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RESEARCH ARTICLE

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A NOVEL RP-HPLC METHOD FOR THE QUANTIFICATION OF ROFLUMILAST IN FORMULATIONS

ABSTRACT

A simple, precise and accurate RP-HPLC method was developed and validated for rapid assay of Roflumilast tablet dosage form. Isocratic elution at a flow rate of 1.0ml/min was employed on a symmetry Chromosil C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of Methanol: Acetonitrile 25:75 v/v. The UV detection wavelength was 244 nm and 20µl sample was injected. The retention time for Roflumilast was 4.4 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Roflumilast tablet dosage form and bulk drug.

Key Words: Roflumilast, RP-HPLC, UV detection, recovery, precise, 244 nm

INTRODUCTION

Roflumilast is a drug which acts as a selective, long-acting inhibitor of the enzyme PDE-4. It hasantiinflammatory effects and is under development as an orally administered drug for the treatment of inflammatory conditions of the lungs such as asthma, and chronic obstructive pulmonary disease (COPD). While roflumilast was found to be effective in clinical trials, it produced several dose-limiting side effects including nausea, diarrhoea and headache, and development is continuing in an attempt to minimise the incidence of side effects while retaining clinical efficacy.

In June 2010, Daxas was approved in the EU for severe COPD associated with chronic bronchitis` In March 2011, Daliresp gained FDA approval in the US for reducing COPD exacerbations.

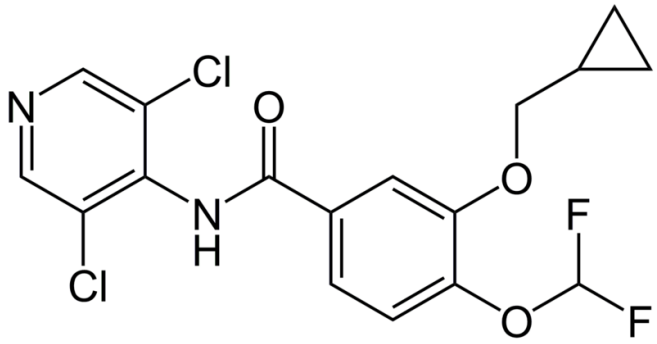

Figure.1 Structure of Roflumilast

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Materials

Working standard of Roflumilast was obtained from well reputed research laboratories. HPLC grade water, Methanol was purchased from E. Merck (Mumbai, India).

Apparatus

A Series HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil C18. 250×4.6mm, Electronic balance-DENVER (SI234), manual Rheodyne injector with a 20 µl loop was used for the injection of sample. PEAK LC software was used. UV 2301 Spectrophotometer was used to determine the wavelength of maximum absorbance

Determination of wavelength of maximum absorbance

The standard solutions of Roflumilast were scanned in the range of 200 -400 nm against mobile phase as a blank. Roflumilast showed maximum absorbance at 244 nm. So the wavelength selected for the determination of Roflumilast was 244 nm.

Chromatographic equipment and conditions

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of Roflumilastan isocratic PEAK HPLC instrument with Zodiac C18 column (250 mm x 4.6 mm, 5µ) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC – 7000 UV-detector. A 20µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software.

The mobile phase consisted of Methanol: Acetonitrile 25:75 v/v. Injections were carried out using a 20 µl loop at room temperature (20 + 2 °C) and the flow rate was 1.0 ml/min. Detection was performed at 244 nm with 10min runtime.

Standard and sample solutions

A 10 mg amount of Roflumilast reference substance was accurately weighed and dissolved in 10 ml mobile phase in a 10 ml volumetric flask to obtain 1000 ppm concentrated solution. Required concentrations were prepared by serial dilution of this solution.

A composite of 20 (Daliresp) tablets was prepared by grinding them to a fine, uniform size powder. 10 mg of Roflumilast was accurately weighed and quantitatively transferred into a 100 ml volumetric flask. Approximately 25 ml mobile phase were added and the solution was sonicated for 15 min. The flask was filled to volume with mobile phase, and mixed**.**

Method validation

Method validation was performed following ICH specifications for specificity, range of linearity, accuracy, precision and robustness.

RESULTS AND DISCUSSION

System Suitability

Having optimized the efficiency of a chromatographic separation,the quality of the chromatograph was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2500. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table.1. Standard chromatogram was given in Figure.2

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Api Concentration	60 ppm
Mobile Phase	Methanol: Acetonitrile 70:30(v/v)
Wavelength	210nm
Column	C_{18} Column
P^H	5.8
Concentration	60ppm
Retention Time	2.83min
Run Time	7min
Area	249699
Theoretical Plates	8256
Tailing Factor	0.63
Pump Pressure	13.4 MPa

Table.1 System suitability parameters of Roflumilast

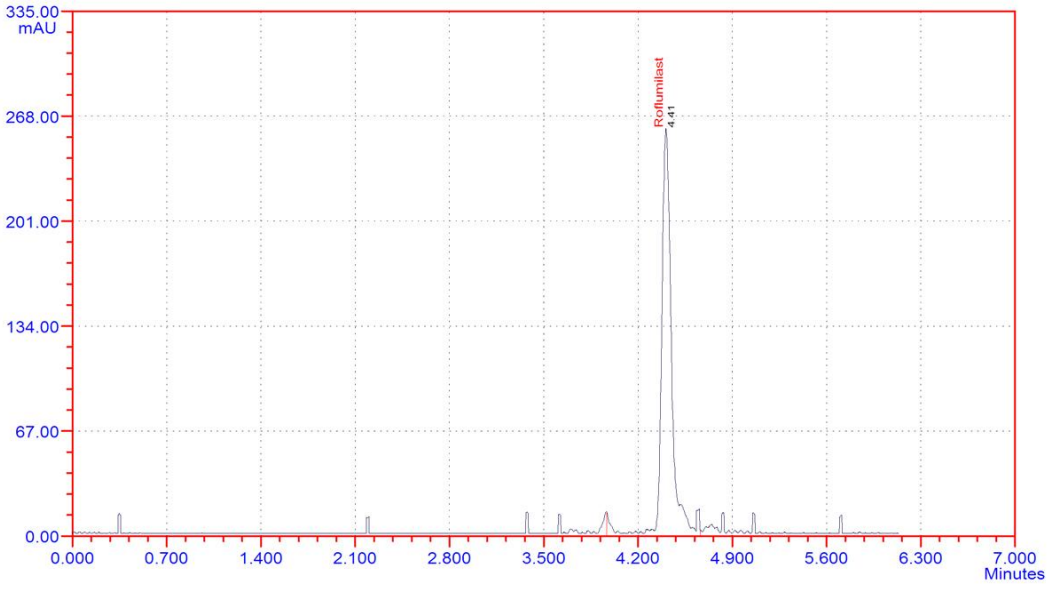

Figure.2: Standard chromatogram of Roflumilast

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Range of linearity

Standard curves were constructed daily, for three consecutive days, using seven standard concentrations in a range of 10, 20, 40, 60, 80 and 100ppm for Roflumilast. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = 7884.705 + 4117.042x (r= 0.999). Linearity values can show in Table: 2

S.No	Concentration (µg/ml)	Area
1	10	49607
2	20	97927
3	40	179362
4	60	249699
5	80	333412
6	100	421469
	Slope	4117.042
	Intercept	7884.705
	CC	0.999249

Table.2: Linearity results of Roflumilast

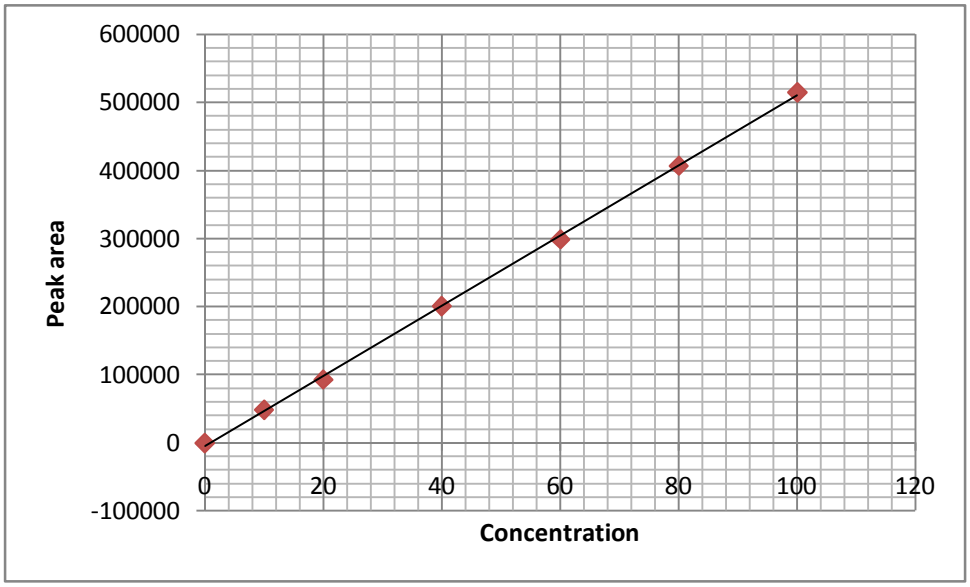

Figure 3: Calibration curve of Roflumilast

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Precision

To study precision, six replicate standard solutions of Roflumilast(60ppm) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated and it was found to be which is well within the acceptance criteria of not more than 2.0%. The % RSD Was found to be both intraday and inter day was 1.23 and 1.12.

Limit of Detection and Limit of Quantification:

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. After 0.80ppm dilution Peak was not clearly observed, based on which 0.01 ppm is considered as Limit of Detection and Limit of Quantification is 0.05 ppm.

Parameter	Measured Value
Limit of Quantification	0.05ppm
Limit of Detection	0.01 ppm

Table.3: LOD and LOQ results of Roflumilast

Robustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. The robustness study was performed by slight modification in flow rate of the mobile phase, composition of the mobile phase and wavelength of the detector. Roflumilast at standard concentration was analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above. Results were shown in table 4.

S.NO	Parameter	Change	Area	% of Change
1	Standard	……	249699	……
2	MP	Methanol:ACN 05:95 45:55	249896 253624	0.11 1.57
3	PH	5.6 6.0	251426 251498	0.69 0.72
4	WL	223nm 219nm	250986 250786	0.51 0.43

Table.4: Robustness results of Roflumilast

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Ruggedness:

Ruggedness was performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different. Ruggedness also expressed in terms of percentage relative standard deviation. The %RSD was found to be 0.66

Recovery

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. Recovery test was performed at 3 different concentrations i.e.60ppm, 80ppm, and 100ppm. The percent recovery was calculated and results are presented in Table. Satisfactory recoveries ranging from 98.6 to 101. 6 were obtained by the proposed method. This indicates that the proposed method was accurate. Results are given in table.5

Table.5: Recovery results of Roflumilast

% Recovery	Roflumilast				
	Target Conc., (ppm)	Spiked conc, (ppm)	Final Conc, (ppm)	Conc., Obtained	% of Recovery
50%	40	20	60	59.93	99.8
	40	20	60	60.2	100.3
	40	20	60	60.8	101.4
100%	40	40	80	79.8	99.7
	40	40	80	81.1	101.3
	40	40	80	80.9	101.1
150%	40	60	100	98.9	98.9
	40	60	100	99.4	99.4
	40	60	100	101.3	101.3

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Table. 6: Formulation Analysis

Formulation	Dosage	Concentration	Amount found	% Assay
Daliresp	500 mcg	80 ppm	79.92	99.9

CONCLUSION

The proposed method for the assay of Roflumilastin tablets is very simple and rapid. It should be emphasized it is isocratic and the mobile phase do not contain any buffer. The method was validated for specificity, linearity, precision, accuracy and robustness. Although the method could effectively separate the drug from its products, further studies should be performed in order to use it to evaluate the stability of pharmaceutical formulations.

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