ANALYTICAL STABILITY INDICATING UPLC ASSAY AND VALIDATION OF CICLESONIDE IN DRY POWDER INHALER DOSAGE FORM

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ABSTRACT

A selective, precise, accurate and stability indicating UPLC method is validated for estimation of Ciclesonide in dry powder inhaler dosage form. The method employed, with Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 μm) column in gradient mode, with mobile phase of Methanol and Acetonitrile in the ratio of 55:45 %v/v. The flow rate was 1.2 ml/min and effluent was monitored at 240nm. Retention time was found to be 7.335±0.005 min. The method was validated in terms of linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) etc. in accordance with ICH guidelines. Linear regression analysis data for the calibration plot showed that there was good linear relationship between response and concentration in the range of 8- 40μg/ml respectively. The LOD and LOQ values for were found to be 0.83836 (μg/ml) and 2.54048 (μg/ml) respectively. No chromatographic interference from dry powder inhaler's excipients and degradants were found. The proposed method was successfully used for estimation of Ciclesonide in dry powder inhaler dosage form.

KEYWORDS: Ciclesonide, UPLC, Validation, dry powder inhaler, stability indicating method.

1. INTRODUCTION

Ciclesonide is a glucocorticoid used to treat asthma. Chemically it is, 2-[(1S,2S,4R,6R,8S,9S,11S,12S,13R)-6-cyclohexyl-11-hydroxy-9,13-dimethyl-16-oxo-5,7-dioxapentacyclo[10.8.0.0^2,^{9}.0^4,^{8}.0^{13},^{18}]icosa-14,17-dien-8-yl]-2-oxoethyl 2-methylpropanoate. Metered dose inhalers (MDI) and Dry powder inhalers (DPI) constitute main-stream therapy regime for Asthma. [1-6] Advantages of inhalation therapy include drug delivery directly to the site of action avoiding drug metabolism in the gastrointestinal tract. Inhaled β2 agonists both short acting (SABA) and Long acting (LABA), Anticholinergic agents and Corticosteroids are the cornerstone in the management of Asthma and COPD. These medications can be given as a liquid inhalation formulation in a metered dose inhaler or as a dry powder inhaler. [7-13] They are available as rescue medication for relief of bronchoconstriction and as preventers for long term therapy. Combination drugs product are available ensuring dose compliance and ease of use for the patient as against taking multiple individual drug products.
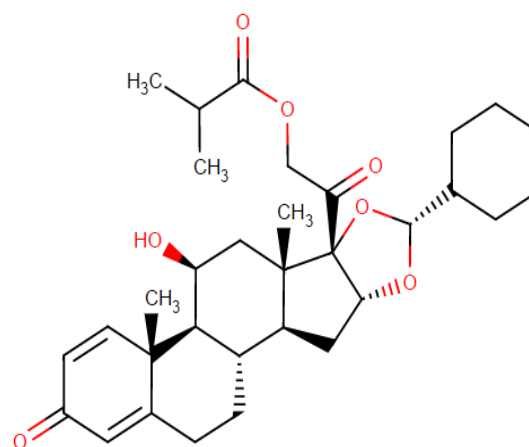

Fig.1: Molecular Structure of Ciclesonide, 2-[(1S,2S,4R,6R,8S,9S,11S,12S,13R)-6-cyclohexyl-11-hydroxy-9,13-dimethyl-16-oxo-5,7-dioxapentacyclo[10.8.0.0^2,^{9}.0^4,^{8}.0^{13},^{18}]icosa-14,17-dien-8-yl]-2-oxoethyl 2-methylpropanoate

Regulatory agencies recommend the use of stability indicating methods (SIMs) for the analysis of stability samples. This requires stress studies in order to generate the potential related impurities under stressed conditions, method development and validation. With the evident of the International Conference on Harmonization (ICH) guidelines, requirements for the establishment of SIMs

have become more clearly mandated. [14] Environmental conditions including light, heat and the susceptibility of the drug product towards hydrolysis or oxidation can play an important role in the production of potential impurities. Stress testing can help identifying degradation products and provide important information

about intrinsic stability of the drug product. [15-16] Therefore, herein we report the results of stability study of Ciclesonide with the aim of determining the extent of the influence of different stress conditions on the stability of the dry powder inhaler product.
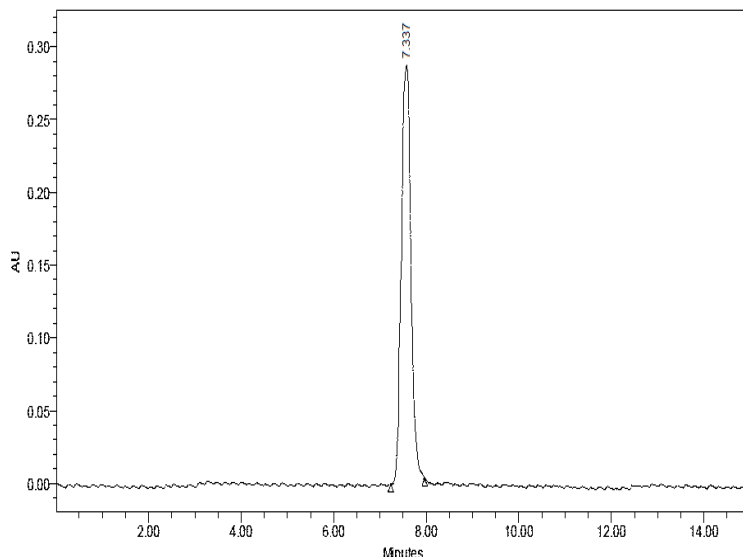

Fig. 2: Standard Chromatogram of Ciclesonide, using mobile phase of Methanol and Acetonitrile in the ratio of 55:45 %v/v

2. EXPERIMENTAL
Materials
Ciclesonide (99.80 % purity) used as analytical standard was procured from Active Pharma Labs (Hyderabad).

HPLC grade methanol, Acetonitrile (HPLC grade) was purchased from Qualigens fine chemicals, Mumbai, India. Distilled, 0.45 μm filtered water used for UPLC quantification and preparation of buffer. Buffers and all other chemicals were analytical grade.

The commercial dry powder inhaler-dosage (Omnaris) labeled to contain 50 mcg/inh of Ciclesonide. All

chemicals used were of pharmaceutical or special analytical grade.

Instrumentation
Acquity, Waters UPLC system consisting of a Water 2695 binary gradient pump, an inbuilt auto sampler, a column oven and Water 2996 wavelength absorbance detector (PDA) was employed throughout the analysis.

The data was collected using Empower 2 software. The column used was Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 μm). A Band line sonerex sonicator was used for enhancing dissolution of the compounds. A Labindia pH System 362 was used for pH adjustment.

Chromatographic Conditions
Table 1: Chromatographic Conditions of the validating method

Parameter	Value
Column	Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 μm)
Mobile Phase	Methanol and Acetonitrile in the ratio of 55:45 %v/v
Flow rate	1.2 mL/min
Run time	14 Min.
Column Temperature	Maintained at ambient temperature
Injection volume	20 μL
Detection wavelength	240nm
Diluent	Mobile Phase

Preparation of Standard Stock Solution
Preparation of Diluent
In order to achieve the separation under the optimized conditions after experimental trials that can be

summarized. Stationary phase like Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 μm) column was most suitable one, since it produced symmetrical peaks with high resolution and a very good sensitivity and with good

resolution. The flow rate was maintained 1.2 mL min-1 shows good resolution. The PDA detector response of Ciclesonide was studied and the best wavelength was found to be 240 nm showing highest sensitivity.

The mixture of two solutions methanol and acetonitrile in the ratio of 55:45%v/v with gradient programming was used as mobile phase at 1.2mL/min was found to be an appropriate mobile phase for separation of Ciclesonide. The column was maintained at ambient temperature.

Preparation of internal standard solution
Weighed accurately about 10 mg of prednisolone working standard and transfer to 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely and then volume was made up to the mark with mobile phase to get 100 µg/ml of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter.

Preparation of Ciclesonide standard solution
Weighed accurately about 10 mg of Ciclesonide and transfer to 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely and then volume was made up to the mark with mobile phase to get 100 µg/ml of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter. Linearity was determined in the range of 8- 40 µg mL-1.

Stability Indicating Studies
Stability Indicating studies like acid hydrolysis, basic hydrolysis, dry heat degradation, wet heat degradation and oxidative degradation were carried out.

3. RESULTS AND DISCUSSIONS
Validation
The analytical method was validated with respect to parameters such as linearity, precision, specificity and accuracy, limit of detection (LOD), limit of quantitation (LOQ) and robustness in compliance with ICH guidelines.

Linearity and Range
The linearity of an analytical procedure is the ability to obtain test results that are directly proportional to the concentration of an analyte in the sample.

The calibration curve showed good linearity in the range of 8 – 40 µg/ml, for Ciclesonide(API) with correlation coefficient (r2) of 0.9985. A typical calibration curve has the regression equation of y = 7622.875x +17157.7 for Ciclesonide. Results are given in Table 2.
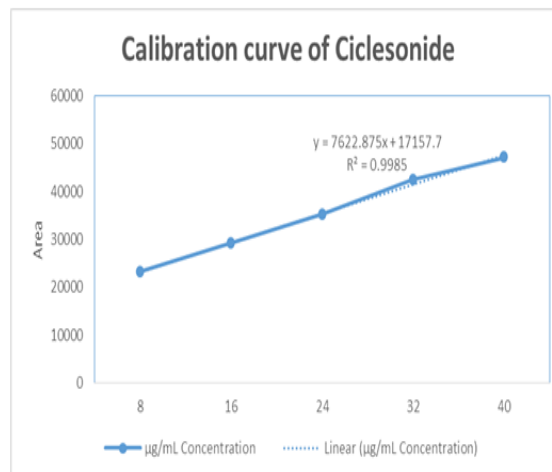
Limit of Detection (LOD) and Limit of Quantitation (LOQ)
The LOD and LOQ of Ciclesonide were calculated by mathematical equation. LOD= 3.3×standard deviation ÷ slope and LOQ=10×standard deviation ÷ slope. The LOD of Ciclesonide was found to be 0.83836 (µg/ml) and the LOQ of Ciclesonide was found to be 2.54048 (µg/ml). Results are given in Table 2.

Table 2: Summary of validation parameters for the proposed method

PARAMETER	CICLESONIDE
Linearity	8 – 40 µg/ml
Intercept (c)	17157.7
Slope (m)	7622.875
Correlation coefficient	0.9985
LOD	0.83836 (µg/ml)
LOQ	2.54048 (µg/ml)

Precision
The Precision of the method was studied in terms of intraday and interday precision of sample injections (16µg/ml). Intraday precision was investigated by injecting six replicate samples of each of the sample on the same day. The % RSD was found to be 0.48%. Interday precision was assessed by analysis of the 6 solutions on three consecutive days. The % RSD obtained was found to be 0.28%. Low % RSD values indicate that the method is precise. The results are given in table 3.

Accuracy
To study the accuracy of method, recovery studies were carried out by spiking of standard drug solution to pre-analyzed sample at three different levels i.e., at 50, 100, and 150%. The resultant solutions were then reanalyzed by the proposed method. At each level of the amount, six determinations were performed. From the data obtained, the method was found to be accurate. The % recovery and %RSD were calculated and presented in Table 4.

Robustness

Small deliberate changes in chromatographic conditions such as change in temperature (± 2°C), flow rate (± 0.1ml/min) and wavelength of detection (± 2nm) were studied to determine the robustness of the method. The results were in favour of (% RSD < 2%) the developed UPLC method for the analysis of Ciclesonide. The results are given in table 5.

Table: 3, Results of Precision Studies

Replicate		Ciclesonide		
S.No.	Concentration Taken (µg/ml)		Area	%LC
1			29234	99.98%
2			29318	99.70%
3	16.18		29421	99.35%
4			29521	99.01%
5			29556	99.90%
6			29581	98.81%
Average				99.45%
Std.Dev				0.4813
% RSD				0.48%
Standard weight				16.18mcg
Standard potency				99.80%

Table: 4, Results of accuracy study

	Ciclesonide					
Level %	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	Mean recovery (%)	Std.Dev	% RSD
50	08.09	08.06	99.62			
100	16.18	16.16	99.87	99.74	0.12503	0.13%
150	24.27	24.21	99.75			

Table: 5, Results of Robustness Studies

Robustness Studies			
Parameter	Value	Peak Area	% RSD
Flow Rate	Low	29541	
	Actual	29546	0.02%
	Plus	29551	
Temperature	Low	29392	
	Actual	29406	0.05%
	Plus	28420	
Wavelength	Low	29604	
	Actual	29609	0.01%
	Plus	29612	

Results of Stability Indicating Studies

According to Singh and Bakshi, the stress testing suggests a target degradation of 20-80 % for establishing stability indicating nature of the method. UPLC study of samples obtained on stress testing of Ciclesonide under different conditions using mixture Methanol and Acetonitrile in the ratio of 55:45 %v/v as a mobile solvent system suggested the following degradation behaviour.

a. Acid hydrolysis

An accurate 10 ml of pure drug sample solution was transferred to a clean and dry round bottom flask (RBF). 30 ml of 0.1 N HCl was added to it. It was refluxed in a water bath at 60°C for 4 hours. Drug became soluble after reflux which was insoluble initially. Allowed to cool at room temperature. The sample was then neutralized using 2N NaOH solution and final volume of the sample was made up to 100ml with water to prepare 100ppm solution. It was injected into the UPLC system against a blank of Methanol and Acetonitrile in the ratio of 55:45 %v/v after optimizing the mobile phase

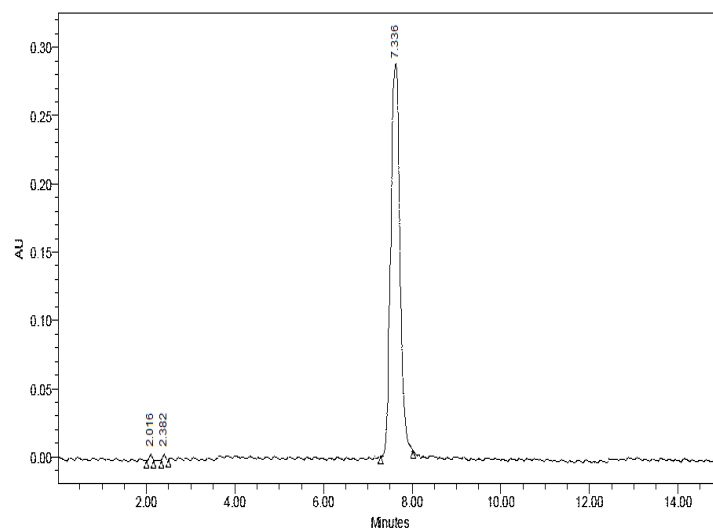composition, chromatogram was recorded and shown in Fig. 3.

Fig. 3: Chromatogram showing the degraded products in Acidic degradation

b. Basic hydrolysis

An accurate 10 ml of pure drug sample solution was transferred to a clean and dry RBF. 30 ml of 0.1N NaOH was added to it. It was refluxed in a water bath at 60°C for 4 hours. Drug became soluble after reflux which was insoluble initially. It was allowed to cool at room temperature. The sample was then neutralized using 2N HCl solution and final volume of the sample was made up to 100ml with water to prepare 100ppm solution. It was injected into the UPLC system against a blank of Methanol and Acetonitrile in the ratio of 55:45 %v/v after optimizing the mobile phase composition, chromatogram was recorded and shown in Fig. 4.
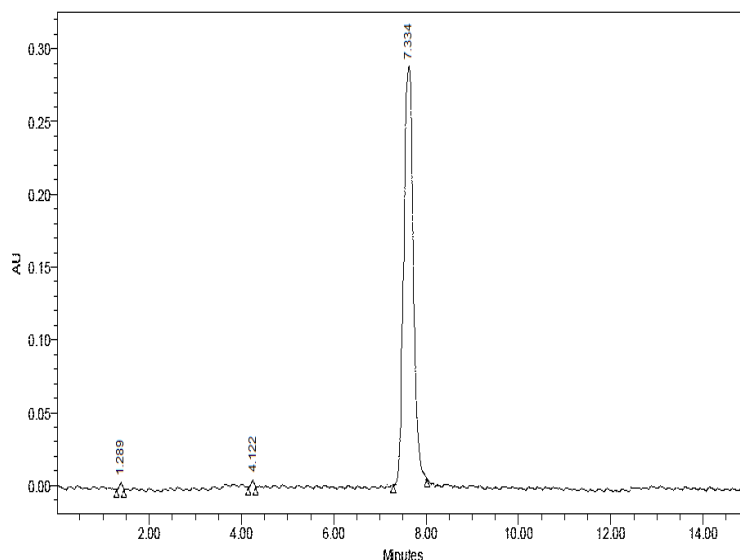

Fig. 4: Chromatogram showing the degraded products in Basic degradation

c. Wet heat degradation

Accurate 10 ml of pure drug sample was transferred to a clean and dry RBF. 30 ml of HPLC grade water was added to it. Then, it was refluxed in a water bath at 60°C for 6 hours uninterruptedly. After the completion of reflux, the drug became soluble and the mixture of drug and water was allowed to cool at room temperature. Final volume was made up to 100 ml with HPLC grade water to prepare 100 ppm solution. It was injected into the UPLC system against a blank of Methanol and Acetonitrile in the ratio of 55:45 %v/v after optimizing the mobile phase composition, chromatogram was recorded and shown in Fig. 5.
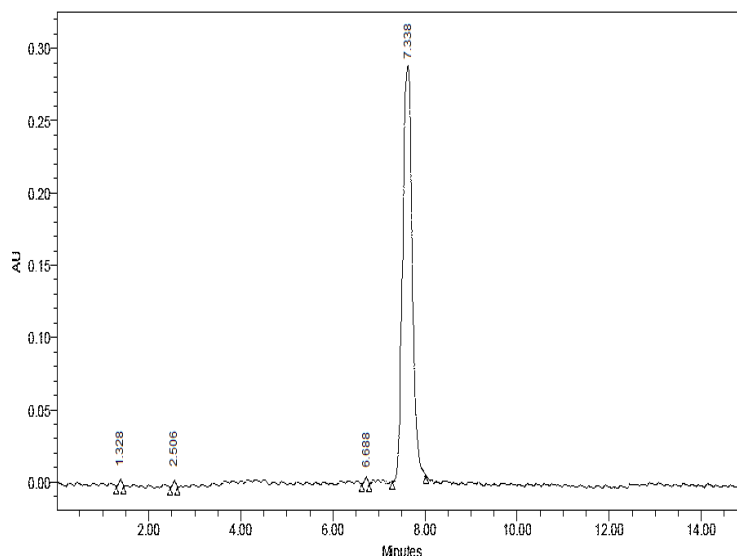
Fig. 5: Chromatogram showing the degraded products in Wet heat degradation

d. Oxidation with (3%) H₂O₂

Approximately 10 ml of pure drug sample was transferred in a clean and dry 100 ml volumetric flask. 30 ml of 3% H_2O_2 and a little methanol was added to it to make it soluble and then kept as such in dark for 24 hours. Final volume was made up to 100 ml using water to prepare 100 ppm solution. The above sample was injected into the UPLC system. The chromatogram was recorded and shown in Fig. 6.
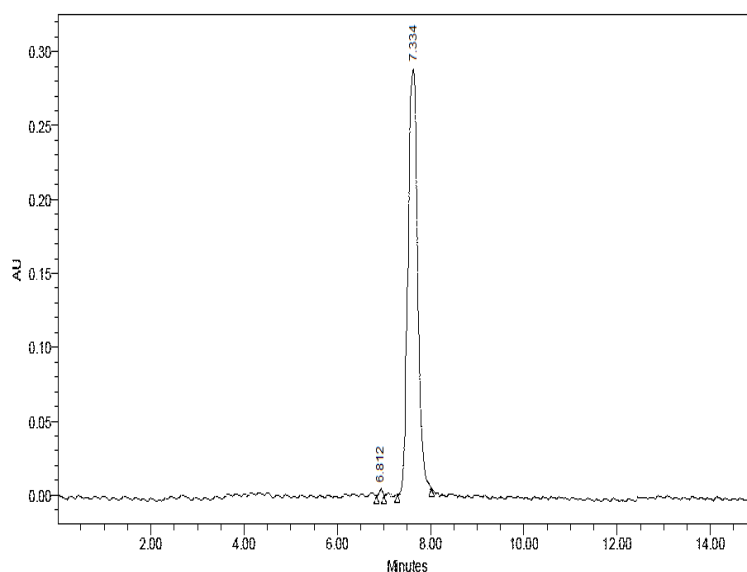

Fig. 6: Chromatogram showing the degraded products in H₂O₂

In all degradation studies, there was a significant formation of degradation products when compared to that of a standard. This indicates that, the drug may be degraded to low molecular weight non-chromophoric compounds.

Table: 6, Stability Indicating study for the developed method

Nature of Stress	Degradation condition	Time(h)	Number of degradation products (Rt)
Acidic	60°C	3	2 (2.016, 2.382)
Basic	60°C	9	2 (1.289, 4.122)
Oxidative	RT	48	1 (6.812)
Wet Heat	105°C	24	3 (1.328, 2.506, 6.688)

4. CONCLUSION

A selective and sensitive stability indicating UPLC method has been validated for the analysis of Ciclesonide in bulk drug and dry powder inhaler dosage form. Based on peak purity results, obtained from the analysis of stability indicating studying samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Ciclesonide indicated that the developed method is specific for the estimation of Ciclesonide in presence of degradation products. Further the proposed UPLC method has excellent precision, sensitivity and reproducibility. Even though no attempt has been made to identify the degraded products, proposed method can be used as stability indicating method for assay of Ciclesonide in commercial formulations.

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