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Research Article

Stability Indicating Rp-HPLC Method Development And Validation For The Estimation Of Indacaterol Maleate In Bulk And Tablet Dosage Form

Trupti Bhalekar*, Kalpana Sable, Jaya Mehetre, Kiran Dhamak

Department of Quality Assurance, PRES College of Pharmacy (Women's), Chincholi, Nashik-422102.

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ABSTRACT

The HPLC method was developed and validated for simultaneous estimation of Indacaterol maleate. The method was developed after the several trials at the composition of Methanol: Phosphate Buffer 6.8 (75:25), flow rate 1.0 ml/min and 260 nm wavelength of detection and retention time was at 4.3 min. The calibration curve was plotted, and regression equation of Indacaterol maleate was found to be y = 38835x + 278291 with correlation coefficient (r2) of 0.9993. From the Accuracy study % recovery was found in the range of 99.97-100.4% which is in the limits according to the ICH guidelines. Intraday and Interday precision assures that % RSD was within the limits of ICH guidelines i.e NMT 2. Limit of detection and limit of Quantitation of Indacaterol is 0.17 µg/ml - 0.52 µg/ml respectively. Robustness was studied by deliberate variation i.e., change in Flow rate and change in Wavelength which was within 2 % of RSD and also the ruggedness study gives results within the limits of 2% in which variation in Analyst was studied. The stress degradation studies demonstrated susceptibility of the drug to acid, base, neutral hydrolysis, oxidative, thermal and photolytic stress conditions. The major degradation of drug was found to be in alkali and acidic stress condition. The result showed that the proposed chromatographic method was suitable for the accurate, precise and rapid simultaneous determination of Indacaterol maleate in its bulk form and pharmaceutical dosage form.

INTRODUCTION

Indacaterol is an inhaled long-acting beta-2 adrenergic agonist used to relax bronchial smooth muscle and improve symptoms and airflow obstruction caused by Chronic Obstructive Pulmonary Disease (COPD) and moderate to severe asthma.

***Corresponding Author:** Trupti Bhalekar
Address: *Department of Quality Assurance, PRES College of Pharmacy (Women's), Chincholi, Nashik-422102 Department of Quality Assurance, PRES College of Pharmacy (Women's), Chincholi, Nashik-422102*
Email : trups_510@yahoo.co.in

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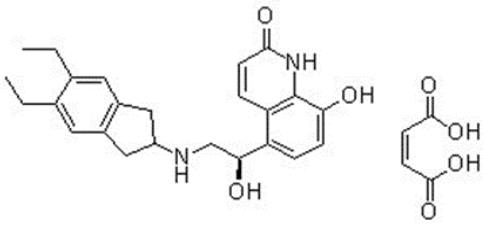

Fig 1: Structure of Indacaterol maleate

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. It was approved by the European Medicines Agency (EMA) on 30 November 2009 and by the FDA on 1 July 2011. It is marketed in Europe as Onbrez and in America as Arcapta Neohaler. Indacaterol is provided as its maleate salt form. Indacaterol is also a chiral molecule but only the pure R-enantiomer is dispensed.

MATERIALS AND METHOD:

Instruments:

The chromatographic method was performed on Shimadzu HPLC system coordinated with a variable wavelength UV detector and a Rheodyne injector outfitted with 20µl fixed circle. An Reverse phase Phenomenex C18 (250mm x 4.6ID, Particle size: 5 micron) was utilized. Model - UV-3000 - M spectrophotometer and Wenser High Precision Balance Model: PGB 100 electronic equilibrium were utilized for Spectrophotometric judgments and gauging purposes individually.

Reagents and chemicals

Indacaterol maleate was procured from PharmaTech Solutions. HPLC grade Acetonitrile and water were acquired from Merck specialities private restricted, Mumbai.

Chromatographic conditions

Phenomenex C18 (250mm x 4.6ID, Particle size: 5 micron) was utilized for the chromatographic method at wavelength of 260 nm. Methanol: Phosphate Buffer (75:25) pH 6.8 was chosen as mobile phase for elution and same solvent was utilized in the preparation of standard and sample solutions. The elution was checked by infusing the 20µl and the flow rate was changed in accordance with 1.0 ml/min.

Preparation of Standard Stock solutions

Accurately 10.0 mg weighed quantity of Indacaterol was transferred to 10.0 mL volumetric flask. That was dissolved by adding 5.0 mL mobile phase and then the drug solution was diluted up to the mark with mobile phase to get the stock solution of 1000 µg/mL of Indacaterol. The working standard solutions of these drugs were obtained by appropriate dilution of the respective stock solution with mobile phase.

Preparation of Mobile Phase

Prepare mobile phase by taking methanol and phosphate buffer in various proportion Methanol: Phosphate Buffer 6.8 (75:25). Mobile phase was filtered through 0.45µm membrane filter and degassed by sonication for 20 min.

Selection of mobile phase

Pure drug solutions of Indacaterol (10µg/mL) were injected into the RP-HPLC system and run in different solvent systems. Different mobile phases systems like phosphate buffer, ACN and methanol were initially tried in the isocratic mode in order to determine the best conditions.

HPLC Method Development

Optimisation of RP-HPLC method

The HPLC method was developed for the simultaneous estimations of Indacaterol maleate. Different mobile phases were gone after for the method optimisation, however satisfactory retention times, hypothetical plates and good resolution were seen with Methanol: Phosphate Buffer (75:25) pH 6.8 using Phenomenex C18 (250mm x 4.6ID, Particle size: 5 micron) by isocratic method.

Table 1: Optimized Chromatographic Conditions

Mobile phase	Methanol: Phosphate Buffer pH 6.8 (75:25)
Selection of column	Phenomenex C18 (4.6mm x 250mm, Particle size: 5µm)
Injection volume	20 µL
Flow rate	1.0 ml/min
Column temperature	Room Temperature
Detection wavelength	260 nm
Run Time	7.0 min
Retention time	4.3 min

Validation of RP-HPLC method

Validation of the optimized RP-HPLC method was performed in accordance with the ICH Q2 (R) guidelines.

Linearity

Test solutions of different concentration were injected separately, and the chromatograms were recorded. A series of test preparations of Indacaterol (10-50 µg/ml) were prepared by taking 0.1-0.5 ml from the stock solution in five 10 ml volumetric flask and final volume make up to the mark with mobile phase. A 20 µl volume of each concentration was injected into HPLC, three times under the optimized chromatographic conditions.

Accuracy

Samples are prepared normally covering 50 % to 150 % of the nominal sample preparation concentration. These samples are analyzed and the recoveries of each are calculated.

Precision

Intraday precision study was carried out by preparing test solution of same concentration and analyzing it at three different times in a day. The same procedure was followed for two different days to determine interday precision. The result was reported as %RSD.

Limit of Quantitation (LOQ) & Limit of Detection (LOD)

The LOD and LOQ were analysed from the slope(s) of the calibration curve and the standard deviation (SD) of the peak areas using the formula LOD = 3.3 s/s and LOQ = 10 s/s.

Robustness

Robustness was calculated by changing the chromatographic conditions like compositions of mobile phase, detection wavelength, flow rate etc. and the % RSD should be reported. In the optimised conditions small changes were allowed and the extent to which the method was robust was determined. A deviation of ± 2 nm in the detection wavelength and ± 0.1 ml/min in the flow rate, were tried individually. Solutions of 100% test concentration with the specified n changes in the optimised conditions were injected to the system in triplicate.

Ruggedness:

Ruggedness is the study to determine effect of external parameters on the method. To evaluate ruggedness of the developed method, parameters were deliberately varied. These parameters included variation of system, different analyst, Atmospheric changes. Test solution prepared as per the test method and injected 3 concentrations of test solution into HPLC system with flow rate 1.0 ml/min by 2 different analysts.

Assay of marketed formulation

20 Capsules of marketed formulation (Onbrex 300mcg) were taken. Average weight of capsule sample was weighed and transferred to 100 mL volumetric flask & diluent was added to make up the volume. Sonicate for 10 min with occasional swirling. The above solution was filtered through 0.45µm membrane filter, The prepared stock solution is of 100 µg/ml of Indacaterol maleate.

For Analysis 1.0 ml solution was withdrawn and diluted upto 10 ml and injected into system.

System suitability

System suitability parameters were measured to verify the system, method and column performance. Standard solution of Indacaterol maleate was injected into the system for five times and system suitability parameters were checked.

Table 2: Forced Degradation Conditions according to ICH guidelines

Test Condition	Acidic degradation	Alkaline degradation	Oxidative degradation	Thermal degradation	Photolytic degradation
Indacaterol maleate	1N HCl, 1 hr. at 60^0c	1N NaOH, 1 hr. at 60^0c	3%H_2O_2, 24hrs	Thermal stress for 24 hrs.	Photolytic stress for 24 hrs.

RESULT AND DISCUSSION

Linearity*:*

It was clarified from the analytical method linearity as the ability of the method to obtain test results that are directly proportional to the analyte concentration, within a specific range. The peak area obtained from the HPLC chromatograph was plotted against corresponding concentrations to obtain the calibration graph. Indacaterol maleate was found to be linear in the concentration range of 5-25µg/ml and Enalapril is in the range of 5-25µg/ml.

Table 3: Summary of results of Linearity

Sr. No.	Conc. (µg/ml)	Area
1	10	685547
2	20	1028449
3	30	1442605
4	40	1837217
5	50	2222926

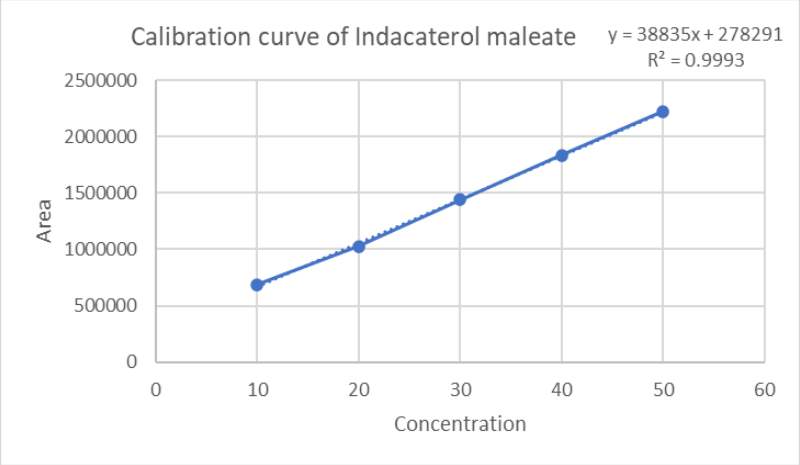

Fig 2: Calibration curve for Indacaterol maleate

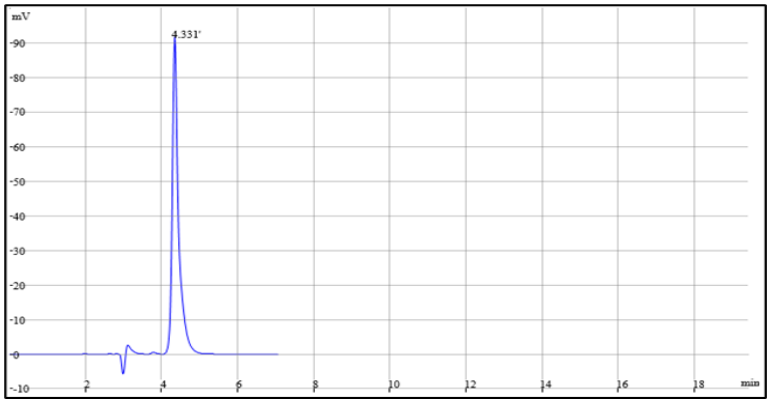

Fig 3: Chromatograph of Indacaterol maleate

Accuracy

The accuracy of the method determines the closeness of results obtained by that method to the true value. From the results of accuracy testing, it was showed that the method is accurate within the acceptable limits. The % RSD is calculated for the Indacaterol maleate and all the results are within limits. Acceptable accuracy was within the range and not more than 2.0% RSD.

Table 4: Statistical validation for accuracy of Indacaterol maleate

Level of addition	% Mean recovery*	SD	% RSD
50%	100.30	0.57	0.57
100%	100.41	0.51	0.51
150%	99.97	0.03	0.03

Precision

Intraday and interday precision assures the repeatability of test results. The % RSD found was below 2 for Indacaterol maleate.

Table 5: Data for intraday precision of Indacaterol maleate

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	10	683528	686095.67	2602.68	0.38
2	10	686027			
3	10	688732			
4	30	1443926	1442858.00	3741.31	0.26
5	30	1443950			
6	30	1440698			
7	50	2225482	2228780.67	9149.41	0.41
8	50	2221738			
9	50	2239122			

Table 6: Data for interday precision of Indacaterol maleate

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	10	685519	687394.67	2008.03	0.29
2	10	689513			
3	10	687152			
4	30	1459633	1447040.33	11794.47	0.82
5	30	1445236			
6	30	1436252			
7	50	2235980	2234477.00	12258.80	0.55
8	50	2221536			
9	50	2245915			

Robustness

Robustness was studied by different deliberate variations in the chromatographic conditions i.e. Change in flow rate and wavelength. From robustness study % RSD was found to be within limit of 2 % for the Indacaterol maleate. Hence it is robust and complies as per ICH guidelines.

Table 7: **Data for Robustness study of Indacaterol maleate**

Sr. No	Parameter	Condition	Area	Mean	SD	%RSD
1	Change in Flow rate (ml/min)	0.9	1026207	1028586.66	2451.40	0.24
2		1	1028449			
3		1.1	1031104			
1	Change in Wavelength (nm)	258	1030728	1030405.33	765.80	0.07
2		260	1029531			
3		262	1030957			

Ruggedness

Ruggedness was studied by different analyst. From robustness study % RSD was found to be within limit of 2 % for the Indacaterol maleate. Hence it is complying as per ICH guidelines.

Table 8: Data for ruggedness study of Indacaterol maleate

Sr. No	Analyst	Conc. (µg/ml)	Area	Mean area*	SD	% RSD
1	Analyst-I	30	1445600	1446497.33	6754.85	0.47
			1453656			
			1440236			
2	Analyst-II	30	1462531	1445908.67	15218.91	1.05
			1442536			
			1432659			

Specificity

Excipients and impurities were not interacting with the standard drugs. Hence the method is specific.

Table 9: Data for specificity study of Indacaterol maleate

Drug conc. (µg/ml)	Excipients (µg/ml)	Total Conc. (µg/ml)	Area	Mean	SD	%RSD
10	20	30	684152	683396.67	748.11	0.11
10	20	30	683382			
10	20	30	682656			
20	20	40	1015694	1024601.00	8453.94	0.83
20	20	40	1025595			
20	20	40	1032514			
30	20	50	1459263	1449270.33	8754.90	0.60
30	20	50	1445600			
30	20	50	1442948			

% Assay of Marketed formulation

The % Assay of Onbrex 300mcg marketed formulation of Novartis was calculated.

Table 10. Data of % Assay of marketed formulation

Sr. NO.	Drug	Area of Sample	Area of Standard	% Assay
1	Indacaterol maleate	1442605	1440359	99.84

System Suitability Parameters:

System suitability parameters were measured to verify the system, method and column performance. Standard solution of Indacaterol maleate was injected into the system for five times and system suitability parameters were checked.

Table 11: System suitability parameter

Sr. No.	Conc. (µg/ml)	Retention Time (min)	Theoretical plates	Asymmetry Factor
1	30	4.35	8129	1.13
2	30	4.33	8203	1.14
3	30	4.35	8230	1.11
4	30	4.35	8359	1.15
5	30	4.35	8252	1.14
6	30	4.36	8032	1.16
Mean		4.35	8200.83	1.14
SD		0.01	111.49	0.02
%RSD		0.22	1.36	1.51

Degradation Studies

Stress testing of the drug substance can help to identify the likely degradation products, the stability and specificity of the analytical procedure. Degradation studies were performed on solutions containing Indacaterol maleate (30µg/ml).

Table 12: Results of Forced Degradation Studies for Indacaterol maleate

Sr. No.	Condition	Area of sample	Area of Standard	% Drug recovered	% Degradation
1	**Water stress**	2158317	2222926	**97.09**	**2.91**
2	**Acid stress**	1823500	2222926	**82.03**	**17.97**
3	**Alkali Stress**	1840374	2222926	**82.79**	**17.21**
4	**Oxidative Stress**	2150953	2222926	**96.76**	**3.24**
5	**Thermal Stress**	2202295	2222926	**99.07**	**0.93**
6	**Photolytic Stress**	2209389	2222926	**99.39**	**0.61**

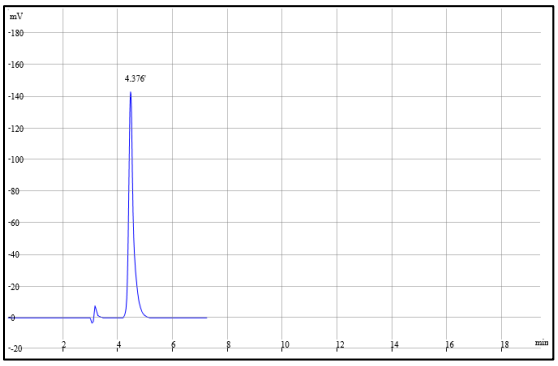

Fig. 4: Water Stressed Chromatogram

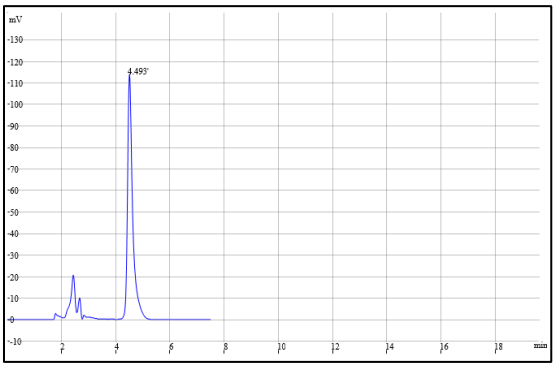

Fig. 5: Acid Stressed Chromatogram

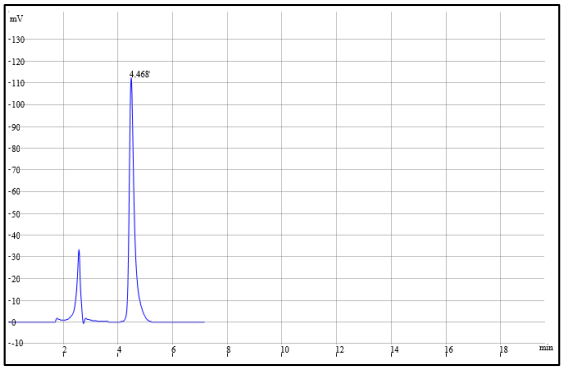

Fig. 6: Alkali Stressed Chromatogram

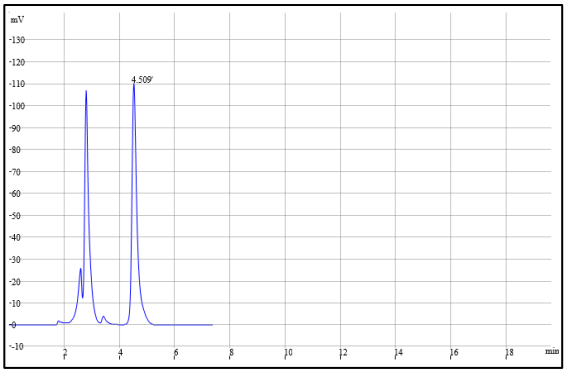

Fig. 7: Peroxide Stressed Chromatogram

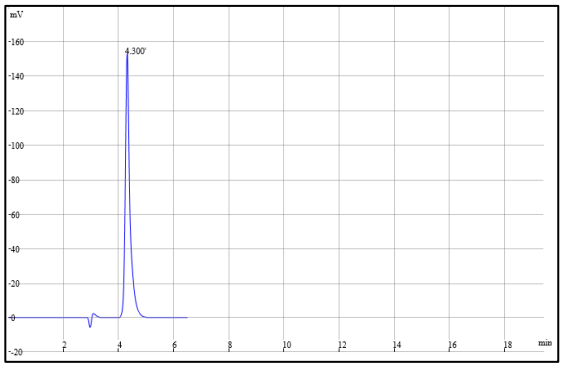

Fig. 8: Thermal Stressed Chromatogram

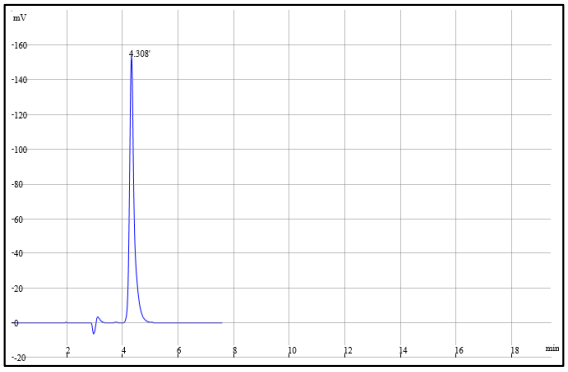

Fig. 9: Photolytic Stressed Chromatogram

SUMMARY

The Indacaterol maleate was found to be linear in the concentration range of 10-50 µg/ml. From Accuracy study % recovery of Indacaterol maleate was found in the range of 99.97-100.4% which is in the limits accordingly the ICH guidelines. Intraday and Interday precision assures that % RSD was within limits of ICH guidelines i.e.,

NMT 2 for both Indacaterol maleate. Limit of detection and limit of Quantitation of Indacaterol maleate is 0.17 µg/ml - 0.52 µg/ml. Robustness was studied by deliberate variation i.e., change in Flow rate and change in Wavelength which was within 2 % of RSD as per ICH guidelines. The ruggedness study gives results within the limits of 2% in which variation in Analyst was studied. The % assay of Onbrex 300mcg was found to be Indacaterol maleate (99.84%).

CONCLUSION

The proposed chromatographic method was found to be simple, precise, accurate, rapid and specific for determination of Indacaterol maleate from pure and its dosage forms. The mobile phase used for method development is very simple to prepare and economical also. The sample recoveries in the formulation were showing good results. This method is economical and run time is relatively short which enables rapid analysis among all the developed methods and hence, this method can be easily and conveniently adopted for *in-vitro* dissolution and routine analysis of Indacaterol maleate in pharmaceutical dosage form.

Degradation studies were performed on solutions containing Indacaterol maleate. The force degradation study was employed on five conditions i.e., Acidic, Alkaline, Oxidative, Thermal and Photolytic degradation. The degradation products produced during the stability study were well separated from the pure drug signifying the specificity of developed procedure. The major degradation of drug was found to be in Acid and Alkali stress condition.

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