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Journal of Chemical and Pharmaceutical Research

ISSN No: 0975-7384
CODEN(USA): JCPRC5

J. Chem. Pharm. Res., 2011, 3(2):770-775

Validation of Rapid Liquid Chromatographic Method for the Determination of Roflumilast

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ABSTRACT

A rapid HPLC method of roflumilast was validated for parameters specificity, system precision, repeatability, detection limit, quantitation limit, linearity and robustness. High linearity of the analytical procedure was confirmed over the concentration range of 15 – 225 µg/ml and 0.065 to 0.25 µg/ml respectively for assay and related substances for roflumilast. Correlation coefficients for linearity of assay and related substances were 0.9999 and 0.9977 respectively. The low value of the RSD expressed the good repeatability and precision of the method. The detection limit and quantitation limit for roflumilast were found to be 0.02 and 0.065 µg/ml respectively. Robustness of the analytical procedure was evaluated using method variables, namely aqueous phase pH, mobile phase flow rate and column temperature. The method has been shown to be robust towards minor changes in the method parameters.

Keywords: COPD, HPLC, Roflumilast, RRHT, Validation.

INTRODUCTION

Roflumilast is a novel, potent, selective phosphodiesterase 4 (PDE4) inhibitor for the treatment of chronic obstructive pulmonary disease (COPD) and asthma. Its empirical formula is $C_{17}H_{14}Cl_2F_2N_2O_3$ and its molecular weight is 403.21.

COPD, a progressive respiratory disease characterized by gradual loss of lung function and airflow obstruction that is not fully reversible, is a leading cause of morbidity and mortality worldwide.

It is estimated that COPD will be the third leading cause of death globally by the year 2020 [1]. An application has been filed for approval in the US for the treatment of patients with symptomatic COPD. PDE4 is found in inflammatory and immune cells and is the primary

enzyme responsible for the regulation of metabolism and inactivation of cellular 3',5'-cyclic adenosine monophosphate (cAMP) in these cells as well as in airway smooth muscles. PDE4 inhibitors offer a novel anti inflammatory mechanism of action that differs from steroidal anti inflammatory medications.

A rapid method for HPLC analysis of roflumilast in the presence of its degradation products was published recently [2]. The published method describes forced degradation behaviour of roflumilast but other analytical parameters were not validated. The objective of this work was to carry out validation of published method which is an essential regulatory requirement.

The method validation confirms that the analytical procedure employed for the analysis is suitable for its intended use.

EXPERIMENTAL SECTION

Materials

The LC system used for validation was Agilent 1100 series liquid chromatographic RRHT (Rapid Resolution High Throughput) system with diode array detector. Data acquisition and processing was carried out using Chemstation software.

Roflumilast was received as a gift sample from MSN laboratories limited. Acetonitrile (Merck) was of HPLC grade. GR grade formic acid and ammonium formate were procured from Merck India limited. HPLC grade water was obtained through milli Q water purification system.

Chromatographic conditions

The chromatographic separation was achieved using 0.005 M ammonium formate buffer pH 3.5 and acetonitrile on a Zorbax SB C18 50 mm×4.6 mm, 1.8 µm column. Gradient elution program (Table 1) with flow rate of 0.5 ml/min was used. The column was thermostated at 25°C and the injection volume was 3 µl. The UV wavelength of detection was 215 nm.

Table 1: Gradient elution program

Time (min)	Ammonium formate buffer pH 3.5 (%)	Acetonitrile (%)
0	90	10
3	47	53
5	43	57
7	40	60
9	10	90
10	10	90
11	90	10
13	90	10

Sample preparation for determination of assay and related substances (RS)

Accurately weighed and transferred about 15 mg of roflumilast in to a 20 ml volumetric flask. Sufficient amount of acetonitrile was added and sonicated to dissolve. The volume was made up with acetonitrile. Further diluted 2 ml of the above solution to 10 ml with acetonitrile (150 µg/ml of roflumilast).

RESULTS AND DISCUSSION

The developed method was validated for specificity, precision, repeatability, limit of detection (LOD), limit of quantitation (LOQ), linearity and robustness [3].

Specificity
Specificity of the method was demonstrated by injecting diluent i.e. acetonitrile into the chromatographic system. No interfering peaks were present in the chromatogram at the retention time of roflumilast.

Forced degradation studies were performed for roflumilast to provide an indication of the stability indicating property and specificity of the proposed method.

Precision
Precision of the method was demonstrated by system precision and repeatability.

System precision
The variability of the measurement itself is addressed in system precision which includes precision of area and retention time.

Six replicate injections of 0.15 µg/ml and 150 µg/ml concentration of roflumilast were analysed for relative standard deviation (RSD) of retention time and peak area.

System precision data is shown in Table 2.

Table 2: System precision

	Assay	Related substances
Concentration of roflumilast	150 µg/ml	0.15 µg/ml
Retention time	8.64 min	8.70 min
% RSD of retention time	0.00	0.02
% RSD of peak area	0.16	2.12

Repeatability
This short-term variability includes, in addition to system precision, the contributions from the sample preparation, such as weighing, solubilizing, aliquoting and dilution. Therefore, it is essential to apply the whole analytical procedure, rather merely to injecting the same sample solution six times.

The repeatability of the method was investigated by analyzing six samples each at 100% of the test concentration. Relative standard deviation obtained at 150 µg/ml concentration of roflumilast was 0.35%. The confidence interval obtained was 99.97 ± 0.28 %.

Table 3: Linear regression analysis

Linear regression analysis	Linearity for assay	Linearity for related substances
Concentration	15 µg/ml to 225 µg/ml	0.065 µg/ml to0.25 µg/ml
Slope	19.978	22.69
Intercept	18.616	0.21
95% confidence interval	-23.21 to 60.44	-0.00023 to 0.42
Residual standard deviation	20.99	0.11
Coefficient of correlation	0.9999	0.9977

Linearity
Six sample solutions of roflumilast dissolved in acetonitrile were prepared in order to obtain a concentration range from 10% to 150% of the theoretical test concentration 150 µg/ml. In addition, the linearity of the test procedure for the determination of related substances was proven in the range of LOQ to 175% of the 0.15 µg/ml concentration roflumilast.

The linear regression analysis is shown in Table 3 and Figure 1-2.

Fig 1: Linearity graph of roflumilast assay determination

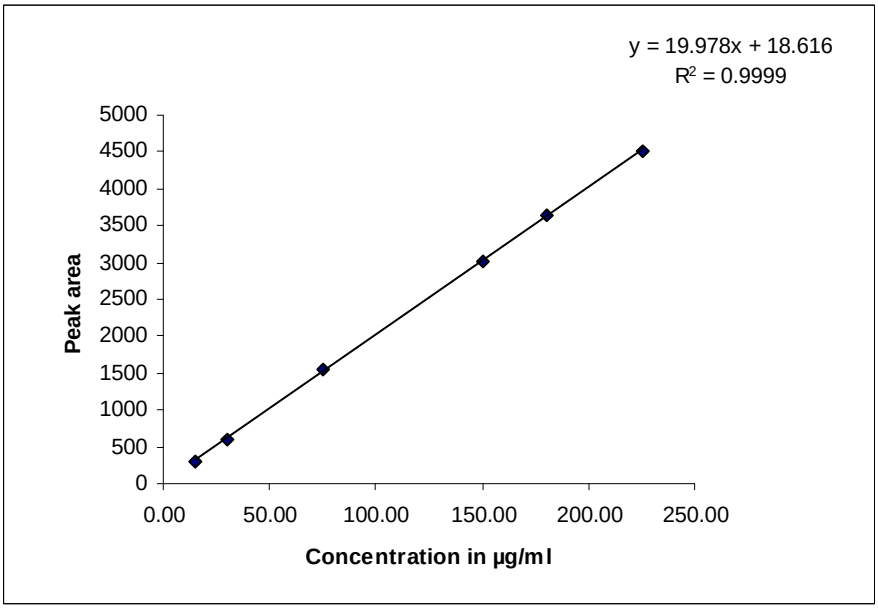
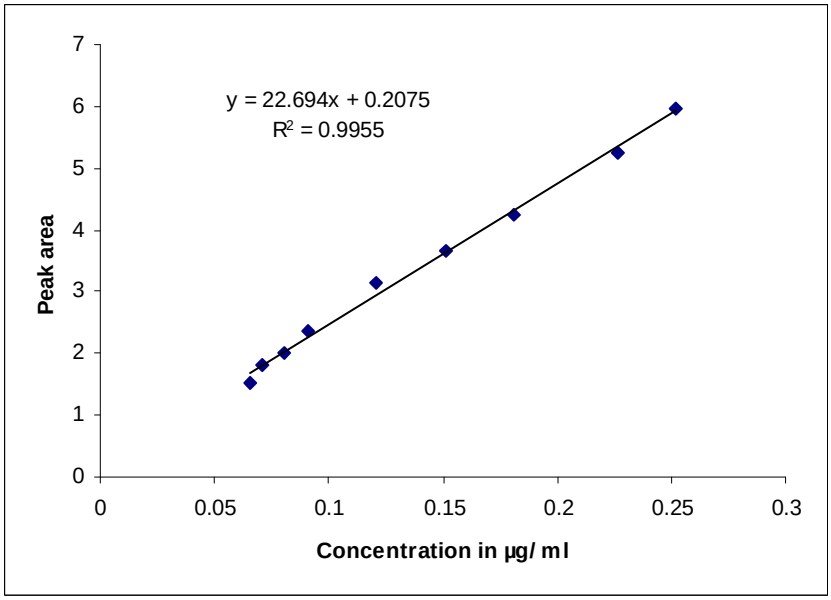


Fig 2: Linearity graph of roflumilast RS determination



These results clearly proved a linear relationship between roflumilast concentration in test solution and its corresponding peak area.

Accuracy of the method was inferred from precision, linearity and specificity studies as per early –phase method validation approach [4].

Limit of detection

The detection limit is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. LOD represents the concentration of analyte that would yield a signal to noise ratio (S/N) of 3. The European pharmacopoeia defines S/N ratio as 2 H/ h where H = height of the peak corresponding to the component concerned, in the chromatogram

with the prescribed (low concentration) reference solution. H was measured from the maximum of the peak to the extrapolated baseline of the signal observed over a distance equal to 20 times the width at half height, and h = range (maximum amplitude) of the background noise obtained after injection of a blank and observed over the above-mentioned interval, situated around the time where the peak would be found.

Different concentrations of roflumilast were injected into the chromatograph & S/N ratios for respective concentrations of solutions calculated using 2 H/ h approach [5].

The minimum concentration at which peak can be detected is 0.020 µg/ml. Presuming that unknown impurities would have response similar to roflumilast, the LOD for unknown impurities can be calculated. The sample solution injected has concentration of roflumilast 150 µg/ml. Hence LOD for unknown impurities was obtained as 0.01 %.

Limit of quantitation

The quantitation limit is the lowest concentration of analyte in a sample which can be quantitatively determined with suitable precision. LOQ represents the concentration of analyte that would yield a signal to noise ratio of 10. S/N ratio of about 10:1 was obtained at concentration of 0.065 µg/ml roflumilast. The minimum concentration at which peak can be quantified is 0.065 µg/ml. In absence of data on unknown impurities it is assumed that unknown impurities would have response similar to roflumilast, the LOQ for unknown impurities can be calculated. The sample solution injected has a concentration 150 µg/ml of roflumilast. Hence LOQ for unknown impurities was obtained as 0.04 % which fits well within ICH [6 -7] reporting threshold for unknown related substances.

To confirm precision at this concentration, 6 injections were made. The precision for roflumilast at LOQ level was satisfactory. The relative standard deviation was found to be 5.79%, indicating that the roflumilast may be quantified to an acceptable degree of precision at 0.065 µg/ml level.

Robustness

The robustness of an analytical procedure is measure of its capability to remain unaffected by small, but deliberate, variations in method parameters and provide an indication of its reliability during normal usage.

In the varied chromatographic conditions viz. aqueous buffer pH, flow rate and column oven temperature, the resolution between roflumilast and unknown impurity at RRT 1.05 was found to be > 2.0 illustrating the robustness of the method.

Table 4: Results from robustness parameters

Chromatographic conditions	pH of buffer	Flow rate ml/min	Temperature °C	Roflumilast		Unknown impurity at RRT 1.05
				Symmetry	Efficiency	Resolution
Standard	3.5	0.5	25	1.012	54394	2.753
Buffer pH change	3.3	0.5	25	1.008	55021	2.746
Buffer pH change	3.7	0.5	25	1.007	55191	2.766
Flow change	3.5	0.4	25	0.994	71567	2.378
Flow change	3.5	0.6	25	1.007	58881	2.879
Column temp change	3.5	0.5	30	1.02	52495	2.909

Solution stability

Solution stability of roflumilast was studied by keeping the 150 µg/ml concentration of roflumilast in tightly capped volumetric flask at temperature 25°C on a laboratory bench for 24 h. Content of impurities was checked after 7 h interval and 24 h interval and compared with freshly prepared solution. Solution stored at 25°C for 24 h did not show any variation for assay and related substances as compared to a freshly prepared solution.

CONCLUSION

The validation study shows that method is precise, reproducible and is suitable for the quality control in the pharmaceutical industry because of its sensitivity, simplicity, selectivity and short run time. LOQ of roflumilast was found to be 0.065 µg/ml. The validated method is easily adaptable for LCMS analysis due to volatile mobile phase buffer.

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