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 \( \lambda_{\text{max}} \) was found to be 312 nm. Standard plot having a concentration range of 1–10 μg/ml showed a good linear relationship with R²=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.

INTRODUCTION

Febuxostat is a novel non-purine selective xanthine oxidase inhibitor, chemically it is 2-[3-cyano-4-(2-methlypropoxy)phenyl]-4- methylthiazole-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 C15H11N2O2S. 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 acylglucuronide 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 Carbopol940 were obtained from CDH Pvt., Ltd. The marketed tablets of febuxostat (Myfeb-80) containing 80 mg of febuxostat, manufactured by Steveds 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 1 cm 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 \( \lambda_{\text{max}} \)

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.
Concentration (µg/ml) of 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 gives 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.

**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

**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.

**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

**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.

**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                    | 98.87±0.246 |
| 2                        | 3                            | 150%              | 5.357                    | 100.49±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

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 R² 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 R² 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 | 0.085±0.036 |
| 4 | Slope | 0.085 |
| 5 | Intercept | 0.036 |
| 6 | Correlation coefficient (R²) | 0.999 |
| 7 | Precision (%RSD) | Intraday 0.126-0.251% | Interday 0.35-0.591% |
| 8 | % Recovery | 10.157±0.332% |
| 9 | LOD (µg/ml) | 0.118 |
| 10 | LOQ (µg/ml) | 0.595 |
| 11 | Assay | Myfeb-80 tablets 99.86% | Hydrogel 97.05% |

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

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

CONFLICTS OF INTEREST

Authors have no conflicts of interest.

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