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A Stability Indicating RP-UPLC Method for Estimation of Febuxostat and its Impurities in Bulk Drugs and Pharmaceutical Dosage forms.

Srihari Molleti*[1], Vinay Rao[2], K. N. Jayaveera[3]

1. Daewoong Pharmaceuticals India Private limited, Balanagar, Hyderabad India.

2. Malla reddy college of pharmacy, Hyderabad

3. Department of chemistry, JNT University, Anantapur. India.

ABSTRACT

This study is aimed at Developing and validating an UPLC method for febuxostat and its related substances that might coexist in bulk drugs and its tablet formulations as impurities that may originate from synthesis process or degradation. A chromatographic system consisting Waters Acquity UPLC HSS C18 (1.8 µm) column, mobile phase of ammonium acetate with pH 4.5 as Buffer phase and Acetonitrile: Methanol in 1:1 ratio as organic phase, with gradient elution at flow of 0.4 mL/min and UV detector set at 315 nm has shown a good chromatographic separation for Febuxostat and its related substances. The developed method was validated as per ICH Guidelines and shown equivalency with API Vendor method. The developed UPLC method has run time of only 10 minutes making the method productive and may be applied for Quality control Testing.

Keywords: Febuxostat, Stability indicating, RP-UPLC, Equivalency.

*Corresponding Author Email: m.hyderabad@gmail.com

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INTRODUCTION

Febuxostat, anti gout agent is chemically 2- [3- cyano-4- (2- methyl propoxy) phenyl]- 4- methyl thiazole- 5 - carboxylic acid. 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].

Ultra performance liquid chromatography (UPLC) is a new category of separation science which builds upon well established principles of liquid chromatography, using sub 2 µm porous particles. These particles operate at elevated mobile phase velocities to produce rapid separations with increased sensitivity and increased resolution. Thus UPLC technology allows analysts time to be drastically reduced while still meeting assay acceptance criteria based on plate count, resolution and analyte retention.

Extensive literature survey revealed that, Ultra performance liquid chromatography method was not reported for the febuxostat and its related substances in its Bulk drugs and its formulations. The aim of the study is to develop a simple, sensitive, accurate, precise and cost effective method for determination of febuxostat and its related substances in bulk drugs and pharmaceutical formulations within 10 minutes.

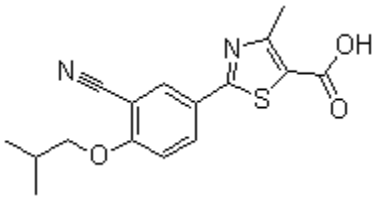

Figure1: Febuxostat chemical structure

MATERIALS AND METHODS:

Instruments:

A Waters acquity ultra performance liquid chromatography equipped with PDA Detector with Binary pump. The column utilised was Acquity UPLC HSS C-18, 2.1x100mm, 1.8µm.

Chemicals:

All the chemicals used were of pharmaceutical grade. Acetonitrile and methanol are chromatographic grade and extra pure ammonium acetate and glacial acetic acid were from Merck. Febuxostat and its impurities were obtained from unimark remedies limited. Uloric tablets were obtained from Takeda pharmaceuticals, America.

The compounds related to febuxostat which could be expected as impurities or might appear as degradation products have been prepared and identified by unimark remedies, vapi, India.

1) Di acid impurity: 2-(3-carboxy-4-isobutoxy phenyl)-4 methyl-1, 3-thiazolo-5-carboxylic acid.

2) Acid amide impurity: 2-(3-carbomoyl-4-isobutoxy phenyl))-4-methyl-1, 3-thiazole-5-carboxylic acid.

3) N-propyl impurity: 2-(3-cyano-4-propoxy phenyl)-4-methyl-1, 3-thiazole-5-carboxylic acid.

4) Sec-butyl impurity: 2-(4-sec-butoxy-3-cyano-phenyl)-4-methyl-1, 3-thiazole-5-carboxylic acid.

5) Des cyano impurity: 2-(4-iso butoxy phenyl)-4-methyl-1, 3-thiazole-3-carboxylic acid.

6) Nitrile impurity: Ethyl-2-(3-cyano-4-iso butoxy phenyl)-4-methyl-1, 3, thiazole-5-carboxylate.

Developing an UPLC Method:

The UPLC method carried out in this study aimed at developing a chromatographic system capable of eluting and resolving febuxostat and its impurities (related substances) from one another and that complies with the general requirements for system suitability.

The preliminary investigations were directed to-wards the effect of various variables on the system suitability of the method. The parameters assessed include the detection wavelength, the type and quantity of the organic modifier, the column, the salt concentration and the pH of the mobile phase.

Febuxostat showed two UV-absorption maxima at 216-315 nm. The 315-nm wavelength showed a better resolution between the chromatographic peaks of febuxostat and its impurities and the absorption measurements at this wavelength were of optimal values for febuxostat and its impurities.

The first trial was carried out by using reversed phase C18column (BEH,C-18,2.1x100mm) and a mixture of acetonitrile and 0.01 M potassium di hydrogen phosphate (7:3, v/v).This system was found to be suitable to elute febuxostat but the retention time was short and the resolution between febuxostat and its impurities was low. As a means to increase the resolution, methanol was added to acetonitrile in organic phase.

The effect of pH on the elution was checked, at the lower pH (2 to3) the retention times and the resolutions are low, at higher pH (6 to7) the retention times and resolution is increased, hence the optimal pH 4.5 was selected.

When the Acquity BEH C18 column of 100mm was then replaced by HSS C18 column of 100mm, the retention times are reduced with better resolution. Changing the buffer from

potassium phosphate to ammonium acetate had resulted in an enhancement to the peak symmetry and resolution.

Consequently, the optimum chromatographic conditions mentioned previously were applied for all measurements. Figure 2 shows the significant separation of febuxostat and its related compounds using the optimised chromatographic system.

Finalized conditions:

Gradient elution technique was utilized with the column maintaining at 45°C, The Buffer phase used was ammonium acetate(0.77 grams per 1000mL), and pH adjusted to 4.5 with glacial acetic acid and organic phase was acetonitrile and methanol in 1:1 ratio with the following gradient programme. The flow rate was 0.4 mL/min. Samples of 1µL was injected into the column and the detector was set at 315 nm with run time of 10 minutes. The relative standard deviation (R.S.D.) of six replicate injections of the standard preparation was not greater than 2.0% for assay standard and 5.0% for impurities diluted standard. The tailing factor was less than 2.0 in both assay and impurities.

Table 1: Gradient programme

Time(min)	Flow(mL/min)	%A	%B
0.01	0.4	75	25
2.00	0.4	70	30
5.00	0.4	45	55
7.00	0.4	10	90
8.00	0.4	75	25
10.00	0.4	75	25

Preparation of solutions:

Preparation of standard solution

An accurately weighed quantity of febuxostat and its impurities (Impurities 1, 2,3,4,5 and 6) was dissolved in the diluent (Water and acetonitrile in 2:8ratio) and diluted quantitatively. Serial dilutions were carried out, using the diluent, to obtain solutions of known concentrations to be used for the standard reparation 80 ppm for assay and 10 ppm for febuxostat diluted standard in impurities.

Preparation of Test solutions (Assay):

Accurately weighed transferred 5 No's of 80 mg febuxostat tablets into 200 ml volumetric flask and added 100 mL of diluent and disintegrated. The solutions were sonicated for 30 min and made up to the volume with diluent, centrifuged at 4000 rpm for 10 min and the supernatant was used to as a test solution for related substances test, the filtered solution diluting 2 mL to 50 mL used for Assay and .

Preparation of Test solutions (Impurities):

Twenty tablets were powdered and accurately weighed portions equivalent to 50 mg Febuxostat was transferred to 50 ml volumetric flasks. 80 mL was added and the solutions were sonicated and centrifuged as above and the supernatant was used as related substances test solution with 1.0 mg Febuxostat per mL.

Quantification:

Equal volumes, (1µL), of the standard preparations and the test preparations that contain Febuxostat and its related substances were injected into the chromatograph and the chromatograms were recorded. The responses (peak area) for the major peaks were measured and the quantity of febuxostat or related substance was calculated from the equation Cs (Au / As) where Au and As are the areas under the corresponding peaks and Cs is the concentration of Febuxostat and its related substance in the standard solution.

Method validation

The method validation was performed as per ICH Guidelines[12].

Linearity, Limit of detection, Limit of quantification

The degree of linearity was assessed by the correlation coefficient, y-intercept, and slope. The limit of detection, LOD and the limit of quantitation LOQ have been estimated for related substances as 3 S.D. and 10 S.D. of the y intercept and slope.

Precision

The precision was performed by preparing six individual preparations as per the method of analysis and evaluated for percentage of Febuxostat and its percentage of individual and total impurities.

Accuracy

The samples were prepared by spiking the Febuxostat and its impurities stock solutions into the Placebo mixture and the percent recovery was estimated.

Solution stability

The solutions prepared was tested at initial, 24hrs and 48Hrs by maintaining at room temperature and estimated for Febuxostat and its impurity content.

Robustness

Robustness was conducted by making the variations in flow rate, Column oven temperature.

Ruggedness

The prepared solutions were filtered through 0.45 µ PVDF syringe filter and 0.45 µ PVDF syringe filter and evaluated against the centrifuged sample.

Intermediate precision

The test was performed with another analyst on different day, different system and different column and the impurity contents were reported.

Forced degradation studies

The forced degradation studies conditions and % degradation s mentioned in the results (Table: 6) section.

Study for Uneluted peaks:

Since the runtimes are lower, a study conducted on all the stressed samples for knowing the retained peaks by increasing the acetonitrile to 90% till 15 minutes.

Equivalency with the API Vendor HPLC method:

The developed UPLC method was tested for equivalency with API Vendor method in three steps.

System suitability equivalence:

The System suitability parameters in the API Vendor method and develop method are compared with the obtained values.

API Analysis equivalence:

The results obtained with the Same API batch analysis with the API Vendor method and the developed method, results were discussed.

Reference Product analysis equivalence:

The results obtained with the Same Uloric tablet batch analysis (Kept on 40/75 for 6M) with the API Vendor method and the developed method, results were discussed.

RESULTS AND DISCUSSIONS

The impurity mix, Blank, Placebo chromatograms was obtained after finalization of method was as below

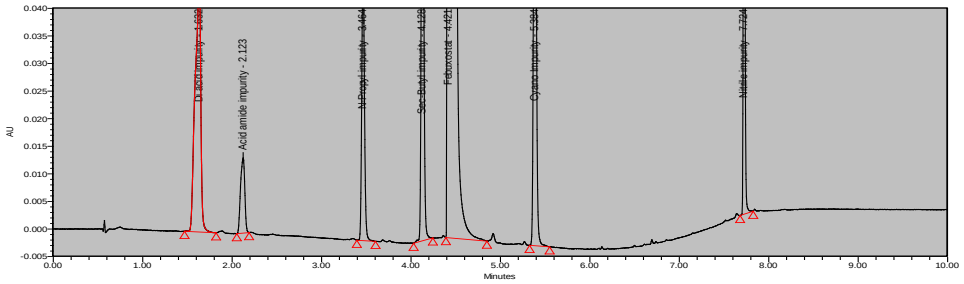

Figure 2: Impurities spiked sample chromatogram.

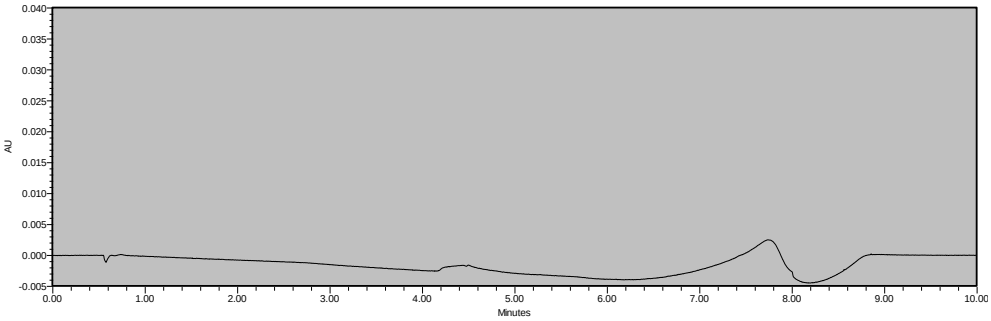

Figure 3: Blank chromatogram.

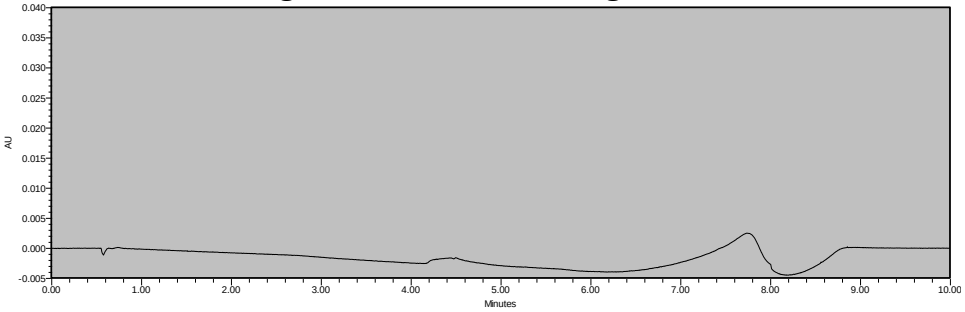

Figure 4: Placebo chromatogram.

Assay method validation results:

Specificity:

Blank interference:

The diluent was injected as a blank; it was found that there was no interference observed in blank preparation with the Febuxostat peak.

Placebo interference:

With the equivalent weight of sample the placebo preparation was prepared and injected into the system and interference checked, it was found that there was no interference observed in placebo preparation with the Febuxostat peak.

Impurity interference:

Impurity solution was prepared at 5 % level of test concentration and injected into the system and checked for the interference, it was found that impurities are not interfering with the Febuxostst peak.

Linearity:

The linearity was performed at 6 levels of the targeted 80 ppm (25%, 50%, 80%, 100%, 200%, 300% levels) and the area results are plotted against the concentration, the correlation coefficient observed was 0.9999.

Precision:

By following the procedure in 2.5.2 section, six sample preparations are prepared and calculated

the assay values and the Percent relative standard deviation was 0.4 shows that the method was precise as per the ICH limits.

Accuracy:

The accuracy was performed in triplicate by spiking the Febuxostat into the placebo mixture at 50%, 100%, and 200% 300% of test concentration; from the area recovery values are calculated. The average recovery values are obtained within 99 to 101% shows that the method was accurate as per the ICH limits.

Solution stability:

The first three solutions prepared in the 2.5.2 section were checked for Febuxostat content at 24 and 48 hours by keeping it in closed container at room temperature, the variation from the initial value was 0.31% at 24 Hours and 0.42% at 48 hours, the results are within 0.5% shows the solution was stable for 48 hours.

Robustness:

To check the effect of deliberate changes in the method, the variation inflow rate (±0.05 mL) and variation in temperature (± 5ºC) are studied; result shows no effect on the method.

Ruggedness:

From the stock solutions, the solution were prepared by filtering through PVDF & PTFE 0.45 µm filter papers and the content of Febuxostst was tested. The results obtained are within 0.5%.

Table 2: Validation results of Febuxostat in assay method

Parameter	Results	
Specificity	Blank interference, Placebo interference, Impurity interference was nil.	
Linearity	Established from 25% to 300% (R^2 value=0.9999)	
Precision:	% RSD of impurity for six preparations= 0.4	
Accuracy	**%Level**	**%Recovery**
	50%	99.7
	100%	100.3
	200%	99.9
	300%	99.3
Solution stability	1)% Difference at 24 Hrs=0.31% 2)%Difference at 48 Hrs=0.42%	
Robustness	Flow rate variation-System suitability passes Temperature variation system suitability passes	
Ruggedness	Filter validation: Variation between PVDF &PTFE 0.45 micron filters=0.32%	
Intermediate precision	% Assay variation=0.11%	

Intermediate precision:

Assay was performed by another analyst on different day, different system, and different

column; the variation between the two analysts was less that 0.2 %, it shows that method was reproducible.

Impurity method validation results:

Specificity:

Blank interference:

The diluent was injected as a blank; it was found that there was no interference observed in blank preparation with the Impurity peaks.

Placebo interference:

With the equivalent weight of sample the placebo preparation was prepared and injected into the system and interference checked, it was found that there was no interference observed in placebo preparation with the Impurity peaks.

Impurity interference:

Impurity solution was prepared at 5 % level with the Febuxostat API at test concentration and injected into the system and checked for the interference, it was found all the impurities are separated with minimum resolution of 3.0, indicating no impurity interference.

Linearity:

The linearity was performed at 8 levels of the targeted diluted standard concentration 10 ppm (10%, 20%, 40%, 50%, 100%, 200% and 400% levels) and the area results are plotted against the concentration, the correlation coefficient observed was above 0.998 shows that the method was linear.

Precision:

By following the procedure in 2.5.3 section, six sample preparations are prepared and calculated the impurity content values and the Percent relative standard deviation for four impurities was below 2% shows that the method was precise.

Accuracy:

The accuracy was performed in triplicate by spiking the impurity stock solutions into the placebo mixture at 10%, 20%, 40%, 50% and 100% 400% of diluted standard concentration; from the obtained area recovery values are calculated. The average recovery values are obtained within 85 to 115% shows that the method was accurate as per the ICH limits.

Solution stability:

The first three solutions prepared in the 3.2.3 section were checked for individual and total impurities contents at 24 and 48 hours by keeping it in closed container at room temperature, the variation from the initial individual impurity and total impurity content value was below 0.04%

shows the solution was stable for 48 hours.

Robustness:

To check the effect of deliberate changes in the method, the variation inflow rate (±0.05 mL) and variation in temperature (± 5ºC) are studied; result shows no effect on system suitability and resolution 3.0 was maintained in all the changes.

Ruggedness:

From the stock solutions of 3.2.3 the solution are prepared by filtering through PVDF & PTFE 0.45 µm filter papers and the content of individual impurities and total impurities content for both filters was tested. The results obtained are within 0.03%.

Intermediate precision:

Impurity test was performed by another analyst on different day, different system, and different column; the variation between the two analysts was less that 0.1 %, it shows that method was reproducible. The validation results obtained with the related compounds are summarized in below (Table 3, 4, 5, 6, 7 and 8)

Table 3: Validation results of Di acid impurity in RS method

Parameter	**Results**	
Response factor	1.22	
Specificity	Blank interference, Placebo interference, Impurity interference was nil.	
Linearity	Established from 1 ppm to 48 ppm (R^2 value=1)	
LOD and LOQ	LOD=1.41 ppm and LOQ=4.26 ppm	
Precision:	% RSD of impurity for six preparations= 1.23	
Accuracy	**%Level**	**%Recovery**
	10%	104.4
	20%	103.7
	50%	96.7
	80%	95.0
	100%	90.7
	120%	89.2
Solution stability	1)% Difference at 24 Hrs=0.01% 2)%Difference at 48 Hrs=0.01%	
Robustness	Flow rate variation-System suitability passes Temperature variation system suitability passes	
Ruggedness	Filter validation: Variation between PVDF &PTFE 0.45 micron filters=0.00%	
Intermediate precision	Individual impurity variation=0.02% Total impurity variation=0.08%	

Table 4: Validation results of acid amide impurity in RS method

Parameter	**Results**
Response factor	1.17
Specificity	Blank interference, Placebo interference, Impurity interference was nil.

Linearity	Established from 1.05 ppm to 42 ppm (R^2 value=1)	
LOD and LOQ	LOD=1.49 ppm and LOQ=4.51 ppm	
Precision:	% RSD of impurity for six preparations=1.32	
Accuracy	**%Level**	**%Recovery**
	10%	104.0
	20%	107.8
	40%	97.8
	50%	95.8
	100%	90.8
	400%	89.3
Solution stability	1)% Difference at 24 Hrs=0.01%	
	2)%Difference at 48 Hrs=0.01%	
Robustness	Flow rate variation-System suitability passes	
	Temperature variation system suitability passes	
Ruggedness	Filter validation:	
	Variation between PVDF &PTFE 0.45 micron filters=0.00%	
Intermediate precision	Individual impurity variation=0.02%	
	Total impurity variation=0.08%	

Table 5: Validation results of N-Propyl impurity in RS method

Parameter	**Results**	
Response factor	1.01	
Specificity	Blank interference, Placebo interference, Impurity interference was nil.	
Linearity	Established from 1.1 ppm to 44 ppm (R^2 value=1)	
LOD and LOQ	LOD=0.64 ppm and LOQ=1.93 ppm	
Precision:	% RSD of impurity for six preparations=1.23	
Accuracy	**%Level**	**%Recovery**
	10%	108.3
	20%	102.3
	40%	98.3
	50%	97.8
	100%	95.9
	400%	94.8
Solution stability	1)% Difference at 24 Hrs=0.03%	
	2)%Difference at 48 Hrs=0.03%	
Robustness	Flow rate variation-System suitability passes	
	Temperature variation system suitability passes	
Ruggedness	Filter validation:	
	Variation between PVDF &PTFE 0.45 micron filters=0.00%	
Intermediate precision	Individual impurity variation=0.01%	
	Total impurity variation=0.08%	

Table 6: Validation results of Sec-butyl impurity in RS method

Parameter	**Results**
Response factor	1.06
Specificity	Blank interference, Placebo interference, Impurity interference was nil.
Linearity	Established from 1.2 ppm to 48 ppm (R^2 value=1)
LOD and LOQ	LOD=0.03 ppm and LOQ=0.10 ppm
Precision:	% RSD of impurity for six preparations=1.37

Accuracy	**%Level**	**%Recovery**
	10%	97.6
	20%	102.5
	40%	100.8
	50%	101.4
	100%	101.1
	400%	100.6
Solution stability	1)% Difference at 24 Hrs=0.01%	
	2)%Difference at 48 Hrs=0.01%	
Robustness	Flow rate variation-System suitability passes	
	Temperature variation system suitability passes	
Ruggedness	Filter validation:	
	Variation between PVDF &PTFE 0.45 micron filters=0.00%	
Intermediate precision	Individual impurity variation=0.02%	
	Total impurity variation=0.08%	

Table 7: Validation results of Des cyano impurity in RS method

Parameter	**Results**	
Response factor	0.98	
Specificity	Blank interference, Placebo interference, Impurity interference was nil.	
Linearity	Established from 1.12 ppm to 46 ppm (R^2 value=1)	
LOD and LOQ	LOD=0.03 ppm and LOQ=0.08 ppm	
Precision:	% RSD of impurity for six preparations=1.56	
Accuracy	**%Level**	**%Recovery**
	10%	97.1
	20%	99.8
	40%	99.8
	50%	100.0
	100%	101.1
	400%	100.7
Solution stability	1)% Difference at 24 Hrs=0.01%	
	2)%Difference at 48 Hrs=0.01%	
Robustness	Flow rate variation-System suitability passes	
	Temperature variation system suitability passes	
Ruggedness	Filter validation:	
	Variation between PVDF &PTFE 0.45 micron filters=0.00%	
Intermediate precision	Individual impurity variation=0.01%	
	Total impurity variation=0.08%	

Table 8: Validation results of Nitrile impurity in RS method

Parameter	**Results**	
Response factor	1.00	
Specificity	Blank interference, Placebo interference, Impurity interference was nil.	
Linearity	Established from 1.2 ppm to 48 ppm (R^2 value=0.999)	
LOD and LOQ	LOD=0.35 ppm and LOQ=1.07 ppm	
Precision:	% RSD of impurity for six preparations=1.48	
Accuracy	**%Level**	**%Recovery**
	10%	106.7
	20%	110.2

	40%	97.6
	50%	97.3
	100%	92.4
	400%	89.1
Solution stability	1)% Difference at 24 Hrs=0.00% 2)%Difference at 48 Hrs=0.00%	
Robustness	Flow rate variation-System suitability passes Temperature variation system suitability passes	
Ruggedness	Filter validation: Variation between PVDF &PTFE 0.45 micron filters=0.02%	
Intermediate precision	Individual impurity variation=0.04% Total impurity variation=0.08%	

3.3 Study of uneluted peak:

The study shows that no peak eluted, proved that there was no uneluted peak with the developed method.

3.4 Forced degradation studies:

To prove the stability indicating power of the method the forced degradation studies are carried out. The degradation reagents were (30 mL) added after the disintegration kept on reflux for the specified time. In each condition the individual % of impurities and total impurities and assay are calculated. The mass balance obtained from the experiment was ranged 99 to 100%. In all the forced degradation conditions peak purity of Febuxostat and major degradent peaks are passed, it shows that the developed method was stability indicating.

3.4.1 Acid degradation:

It was performed with 1 N Hydrochloric acid for 5 Days and the degradation observed was 0.22% and the assay value was 99.8,In Impurities test the peak purity of febuxostat and unknown and known impurities was passed, this proves that the method was stability indicating in acidic condition.

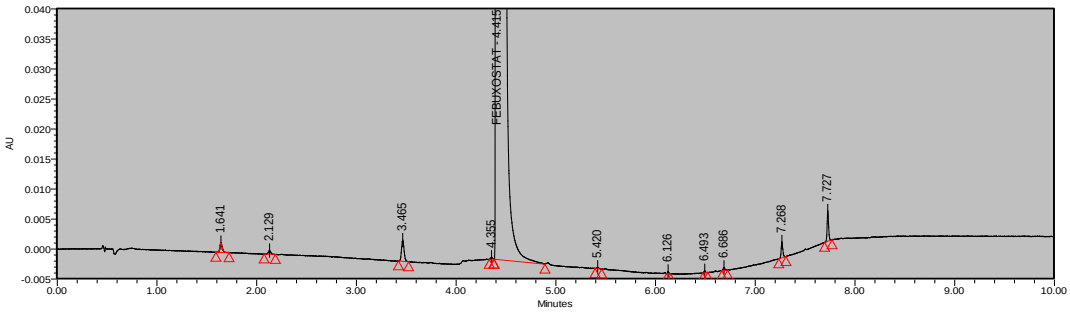

Figure 5: Acid degraded sample chromatogram

3.4.2 Base degradation:

It was performed with 1 N Sodium Hydroxide for 5 Days and the degradation observed was 8.94% and the assay value was 91.2%,In Impurities test the peak purity of febuxostat and

unknown and known impurities was passed, this proves that the method was stability indicating in Base degradation.

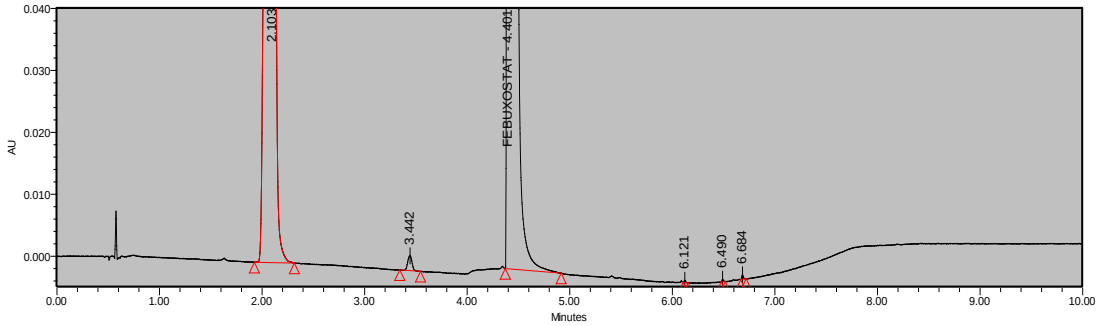

Figure6: Base stressed sample chromatogram

3.4.3 Peroxide degradation:

It was performed with 10% Hydrogen peroxide for 5 Days and the degradation observed was 0.57% and the assay value was 99.4%,In Impurities test the peak purity of febuxostat and unknown and known impurities was passed, this proves that the method was stability indicating in Peroxide degradation.

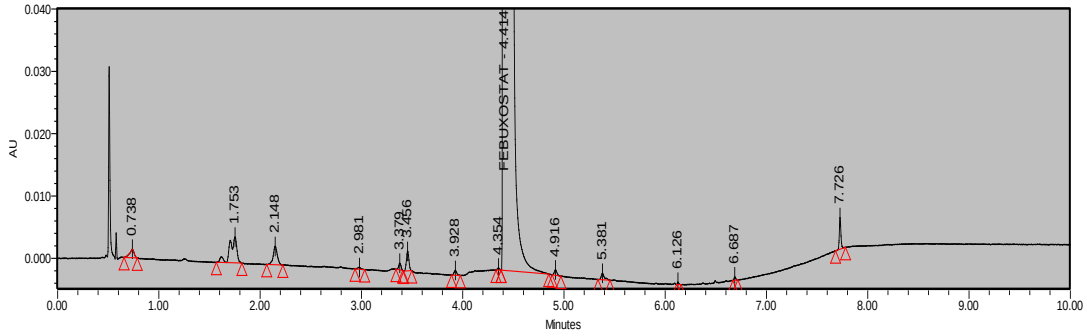

Figure7: Peroxide stressed sample chromatogram

3.4.4 Water degradation:

It was performed with Milli Q water for 5 Days and the degradation observed was 0.19% and the assay value was 99.7%,In Impurities test the peak purity of febuxostat and unknown and known impurities was passed, this proves that the method was stability indicating in Water degradation.

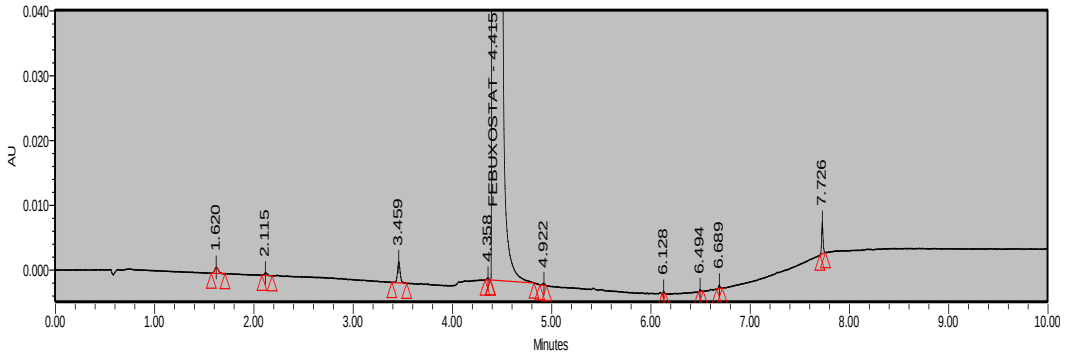

Figure8: Water stressed sample chromatogram

3.4.5 Thermal degradation:

It was performed at 50ºC water for 5 Days and the degradation observed was 0.20% and the assay value was 99.8%,In Impurities test the peak purity of febuxostat and unknown and known impurities was passed, this proves that the method was stability indicating in Thermal degradation.

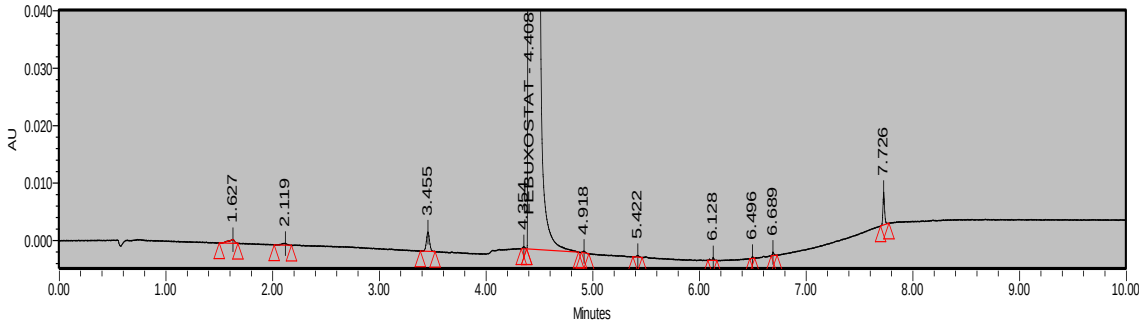

Figure9: Thermal stressed sample chromatogram

3.4.6 Photo degradation:

It was performed till 1.2 million Lux hours visible light and 200 Watts UV exposure, the degradation observed was 0.13% and the assay value was 99.7%,In Impurities test the peak purity of febuxostat and unknown and known impurities was passed, this proves that the method was stability indicating in Photo degradation.

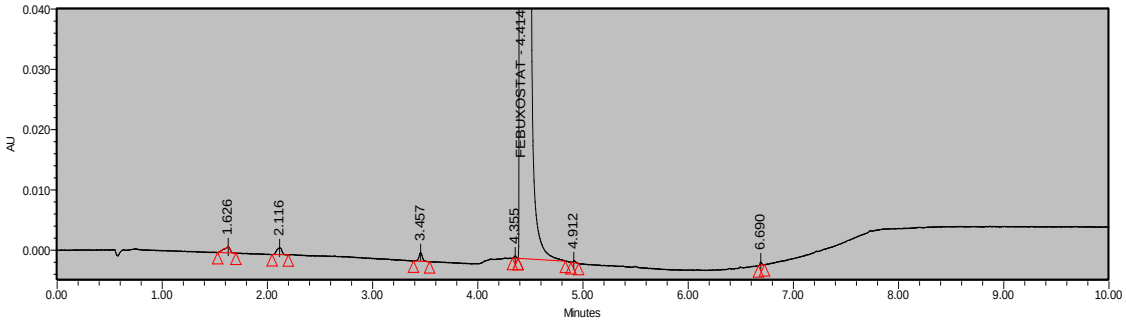

Figure 10: Light stressed sample chromatogram.

The overall summery of forced degradation results was as below.

Table 9: Forced degradation study results compilation

Type	Condition &Duration	% Degradation	%Assay	Peak purity
Acid	1N Hcl,5 days,50°C	0.22	99.8	Passes
Base	1N NaoH,5 days,50°C	8.94	91.2	Passes
Peroxide	10% H_2O_2,5 days,50°C	0.57	99.4	Passes
Water	Water,5 days,50°C	0.19	99.7	Passes
Thermal	5 days,50°C	0.20	99.8	Passes
Photo	1.2 million Lux hours	0.13	99.7	Passes

3.5 System suitability equivalence:

The difference in the results between developed method and API Vendor method was much

lesser. The critical pair resolution was maintained above 2.0 in the developed method, it shows that the results are comparable to that of API Vendor method results.

Table 10: System suitability equivalence table

Parameter	API Vendor method	UPLC Method
Assay Standard %RSD	Not more than 2%	Not more than 2%
RRT	1)Diacid-0.46	1)Diacid-0.36
	2)Acid amide-0.59	2)Acid amide-0.46
	3)N-Propyl-0.83	3)N-Propyl-0.77
	4)Sec-Butyl-0.95	4)Sec-Butyl-0.92
	5)Des cyano-1.19	5)Des cyano-1.20
	6)Nitrile-1.77	6)Nitrile-1.72
Response factors	1)Diacid-1.22	1)Diacid-1.22
	2)Acid amide-1.12	2)Acid amide-1.12
	3)N-Propyl-1.01	3)N-Propyl-1.01
	4)Sec-Butyl-1.06	4)Sec-Butyl-1.06
	5)Des cyano-0.98	5)Des cyano-0.98
	6)Nitrile-1.00	6)Nitrile-1.00
Critical pair resolution (Between Sec butyl imp &Febuxostat)	2.8	4.1

3.6 API Batch analysis results equivalence:

The results obtained with the developed method was compared with the API Vendor method results, the variation in assay and impurities results was below 0.1%, proves that the method was equivalent to the API Vendor method with 10 minutes runtime.

Table 11: API Analysis results equivalence table

Details	API Vendor method Results	UPLC Method results
B.No:FBT-0011011		
1) Known impurity	1)0.07%	1)0.08%
2) Any unknown individual impurity	2)0.03%	2)0.03%
3) Total impurity	3)0.17%	3)0.18%
4) Assay	4)99.9%	4)99.9%

3.7 Reference product analysis results equivalence:

The results obtained with the developed method shows the difference less than 0.05% with API Vendor method, shows that the developed method is equivalent to that of API Vendor method with 10 minutes runtime.

Table 12: Reference product analysis equivalence table

Details	API Vendor method Results	UPLC Method results
B.No:08029AF		
1)Known impurity	1)0.01%	1)0.01%
2)Any unknown individual impurity	2)0.06%	2)0.06%
3)Total impurity	3)0.14%	3)0.14%
4) Assay	4)100.1%	4)100.0%

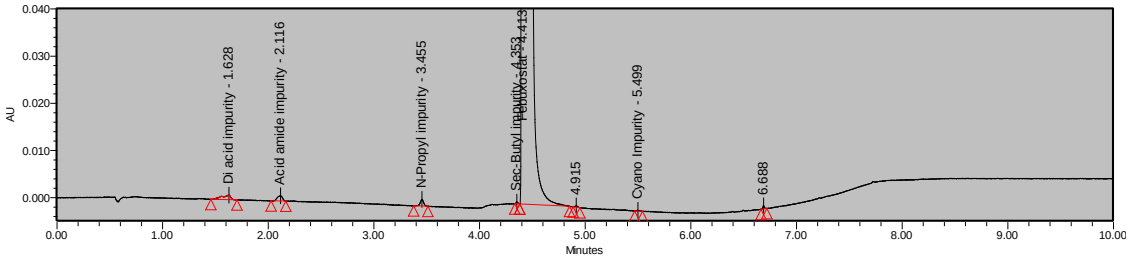

Figure 11: Uloric tablets chromatogram

CONCLUSION:

An UPLC method for related compounds in the commercial drug products and in the tablet formulation was validated in this study. Febuxostat, Febuxostat degradents and impurities gave chromatograms of very well resolved peaks which indicate the specificity of the method and the possibility of using it as an indicator of stability. Slight changes in the experimental conditions did not affect significantiy the resolution of the compounds of interest or their percent recoveries indicating the robustness of the method. All the statistical values (percent recovery, RSD, %, the slope and the intercept, LOD and LOQ) calculated were within the acceptable limits and shown equivalent to the API Vendor method. The method can be used for estimation of Febuxostat and its related impurities in bulk drugs and its tablet dosage forms for quality control purposes.

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