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Development and Validation of Simple Simultaneous Analysis for Indacaterol Maleate and Glycopyrronium Bromide by RP-HPLC

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ABSTRACT

Simple, Accurate, precise method was developed for the simultaneous estimation of the Indacaterol and Glycopyrrolate in Bulk and pharmaceutical dosage form. Chromatogram was run through Kromasil C18 150 x 4.6 mm, 5µ. Mobile phase containing Buffer 0.1% OPA: Acetonitrile taken in the ratio 55:45 was pumped through column at a flow rate of 1.0ml/min. Buffer used in this method was 0.1% OPA buffer Retention time of Indacaterol and Glycopyrrolate were found to be 2.319min and 2.830 min. %RSD of the Indacaterol and Glycopyrrolate were and found to be 0.8 and 1.1 respectively. %Recovery was obtained as 99.68% and 100.08% for Indacaterol and Glycopyrrolate respectively. LOD, LOQ values obtained from regression equations of Indacaterol and Glycopyrrolate were 0.60, 0.23 and 1.82, 0.70 respectively. Regression equation of Indacaterol is y = 21685x + 6734.3, and y = 21025x + 3152.8 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

I. INTRODUCTION

Indacaterol Is a Novel, Ultra-Long-Acting, Rapid Onset B (2)-Adrenoceptor Agonist [1,2] Developed for Novartis for The Once-Daily Management of Asthma and Chronic Obstructive Pulmonary Disease [3,4]. It was approved on 30 November 2009 by the European Medicines Agency [5,6] (EMA), and by the FDA on 1 July 2011. It is sold as Onbrez[6] and as Arcapta Neohaler[7] in America in Europe. Indacaterol[8,9] is supplied as the form of its maleate salt. Indacaterol Is also a chiral molecule but the pure R-Enantiomer[10,11] is dispensed only.

Glycopyrronium (as the bromide salt glycopyrrolate) is a synthetic anticholinergic[12] agent with a quaternary ammonium structure[13]. A strong muscarinic antagonist used as an antispasmodic[14] in certain gastrointestinal tract disorders[15,16] and with some anaesthetics to suppress salivation. The FDA approved glycopyrrolate[17] as a stand-alone treatment for chronic obstructive pulmonary disease[18,19] (COPD) in October 2015 as Seebri Neohaler[20].

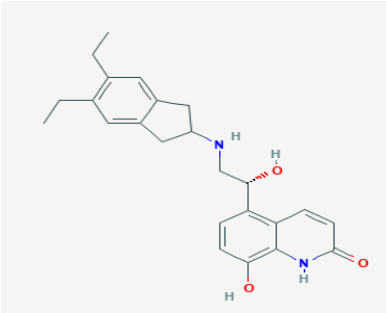

Fig 1: Structure of Indacaterol

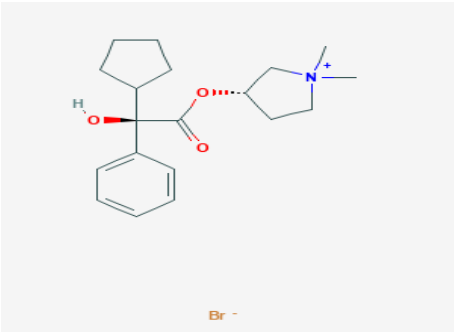

Fig 2: Structure of Glycopyrronium

II. MATERIALS AND METHOD

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Materials:

- 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:

- Electronics Balance-Denver, p^H 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 was used for measuring absorbances of Indacaterol and Glycopyrrolate solutions.

Preparation of buffer:

0.1%OPA Buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

Preparation of Standard stock solutions: 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 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. (55µg/ml of Indacaterol and 25µg/ml of Glycopyrrolate)

III. RESULTS AND DISCUSSION

The main analytical challenge during development of a new method was to separate active Pharma ingredients. In order to provide a good performance, the chromatographic conditions were optimized.

Method validation

The optimized RP-HPLC validated method according to ICH guidelines in terms of system suitability, linearity, accuracy, precision and robustness.

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.

Table 1: SYSTEM SUITABILITY PARAMETERS FOR INDACATEROL AND GLYCOPYRROLATE

S no	Indacaterol			Glycopyrrolate			
Injection	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	2.319	5692	1.30	2.830	6228	1.33	3.8
2	2.319	5690	1.31	2.833	6097	1.32	3.8

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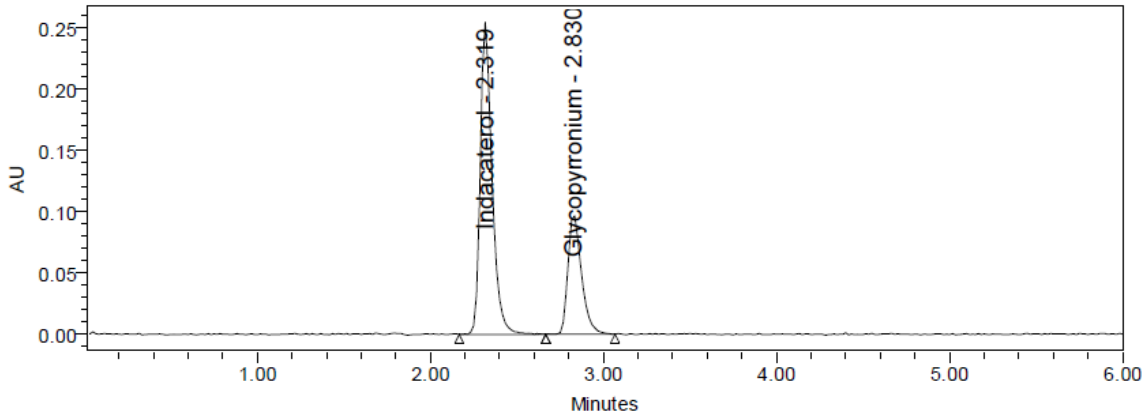

Fig 3: System suitability Chromatogram

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 & 500µg/ml. It was centrifuged for 20 min. Then the supernatant was collected and filtered using 0.45 µm filters using (Millipore, Milford, PVDF)

0.5ml from sample stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (55µg/ml of Indacaterol and 27.5µg/ml of Glycopyrrolate)

Table 2: SYSTEM PRECISION TABLE OF INDACATEROL AND GLYCOPYRROLATE

S. No	Area of Indacaterol	Area of Glycopyrrolate
1.	1203206	528596
2.	1201745	523155
3.	1222702	536672
Mean	1204284	530651
S. D	9278.5	5857.3
%RSD	0.8	1.1

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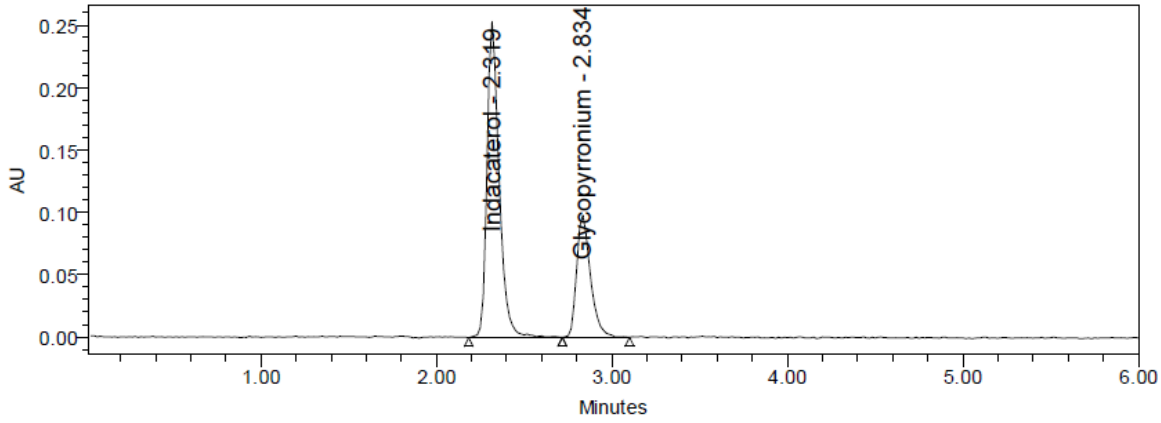

Fig 4: System precision chromatogram

Linearity:

Preparation of Standard stock solutions: 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)

Table 3: LINEARITY TABLE FOR INDACATEROL AND GLYCOPYRROLATE

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	916482	18.75	407184
55	1199024	25	533343

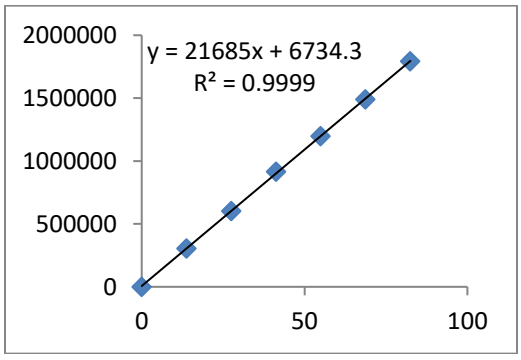

Fig 5: Calibration curve of Indacaterol

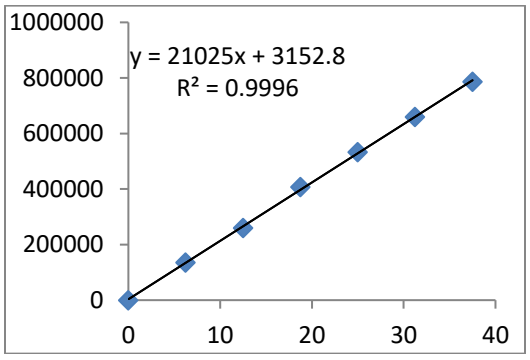

Fig 6: Calibration curve of Glycopyrrolate

Accuracy:

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Preparation of Standard stock solutions: 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)

Table 4: RESULTS OF ACCURACY

S. No.	% Level	Indacaterol % Recovery	Glycopyrrolate % Recovery
1	50	98.4	100.6
2	100	100.0	99.4
3	150	98.1	100.3

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there was 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.

Table 5: ROBUSTNESS DATA FOR INDACATEROL AND GLYCOPYRROLATE

S.no	Condition	%RSD of Indacaterol	%RSD of Glycopyrrolate
1	Flow rate (-) 0.9ml/min	1.2	1.7
2	Flow rate (+) 1.1ml/min	0.9	0.3
3	Mobile phase (-) 60B:40A	0.4	0.5
4	Mobile phase (+) 50B:50A	1.4	1.2
5	Temperature (-) 25°C	0.6	0.7
6	Temperature (+) 35°C	0.9	1.0

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.

Table 6: SENSITIVITY TABLE OF INDACATEROL AND GLYCOPYRROLATE

Molecule	LOD	LOQ
Indacaterol	0.60	1.82
Glycopyrrolate	0.23	0.70

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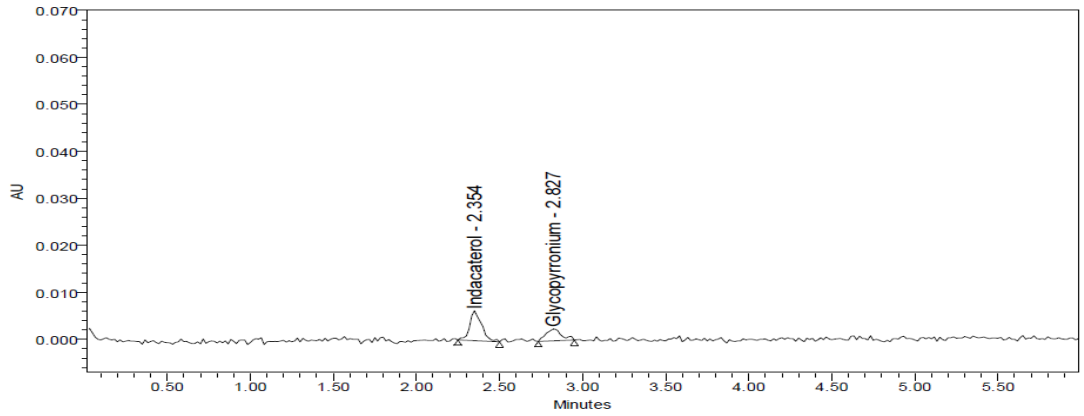

Fig 7: LOD Chromatogram of Standard

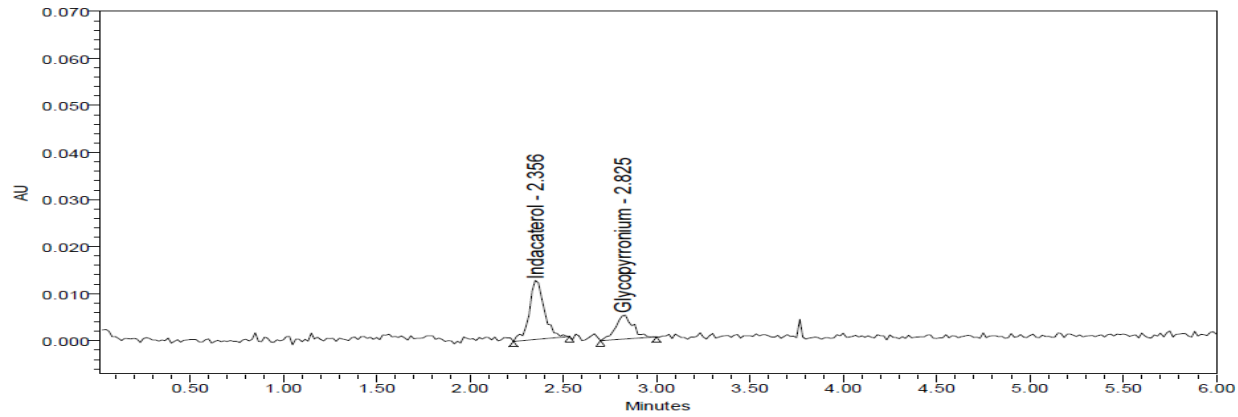

Fig 8: LOQ Chromatogram of Standard

Degradation studies:

Oxidation:

To 1 ml of stock solution of Indacaterol and Glycopyrrolate, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 55µg/ml & 25µg/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 ssolution Indacaterol and Glycopyrrolate, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 55µg/ml & 25µg/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 60^0c. The resultant solution was diluted to obtain 55µg/ml & 25µg/ml solution and 10 µl 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 1 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 55µg/ml & 25µg/ml solution and 10µl were injected into the system and

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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 550µg/ml & 250µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1days or 200-Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 55µg/ml & 25µg/ml solutions and 10 µl 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 1hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to 55µg/ml & 25µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Table 7: DEGRADATION DATA

Type of degradation	Indacaterol			Glycopyrrolate		
	Area	%Recovered	% Degraded	Area	%Recovered	% 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	96.40	3.60
Thermal	1174121	97.30	2.70	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

IV. CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Indacaterol and Glycopyrrolate in bulk and dosage form. Retention time of Indacaterol and Glycopyrrolate were found to be 2.319min and 2.830 min. %RSD of the Indacaterol and Glycopyrrolate were and found to be 0.8 and 1.1 respectively. %Recovery was obtained as 99.68% and 100.08% for Indacaterol and Glycopyrrolate respectively. LOD, LOQ values obtained from regression equations of Indacaterol and Glycopyrrolate were 0.60, 0.23 and 1.82, 0.70 respectively. Regression equation of Indacaterol is y = 21685x + 6734.3, and y = 21025x + 3152.8 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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CONFLICTS OF INTEREST
Author declares that there have been no conflicts of interest.
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None

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