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Development and Validation of Spectrophotometric Method for Simultaneous Determination of Roflumilast and Salmeterol in Synthetic Mixture

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ABSTRACT

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical spectrophotometric method based on simultaneous equation method for the simultaneous determination of Roflumilast and Salmeterol in synthetic mixture. The method is based on the simultaneous equations for analysis of both the drugs using methanol as solvent. Roflumilast has absorbance maxima at 213nm and Salmeterol has absorbance maxima at 252nm in methanol. The linearity was obtained in the concentration range of 2-14 µg/ml for both Roflumilast and Salmeterol. The concentrations of the drugs were determined by using simultaneous equations method. The mean recovery was 99.41±0.79 and 100.1±1.58 for Roflumilast and Salmeterol respectively. The method was found to be simple, sensitive, accurate and precise and was applicable for the simultaneous determination of Roflumilast and Salmeterol in synthetic mixture. The results of analysis have been validated statistically and by recovery studies.

INTRODUCTION

Roflumilast (ROF) is chemically3-(cyclopropylmethoxy)-N-(3, 5-dichloropyridin-4yl)-4(difluoromethoxy) benzamide(Figure 1), is a Phosphodiesterase-4 (PDE-4) inhibitor orally acting Anti-inflammatory[1]. It is official in Indian Pharmacopeia[2]. Literature survey reveals UV Spectrophotometry[3] and HPLC[4] methods for determination of ROF in pharmaceutical dosage forms. Salmeterol (SAL)is chemically(RS)-2-(hydroxymethyl)-4-{1-hydroxy-2-[6-(4-phenylbutoxy) hexylamino] ethyl} phenol (Figure 2), Long-acting beta$_2$ adrenoreceptor agonist[5]. It is official in Indian Pharmacopeia[6], United State Pharmacopeia[7], European Pharmacopeia[8], and British Pharmacopeia[9]. Literature survey reveals UV Spectrophotometry[10], HPLC[11] and UPLC[12] methods for determination of SAL in pharmaceutical dosage forms. Literature survey also reveals Spectrophotometric[13], HPLC[14] and HPTLC[15] methods for determination of SAL with other drugs in combination.The combination of these two drugs is not official in any pharmacopoeia; hence, no official method is available for the simultaneous estimation of ROF and SAL in their combined mixture. Literature survey does not reveal any simple spectrophotometric or another method for simultaneous estimation of ROF and SAL in combined dosage forms. The combination of Roflumilast and Salmeterol are studied under clinical trial phase Identifier No: NCT00313209 by Takeda Pharmaceuticals[16]. This combination is used in the treatment of chronic obstructive pulmonary disease (COPD). The present communication describes simple, sensitive, rapid, accurate and economical spectrophotometric method for simultaneous estimation of both drugs in their synthetic mixture.

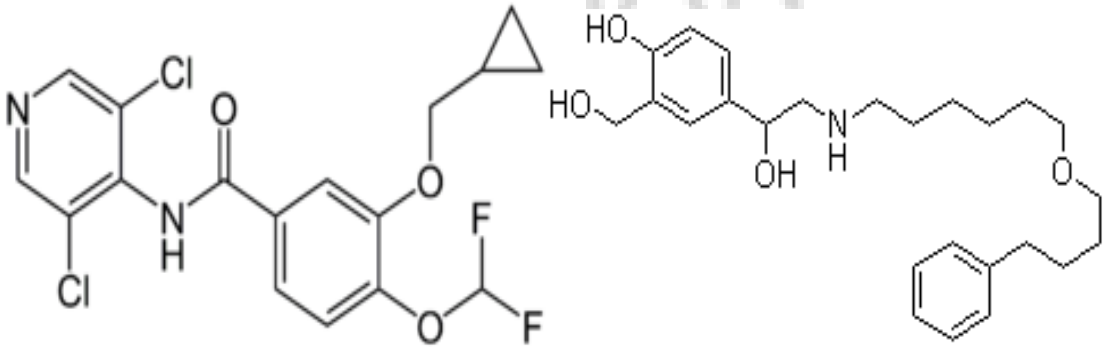

Figure 1: Chemical structure of Roflumilast Figure 2: Chemical structure of Salmeterol

MATERIALS AND METHODS

Apparatus

A Shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2nm, wavelength accuracy of 0.5nm and a pair of 10mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

Reagents and Materials

ROF and SAL bulk powder were kindly gifted by Cadila Zydus Ltd. Ahmedabad, Gujarat, India. Methanol AR Grade was procured from S. D. Fine Chemicals Ltd., Mumbai, India. Whatman filter paper no. 41 (Millipore, USA) was also used in the study.

Preparation of standard stock solutions

Standard stock solution of ROF and SAL (100 µg/ml) was prepared separately by dissolving an accurately weighed quantity of ROF (10 mg) and SAL (10 mg) in a separate 100 ml volumetric flask and diluted to the mark with methanol to obtain standard solution having concentration of ROF (100 µg/ml) and SAL (100 µg/ml). For SAL preparation amber colored volumetric flask was used.

Preparation of synthetic mixture

Synthetic mixture (130mg) was prepared by using ROF (50mg) and SAL (30mg) and excipients (50mg) like Starch, Magnesium stearate, Lactose and Talc.

Development of Method

Standard drug solution having concentration 10ug/ml was scanned separately in the range of 200nm to 400nm. Maximum absorbance was observed at 213nm and 252nm by Roflumilast and Salmeterol respectively. So these two wavelengths were selected as an analytical wavelength. In this method, the absorbances of the solutions were measured at the λ_{max} of both the drugs. The

criteria are that the ratios $[(A_2/A_1) / (ax_2/ax_1)]$ and $[(ay_2/ay_1) / (A_2/A_1)]$ should lie outside the range 0.1-2.0. For this measurement, the standard solutions of ROF and SAL (100 µg/ml) were scanned separately in the range of 200-400nm against methanol as a blank. Data were recorded at an interval of 1nm. (Figure 3) indicates the overlain spectra of the two drugs. Absorbance was measured at selected wavelengths i.e. 213nm and 252nm absorption maxima for ROF and SAL respectively. Absorbance and absorptivity values at the particular wavelengths were calculated and substituted into the following equation to obtain the concentration.

$$C_x = (A_2\, Ay_1 - A_1\, Ay_2) / (Ay_1\, Ax_2 - Ay_2\, Ax_1) \text{ ----- (1)}$$

$$C_y = (A_1\, Ax_2 - A_2\, Ax_1) / (Ay_1\, Ax_2 - Ay_2\, Ax_1) \text{ ------ (2)}$$

Where, A_1, A_2= Absorbances of mixture at λ_1& λ_2 respectively,

ax_1 = Absorptivity of ROF at 213 nm

ax_2 = Absorptivity of ROF at 252 nm

ay_1 = Absorptivity of SAL at 213 nm

ay_2 = Absorptivity of SAL at 252 nm

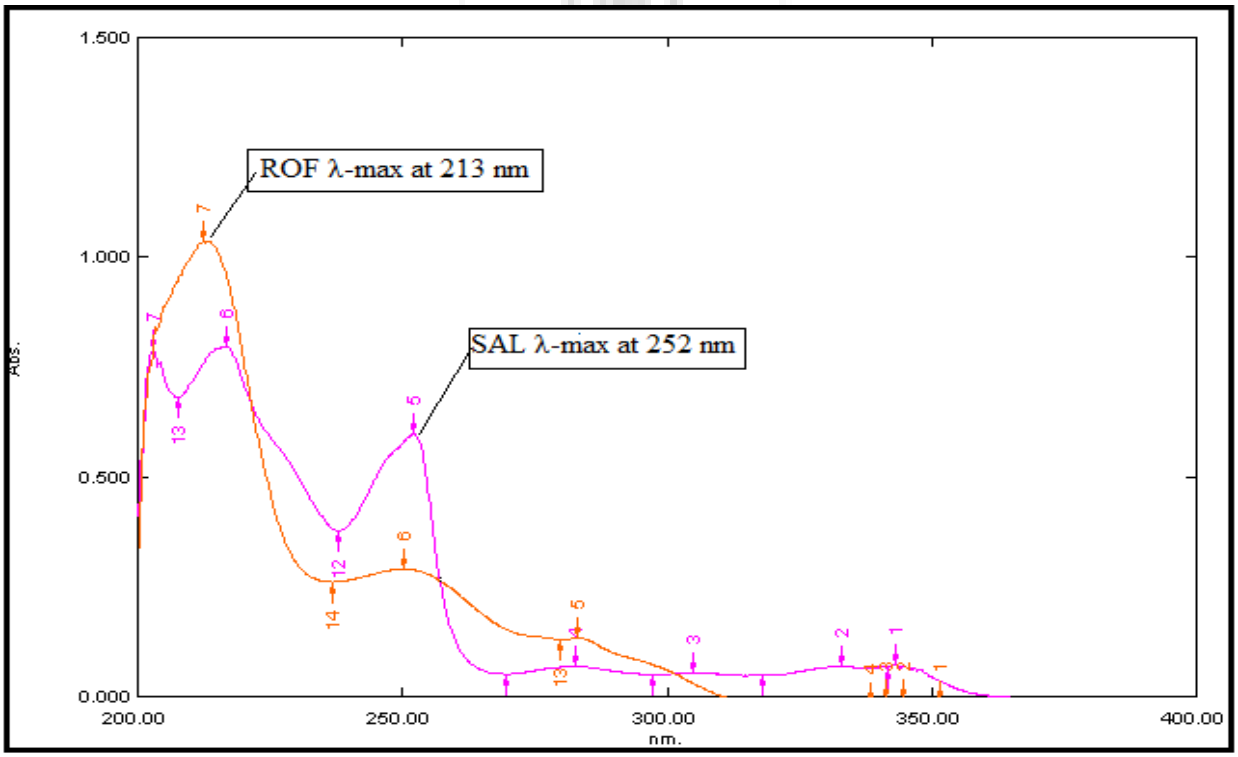

Figure 3: Overlain absorption spectra of ROF (213nm) and SAL (252nm) in methanol

METHOD VALIDATION

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[17].

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 2-14 µg/ml for each RPF and SAL. Accurately measured standard stock solutions of each ROF and SAL (0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 ml) were transferred to a series of 10 ml volumetric flask separately and diluted up to the mark with methanol. The absorbances of solution were then measured at 213nm and 252nm. The calibration curves were constructed by plotting absorbances versus concentration and the regression equations were calculated.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of the absorbances of solutions (n = 6) of ROF and SAL (6 µg/ml for both drugs) without changing the parameters of the proposed method.

Intermediate precision (reproducibility)

The intraday and interday precisions of the proposed method was determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for 3 different concentrations of standard solutions of ROF and SAL (3, 5 and 7 µg/ml). The results were reported in terms of relative standard deviation (% RSD).

Accuracy (recovery study)

The accuracy of the method was determined by calculating recoveries of ROF and SAL by the standard addition method. Known amounts of standard solutions of ROF and SAL were added at 50, 100 and 150% level of prequantified sample solutions of ROF and SAL 5 µg/ml and 3 µg/ml respectively. The amounts of ROF and SAL were estimated by putting obtained values in the equation (1) and (2). The experiment repeated three times.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines[17]

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

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Analysis of ROF and SAL in synthetic mixture

ROF (50mg) and SAL (30mg) standard drug powders were weighed and then mixed with commonly used formulation additives like starch, lactose, magnesium stearate and talc. The synthetic mixture was then transferred to 100 ml volumetric flask containing 50 ml methanol and sonicated for 15 minutes. The solution was filtered through Whatman filter paper No.41 and the volume was adjusted up to mark with methanol. The solution was suitably diluted with methanol to get a final concentration of 5 µg/ml of ROF and 3 µg/ml of SAL. The absorbances of the sample solution i.e. A_1 and A_2 were recorded at 213nm (λ-max of ROF) and 252nm (λ-max of SAL) respectively. The amounts of ROF and SAL were estimated by applying obtained values to the respective regression line equations and relative concentration of both drugs in the synthetic mixture was calculated using above equation (1) and (2). The experiment was repeated three times.

RESULTS AND DISCUSSION

Simultaneous equations method, the primary requirement for developing a method of analysis is that the entire spectra should follow the Beer's law at all the wavelength[18], which was fulfilled in case of both these drugs. The two wavelengths were used for the analysis of drugs were 213nm (λ-max of ROF) and 252nm (λ-max of SAL) at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of ROF (213nm) and SAL (252nm) showing in methanol is shown in (Figure 3).

The validation parameters were studied at all the wavelengths for the proposed method. Accuracy was determined by calculating the recovery, and the mean was determined (Table 1). The method was successfully used to determine the amounts of ROF and SAL present in the synthetic mixture. The results obtained were in good agreement with the corresponding labeled amount (Table 2). Precision was calculated as repeatability and intra and interday variations (% RSD) for both the drugs. Optical characteristics and summary of validation parameters for method are given in (Table 3). By observing the validation parameters, the method was found to be simple, sensitive, accurate and precise. Hence, the method can be employed for the routine analysis of these two drugs in synthetic mixture.

Table 1: Recovery data of proposed method

Drug	Level	Amount taken (µg/ml)	Amount added (%)	% Mean recovery ± S.D. (n = 3)
ROF	I	5	50	98.93 ± 1.28
	II	5	100	98.48 ± 0.69
	III	5	150	100.9 ± 0.37
SAL	I	3	50	99.33 ± 1.76
	II	3	100	99.67 ± 1.32
	III	3	150	101.3 ± 1.67

S.D. is Standard deviation and n is number of replicates.

Table 2: Analysis of ROF and SAL by proposed method

Synthetic mixture	Label claim (µg)		Amount found (µg)		% Label claim ± S. D. (n = 5)	
	ROF	SAL	ROF	SAL	ROF	SAL
I	500	100	500.25	99.93	100.1 ± 0.14	100.1 ± 0.73

S.D. is Standard deviation and n is number of replicates.

Table 3: Regression analysis data and summary of validation parameters for the proposed method

PARAMETERS	ROF		SAL	
	At 213 nm	At 252 nm	At 213 nm	At 252 nm
Beer's Law limit (µg/ml)	2-14		2-14	
Regression equation (y = mx + c) Slope (m) Intercept (c)	y = 0.1055x - 0.0144 0.1055 0.0144	y = 0.0309x - 0.0164 0.0309 0.0164	y = 0.0805x - 0.0183 0.0805 0.0183	y = 0.0622x - 0.0197 0.0622 0.0197
Correlation Coefficient (R^2)	0.9999	0.9999	0.9996	0.9998
Method precision (Repeatability) (% RSD, n = 6)	0.56	0.96	1.73	0.18
Intraday (n = 3) (% RSD)	0.22 – 0.58	0.41 – 1.61	0.33 – 1.35	0.35 – 1.01
Interday (n = 3) (% RSD)	0.22 – 0.66	0.39 – 1.78	0.58 – 1.69	0.13 – 0.75
LOD (µg/ml)	0.037	0.255	0.101	0.082
LOQ (µg/ml)	0.113	0.773	0.308	0.251
Accuracy (Mean % Recovery ± S.D) (n = 3)	99.41 ± 0.79		100.1 ± 1.58	
% Assay ± S.D. (n = 5)	99.82 ± 0.59		99.78 ± 0.6	

RSD = Relative standard deviation. LOD = Limit of detection. LOQ = Limit of quantification. SD = Standard deviation.

CONCLUSION

The proposed simultaneous equation method was found to be simple, sensitive, accurate, precise and economical and can be employed for the routine analysis of these two drugs in combined synthetic mixture.

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