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A Validated RP-HPLC Method for the Estimation of Febuxostat in Bulk Drugs

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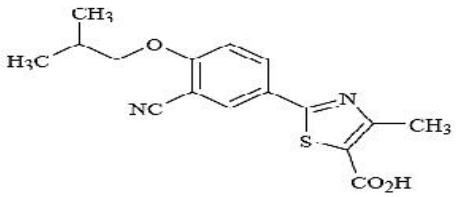
Abstract: An accurate, sensitive, precise and robust reverse phase high performance liquid chromatographic (RP-HPLC) method for the estimation of Febuxostat in bulk forms has been developed and validated. Chromatographic separation is conducted on Nucleosil C18 (250 x 4.6mm, 5µm) column at ambient temperature using mixture of 10 mM ammonium acetate buffer (buffer of pH 4.0 adjusted with 0.2% triethyl amine) and acetonitrile in the ratio (15: 85, v/v) as a mobile phase and at a flow rate of 1.2 ml / min, while UV detection is performed at 275nm. The retention time for Febuxostat is found to be 3.45 ± 0.05 min. The method is found to be linear in the range of 50.0 – 400.0 µg/mL. The limit of detection and quantization for Febuxostat are found to be 9.98 and 30.23 µg /mL respectively. Analytical recovery is 99.29 %. The percentage RSD for precision and accuracy of the method is found to be less than 2%. The method is validated as per the ICH guidelines and applied for the quantitative analysis of Febuxostat in bulk forms.
Keywords: Febuxostat, RP-HPLC method, Validation, Acetonitrile, Ammonium acetate buffer.

Introduction and Experimental

The Febuxostat chemically is 2-[3-cyano-4-(2-methylpropoxy) phenyl]-4-methylthiazole-5-carboxylic acid[1] (Fig. 1) with a molecular weight of 316.38. The molecular formula is $C_{16}H_{16}N_2O_3S$. Febuxostat is a non-hygroscopic, white crystalline powder that is freely soluble in dimethylformamide; soluble in dimethylsulfoxide; sparingly soluble in ethanol; slightly soluble in methanol and acetonitrile; and practically insoluble in water. The melting range is 205^0c to 208^0c. Tablets for oral use contain the active ingredient, febuxostat, and are available in two dosage strengths; 40 mg and 80 mg. Inactive ingredients include lactose monohydrate, microcrystalline cellulose, hydroxypropyl cellulose, sodium croscarmellose, and silicon dioxide and magnesium stearate [2].

Figure 1: Chemical Structure of Febuxostat



Febuxostat (TEI-6720; TMX-67) is a novel, orally administered, potent and non–purine analogue being developed by Teijin, with licensees Ipsen and TAP Holdings, for the treatment of hyperuricemia in gout [3]. It completely inhibits activity of both oxidized and reduced forms of xanthine oxidase (XO) by obstructing substrate binding [4,5] and also inhibits with minimal effects on activity of other enzymes in purine and pyrimidine metabolism[2,6]. It appears to be safe and well tolerated in different renal function groups and does not appear to require any dose adjustment based on differences in renal function[7,8]. It can be administered regardless of food or antacid intake[9]. Its doses were more efficacious than allopurinol[10]. The residual organic solvents (acetone, ethyl acetate, ethanol and N,N-DMF) in Febuxostat were quantitatively determined by GC [11] and the impurities (amide, sec-butyl, des-cyano and des-acid) were separated by reverse phase gradient system[12]. Febuxostat was estimated by spectrophotometric method[13] and HPLC method [14]. Febuxostat in human plasma was determined by using ultra-performance liquid chromatography tandem mass spectrometry[15] and HPLC-FLU method[16].

The present study is aimed at developing a rapid, sensitive, precise and accurate RP-HPLC method for the validation of Febuxostat in bulk forms by conducting systematic trails.

Materials and Instruments

HPLC grade acetonitrile (ACN) and ammonium acetate are purchased from Merck Pvt. Ltd., India. The analysis of drug is carried out on a SHIMADZU LC2010 series HPLC system equipped with a reverse phase Nucleosil C18 column (250x4.6mm, 5µm in particle size), a LC 20AT isocratic pump, a 20µl injection loop and a SPD – 20A Promenence UV-Visible Detector and running on Spinchrom Chromatographic Software version. Isocratic elution with 10 mM ammonium acetate buffer (buffer of pH adjusted to 4.0 with 0.2% triethyl amine): acetonitrile (15 : 85, v/v) is used at a flow rate of 1.2 ml / min. Milli-Q water is used for buffers and other reagents preparation.

Mobile phase

A mixture of 10 mM ammonium acetate buffer (buffer of pH 4.0 adjusted with 0.2% triethyl amine) and acetonitrile in the ratio (15: 85, v/v) is used as mobile phase. It is filtered through a 0.45 µ nylon membrane filter and degassed prior to use.

Standard solution of Febuxostat

About 100 mg of Febuxostat is weighed accurately and transferred to 100 ml standard volumetric flask. It is dissolved in acetonitrile and then made up to the volume with the same acetonitrile. This solution is sonicated for about 30 min and it is the standard stock solution with known concentration of 1000 µg/ml (Stock solution-A).

Results and Discussion

The purpose of the present study is to develop a rapid, sensitive, precise and accurate RP-HPLC method for the analysis of febuxostat in bulk forms using Nucleosil C18 (250 x 4.6mm, 5µm particle size) analytical column with UV detection.

Scanning and determination of maximum wavelength (λ_{max})

In order to ascertain the wavelength of maximum absorption (λ_{max}) of the drug, qualitative solution of the drug is prepared in mobile phase and scanned using UV spectrophotometer within the wavelength region of 200-400 nm against mobile phase as blank. The resulting spectrum is shown below (Fig.2) and the absorption curve showed characteristic absorption maxima at 275 nm for Febuxostat.

Method development

To optimize the operating conditions for isocratic RP-HPLC detection of analyte, a number of parameters such as the solvent, mobile phase composition and pH are varied and measurement of active ingredient is carried out at the wavelength in UV region at 275 nm. Two organic solvents (methanol and acetonitrile) are taken and their effects on the elution of Febuxostat are investigated. Six trails are carried out with varying solvent / composition of solvent and buffer / pH of mobile phase (**Table 1).** Three types of buffer, ammonium acetate, ammonium formate and potassium phosphate are considered for studies.

Figure 2: U.V. spectrum of Febuxostat

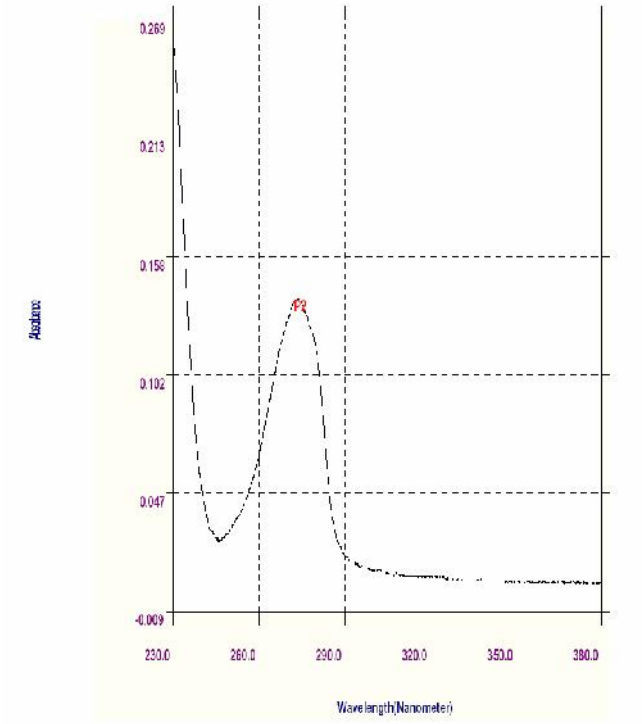


Table 1. Method development trials of Febuxostat showing composition of mobile phase pH of buffer

Trail	Composition of Mobile Phase	pH of Buffer
1	10mM KH_2PO_4 : CH_3OH (50:50)	4.2
2	5mM KH_2PO_4 : CH_3CN (50:50)	6.2
3	10mM CH_3COONH_4 : CH_3OH (60:40)	4.2
4	5mM CH_3COONH_4 : CH_3OH (50:50)	6.5
5	5mM $HCOONH_4$: CH_3OH (50:50)	6.4
6	10mM CH_3COONH_4 : CH_3CN (15:85)	4.0

No peak for Febuxostat is observed in the mobile phase containing methanol and different buffers (Trails – 1, 3, 4 and 5) (Fig. 3, 5, 6 and 7). Peak for Febuxostat in chromatogram is obtained (Figure 4) from the mobile phase containing acetonitrile (50%) and 10mM ammonium acetate buffer (50%) (Trail – 2). The variation in the mobile phase led to considerable changes in the chromatographic parameters like symmetry, capacity factor and retention time. Therefore, acetonitrile is selected for further study with varying buffer and composition. High concentration of buffer has good efficiency to control pH but the precipitation in organic solvent can occur. Low concentration of buffer can avoid precipitation problem but this may result in the irreproducible retention time. The best result for this study is obtained from the condition containing 10mM ammonium acetate buffer (15%) and acetonitrile (85%) (Trail-6, Fig. 8). For quantitative determination of febuxostat in formulations initially standard solution of febuxostat is injected into the column six times and the retention time is found to be 3.45 ± 0.05 min.

The present method is found to be the most suitable as the chromatographic peaks obtained with this system are better defined, resolved and all almost free from tailing by using a mobile phase 10 mM ammonium acetate buffer and acetonitrile in the ratio 15:85 (v/v). Therefore, combination of 10 mM ammonium acetate buffer and acetonitrile in the ratio 15:85 (v/v) with a flow rate of 1.2 ml / min is selected as a suitable mobile phase.

Figure 3: Chromatogram of Trial – 1

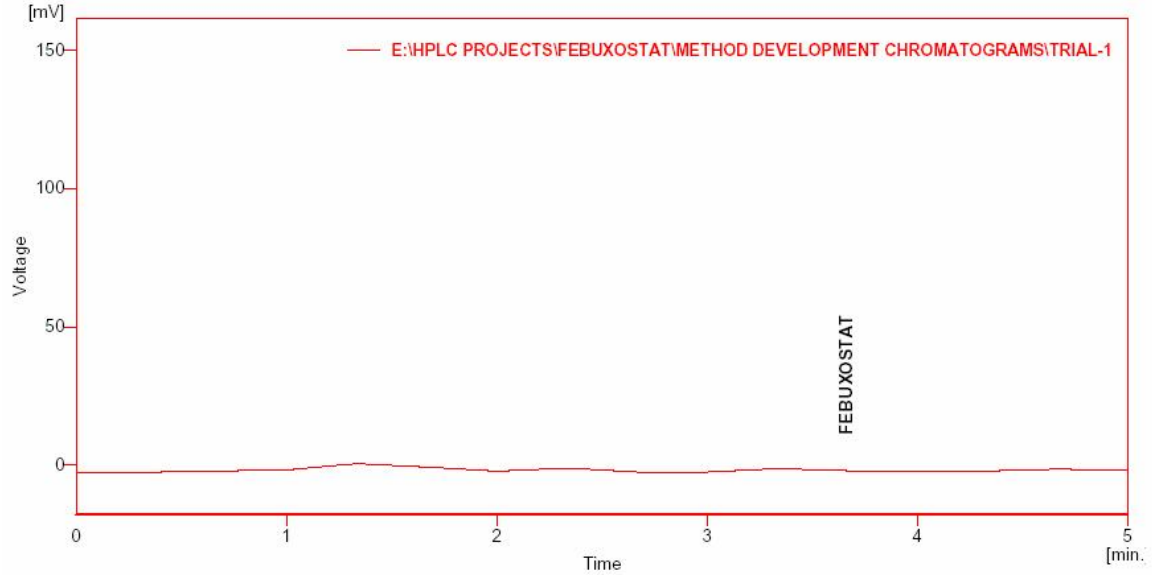


Figure 4: Chromatogram of Trial – 2

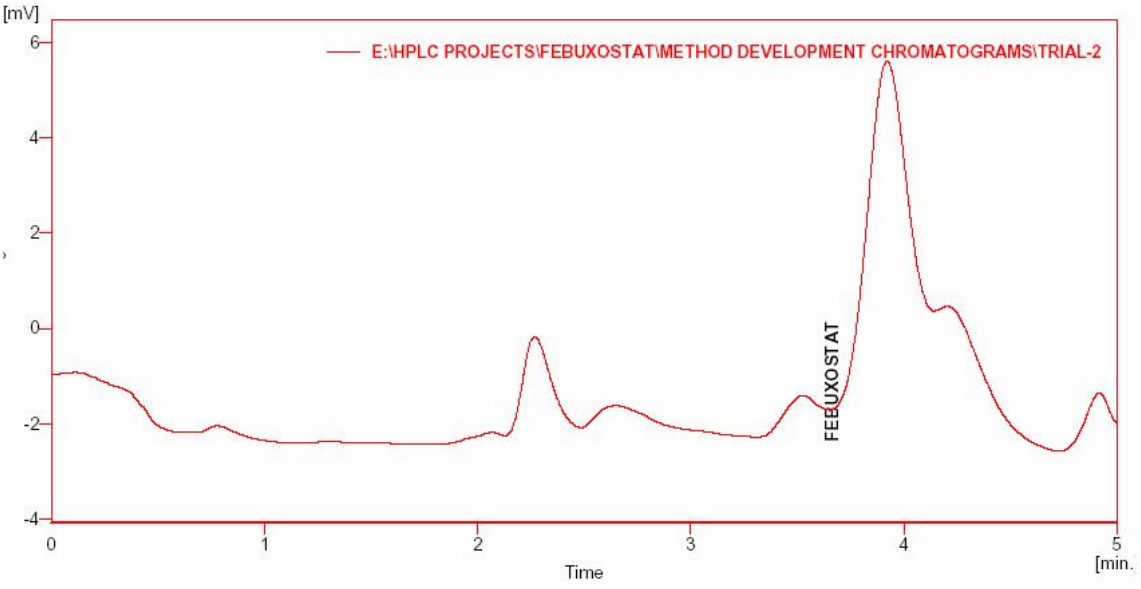


Figure 5: Chromatogram of Trial – 3

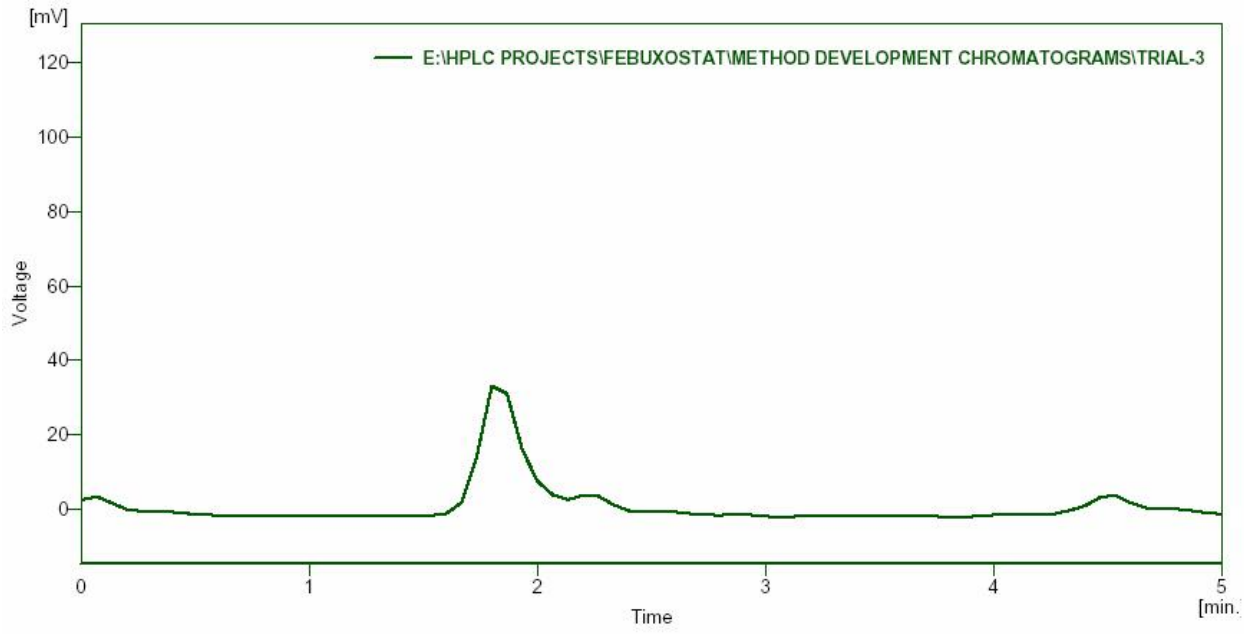


Figure 6: Chromatogram of Trial – 4

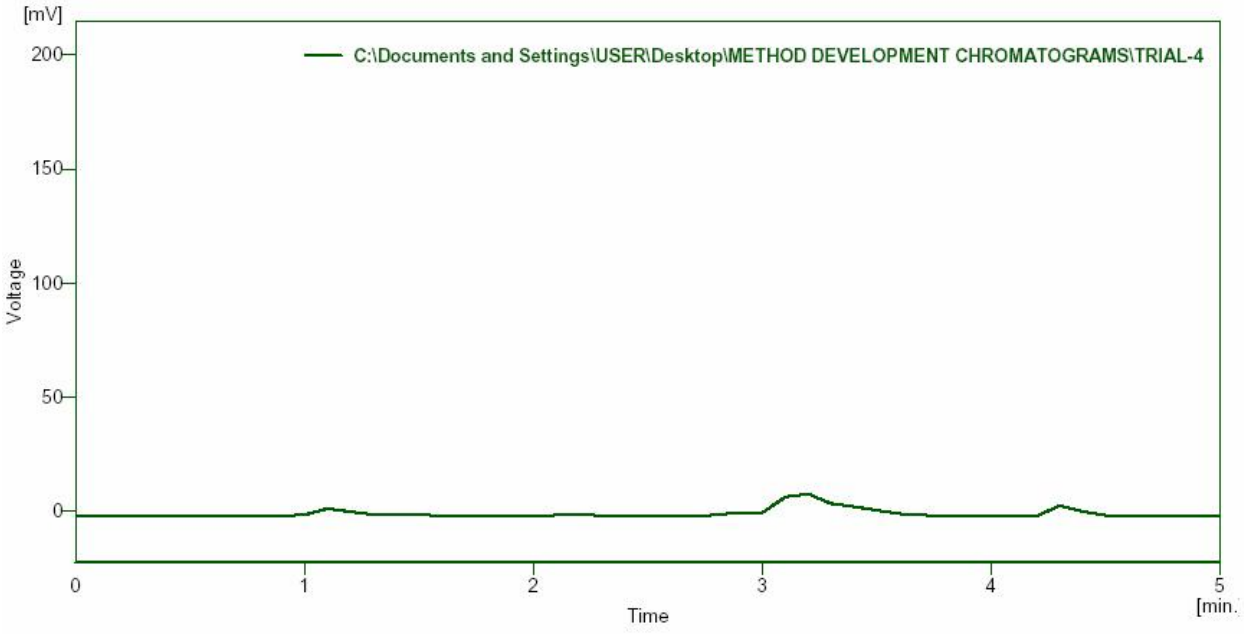


Figure 7: Chromatogram of Trial – 5

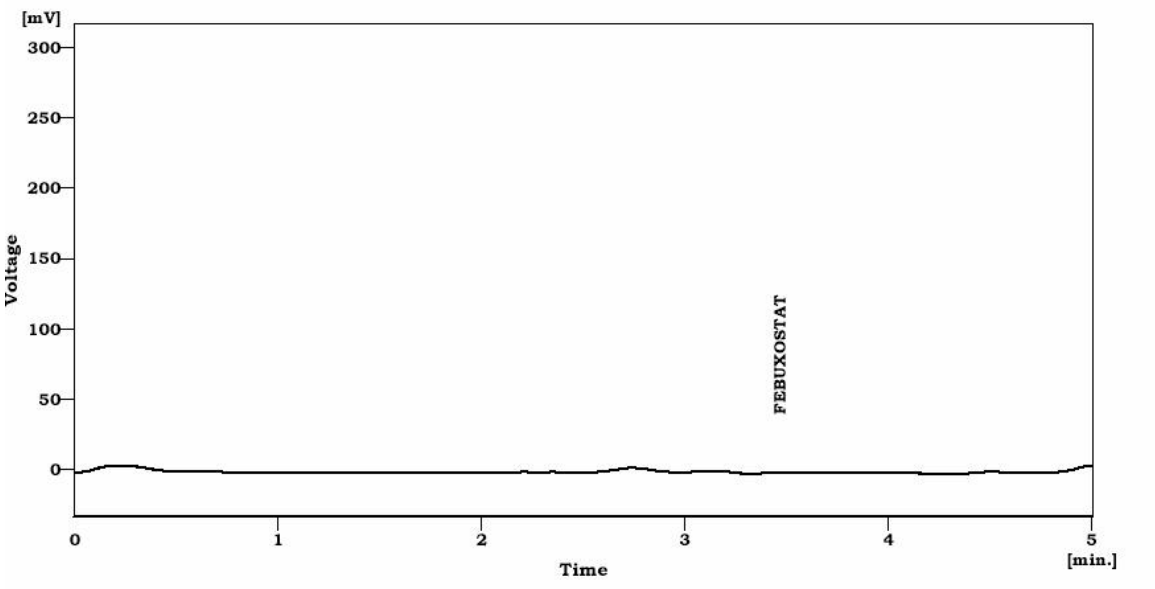


Figure 8: Chromatogram of Trial – 6

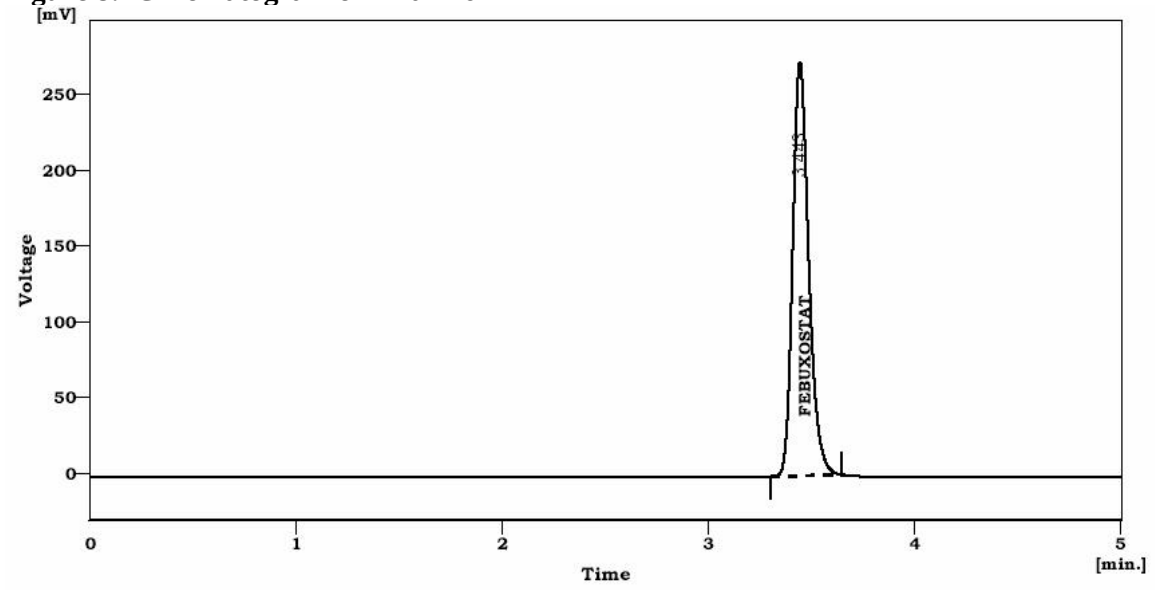


Table 2. System Suitability (Concentration 200 µg/mL)

	Retention Tine (in min.)	**Peak area(mV.s)**
	3.45	3204.188
	3.45	3189.142
	3.45	3109.923
	3.45	3219.218
	3.45	3162.849
	3.45	3242.353
Mean	**3.45**	**3208.14**
SD	**0.00**	**40.89**
%RSD	**0.00**	**1.27**

Table 3. Recovery of febuxostat using the proposed HPLC method

Sl. No.	Amount of drug added (µg/mL)	Mean (± SD)* amount found (µg/ml)	Mean (± SD)* % of recovery
1.	50	148.47 ± 0.74	98.98 ± 0.74
2.	100	199.07 ± 0.18	99.43 ± 0.18
3.	150	248.77 ± 0.44	99.51 ± 0.44

* Average of three determinations

Table 4. Intra and inter day precision for febuxostat assay in pharmaceutical dosage forms by the proposed HPLC method

Concentration	Intraday variation *		Interday variation *	
	Area Mean	%RSD	Area Mean	%RSD
300µg / ml	4213.324	1.070	4220.960	0.907

* Averages of six determinations

Table 5. Calibration Values for Febuxostat

Concentration (µg/mL)	Peak area(mV.s)	Retention time (in min.)
0	0	0
50	753.815	3.450
100	1474.857	3.443
150	2219.500	3.390
200	3247.479	3.450
250	4035.424	3.410
300	4244.826	3.413
350	5150.732	3.440
400	5883.203	3.383

Validation[17]

The described method has been validated for the assay of febuxostat using following parameters.

Accuracy

Accuracy of the method is demonstrated at three different concentration levels (50 – 150 %) by spiking a known quantity of febuxostat into a previously analyzed sample (100µg /ml) in triplicate. The results of accuracy (Table 3) revealed that the method is more accurate.

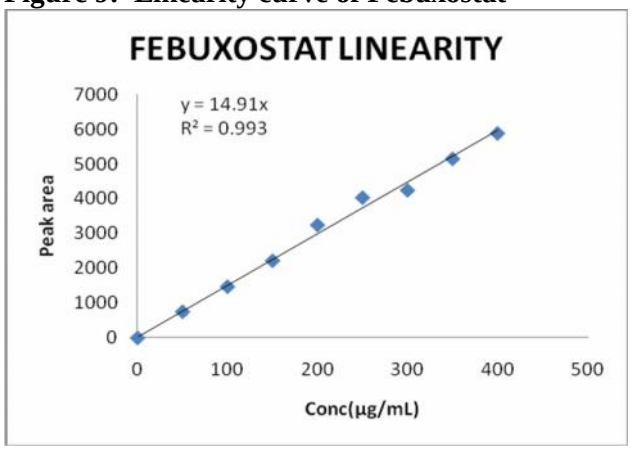
Precision

For the precision of the method, three replicates are injected into the system on two different non consecutive days and results are reported in terms of relative standard deviation in Table 4.

Linearity

To establish linearity of the proposed method, drug solutions of eight separate concentrations are prepared by transferring different aliquots of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 ml of standard stock-A solution of Febuxostat into a series of 10 ml standard volumetric flasks and are made up to the mark with mobile phase. The standard solutions prepared as above are filtered through 0.45 µ membrane filter. After stabilization for 30 min all the standard solutions are injected separately using rheodyne injector and the chromatograms are recorded. Standard curve is constructed in the concentration range of 50 – 400 µg / ml from which slope, intercept and the correlation coefficient are determined.

Figure 9: Linearity curve of Febuxostat



Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) for febuxostat are calculated for the sensitivity of the method. LOD and LOQ are calculated according to the ICH guidelines by using relative standard deviation of the response (S) and slope of the calibration curve (σ) for febuxostat.

$LOD = 3.3 \times \sigma / S$

$LOQ = 10 \times \sigma / S$

The LOD of a compound is defined as the lowest concentration of analyte that can be detected. LOD value is found to be 9.98 µg /ml for febuxostat. The limit of quantification is the lowest concentration of a compound that can be quantified with acceptable precision and accuracy. LOQ value is found to be 30.23 µg /ml for febuxostat.

Robustness and Ruggedness

In order to demonstrate the robustness of the method, system suitability parameters are verified by making deliberate changes in the chromatographic conditions, viz. change in flow rate by ± 0.05 ml / min, change in pH of the buffer by ± 0.1 unit and change in the ratio of mobile phase (± 2% absolute). The method is demonstrated to be robust over an acceptable working range of its HPLC operational parameters. Ruggedness is being determined by the varying the analyst, instrument and different column of different grades. The relative standard deviation of the results obtained from different analysts instruments is < 1.0 %. To ascertain the system suitability for the proposed method a number of statistical values such as theoretical plates, HETP, peak asymmetry, have been calculated with the observed readings and the results complies with in specification limits.

Conclusion

The proposed method is found to be simple, fast, robust, more precise and accurate under the present experimental conditions. Therefore the developed method can be used for routine analysis for estimation of febuxostat in bulk forms.

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