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RP HPLC Estimation of Indacaterol and Glycopyrrolate in Bulk and Pharmaceutical Dosage form Simultaneously

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Abstract

Simultaneous estimation of Indacaterol and Glycopyrrolate was done simultaneously which is simple, accurate and precise. It was developed with Kromasil C18 with 150x4.6mm, 5 dimensions with 0.1% Ortho phosphor acetate and acetonitrile in the ratio of 55: 45 was pumped through the column at 1.0 ml/min flow rate. Retention time of Indacaterol and Glycopyrrolate was found be 2.319 and 2.830 min respectively. Regression equation of Indacaterol and Glycopyrrolate is y = 21685x + 6734.3, and y = 21025x + 3152.8 respectively. % RSD ofIndacaterol and Glycopyrrolate was 0.8 and 1.1 respectively. % Recovery obtained as 99.68% and 100.058% for Indacaterol and Glycopyrrolate respectively. LOD and LOQ of Indacaterol and Glycopyrrolate are 0.60, 0.23; and 1.82, 0.70 respectively. 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 various industries.

Keywords: Indacaterol; Glycopyrrolate; RP-HPLC; Method Development; ICH Guidelines; Validation

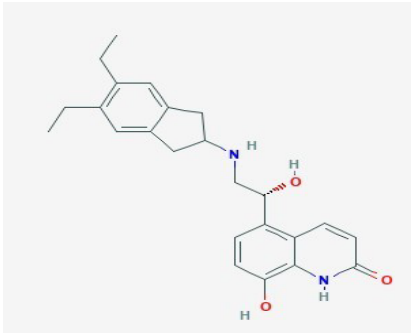
Introduction

The quality of a drug plays an important role in ensuring the safety and efficacy of the drugs [1]. Quality assurance and control of pharmaceutical and chemical formulations is essential for ensuring the availability of safe and effective drug formulations to consumers. Hence Analysis of pure drug substances and their pharmaceutical dosage forms occupies a pivotal role in assessing the suitability to use in patients [2].

The wide variety of challenges is encountered while developing the methods for different drugs depending on its nature and properties [3]. This along with the importance of achieving the selectivity, reproducibility and accuracy of results gives an opportunity for researchers to come out with solution to address the challenges in getting the new methods of analysis to be adopted by the pharmaceutical industry and chemical laboratories [4] Figure 1.

Drug Profile

Indacaterol



IUPAC Name: 5-[(1R)-2-[(5,6-diethyl-2,3-dihydro-1H-inden-2-yl)amino]-1-hydroxyethyl]-8-hydroxy-1H-quinolin-2-one

Figure 1: Structure of Indacaterol.

Mechanism of action: Indacarerol works by stimulating adrenergic beta-2 receptors. They cause relaxation of the muscle by increasing the diameter of airway which beomesconstricted. The pharmacological effects of beta 2 adrenergic agonist drugs which includes indacaterol partly due to intracellular adenyl cyclase stimulation and the enzyme catalyzes the conversion of ATP to cyclic adenosine monophosphate.

Glycopyrroniumbromide: It is a synthetic anticholinergic agent with quaternary ammonium structure, strong muscarinic antagonist used as antispasmodic Figure 2.

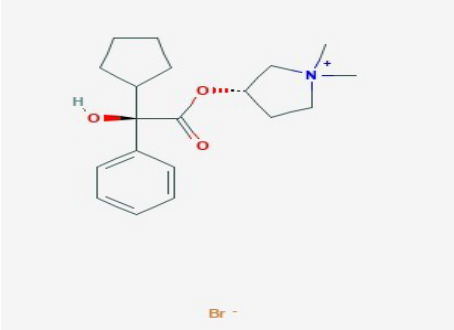


Figure 2: Structure of Glycopyrronium.

IUPAC Name: (1,1-dimethylpyrrolidin-1-ium-3-yl)2-cyclopentyl-2-hydroxy-2-phenylacetateC

Mechanism of Action: Glycopyrrolate binds to the muscarinic acetylcholine receptor in a competitive way. It inhibit the acetyl cholinergic nerve innervated structures, and on smooth muscles that respond to acetylcholine. The peripheral cholinergic receptors are present in smooth muscle, cardiac muscle, and also reduces the volume of gastric secretions.

Method Development

Diluent:

Acetonitrile and water in the ratio of 50:50

Preparation of Standard stock solutions

Weigh 27.5 mg of Indacaterol, 12.5 mg of Glycopyrrolate and transfer into 50 ml volumetric flask , sonicate for 10 min . Prepare 550 µg/ml of Indacaterol and 250 µg/ml Glycopyrrolate.

Preparation of Standard working solutions(100%solution)

1 ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (55µg/ml of Indacaterol and 25 µg/ml of Glycopyrrolate)

- **Preparation of Sample solutions**

❖ The contents of nasal spray deliveried by 50 actuations (110&55 mcgeach) were collected in 100ml volumetric flask. Then 20ml acetonitrile was added , sonicated for 25 min and made up to mark to yield 110 & 500µg/ml. It was centrifuged for 20 min. Then the supernatant was collected and filtered using 0.45 µm filters using (Millipore, Milford, PVDF)

❖ 0.5 ml from sample stock solution was pipette out and taken into a 10ml volumetric flask and made up with diluent. (55µg/ml of Indacaterol and 25 µg/ml of Glycopyrrolate)

- **Preparation of buffer**

% OPA Buffer:

❖ 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

Method Validation

- System suitability

❖ The system suitability parameters were determined by preparing standard solutions of Indacaterol (55ppm) and Glycopyrrolate (25ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined Table 1-10 & Figures 3-21.

Sno	Indacaterol			Glycopyrrolate			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.319	5692	1.3	2.83	6228	1.33	3.8
2	2.319	5690	1.31	2.833	6097	1.32	3.8
3	2.319	5457	1.3	2.834	5982	1.34	3.8
4	2.325	5553	1.32	2.838	6464	1.35	3.8
5	2.325	5516	1.33	2.838	6476	1.41	3.7
6	2.329	5511	1.29	2.84	6576	1.39	3.7

Table 1: System suitability parameters for Indacaterol and Glyco pyrrolate

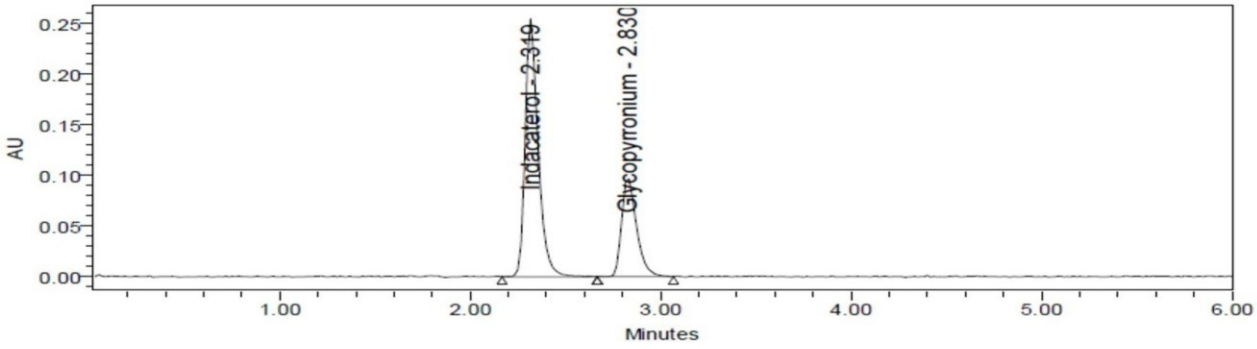


Figure 3: System suitability Chromatogram.

Selectivity/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.

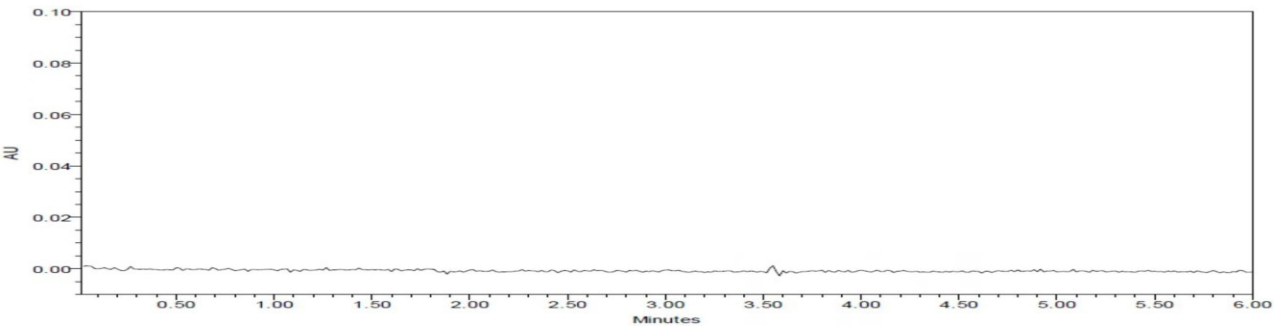


Figure 4: Chromatogram of blank.

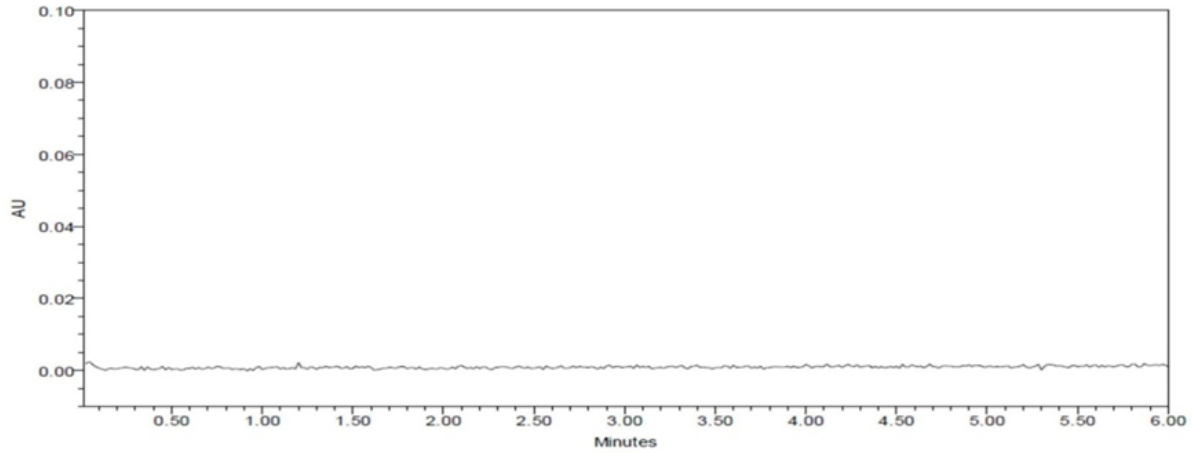


Figure 5: Chromatogram of Placebo.

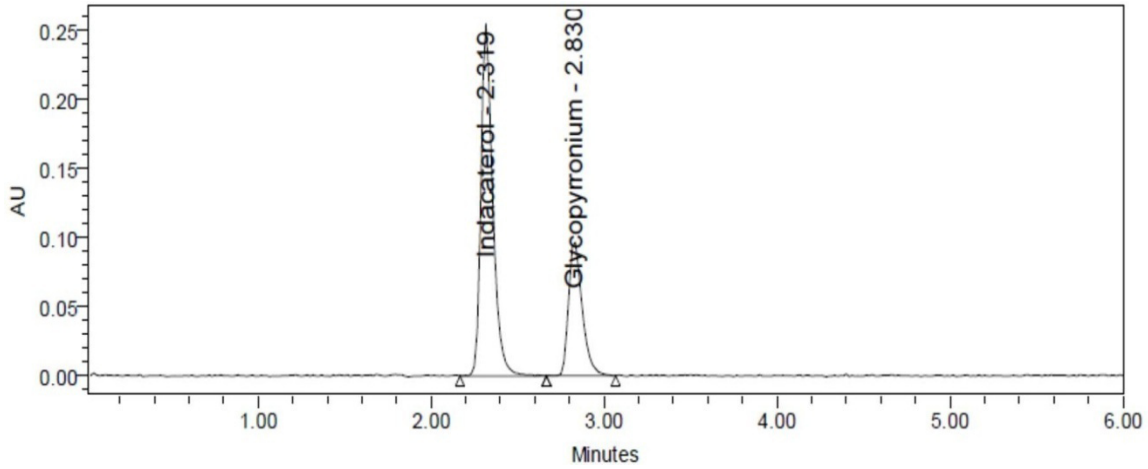


Figure 6: Typical Chromatogram.

Linearity

- **Preparation of Standard stock solutions** Table 2 & Figures 7-14**:**

Indacaterol		Glycopyrrolate	
Conc (µg/ml)	**Peak Area**	**Conc (µg/ml)**	**Peak Area**
0	**0**	**0**	**0**
13.75	304777	6.25	135350
27.5	603612	12.5	260271
41.25	916412	18.75	407184
55	1199024	25	533343
68.75	1491566	31.25	659408
82.5	1793262	37.5	786075

Table 2: Linearity table for Indacaterol and Glycopyrrolate.

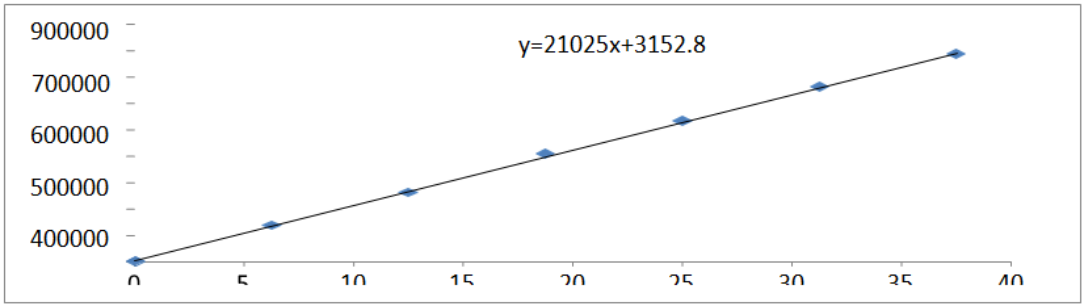


Figure 7: Calibration curve of Indacaterol.

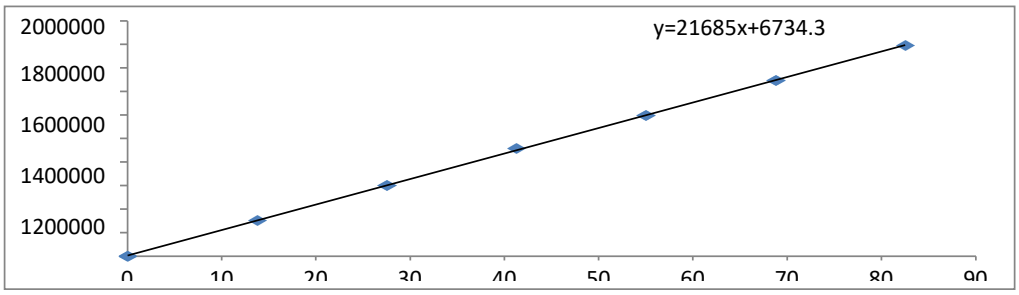


Figure 8: Calibration curve of Glycopyrrolate.

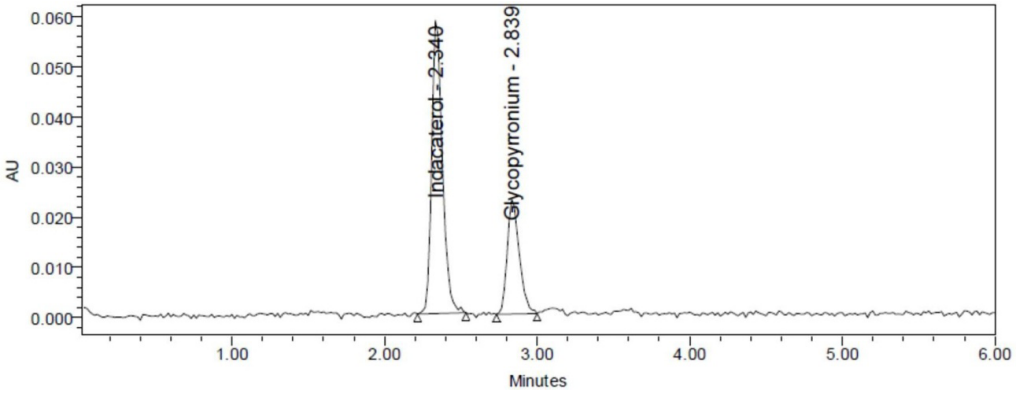


Figure 9: Linearity25% Chromatogram of Indacaterol and Glycopyrrolate.

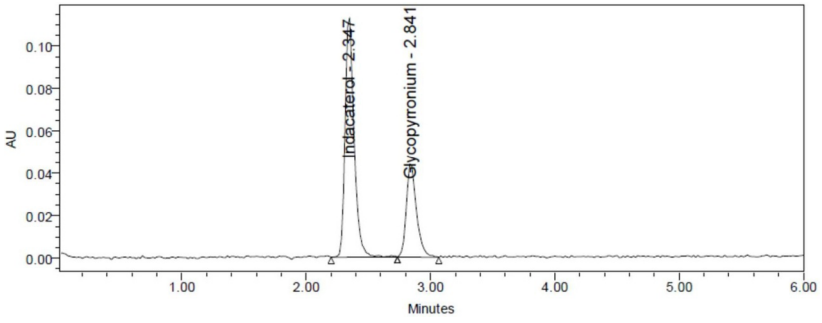


Figure 10: Linearity 50% Chromatogram of Indacaterol and Glycopyrrolate.

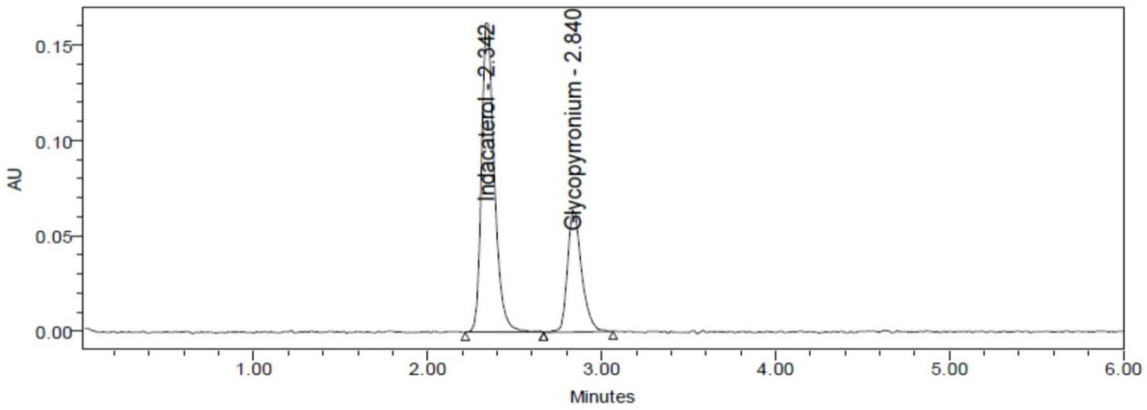


Figure 11: Linearity 75% Chromatogram of Indacaterol and Glycopyrrolate.

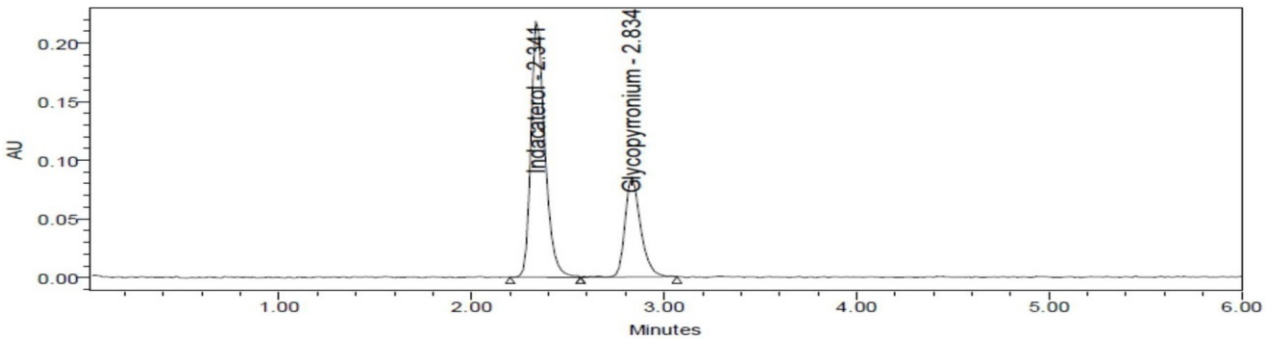


Figure 12: Linearity100% Chromatogram of Indacaterol and Glycopyrrolate.

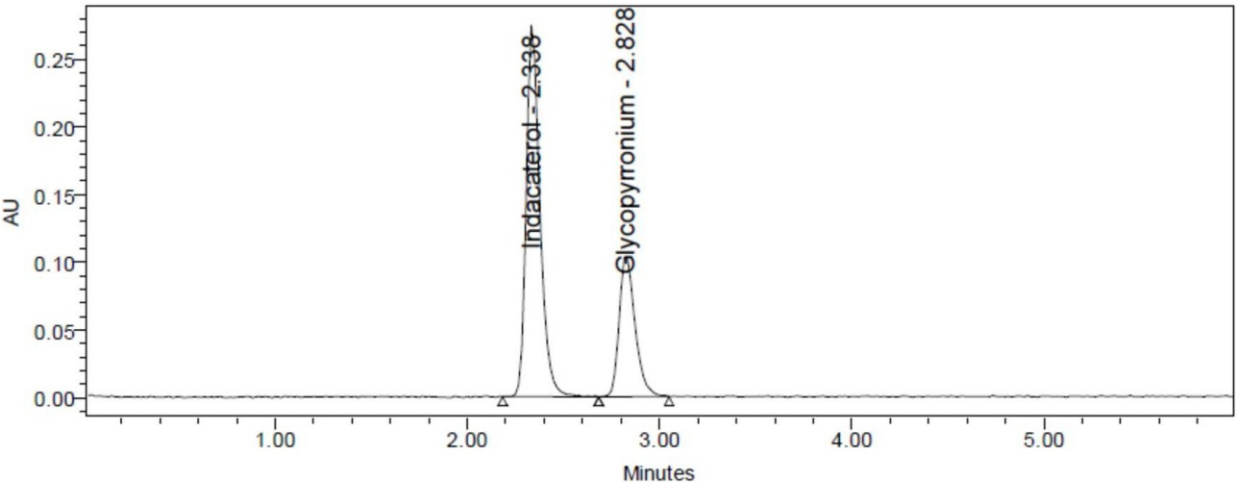


Figure 13: Linearity 125% Chromatogram of Indacaterol and Glycopyrrolate.

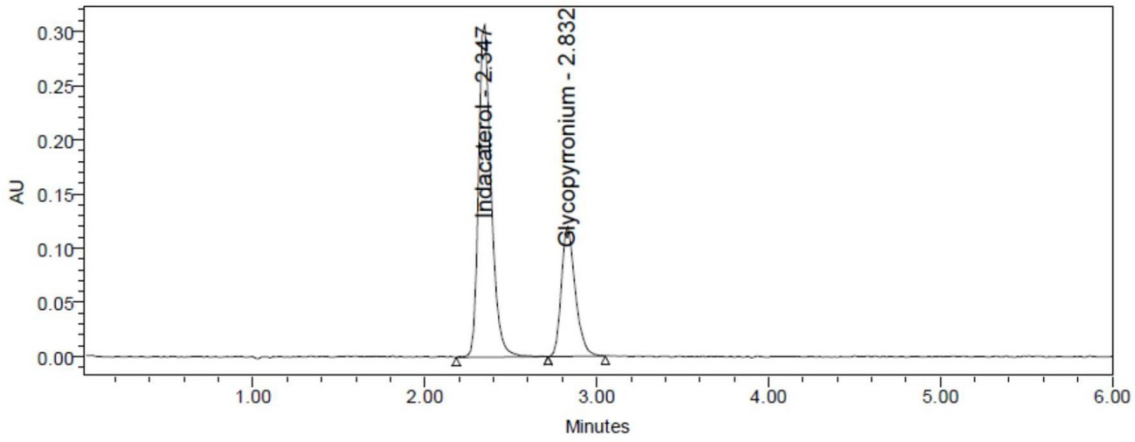


Figure 14: Linearity150% Chromatogram of Indacaterol and Glycopyrrolate.

Precision

- **System Precision**

S.No	Area of Indacaterol	Area of Glycopyrrolate
1	1203206	528596
2	1201745	523155
3	1222702	536672
4	1201920	526263
5	1198667	538094
6	1197466	531124
Mean	1204284	530651
S.D	9278.5	5857.3
%RSD	0.8	1.1

Table 3: System precision table of Indacaterol and Glycopyrrolate

- **Intermediate Precision**

S.No	Area of Indacaterol	Area of Glycopyrrolate
1	1187345	514303
2	1158491	512366
3	1171746	507554
4	1163121	503856
5	1158474	496384
6	1148341	500444
Mean	1164586	505818
S.D	13489.2	6922.6
%RSD	1.2	1.4

Table 4: Intermediate precision table of Indacaterol and Glycopyrrolate.

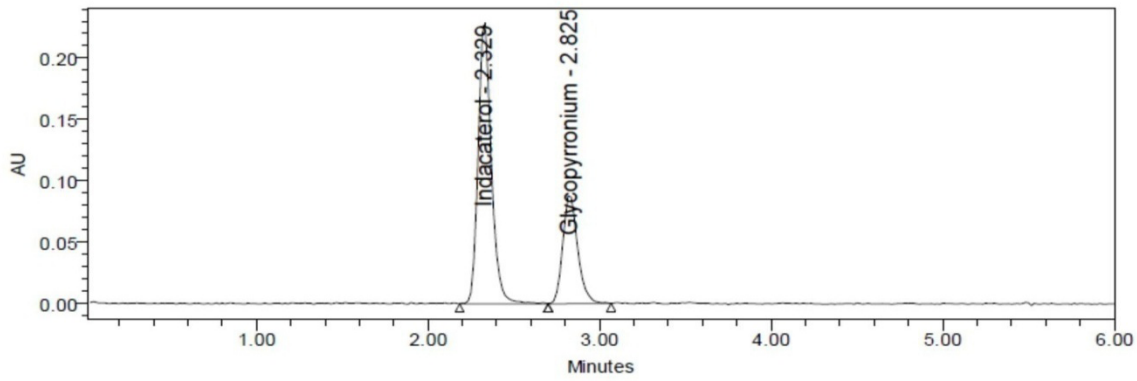


Figure 15: Intra day precision 1.

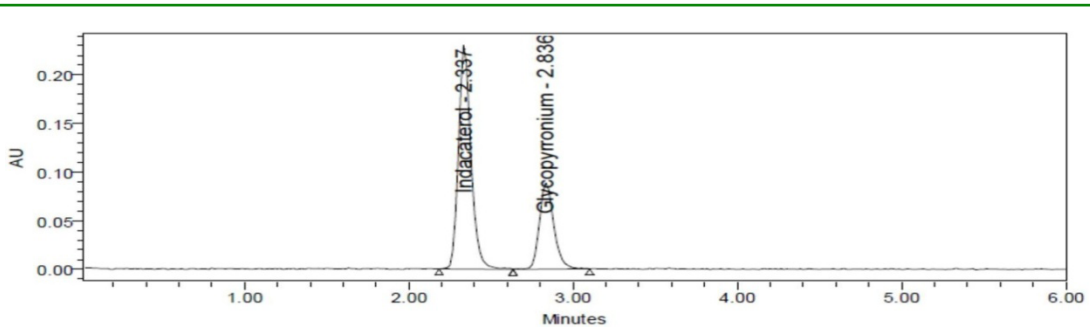


Figure 16: Intraday precision 2.

Accuracy:

%Level	Amount Spiked (µg/mL)	Amount Recovered (µg/mL)	%Recovery	Mean %Recovery
50%	27.5	27.66	100.57	99.68%
	27.5	27.1	98.54	
	27.5	27.34	99.43	
100%	55	54.62	99.32	
	55	55.48	100.87	
	55	55.64	101.16	
150%	82.5	81.04	98.23	
	82.5	81.81	99.16	
	82.5	82.35	99.82	

Table 5: Accuracy table of Indacaterol.

%Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	%Recovery	Mean %Recovery
50%	12.5	12.35	98.8	100.08%
	12.5	12.57	100.53	
	12.5	12.65	101.16	
100%	25	24.88	99.51	
	25	24.76	99.05	
	25	24.98	99.93	
150%	37.5	37.89	101.05	
	37.5	37.66	100.42	
	37.5	37.61	100.29	

Table 6: Accuracy table of Glycopyrrolate.

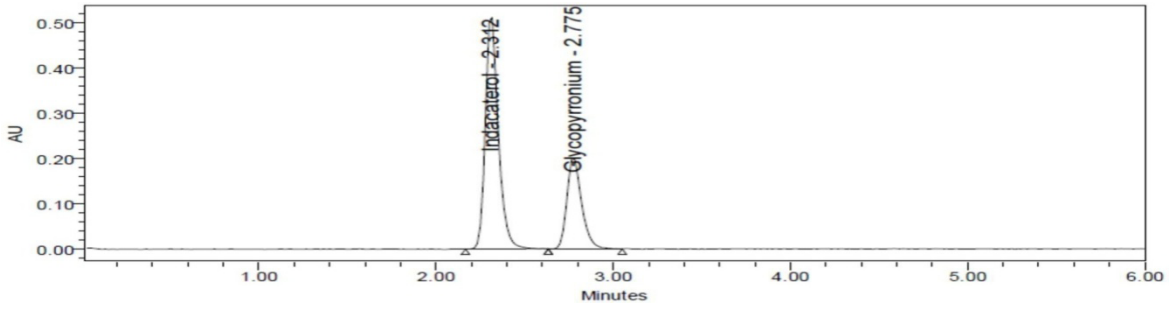


Figure 17: Accuracy 150% Chromatogram of Indacaterol andGlycopyrrolate.

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

S.no	Condition			%RSD of Umeclidinium	% RSD of Vilanterol
1	Flow rate (-)0.9ml/min			1.2	1.7
2	Flow 1.1ml/min	rate	(+)	0.9	0.3
3	Mobile 60B:40A	phase	(-)	0.4	0.5
4	Mobile 50B:50A	phase	(+)	1.4	1.2
5	Temperature(-)25°C 0.6 0.7				
6	Temperature(+)35°C 0.9 1				

Table 7: Robustness data for Indacaterol and Glycopyrrolate.

- **LOD and LOQ**

Molecule	LOD	LOQ
Indacacetarol	0.6	1.8
Glycopyrrolate	0.2	0.7

Table 8: LOD and LOQ of Indacaterol and Glycopyrrolate.

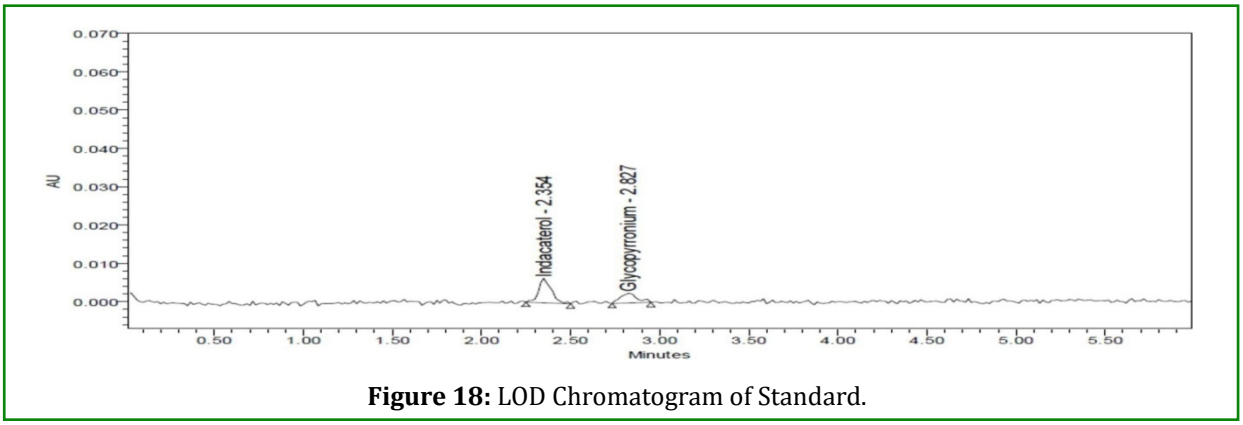


Figure 18: LOD Chromatogram of Standard.

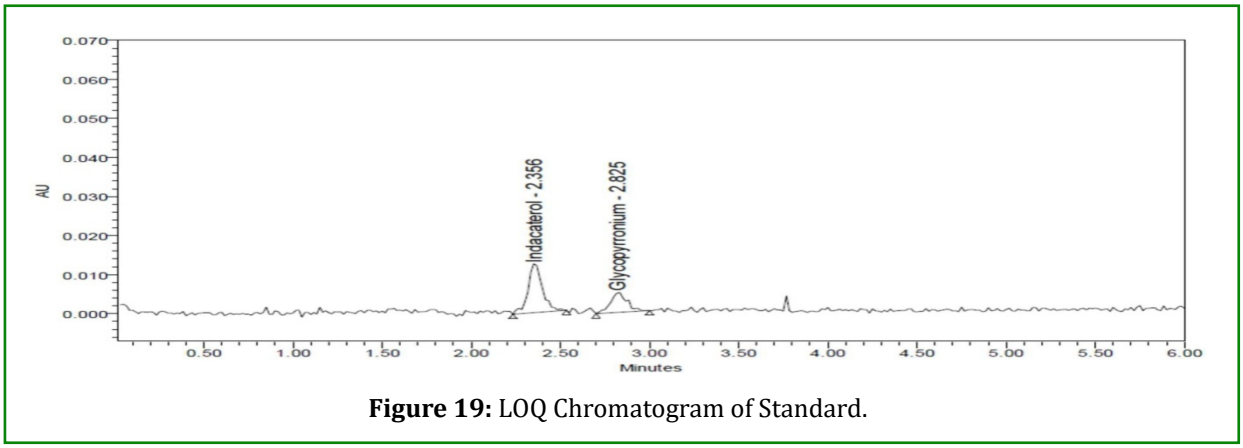


Figure 19: LOQ Chromatogram of Standard.

Assay:

S.no	Standard Area	Sample area	% Assay
1	1203206	1190352	98.65
2	1201745	1215405	100.72
3	1222702	1196663	99.17
4	1201920	1212294	100.46
5	1198667	1214045	100.61
6	1197466	1208358	100.14
Avg	1204284	1206186	99.96
St dev	9278.5	10297.4	0.85
%RSD	0.8	0.9	0.9

Table 8: Assay Data of Indacaterol.

S.no	StandardArea	Samplearea	%Assay
1	528596	528813	99.45
2	523155	531619	99.98
3	536672	533624	100.36
4	526263	540245	101.6
5	538094	531503	99.96
6	531124	529065	99.5
Avg	530651	532478	100.14
St dev	5857.3	4205	0.8
%RSD	1.1	0.8	0.8

Table 9: Assay Data of Glycopyrrolate.

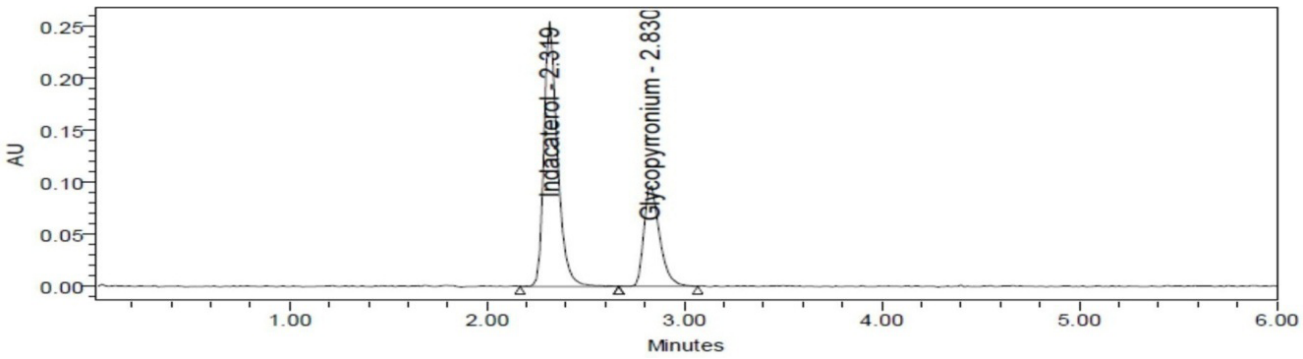


Figure 20: Chromatogram of working standard solution.

Degradation studies:

Type of degradation	Indacaterol			Glycopyrrolate		
	Area	%recovered	% Degraded	Area	%recovery	% Degraded
Acid	1129065	93.57	6.43	498599	93.77	6.23
Base	1146857	95.04	4.96	508159	95.57	4.43
Peroxide	1161643	96.27	3.73	512594	96.4	3.6
Thermal	1174121	97.3	2.7	518082	97.44	2.56
UV	1193866	98.94	1.06	524439	98.63	1.37
Water	1197893	99.27	0.73	528741	99.44	0.56

Table 10: Degradation data.

Dry Heat Degradation Studies:

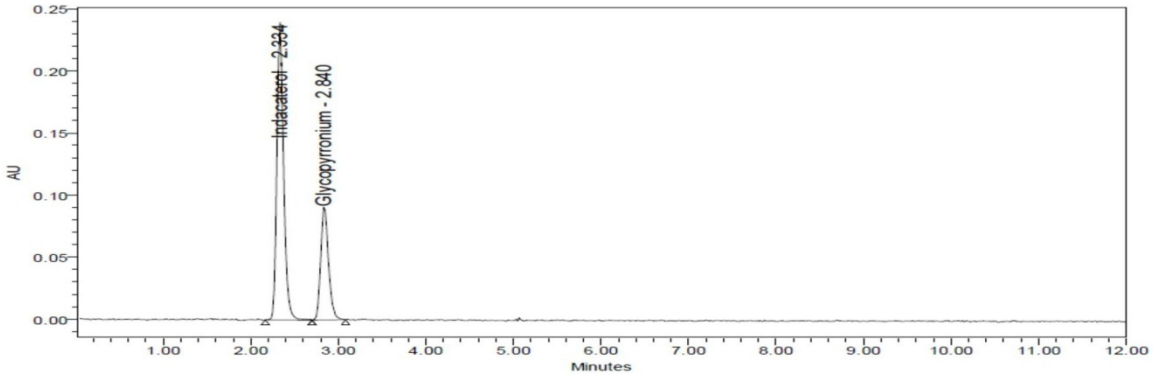


Figure 21: Thermal degradation chromatogram.

Results and Discussion

Retention times of Indacaterol and Glycopyrrolate were 2.319 min and 2.830 min respectively. Six linear concentrations of Indacaterol (13.75-82.5µg/ml) and Glycopyrrolate (6.25-37.5µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Indacaterol was y=21685x+6734.3 and of Glycopyrrolate was y=21025x+ 3152.8. Correlation coefficient to obtained was 0.999 for the two drugs. Six samples were prepared and injected [5-10]. Average area, standard deviation and %RSD were calculated for two drugs and obtained as 1.2% and 1.4% respectively for Indacaterol and Glycopyrrolate. As the limit of Precision was less than "2" the system precision was passed in this method. Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %. Recovery was obtained as 99.68% and 100.08 % for Indacaterol and Glycopyrrolate respectively. Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (60B:40A), mobile phase plus (50B:50A), 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 Table 11.

Parameters	Indacaterol	Glycopyrrolate
Linearity Range (µg/ml)	13.75 - 82.5µg/ml	12.5-37.5 µg/ml
Regression coefficient	0.999	0.999
Slope(m)	21685	21025
Intercept(c)	6734.3	3152.8
Regression equation	y = 21685x	y = 21025x +
(Y=mx+c)	6734.3	3152.8
Assay(%mean assay)	99.96%	100.14%
Specificity	Specific	Specific
System precision% RSD	1.2	1.1
Method precision %RSD	0.7	1.1
Accuracy % recovery	99.68%	100.08%
LOD	0.6	0.23
LOQ	1.82	0.7

Robustness	FM	1.2
	FP	0.9
	MM	0.4
	MP	1.4
	TM	0.6
	TP	0.9

Table 11: Summary of Results.

Conclusion

Simultaneous estimation of Indacaterol and Glycopyrrolate was done simultaneously which is simple, accurate and precise. Retention time of Indacaterol and Glycopyrrolate was found be 2.319 and 2.830 min respectively. It was developed with Kromasil C18 with 150x4.6mm, 5 dimensions with 0.1% Ortho phosphor acetate and acetonitrile in the ratio of 55: 45 was pumped through the column at 1.0 ml/min flow rate. Regression equation of Indacaterol and Glycopyrrolate is y =21685x + 6734.3, and y = 21025x + 3152.8 respectively. %RSD of Indacaterol and Glycopyrrolate was 0.8 and 1.1 respectively [11-13]. % Recovery obtained as 99.68% and 100.058% for Indacaterol and Glycopyrrolate respectively. LOD and LOQ of Indacaterol and Glycopyrrolate are 0.60,0.23; and 1.82, 0.70 respectively. 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 various industries.

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