

RESEARCH ARTICLE**Analytical Method Development and Validation of RP-HPLC for Estimation of Roflumilast in Bulk drug and Tablet Dosage Form**Ladani JJ*¹, Bhimani RD¹, Vyas KB², Nimavat KS³¹Research Scholar, JJT University, Jhunjhunu, Rajasthan, India.²Sheth L. H. Science College, Mansa, Gujarat, India.³Government Science College, Gandhinagar, Gujarat, India.

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ABSTRACT

A new simple, specific, sensitive, rapid accurate and precise RP-HPLC method was developed for the estimation of Roflumilast in bulk and pharmaceutical formulation. Roflumilast was chromatographed on zorbex XDB C18 column (150 mm × 4.6 mm, 5 μ m) in a mobile phase consisting of mixture of ammonium acetate buffer and a solvent mixture (Acetonitrile : Methanol : 80 : 20) in the ration of 40:60v/v. The mobile phase was pumped at a flow rate of 1.0 ml/min with detection at 245 nm. The detector response was linear in the concentration of 1-15 μ g/ml. the intra and inter day variation was found to be less than 1.0%. The mean recovery of the drug from the solution was 100.1%. Hence it can be applied for routine quality control analysis of Roflumilast in bulk and pharmaceutical formulation.

KEYWORDS

Roflumilast, RP-HPCL, Accuracy, Precision.

INTRODUCTION

Roflumilast, which is an anti-inflammatory medicine called phosphodiesterase 4 inhibitor. Roflumilast reduces the activity of phosphodiesterase 4, a protein occurring naturally in body cells. It is chemically 3-(cyclopropylmethoxy)-N-(3,5-dichloro-4-pyridinyl)-4-(difluoromethoxy)-benzamide.

Roflumilast is used to treat severe COPD in adults. The typical dose of Roflumilast 0.5 microgram per day. The present study was to develop a RP-HPLC method for estimation of Roflumilast.^(2,3,4) A literature survey reveals a few analytical method have been reported for the quantitative estimation for the Roflumilast. Hence an attempt has been made to develop new HPLC method for its estimation in bulk and

pharmaceutical formulation with good precision, accuracy, linearity, and reproducibility. It is not official in any pharmacopoeia.

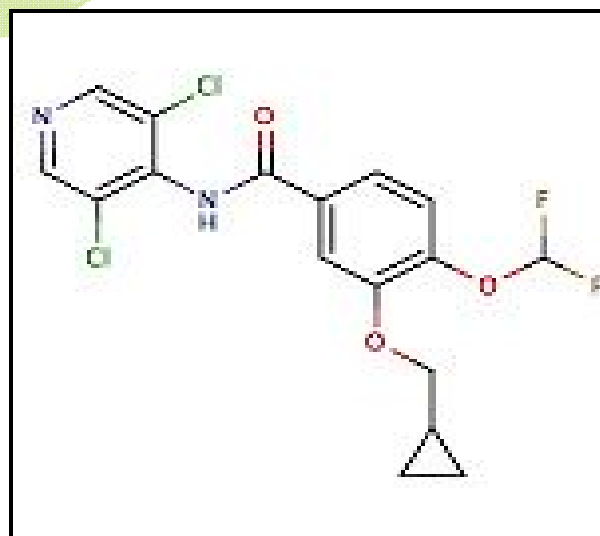


Figure 1: Chemical structure of Roflumilast

***Address for Correspondence:**

Jatin Ladani

Research Scholar,
JJT University, Jhunjhunu,
Rajasthan, India.E-Mail Id: jatin_ladani@yahoo.co.in

MATERIALS AND METHODS

MATERIALS

Roflumilast and its tablet (0.5 microgram per tablet) were generous gift from Zydus cadila. All solvents and reagents were of analytical or HPLC grade. HPLC- grade water was prepared by using MILLI-Q water purification system. All the reagents used in this method were of analytical reagent grade and HPLC grade methanol was used. Distilled water, filtered through 0.45 µm filter (Millipore) was used to prepare solutions. Mobile phase consist of mixture of Buffer (1.2 gm of Ammonium acetate in 2000 ml of water and filter through 0.45 µm filter paper.) and solvent mixture (Acetonitrile: Methanol:: 80: 20) in the ratio of 40:60. Methanol was used as diluents for the sample preparation. An isocratic high pressure liquid chromatography-mass with one LC-10 AT VP pumps, with UV/VIS detector, and a Zorbex eclipsed XDB C18, 5µm; 150 mm × 4.6 mm was equipped with the software class class- vp (shimadzu).

DETERMINATION OF λ MAX

The standard solution of 10 µg/ml of Roflumilast was scanned in the range of 200-400 nm and the λ max was determined. The overlain spectra of the drug are also run.

CALIBRATION CURVE FOR THE ROFLUMILAST (4-12 µg/ml)

Appropriate volumes of aliquots from standard Roflumilast stock solution were transferred to different volumetric flasks of 50 ml capacity. The volume was adjusted with the mark with the methanol to obtain a concentration of 4, 6, 8, 10, and 12µg/ml. The curve of the each solution against the methanol was recorded. Absorbances were recorded at 245 nm and the plot of absorbance vs. concentration was plotted. The straight- line equation was determined.

PREPARATION OF STOCK SOLUTION

An accurately weighed quantity of Roflumilast working/reference standard about 20 mg and transferred in to 200 ml volumetric flask. About 50 ml of methanol was added and sonicated to

dissolve. The solution was cooled to the room temperature and made up to mark with methanol. All solutions were freshly prepared prior to analysis. This stock solution is used for making dilutions for calibration curve.

PREPARATION OF STANDARD SOLUTION

5 ml of stock solution of Roflumilast was pipette out and transferred to 50 ml volumetric flask and made volume up to mark with diluent.

PREPARATION OF SAMPLE SOLUTION

10 tablets were accurately weighed and crushed. Weighed accurately powder which is equivalent to 10 mg Roflumilast and transferred into 100 ml volumetric flask. Approximate 50 ml methanol was added and sonicated for 20 minutes. The solution was cooled to the room temperature and make up to volume with methanol. The solution was filtered through 0.45 µm filter. First 5 ml of the filtrate was discarded and the remaining solution was filtered.

PROCEDURE

Separately injected 5 replicated of about 10 µl of the standard preparation and two replicate of the sample solution, the chromatograms was recorded for 10 min and the peak areas was measured and the % amount of Roflumilast was calculated with respect to the individual average of standard area.

CALCULATION

$$\% \text{ of Amount present in Tablet} = \frac{A \times C \times E \times P \times C}{B \times D \times L.A}$$

Where,

A: Sample area

B: Standard area

C: Dilution factor of standard

D: dilution factor of sample

E: weight of standard

P: Potency of standard

L.A: Labeled amount

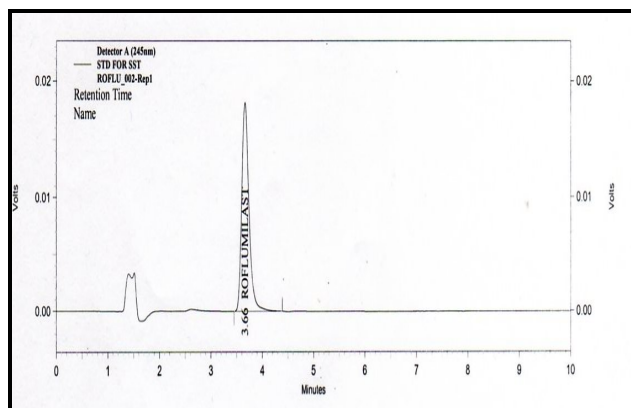


Figure 2: Chromatogram of Standard Roflumilast

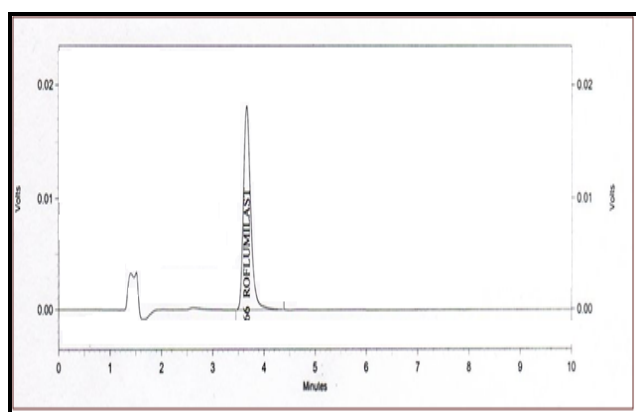


Figure 3: Chromatogram of Sample Roflumilast

DEVELOPMENT OF OPTIMUM MOBILE PHASE

The present investigation was aimed to develop a simple, precise and accurate HPLC method to estimate Roflumilast tablet by using RP-HPLC C18 column. Initially different mobile phase compositions were tried and finally the mobile phase was optimized with acetate buffer and solvent mixture (Acetonitrile: Methanol:: 80:20) in the ratio of 40:60. With above mobile phase good peak shape with a reasonably short run time of 10 minute. UV detection was carried out at 245 nm.

VALIDATION OF METHOD

System Suitability Parameter

Retention time of Roflumilast was found to be 3.66 min. The peak shape of Roflumilast was symmetrical and the asymmetry factor was less than 1.5. The proposed method was validated as

per standard analytical procedure. The sample was repeated 6 times and the same retention time was observed in all the cases. System suitability parameter of Roflumilast is given in table.

Linearity

Linearity experiment was performed by giving 6 replicate for the Roflumilast and the response was found to be linear in the range of 1-15 µg/ml for Roflumilast. Linearity of Roflumilast was plotted by a graph of response factor versus concentration. The correlation coefficient 'r' values (n=6) for Roflumilast was 0.999.

Precision

The precision was assessed in terms of Intra - day and Inter - day variation. The Intra - day and Inter day variation in the peak area of drug solution was calculated in terms of coefficient of variation (C.V).

Limit of Detection and Limit of Quantification

The LOD and LOQ for Roflumilast were calculated from linearity of the response data of Roflumilast.

Table 1: System suitability parameter

No	Parameter (n=6)	Roflumilast
1	Retention time	3.66
2	Theoretical plates	3308.11
3	Asymmetry	1.29
4	Linearity range	1-15 µg/ml
5	Correlation coefficient	0.9997
6	Slope	18407.17
7	Intercept	970.74
8	Regression coefficient	0.9995
9	LOD	0.049 µg/ml
10	LOQ	0.151 µg/ml

Robustness

The robustness was checked by changing the flow rate to 0.9 and 1.1 ml/min, temp to 20°C and 30°C and mobile phase ration 38:62 and 42:58.

Table 2: Result of Robustness study

Compound	% RSD (n= 6)		
	Normal Condition	Changed Condition	
Temp	25°C	20C	30C
	0.1	0.4	0.3
Flow Rate	1 ml/min	0.9 ml/min	1.1 ml/min
	0.1	1.3	0.1
Mobile Phase Ration	40:60	38:62	42:58
	0.1	0.4	0.3

Accuracy

The accuracy was carried out in triplicate. The accuracy was expressed in terms of recovery at three levels 50%, 100% and 150%. The result is furnished in Table 3.

Present recovery at each level was calculated. Table 2.shows data from the recovery study for Roflumilast was 100.1%. High percentage of recovery showed that the method was free from interference of excipients used in formulation.

The method was simple and had short run time of 10 min, which make the method rapid. The result of the study indicate that the proposed HPLC method was simple, Precise, highly accurate, specified and less time consuming. The developed method was validated based on ICH guidelines.

Table: 3 Result of Accuracy data

Level	Area	Amt of drug Added (mg)	Amt of drug recovered (mg)	Recovery (%)	Mean (%)	% RSD
50%	92181	0.52	0.52	100%		
50%	92458	0.52	0.52	98.1%	98.7%	1.1%
50%	91609	0.53	0.51	98.1%		
100%	182804	1.00	1.00	102.0%		
100%	183964	1.02	1.02	101.0%	101.3%	0.6%
100%	184420	1.02	1.02	101.0%		
150%	278451	1.58	1.56	99.4%		
150%	282811	1.57	1.58	101.3%	100.2%	1.0%
150%	277445	1.56	1.55	100.0%		

RESULTS AND DISCUSSION

Optimization of the chromatographic condition was carried out with various combinations of buffer and solvent and observing the peak parameters, the run time of the method was set at 10 min., Roflumilast appeared on the typical chromatogram at 3.66 min, which indicates a good base line. When the same drug solution was injected 3 times, the retention time of the drug was same. Linearity range was observed in the concentration range of 1-15 μ g/ml. The regression equation of Roflumilast concentration over its peak area ration was found to be $Y = 18407X + 970.74$ ($r^2 = 0.9995$) where Y is the peak area ratio and X is the concentration of Roflumilast. The proposed HPLC method was also validated for Intra-day and Inter-day variation. The coefficient of variation in the peak area of the drug for 3 replicate injections was found to be less than 2%. The tailing factor was found to be 1.29, which indicates good shape of peak. The number of theoretical plates was found to be 3308, which indicates efficient performance of the column. The limit of detection and limit of quantification was found to be 0.049 μ g/ml and 0.151 μ g/ml which indicates the sensitivity of the methanol. The use of buffer and solvent mixture in the ratio of 40:60 resulted in peak with good shape and resolution. The high percentage of recovery of Roflumilast ranging from 98.7 - 101.3 indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulation did not interfere with the estimation of the drug by proposed HPLC method.

CONCLUSION

The assay experiment showed that the Roflumilast estimated in the tablet dosage form was free from the interference of excipients. Present work describes simple, accurate and economical method for estimation of Roflumilast in pharmaceutical formulation. Hence this method can easily and conveniently be adopted for routine quality control analysis

of Roflumilast in bulk and pharmaceutical formulation.

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