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DEVELOPMENT AND APPLICATION OF DIFFERENCE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF FEBUXOSTAT IN TABLETS

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ABSTRACT

A simple, rapid, sensitive and cost effective difference spectrophotometric method have been developed for the determination of Febuxostat in its tablet dosage form. The method is based on the induced spectral changes upon changing the pH of the medium that differ in their UV spectra. Difference spectrum, obtained by keeping Febuxostat in 0.1N NaOH in reference cell and same in 0.1N HCl in sample cell, showed two characteristic peaks at 260nm and 315nm with positive and negative absorbance respectively and viceversa. Difference of absorbance between these two maxima was calculated to find out the amplitude, which was plotted against concentration. The calibration curve is linear over the concentration range of 5-25 µg/ml (r2 = 0.999), with a detection limit of 0.58µg/ml. The method was successfully applied to the commercial pharmaceutical drug without interference from common ingredient accompanying the drug along with its dissolution profile and very cost effective as its prevents the use of hazardous and costly organic solvents.

INTRODUCTION: Febuxostat, antigout agent is chemically 2- [3- cyano-4- (2- methlypropoxy) phenyl]- 4- methlythiazole- 5 - carboxylic acid (**fig. 1**). It is a non purine selective inhibitor of xanthine oxidase [1]. It inhibits both oxidized and reduced forms of xanthine oxidase [2, 3] and has very less effects on other enzymes of purine and pyrimidine metabolism [3, 4]. Based on the literature survey it shows that very few analytical methods have been reported for the estimation of Febuxostat which includes UV (Drug dissolution [5] and determination [6]), HPLC [7], GC [8] and LC/MS/MS (Impurity profiling) [9].

The aim of the study is to develop a simple, sensitive, accurate, precise and cost effective method for determination of febuxostat in pharmaceutical formulations and bulk drugs using UV spectro-photometer without using costly and hazardous organic solvents.

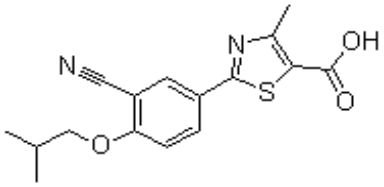

FIG. 1: STRUCTURE OF FEBUXOSTAT

Difference spectrophotometric assay measure the difference absorbance between two equimolar solutions of the analyte in different chemical forms, which exhibit difference spectral characteristics. The simplest and most commonly employed technique for altering the spectral properties of analyte is the adjustment of the pH by means of aqueous solution of acids and alkali.

MATERIALS AND METHOD: A Simadzu UV Spectro-photometer 1800 with 1.0 cm matched quartz cells was used. Febuxostat bulk drug was obtained from Cadila Healthcare Ltd., Ahmedabad. Tablets (Urifix 40mg) were obtained from the market (manufactured by Precise Biopharma Ltd.) Sodium hydroxide and Hydrochloric acid (were from Merck, Mumbai, India) (0.1M Solution), Water was always distilled. The year of experimentation was 2012, at K.B.Raval College of Pharmacy, Shertha, Dist. - Gandhinagar-382423, Gujarat, India.

RESULT AND DISCUSSION:

Calibration: Stock Febuxostat solution was prepared by dissolving 10 mg of working standard in 10 ml of methanol. Working standard solutions with concentration ranging from 5-25 µg/ml were prepared by transferring appropriate volume of stock solution to 10ml volumetric flask in duplicate. The volume was then adjusted with 0.1M HCl and 0.1M NaOH to give a series of equimolar solutions of Febuxostat. Difference spectra were obtained by keeping acidic form (in 0.1M HCl) in reference cell and basic form (in 0.1M NaOH) in sample cell and vice versa. Difference of absorbance between 260nm and 315nm was calculated to find out the amplitude (**table 1**)

TABLE 1: DIFFERENCE STANDARD CURVE OF FEBUXOSTAT IN 0.1M HCl WITH RESPECT TO 0.1M NaOH

Conc. (µg/ml)	Absorbance		Amplitude
	λmax	λmin	
05	0.0259	-0.3442	0.3701
10	-0.0173	-0.6903	0.6730
15	-0.1306	-1.1057	0.9751
20	-0.1758	-1.4776	1.3018
25	-0.2310	-1.8298	1.5988

TABLE 2. DIFFERENCE STANDARD CURVE OF FEBUXOSTAT IN 0.1M NaOH WITH RESPECT TO 0.1M HCl

Conc. (µg/ml)	Absorbance		Amplitude
	λmax	λmin	
05	-0.024	0.3458	0.3698
10	0.0287	0.6914	0.6627
15	0.1221	1.0899	0.9678
20	0.1830	1.4908	1.3078
25	0.2383	1.8470	1.6087

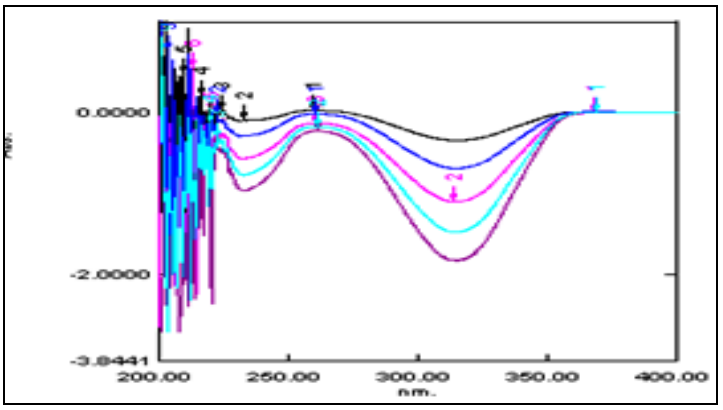

FIG. 2: OVERLAY SPECTRA OF FEBUXOSTAT IN 0.1M HCl WITH RESPECT TO 0.1M NaOH

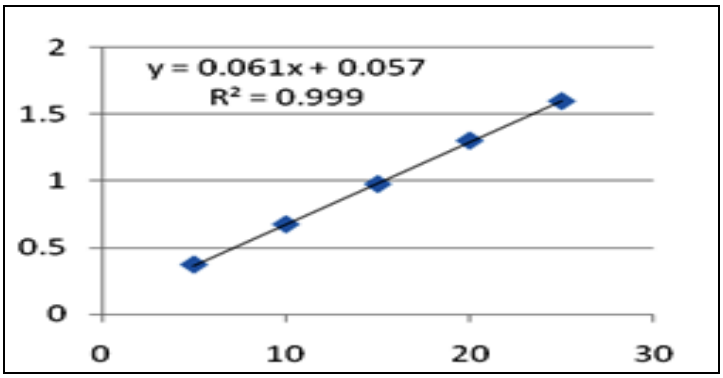

FIG. 3. CALIBRATION CURVE OF FEBUXOSTAT IN 0.1M HCl W.R.T. 0.1M NaOH

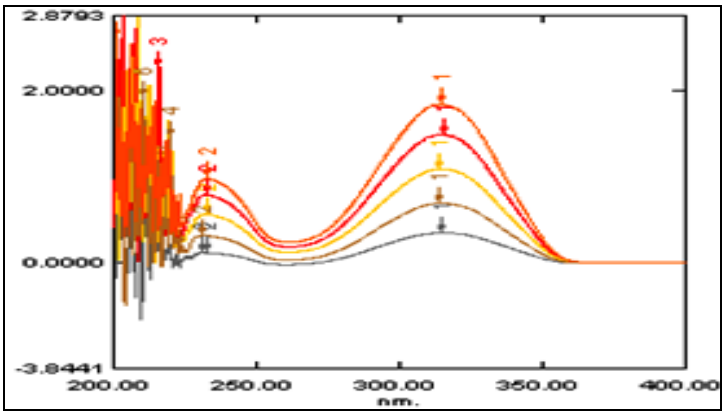

FIG. 4. OVERLAY SPECTRA OF FEBUXOSTAT IN 0.1M NaOH WITH RESPECT TO 0.1M HCl

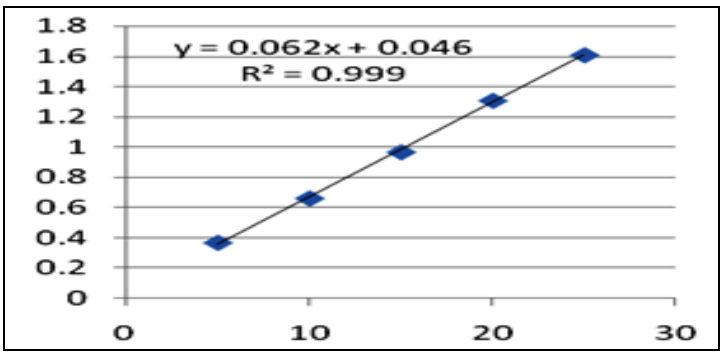

FIG. 5. CALIBRATION CURVE OF FEBUXOSTAT IN 0.1M NaOH W.R.T. 0.1M HCl

Procedure for the assay of Febuxostat from tablet: The average mass of 10 tablets was determined and was ground in a mortar. An amount of powder (accurately weighed) equivalent to 10mg Febuxostat was transferred in 10ml volumetric flask and made up to the mark with methanol. The content of the flask was sonicated for 10min and then the solution was filtered through Whatmann filter paper.

Then 1ml of the filtrate was transferred to 10ml volumetric flask in Methanol. From the above solution 1 ml of solutions were transferred to 10 ml volumetric flask in duplicate and made up to mark with 0.1M NaOH and 0.1M HCl respectively. The absorbance difference between the 0.1M HCl solution and equimolar 0.1M NaOH solution was measured at 206nm and 315nm by placing basic solution as reference and acidic solution as sample. The content of the tablet is calculated from the calibration curve or using the corresponding regression equation in (**table 3**)

TABLE 3: ASSAY OF FEBUXOSTAT FROM MARKET FORMULATION

Brand	Amount taken	Amount found	% Assay
Urifix(40mg)	10 mcg	10.22 mcg	102.22%

Validation Parameters: Estimation of Febuxostat was validated as per ICH guidelines [10].

Accuracy: To check the accuracy of the developed methods and to study the interference of formulation additives, analytical recovery experiments was carried out by standard addition method. Total amount of drug found and percentage recovery was calculated by adding 0%, 80%, 100% and 120% standard and results were reported in **table 4**.

TABLE 4. RECOVERY STUDY OF FEBUXOSTAT

	Amount added (mcg/ml)	Amount found (mcg/ml)	%Recovery
0%	10	10.22	102.2
80%	18	18.16	100.88
100%	20	20.35	101.75
120%	22	22.34	101.54

Precision (inter-day and intra-day precision): precision of the method was checked by assay the sample solution on sameday at an interval of one hour(intraday precision) for three hours and on three different days (interday precision) the result was reported in **table 5**. This study indicates that the solutions can be analyzed within 48-72 hr without having any adverse effect on chemical stability of the drug in acidic and basic medium**.**

TABLE 5. INTERDAY AND INTRADAY PRECISION STUDY OF FEBUXOSTAT

Conc. µg/ml	RSD		LOD	LOQ
	Intraday	Interday		
10	0.8380	1.1683		
15	0.3657	0.6392	0.5871	1.7793
20	0.7843	0.5062		
mean	0.6627	0.7712		

Limit of detection (LOD) and limit of quantitation (LOQ): The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve. The standard deviation of Y-intercept and slope of the calibration curves were used to calculate the LOD and LOQ by using the equations 3.3σ/s for LOD and 10σ/s for LOQ, where σ stands for standard deviation of Y-intercept and S stands for slope of the calibration curve. The results of the same were given in table 5.

Application of developed difference spectroscopic method for dissolution study of Febuxostat: Dissolution is an official test used by pharmacopoeias for drug evaluation release of solid and semisolid dosage forms, and it is routinely used in Quality Control (QC) and Research & Development (R&D).

The purpose of *in vitro* dissolution studies in QC is batch to batch consistency and detection of manufacturing deviation while in R&D the focus is to provide some predictive estimate of the drug release in respect to the in vivo performance of a drug product.

For QC, an over-discriminatory test might be suitable to detect even small production deviations. However, for prediction of the in vivo performance of drug product a dissolution test should be sensitive and reliable [11]. A review of the literature found a simple UV spectrophotometric method reported for the determination of Febuxostat in

raw materials and in vitro drug dissolution studies [5], but, it is costly as it require methanol as the solvent. So, it is better to use another cost effective method for the same purpose. The above developed difference spectroscopic method is suitable for the dissolution purpose. Febuxostat from tablets containing 40 mg of this API, using Phosphate buffer pH 6.8 with 0.0675% SLS as the dissolution medium. RPM was set at 75 and the temperature was maintained at 37±0.5°C. Final dilution of the samples were done in 0.1M NaOH and 0.1M HCl and % cumulative Release of the drug was determined.

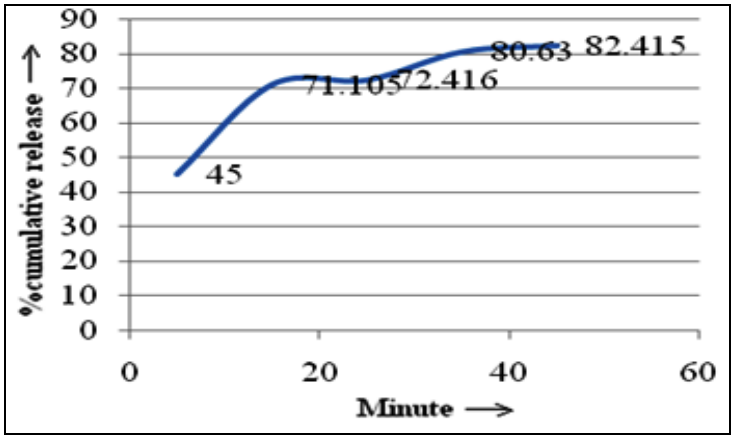

FIG. 6. DISSOLUTION PROFILE OF FEBUXOSTAT

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