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Research Article

Stability Indicating RP-HPLC Method Development and Validation for simultaneous estimation of Indacaterol and Glycopyrrolate and in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Indacaterol and Glycopyrrolate in API and pharmaceutical dosage form. Chromatogram was run through Agilent C18 (150 x 4.6 mm, 5µ). Mobile phase containing Buffer 0.01N KH_2PO_4: Acetonitrile taken in the ratio 50:50 was pumped through column at a flow rate of 1.0ml/min. Buffer used in this method was 0.01N KH_2PO_4 buffer. Retention time of Indacaterol and Glycopyrrolate were found to be 2.285min and 2.992 min. %RSD of the Indacaterol and Glycopyrrolate were and found to be 0.3 and 0.6 respectively. %Recovery was obtained as 100.73% and 99.54% for Indacaterol and Glycopyrrolate, respectively. LOD, LOQ values obtained from regression equations of Indacaterol and Glycopyrrolate were 0.45, 1.36 and 0.19, 0.59 respectively. Regression equation of Indacaterol is y = 17065x + 3029, and y = 16589x + 4026, 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.

Key Words: Indacaterol, Glycopyrrolate, RP-HPLC

INTRODUCTION

Indacaterol is a novel, Ultra-Long-Acting, rapid onset β (2)-Adrenoceptor agonist developed for Novartis for the once-daily management of Asthma and Chronic Obstructive Pulmonary Disease. **Glycopyrronium** (as the bromide salt glycopyrrolate) is a synthetic anticholinergic agent with a quaternary ammonium structure. A strong muscarinic antagonist used as an antispasmodic in certain gastrointestinal tract disorders and with some anaesthetics to suppress salivation. The FDA approved glycopyrrolate as a stand-alone treatment for chronic obstructive pulmonary disease (COPD). Literature survey revealed that there were few analytical methods reported for Indacaterol and Glycopyrrolate in RP-HPLC. However, an

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extensive literature search didn't reveal any estimation method for Indacaterol and Glycopyrrolate in API & Pharmaceutical dosage form. Therefore, an attempt has been made to develop and validate simple, precise, accurate economical RP-HPLC method as per ICH guidelines for the estimation of Indacaterol and Glycopyrrolate in Bulk and Pharmaceutical dosage form.

MATERIALS AND METHOD

Indacaterol and Glycopyrrolate pure drugs (API), Combination Indacaterol and Glycopyrrolate bromide inhaler (Sequadra), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

Instruments and Chromatographic Conditions Electronics Balance-Denver, pH meter -BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Indacaterol and Glycopyrrolate solutions.

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

0.01N Potassium dihyrogen Ortho phosphate (KH_2PO_4): Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate **(KH_2PO_4)** 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 then added 1ml of Triethylamine then PH adjusted to 3.5 with dil. Orthophosphoric
acid solution.

Standard stock solution Preparation: Accurately weighed 27.5mg of Indacaterol, 12.5mg of Glycopyrrolate and transferred to 50ml volumetric flask and 3/4th of diluents was added to this flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (550µg/ml of Indacaterol and 250µg/ml of Glycopyrrolate)

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

Sample stock solution Preparation: The contents of nasal spray delivered by 50 actuations (110 mcg &55 mcg each) were collected in 100 ml volumetric flask. Then 20ml acetonitrile was added, sonicated for 25 min and made up to mark to yield 1100 & 500µg/ml. It was centrifuged for 20 min. Then the supernatant was collected and filtered using 0.45 µm filters using (Millipore, Milford, PVDF)

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

Method Validation

As per ICH guidelines the method was validated and the parameters like Linearity, Specificity, Accuracy, Precision, Limit of Detection (LOD) and Limit of Quantitation (LOQ) were assessed.

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.

Linearity: Accurately weighed 27.5mg of Indacaterol, 12.5mg of Glycopyrrolate and transferred to 50ml volumetric flask and 3/4th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (550µg/ml of Indacaterol and 250µg/ml of Glycopyrrolate). Stock solutions of Indacaterol and Glycopyrrolate is taken into 6 different volumetric flasks and diluted to 10ml with diluents. Linearity solutions are prepared such that 0.25, 0.5, 0 .75, 1, 1.25, 1.5ml.

Accuracy: Accurately weighed 27.5mg of Indacaterol, 12.5mg of Glycopyrrolate and transferred to 50ml volumetric flask and 3/4th of diluents was added to these flasks and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (550µg/ml of Indacaterol and 250µg/ml of 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.

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 effected and all the parameters were passed. %RSD was within the limit.

LOD: 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 Indacaterol, Glycopyrrolate, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ: 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 Indacaterol, Glycopyrrolate solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

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System suitability parameters: 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. The % RSD for the area of six standard injections results should not be more than 2%.

Assay: (Sequadra) bearing the label claim Indacaterol 110mcg, Glycopyrrolate 50mcg. Assay was performed with the above formulation. by injecting sample corresponding to equivalent weight into HPLC system.

RESULTS & DISCUSSIONS

Optimization of Chromatographic Conditions: To develop and establish a suitable RP-HPLC method for estimation of Indacaterol 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 0.01N Potassium dihyrogen Ortho phosphate : Acetonitrile (50:50) at a flow rate of 1.0ml/min, samples were analyzed at 210 nm detector wave length and at an injection volume of 10µL using Agilent C18 (4.6 x 150mm, 5µm) with run time of 5min. The proposed method was optimized to give sharp peak and both peaks have good resolution, tailing factor, theoretical plate count and resolution for Indacaterol and Glycopyrrolate, the optimized chromatogram was obtained as shown in (Figure- 3).

Linearity: Linearity was established for six linear concentrations of Indacaterol (13.75-82.5µg/ml) and Glycopyrrolate (6.25-37.5µg/ml) were injected in a duplicate manners Average areas were determined and linearity equations obtained for Indacaterol was y = 17065x + 3029. and of Glycopyrrolate was y = 16589x + 4026 respectively. Correlation coefficient obtained was 0.999 for the two drugs. The Linearity calibration curves were plotted as shown in (Figure-4, 5).

Specificity: Retention times of Indacaterol and Glycopyrrolate are 2.285 min and 2.992 min where no interfering peaks in blank and placebo were found in this method. So, this method holds its specificity.

Accuracy: 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 100.73% and 99.54% for Indacaterol and Glycopyrrolate.

Precision: Intraday precision (Repeatability) with % RSD calculated from the corresponding peaks obtained by injecting six times a known concentration of both Indacaterol and Glycopyrrolate was obtained as 0.5% and the % RSD for Interday precision was obtained as 0.4% and 0.8% for Indacaterol and Glycopyrrolate respectively. Low % RSD values indicates that the method developed was precise as shown in table.

LOD & LOQ: The LOD and LOQ values were evaluated based on Relative standard deviation (%RSD) of response and slope of the calibration curve of the two drugs. The detection limit (LOD) value was obtained as 0.45, 0.19 for Indacaterol

and Glycopyrrolate respectively. Quantitation limit (LOQ) was found to be 1.36 & 0.59 for Indacaterol and Glycopyrrolate respectively as given in (Table-4).

Robustness: Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (55:45), mobile phase plus (45:55), temperature minus (25°C) and temperature plus (35°C) were 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).

Assay: Indacaterol and Glycopyrrolate pure drug (API) was obtained from Spectrum Pharma research solutions, combination dosage form (Sequadra) bearing the label claim Indacaterol 110mcg, Glycopyrrolate 50mcg. Assay was performed with the above formulation. Average % Assay obtained for Indacaterol and Glycopyrrolate was 100.57% and 99.85% and the chromatogram of standard drugs and pharmaceutical dosage forms were shown in (Figure-6,7) respectively.

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).

Conclusion

Chromatographic conditions used are stationary phase Agilent C18 (150mmx 4.6mm, 5µ). Mobile phase is 0.01N Potassium dihyrogen Ortho phosphate **(KH_2PO_4)** : Acetonitrile in the ratio of 50:50 and flow rate was maintained at 1.0ml/min, detection wave length was 210 nm, column temperature was set to 30ºC. Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to150 % levels, R^2 values for Indacaterol and Glycopyrrolate were found to be 0.999 and 0.999 respectively. Precision was found to be 0.4% and 0.8% for Indacaterol and Glycopyrrolate respectively. LOD and LOQ are 0.45 & 0.19 and 1.36 & 0.59 for Indacaterol and Glycopyrrolate respectively. By using above method assay of marketed formulation was carried out 100.57% and 99.85% for Indacaterol and Glycopyrrolate respectively. Degradation studies of Indacaterol and Glycopyrrolate were done, in all condition's purity threshold was more than purity angle and within the acceptable range. Retention times are 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.

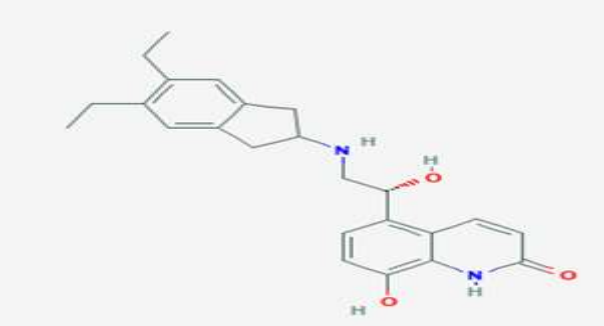

Figure-1: Chemical Structure of Indacaterol

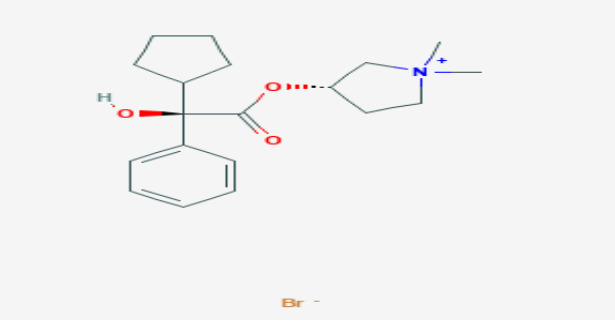

Figure-2: Chemical Structure of Glycopyrrolate

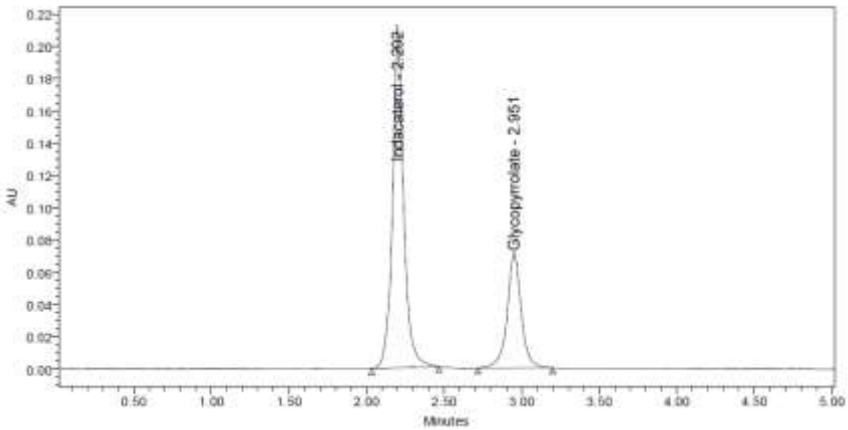

Figure-3: Optimized Chromatogram of Indacaterol and Glycopyrrolate

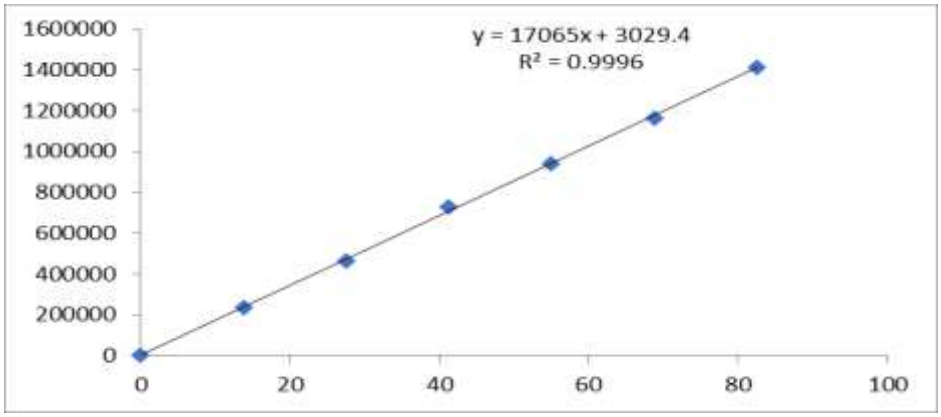

Figure -4: Linearity curve of Indacaterol

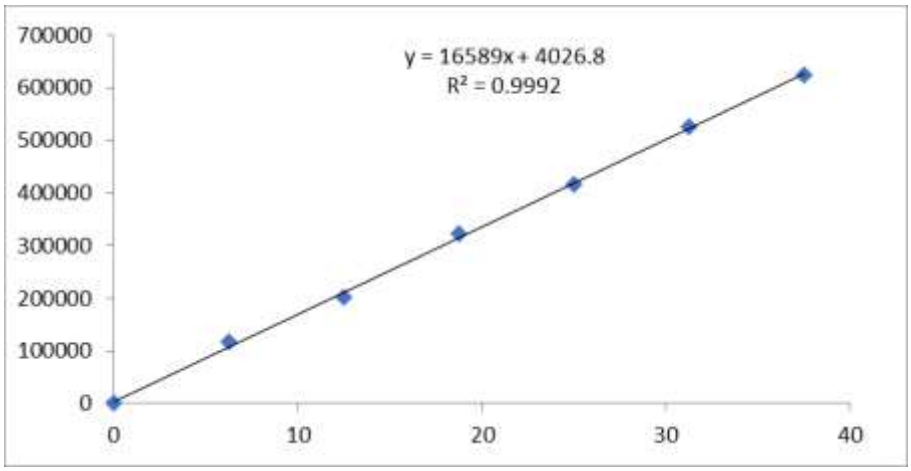

Figure -5: Linearity curve of Glycopyrrolate

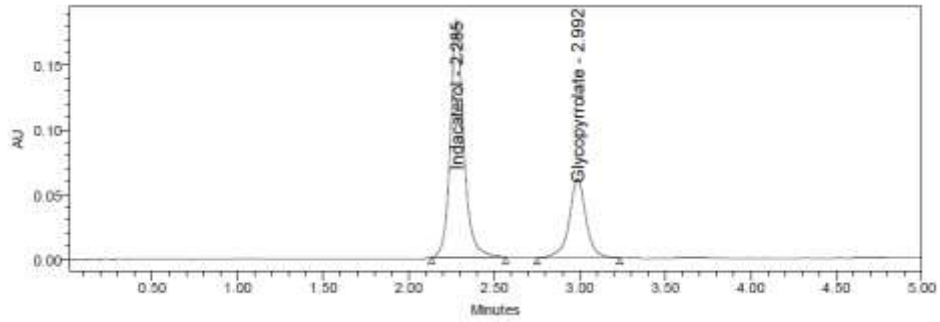

Figure -6: Standard Chromatogram of Indacaterol and Glycopyrrolate

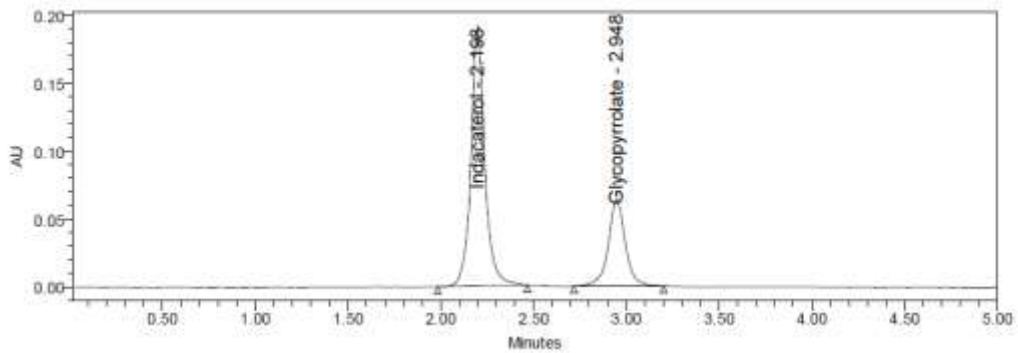

Figure -7: Sample Chromatogram of Indacaterol and Glycopyrrolate

Table 1: Optimized Chromatographic Conditions

Parameter	Conditions
RP-HPLC	WATERS HPLC SYSTEM equipped with quaternary pumps with PDA detector
Mobile Phase	0.01N KH_2PO_4 : Acetonitrile(50:50)
Flow rate	1.0ml/min
Column	Agilent C18(4.6x150mm,5µm)
Injection Volume	10µL
Run Time	5 min
Diluent	Water and Acetonitrile in ratio 50:50
Retention Time	Indacaterol - 2.285 min and Glycopyrrolate - 2.992 min
Theoretical Plates	Indacaterol -4553, Glycopyrrolate - 5050

Table-2: Precision Results of Indacaterol and Glycopyrrolate

s.no	Repeatability (Intraday)		Intermediate Precision (Day-Day Precision)	
	Indacaterol	**Glycopyrrolate**	**Indacaterol**	**Glycopyrrolate**
1	935494	417178	942577	422446
2	940812	419887	933513	418150
3	947658	423602	935320	413101
4	945698	421186	930494	414178
5	942712	419832	936192	419186
6	936843	420892	935698	415908
mean	941536	420430	935632	417162
S.D	4800.8	2101.2	3988.4	3463.0
%RSD	0.5	0.5	0.4	0.8

Table-3: Accuracy results of Indacaterol (Drug 1) and Glycopyrrolate (Drug 2):

%Level	Amount Spiked(µg/ml)		Amount Recovery(µg/ml)		% Recover		Mean % Recovery	
	Drug 1	**Drug 2**	**Drug 1**	**Drug 2**	**Drug 1**	**Drug 2**	**Drug 1**	**Drug 2**
50%	27.5	12.5	27.88	12.43	101.36	99.41	100.73%	99.54%
	27.5	12.5	27.42	12.45	99.70	99.64		
	27.5	12.5	27.72	12.40	100.79	99.24		
100%	8	25	55.70	24.88	101.27	99.53		
	55	25	55.70	24.88	101.47	99.53		
	55	25	55.81	24.98	101.12	99.90		
	55	25	55.62	25.00	100.06	100.00		
150%	82.5	37.5	82.55	37.20	100.00	99.21		
	82.5	37.5	82.50	37.20	100.84	99.21		
	82.5	37.5	83.19	37.41	101.36	99.75		

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

Molecule	LOD	LOQ
Indacaterol	0.45	1.36
Glycopyrrolate	0.19	0.59

Table-5 Robustness Data of Indacaterol and Glycopyrrolate

S.no	Condition	%RSD of Indacaterol	%RSD of Glycopyrrolate
1	Flow rate (-) 0.9ml/min	0.1	1.1
2	Flow rate (+) 1.1ml/min	0.3	0.7
3	Mobile phase (-) 55B:45A	0.2	0.5
4	Mobile phase (+) 45B:55A	0.3	0.8
5	Temperature (-) 25°C	0.6	0.8
6	Temperature (+) 35°C	0.1	0.2

Table-6 System Suitability Parameters for Indacaterol and Glycopyrrolate

S no	Indacaterol			Glycopyrrolate			
Inj	RT(min)	USP Plate Count	Inj	RT(min)	USP Plate Count	Inj	RT(min)
1	2.189	4783	1	2.189	4783	1	2.189
2	2.190	4628	2	2.190	4628	2	2.190
3	2.201	5122	3	2.201	5122	3	2.201
4	2.202	4900	4	2.202	4900	4	2.202
5	2.204	4736	5	2.204	4736	5	2.204
6	2.285	4553	6	2.285	4553	6	2.285

Table-7 Assay Results of Indacaterol and Glycopyrrolate

S.no	% Assay Indacaterol	% Assay Glycopyrrolate
1	99.92	100.27
2	100.49	100.92
3	101.22	101.81
4	101.01	101.23
5	100.69	100.91
6	100.07	101.16
Avg	100.57	101.05
SD	0.51	0.5
%RSD	0.5	0.5

Table-8 Degradation Data for Indacaterol and Glycopyrrolate

Type of Degradation	Indacaterol		Glycopyrrolate	
	% Degraded	% Recorded	% Degraded	% Recorded
Acid	94.16	5.84	94.30	5.70
Alkali	95.87	4.13	95.91	4.09
Oxidation	95.88	4.12	95.48	4.52
Thermal	98.10	1.90	96.54	3.46
UV	99.36	0.64	99.09	0.91
Water	99.71	0.29	99.33	0.67

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