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A validated stability indicating RP-HPLC method development and validation of for simultaneous estimation of indacaterol and glycopyrrolate in pharmaceutical dosage form

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## Abstract

A simple, Accurate, precise method was developed for the simultaneous estimation of the Indacaterol and Glycopyrronium in the bulk and pharmaceutical dosage form. The chromatogram was run through Std Denali C18150 x 4.6 mm, 5m. Mobile phase containing Buffer 0.1%OPA: Acetonitrile taken in the ratio 55:45 was pumped through the column at a flow rate of 0.8 ml/min. The buffer used in this method was 0.1% OPA buffer. The temperature was maintained at 30°C. The optimized wavelength selected was 230.0 nm. The retention time of Indacaterol and Glycopyrronium were found to 2.323min and 3.140 min. %RSD of the Indacaterol and Glycopyrronium were %RSD found to be 0.2% and 0.2% respectively. %Recovery was obtained as 99.39% and 99.41% for Indacaterol and Glycopyrronium respectively. LOD, LOQ values obtained from regression equations of Indacaterol and Glycopyrronium were 1.08, 3.28, and 0.25, 1.47 respectively. Regression equation of Indacaterol is  $y = 24501x + 7142.3$ ,  $y = 26335x + 7822.7$  of Glycopyrronium. 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 tests in Industries.

**Keywords:** Indacaterol, Glycopyrronium, RP-HPLC.

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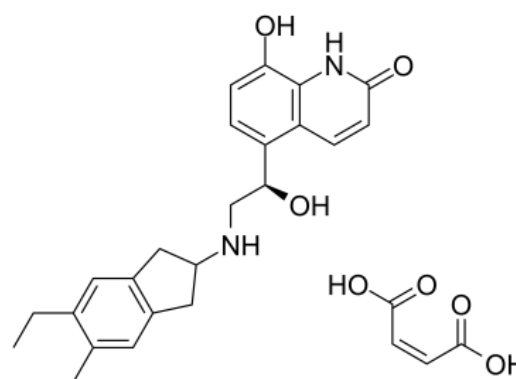
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## Introduction

Indacaterol Maleate (IND) is chemically known as 2-[(5,6-Diethyl-2,3-dihydro-1H-inden-2-yl)amino]-1-hydroxyethyl]-8-hydroxyquinolin-2(1H)-one (Figure 1). IND stimulate adrenergic  $\beta_2$  receptors in the smooth muscle of the airways. IND prevents airway spasms caused by chronic obstructive pulmonary disease (COPD). This drug is indicated for the treatment of COPD. This causes relaxation of the muscle, thereby increasing the diameter of the airways, which becomes constricted in asthma and COPD [1,2,3].



**Fig 01: chemical structure of Indacaterol Maleate**

Glycopyrronium bromide (GLY) is a chemically 1, 1 - dimethylpyrrolidin-1-ium-3-yl 2- cyclopentyl-2-hydroxy phenyl acetate bromide (Figure 2). GLY is a synthetic anticholinergic agent with a quaternary ammonium structure. It reduces secretions in the mouth, throat, airways, and stomach before surgery [4,5]. It used along with other medicines to treat peptic ulcers. The combination of Indacaterol Maleate and

Glycopyrronium Bromide mainly used as  $\beta_2$  adrenoreceptor -agonist and anticholinergic agent with a quaternary ammonium structure and widely used in COPD [6].

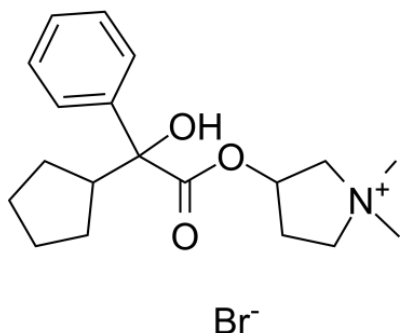


Fig 02: chemical structure of Glycopyrronium bromide

The deep literature review revealed that various analytical methods like spectrophotometric, HPLC, HPTLC, stability indicating HPLC, LC-MS and other methods are reported for estimation of IND and GLY individually and in combined with other dosage form and in biological fluids but none of the analytical method is reported for simultaneous estimation of both the drugs in combined pharmaceutical dosage form [7-13]. Therefore, there is a challenge to develop RP-HPLC and UV spectrophotometric method for the simultaneous estimation of Indacaterol maleate and Glycopyrronium bromide. The present study was involved in a research effort aimed at developing and validating a simple, specific, accurate, economical, and precise RP-HPLC and Absorption correction UV spectrophotometric method for the simultaneous estimation of two drugs in pharmaceutical dosage form.

## Experimental work

### Materials

Indacaterol and Glycopyrrate pure drugs (API), Combination Indacaterol and Glycopyrrate 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

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 Glycopyrrate solutions.

## Methods

### Diluent

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

### Preparation of Standard stock solutions

Accurately weighed 55mg of Indacaterol, 25mg of Glycopyrrate 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. (1100 $\mu$ g/ml of Indacaterol and 500 $\mu$ g/ml of Glycopyrrate).

### Preparation of Standard working solutions (100% solution)

1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (110 $\mu$ g/ml of Indacaterol and 50 $\mu$ g/ml of Glycopyrrate).

### Preparation of Sample solutions

The contents of nasal spray delivered by 50 actuations (110&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 & 500 $\mu$ g/ml. It was centrifuged for 20 min. Then the supernatant was collected and filtered using 0.45  $\mu$ m filters using (Millipore, Milford, PVDF).

1ml from sample stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (110 $\mu$ g/ml of Indacaterol and 50 $\mu$ g/ml of Glycopyrrate).

### Preparation of buffer

#### 0.1%OPA Buffer

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

### Validation [14,15]

#### System suitability parameters

The system suitability parameters were determined by preparing standard solutions of Indacaterol (110ppm) and Glycopyrrate (50ppm) 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 Sample solutions**

The contents of nasal spray delivered by 50 actuations (110 & 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 & 500 µg/ml. It was centrifuged for 20 min. Then the supernatant was collected and filtered using 0.45 µm filters using (Millipore, Milford, PVDF). 1ml from sample stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (110 µg/ml of Indacaterol and 50 µg/ml of Glycopyrrolate)

### **Linearity**

#### **Preparation of Standard stock solutions**

Accurately weighed 55mg of Indacaterol, 25mg 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. (1100 µg/ml of Indacaterol and 500 µg/ml of Glycopyrrolate)

#### **25% Standard solution**

0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (27.5 µg/ml of Indacaterol and 12.5 µg/ml of Glycopyrrolate)

#### **50% Standard solution**

0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (55 µg/ml of Indacaterol and 25 µg/ml of Glycopyrrolate)

#### **75% Standard solution**

0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (82.5 µg/ml of Indacaterol and 37.5 µg/ml of Glycopyrrolate)

#### **100% Standard solution**

1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (110 µg/ml of Indacaterol and 50 µg/ml of Glycopyrrolate)

#### **125% Standard solution**

1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (137.5 µg/ml of Indacaterol and 62.5 µg/ml of Glycopyrrolate)

#### **150% Standard solution**

1.5ml each from two standard stock solutions was pipetted out and made up to 10ml (165 µg/ml of Indacaterol and 75 µg/ml of Glycopyrrolate)

### **Accuracy**

#### **Preparation of Standard stock solutions**

Accurately weighed 55mg of Indacaterol, 25mg 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. (1100 µg/ml of Indacaterol and 500 µg/ml of Glycopyrrolate).

#### **Preparation of 50% Spiked Solution**

1ml 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:** 2.0ml 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**

2.5ml 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

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

### Degradation studies [16]

#### Oxidation

To 1 ml of stock solution of Indacaterol and Glycopyrrolate, 1 ml of 20% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was added separately. The solutions were kept for 30 min at 60°C. For HPLC study, the resultant solution was diluted to obtain 110µg/ml & 50µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

#### Acid Degradation Studies

To 1 ml of stock solution Indacaterol and Glycopyrrolate, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60°C. The resultant solution was diluted to obtain 110µg/ml & 50µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

#### Alkali Degradation Studies

To 1 ml of stock solution Indacaterol and Glycopyrrolate, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60°C. The resultant solution was diluted to obtain 110µg/ml & 50µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

#### Dry Heat Degradation Studies

The standard drug solution was placed in oven at 105°C for 1h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 110µg/ml & 50µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

#### Photo Stability studies

The photochemical stability of the drug was also studied by exposing the 110µg/ml & 50µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/m<sup>2</sup> in photo stability chamber For HPLC study, the resultant solution was diluted to obtain 110µg/ml & 50µg/ml solutions and 10µl were injected into the system and the chromatograms were recorded to

assess the stability of sample.

### Neutral Degradation Studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 1hr s at a temperature of 60°. For HPLC study, the resultant solution was diluted to 110µg/ml & 50µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

### Results and Discussion

Optimized wavelength selected was 230nm.

#### Method development

Method development was done by changing various, mobile phase ratios, buffers etc.

#### Optimized method

##### Chromatographic conditions

**Mobile phase :** 55% OPA (0.1%): 45% Acetonitrile

**Flow rate :** 1ml/min

**Column :** Agilent C18 (4.6 x 150mm, 5µm)

**Detector wave length :** 230nm

**Column temperature :** 30°C

**Injection volume :** 10µL

**Run time :** 6 min

**Diluent :** Water and Acetonitrile in the ratio 50:50

#### Results

Both peaks have good resolution, tailing factor, theoretical plate count and resolution.

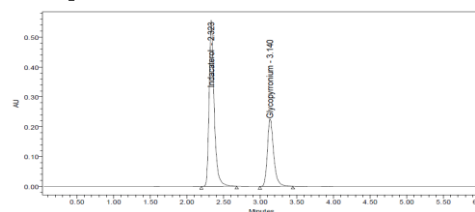


Fig 03: Optimized Chromatogram

#### Observation

Indacaterol and Glycopyrronium were eluted at 2.323 min and 3.140 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

#### System suitability

All the system suitability parameters were within the range and satisfactory as per ICH guidelines

Table 01: System suitability parameters for Indacaterol and Glycopyrronium

S n o	Indacaterol			Glycopyrronium			USP Resol
	RT(	USP	Tail	RT(	US	Tail	
I	RT(	USP	Tail	RT(	US	Tail	USP Resol

n j	min)	Plate Cou nt	ing	min)	P Pla te Co unt	ing	ution
1	2.33 4	5417	1.33	3.13 6	771 4	1.26	5.7
2	2.33 5	5677	1.32	3.13 8	745 1	1.27	5.6
3	2.33 6	5443	1.32	3.14 2	738 6	1.26	5.7
4	2.33 6	5602	1.33	3.14 2	723 3	1.27	5.7
5	2.33 6	5273	1.36	3.14 2	694 9	1.26	5.8
6	2.33 8	5383	1.31	3.14 3	702 9	1.27	5.7

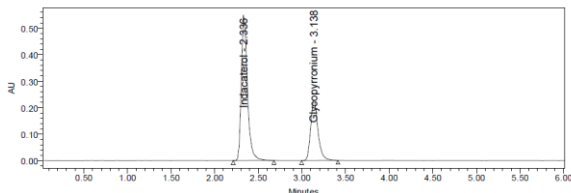


Fig 04: System suitability Chromatogram

**Discussion**

According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

**Validation**

**Specificity**

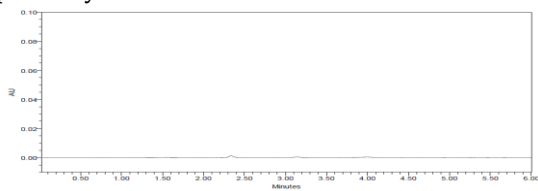


Fig 05: Chromatogram of blank

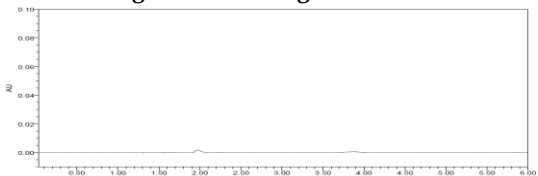


Fig 06: Chromatogram of placebo

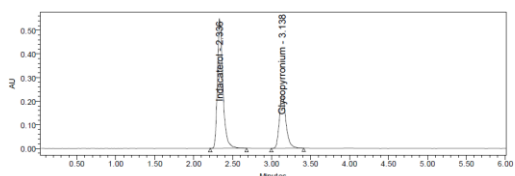


Fig 07: Typical Chromatogram

**Discussion**

Retention times of Indacaterol and Glycopyrronium were 2.336 min and 3.138 min respectively. We did not find and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

**Linearity**

Table 02: Linearity table for Indacaterol and Glycopyrronium

Indacaterol		Glycopyrronium	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
27.5	715271	12.5	342706
55	1311494	25	689039
82.5	2013645	37.5	962862
110	2690775	50	1340523
137.5	3402736	62.5	1646639
165	4040614	75	1985997

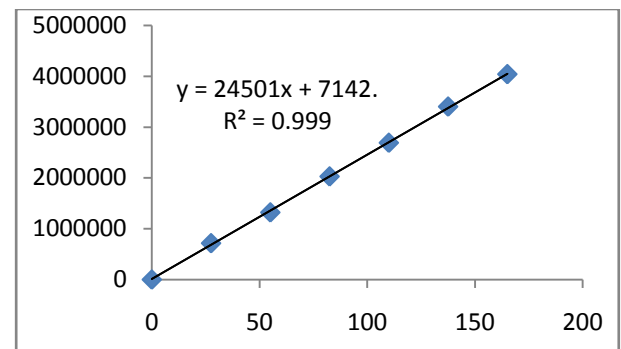


Fig 08: Calibration curve of Indacaterol

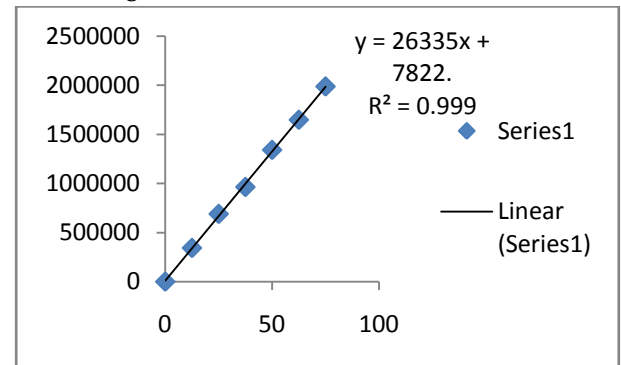


Fig 09: Calibration curve of Glycopyrronium

**Discussion**

Six linear concentrations of Indacaterol (16.25\_97.5µg/ml) and Glycopyrronium (7.25-43.5µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Indacaterol was  $y = 24501x + 7142.3$ .and of Glycopyrronium was  $y = 26335x + 7822.7$ .Correlation coefficient obtained was 0.999 for the two drugs.

**Precision****System Precision****Table 03: System precision table of Indacaterol and Glycopyrronium**

S. No	Area of Indacaterol	Area of Glycopyrronium
1.	2694165	1331679
2.	2675402	1326526
3.	2665723	1351324
4.	2678464	1351240
5.	2703242	1340170
6.	2695349	1349125
Mean	2685391	1341677
S.D	14348.5	10693.7
%RSD	0.5	0.8

**Discussion**

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.5% and 0.8% respectively for Indacaterol and Glycopyrronium. As the limit of Precision was less than "2" the system precision was passed in this method.

**Repeatability****Table 04: Repeatability table of Indacaterol and Glycopyrronium**

S. No	Area of Indacaterol	Area of Glycopyrronium
1.	2682197	1341905
2.	2678407	1339524
3.	2678537	1335420
4.	2679682	1336952
5.	2666181	1339277
6.	2671939	1335590
Mean	2676157	1338111
S.D	5948.9	2557.0
%RSD	0.2	0.2

**Discussion**

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each

working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.2% and 0.2% respectively for Indacaterol and Glycopyrronium. As the limit of Precision was less than "2" the system precision was passed in this method.

**Intermediate precision (Day\_Day Precision):****Table 05 Intermediate precision table of Indacaterol and Glycopyrronium**

S. No	Area of Indacaterol	Area of Glycopyrronium
1.	2502753	1318488
2.	2517263	1314818
3.	2503124	1316101
4.	2493270	1324397
5.	2498013	1341534
6.	2502015	1317768
Mean	2502740	1322184
S.D	8045.1	10037.4
%RSD	0.3	0.8

**Discussion**

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.3% and 0.8% respectively for Indacaterol and Glycopyrronium. As the limit of Precision was less than "2" the system precision was passed in this method.

**Accuracy****Table 06: Accuracy table of Indacaterol**

% Level	Amount Spiked ( $\mu\text{g/mL}$ )	Amount recovered ( $\mu\text{g/mL}$ )	% Recovery	Mean % Recovery
50%	55	54.70	99.45	99.39%
	55	54.10	98.36	
	55	54.65	99.37	

100%	110	110.24	100.22
	110	109.65	99.68
	110	109.51	99.55
150%	165	163.32	98.98
	165	164.23	99.53
	165	163.99	99.39

**Table 07: Accuracy table of Glycopyrronium**

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	25	24.88	99.51	99.41%
	25	24.81	99.25	
	25	24.90	99.60	
100%	50	49.69	99.39	
	50	49.76	99.51	
	50	49.54	99.08	
150%	75	74.46	99.28	
	75	74.34	99.11	
	75	74.96	99.95	

**Discussion**

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 99.39% and 99.41% for Indacaterol and Glycopyrronium respectively.

**Sensitivity**

**Table 08: Sensitivity table of Indacaterol and Glycopyrronium**

Molecule	LOD	LOQ
Indacaterol	1.08	3.28
Glycopyrronium	0.48	1.47

**Robustness**

**Table 09: Robustness data for Indacaterol and Glycopyrronium**

S.no	Condition	%RSD of Indacaterol	%RSD of Glycopyrronium
1	Flow rate (-) 0.7ml/min	0.2	0.2
2	Flow rate	0.4	0.3

	(+) 0.9ml/min		
3	Mobile phase (-) 50B:50A	0.4	0.5
4	Mobile phase (+) 60B:40A	0.7	0.6
5	Temperature (-) 25°C	0.3	0.3
6	Temperature (+) 35°C	0.4	0.6

**Discussion**

Robustness conditions like Flow minus (0.7ml/min), Flow plus (0.9ml/min), mobile phase minus (50B:50A), mobile phase plus (60B:40A), 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.

**Degradation [16]**

**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 10: Degradation data for Indacaterol and Glycopyrronium**

S. NO	Degradation Condition	Indacaterol			Glycopyrronium		
		% Drug Degraded	Purity Angle	Purity Threshold	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	7.72	1.124	3.093	6.79	0.415	0.415
2	Alkali	5.10	0.217	0.299	4.61	0.873	1.424
3	Oxidation	4.73	0.107	0.303	4.34	0.252	0.416
4	Thermal	3.12	1.120	1.488	2.81	0.177	0.362
5	UV	1.72	0.635	1.391	2.06	0.146	1.278
6	Water	1.13	0.565	1.298	1.07	0.237	1.413

## Discussion

Regarding the pH adjustment in mobile phase for the acid and base degradation studies have movement in retention time of drugs. But due to neutralized acid sample with 2N Base solution and base sample with 2N Acid solution there will be no change in retention time

## Conclusion

A simple, Accurate, precise method was developed for the simultaneous estimation of the Indacaterol and Glycopyrronium in injection form. Retention time of Indacaterol and Glycopyrronium were found to 2.323min and 3.140 min. % RSD of the Indacaterol and Glycopyrronium were found to be 0.2% and 0.2% respectively. % Recovery was obtained as 99.39% and 99.41% for Indacaterol and Glycopyrronium respectively. LOD, LOQ values obtained from regression equations of Indacaterol and Glycopyrronium were 1.08, 3.28 and 0.25, 1.47 respectively. Regression equation of Indacaterol is  $y = 24501x + 7142.3$ ,  $y = 26335x + 7822.7$  of Glycopyrronium. 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.

## Author Contribution

All authors are Contributed Equally.

## Funding

No Funding.

## Conflict of Interest

Authors are Declared no Conflict of Interest.

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