DEVELOPMENT AND VALIDATION OF METHOD FOR DETERMINATION OF CICLESONIDE INN (MICRONIZED) BY HPLC

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ABSTRACT

A simple, selective and rapid reversed phase high performance liquid chromatographic (RPHPLC) method for the analysis of Ciclesonide has been developed and validated. The separation was achieved from HPLC Column (Waters Symmetry C18 5 μ ; 4.6 mm x 250 mm) with a mobile phase consisting of 85 volume of Ethanol with 15 volume of Millie-Q-Water at a flow rate of 1.10 ml/min with UV detection at 242 nm. The method is specific and it is observed that no interference with diluents. The proposed method is accurate with 99.6 % recovery for Ciclesonide and precise (% RSD of area of system precision, % RSD of assay of method precision and Intermediate precision are found to be 0.20 %, 0.20 % and 0.20 % respectively). From the linearity study the correlation coefficient is found to be 0.9999, which indicates that the method is linear over 10 % to 150 % range. The method is found robust for possible changes. Therefore, this method can be used as a more convenient and efficient option for the analysis of Ciclesonide to establish the quality of the drug substance during routine analysis with consistent and reproducible results.

Keywords: Validation, HPLC, Development, Ciclesonide.

INTRODUCTION

The Ciclesonide is a non-halogenated glucocorticoid having the chemical name pregna-1, 4-diene-3, 20-dione, 16, 17-[[[(R)-cyclohexylmethylene] bis (oxy)]-11-hydroxy-21-(2-methyl-1-oxopropoxy), (11 β , 16 α). The empirical formula is C₃₂H₄₄O₇ and its molecular weight is 540.7. Ciclesonide is a white to yellow-white powder. It is soluble in dehydrated alcohol, acetone, dichloromethane and chloroform. Ciclesonide is used to treat asthma and allergic rhinitis. The side effects of Ciclesonide include headache, nosebleeds and inflammation of the nose and throat linings.¹ In the lung, Ciclesonide is metabolized to its active metabolite which has a higher affinity for glucocorticoid receptors. Although Ciclesonide has a different structure, it still acts like other inhaled corticosteroids by reducing bronchial hyper-reactivity and inflammation in the airways. Validation is a fast growing and evolving subject. Validation is a requirement that has always made sense from both a regulatory and quality perspective.^{2,3} As the analytical process varies so widely there is no universal approach to validation regulatory bodies such as FDA and EC for medicinal products have developed general non-mandatory guidelines.^{4,5} The most common reason for validation is to guarantee as far as possible that all processes and machinery in the Pharmaceutical manufacturing process are being used in a way which will ensure safety, integrity, quality and strength of the product for use by the general public.^{6,7} The official methods for determination of Ciclesonide by liquid chromatography include in Indian pharmacopoeia – 2007.^{8,9} other methods available for identification as well as determination of Ciclesonide include LC-MS/MS¹⁰ and UV-visible spectrophotometry.¹¹ But these methods are for the combined preparation and only few methods are available for determination of Ciclesonide in bulk drugs, so the present work was undertaken with the aim to develop and validate a

rapid and consistent reversed-phase high performance liquid chromatographic method for determination for Ciclesonide in bulk drug according to ICH guideline.¹²

MATEREALS AND METHODS

Reagents and Chemicals

HPLC grade Ethanol from Merck, Germany; Ciclesonide (INN) working standard (WS) from Cipla Ltd. India were used in this study. Purified water was used for the analytical purpose.

Instruments

A Waters Alliance 2695 separation module with PDA detector, USA was used. The HPLC method used a Water symmetry C18 5 μ , 4.6 mm x 250 mm column. Analytical Balance, pH meter from Mettler, UK and Micropipette from Fischer, Germany was used.

Method Development

Preparation of Mobile Phase

A mixture of Ethanol and purified water in the ratio of 85:15 was prepared and the mixture was filtered through 0.45 μ nylon membrane and then degassed.

Preparation of Diluent

HPLC Grade Absolute Ethanol

Chromatographic Conditions

Column used in this method: Waters Symmetry C18 5 μ ; 4.6 mm x 250 mm, injection volume was 50 μ l. Detection was carried out at 242 nm and the flow rate was 1.10 ml/min.

Reference Solution

16.0 mg of Ciclesonide working standard was taken in to a 100 ml volumetric flask. Diluent was added and sonicated to

dissolve. Then 5 ml of this solution was diluted to 50 ml with diluent.

Preparation of Test Solution

16.0 mg of the test sample was taken in to a 100 ml volumetric flask. Diluent was added and sonicated to dissolve. Diluent was added to make the volume 100 ml and was mixed well. Then 5 ml of this solution was diluted to 50 ml with diluent.

System Suitability solution

The Reference solution is used as a system suitability solution and 50 μ l of five replicate injections of Reference solution were injected. The chromatogram was recorded and the system suitability parameters for each of the injection was checked and also was checked for % RSD of area within 1.0 % and Tailing factor not more than 2.0, Plate count not less than 1000.

Method Validation

System Suitability

The system was deemed suitable if the following acceptance criteria were satisfied. The relative standard deviation (% RSD) of the peak area responses for Ciclesonide from five replicate injections of Reference solution is not more than 2.0 %, The tailing factor is not more than 2.0, theoretical plate counts in is not less than 1000.

Specificity

For specificity study identification, placebo (diluent) interference and RT ratio of Sample and Standard were observed.

Linearity

The linearity was carried out by observing the correlation coefficient (r) of standard solution.

System Precision

System Precision was carried out by performing six replicate Injections of standard at 100 % of the test concentration and calculating the % RSD of the measured area.

Method Precision

To demonstrate method precision, six replicate of sample against standard at 100 % of test concentration was carried out and the precision of method was calculated by computing % RSD of six measurements.

Intermediate Precision (Ruggedness)

Intermediate precision or Ruggedness study of an analytical method is the degree of reproducibility of the test results obtain by the analysis of the same samples under a variety of normal test conditions. Test sample of Ciclesonide representing single batch was analyzed by two different analysts on two different equipments and on two different days. The ruggedness of the test method was calculated by measuring % RSD of six results and % RSD of results of two analysts.

Accuracy

Study was carried out over a range of 80 %, 100 % and 120 % (3 replicates each) of the test concentration. The % recovery and RSD of % recovery for each concentration was also measured.

Range

Data generated in linearity, precision and accuracy were considered for establishing the range of the analytical method.

Robustness

Robustness of the method was investigated by changing flow rate (± 0.1 %), changing column temperature ($\pm 5^\circ\text{C}$), and ratio of components of mobile phase.

Stability Study

The solution stability experiments were carried out under room temperature at intervals of 0 h, 6 h, 12 h, 18 h, 24 h, 30 h and 48 h.

RESULTS AND DISCUSSION

System Suitability

System suitability is an integral part of analytical procedures. In optimized chromatographic conditions Relative Standard Deviation (% RSD) of area of Ciclesonide was 0.08 (NMT 1.0 %), % RSD of retention time was 0.27, Average Tailing factor was 1.4 (NMT 2.0) and theoretical plate count was 1433 (NLT 1000) (Table 1).

Specificity

Specificity of an analytical method is its ability to assess unequivocally the analyte in the presence of components that may be expected to be present. Lack of specificity of an Individual analytical procedure may be compensated by other supporting analytical Procedures.¹³ From the specificity study it is observed that the chromatogram for Ciclesonide Sample with Ciclesonide reference Standard showed positive response and Blank (diluent) had no response. So the method was specific.

Linearity

The linearity of an analytical method is its ability to elicit test results directly proportional to the concentration of the analyte in samples within given range.¹⁴ Linearity of the method was evaluated from the correlation coefficient of calibration curves that were constructed from mean peak area of Ciclesonide at different concentrations level (10 %, 20 %, 30 %, 50 %, 80 %, 90 %, 100 %, 120 %, 130 % and 150 %). Correlation coefficient was 0.9999 (Table 2) (Figure 1).

System Precision

System Precision was carried out by performing six replicate Injections at 100 % of the test concentration and calculating the % RSD of the measured area. Table 3 shows the system precision data. From the data it is observed that the % Relative Standard Deviation of area was 0.20 % which was well within the acceptance limit of 2.0 %.

Method Precision

The result shows that the % RSD of six sample assay results was found to be 0.20 % which was within the acceptance limit (Table 4).

Intermediate Precision or Ruggedness

Assay results by two different analysts at different days have been found very much close to each other and with a difference of only 0.2 % and the % RSD of two analysts (12 samples) is 0.20 % which was within acceptance criteria. So the method can be considered to be rugged enough (Table 5).

Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The result shows that Average % Recovery at different Accuracy level is 99.6 % and % RSD for individual % recovery meets the acceptance criteria (Table 6).

Range

The specified range is normally derived from linearity studies and depends on the intended application of the procedure. It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of analyte within the extremes of the specified range of the analytical procedure. Based on the Linearity, precision and accuracy results, the Range of the method was determined as 80 % to 120 % of the target concentration (Table 7).

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage.¹⁵ Robustness of the method was investigated by changing flow rate (± 0.1), changing column temperature ($\pm 5^\circ\text{C}$), and ratio of components of mobile phase. From the results presented on Table 8, it is clear that the system suitability criteria meet with the acceptance limit. Hence the method is robust.

Stability Study

From the stability study data, it was observed that the test sample solution is found to be stable up to 48 h at ambient condition. (Table 9)

Table 1: System Suitability Study

Injection	Area	Retention Time	Tailing Factor	Plate Count
1.	1240021	3.75	1.4	1430
2.	1240063	3.74	1.4	1441
3.	1240044	3.75	1.4	1445
4.	1241711	3.74	1.4	1420
5.	1239073	3.74	1.4	1430
Average (n = 5)	1240182	3.74	1.4	1433
Standard deviation	952.21	0.01		
% Relative standard deviation	0.08	0.27		

Table 2: Linearity Study

Level	Concentration in % (X -axis)	Area (Y - axis)
1.	10	128821
2.	20	260444
3.	30	381825
4.	50	640525
5.	80	1013319
6.	90	1143045
7.	100	1245226
8.	120	1532850
9.	130	1658616
10.	150	1916998
Correlation coefficient (r)		0.9999
Acceptance Criteria : Correlation coefficient, $r \geq 0.9990$

Correlation Coefficient, R	0.9999
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Figure 1: Graphical Representation of Linearity

Table 3: System Precision Study

Sr. No.	Weight of Sample (mg)	Area	Weight of Standard (mg)	Area of Standard
01	15.9	1236924	16.13	1240621
02		1240619		
03		1237180		
04		1234788		
05		1239419		
06		1234564		
Average n = 6		1237249		
Standard deviation		2426.60		
% Relative Standard deviation (% RSD)		0.20		

Table 4: Method Precision

Sr. No.	Weight of Sample (mg)	Area	Weight of Standard (mg)	Area of Standard	% of Assay
01	16.0	1241676	16.13	1240621	99.5
02	16.0	1239519			99.3
03	16.0	1241798			99.5
04	16.0	1239373			99.3
05	15.9	1237813			99.8
06	16.0	1239454			99.3
Average n = 6					99.5
Standard deviation					0.20
% Relative Standard deviation (% RSD)					0.20

Table 5: Table for Intermediate Precision or Ruggedness Study

Analyst Name	Analyst 1			Analyst 2		
Area of Std.	1240621			1237999		
Sr.No.	Weight of sample (mg)	Area	Assay (%)	Weight of sample (mg)	Area	Assay (%)
1.	16.0	1241676	99.5	16.0	1239646	99.2
2.	16.0	1239519	99.3	16.0	1241997	99.4
3.	16.0	1241798	99.5	16.0	1236375	99.0
4.	16.0	1239373	99.3	15.9	1235437	99.5
5.	15.9	1237813	99.8	16.0	1240284	99.3
6.	16.0	1239454	99.3	16.0	1237772	99.1
	Mean Assay n = 6		99.5	Mean Assay n = 6		99.3
	Standard deviation n = 6		0.20	Standard deviation n = 6		0.19
	% Relative standard deviation n = 6		0.20	% Relative standard deviation n = 6		0.19
Difference (Difference between mean assay of Two different analysts) → 0.2 %						

Table 6: Accuracy Study

Wt of Std.(mg)	Level	Weight of sample (mg)	Amount recovered (%)
16.13	80 %	12.9	99.5
		12.8	99.8
		12.8	99.8
	100 %	16.0	99.5
		16.1	99.2
		16.0	99.4
	120 %	19.0	99.7
		18.9	100.2
		19.0	99.7
Average (n = 9)			99.6
Standard Deviation (SD)			0.29
% Relative Standard Deviation (% RSD)			0.29

Table 7: Range Study

Parameter	Concentration Range	Acceptance limit	Result
Linearity	10 % to 150 %	R NLT 0.9990	R = 0.9999
Method Precision	100 %	% RSD of Assay NMT 2.0 %	0.20 %
Intermediate Precision	100 %	% RSD of two analyst NMT 2.0 ± 2 % Difference of each other	% RSD of 2 analyst = 0.20 % Difference of assay = 0.2 %
Accuracy	80 % to 120 %	% Recovery: (98 – 102) % and % RSD: NMT 1.0 %	% Recovery: 99.6 %, % RSD: NMT 0.29 %

Table 8: Data of System Suitability (Robustness Study)

Parameter	% RSD of Area	Theoretical Plate Number	Tailing Factor
Flow rate + 0.1 %	0.08 %	1433	1.4
Flow rate – 0.1 %	0.09 %	1512	1.4
Column Temperature at 40°C	0.08 %	1442	1.4
Column Temperature at 50°C	0.07 %	1447	1.4
Ethanol : Milli-Q-Water (90 : 10)	0.09 %	1478	1.4
Ethanol : Milli-Q-Water (80 : 20)	0.11 %	1432	1.4

Table 9: Solution Stability Study

Measurements	% of Assay	% Difference from Initial
At 0 h	99.6	-
After 6 h	99.3	- 0.3
After 12 h	99.3	- 0.3
After 24 h	99.1	- 0.5
After 36 h	99.2	- 0.4
After 48 h	99.4	- 0.2

CONCLUSION

The method adopted for estimation of Ciclesonide by HPLC is precise, linear, accurate, rugged and robust enough. The sample solution is found to be stable up to 48 h at ambient condition. Hence this method can be considered validated for its intended purpose to establish the quality of the drug substance during routine analysis with consistent and reproducible results.

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