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RP-HPLC Method Development and Validation for the Simultaneous Estimation of Formoterol and Glycopyrrolate in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A simple, accurate, precise method was developed for the simultaneous estimation of the Formoterol and Glycopyrrolate in tablet dosage form. Chromatogram was run through STD BDS C18 150 x 4.6 mm, 5μ. Mobile phase containing Buffer0.1% OPA: Acetonitrile taken in the ratio 50:50 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 0.1%OPA buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 280 nm. Retention time of Formoterol and Glycopyrrolate were found to be 2.308min and 3.189. %RSD of the Formoterol and Glycopyrrolate were and found to be 1.8 and 0.4 respectively. %Recovery was obtained as 100.56% and 100.37% for Formoterol and Glycopyrrolate respectively. LOD, LOQ values obtained from regression equations of Formoterol and Glycopyrrolate were 0.05, 0.04 and 0.15, 0.11 respectively. Regression equation of Formoterol is y = 35768x + 1043, y = 62509x + 1549 of Glycopyrrolate. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in industries.

Keywords: Formoterol, Glycopyrrolate, RP-HPLC, BDS.

1. 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. 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.

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.

The main aim of the present study is to develop an accurate, precise, sensitive, selective, reproducible and rapid analytical technique for simultaneous estimation of Formoterol, Glycopyrrolate in bulk and its formulation.

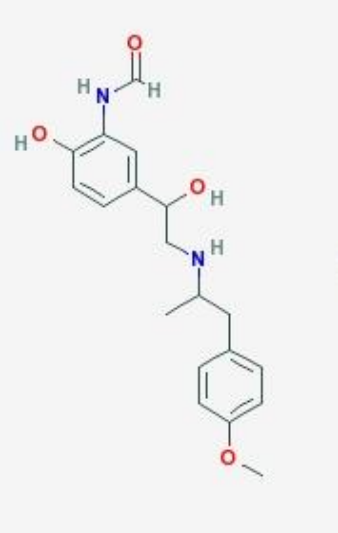

Figure-1: Chemical Structure of Formoterol

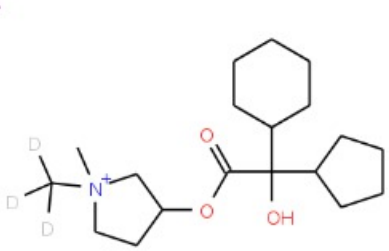

Figure-2: Chemical Structure of Glycopyrrolate

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Formoterol and Glycopyrrolate pure drugs (API), Combination Formoterol and Glycopyrrolate Eye drops (**Formosone**), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

2.2 Instruments and Chromatographic Conditions

Electronics Balance-Denver, P^H meter -BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS HPLC Aquity system equipped with quaternary pumps, UV detector and Auto sampler integrated with Empower 2 Software was used for LC peak integration and Data processing. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz cells integrated with UV-win 6 Software was used for measuring absorbance of Formoterol and Glycopyrrolate solutions. The mobile phase used was 50% 0.1%OPA: 50% Acetonitrile at a flow rate of 1ml/min. samples were analyzed at 220 nm detector wave length and at an injection volume of 10 µL using BDS C8 (4.6 x 150mm, 5µm) with run time of 6 min.

2.3 Preparation of Solvents and Solution Methods:

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

Buffer 0.1%OPA: Accurately 1ml of OPA in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water.

Preparation of Standard stock solutions: Accurately Weighed and transferred 2.4mg&4.5mg of Formoterole and Glycopyrrrolate working Standards into a 50ml clean dry volumetric flask, add 25ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents .From the above stock solution. (48µg/ml of Formoterol and 90µg/ml Glycopyrrolate)

Preparation of Standard working solutions (100% solution): 1ml from stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (4.8µg/ml of Formoterol and 9µg/ml of Glycopyrrolate)

Preparation of Sample solutions: The contents of nasal spray deliveried by 50 actuations (4.8&9 µg each) were collected in 10 ml volumetric flask. Then 8ml acetonitrile was added , sonicated for 25 min and made up to mark to yield 240&450 µg/ml. It was centrifuged for 20 min. Then the supernatant was collected and filtered using 0.45 µm filters using (Millipore, Milford, PVDF)

2ml from sample stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (4.8µg/ml of Formoterol and 9µg/ml of Glycopyrrolate).

2.4 Validation:

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Formoterol (6ppm) and Glycopyrrolate (100ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Precision:

Preparation of Standard stock solutions: Accurately Weighed and transferred 2.4mg&4.5mg of Formoterole and Glycopyrrrolate working Standards into a 50ml clean dry volumetric flask, add 25ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents .From the above stock solution. (48µg/ml of Formoterol and 90µg/ml Glycopyrrolate)

Preparation of Standard working solutions (100% solution): 1ml from stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (6µg/ml of Formoterol and 100µg/ml of Glycopyrrolate)

Linearity:

25% Standard solution: 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (1.2µg/ml of Formoterol and 2.25µg/ml of Glycopyrrolate)

50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (2.4µg/ml of Formoterol and 4.5µg/ml of Glycopyrrolate)

75% Standard solution: 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (3.6µg/ml of Formoterol and 6.75µg/ml of Glycopyrrolate)

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (4.8µg/ml of Formoterol and 9µg/ml of Glycopyrrolate)

125% Standard solution: 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (6.0µg/ml of Formoterol and 11.25µg/ml of Glycopyrrolate)

150% Standard solution: 1.5ml each from two standard stock solutions was pipettede out and made up to 10ml (7.2µg/ml of Formoterol and 13.5µg/ml of Glycopyrrolate)

2.5 Accuracy:

Preparation of Standard stock solutions: Accurately Weighed and transferred 2.4mg&4.5mg of Formoterole and Glycopyrrrolate working Standards into a 50ml clean dry volumetric flask, add 25ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solution. (48µg/ml of Formoterol and 90µg/ml Glycopyrrolate)

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102

2.6 Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Formoterol, Glycopyrrolate, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents.

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Formoterol, Glycopyrrolate, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

2.7 System suitability parameters:

The system suitability parameters were determined by preparing standard solutions of Formoterol (6mcg) and Glycopyrrolate (100mcg) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined to check whether the results complies with Recommended limits.

2.8 Assay of Formoterol and Glycopyrrolate

An Accurately measured weight equivalent to (Bevespi Aerosphere) 0.24 mg and 0.45mg of Formoterol and

Glycopyrrolate respectively was used to perform assay by utilizing the method developed and under the optimized chromatographic conditions. Sample solutions were injected in to the HPLC system and scanned at 220 nm from which the % of drug was estimated.

3. RESULTS AND DISCUSSION

3.1 Optimization of Chromatographic Conditions:

To develop and establish a suitable RP-HPLC method for simultaneous estimation of Formoterol and Glycopyrrolate in bulk and Tablet dosage forms, different preliminary tests were performed and different chromatographic conditions were tested and optimized chromatographic conditions were developed which were given in Table-1.The final analysis was performed by using 50% Ortho phosphoric acid:50% Acetonitrile at a flow rate of 1ml/min. samples were analyzed at 220 nm detector wave length and at an injection volume of 10 µL using BDS C18 4.6 x 150mm, 5µm.with run time of 6 min. The proposed method was optimized to give sharp peak with good resolution and minimum tailing effect for Formoterol and Glycopyrrolate, the optimized chromatogram was obtained as shown in (Figure-3).

Table-1: Optimized Chromatographic Conditions

Parameter	Condition
RP-HPLC	WATERS HPLC SYSTEM equipped with quaternary pumps with PDA detector
Mobile phase	50% OPA (0.1%) : 50% Acetonitrile
Flow rate	1ml/min
Column	BDS C18 (4.6 x 150mm, 5µm)
Detector wave length	220nm
Column temperature	30°C
Injection volume	10µL
Run time	6 min
Diluent	Water and Acetonitrile in the ratio 50:50
Retention Time	Formoterol 2.383 min and Glycopyrrolate 3.216min
Theoretical Plates	Formoterol 6235 and Glycopyrrolate 8354

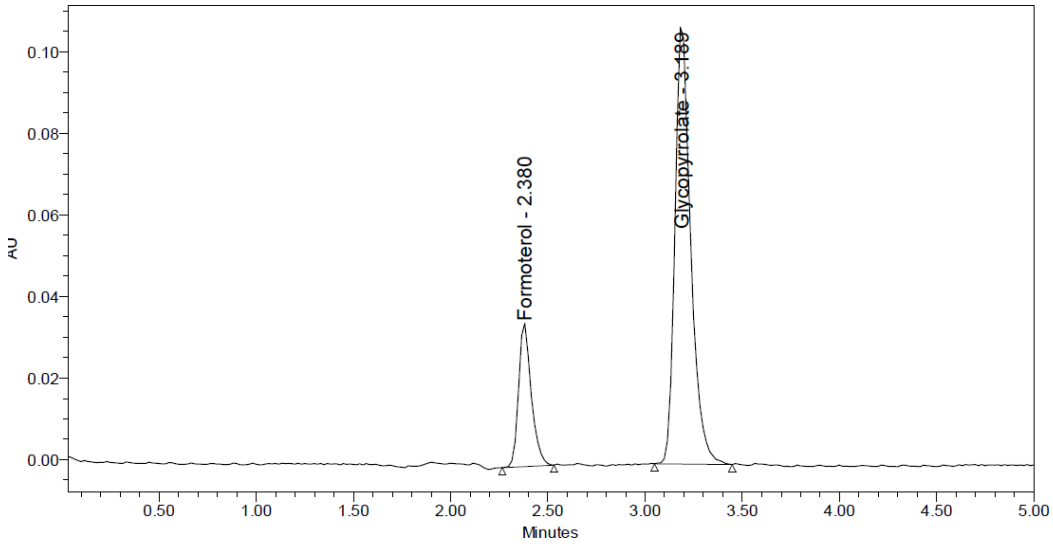

Figure-3: Optimized Chromatogram of Formoterol and Glycopyrrolate

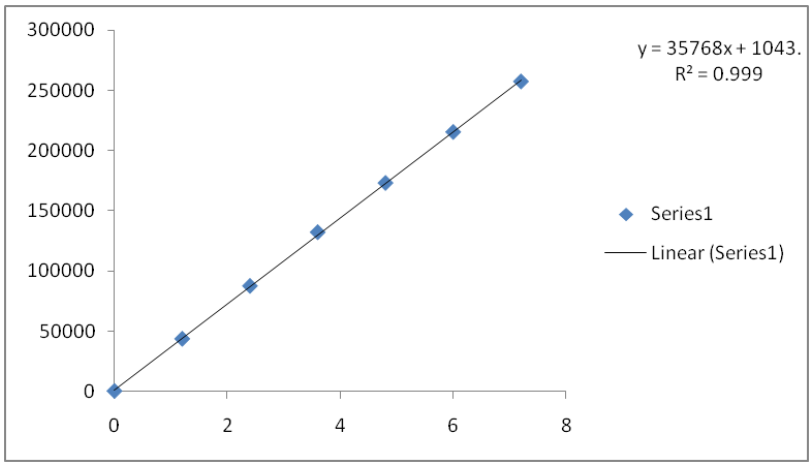

Figure-4: Linearity Curve of Formoterol

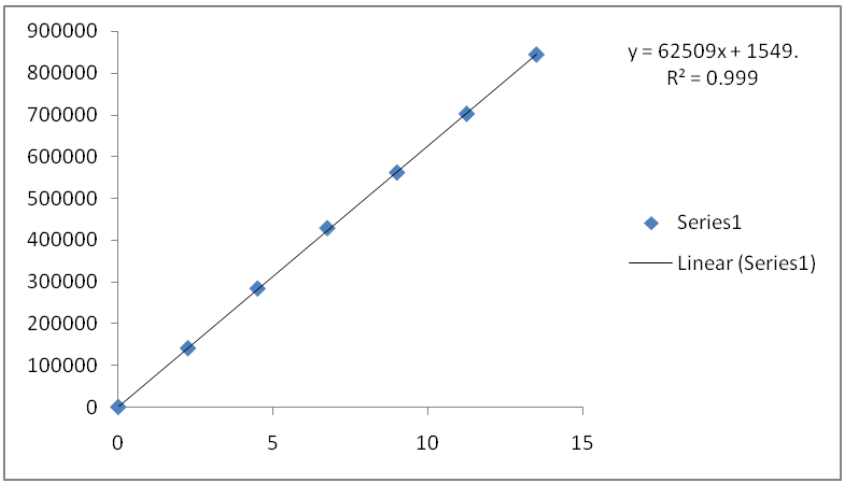

Figure-5: Calibration Curve of Glycopyrrolate

Table-2: Accuracy results of Formoterol and Glycopyrrolate

Conc.	Formoterol			Glycopyrrolate		
	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery
	2.4	2.44	101.67	4.5	4.50	99.92
50%	2.4	2.38	99.37	4.5	4.50	100.09
	2.4	2.41	100.54	4.5	4.51	100.23
	4.8	4.83	100.68	9	8.98	99.77
100%	4.8	4.75	99.04	9	9.13	101.48
	4.8	4.81	100.31	9	9.06	100.63
	7.2	7.21	100.07	13.5	13.56	100.41
150%	7.2	7.15	99.24	13.5	13.62	100.87
	7.2	7.24	100.56	13.5	13.49	99.89
Mean % Recovery			100.16%	Mean % Recovery		100.37%

Table-3: Precision Results of Formoterol and Glycopyrrolate

S.No	Repeatability		Intermediate precision	
	Area of Formoterol	Area of Glycopyrrolate	Area of Formoterol	Area of Glycopyrrolate
1.	168558	524879	171108	559397
2.	165982	511810	172422	558475
3.	166746	522382	170301	559059
4.	167893	519410	171308	561285
5.	170480	519704	169413	560946
6.	169943	505707	170979	558272
Mean	168267	517315.3	170922	559572.3
S.D	1758.7	7183.8	1009.9	1265.7
%RSD	1.0	1.4	0.6	0.2

Table-4: LOD and LOQ values of Formoterol and Glycopyrrolate

Molecule	LOD	LOQ
Formoterol	0.05	0.15
Glycopyrrolate	0.04	0.11

3.2 Validation

Linearity was established for **Formoterol** (1.2-7.2µg/ml) and **Glycopyrrolate** (2.25-13.5µg/ml) at six different concentrations each were injected in a duplicates and average areas were determined and linearity equations were obtained as y = 35768x + 1043 for Formoterol and y = 62509x + 1549 for Glycopyrrolate, Correlation coefficient (R^2) was determined as 0.999 for the two drugs. The Linearity calibration curves were plotted as shown in (Figure-4&5) for Formoterol and Glycopyrrolate, respectively. Retention times of Formoterol and Glycopyrrolate were

2.415min and 3.246 min respectively where no interfering peaks in blank and placebo at retention times of these drugs were not found in this method. So this method holds its specificity. Three levels of Accuracy samples 50%, 100%, 150% were prepared and Triplicates of injections were given for each level of accuracy and mean %Recovery was obtained as 100.16% and 100.37% for Formoterol and Glycopyrrolate respectively were shown in (Table-2).% RSD was calculated from the corresponding peaks obtained by injecting six times a known concentration of Formoterol and Glycopyrrolate the repeatability was obtained as 0.9% and 0.6% respectively for Formoterol and Glycopyrrolate and the % RSD for intermediate Precision was obtained as 1.0%, 1.4% for Formoterol and Glycopyrrolate, Low % RSD values indicates that the method developed was precise as shown in (Table-3). The LOD and LOQ values were evaluated based on Relative standard deviation of response and slope of the calibration curve Formoterol and Glycopyrrolate. The detection limit values were obtained as 0.05 and 0.04 and Quantitation limit were fund to be 0.15 and 0.11 for Formoterol and Glycopyrrolate Respectively as given in (Table-4).

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (65:35) mobile phase plus (55:45) temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner Table -5).. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit (Table -6). Formoterol and Glycopyrrolate pure drugs (API) were obtained from spectrum Pharma research solutions and Rhodes pharmaceuticals, bearing the label claim Formoterol 6mcg, Glycopyrrolate 100mcg. Assay was performed with the above formulation. Average % Assay for Formoterol and Glycopyrrolate obtained was 99.37% and 99.94% respectively the results were shown in (Table-7) and the chromatograms for Formoterol and Glycopyrrolate standard drugs and pharmaceutical dosage forms were shown in (Figure-6, 7) respectively.

Table-5: Robustness Data of Formoterol and Glycopyrrolate

S.No.	Condition	%RSD of Formoterol	%RSD of Glycopyrrolate
1	Flow rate (-) 0.9ml/min	1.1	1.5
2	Flow rate (+) 1.1 ml/min	1.5	0.9
3	Mobile phase (-) 60B:40A	1.3	1.3
4	Mobile phase (+) 50B:50A	1.0	0.7
5	Temperature (-) 25°C	1.4	1.3
6	Temperature (+) 35°C	0.8	1.3

Table-6: System Suitability Parameters Results of Formoterol and Glycopyrrolate

S No	Formoterol			Glycopyrrolate			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.380	6368	1.17	3.216	7739	1.30	6.2
2	2.383	6195	1.25	3.218	8481	1.33	6.1
3	2.385	5460	1.32	3.219	7778	1.25	6.2
4	2.388	6324	1.37	3.225	7206	1.32	6.2
5	2.422	6263	1.02	3.254	9412	1.31	6.4
6	2.429	6802	1.38	3.255	9511	1.30	6.3

Table-7: Assay Results of Formoterol and Glycopyrrolate

S.No	Formoterol			Glycopyrrolate		
	Standard Area	Sample area	% of Drug	Standard Area	Sample area	%of Drug
1.	174237	171108	99.48	561074	559397	101.23
2.	169307	172422	100.2	559198	558475	99.22
3.	168437	170301	99.01	565813	559059	100.03
4.	173893	171308	99.59	560559	561285	99.57
5.	169607	169413	98.49	559024	560946	99.53
6.	175535	170979	99.40	562447	558272	100.05
Mean	171836	170922	99.37	561353	559572.3	99.94
S.D	3052.7	1009.9	0.587	2524	1265.7	0.71
%RSD	1.8	0.6	0.59	0.4	0.2	0.71

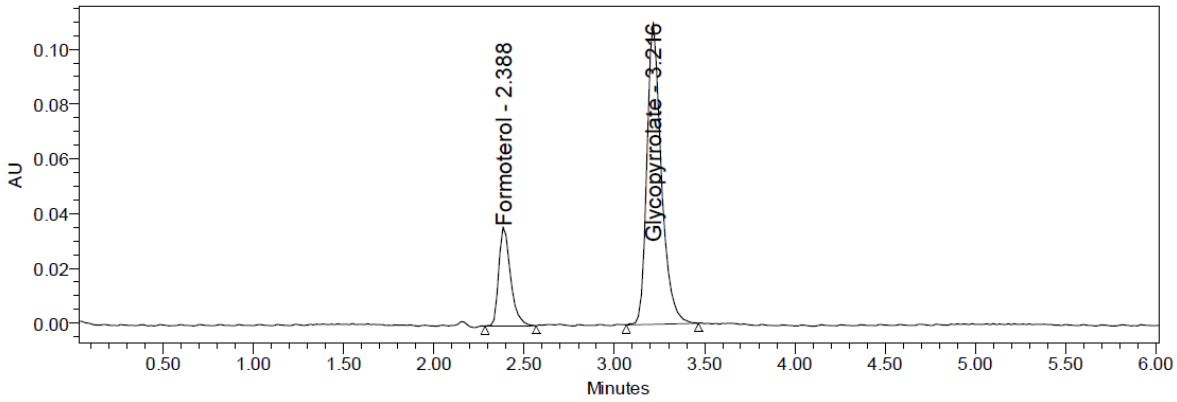

Figure-6: Standard Chromatogram of Formoterol and Glycopyrrolate

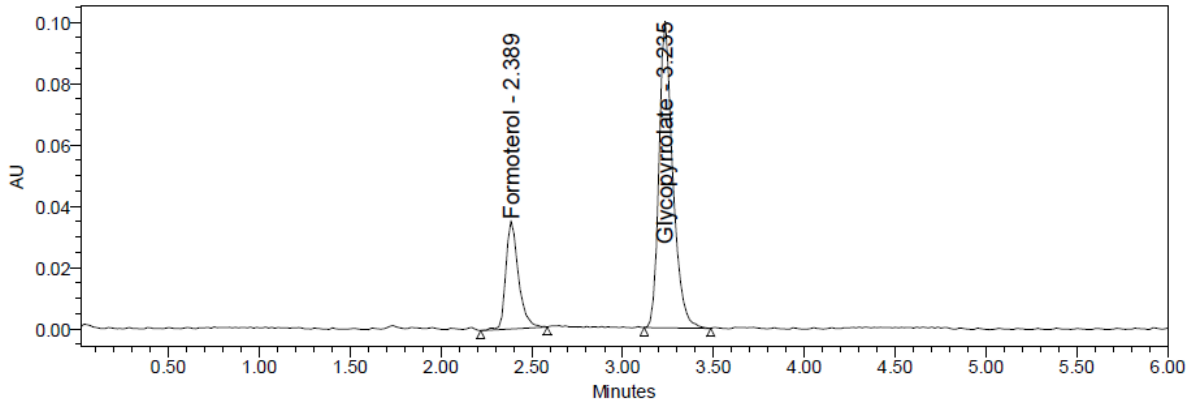

Figure-7: A Sample Chromatogram of Formoterol and Glycopyrrolate in Pharmaceutical Dosage Form

3.3 Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation (Table 8).

Table 8. Degradation Data of Formoterol & Glycopyrrolate

S.No	Degradation Condition	%Drug Degraded	
		Formoterol	Glycopyrrolate
1	Acid	3.58	3.81
2	Alkali	2.97	3.40
3	Oxidation	4.24	2.84
4	Thermal	2.54	2.13
5	UV	0.77	1.98
6	Water	0.75	0.53

4. CONCLUSION

A simple, accurate, precise method was developed for the simultaneous estimation of the Formoterol and Glycopyrrolate in Tablet dosage form. Retention time of Formoterol and Glycopyrrolate were found to be 2.308 min and 3.189. %RSD of the Formoterol and Glycopyrrolate were and found to be 1.8 and 0.4 respectively. %Recovery was obtained as 100.56% and 100.37% for Formoterol and Glycopyrrolate respectively. LOD, LOQ values obtained from regression equations of Formoterol and Glycopyrrolate were 0.05, 0.04and 0.15, 0.11 respectively. Regression equation of Formoterol is y = 35768x + 1043, y = 62509x + 1549 of Glycopyrrolate. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in industries.

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