Research Article

SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF FEBUXOSTAT AND ALLOPURINOL IN SYNTHETIC MIXTURE

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ABSTRACT

The present manuscripts describe simple, sensitive, rapid, accurate, precise and economical absorbance ratio method for the simultaneous determination of Febuxostat and Allopurinol in synthetic mixture. Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the λ -max of one of the two components. Febuxostat and Allopurinol show an isoabsorptive point at 274 nm in methanol. The second wavelength used is 250 nm, which is the λ -max of Allopurinol in methanol. The linearity was obtained in the concentration range of 2-12 μ g/ml for both Febuxostat and Allopurinol. The concentrations of the drugs were determined by using ratio of absorbances at isoabsorptive point and at the λ -max of Allopurinol. The method was successfully applied to synthetic mixture because no interference from the mixture excipients was found. The results of analysis have been validated statistically and by recovery studies according to ICH guidelines.

Keywords: Febuxostat, Allopurinol, Absorbance ratio method, Spectrophotometric, Synthetic mixture, Validation.

INTRODUCTION

Allopurinol (ALO) is chemically 1,2-dihydropyrazolo[3,4-d]pyrimidin-4-one¹, is a xanthine oxidase inhibitor, used in the treatment of gout.² It is official in Indian Pharmacopeia³, United State Pharmacopeia⁴, British Pharmacopeia⁵ and European Pharmacopeia⁶. Various methods like Potentiometry⁷, UV spectrophotometry⁸ and RP-HPLC⁹ have been reported for estimation of ALO with other drugs. Febuxostat (FEB) is chemically 2-[3-cyano-4-(2-methylpropoxy) phenyl]-4-methylthiazole-5 carboxylic acid.¹ FEB is not official in any Pharmacopeia. Literature shows various methods like spectrophotometry¹⁰ and RP-HPLC^{11,12} for estimation of FEB with other drugs. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of ALO and FEB in their combined mixture. The combination of ALO and FEB are study under clinical trial phase by Takeda Pharmaceuticals Ltd¹³. The present manuscript describes simple, sensitive, accurate, precise, rapid and economic Spectrophotometric method based on Absorbance ratio method for estimation of ALO and FEB in synthetic mixture.

MATERIALS & METHODS

Apparatus

A Shimadzu model 1800 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software (UV Probe version 2.31). A Sartorius CP224S analytical balance (Göttingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

Reagents and Materials

ALO bulk powder was kindly gifted by Zydus Cadila Healthcare, Ahmedabad, Gujarat, India and FEB bulk powder was kindly gifted by Sun Pharma Ltd, Baroda, Gujarat, India. Methanol (AR Grade, Finar Chemicals Ltd., Ahmedabad, India) and Whatman filter paper no. 41 (Millipore, USA) were used in the study.

Preparation of standard stock solutions

Standard stock solution of FEB and ALO (100 μ g/ml) was prepared separately by dissolving an accurately weighed quantity of FEB (10 mg) and ALO (10 mg) to a separate 100 ml volumetric flask and diluted to the mark with methanol and the flask were shaken and sonicated to 20 min. to obtain standard solution having concentration of FEB (100 μ g/ml) and ALO (100 μ g/ml). For both of preparations amber colored volumetric flask were used.

Preparation of synthetic mixture

Synthetic mixture (300 mg) was prepared by using FEB (80 mg) and ALO (100 mg) and excipients (120 mg) like starch, magnesium stearate, lactose and talc.

Development of method¹⁵

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the λ -max of one of the two components. From the overlay spectra of two drugs, it is evident that FEB and ALO show an isoabsorptive point at 274 nm. The second wavelength used is 250 nm, which is the λ -max of ALO. Six working standard solutions having concentration 2, 4, 6, 8, 10, and 12

µg/ml for FEB and 2, 4, 6, 8, 10, and 12 µg/ml for ALO were prepared in methanol, and the absorbances at 274 nm (isoabsorptive point) and 250 nm (λ -max of ALO) were measured and absorptivity coefficients were calculated using calibration curve.

The concentration of two drugs in the mixture can be calculated using following equations

$$C_X = [(Q_M - Q_Y) / (Q_X - Q_Y)] \times A_1 / aX_1 \quad \text{..... (1)}$$

$$C_Y = (A_1 / aX_1) - C_X \quad \text{..... (2)}$$

Where, A_1 and A_2 are absorbances of mixture at 274 nm and 250 nm; and aX_1 and aY_1 are absorptivities of FEB and ALO at 274 nm; aX_2 and aY_2 are absorptivities of FEB and ALO

respectively at 250 nm; and $Q_M = A_2 / A_1$, $Q_X = aX_2 / aX_1$ and $Q_Y = aY_2 / aY_1$.

METHOD VALIDATION

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines.¹⁶

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 2-12 µg/ml for each FEB and ALO. Accurately measured standard stock solutions of each FEB and ALO (0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 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 274 nm and 250 nm. 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 FEB and ALO (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 FEB and ALO (6, 8 and 10 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 the recoveries of FEB and ALO by the standard addition method. Known amounts of standard solutions of FEB and ALO were added at 80, 100 and 120 % level to prequantified sample solutions of FEB and ALO 4 µg/ml and 5 µg/ml respectively. The amounts of FEB and ALO 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) guidelines¹⁶

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

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

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

Analysis of ALO and FEB in synthetic mixture

FEB (80 mg) and ALO (100 mg) standard drug powder 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 4 µg/ml of FEB and 5 µg/ml of ALO. The absorbances of the sample solution i.e. A_1 and A_2 were recorded at 274 nm (isoabsorptive point) and 250 nm (λ -max of ALO) respectively, and ratios of absorbance were calculated, i.e. A_2/A_1 . Relative concentration of both drugs in the synthetic mixture was calculated using above equation (1) and (2).

RESULTS & DISCUSSION

In absorbance ratio method (Q-analysis), the primary requirement for developing a method for analysis is that the entire spectra should follow the Beer's law at all the wavelength¹⁴, which was fulfilled in case of both these drugs. The two wavelengths were used for the analysis of the drugs were 274 nm (isoabsorptive point) and 250 nm (λ -max of ALO) at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of FEB (315 nm) and ALO (252 nm) showing isoabsorptive point (274 nm) 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 FEB and ALO 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 inter day variations (% RSD) for both the drugs. Optical characteristics and summary of validation parameters for method is 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)
FEB	I	4	80	99.99 ± 0.01
	II	4	100	100.5 ± 0.70
	III	4	120	100.1 ± 0.14
ALO	I	5	80	100.3 ± 0.50
	II	5	100	101.4 ± 0.84
	III	5	120	100.1 ± 0.70

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

Table 2: Analysis of FEB and ALO by proposed method

Synthetic mixture	Label claim (µg)		Amount found (µg)		% Label claim ± S. D. (n = 5)	
	FEB	ALO	FEB	ALO	FEB	ALO
I	80	100	81.1	99.98	101.3 ± 0.59	99.78 ± 0.60

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

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

PARAMETERS	FEB	ALO	ISOABSORPTIVE POINT
Wavelength range (nm)	250	250	274
Beer's law limit (µg/ml)	2 - 12	2 - 12	2 - 12
Regression equation ($y = mx \pm c$)	$y = 0.0157x - 0.0149$	$y = 0.058x - 0.0281$	$y = 0.0191x - 0.0074$
Correlation Coefficient (r^2)	0.9943	0.9905	0.9963
Method precision (Repeatability) (%RSD, n = 6)	0.16	0.03	0.10
Intraday (n = 3) (%RSD)	0.16 - 0.30	0.10 - 0.13	0.15 - 0.20
Interday (n = 3) (%RSD)	0.17 - 0.29	0.10 - 0.13	0.10 - 0.26
LOD (µg/ml)	0.07	0.20	0.06
LOQ (µg/ml)	0.23	0.60	0.18
Accuracy (Mean % Recovery ± S.D) (n = 3)	100.1 ± 0.28	100.9 ± 0.68	-
% Assay ± S.D. (n = 5)	101.3 ± 0.59	99.78 ± 0.60	-

LOD = Limit of Detection. LOQ = Limit of Quantification. S.D = Standard Deviation. RSD = Relative Standard Deviation.

Figure 1: Chemical structure of Allopurinol

Figure 2: Chemical structure of Febuxostat

Figure 3: Overlain absorption spectra of FEB (315 nm) and ALO (250 nm) showing isoabsorptive point (274 nm) in methanol.

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