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A NEW VALIDATED RP-HPLC METHOD FOR THE DETERMINATION OF GLYCOPYRROLATE AND FORMETEROL FUMARATE IN ITS BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A New method was established for simultaneous estimation of Glycopyrrolate and Formoterol fumarate by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Glycopyrrolate and Formoterol fumarate using Xterra column (4.6×150mm) 5µ, flow rate was 1ml/min, mobile phase ratio (40:60v/v) Methanol,1ml of OPA in 1000ml waterpH 3 (pH adjusted with triethylamine), detection wavelength used by WATERS HPLC Auto Sampler, Separation module 2695, UV detector 2489, Empower-software version-2. The retention times were found to be 3.0 mins and 4.20mins. The % purity of Glycopyrrolate and Formoterol fumarate were found to be 99.80 and 99.70 respectively. The present analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Glycopyrrolate and Formoterol fumarate was found in the concentration range 9µg/ml-45µg/mland 4.8µg/ml-24µg/ml and correlation coefficient (R^2) be 0.999 and 0.999, % recovery was found to be 100.01 and 100.34, % RSD for repeatability 0.3 and 0.6, % RSD for intermediate precision was 0.3 and 0.4respectively. The precision study was precision, robustness and repeatability. It is a convenient, simple and quick method for the determination of Glycopyrrolate and Formoterol fumarate its bulk and pharmaceutical dosage forms.

KEYWORDS: Glycopyrrolate, Formoterol fumarate, HPLC, Methanol.

INTRODUCTION

Formoterol is a long-acting bronchodilator used as a long-term (maintenance) treatment to prevent or decrease wheezing and trouble breathing caused by asthma or ongoing lung disease (chronic obstructive pulmonary disease-COPD, which includes chronic bronchitis and emphysema). It should only be used long-term if your asthma symptoms are not controlled by your other asthma medications (such as inhaled corticosteroids). Formoterol must not be used alone to treat asthma. (See also Warning section.) It works in the airways by relaxing muscles and opening air passages to improve breathing. Controlling symptoms of breathing problems can decrease time lost from work or school.

Glycopyrrolate solution is used to reduce excessive drooling caused by medical conditions (such as cerebral palsy). This medication works by decreasing the amount of saliva you make. Glycopyrrolate belongs to a class of drugs known as anticholinergics

Literature review reveals that there fewHPLC[7-13]and HPTLC[14-15] methods are available forthe determination of Glycopyrrolate and Formoterol fumarate in different dosage forms.

For Glycopyrrolate and Formoterol fumarate there are several HPLC[17-21]methods available in combined dosage forms.

The structures of Glycopyrrolate and Formoterol fumarate were shown in figures 1 and 2.

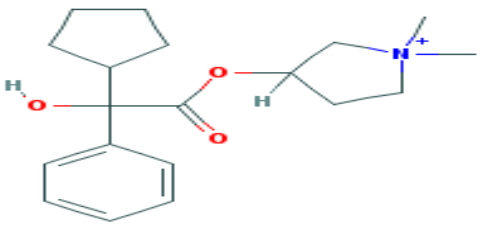

Fig 1: Structure of Glycopyrrolate

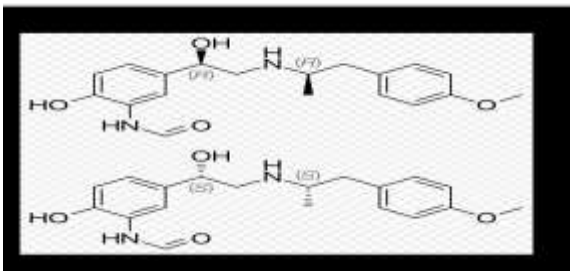

Fig 2: Structure of Formoterol Fumarate

MATERIALS AND METHODS

Instrumentation

The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. The analysis was carried out at 220 nm with an Xterra Coloumn (4.6 x 150mm, 5µm) dimensions at ambient temperature (25^0c).

Chemicals and reagents

Glycopyrrolate and Formoterol fumarate were supplied from Mylon laboratories, Hyderabad. Orthophosphoric acid (OPA) (Merck), Methanol (MERCK HPLC grade) Acetonitrile (Molychem, HPLC grade) and Water for HPLC (LICHROSOLV (MERCK). were employed in the present work.

Preparation of solutions

Preparation of buffer

1ml of Orthophosphoric acid in1000 ml of HPLC water. The $P^H$is adjusted to 3.0 with TEA. The final solution is filtered through 0.45µm membrane filter and sonicate it for 10 mins.

Preparation of mobile phase

Accurately measured 600 ml (60%) of P^H=3.0 buffer and 400 ml (40%) of Methanol. mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45µm membrane filter under vacuum filtration. Figure4 represents the Chromatograms of mobile phase (blank solution).

Diluent Preparation

The Mobile phase was used as the diluent.

Preparation of standard stock solution

Accurately weigh and transfer 9mg of Glycopyrrolate and 4.8 mg of Formoterol fumarate working standard into a 10ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Sample Solution

Accurately weigh and transfer 9 mg of Glycopyrrolate and 4.8 mg of Formoterol fumarate working standard into a 10 ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

20µL of the standard, stock and sample solution are injected into the chromatographic system. The areas are measured for Glycopyrrolate and Formoterol fumarate peaks are calculated. The %Assay by using the standard formula.

Method development and selection of wavelength

UV spectrum of 10 µg/ml Glycopyrrolate and Formoterol fumarate in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 220nm. At this wavelength both the drugs show good absorbance.

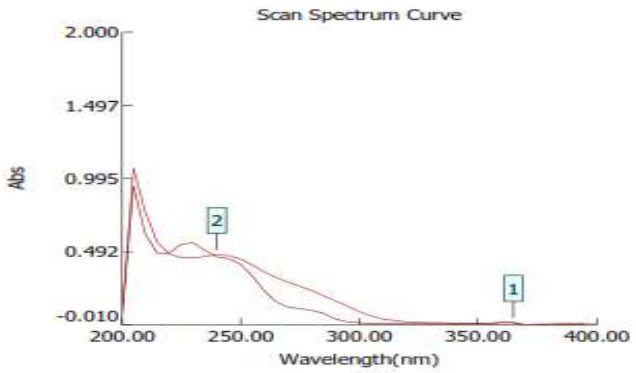

Fig 3: UV Spectra of Glycopyrrolate and Formoterol fumarate for Selection of Wavelength

Construction of calibration curve

Aliquots of different concentrations of standard solution were prepared and their chromatograms were recorded at the optimized chromatographic conditions. The mean peak areas at different concentration levels were calculated from the chromatograms. Then the linearity plot was constructed using the mean peak areas at their respective concentrations. (Figures 8 &9)

Method Of validation

The developed method was validated for linearity, accuracy, precision, and limit of detection, limit of quantitation, robustness and system suitability parameters as described in ICH guidelines.

Linearity

From the stock solution, 4.8,9.6,14.4,19.42,24 ug/ml solutions for Formoterol fumarate and the stock solution, 9,18,27,36,45µg/ml solutions for Glycopyrrolate were made and their chromatograms were recorded. From the recorded chromatograms, their respective mean peak areas were calculated and the linearity plot was constructed using the mean peak areas at their respective concentrations. The correlation coefficient was found to be 0.999. The linearity data of Formoterol fumarate and Glycopyrrolate was shown in Table 2 and table 3, the calibration plot.

Table 1: Showing assay results

S. No	Name of compound	Amount taken (mg)	% purity
1	Glycopyrrolate	9	99.80
2	Formoterol fumarate	4.8	99.72

Table: 2 Linearity results for Formoterol fumarate

S. No	Linearity Level	Concentration (µm/ml)	Area
1	I	4.8	12774
2	II	9.6	228918
3	III	14.4	131638
4	IV	19.2	465502
5	V	24	607979
Correlation Coefficient			0.999

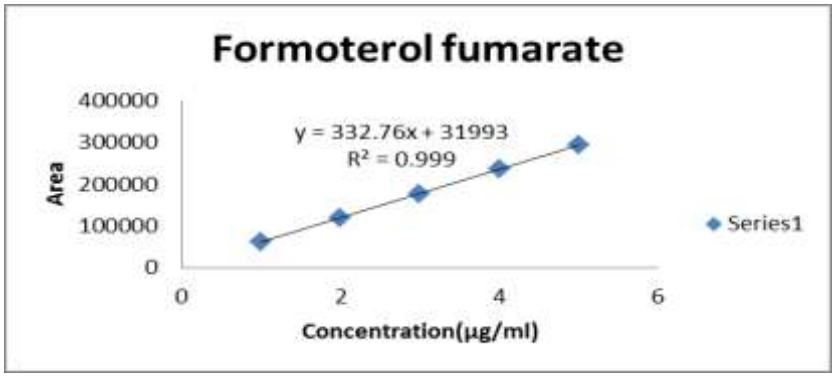

Fig4: Showing calibration graph for Formoterol fumarate

TabeL: 3 Linearity results for Glycopyrrolate

S. No	Linearity Level	Concentration(ppm)	Area
1	I	9	61241
2	II	18	119943
3	III	27	176636
4	IV	36	235363
5	V	45	293580
Correlation Coefficient			0.999

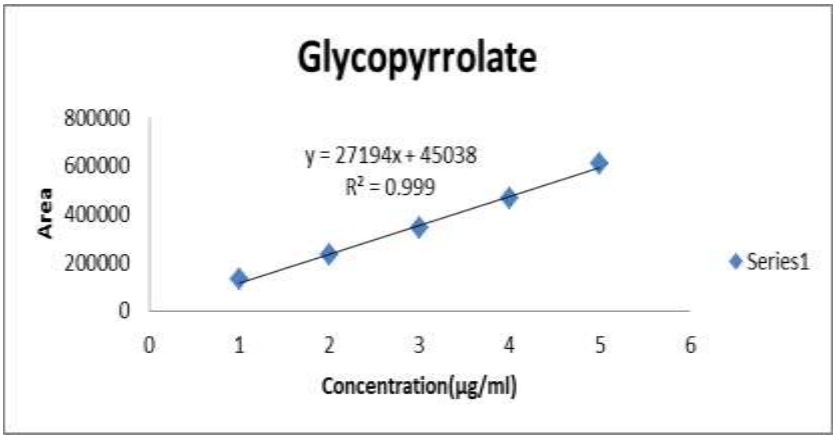

Fig: 5 Showing calibration graph for Glycopyrrolate

RESULTS AND DISCUSSION

The present investigation reported by the authors are to develop a new validated method for the simultaneous estimation of Glycopyrrolate and Formoterol fumarate by RP-HPLC method. Mobile phase contains the mixture of 60% PH 3 Buffer(1ml OPA in 1000ml water) and 40% of Methanol It is used as diluent in the present study. An Xterra column of 5µ (4.6X150mm) is employed for the simultaneous determination of Glycopyrrolate and Formoterol fumarate by RP-HPLC method. A flow rate of 1ml for minute is used in this method. UV detection wavelength at 220mm and temperature of 25^{o}C were maintained. Two sharp peaks were obsorbed at 3.0min and 4.2 min for Formoterolfumarate and Glycopyrrolate respectively. The representative chromatograms of blank solution shown in this figure 6. Glycopyrrolate and Formoterol fumarate Chromatograms of assay of sample injection and standard of sample injection are shown in the figures 7&8 and assay results of purity in the table 1. The % purity of Glycopyrrolate and Formoterol fumarate were found to be 99.80 and 99.72 respectively.

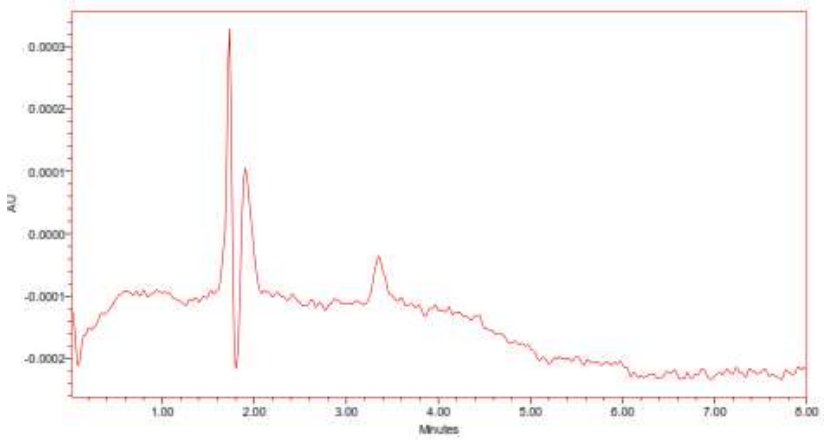

Fig6: Chromatogram showing blank Solution (mobile phase)

Linearity

Figures 9a to 9e represent the chromatograms showing different linearity levels with different concentrations of Glycopyrrolate and Formoterol fumarate and results of given in the tables 2 & 3. Both Glycopyrrolate and Formoterol fumarate obey Beer Lamberts Law in the range of concentrations 4.8µg /ml to 24 µg /ml and 9µg /ml to 45µg/ml respectively with regression equations Y= 27194X+45038(correlation and coefficient) R^2= 0.999 for Formoterrol fumarate and Y= 332.76 X + 31993 , R^2= 0.999. for Glycopyrrolate.

Precision

This validated method is more precise and the percentage of relative standardization (%RSD) and intermediate precision / Ruggedness were found to be 0.3 and 0.3 for Glycopyrrolate and 0.3 and 0.4 for Formoterrol Fumarate. The results are given the in the tables 6 and 7.

System suitability

The results for Glycopyrrolate and Formoterol fumarate are given in the Inhalation. It was performed to ensure that complete testing system was suitable for the intended application. The USP tailing factor for Glycopyrrolate and Formoterol fumarate were 1.87 and 1.84 which is <2 and the USP plate found were 2940 and 3415 which is >2000 the results for actual flow of 1.0ml/min is considered from assay standard. Samples for all shows system suitability results with change in the organic composition in the mobile phase for Glycopyrrolate and Formoterol fumarate chromatograms of and Glycopyrrolate and Formoterol fumarate show in the figures 12 and 13.

Assay

Accurately weigh and transfer 9 mg of Glycopyrrolate and 4.8 mg of Formoterol fumarate working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents

Procedure

20µL of the standard, stock and sample solution are injected into the chromatographic system. The areas are measured for Glycopyrrolate and Formoterol fumarate peaks are calculated.

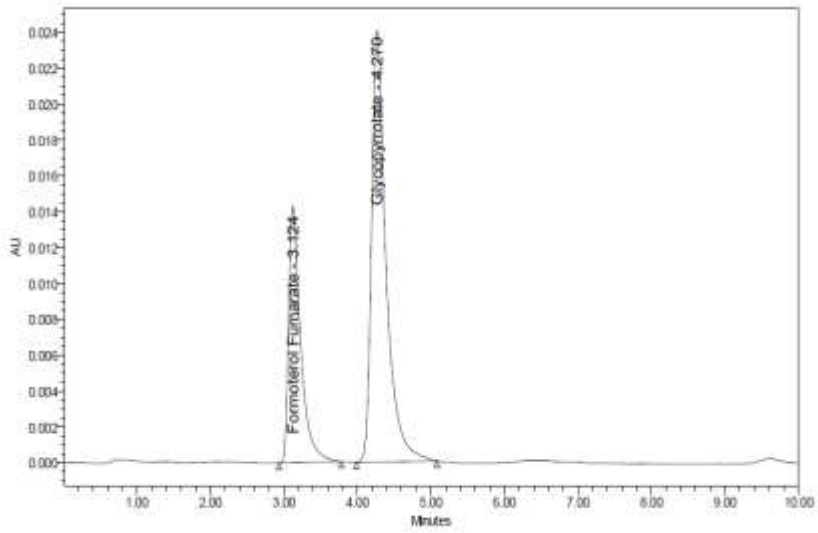

Fig7: Chromatogram showing assay of sample injection

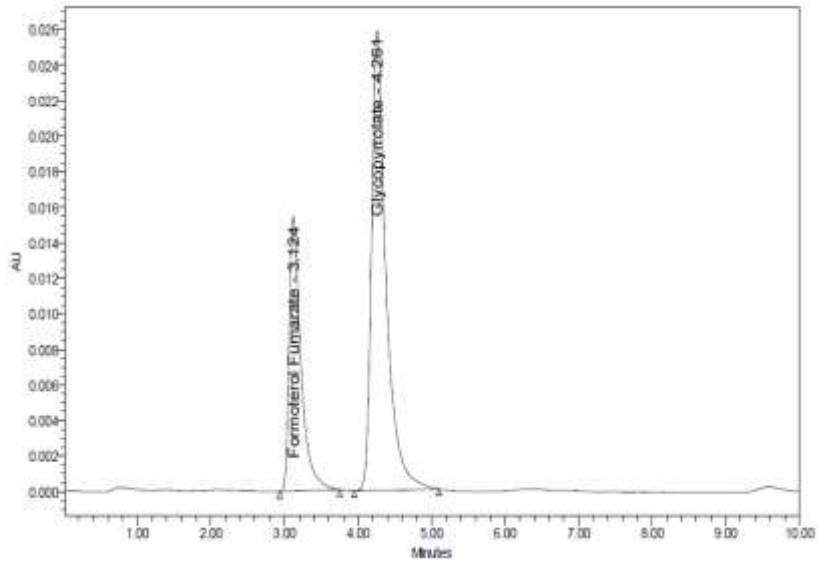

Fig8: Chromatogram showing standard of sample injection

Linearity

From the stock solution, 4.8,9.6,14.4,19.42,24 ug/ml solutions for Formoterol fumarate and the stock solution, 9,18,27,36,45µg/ml solutions for Glycopyrrolate were made and their chromatograms were recorded.

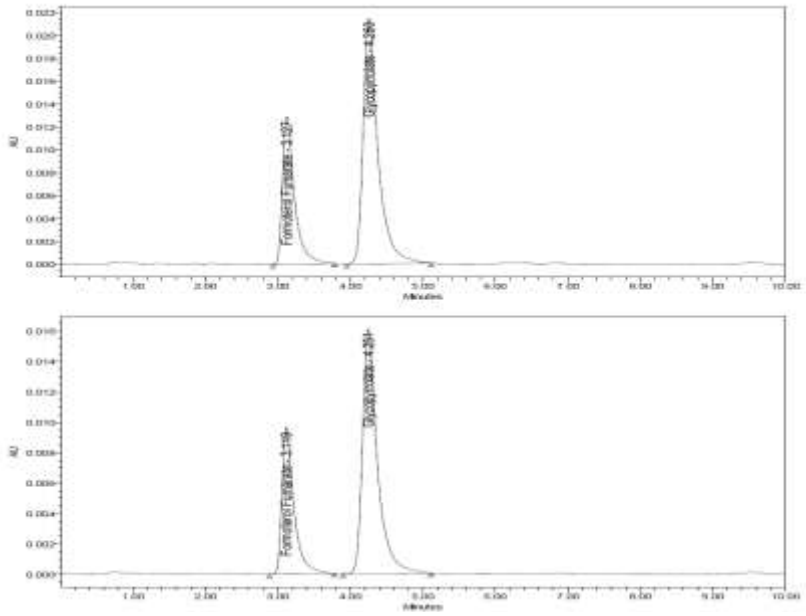

Fig 9.a,b Level 1,2 Chromatograms showing Linearity of Glycopyrrolate and Formoterol fumarate

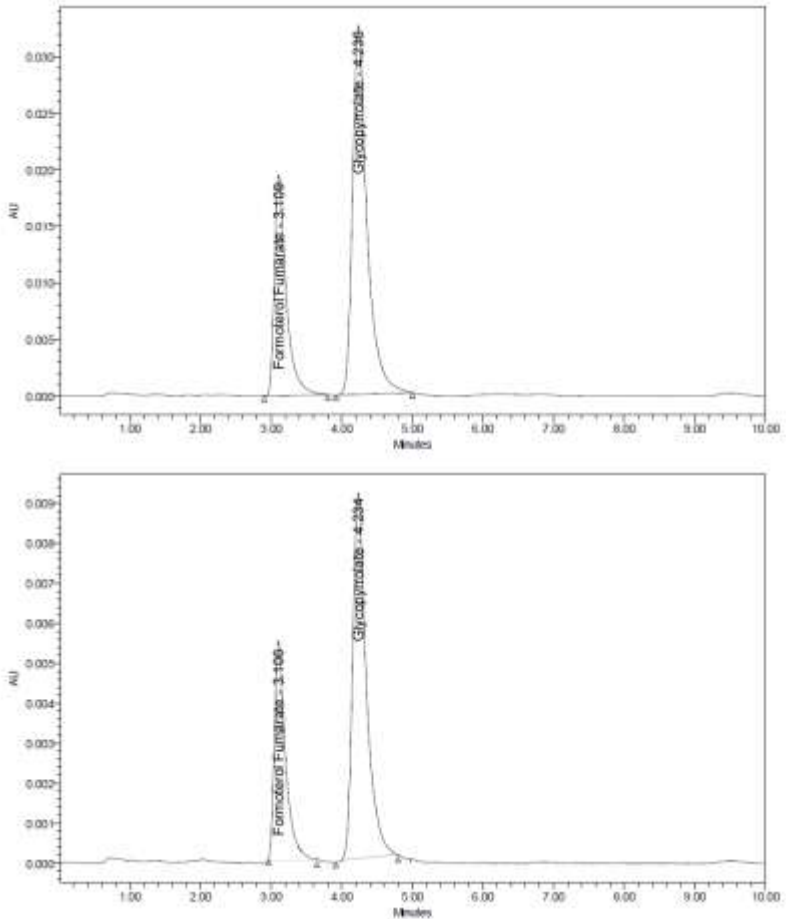

Fig 9c,d. Level 3,4Chromatograms showing Linearity of Glycopyrrolate and Formoterol fumarate

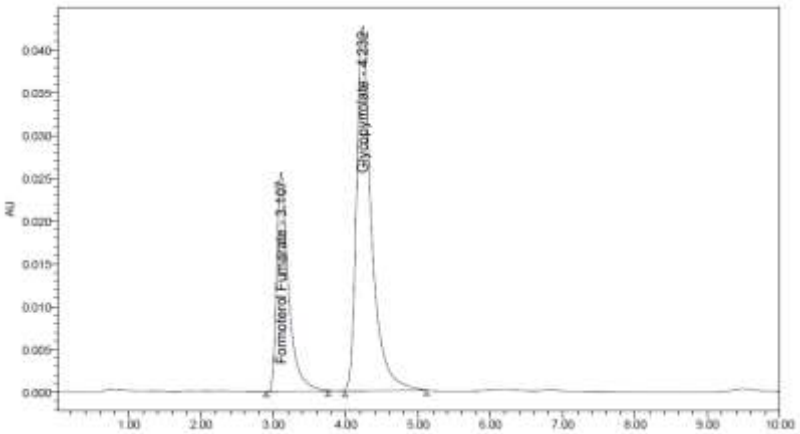

Fig 9.e. Level 5 Chromatograms showing Linearity of Glycopyrrolate and Formoterol fumarate

Accuracy

The accuracy study was performed for 50%, 100% and 150% for Glycopyrrolate and Formoterol fumarate. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery. Shown in Tabel no 4&5

Table 4: Showing accuracy results for Glycopyrrolate

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	172505	4.5	4.47	99.38	100.01
100%	346412	9	8.98	99.78	
150%	525309	13.5	13.62	100.88	

Table 5: Showing accuracy results for Formoterol fumarate

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	85620	2.4	2.40	99.85	100.34
100%	171845	4.8	4.81	100.21	
150%	259676	7.2	7.27	100.95	

Precision

Table 6: Showing% RSD results for Glycopyrrolate and Formoterol fumarate

Injection	Area for Glycopyrrolate	Areafor Formeterolfumarate
Injection-1	341368	178876
Injection-2	340717	177224
Injection-3	342655	179055
Injection-4	343939	178739
Injection-5	343013	176699
Injection-6	342282	179220

Average	342329.0	178302.2
Standard Deviation	1156.8	1064.1
%RSD	0.3	0.6

Intermediate precision/Ruggedness

Table 7: Showing results for intermediate precision of Glycopyrrolate and Formoterol fumarate

Injection	Area for Glycopyrrolate	Areafor Formeterolfumarate
Injection-1	349453	172535
Injection-2	347162	171224
Injection-3	349458	172915
Injection-4	348377	173391
Injection-5	348482	173108
Injection-6	349771	172959
Average	348783.8	172688.7
Standard Deviation	976.1	769.7
%RSD	0.3	0.4

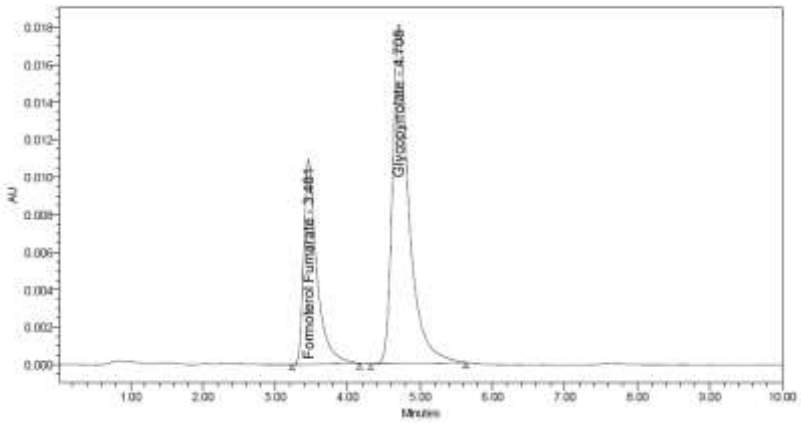

Fig 10: Chromatogram showing less flow rate

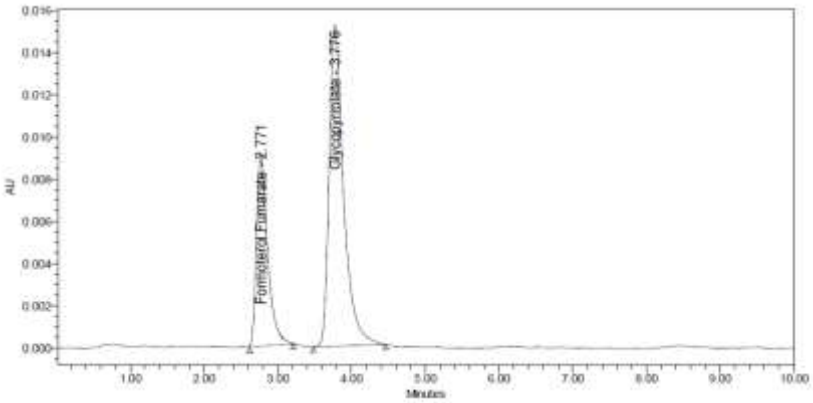

Fig 11: Chromatogram showing more flow rate.

Table: 8 System suitability results for Glycopyrrolate:

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	2452	1.12
2	1.0	2718.66	1.64
3	1.1	2255	1.22

Table: 9 System suitability results for Formoterol fumarate

S. No	Flow Rate (ml/min)	System Suitability Results		
		USP Plate Count	USP Tailing	USP Resolution
1	0.9	2025.5	1.18	3.63
2	1.0	3961.26	1.15	3.82
3	1.1	2644.17	1.13	3.45

***** Results for actual flow (1.0ml/min) have been considered from Assay standard.

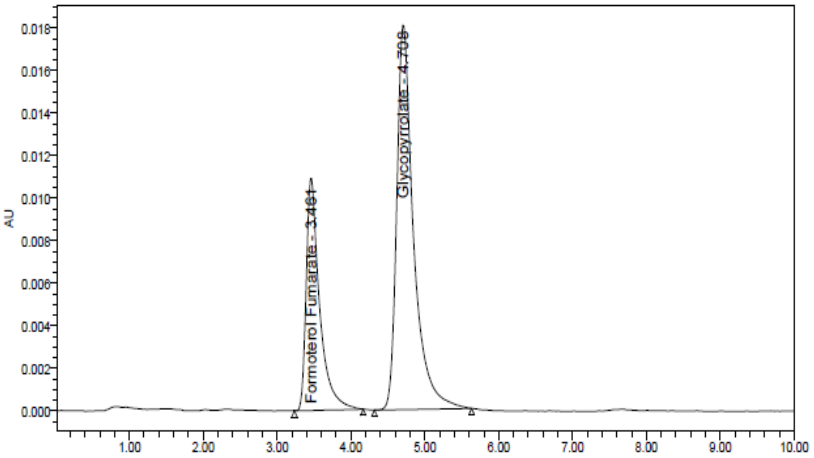

Fig12: Chromatogram showing less organic composition in the mobile phase

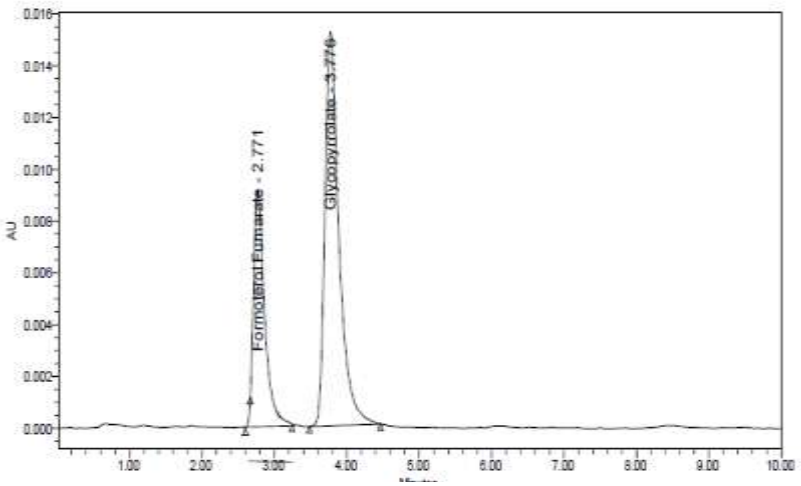

Fig 13: Chromatogram showing more organic composition in the mobile phase

Table 10: Showing system suitability results for Glycopyrrolate

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2452	1.10
2	*Actual	2718.66	1.64
3	10% more	2055.73	1.13

Table 11: Showing system suitability results for Formeterol Fumarate

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results		
		USP Plate Count	USP Tailing	USP Resolution
1	10% less	2025	1.18	3.62
2	*Actual	3961.26	1.15	3.82
3	10% more	3644	1.10	3.45

Detection limit

As per ICH guidelience S/N Ratio value shall be 3 for LOD solution.

As per ICH guidelience S/N Ratio value shall be 10 for LOQ solution.

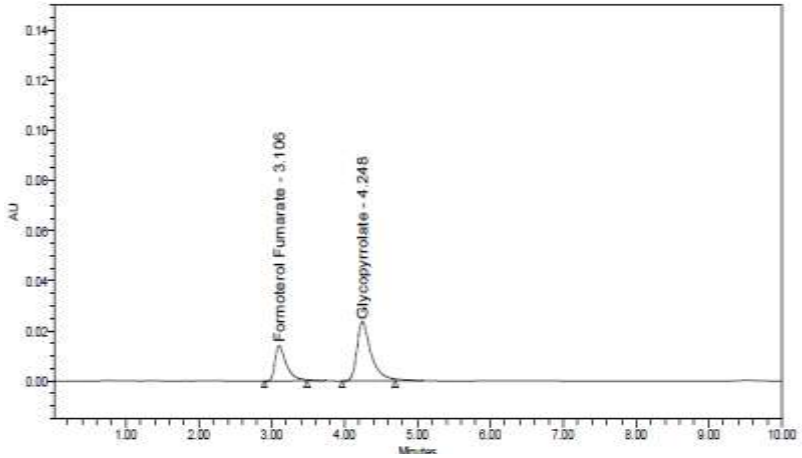

Fig14: Chromatogram showing LOD

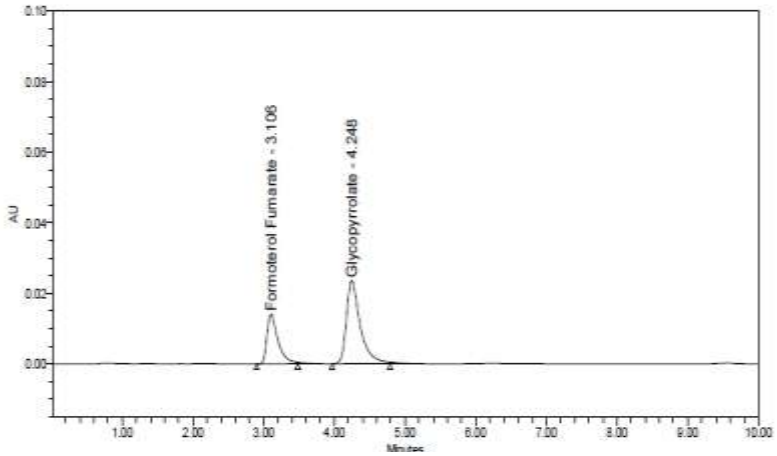

Fig15: Chromatogram showing LOQ

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Glycopyrrolate and Formoterol fumarate pharmaceutical dosage forms. The results are accordance with ICH guidelines. Hence, this method can easily and conveniently adopt for routine quality control analysis of Glycopyrrolate and Formoterol fumarate in pure and its pharmaceutical dosage forms.

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