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Research Article

DEVELOPMENT AND VALIDATION OF ULTRAVIOLET-SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF FEBUXOSTAT FOR CONDUCTING *IN-VITRO* QUALITY CONTROL TESTS IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Objective: A simple, accurate, and selective ultraviolet-spectrophotometric method has been developed for the estimation of febuxostat in the bulk and pharmaceutical dosage forms.

Method: The method was developed and validated according to International Conference on Harmonization (ICH Q2 R1) guidelines. The developed method was validated statistically with respect to linearity, range, precision, accuracy, ruggedness, limit of detection (LOD), limit of quantitation (LOQ), and recovery. Specificity of the method was demonstrated by applying different stressed conditions to drug samples such as acid hydrolysis, alkaline hydrolysis, oxidative, photolytic, and thermal degradation.

Results: The study was conducted using phosphate buffer pH 6.8 and λ_{max} was found to be 312 nm. Standard plot having a concentration range of 1–10 µg/ml showed a good linear relationship with R^2=0.999. The LOD and LOQ were found to be 0.118 µg/ml and 0.595 µg/ml, respectively. Recovery and percentage relative standard deviations were found to be 100.157±0.332% and <2%, respectively.

Conclusion: Proposed method was successfully applicable to the pharmaceutical formulations containing febuxostat. Thus, the developed method is found to be simple, sensitive, accurate, precise, reproducible, and economical for the determination of febuxostat in pharmaceutical dosage forms.

Keywords: Ultraviolet-spectrophotometric, Febuxostat, Validation.

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INTRODUCTION

Febuxostat is a novel non-purine selective xanthine oxidase inhibitor, chemically it is 2-[3-cyano-4-(2-methlypropoxy)phenyl]-4-methlythiazole-5-carboxylic acid. It is approved in the Indian market by the year 2009 by Central Drugs Standard Control Organization [1]. It is available in the dose of 80 or 120 mg. It has been found as highly protein bound drug (98%) mainly at diazepam binding site. It is highly effective in the long-term management of hyperuricemia in patients with gout and chronic tophaceous gout [2]. It has a molecular weight of 319.68 g/mol with an empirical formula (Fig. 1) of $C_{16}H_{16}N_2O_3S$. It has crystalline and nonhygroscopic nature. It has poor solubility profile in water but freely soluble in dimethylformamide, soluble in dimethylsulfoxide, sparingly soluble in ethanol, and slightly soluble in methanol and acetonitrile [3]. It gets metabolized by uridine diphosphate glucuronosyltransferase enzymes to its acyl glucuronide metabolite and lesser extent to its oxidative metabolite such as 67M-1, 67M-2, and 67M-4 through cytochrome P450 enzymes. It has been recommended as a potential alternative to allopurinol in those patients not tolerating or having an inadequate reduction in the level of serum uric acid when treated with allopurinol [4].

METHODS

Chemicals and reagents

Standard sample of febuxostat was a generous gift sample from Ami Lifesciences, Gujarat, India. Guar gum and Carbopol 940 were obtained from CDH Pvt., Ltd. The marketed tablets of febuxostat (Myfeb-80) containing 80 mg of febuxostat, manufactured by Stevels Pharma Ltd., India, were purchased from the market. All other chemicals used were of analytical grade.

Instrumentation

A double beam Systronics ultraviolet (UV)-visible spectrophotometer, model UV-2201 (India) having a spectral bandwidth of 1 nm, wavelength accuracy of ±0.5 nm, and a pair of 1cm quartz cells were used to measure the absorbance of the resulting solutions.

Preparation of standard stock solution

Accurately weighed the quantity of 10 mg febuxostat was transferred into 10 ml volumetric flask, and then 1 ml methanol was added as a cosolvent. The volume was adjusted up to the mark with phosphate buffer pH 6.8. The prepared solution was found to be a clear solution having the strength 1000 µg/ml.

Preparation of sample stock solution

1 ml stock solution was taken in the 100 ml volumetric flask and then diluted up to the mark with phosphate buffer pH 6.8 to get the sample stock solution having the strength 10 µg/ml. Then, further dilutions were made from 1 to 10 µg/ml.

Determination of λ_{max}

The standard solution of febuxostat (10 µg/ml) was scanned in the wavelength region of 200–400 nm.

Validation [5-14]

In the method, development validation plays an important role in analytical determination. Validation covers six main parameters for method development such as linearity and range, precision and accuracy, limit of detection (LOD), limit of quantitation (LOQ), recovery, and ruggedness.

Linearity and range

To determine the linearity, Three independent levels of calibration curve were analyzed in the range of 1–10 µg/ml. The absorbance of each solution was recorded at 312 nm against phosphate buffer pH 6.8. The calibration curve was plotted, and the correlation coefficient and regression line equation for febuxostat were determined.

Precision
Determination of intraday precision was done by analyzing febuxostat (1–10 µg/ml) at 3 different time points of the same day, and the determination of the interday precision was done by analyzing febuxostat (1–10 µg/ml) at 3 different time points on different days.

Accuracy
The accuracy of the method was determined by percentage recovery experiments performed at three different levels such as 50%, 100%, and 150%. A known amount of standard febuxostat solutions were added to the pre-analyzed sample solutions. Absorbance was recorded, and percentage recovery was estimated using the calculated amount of drug in the following formula.

$$\% \text{ Recovery} = A\text{-}B/C*100$$

Where, A represents total amount of drug estimated
B represents amount of drug found on pre-analyzed basis
C represents amount of pure drug added.

Ruggedness
The ruggedness of the purposed method was evaluated by applying the same developed procedure to assay 10 µg/ml of febuxostat using the same instrument by two different analysts at different days under the same conditions. The obtained results were evaluated for the reproducibility.

LOD and LOQ [15]
LOD and LOQ were calculated using following formula:

$$LOD = 3.3*\sigma/S \text{ and } LOQ = 10*\sigma/S$$

Where, σ is the standard deviation of y-intercepts of regression lines
S is the slope of the calibration curve.

Forced degradation study (stressed conditions) [16]
A 2 ml of the standard stock solution (1000 µg/ml) of Febuxostat was taken as four replicates in four different volumetric flasks of 100 ml and mixed with 10 ml of following (1–3) solutions. Then, all the flasks were set aside for 1 h at room temperature. The solution was diluted up to mark with double distilled water.

1. 0.1N HCl for acid hydrolysis.
2. 0.1N NaOH for alkaline hydrolysis.
3. 5% H_2O_2 for oxidative degradation.
4. For photolytic degradation, a solution of drug (10 µg/ml) was exposed to UV radiation of wavelength 254 nm and of 1.4 flux intensity for 24 h in a UV chamber.
5. For thermal degradation, solid pure drug was kept in an oven at 100°C for 24 h. After cooling to room temperature, 10 µg/ml solution was prepared.

The absorbance of all the solutions from acid hydrolysis, alkaline hydrolysis, oxidative degradation, photolytic degradation, and thermal degradation was measured at 312 nm against respective solvent as blank in each case

Formulation of febuxostat loaded hydrogel [17,18]
The hydrogel was prepared using hydrophilic polymer guar gum (0.75%) and Carbopol 940 (1%).

Carbopol 940 is water soluble while guar gum produces colloidal dispersion with water. Dispersion of polymers was made using magnetic stirrer (500 rpm). For complete swelling of both polymers, dispersions were kept in the dark for 24 h. Dispersing Carbopol 940 in distilled water was followed by slow addition of guar gum dispersion under continuous stirring. Aqueous drug solution was added to the polymeric dispersion after addition of 0.1 N sodium hydroxide solution which acts as a cross-linking agent. Finally, the remaining distilled water was added to obtain a homogeneous dispersion of gel under magnetic stirring

Comparative study of calibration curve with standard plot or analysis of marketed formulation (Myfeb-80) and prepared hydrogel by UV-spectrophotometric method
Ten marketed tablets of febuxostat (Myfeb-80) containing 80 mg drug per tablet were taken in pestle and mortar. Weighed amount of the powder equivalent to 10 mg of febuxostat was transferred into the 10 ml volumetric flask, 1 ml methanol was added as a cosolvent and finally, diluted up to the mark with phosphate buffer pH 6.8 to produce a solution of 10 ml. The prepared solution was found to be a clear having the strength 1000 µg/ml, which was a stock solution. From this stock solution, 1 ml of solution was taken and transferred to 100 ml volumetric flask and further diluted with pH 6.8 phosphate buffer to get a concentration of 10µg/ml. From this solution, dilutions were prepared in the range 1–10 µg/ml and were analyzed at 312 nm.

Assay of marketed formulation Myfeb-80 by developed method [19]
Ten marketed tablets of febuxostat (Myfeb-80) were taken and average weight was determined. Then, the mixture was ground and mixed well. The powder of the sample equivalent to 10 mg of febuxostat was accurately weighed (22.62 mg powder) and transferred into 10 ml volumetric flask. Then, 1 ml of methanol was added, and final volume was adjusted using phosphate buffer pH 6.8 sonicated for 5 min to get a clear solution of strength 1000 µg/ml. Then, the content of the flask was filtered using Whatman filter of pore size 0.45 µ. From this stock solution, 1 ml of solution was taken and transferred to 100 ml volumetric flask by adjusting the volume up to 100 ml, using phosphate buffer pH 6.8 which gives a solution of strength 10 µg/ml. This solution was prepared 5 times, and the absorbance of each solution was determined at 312 nm, and the concentration of drug present in sample solution was calculated; hence, drug present per tablet was also calculated.

Assay of prepared hydrogel [20]
50 mg of prepared gel (equivalent to 1.0 mg of febuxostat) was weighed accurately and it was dissolved in 100 ml of phosphate buffer of pH 6.8. The conical flask containing gel was shaken for 2 h on mechanical shaker to get complete solubility of febuxostat.

The resulting solution is filtered through Whatman filter paper; the febuxostat content was analyzed spectrophotometrically at 312 nm using a UV spectrophotometer (Systronics, India). The measurement was carried out in triplicate, and the average febuxostat content in the topical gel was calculated.

RESULTS

Determination of λ_{max}
When a standard solution having concentration 10 µg/ml was scanned, the λ_{max} was found to be 312 nm as shown in Fig. 2.

Linearity and range
The linearity of febuxostat was found to be in the range of 1–10 µg/ml with correlation coefficient 0.999. Standard plot data and curve are given in Table 1 and Fig. 3.

Table 1: Standard plot of pure drug (Febuxostat)

Sr. no.	Concentration (µg/ml)	Absorbance*±SD
1	1	0.120±0.011
2	2	0.214±0.022
3	3	0.282±0.019
4	4	0.379±0.019
5	5	0.465±0.014
6	6	0.541±0.017
7	7	0.625±0.012
8	8	0.726±0.017
9	9	0.808±0.016
10	10	0.879±0.026

*Average of three observations. SD: Standard deviation

The standard plot was successfully prepared, the equation of straight line give regression coefficient value near to one which confirms data fits into the equation of straight line.

Precision

Intraday precision

The percentage relative standard deviations (RSD) was found to be in the range of 0.126–0.251% (Table 2) which is <1 which confirms the reliability of the method.

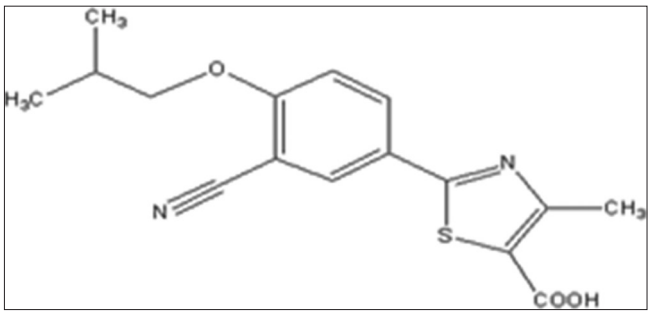

Fig. 1: Structure of febuxostat

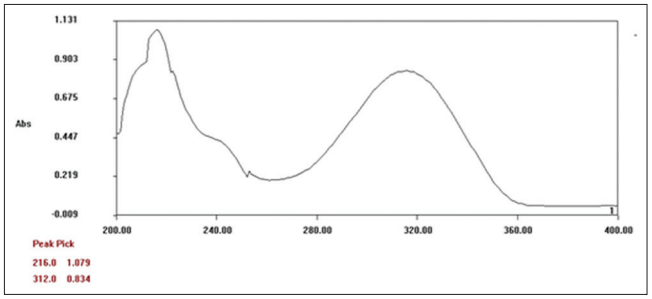

Fig. 2: Ultraviolet spectrum of pure drug (Febuxostat)

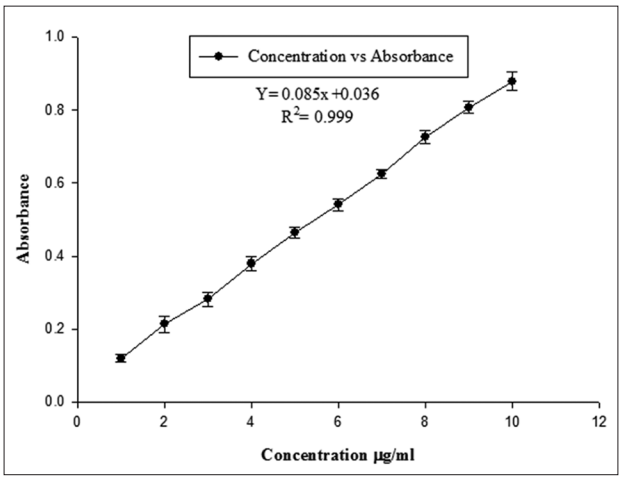

Fig. 3: Standard plot of pure drug (Febuxostat)

Interday precision

The percentage RSD was found to be in the range of 0.135–0.591% (Table 3) which is <1 which strongly confirms the reliability of the method.

Accuracy

The accuracy of the method was checked by the recovery studies at three different levels, i.e., 50%, 100%, and 150%. The mean of the recovery for febuxostat was found to be 100.157±0.332%. The results obtained were shown in Table 4.

Ruggedness

The obtained results were found to be reproducible (Table 5) since there was no significant difference between analysts. Thus, the proposed method was considered to be rugged.

LOD and LOQ

The sensitivity of the method was assessed by determining the LOD and LOQ. The LOD and LOQ for Febuxostat were found to be 0.118 µg/ml and 0.595 µg/ml, respectively.

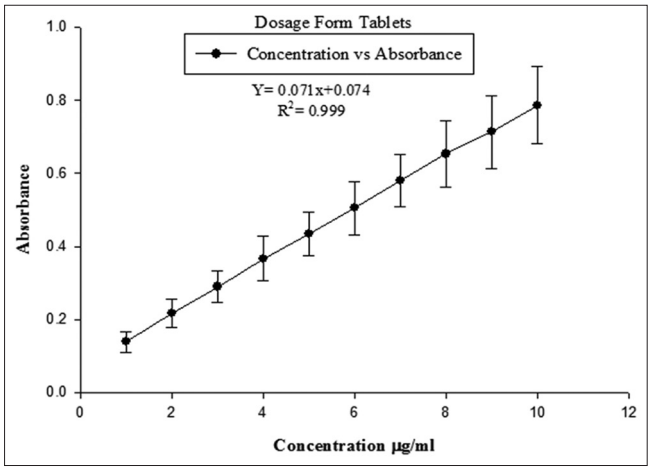

Fig. 4: Calibration curve of febuxostat drug-containing tablet by simple ultraviolet-spectrophotometer

Table 2: Results of intraday precision

Concentration (µg/ml)	Absorbance*	SD	%RSD
2	0.223	0.003	0.142
4	0.377	0.003	0.126
6	0.540	0.005	0.251

*Average of three observations. SD: Standard deviation, RSD: Relative standard deviations

Table 3: Results of interday precision

Concentration (µg/ml)	Absorbance*	SD	%RSD
2	0.238	0.003	0.135
4	0.397	0.014	0.591
6	0.543	0.005	0.192

*Average of three observations. SD: Standard deviation, RSD: Relative standard deviations

Table 4: Results of recovery studies

Amount of sample* (µg/ml)	Amount of drug added* (µg/ml)	% of spiked sample	Amount recovered (µg/ml)	% Recovery
2	1	50%	3.245	101.11±0.398
2	2	100%	4.271	98.876±0.246
2	3	150%	5.357	100.484±0.352

*Average of three observations

Table 5: Ruggedness data at 10 µg/ml of febuxostat by two analysts at different days

Test concentration (µg/ml)**	Analyst-I	Analyst-II
10 µg/ml	0.887	0.885
SD	0.085	0.091
%RSD	0.838	0.897

**Average of five observations. SD: Standard deviation, RSD: Relative standard deviations

Table 6: Results of the drug under stressed conditions

Parameter studied	Concentration taken* (µg/ml)	Concentration obtained* (µg/ml)	% Drug degradation*	% Drug recovered*
Acid hydrolysis	10	8.93	10.7	89.3
Alkaline hydrolysis	10	9.08	9.2	90.8
Oxidative degradation	10	9.63	3.7	96.3
Photolytic degradation	10	8.92	10.8	89.2
Thermal degradation	10	8.61	13.9	86.1

*Average of three observations

Table 7: Calibration data of febuxostat tablet by simple UV-Spectrophotometer

Sr. no.	Concentration (µg/ml)	Absorbance*±SD
1	1	0.139±0.028
2	2	0.218±0.039
3	3	0.290±0.044
4	4	0.366±0.061
5	5	0.435±0.059
6	6	0.506±0.073
7	7	0.580±0.072
8	8	0.654±0.091
9	9	0.713±0.100
10	10	0.786±0.106

*Average of three observations, UV: Ultraviolet, SD: Standard deviation

Forced degradation study

From the result given in Table 6, it was found that there were no significant changes in absorbance after performing acid hydrolysis, alkaline hydrolysis, photolytic degradation, and thermal degradation.

Analysis of marketed formulation (Myfeb) by UV-spectrophotometric method

The linearity of the calibration data showed (Table 7) that the proposed method can be successfully applied to the pharmaceutical dosage form without any interference from common excipients. As the drug was same as that of the standard plot so same concentrations were reproduced (Fig. 4), and values were fit into the equation of a straight line, the values were successfully obtained.

Assay of pharmaceutical formulations containing drug febuxostat

Validated UV-spectrophotometric method was applied to estimate the amount of drug present in both marketed (Myfeb-80 tablets) and prepared formulation (Hydrogel), obtained results were shown in Tables 8 and 9.

In both prepared and marketed formulations, we were successful to estimate drug accurately that was claimed in the formulations.

Statistical analysis of standard plot and calibration curve

As we considered null hypothesis that is no significant difference in obtained absorbance of standard plot and calibration plot, Ha is one-sided, so we had applied one-tail t-test for determining the rejection region at 5% level, which comes as 1.833 under the table of t-distribution for 9° of freedom. The calculated value of t comes out to be 0.201, which is in the acceptance region thus we concluded that difference between two sample data is insignificant.

All the validation parameters are summarized in Table 10, shows that the proposed UV spectrophotometric method is found to be accurate, rapid, simple, precise, selective, reproducible, and economical.

DISCUSSION

The standard plot was successfully prepared thrice with a very low error which indicates that method is quite accurate as there is precision in method followed and the readings were fit to the line of best fit in the equation of the straight line with high R^2 value. All other validation parameters were also in reasonable range confirming the followed method of estimation is accurate, precise, and reproducible. To confirm the reproducibility of the standard curve, same drug concentrations were prepared from marketed formulations in a repeated manner. The observations were obtained. Thereafter, "t-test" was applied considering the null hypothesis. After calculation of "t" value, it was confirmed that there was not a significant difference in observation of prepared standard curve with that of the calibration curve. However, from forced degradation study, it is also revealed that drug is quite stable at diverse conditions.

CONCLUSION

From above study, it has been concluded that the developed method for the determination of febuxostat in pharmaceutical formulations was found to be simple, sensitive, accurate, precise, reproducible, and economical. The purity of the drug peak was assessed by analyzing the spectra. High R^2 value in linearity and percentage RSD<2 indicate good results. The interday precision at three level of concentration on 3 different days also provides evidence about the ruggedness of the analytical method due to the low value of percentage RSD. Thus, the method was found to be specific. The lowest value of LOD obtained by proposed method proved that the method followed was the most sensitive method. Small but deliberate changes do not affect the method which indicates that the proposed method was found to be robust. Percentage RSD of the pharmaceutical formulation was found to be 0.0725% which showed that there was no interference from the excipients used in the formulation which indicates the accuracy and reliability of the method. To ensure the specificity of the developed method, it was calibrated with the market formulation of febuxostat, two evaluated parameters that were calibration and assay ensures that the developed method can be used for routine analysis for estimation of febuxostat in bulk and pharmaceutical dosage form.

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AUTHOR'S CONTRIBUTION

Designing of experiment, forced degradation study, hydrogel preparation and its analysis, comparative study, and statistical analysis was carried out by Jaspreet Kaur. Whereas, Daljit Kaur had arranged gift sample of drug and other chemicals required to carry out the study.

Table 8: Assay of pharmaceutical formulations Myfeb-80

Formulation	Label Claimed* (mg)	Amount recovered (mg)	% Drug recovered	% RSD
Myfeb-80 (tablet)	80	79.890±0.024	99.862±0.03	0.0725

*Average of three observations. RSD: Relative standard deviations

Table 9: Assay of prepared hydrogel

Formulation	Amount taken equivalent to (µg)	Amount recovered (µg)	% Drug recovered	% RSD
Hydrogel	10	9.705±0.038	97.050±0.03	0.0973

*Average of three observations. RSD: Relative standard deviations

Table 10: Summary of the validation parameters of UV-spectrophotometry

Sr. No.	Parameter	Result
1	λ_{max} (nm)	312
2	Beer's law limit(µg/ml)	1–10
3	Regression equation	0.085x+0.036
4	Slope	0.085
5	Intercept	0.036
6	Correlation coefficient (R^2)	0.999
7	Precision (%RSD)	
	Intraday	0.126–0.251%
	Interday	0.135–0.591%
8	% Recovery	100.157±0.332%
9	LOD (µg/ml)	0.118
10	LOQ (µg/ml)	0.595
11	Assay	
	Myfeb-80 tablets	99.862%
	Hydrogel	97.050%

UV: Ultraviolet, LOD: Limit of detection, LOQ: Limit of quantitation

Validation study parameters were carried by Daljit and Sukhmeet Singh Kamal. The manuscript had been written by first two authors.

CONFLICTS OF INTEREST

Authors have no conflicts of interest.

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