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Analytical Method Development and Validation for Simultaneous Estimation of Indacaterol and Glycopyrrolate in Pharmaceutical Dosage Forms by RP-HPLC

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ABSTRACT

A new method was established for simultaneous estimation of Indacaterol and Glycopyrrolate by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Indacaterol and Glycopyrrolate by using Xterra C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 255nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2.The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Indacaterol and Glycopyrrolate was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100% and 100.5%, %RSD for repeatability was0.7 and 0.4, % RSD for intermediate precision was 0.18 and 0.39 respectively.

Key Words: Indacaterol, Glycopyrrolate, RP-HPLC, validation.

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1. Introduction

Indacaterol maleate is 5-{(1R)-2-[(5,6-diethyl-2,3-dihydro-1H-inden-2-yl)amino]-1-hydroxyethyl}-8-hydroxy-2(1H)-quinolinone maleate. It's white to very slightly grayish or very slightly yellowish not hygroscopic powder. It is

soluble in methanol, slightly soluble in water, insoluble in 0.9% NaCl.

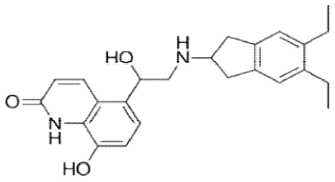


Fig 1: Chemical structure of Indacaterol maleate

Glycopyrronium bromide is 3-(2-cyclopentyl-2-hydroxy-2-phenylacetoxy)-1,1- dimethylpyrrolidinium bromide . It's a white, non-hygroscopic powder, freely soluble in water, soluble in methanol (96%), very slightly soluble in methylene chloride.

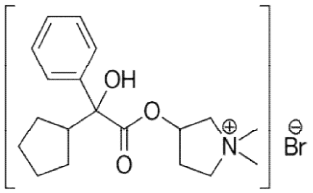


Fig 2: Chemical structure of Glycopyrronium bromide

2. Materials and Methods

Instrumentation:

HPLC Auto Sampler : Shimadzu Model number SPD20A, Software LC Solutions, Detector: Photo diode array detector, Thermosil C18 Column (4.0×1.25mm, 5µ), Sonicator: Model number SE60US Enertech , U.V double beam spectrophotometer: PG Instrument Model number T60 Software UV Win5, pH meter: ADWAModel number AD102U, Digital Weighing machine:a Model number ER200A .

Chemicals:

Indacaterol and Glycopyrrolate, KH_2PO_4, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid, K_2HPO_4.

Optimized Chromatographic conditions:

Column : Xterra C18 5µm (4.6*250mm)

Mobile phase ratio: Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v)

Detection wavelength: 255 nm

Flow rate : 1ml/min

Injection volume : 20µl

Column temperature : Ambient

Auto sampler temperature : Ambient

Run time : 10min

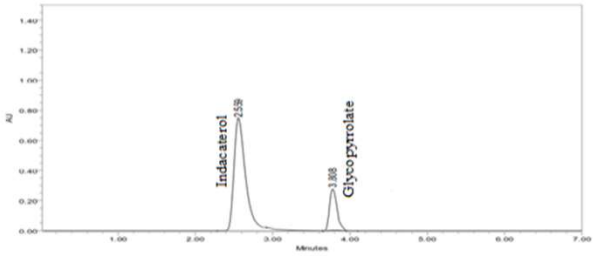


Fig 3: Optimized Chromatogram

Preparation of Sample Solution :(Tablet)

Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to 10 mg of Glycopyrrolate and Indacaterol (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL of Diluents is added and sonicated to dissolve it completely and made volume upto the mark with the same solvent. (Stock solution) Further 3 ml of above stock solution was pipetted into a10ml volumetric flask and diluted upto the mark with diluant.

Method Validation

- Linearity
- Accuracy
- Precision
- Intermediate Precision
- Limit of Detection
- Limit of Quantification
- Robustness
- System suitability testing

3. Results and Discussion

Table1: Accuracy results of Glycopyrrolate

%Concentration (at specification Level)	Area	Amount added(mg)	Amount found(mg)	% Recovery	Mean Recovery
50%	544711	5	5.10	101.8%	100.5%
100%	675935	10	9.99	99.9%	
150%	812764	15	14.9	99.1%	

Table 2: Accuracy results of Indacaterol

%Concentration (at specification level)	Area	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	644765	5	5.0	101.3%	100.0%
100%	803722	10	9.94	99.4%	

150%	962917	15	14.8	99.2%	

Table 3: Repeatability results of Indacaterol

	Name	RT	Area
1	Indacaterol	3.019	691143
2	Indacaterol	3.011	685431
3	Indacaterol	3.004	683543
4	Indacaterol	2.997	683564
5	Indacaterol	2.994	683532
Mean			685443
Std.Dev.			3289.7
%RSD			0.48

Table 4: Repeatability results of Glycopyrrolate

	Name	RT	Area
1	Glycopyrrolate	3.557	819305
2	Glycopyrrolate	3.547	807157
3	Glycopyrrolate	3.544	804070
4	Glycopyrrolate	3.537	808474
5	Glycopyrrolate	3.534	804505
Mean			808702
Std.Dev.			6203.7
%RSD			0.77

Table 5: Ruggedness results of Indacaterol

	Name	RT	Area
1	Indacaterol	3.001	673725
2	Indacaterol	3.009	672535
3	Indacaterol	3.010	676216
4	Indacaterol	2.997	679037
5	Indacaterol	3.007	677101
Mean			675723
Std.Dev.			2611.5
% RSD			0.39

Table 6: Ruggedness results of Glycopyrrolate

	Name	RT	Area
1	Glycopyrrolate	3.524	813507
2	Glycopyrrolate	3.533	817673
3	Glycopyrrolate	3.533	815189
4	Glycopyrrolate	3.517	815816
5	Glycopyrrolate	3.530	815356
Mean			815508
Std.Dev.			1492.7
% RSD			0.18

Table 7: Linearity results of Glycopyrrolate

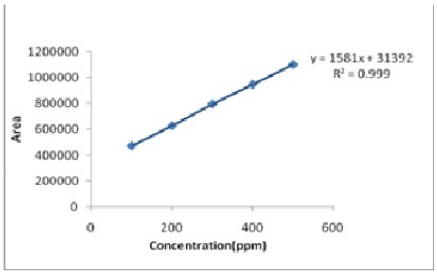
S.No	Linearity Level	Concentration	Area
1	I	100ppm	226418
2	II	200ppm	432920
3	III	300ppm	677256
4	IV	400ppm	869825
5	V	500ppm	1095759
Correlation Coefficient			0.999

Table 8: Linearity results of Indacaterol

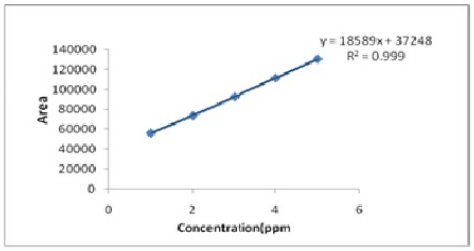
S.No	Linearity Level	Concentration	Area
1	I	1ppm	277182
2	II	2ppm	521695
3	III	3ppm	808274
4	IV	4ppm	1033875
5	V	5ppm	1285804
Correlation Coefficient			0.999

Table 9: Area of different concentration of Melitracen

S.No	Linearity Level	Concentration	Area
1	I	1ppm	34510
2	II	2ppm	71701
3	III	3ppm	108802
4	IV	4ppm	142731
5	V	5ppm	179732
Correlation Coefficient			0.999



Calibration curve of Glycopyrrolate



Calibration curve of Indacaterol

Fig 4: Calibration graphs

Table 10: System suitability results For Glycopyrrolate (Flow rate)

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	2690	0.9
2	1.0	3115	1.1
3	1.2	2503	0.9

Table 11: System suitability results for Indacaterol (Flow rate)

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	2716	0.9
2	1.0	3527	1.0
3	1.2	2685	0.9

Table 12: System suitability results for Glycopyrrolate (Mobile phase)

S.No	Changein Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10% Less	2818	1.1
2	Actual	3125	1.1
3	10% More	2707	1.1

Table 13: System suitability results for Indacaterol (Mobile phase)

S.No	Changein Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10% Less	3107	1.0
2	Actual	3526	1.0
3	10% More	3001	1.0

4. Conclusion

Simple, rapid, accurate and precise RPHPLC as well as spectrophotometric methods have been developed and validated for the routine analysis of IND and GLY in API pharmaceutical dosage forms.

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