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METHOD DEVELOPMENT AND VALIDATION OF ROFLUMILAST IN SPIKED HUMAN PLASMA BY USING UV SPECTROSCOPY

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ABSTRACT

Method development and validation of Roflumilast in spiked human plasma by using UV spectroscopy. The UV spectroscopic method developed for the estimation of Roflumilast in spiked human plasma is based on measurement at maximum wavelength 255 nm using 0.2 M HCl as a solvent. The stock solution of Roflumilast was prepared and subsequent suitable dilution was prepared in distilled water to produce calibration curve. The standard solution of Roflumilast shows absorption maxima at 255 nm. The Roflumilast obeyed Beer Lamberts law in the concentration range of 40-88 µg/mL with regression 0.999 at 255 nm. The overall percentage recovery was found to be 98.36% which reflects that the method was free from the interference. The low value of percent relative standard deviation was indicative of accuracy and reproducibility of the method. The percent relative standard deviation for intra-day and inter-day precision was found to be 0.019 and 0.018, respectively, which is less than 2 hence proved that method is precise.

Key words: Roflumilast, Method, Plasma, UV, Estimation, HCl.

INTRODUCTION

Roflumilast (Fig. 1) is a chemically 3-(cyclopropylmethoxy)-N-(3,5-dichloropyridin-4-yl)-4-(difluoromethoxy)benzamide and it is a selective, long acting phosphodiesterase-4 inhibitor. Roflumilast is used for the treatment of chronic obstractive pulmonary disease[1,2] Roflumilast is freely soluble in methanol and lower alcohols.

A few spectrophotometric[3-5] and HPLC[6,7] methods were reported earlier for the estimation of Roflumilast in bulk and pharmaceutical dosage forms and this method was validated as per ICH guidelines[8]. No method is reported for the estimation of Roflumilast in spiked human plasma in pharmaceutical dosage forms. The present study the authors

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reported a simple, sensitive, accurate and precise for estimation of Roflumilast in spiked human plasma by using UV spectroscopy.

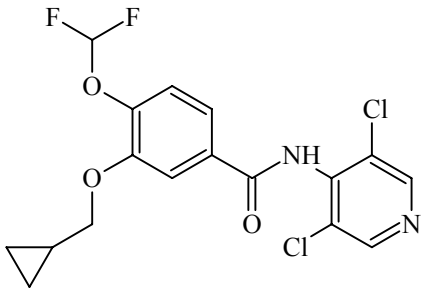

Fig. 1: Chemical structure of Roflumilast

EXPERIMENTAL

Instrument

Elico SL164 UV-visible spectrophotometer with double beam detector configuration. The above instruments had automatic wavelength accuracy 0.1 nm and matched quartz cells and weighing balance (Elico, India).

Materials

Blank human plasma, Roflumilast pure sample was obtained from Spectrum Pharma Research Solutions, Hyderabad, India. Hydrochloiric acid and water obtained from Qualigens Fine Chemicals, Mumbai, India.

Method development

Preparation of standard solution

A standard stock solution was prepred by accurately weighed 25 mg of Roflumilast in 25 mL of volumetric flask and dissolved in 0.2 N of HCl to obtain a concentration 1000 µg/mL. Further diluting 2.5 mL of stock solution to 25 mL with distilled water to get desired concentration of 25 µg/mL. Further diluting 0.4 mL of stock solution to 100 mL with distilled water to get desired concentration of 40 µg/mL.

Selection of wavelength for analysis of Roflumilast

Accurately measured 0.4 mL of standard stock-III was transferred in to 100 mL volumetric flask and diluted to 100 to give concentration of 40 µg/mL and it was used for

initial spectral scan in the range of 200-400 nm to detect maximum wavelength and further dilutions for linearity were prepared from the stock solution by allegation method. The maximum wavelength was found at 255 nm.

Preparation of calibration plot

5 mL of plasma was taken in a 250 mL separating funnel and spiked with 10 mL aqueous solution containg 40-88 µg/mL of Roflumilast (40, 48, 60, 68, 88 µg/mL). To the same solution add 20 mL of methyl acetate. The content was shaken for 15 min and the liquids were allowed to separate in to two immiscible phases. The lower aqueous layer was discarded and the upper organic layer was collected in a beaker. Finally water free organic layer was trancferred in to a dried beaker and evaporated to dryness in a hot water bath. The dry residue was reconstituted with 2 mL of 0.2 M HCl and transferred in to a 25 mL calibrated flask. The volume was made upto mark with water.

Method validation

The developed method was validated for various parameters like linearity, precision, accuracy, limit of detection, limit of quantitation, robustness and ruggedness according to ICH guidelines.

Linearity

The linearity of an analytical procedure is ts ability to obtain test result, which are directly proportional to the concentration of an analyte in the sample. The manifest linear relationship in the range of 40-84 µg/mL of Roflumilast.

Precision

The precision of analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the homogeneous sample under the prescribed conditions. The precisin of the method was demonstrated by intra-day and inter-day varation studies. In the intra day precision analysed in the same day and inter day precision analysed for three consecutive days. The results was indicated by calculated percent relative standard deviation.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted value

and the value found. Recovery study was carried out at three different levels 50%, 100% and 150%. The percentage recovery was calculated as mean ± standard deviation.

Limit of detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in the sample, which can be detected, but not necessarily quantitated as an exact value. Formula for measuring of limit of detection.

$$LOD = 3.3\ \sigma/S$$

Where, σ = Standard deviation

S = Slope

Limit of quantification (LOQ)

The lowest amount of analyte in a sample, which can be detected but not quantitates.

$$LOD = 10\ \sigma/S$$

Where, σ = Standard deviation

S = Slope

Robustness

The robustness of an analytical procedure is a measure of its capacity unaffected by a small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Ruggedness

The ruggedness is a degree of reproducibility of test results verification of condition like a different analyst, different instruments and different days.

RESULTS AND DISCUSSION

Selection of wavelength

The spectra of Roflumilast in 0.2 M hydrochloric acid showed absorption at 255 nm

shown in Fig. 2, which is complying with reported λ_{max}. Hence, it was selected as λ_{max} of Roflumilast in 0.2 M hydrochloric acid for further use.

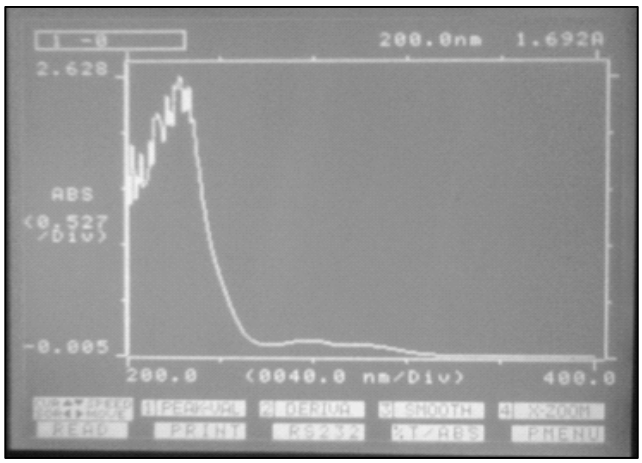

Fig. 2: UV spectrum of Roflumilast

Linearity

The linearity for the proposed method was investigated at five concentration levels (40-88 µg/mL) of reference standard Roflumilast. Graph was plotted absorbance vs concentration. It shows good linear correlation coefficient. The linearity is shown in Table 1 and Fig. 3.

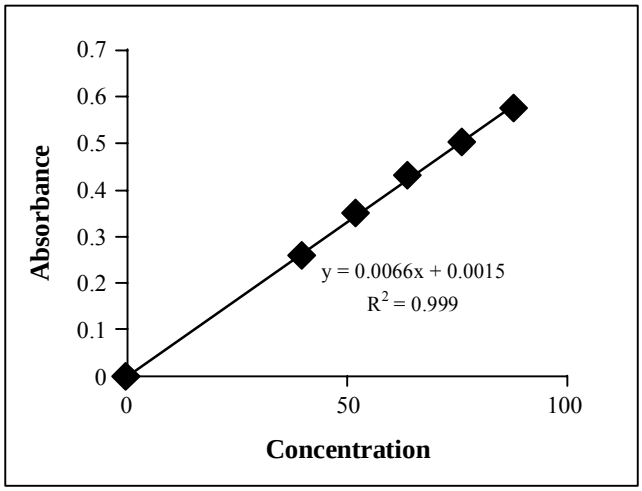

Fig. 3: Calibration plot of Roflumilast

Table 1: Calibration data of Roflumilast

S. No.	Concentration (mcg/mL)	Absorbance
1	40	0.258
2	52	0.352
3	64	0.432
4	72	0.502
5	88	0.574

Precision

The precision of the proposed method was measured by intra-day and inter-day precision, and it was expressed in terms of percent relative standard deviation (% RSD). % RSD value of intra day and inter day precision were found to be 0.019 and 0.018 respectively as shown in Table 2.

Table 2: Precision data of Roflumilast

S. No.	Assay of Roflumilast	Assay of Roflumilast
	Intra-day precision	Inter-day precision
1	98.45	98.44
2	98.44	98.48
3	98.46	98.45
4	98.47	98.42
5	98.48	98.46
6	98.43	98.41
Mean	98.45	98.44
RSD	0.019	0.018

Accuracy

The accuracy was determined in triplicate by analysing percentage recovery of Roflumilast by standard addition method. The percentage of recovery of Roflumilast was found to be 99.82 %. The results are shown in Table 3.

Table 3: Accuracy data of Roflumilast

Ingredient	Tablet amount (mg/mL)	Level of addition (%)	Amount added (mg)	Drug found (mg/mL)	% Recovery	Average % recovery
Roflumilast	500	50	5	4.99	99.80	
	500	100	10	9.98	99.80	99.82 ± 0.18
	500	150	15	14.98	99.86	

Limit of detection

The limit of detection (LOD) value obtained was 1.09 µg/mL, which indicates the high sensitivity of the proposed method.

Limit of quantitation

The limit of quantitation (LOQ) obtained was 3.3 µg/mL, which indicates the high sensitivity of the proposed method.

Table 4: Validation parameters of Roflumilast

S. No.	Parameter	Roflumilast
1	Absorption maxima (nm)	255
2	Linearity (mcg/mL)	40-88
3	Standard regression equation	Y = 0.0066X – 0.0015
4	Correlation coefficient (r2)	0.999
5	Molar extinction coefficient	0.006
6	Accuracy (% recovery ± SD)	99.82 ± 0.18
7	Precision	98.45% (Intra-day precision) and 98.44% (Inter-day precision)
8	Sandell's sensitivity (mg/cm^2/0.001 absorbance unit) 8	0.155
9	LOD (mg/mL)	1.15
10	LOQ (mg/mL)	3.48

Robustness

Percent relative standard deviation of Roflumilast was found to be 0.89. The robustness of results are shown in Table 5.

Table 5: Robustness data of Roflumilast

S. No.	Roflumilast
Assay-1	98.95
Assay-2	98.97
Assay-3	98.99
Assay-4	98.89
Assay-5	98.86
Assay-6	98.83
Mean	98.80
S.D	0.140
%RSD	0.89

Ruggedness

Percent relative standard deviation of Roflumilast was found to be 0.062. The ruggedness of results are shown in Table 6.

Table 6: Ruggedness data of Roflumilast

S. No.	Roflumilast
Assay-1	97.45
Assay-2	97.46
Assay-3	97.49
Assay-4	97.46
Assay-5	97.48
Assay-6	97.47
Mean	97.46
S.D	0.061
%RSD	0.062

CONCLUSION

The proposed method was simple, linear, precise, accurate and robust developed for the estimation of Roflumilast in human plasma by using UV spectroscopy. The method was validated as per the ICH guidelines. Hence, The present method can be adopted for routine in quality control and reaserch industries.

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