the testing of content of 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide in Glycopyrrolate oral solution 1 mg/5 mL routine quality control and stability testing.

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INTRODUCTION:

Glycopyrrolate solution is used to reduce excessive drooling caused by medical conditions (such as cerebral palsy)[1-2]. This medication works by decreasing the amount of saliva you make. Chemical name is 3-[2-Cyclopentyl (hydroxy) phenylacetoxy]-1,1-dimethylpyrrolidinium bromide[3-5]. Glycopyrrolate belongs to a class of drugs known as anticholinergics. Glycopyrrolate reduces the secretions of certain organs in the body. Glycopyrrolate helps to control conditions such as peptic ulcers that involve excessive stomach acid production[6]. Glycopyrrolate is also used to reduce drooling in children ages 3 to 16 who have certain medical conditions, such as cerebral palsy[7]. Glycopyrrolate is an anticholinergic agent used to treat gastrointestinal conditions associated with intestinal spasm and to decrease secretions[8]. Side effects are, Decreased sweating, Dry mouth, Constipation, Mild dizziness, Drowsiness, Headache, Loss of taste and Nervousness

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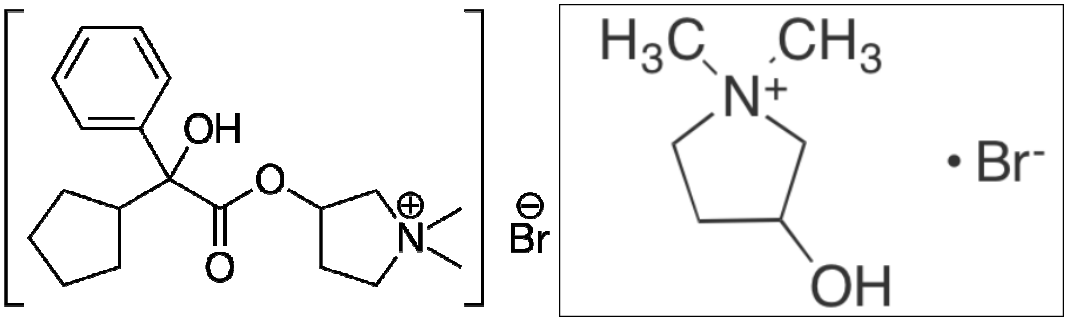

Glycopyrronium bromide Impurity

Figure-1: Structures of Glycopyrronium bromide and 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium bromide

Literature survey reveals that very few analytical methods have been established for the determination of Glycopyrronium bromide and 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium bromide. Reported methods are Kishor Kumar Erukulla (2018) [9] HPLC method, Nasr Mohamed A. El-Abasawy (2018) [10] and team RP-HPLC method for the simultaneous quantification of Indacaterol and Glycopyrronium, Yomna A. Salem (2019) [11] and team HPLC method for formoterol and glycopyrronium, Yomma A Salem (2018) [12] and team ion pair chromatographic method, Liu Y (2018) [13] and team was developed the micro-solid-phase extraction combined with online preconcentration by capillary electrophoresis, Muneer FADR (2018) [14] and team was developed the method on Voltammetric determination method.

To the best of our knowledge, there was no reported LC-MS/MS method in previous reported literature. Thus, efforts were made to develop fast, selective and sensitive analytical method using LC-MS/MS. In the current work author developed a simple, reliable and reproducible LC-MS/MS method which was duly validated by statistical parameters precision, accuracy and recovery.

MATERIALS AND METHODS

Glycopyrrolate oral solution and 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium bromide was the generous gift from Ranbaxy Pharmaceuticals Limited, Paonta Sahib, India. HPLC grade acetonitrile was procured from Qualigens, India. Ammonium formate and Formic acid were purchased from Loba Chemie, Mumbai, India. All other chemicals and solvents used were of analytical grade. Water used in the LC/MS analysis was prepared by the water purifier (Arium®, 611UF, Sartorius, Germany). The mobile phase and all the solutions were filtered through a 0.45 µm Ultipor® N66® membrane filter (Pall Life Sciences, USA) prior to use.

Preparation of mobile phase:

Mobile phase A:

Preparation of 10mM Ammonium Formate with 0.2% Formic acid:

Weigh and transfer about 0.6306 g of Ammonium Formate into 1000-mL of Milli-Q- Water, to this add 2 mL of formic acid.

Mobile phase B: 100% Acetonitrile

Diluent: 0.2% formic acid: Acetonitrile (70:30 v/v).

Chromatographic conditions:

Instrument Name: LC-MS/MS

Make & Model: Agilent 1100 Series & ABSCIEX API 4000 or Equivalent

Column: XBridge HILIC 100X 4.6mm, 5µm

Flow rate (µL/min): 1200

Split ratio: 90:10

Injection volume: 5 µL

Column oven temperature: 45.0 ºC ± 1.0 ºC

Gradient program:

Table-1: Gradient program

Step	Time (min)	Flow rate (µL/min)	%A	%B
0	0.5	1200	2	98
1	1.0	1200	30	70
2	2.2	1200	30	70
3	2.5	1200	2	98
4	5.0	1200	2	98

Auto sampler temperature: 5.0 ºC ± 0.5 ºC

Run Time: 5.00 minutes

Retention time of 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium is about 3.2 minutes ± 0.3 minutes.

MRM Conditions

Ion Source: Turbo spray

Polarity: Positive

MRM Transitions

1,1-Dimethyl-3-Hydroxy-Pyrrolidinium: 116.10 ± 0.5 (parent), 88.10 ± 0.5 (product)

Table-2: MRM instrument conditions

Collision Gas (CAD)	9.0	
Curtain Gas (CUR)	20.0	
Ion Source Gas 1 (GS1)	30.0	
Ion Source Gas 1 (GS2)	30.0	
Ion Spray Voltage (IS)	5500	
Temperature (TEM)	400	
Entrance Potential (EP)	10.0	
Resolution	Unit	
1,1-Dimethyl-3-Hydroxy-Pyrrolidinium	Declustering Potential (DP)	46.00
	Collision Energy (CE)	27.00
	Collision Cell Exit Potential (CXP)	6.00

Preparation of standard solution: (1µg/mL)

Weight and transfer about 2.5 mg of 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide impurity standard or reference standard into a 100 mL volumetric flask, add 50 mL of diluent, sonicate to dissolve and make up to volume with diluent. Transfer 1.0 mL of above solution into 25 mL volumetric flask and dilute to volume with placebo solution and mix. Dilute the

above solution to 50 folds with diluent and inject into LC-MS/MS.

Preparation of test sample solution:

Take as such test sample solution, dilute to 50 folds with diluent and inject in to LC-MS/MS.

Preparation of placebo preparation: (Placebo for Glycopyrrolate oral solution)

Take as such placebo solution, dilute to 50 folds with diluent and inject in to LC-MS/MS.

RESULTS AND DISCUSSION:

Method development and Optimization:

Initially the method development was started with HPLC with RI detector and the mobile phase was used 0.05m phosphate buffer with Inertsil ODS 250 X 4.6 mm, 3.5µm column, 0.8mL/min flow and 40° C column temperature. This method the 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium peak was eluted at void volume means 1.5 minutes and this peak response is not reproducible. Later the method was developed on LC-MS/MS by using mobile phase A as ammonium formate buffer with 0.2% formic acid and mobile phase B as acetonitrile, 1.2mL /min flow with gradient elution through XBridge HILIC 100X 4.6mm, 5µm column. The retention time of 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium is about 3.2 minutes and peak response also good and reproducible. Hence the method validation was performed as per ICH guidelines and the results are given below.

Method Validation:

System suitability:

System suitability test is an integral part of method validation and are used to ensure adequate performance of the chromatographic system.Established system suitability/System precision by injecting standard solution for six times and calculated %RSD for the peak areas of 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide. System suitability parameters were captured in table-3.

Table-3: System suitability

Replicate injections	Peak Area
	1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide
1	84331
2	82720
3	81840
4	82992
5	84789
6	82817
Mean	**83248**
%RSD	**1.3**

Specificity:

An interference study was evaluated with blank. Injected blank and 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide standard and no interference was detected at the retention time of 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide. This shows that the blank do not interfere with the analyte. The chromatograms for specificity parameter were shown from Figure.2 to Figure.5.

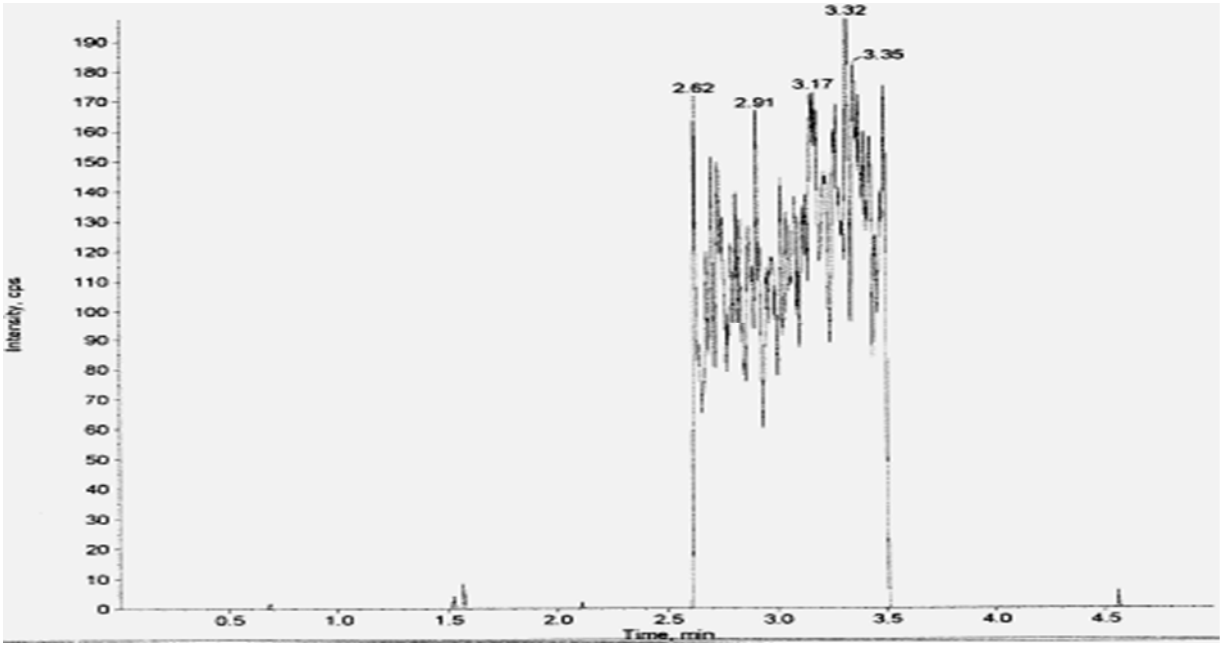

Figure-2: Blank Chromatogram

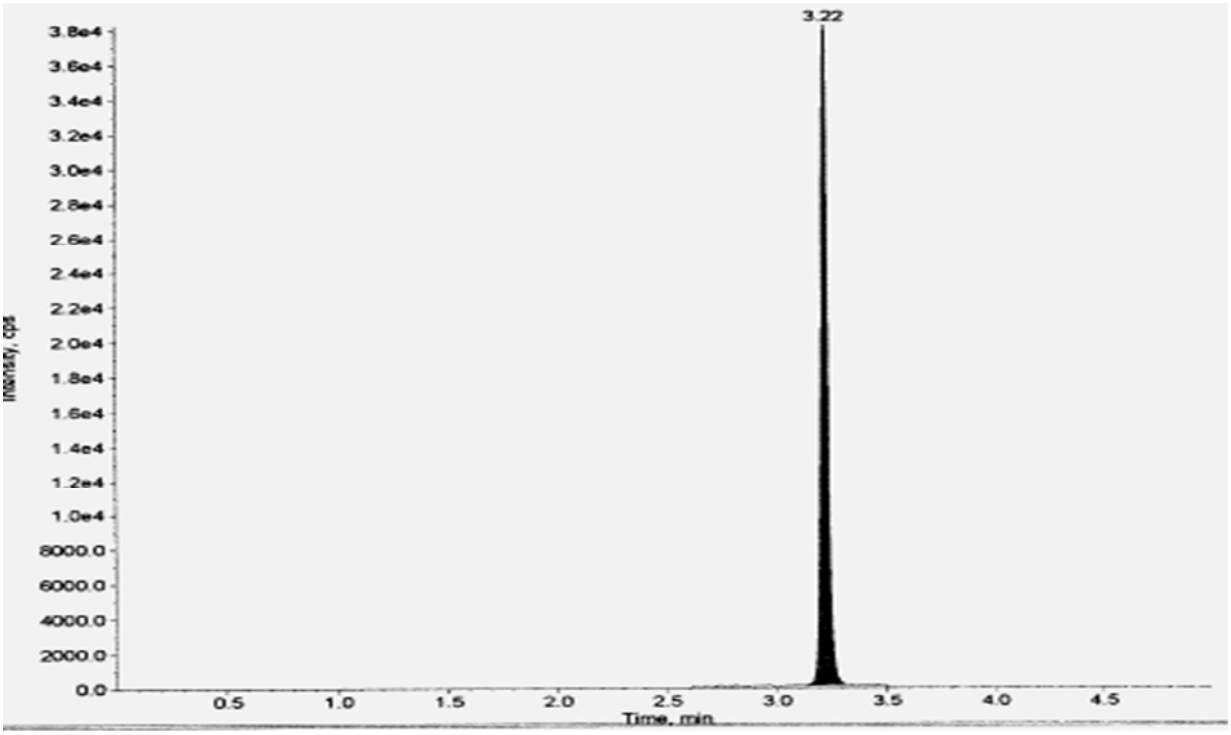

Figure-3: Standard chromatogram

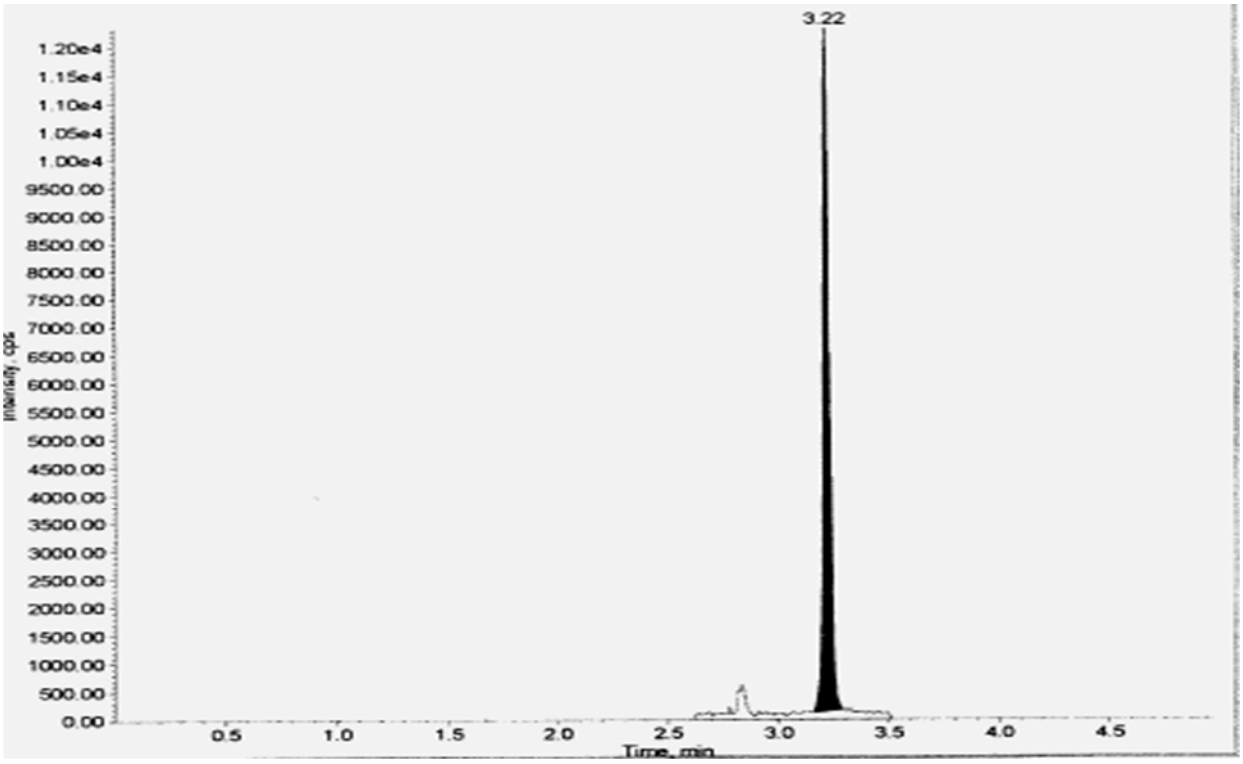

Figure-4: Test Sample chromatogram

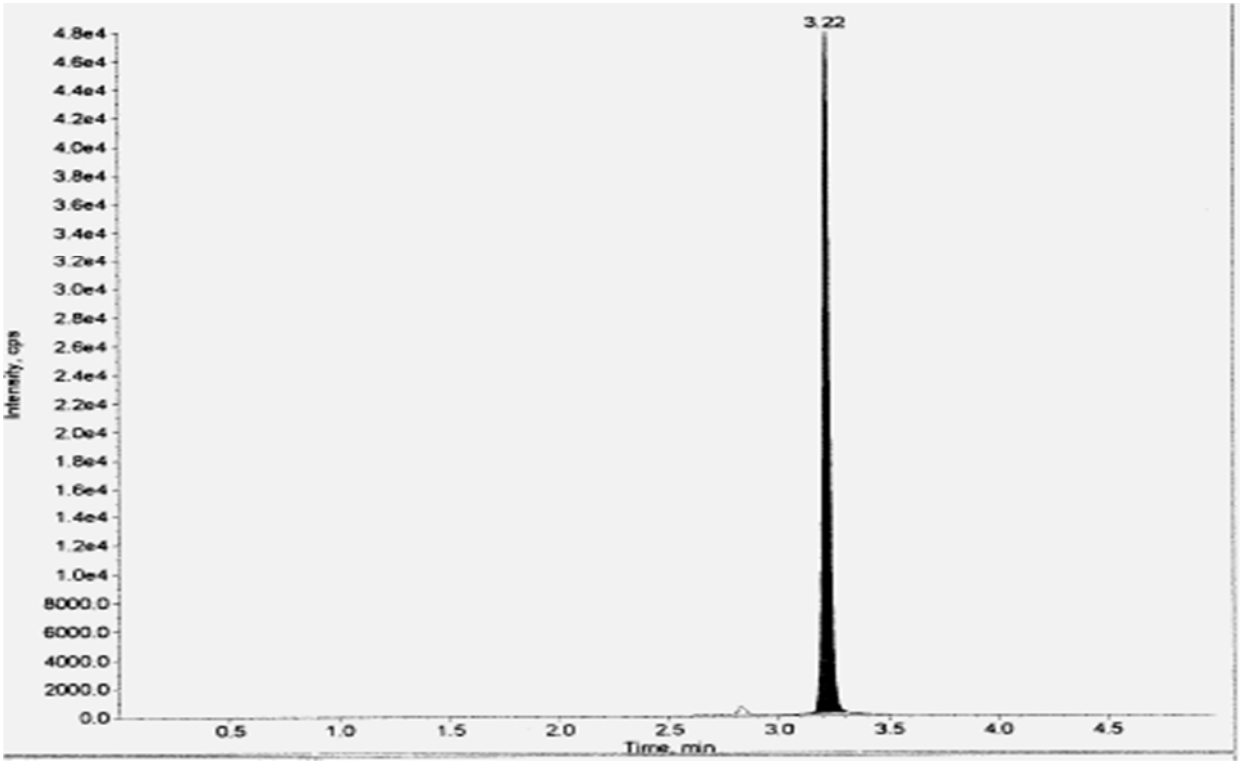

Figure-5: Spiked Sample chromatogram

Forced Degradation study:

Forced degradation of Glycopyrrolate oral solution shall be carried out, to confirm that during stability study or throughout the shelf life, 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide impurity peak degrading or not and also the forced degradation study will help to identify the type of degradation pathway (whether oxidative, alkali hydrolysis, acid hydrolysis,

photolytic, dry heat and humidity) for 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide impurity peak. The forced degradation study details are presented in table-4.

Table-4: Forced degradation study table

Stressed sample	**% Impurity**
Control Sample	0.163
Acid hydrolysis (5N HCL at 60°C_2Hrs)	4.744
Alkali hydrolysis (0.5N NaOH at 60°C_1Hr)	24.677
Peroxide oxidation (30% Peroxide_BT_2hrs)	0.148
Water hydrolysis (Water at 60°C_2Hrs)	0.149
Humidity degradation (90%RH for 44Hrs)	0.144
Thermal degradation (80°C for 43Hrs)	0.290
UV light Degradation (200watts-hours/ sq.meter)	0.147
Photolytic light Degradation (1.2million lux hours)	0.136

The above results are shows, 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide impurity is acid and alkali degradants and it can be concluded that the method is specific.

LOD and LOQ:

The LOD (Figure.6) and LOQ (Figure.7) are expressed as a known concentration of 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide at a specified signal to noise ratio, usually for LOQ 10:1, for LOD 3:1 can be quantitated or detected under the stated LCMS/MS method. The LOQ S/N for 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide was 10.9 at concentration with respect to test sample 0.0256%, LOD S/N for 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide was 3.46 at concentration with respect to test sample 0.0085%. LOQ precision results were captured in table-5.

Table-5: Results of LOQ Precision

Injection	1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide Peak Areas
1	**3514**
2	**3614**
3	**3644**
4	**3870**
5	**3684**
6	**3914**
Mean	3707
%RSD	4.2

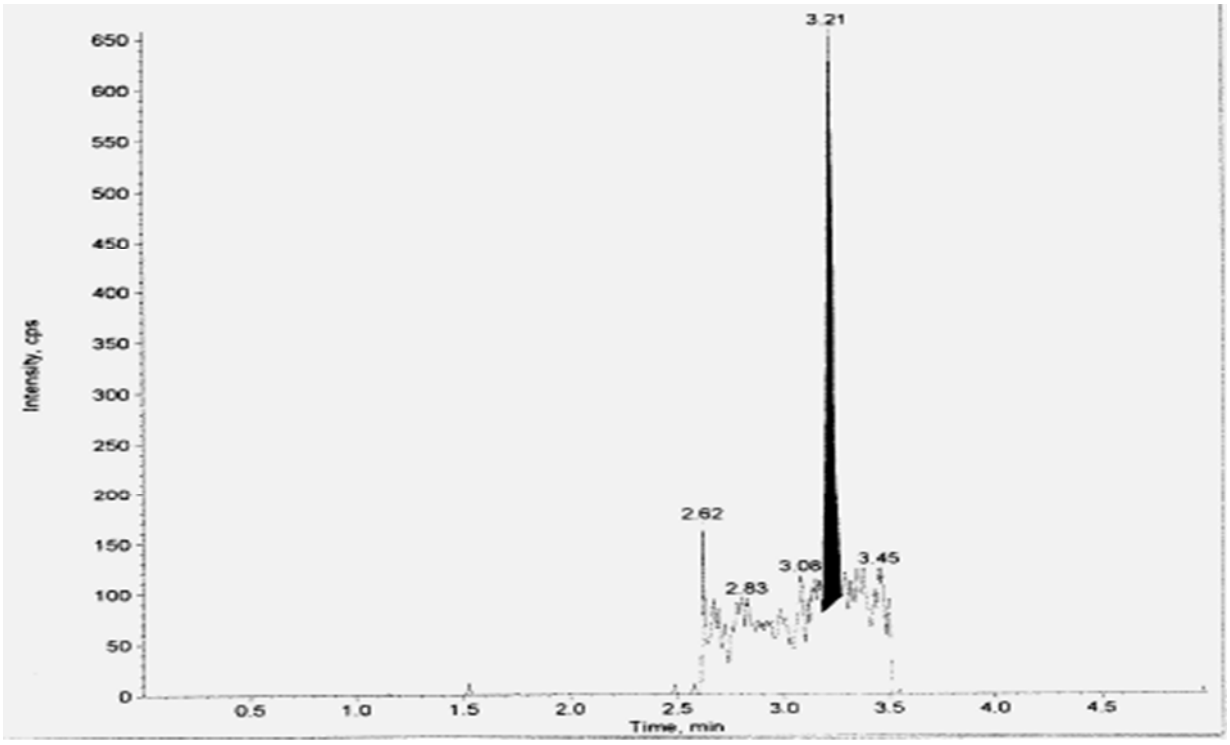

Figure-6: LOD Chromatogram

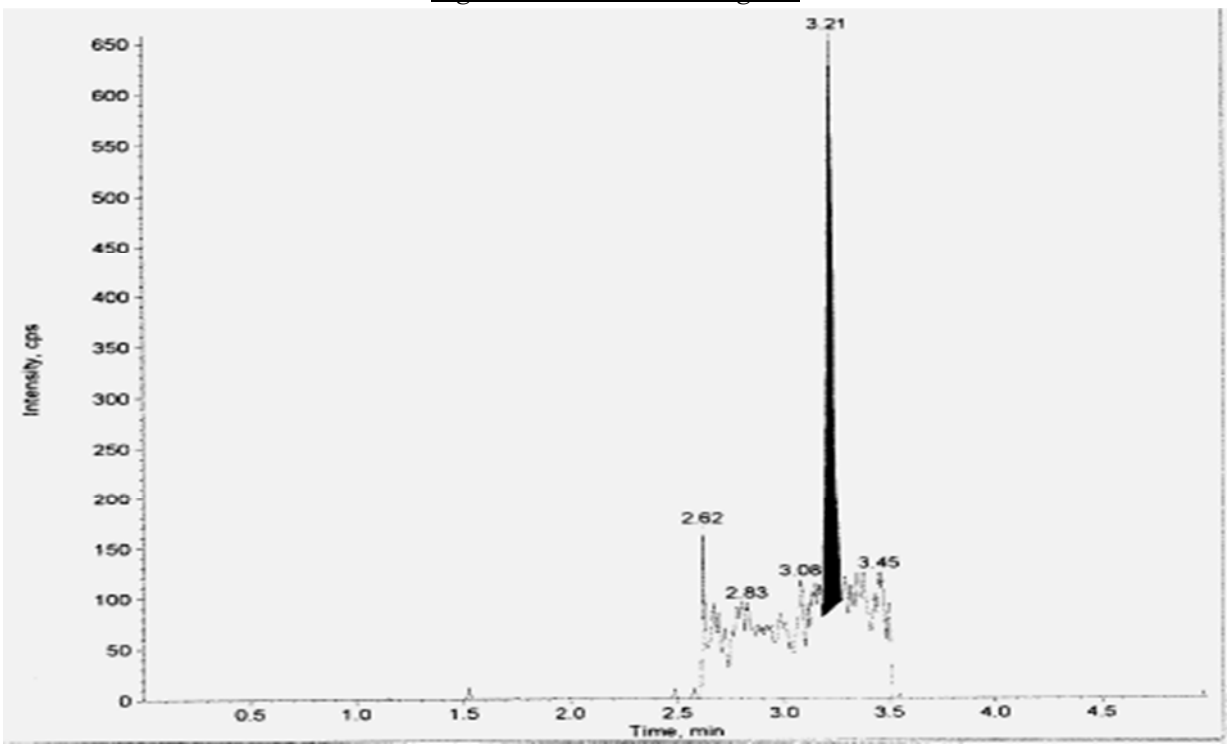

Figure-7: LOQ Chromatogram

Linearity:

Linearity study was conducted by preparing the six levels of linearity solutions for 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide (n=3) from 0.0497 µg/mL (LOQ), to 1.9889 µg/mL. Linearity Plot of peak area against concentration see the Figure.8 and Linearity results were captured in table.6.

Table-6: Linearity of 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide

Linearity levels	1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide	
	Concentration (%)	**Area response**
LOQ level	0.0497	3856
25% level	0.2486	19557
50% level	0.4972	39471
100% level	0.9945	74266
150% level	1.4917	112950
200% level	1.9889	150074
Correlation Coefficient (r)	1.000	
Y-Intercept at 100% Level	777.84	
Slope	75040.22	
Residual sum of square	**3708320.8069**	

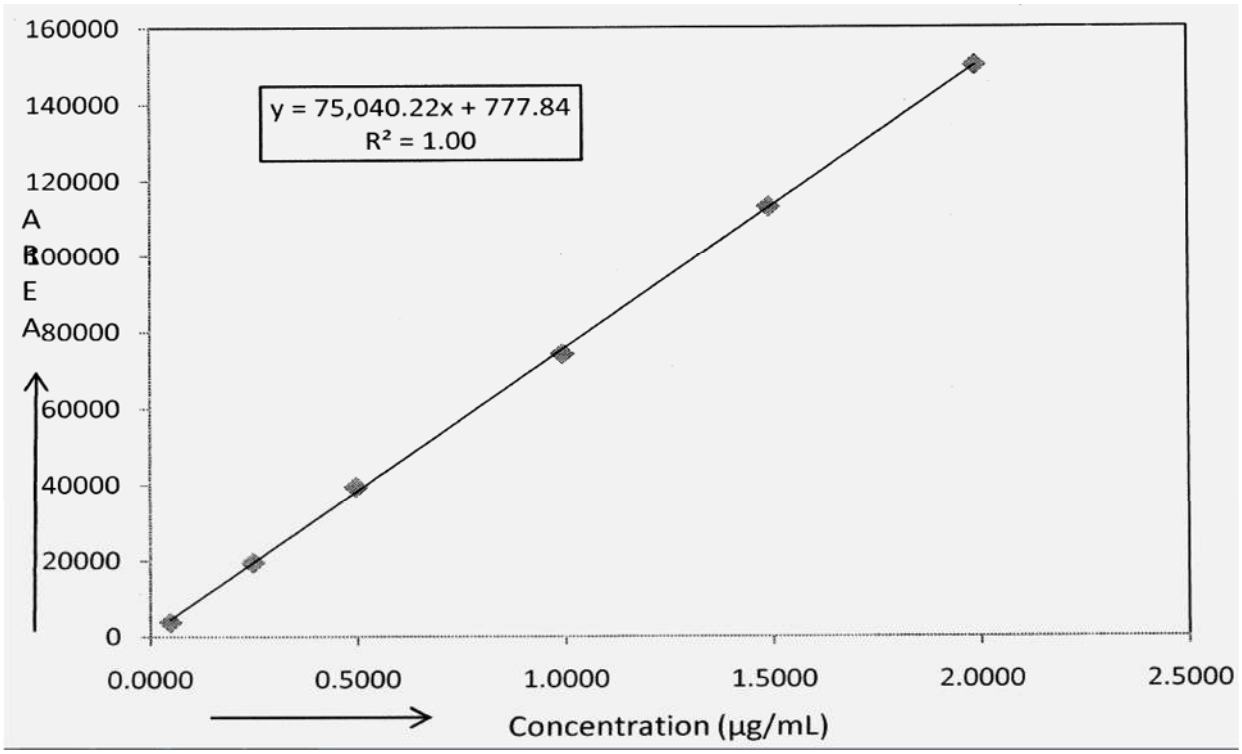

Figure-8: Linearity graph for 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide

Precision:

Method precision was evaluated by test sample spiked with1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide at specification level with respect to the test sample concentration by injecting six different test preparations. The results of Precision and Intermediate precision are reported in Table-7. % RSD values for both Precision study and Intermediate precision study are 1.2 and 1.2 and cumulative %RSD was 2.3. The data demonstrated that the values are met the acceptance criteria. Hence the method was Precise.

Table-7: Method precision / Intermediate precision

Precision& Intermediate Precision	**Tetra butyl ammonium bromide (%)**	
	Analyst 1	**Analyst 2**
1	0.646	0.691
2	0.655	0.686
3	0.661	0.671
4	0.663	0.680
5	0.663	0.693
6	0.669	0.686
Mean	**0.660**	**0.685**
%RSD	**1.2**	**1.2**
Cumulative Mean (12 determinations)	**0.672**	
Cumulative %RSD (12 determinations)	**2.3**	

Accuracy:

The accuracy of the method was proved by checking the recovery of ,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide. Test solution was spiked with 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide at LOQ, 50%, 100% and 200% level and the obtained results were addressed in table-8.

Table-8: Accuracy of Tetra butyl ammonium bromide

Level	**% Recovery**	**Overall Mean %Recovery**	**Overall %RSD**
LOQ	93.5	92.22	0.76
	92.5		
	91.8		
	91.8		
	91.6		
	92.1		
50%	100.2	100.52	0.38
	100.6		
	100.2		

	100.8		
	100.2		
	101.1		
100%	100.5	100.45	0.24
	100.6		
	100.8		
	100.1		
	100.4		
	100.3		
150%	101.6	101.28	0.30
	101.0		
	101.6		
	101.2		
	101.4		
	100.9		

Stability of the analytical solutions:

The bench top stability of 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide in spiked sample established up to 48 Hours, after preparation (n = 1) at bench top condition. The results were addressed in table-9.

Table-9: Solution Stability

Sample number	**Solution stability (Hours)**	1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide **(%)**	**Variation (%)**
Sample-1	Initial	0.646	-----
	1ST Interval (24Hrs)	0.668	0.022
	2nd Interval (48Hrs)	0.604	0.042
Sample-2	Initial	0.656	-----
	1ST Interval (24Hrs)	0.639	0.017
	2nd Interval (48Hrs)	0.624	0.032
Sample-3	Initial	0.625	-----
	1ST Interval (24Hrs)	0.636	-0.011
	2nd Interval (48Hrs)	0.645	-0.02
Sample-4	Initial	0.651	-----
	1ST Interval (24Hrs)	0.665	-0.014
	2nd Interval (48Hrs)	0.625	0.026
Sample-5	Initial	0.632	-----
	1ST Interval (24Hrs)	0.642	-0.01
	2nd Interval (48Hrs)	0.652	-0.02
Sample-6	Initial	0.645	-----
	1ST Interval (24Hrs)	0.632	0.013
	2nd Interval (48Hrs)	0.656	-0.011

Robustness:

The robustness of the method was evaluated by deliberately altering the test method conditions from the original method parameters on different and verifying concentration of the 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide. Test sample was analyzed with three different preparations. Robustness of the method was assessed by varying the instrumental conditions such as flow rate (± 10%) and column temperature (± 5°C).The deliberate changes in the method have no significant changes in the % content of 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide in spiked sample. The robustness results were addressed in table-10.

Table- 10: Robustness studies

parameter	**Variation**	1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide **(%)**					
		Test-1	**Test-2**	**Test-3**	**Test-4**	**Test-5**	**Test-6**
Flow Rate mL/min	Low Flow	0.643	0.651	0.648	0.652	0.654	0.653
	High Flow	0.649	0.649	0.651	0.643	0.646	0.654
Column temperature	40°C	0.651	0.641	0.644	0.634	0.648	0.646
	50°C	0.654	0.648	0.657	0.654	0.652	0.643
As such condition		0.646	0.655	0.661	0.651	0.654	0.656

CONCLUSION

The results obtained in the study demonstrate that the determination of 1,1-Dimethyl-3-Hydroxy-Pyrrolidinium Bromide by LC-MS/MS method of Glycopyrrolate oral solution is specific, linear, precise, accurate and rugged. Therefore the method is suitable for its intended use.

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