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Research Article

Method Development and Validation of Indacaterol Maleate By RP-HPLC In Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A simple, sensitive and accurate RP-HPLC method has been developed for the determination of Indacaterol maleate in bulk and marketed formulation. The λmax of the Indacaterol maleate was found to be 260 nm in Methanol: water (90:10) pH 3.0. The method shows high sensitivity with linearity 10 to 50µg/ml (regression equation: y = 38835x + 278291; r^2 = 0.9993). The various parameters according to ICH guidelines and USP are followed for validating and testing of this method. The Detection limit and quantitation limit were found to be 0.146 µg ml –1 and 0.442 µg ml–1 respectively. The results demonstrated that the procedure is accurate, specific and reproducible (RSD <2%), and also being simple, cheap and less time consuming and appropriate for the determination of Indacaterol maleate in bulk and Table dosage form.

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. It was approved by the European Medicines Agency (EMA) on 30 November 2009 and by the FDA on 1 July 2011. By synthetically Indacaterol maleate is 4(*Z*)-but-2-enedioic acid;5-[(1*R*)-2-[(5,6-diethyl-2,3-dihydro-1*H*-inden-2-yl)amino]-1-hydroxyethyl]-8-hydroxy-1*H*-quinolin-2-one and having atomic equation is $C_{28}H_{32}N_2O_7$. The construction of Indacaterol maleate is appearing in fig 1.

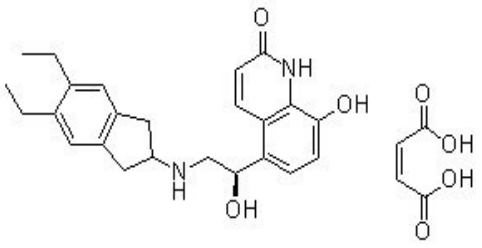

Figure 1: Structure of Indacaterol maleate

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Indacaterol maleate is having atomic mass of 508.6 g/mol. 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 METHODS

Instruments:

The chromatographic partition was performed on Analytical Technologies HPLC-3000 arrangement smaller fluid chromatographic framework coordinated with a variable frequency programmable UV identifier and a Rheodyne injector outfitted with 20µl fixed circle. An opposite stage C18 [Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)] was utilized. Model - UV 2012 twofold shaft UV obvious spectrophotometer and Wenser High Precision Balance Model: PGB 100 electronic equilibrium were utilized for Spectrophotometric judgments and gauging purposes individually.

Reagents and chemicals

Drug grade unadulterated Indacaterol maleate sample was secured from PharmaTech solutions. HPLC grade Methanol and water were acquired from Merck specialities private restricted, Mumbai.

Chromatographic conditions

C18 [Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)] was utilized for the chromatographic separation at a discovery frequency of 260 nm. Methanol: water (90:10) pH 3.0was chosen as mobile phase for elution and same blend was utilized in the arrangement of standard and sample solutions. The elution was checked by infusing the 20µl and the stream rate was changed in accordance with 1.0 ml/min.

Preparation of Standard solutions

10mg Indacaterol maleate was precisely gauged and moved into 10 ml volumetric cups, broken up utilizing portable stage and the volume was made up with a similar dissolvable to acquire essential stock arrangement of focus 1000µg/ml of the medication. (Working stock arrangement).

Optimisation of RP-HPLC method

The HPLC technique was streamlined with an expect to build up an assessment 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:Water (90:10) utilizing C18 column [Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)] and a run of the chromatograph of Indacaterol maleate was appeared in figure 3.

Table 1: Optimized parameter

Parameter	Condition
Column	Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)
Mobile Phase	Methanol: water (90:10) pH 3.0
Flow Rate	1.0 ml/min
Wavelength	260 nm
Injection Volume	20 µl
Detector	UV-3000-M
Retention Time	Approx. 4.3 min

Validation of RP-HPLC method

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

1. Linearity

For the determination of linearity, appropriate sample solutions were pipetted out from 1000µg/ml stock solution. 0.1 – 0.5 ml was pipetted out in to five of 10ml volumetric flasks respectively and volume was made with the mobile phase to obtain concentration ranging from 10-50µg/ml of Indacaterol maleate. Each solution from flask was injected in triplicate in system. Calibration curves were plotted with concentration of solutions against observed peak areas made by them followed by the determination of regression factor and calculation of the correlation coefficients. The calibration curves of Indacaterol maleate sample was shown in figure 2 and their related linearity parameters given in table 2.

2. Accuracy

To make sure the reliability and accuracy of the recovery study data were carried out by % recovery method which is also called as standard addition method. A known quantity of pure drug of Indacaterol maleate was mixed to pre-analysed sample and contents again undergoes analysis by the optimised method and the % recovery was reported in table 3.

3. Precision

The repeatability study of the proposed method was verified by calculating the percentage RSD of three replica injections of 100% concentration i.e., 30µg/ml of Indacaterol maleate on the same day and for intraday precision % RSD was calculated from repetition. The results were shown in table 5.

4. 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.

5. 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. percentage RSD was shown in the table 7.

6. System suitability

It was made sure that from the system suitability parameters, the method can give results of accuracy and precision**.** System suitability was performed with three replicate injections of solution of 30 µl/ml of Indacaterol maleate into the chromatographic system. Tailing factor (T) Number of theoretical plates (N) obtained was reported in table 8.

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. The results of linearity study (Figure 1) gave linear relationship over the concentration range of 0.1 - 0.5 µg/ml for Indacaterol maleate. From the regression analysis, a linear equation was obtained y = 38835x + 278291, and the goodness-of-fit (r^2) was found to be 0.9992, indicating a linear relationship between the concentration of analyte and area under the peak.

Table 2: 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

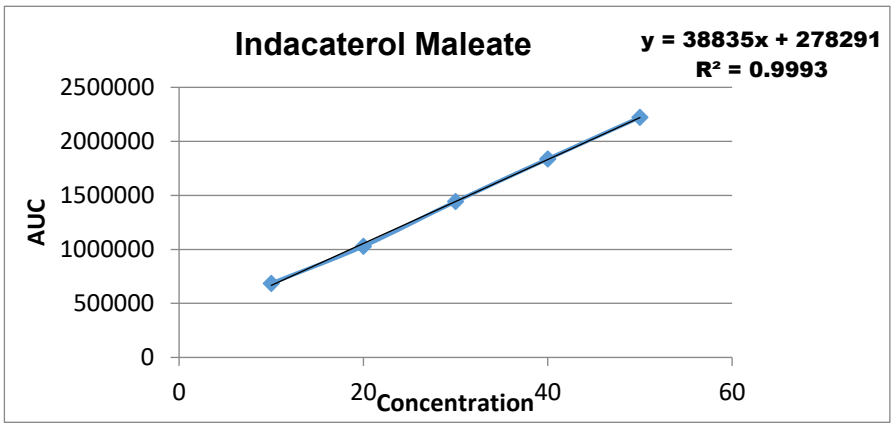

Figure 2: Linearity

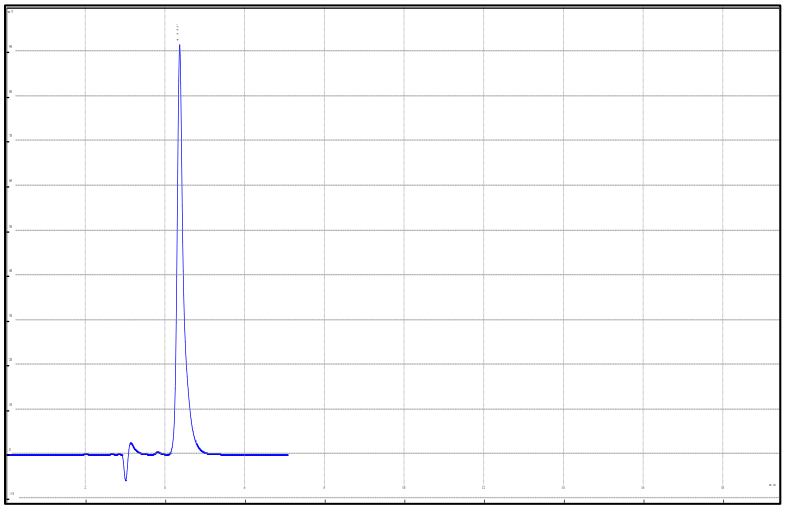

Figure 3: Typical chromatograph of Indacaterol maleate

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, as demonstrated in Table -3.

Table 3: summary of Results of Accuracy

Level of addition	Standard added (µg/ml)	conc. (µg/ml)	Total conc. (µg/ml)	Area obtained*	Std Area	Drug recovered (µg/ml)	%Recovery
50%	10	20	30	1456205	1442605	30.282822	100.942739
	10	20	30	1443950		30.02797	100.093234
	10	20	30	1440698		29.960343	99.8678086
100%	20	20	40	1836318	1837217	39.980427	99.9510673
	20	20	40	1843264		40.131656	100.329139
	20	20	40	1854932		40.385692	100.96423
150%	30	20	50	2223126	2222926	50.004499	100.008997
	30	20	50	2221922		49.977417	99.9548343
	30	20	50	2221738		49.973278	99.9465569

Table 4: % recovery data

Level of addition	% Mean recovery*	SD	% RSD
50%	100.3	0.5669	0.565153
100%	100.4	0.512	0.509871
150%	99.97	0.0339	0.033924

Precision

Precision is "the closeness of results obtained from multiple sampling of the same homogeneous sample under the prescribed conditions," and it is expressed in the form of relative standard deviation. The repeatability, intra-day and inter-day precision results are shown in the table 5. The RSD were calculated for all the results are within limits. Precision was not more than 2.0% RSD, as demonstrated in Table 5 and 6.

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Table-5: summary of Intraday Precision

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	10	685547	686768.667	1717.151	0.250033
2	10	686027			
3	10	688732			
4	30	1442605	1442417.67	3268.147	0.226574
5	30	1443950			
6	30	1440698			
7	50	2222926	2227928.67	9711.893	0.435916
8	50	2221738			
9	50	2239122			

Table-6: summary of Interday Precision

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	10	685519	687394.667	2008.02747	0.29212148
2	10	689513			
3	10	687152			
4	30	1459633	1447040.33	11794.4692	0.81507536
5	30	1445236			
6	30	1436252			
7	50	2235980	2234477	12258.7996	0.54862053
8	50	2221536			
9	50	2245915			

LOD and LOQ

The LOD and LOQ were calculated by the equations $\text{LOD} = \frac{3.3 \times \text{std.Deviation}}{\text{slope}}$ and $\text{LOQ} = \frac{10 \times \text{std.Deviation}}{\text{slope}}$ where, std. Deviation taken from accuracy and slope is from linearity. Based on these equations, the calculated LOD and LOQ values for Indacaterol maleate were 0.1094 and 0.3316 µg/ml, respectively.

Robustness

Robustness of the method reflects that the results are unaffected or reliable even if the minute changes in the method parameters. Here, the flow rate and wavelength were slightly changed to lower and higher sides of the actual values to find if the change in the peak area and retention time were within limits. The results obtained with changes in the parameters on a 30µg/mL solution are as shown in Table No. 7

Table 7: robustness

Sr.No	Parameter	Condition	Area	Mean	SD	%RSD
1	Change in Flow rate (ml/min)	0.8	1026207	1028587	2451.4	0.23833
2		1.0	1028449			
3		1.2	1031104			
1	Change in Wavelength (nm)	258	1030728	1030045	1386.62	0.13462
2		260	1028449			
3		262	1030957			

Ruggedness

Ruggedness was studied by different analyst.

Table 8: Data for ruggedness study of Indacaterol by HPLC method

Sr.No	Analyst	Conc. (µg/ml)	Area	Mean area*	SD	% RSD
1	Analyst-I	30	1445600	1446497.33	6754.8505	0.46697981
			1453656			
			1440236			
2	Analyst-II	30	1462531	1445908.67	15218.9111	1.05254996
			1442536			
			1432659			

% Assay:

% Assay was carried out to calculate the amount of drug present in the marketed Indacaterol maleate formulation i.e. Onbrez Capsules

Table 9: % Assay of Indacaterol by HPLC method

Sr. NO.	% Composition	Area of Standard	Area of Sample	% Assay
1	**% Assay**	685547	683292	**99.67**

System Suitability Parameters:

System suitability was performed by injecting three replicate injections of 100% test concentration, number of theoretical plate, asymmetry factor are satisfactory. The chromatographs confirm the presence of Indacaterol maleate at 4.3 min without any interference.

Table 10: System suitability parameter

Sr. No.	Conc. (µg/ml)	Retention Time (min)	Theoretical plates	Asymmetry Factor
1	30	4.353	8129	1.13
2	30	4.331	8203	1.14
3	30	4.353	8230	1.11
4	30	4.351	8359	1.15
5	30	4.35	8252	1.14
6	30	4.359	8032	1.16
Mean		4.3495	8200.83333	1.1383333
SD		0.009586449	111.494245	0.017224
%RSD		0.220403466	1.35954775	1.5130906

CONCLUSION

The proposed 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. And hence, this method can be easily and conveniently adopted for routine analysis of Indacaterol maleate in pure form.

ACKNOWLEDGEMENT

No conflict of interest.

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