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Validated First-Order Derivative Spectrophotometry for Simultaneous Determination of Febuxostat And Diclofenac In Pharmaceutical Dosage Form

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ABSTRACT

A simple, precise, accurate, and economical spectrophotometric method has been developed for simultaneous estimation of Febuxostat(FEB) andDiclofenac(DIC) by employing first-order derivative spectrophotometric method. The first derivative spectrum is a plot of the rate of change of absorbance with wavelength against wavelength (dA /dλ versus λ). It is characterized by a maximum, minimum and a cross- over point at the λ_{max} of the absorption band. The linearity was established over the concentration range of 2-10µg/mL for FEB and DIC with correlation coefficients 0.9985and 0.999, respectively. The mean percentage recoveries were found to be 99.99% for FEB and 100.03%for DIC. The proposed method has been validated as per ICH guidelines and successfully applied for the simultaneous estimation of FEB and DIC in combined tablet dosage form.

Keywords: Febuxostat, Diclofenac, First derivative, Validation

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INTRODUCTION

Febuxostat(FEB) is chemically known as 2-(3-Cyano-4-isobutoxyphenyl)-4-methyl-1,3-thiazole-5-carboxylic acid[1]. The chemical structure of febuxostat is shown in Figure 1. Febuxostat is a non-purine xanthine oxidase inhibitor used in the treatment of hyperuricaemia with chronic gout. [2]. Diclofenac(DIC) is chemically 2-(2,6-Dichloranilino) phenylacetic acid[1]. The chemical structure of diclofenac is shown in Figure 2. It is is a nonsteroidal anti-inflammatory drug (NSAID) taken or applied to reduce inflammation and as an analgesic for reducing pain in certain conditions[2]. Literature review revealed that spectrophotometric method[3], HPLC methods[4-7] and stability indicating studies[8-12] have been reported for estimation of Febuxostat and HPLC[13,14] and spectrophotometric[15-17] methods have been reported for Diclofenac alone. Further a validated HPLC method[18] for simultaneous estimation of febuxostat and diclofenac potassium in bulk and tablet dosage form and validated simultaneous equation and absorbance ratio methods[19,20] for the simultaneous estimation of febuxostat and diclofenac in tablet dosage form have been reported. The present work describes the development of a simple, precise, accurate and reproducible spectrophotometric method for the simultaneous estimation of FEB and DIC in combined dosage form. The developed method was validated in accordance with ICH guideline and successfully employed for the assay of FEB and DIC in combined tablet dosage form.
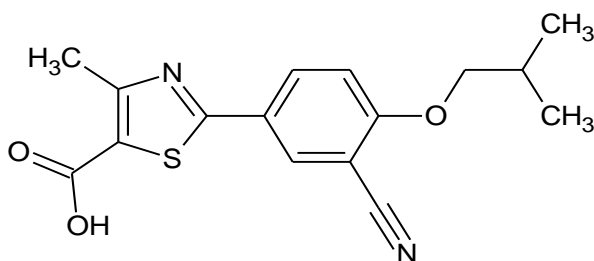

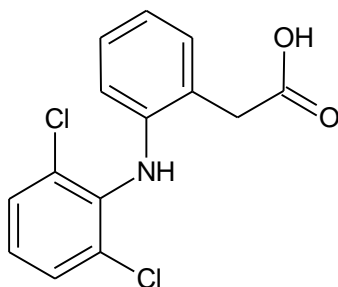**Figure 1: Structure of Febuxostat**

Figure 2: Structure of Diclofenac

EXPERIMENTAL

Chemicals and Reagents

Working standards of FEB and DIC were provided as gift samples by M/s Centaur Pharmaceuticals Ltd., Pune. The tablet XANFEB DSR was procured from the local pharmacy. All the chemicals used were of AR grade and obtained from Merck, Mumbai, India Limited. Double distilled water and Whatmann filter paper (0.45μm) were used for filtration.

Instrumentation

SHIMADZU double beam UV visible spectrophotometer (model 1800) with 1 cm matched quartz cells were usedfor all absorbance measurements. Shimadzu AUX 220 balance was used for weighing the samples.All statistical calculations were carried out using Microsoft Excel 2007 analytical tool.

Preparation of standard stock solution

A stock solution of 1000μg/ml each of Febuxostat(FEB) and Diclofenac(DIC) were prepared by dissolving accurately weighed quantities of both drugs in methanol. Further dilution of standard stock solutions of both drugs were made with methanol to get working standard solution of 100μg/ml concentration.

Selection of scanning range and sampling wavelength

4 μg/ml solutions were prepared from working standard solution for both drugs and were scanned in the UV range of 400-200nm. The wavelength maxima for Febuxostat andDiclofenac sodium were found to be 314 nm and 282 nm respectively. The Zero Crossing Point (ZCP) for Febuxostat andDiclofenac sodium were found to be 261 nm and 282 nm respectively.

Calibration Curve Procedure

Aliquots of standard stock solutions of FEB and DIC were taken in volumetric flasks and diluted with methanol to get final concentrations in range of 2-10μg/ml of FEB and DIC. The solution was scanned in the range of200 to 400 nm against methanol as blank to obtain the overlain spectrum. All zero-order spectrums (D^0) were converted to first derivative spectrums (D^1) The overlain first derivative spectrums of FEB and DIC at different concentrations were recorded (Figure 3). The solutions were scanned for their derivativeabsorbance at 261nm and 282nm.The calibration curve of responses against concentration was plotted. Correlation coefficient and regression line equations for FEB and DIC were calculated.
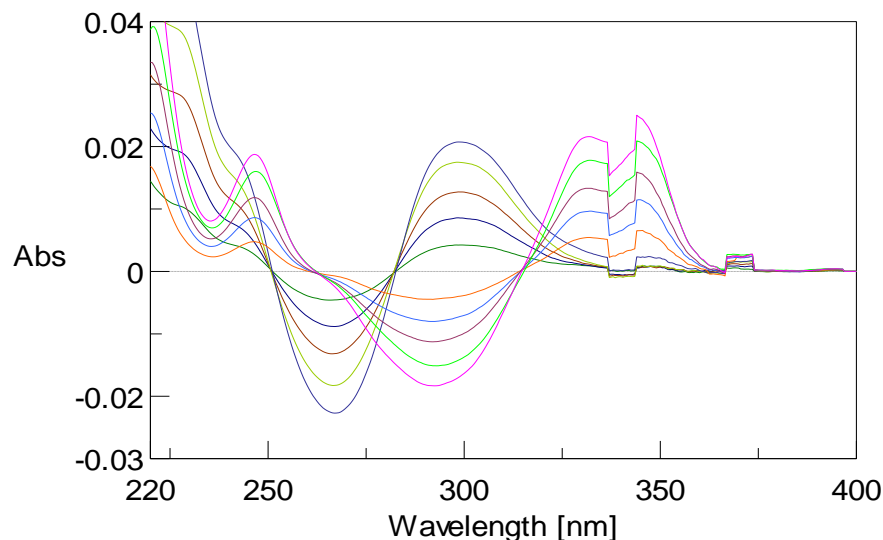
Figure 3: Overlain first derivative spectrum of FEB and DIC

Preparation and analysis of formulation

Twenty tablets of brand xanfeb DSR (indigo remedies ltd) containing 40 mg of Feb and 100 mg of DIC were weighed, average weight determined and finely powdered. Appropriate quantity of powder equivalent to 4 mg of FEB and 10 mg of DIC was accurately weighed, transferred to a 100 ml volumetric flask and volume was made up to 100 ml with methanol and shaken for 15 minutes. The solution was filtered through whatman filter paper 0.45µm pore size, necessary dilution of filtrate was made with methanol to get final concentration of 4 µg/ml of FEB and 10 µg/ml of DIC. Absorbance of this solution was measured at 282 nm (ZCP of DIC) and 261 nm (ZCP for FEB).

Method Validation

The proposed method was validated for parameters like linearity, accuracy, precision,specificity, limit of detection and limit of quantitation as per ICH guidelines[21].

Linearity

Linearity graph was plotted over a concentration range of 2-10 µg/ml of FEB and DIC. The absorbance of the solutions were measured at 261nm and 282nm against methanol as blank. The calibration curve was constructed by plotting absorbance versus concentration.

The regression analysis was carried out for the calibration graph to find out correlation coefficient, y-intercept and slope of the regression line which estimates the degree of linearity. The correlation coefficient was found to be 0.9985 for FEB and 0.999 for DIC.

Precision

The precision was determined with standard samples of both drugs prepared in triplicates at three different concentration levels covering the entire linearity range. The precision was calculated both at intraday and interdaylevels and reported as % RSD.

Accuracy

To study the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels. A known amount of drug was added to pre-analyzed tablet powder and percentage recoveries were calculated.

Specificity

The specificity of an analytical method is ability to measure accurately an analyte in presence of interferences like synthetic precursor, excipients, degradants, or matrix component. Comparison of first derivative UV spectrum of standard mixture and formulation shows the specificity of method. The proposed derivative spectrophotometric method is able to assess the analyte in presence of excipients, and, hence, it can be considered specific.

Limit of Detection(LOD) and Limit of Quantitation(LOQ)

The lowest amount of the analyte in the sample which can be detected and the lowest amount of analyte which can be quantitatively determined were studied and LOD & LOQ values were recorded.

RESULTS AND DISCUSSION

First-order derivative spectrophotometric method was developed for determination of FEB and DIC.The optical characteristics such as absorption maxima, Beer's law limits and the regression characteristics like slope(b),intercept(c), correlation co-efficient(r), percent relative standard deviation(% RSD) and standard error(SE) were calculated and the results are summarized in Table-1.The results of sample analysis showed that the drug determined by the proposed methods was in good agreement with the label claim proving the accuracy of the proposed method.To study the accuracy and reproducibility of the proposed methods, recovery experiments were carried out by adding a known amount of drug to preanalysed sample and the percentage recovery was calculated. The results are furnished in Table-2. The results indicate that there is no interference of other ingredients present in the formulations. The results of precision study are shown in Table-3.The proposed method is found to be simple, sensitive, economical, accurate and reproducible and useful for the simultaneous estimation of FEB and DIC in combined tablet dosage form.

Table 1: Optical and Statistical parameters

Parameters	FEB	DIC
Absorption maximum/ Wavelength range(nm)	200-400	200-400
Linearity Range(µg/mL)	2-10	2-10
Correlation coefficient	0.9985	0.999
Standard Error(SE)	0.0424	0.0122
Regression Equation y=mx+c	0.021x + 0.034	0.029x + 0.013
Intercept (c)	0.034	0.013
Slope (m)	0.021	0.029
LOD(µg/mL)	0.1180	0.1212
LOQ(µg/mL)	0.3601	0.3672

Table 2: Assay and recovery of FEB and DIC

Method	Labelled amount(mg)	Amount obtained(mg)*	Percentage recovery*
FEB	40	39.78	99.99%
DIC	100	99.34	100.03%

*Average of six determinations

Table 3: Precision study

Drug	Conc. of drug(µg/ml)	% RSD	
		Intraday	Interday
FEB	4	0.7094	0.3657
	6	0.2841	0.3031
	8	0.3111	0.3546
DIC	4	0.2679	0.2576
	6	0.2839	0.4853
	8	0.5039	0.6968

CONCLUSION

The proposed first-order derivative method provides simple, specific, precise and accurate quantitative analysis for simultaneous determination of FEB and DIC in combined tablet dosage form. The method was validated as per ICH guidelines in terms of linearity, accuracy, precision, limits of detection (LOD) and quantitation (LOQ) and reproducibility. The proposed method can be used for routine analysis of FEB and DIC in combined dosage form.

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