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Development and Validation of Reverse Phase-Ultra Performance Liquid Chromatographic Method for Estimation of Related Substances in Febuxostat Drug Substance

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Abstract

A novel high speed, high resolution Reverse phase-UPLC method was developed for the quantification of related substances in Febuxostat drug substance. The separation of drug from the possible impurities was achieved on a Halo C18 column. The innovative approach of using stationary phase with sub 2 µ particles provides a comprehensive combination of selectivity and speed. 10 mM mono basic potassium phosphate buffer at pH 2.7 and acetonitrile mixture was selected as mobile phase. Flow rate and detection were kept at 0.8 mL/min and 320 nm respectively. The developed UPLC method was subjected to validation parameters. System precision, accuracy, specificity, limit of detection, limit of quantification and linearity were established as per the guidelines recommended by ICH. Stability indicating nature of the method was also performed by exposing the sample under various conditions like acid, base, peroxide and photo stability exposures. Total analysis run time 7.0 minutes indicates the speed and cost saving initiation of the developed method. Using the method one can carry out the quantitative estimation of related substances in Febuxostat drug substance, further the same method can be adopted for determination of drug substance assay also.

Keywords: Febuxostat; Ultra performance liquid chromatography; Hyperuricemia with gout; Related compounds; Xanthine oxidase inhibitor

Introduction

Febuxostat is a xanthine oxidase inhibitor indicated for the chronic management of hyperuricemia in patients with gout [1]. The recommended starting dose of drug is 40 mg once daily. For patients who do not achieve a serum uric acid less than 6 mg per dL after 2 weeks with 40 mg, Uloric 80 mg is recommended. The primary mode of action of a xanthine oxidase inhibitor, achieves its therapeutic effect by decreasing serum uric acid.The objective of this work was to develop a cost effective ultra fast reverse phase UPLC method, the developed method was validated as per regulatory guidelines and transfer the method to quality control lab for analysis of related substances in Febuxostat. As per the literature Febuxostat Determination was done by spectrophotometric method [2] and RP-HPLC [3,4] methods. LC-MS/MS [5-7] assay method was proposed for bioequivalence and pharmacokinetics evaluation. Since this drug is being marketed in domestic and international market the present investigation by the author was to develop a rapid, accurate and precise RP-UPLC method [8-10] for the determination of related substances. The aim of this paper is to develop a cost effective and fast method. The run time for the newly developed method was 7.0 minutes. The innovative approach of using stationary phase with sub 2 µ particles [11-14] provides a comprehensive combination of selectivity and speed. The validation parameters [15,16] provide valuable information on precision accuracy, limit of detection, limit of quantification and linearity of related substances. The method was subjected to validation according to ICH requirements [17,18] (Figure 1).

Materials and Methods

Instrumentation and reagents

Test samples and reference standards of Febuxostat were donated by Apotex India pvt ltd. Acetonitrile was purchased from Ultra Scan Ltd. Ultra performance liquid chromatography from Waters with gradient elution. MilliQ purification system was used to get HPLC grade water. Halo C18, 100 x 2.1 mm 2.7 µm column purchased from advanced materials technology.

Chromatographic conditions

The chromatographic separation was achieved on a Halo C18, 100 x 2.1 mm 2.7 µm Column. Mobile phase consists of 10 mM monobasic phosphate buffer with 0.2% Triethyl amine and pH of the solution was adjusted to 2.7. HPLC grade acetonitrile was used as organic modifier. Mobile phase flow rate was kept at 0.8 mL/min. Gradient program was set as Time/% of solution B: 0/40, 3/40, 3.5/70, 4/75, 5/80, 5.5/40, 7/40. Column temperature was maintained at 50°C and detection was carried at 320 nm. Sample compartment was maintained at 10°C with an injection volume of 1 µL.

Preparation of standard and sample

A mixture of standard solution was prepared by weighing febuxostat and its related compounds to yield a final concentration of 0.10% of febuxostat and 0.15% of each Impurity A, Impurity B, Impurity C and Impurity D with respect to the sample concentration

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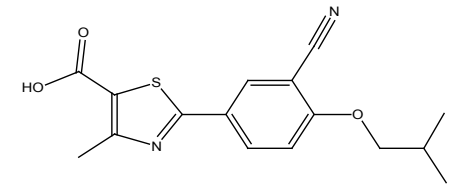
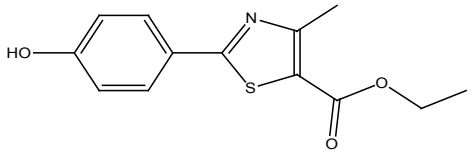


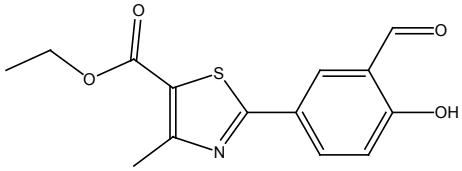
2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid

(a) Febuxostat



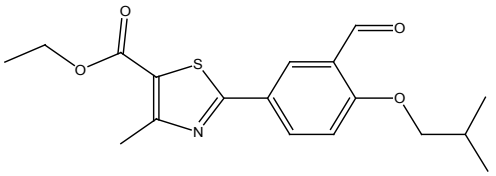
Ethyl 2-(4-hydroxy phenyl)-4-methylthiazole-5-carboxylate

(b) Febuxostat Impurity A



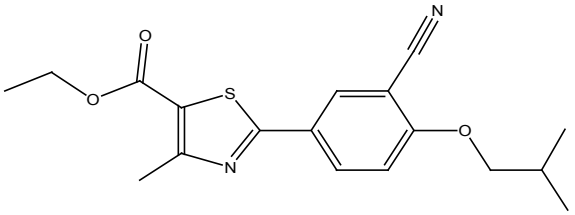
Ethyl 2-(3-formyl-4-hydroxyphenyl)-4-methylthiazole-5-carboxylate

(c) Febuxostat Impurity B



Ethyl 2-(3-formyl-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylate

(d) Febuxostat Impurity C



Ethyl 2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylate

(e) Febuxostat Impurity D

Figure 1: Chemical structures of febuxostat and its related substances.

of 0.5 mg/ml. Buffer and acetonitrile in the ratio 1:4 was used as the diluent for preparation of sample and standard solution.

Method validation

Specificity: Specificity is the ability of method to measure the analyte response in the presence of its potential impurities. The specificity of the developed RP-UPLC method was demonstrated in presence of sample diluent and its four potential impurities.

System suitability: To ensure system suitability, a standard solution was injected on to the system and verified spectral purity of individual peaks to ensure that no co elution has occurred. Tailing factor (T) column efficiency (N) and resolution (R) were calculated for febuxostat and its related substances.

Precision: Precision of the method was reported by injecting six replicates of standard solution consecutively under the same analytical conditions. The %RSD of individual peaks was calculated. Intermediate precision of the method was also evaluated using different analyst, different day and different make of instrument in the same laboratory.

Limit of detection (LOD) and limit of quantification (LOQ): The LOD and LOQ for Febuxostat and its related compounds were determined by injecting series of diluted impurity standard solution to a level such that % RSD was not more than 10% at LOQ level. Precision carried out at LOQ level by injecting six individual preparations and calculated the % RSD.

Linearity: Linearity for the related substances method was prepared by serially diluting the impurity stock solution to required concentration levels. The solutions were prepared at five different concentration levels ranging from LOQ to 160% with respect to specification limits. The calibration curve was drawn by plotting the peak areas of impurities versus its corresponding concentrations. Correlation coefficient of the calibration curve, slope and relative response factors were reported.

Accuracy: Febuxostat sample solution was spiked with impurity standard solutions at three concentration levels corresponding to LOQ, 100% and 160% of impurity concentration. The % recovery was calculated.

Results and Discussion

Method development and optimization

Febuxostat and related substances are closely related structures so the main target of the method was to have more specific method to achieve the separation between all impurities from the drug substance effectively and to support routine quality check at competitive time period. The wavelength of detection was selected at 320 nm as all the related impurities and febuxostat shown maximum absorbance at selected wavelength. Resolution and peak symmetry were good in

Halo C18. A simple gradient was selected to resolve all the identified impurities in the standard solution and to eliminate interference with diluent and unidentified peaks from sample.

System suitability results

The peak shape of febuxostat drug substance was found symmetric and well separated by its potential process impurities. A typical system suitability chromatogram of sample diluent, standard solution and test solution chromatograms are shown in Figure 2a-2c.

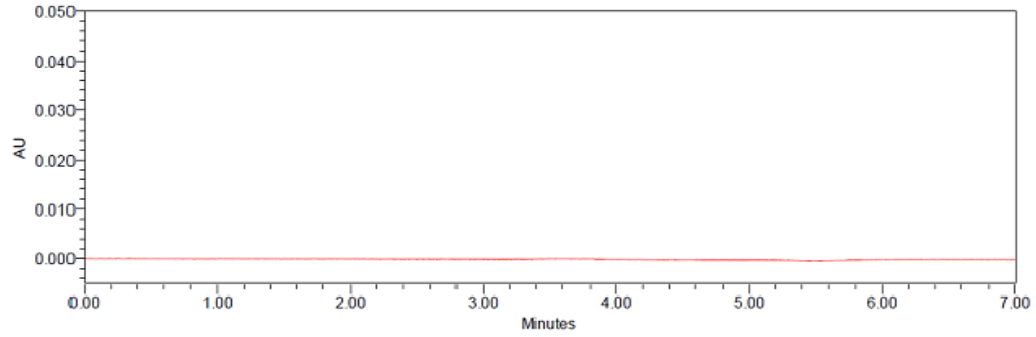
In the optimized conditions, febuxostat and its related substances were well resolved with a resolution of more than 2.0.The tailing factor is in the range of 1.0-1.2 which indicates symmetry of peaks. Theoretical plates more than 10000 show the efficiency of the column. System suitability parameters for febuxostat and its related substances are tabulated in Table 1.

Method validation results

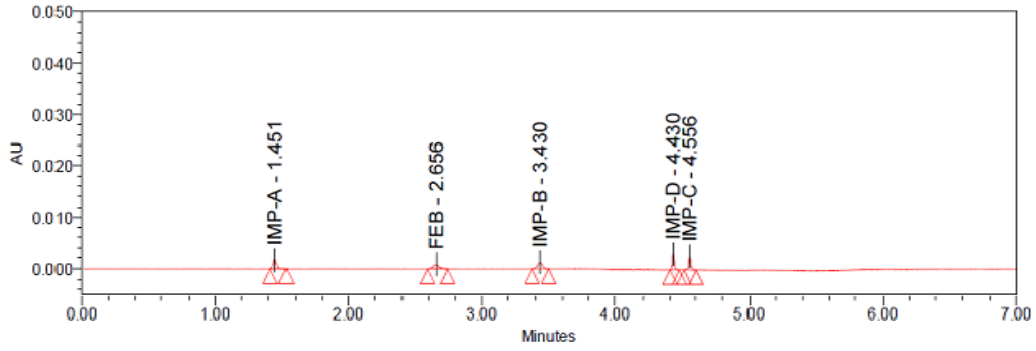
Precision: System precision was evaluated by performing six replicate injections for the standard solution at specification level. The % relative standard deviation of 6 injections was within the acceptable limit.

The obtained results are in the range of 1.4-3.2 which indicates the precision of the system to proceed for analysis. Results are tabulated in Table 2.

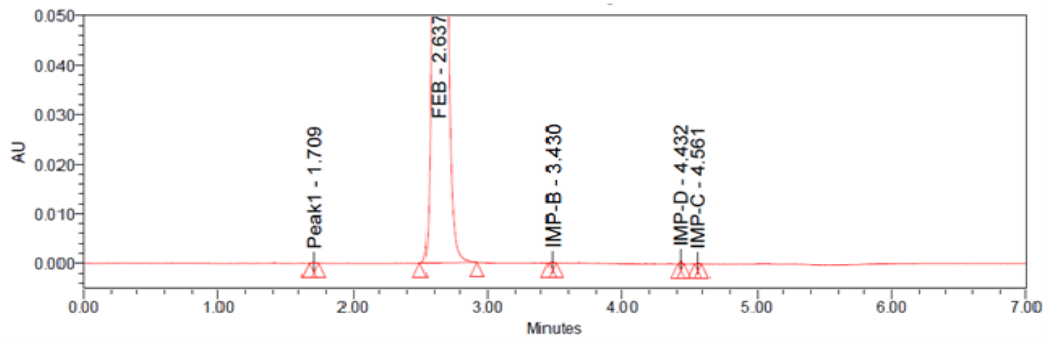
Limit of detection (LOD) and limit of quantification (LOQ): Data obtained for limit of detection (LOD) and limit of quantification (LOQ) of febuxostat drug substance and its related substances are tabulated in Table 3. Sensitivity is the ability of method to detect and quantify the impurities present in the sample accurately. %RSD for area count of 6 consecutive injections should not be more than 10.0% at



a) Sample diluent



b) Standard solution



c) Test solution

Figure 2: A typical chromatogram showing the febuxostat (a) sample diluent (b) Standard solution

Component	RT	Resolution	Tailing factor	Plate count	Peak angle	Peak threshold
Impurity A	1.451	-	1.2	10800	3.118	5.232
Febuxostat	2.656	15.8	1.0	13293	5.523	9.151
Impurity B	3.430	9.2	1.0	48116	4.140	6.438
Impurity D	4.430	20.3	1.2	387969	3.243	3.632
Impurity C	4.556	4.1	1.1	341520	2.903	4.296

Table 1: System suitability results.

Inj.#	Febuxostat	Impurity A	Impurity B	Impurity C	Impurity D	Criteria
1	2811	3659	3386	3195	3367	NMT 5%
2	2933	3503	3431	3089	3443	
3	2957	3538	3311	3142	3591	
4	3075	3601	3211	3091	3467	
5	2865	3340	3449	3131	3380	
6	2964	3463	3281	3171	3348	
Mean	2934	3517	3345	3136	3433	
SD	90.93	111.56	92.71	42.51	90.33	
%RSD	**3.1**	**3.2**	**2.8**	**1.4**	**2.6**	

Table 2: System precision results.

Inj.#	Febuxostat	Impurity A	Impurity B	Impurity C	Impurity D
LOQ (µg/ml)	0.404	0.426	0.420	0.424	0.428
LOD (µg/ml)	0.13	0.142	0.140	0.141	0.142

LOQ-Limit of Quantitation; LOD-Limit of Detection

Table 3: Limit of quantitaion and detection.

Level	Febuxostat		Impurity A		Impurity B		Impurity C		Impurity D	
	Conc. (µg/mL)	Area (µV* sec)	Conc. (µg/mL)	Area (µV* sec)	Conc. (µg/mL)	Area (µV* sec)	Conc. (µg/mL)	Area (µV* sec)	Conc. (µg/mL)	Area (µV* sec)
LOQ	0.4040	1237	0.4256	1310	0.4200	1281	0.4240	1197	0.4280	1365
80%	0.8080	2453	0.8512	2692	0.8400	2708	0.8480	2360	0.8560	2744
100%	1.0100	3143	1.0640	3712	1.0500	3532	1.0600	3185	1.0700	3383
120%	1.2120	3576	1.2768	4270	1.2600	4242	1.2720	3940	1.2840	4027
160%	1.6160	4749	1.7024	5524	1.6800	5296	1.6960	5073	1.7120	5437
Slope	2886		3341		3233		3115		3154	
R^2	0.9990		0.9960		0.9960		0.9980		1.0000	
RRF	1.00		0.86		0.89		0.93		0.92	

RRF-Relative Response factor, R^2- Correlation Coefficient

Table 4: Linearity results.

LOQ level. The Accuracy at LOQ level should be in the range of 80 to 120%. The Precision at LOQ level and accuracy at LOQ level were found to be within the specified limits.

Linearity: Linearity of the method is to establish a linear relationship of concentration against response. Solutions of febuxostat and its related substances are prepared from LOQ level to 160% of the specification limit. The correlation coefficient obtained was greater than 0.99. The regression statistics for febuxostat drug substance and its related substances are tabulated in Table 4.

The result shows that an excellent correlation existed between the peak response and concentration of the analyte and impurities. Relative response factor is established to estimate the factor of impurity response against drug substance response to calculate the content of impurity present in the drug substance.

Accuracy: Accuracy of the method can be determined by spiking known concentrations of standard solution to the sample. The obtained recovery value indicates the trueness of the method to estimate impurities. Related substances of febuxostat spiked to the sample over a concentration range varying from QL to 160% of their respective target analyte concentrations. The Acceptance criteria for the accuracy are 80% to 120%.

The obtained percentage recovery value of related substances is in the range of 95.2 % to 102.3% which declares the method accuracy. Accuracy results are reported in Table 5.

Forced degradation study: Degradation studies were performed to demonstrate stability indicating nature of the method. Febuxostat test sample was exposed to various stress conditions like heat and humidity (40°C and 70% RH for 7 days), thermal (60°C for 7 days) and photolytic conditions of fluorescent light (1.2 x 10^6 LUX hours), UV light for a total exposure of 200 W·hr/m^2, acid hydrolysis (0.1 N HCl 80°C for 24 Hrs), base hydrolysis (0.1 N NaOH, 80°C for 24 Hrs) and oxidative stress. Testing of peak purity concludes the homogeneity and interference of unidentified impurities with peak of interest. The obtained peak purity value gives a clear indication of separation between stressed impurities with related substances of febuxostat.

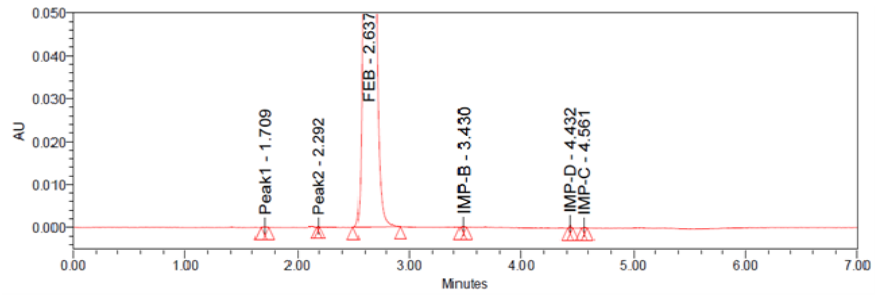
Peak obtained in all the stress conditions was homogenous and

S.NO	Accuracy Level	Impurity A	Impurity B	Impurity C	Impurity D	Criteria
1	LOQ	102.4%	87.5%	105.2%	101.3%	80% to 120%
2	100%	100.5%	85.6%	103.1%	100.0%	
3	160%	101.8%	95.2%	102.3%	99.4%	

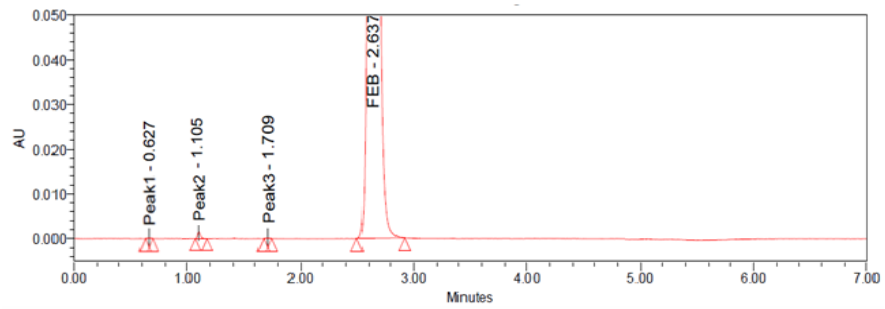
Table 5: Accuracy results.

Stress condition	Conc. µg/mL	Match angle	Match threshold	Purity angle	Purity Threshold	Mass balance
Non stressed	504	2.880	10.550	0.075	10.123	100
Acid hydrolysis	520	2.542	10.235	0.012	10.225	99.8
Base hydrolysis	510	2.325	10.835	0.085	10.522	98.9
Oxidation	530	1.987	11.123	0.023	10.995	99.9
Heat and humidity	525	1.897	11.556	0.093	10.789	99.9
Photo stability	515	2.552	11.005	0.075	10.123	99.9
Dry heat	518	2.342	11.123	0.092	10.889	99.8

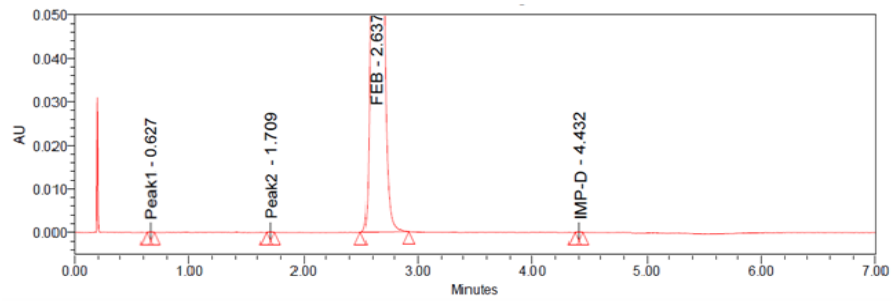
Table 6: Forced degradation studies.



a) Acid Hydrolysis



b) Base Hydrolysis



c) Oxidative degradation

Figure 3: A typical chromatogram showing the febuxostat (a) Acid hydrolysis (b) Base Hydrolysis (c) Oxidative degradation.

unaffected by the presence of its degradation impurities, confirming the stability indicating nature of the method. Mass balance also established to match up the sum of impurities with its assay value against reference unstressed sample. The results from forced degradation studies are summarized in Table 6. Figure 3a-3c indicates stressed sample chromatograms under acidic, basic and peroxide conditions.

Conclusion

A fast Reverse phase-UPLC method was developed for the

estimation of related substances in Febuxostat drug substance. The developed method was subjected to method validation parameters as recommended by ICH. Stability indicating nature of the method is also established by assessing forced degradation studies. The shorter run time demonstrates that the method is cost effective, time effective and even uses lesser quantity of solvents aiming towards green chemistry. The developed method was specific, precise, accurate and linear to estimate accurate amount of impurities present in the sample. Degradation studies confirmed the homogeneity and free of interferences with the peak of interest.

The method can be adopted to determine the related substances of drug substance in quality control labs. The same procedure can also be used to perform assay of drug substance.

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