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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING METHOD FOR SIMULTANEOUS ESTIMATION OF FEBUXOSTAT AND DICLOFENAC POTASSIUM IN BULK AND TABLET DOSAGE FORM USING RP-HPLC

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ABSTRACT: A novel method for the simultaneous estimation of Febuxostat and Diclofenac Potassium in combine dosage form was developed and validated by reverse phase high performance liquid chromatography. The determination was performed on an Inertsil C_{18} column (100mm x 4.6 mm ID, 5µm particle size) and the mobile phase consisting a mixture of 0.01M Ammonium di-hydrogen phosphate buffer (pH adjusted to 5 with orthophosphoric acid) and Acetonitrile (60:40, v/v) was delivered at a flow rate of 1 ml/min and detector wavelength at 287nm. The retention time of Febuxostat and Diclofenac Potassium was found to be 2.303 and 4.105min respectively. The linearity for Febuxostat and Diclofenac Potassium was obtained in the concentration range of 10-60µg/ml and 25-150µg/ml with correlation coefficients (r^2) of the regression equations greater than 0.9999 in all cases respectively. Results of assay, accuracy and precision were statistically evaluated as per ICH guidelines. Febuxostat and Diclofenac Potassium were subjected to acid and alkali hydrolysis, thermal and photolytic forced degradation. In the forced degradation study Febuxostat and Diclofenac Potassium showed maximum degradation in acid hydrolysis followed by less degradation in alkali hydrolysis, thermal and photolytic forced degradation. The developed method was simple, specific, sensitive, rapid, and economic and can be used for simultaneous estimation of Febuxostat and Diclofenac Potassium in bulk and their combined dosage form for routine analysis and stability studies.

INTRODUCTION: Febuxostat is chemically known as 2-(3-cyano-4-isobutoxyphenyl)-4methyl-1, 3-thiazole-5-carboxylic acid is used in the treatment of gout **Figure 1**. Febuxostat is a non-purine selective inhibitor of xanthine oxidase, therefore reducing production of uric acid [1].

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Diclofenac Potassium is chemically known as 2-((2, 6-dichlorophenyl) amino) benzene acetic acid, monopotassium salt is used in the treatment of pain, inflammatory disorders and dysmenorrhoea **Figure 2**. The primary mechanism responsible for its anti-inflammatory, antipyretic, and analgesic action is thought to be inhibition of prostaglandin synthesis by inhibition of cyclooxygenase (COX). It also appears to exhibit bacteriostatic activity by inhibiting bacterial DNA synthesis [2]. Literatures survey reveals Spectrophotometry [3], first derivative spectrophotometry [4], RP-HPLC [5, 6], HPTLC [7] and Spectroflourimetry [8] methods have been reported as a single as well as combination with other drugs. However, there is few work was reported for the

simultaneous estimation of these drugs by RP-HPLC method with degradation studies. Hence, in the present study an attempt has been made to develop simple, accurate, sensitive, precise and repeatable RP-HPLC method for the simultaneous estimation of Febuxostat and Diclofenac Potassium in bulk and tablet dosage form.

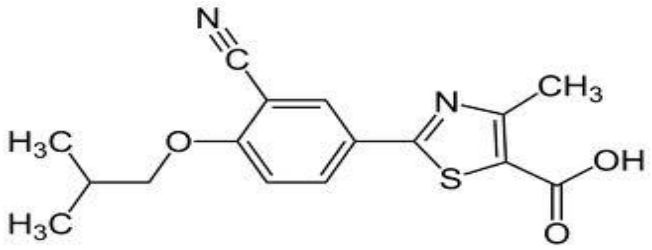

FIG. 1: STRUCTURE OF FEBUXOSTAT

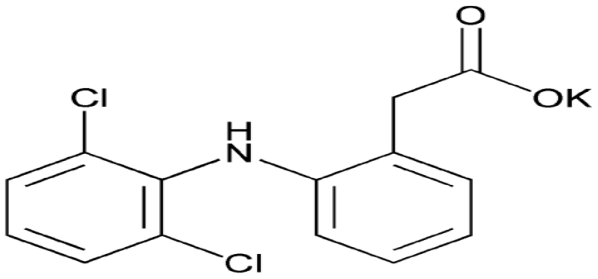

FIG. 2: STRUCTURE OF DICLOFENAC POTASSIUM

MATERIALS AND METHODS:

Apparatus: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, PDA detector and Empower 2 software, Inertsil C_{18} column (100 mm x 4.6 mm ID, 5µm) was used as stationary phase. Semi-micro analytical balance (India), an Ultrasonic bath sonicator (Frontline FS 4, Mumbai, India) and Whatmann filter paper No. 41 (Whatmann International Ltd., England) were used in the study.

Reagents and materials: Febuxostat and Diclofenac Potassium bulk powder was obtained from Orbit Life science Pvt. Ltd., India. The commercial fixed dose combination product.

Xanfeb DSR tablet was procured from the Indoco Remedies Ltd., India. Acetonitrile (HPLC grade, Merck, India), Ammonium di-hydrogen phosphate (AR, Finar Reagent, Ahmedabad, India), Ortho phosphoric acid (AR, Finar Reagent, Ahmedabad, India) and purified water (HPLC grade, Rankem, India) was used in the study.

Chromatographic condition: In this work, a reverse phase Inertsil C_{18} column (100 mm x 4.6 mm ID, 5µm) was used as a stationary phase and a mobile phase consisting a mixture of 0.01M Ammonium dihydrogen phosphate buffer (pH adjusted to 5 with ortho phosphoric acid) and Acetonitrile taken in the ratio 60:40 (v/v) delivered at a flow rate of 1 ml/min, injection volume of 20µl and detector wavelength at 287nm..

Preparation of mobile phase:

Solvent A: Accurately weighed about 1.15 grams of Ammonium di-hydrogen phosphate was taken into a 1000ml beaker and dissolved and diluted to 1000ml with HPLC water and degassed in ultrasonic water bath and filtered through 0.45µm filter using vacuum filtration and the pH of 5 was adjusted by using diluted ortho phosphoric acid.

Solvent B: Acetonitrile HPLC grade

Mobile phase: Volume of solvent (A) and solvent (B) taken in the ratio 60:40 (v/v) and mixed well and filter through 0.45 µm membrane filter and degas for 10 minutes.

Preparation of standard stock solutions: An accurately weighed 40 mg of Febuxostat and 100mg of Diclofenac Potassium were transferred to 100 ml volumetric flask, dissolved in 50 ml with Mobile phase and sonicated to dissolve it completely and diluted up to mark with Mobile phase to get 400 µg/ml solution of Febuxostat and 1000µg/ml solution of Diclofenac Potassium.

Preparation of Marketed sample solution for Assay: Twenty tablets were accurately weighed and powdered and powder equivalent to 40 mg of Febuxostat and 100mg of Diclofenac Potassium sample were taken into 100ml clean dry volumetric flask, mobile phase was added and sonicated to dissolve it completely and volume was made up to the mark with the same mobile phase. 1ml was pipette out from the above Febuxostat & Diclofenac Potassium sample stock solution into a 10ml volumetric flask and diluted up to the mark with mobile phase to get a concentration of 40µg/ml solution of Febuxostat and 100µg/ml solution of Diclofenac Potassium. From the standard solution 40µg/ml of Febuxostat and 100µg/ml of Diclofenac Potassium and from the

sample solution 40µg/ml of Febuxostat and 100µg/ml of Diclofenac Potassium, 20µL from standard and sample solution were injected into the chromatographic system and the peak areas was measured for Febuxostat and Diclofenac Potassium and the % Assay was calculated by comparing the peak area of standard and sample chromatogram was shown in **Table 1** and **Figure 3** and **4**.

TABLE 1: ANALYSIS OF MARKETED FORMULATION OF FEBUXOSTAT AND DICLOFENAC POTASSIUM

Xanfeb DSR Label Claim (mg)		Amount Found (mg)		% Label Claim ± % RSD (n=3)	
Febuxostat	**Diclofenac Potassium**	**Febuxostat**	**Diclofenac Potassium**	**Febuxostat**	**Diclofenac Potassium**
40	100	39.90	99.74	99.75±0.06	99.74±0.07

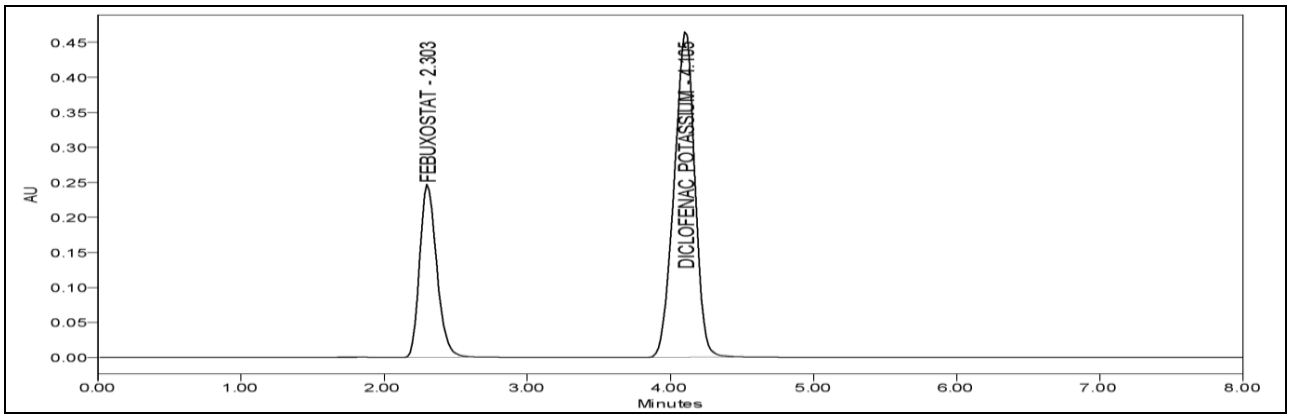

FIG. 3: STANDARD CHROMATOGRAM OF FEBUXOSTAT AND DICLOFENAC POTASSIUM

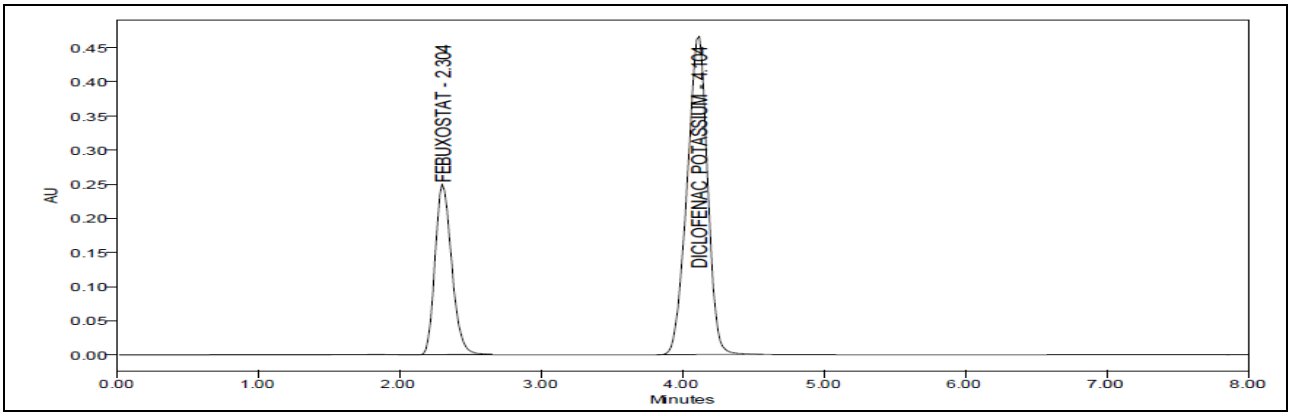

FIG. 4: SAMPLE CHROMATOGRAM OF FEBUXOSTAT AND DICLOFENAC POTASSIUM

Method Validation: The method was validated in compliance with ICH guidelines [9, 10].

Preparation of calibration curve (Linearity): Aliquots of 0.25,0.5,0.75,1,1.25 and 1.5ml of mixed standard working solutions (equivalent to 10,20,30,40,50 and 60µg/ml of Febuxostat and 25,50,75,100,125 and150µg/ml of Diclofenac Potassium) were transferred in a series of 10 ml volumetric flasks, and the volume was made up to the mark with Mobile phase. Each solution was injected under the operating chromatographic condition as described above and responses were recorded. Calibration curves were constructed by plotting the peak areas versus the concentration, and the regression equations were calculated **Table 2** and **3** and **Figure 5** and **6.**

Accuracy (recovery study): The accuracy of the method was determined by calculating the recoveries of Febuxostat and Diclofenac Potassium by the standard addition method. Known amounts of standard solutions of Febuxostat and Diclofenac Potassium were added at 50, 100 and 150 % level to pre-quantified sample solutions of Xanfeb DSR tablet.

TABLE 2: LINEARITY OF FEBUXOSTAT

Concentration(µg/ml)	Peak Area
10	517519
20	1028504
30	1547587
40	2047754
50	2564870
60	3070745

TABLE 3: LINEARITY OF DICLOFENAC POTASSIUM

Concentration(µg/ml)	Peak Area
25	1164535
50	2327768
75	3509529
100	4655523
125	5850695
150	7024588

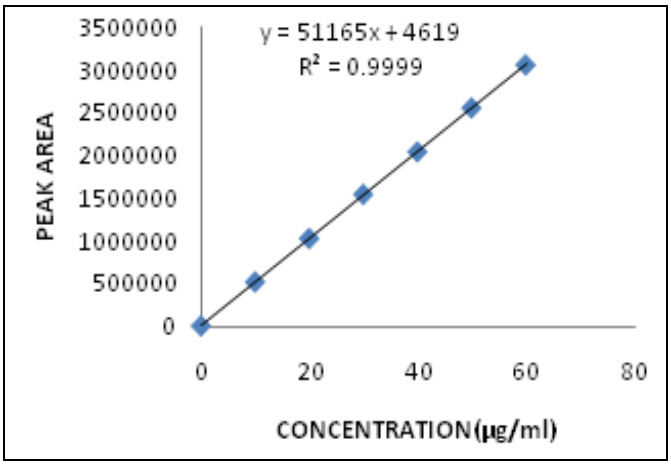

FIG. 5: LINEARITY OF FEBUXOSTAT

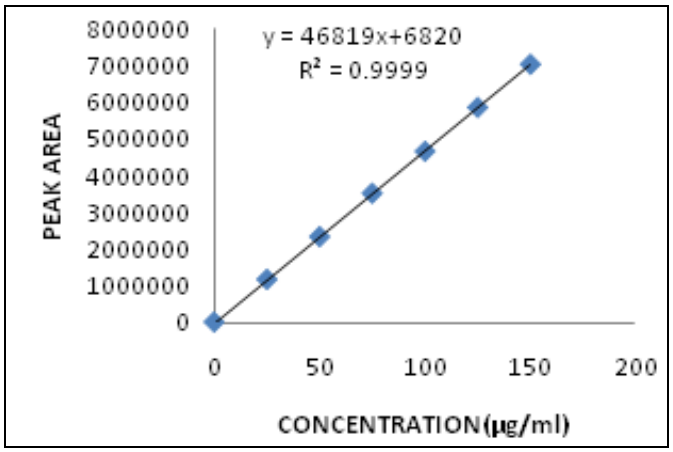

FIG. 6: LINEARITY OF DICLOFENAC POTASSIUM

Preparation of Standard Stock Solution:

Accurately weighed 40 mg of Febuxostat and 100mg of Diclofenac Potassium working standard were taken into 100ml clean dry volumetric flasks, mobile phase was added and sonicated to dissolve it completely and volume was made up to the mark with the mobile phase. 1ml was pipette out from the above Febuxostat & Diclofenac Potassium stock solutions into a 10ml volumetric flask and diluted up to the mark with mobile phase to get a concentration of 40µg/ml solution of Febuxostat and 100µg/ml solution of Diclofenac Potassium.

Preparation of Sample Solutions:

For preparation of 50% solution:

Accurately weighed quantity of 20 mg of Febuxostat and 50mg of Diclofenac Potassium sample was taken into 100ml clean dry volumetric flask, mobile phase was added and sonicated to dissolve it completely and volume was made up to the mark with the same mobile phase. 1ml of above solution was pipetted out into 10ml volumetric flask and made up to the mark with mobile phase to get a concentration of 20µg/ml solution of Febuxostat and 50µg/ml solution of Diclofenac Potassium.

For preparation of 100% solution:

Accurately weighed quantity of 40 mg of Febuxostat and 100mg of Diclofenac Potassium sample was taken into 100ml clean dry volumetric flask, mobile phase was added and sonicated to dissolve it completely and volume was made up to the mark with the same mobile phase. 1ml of above solution was pipetted out into 10ml volumetric flask and made up to the mark with mobile phase to get a concentration of 40µg/ml solution of Febuxostat and 100µg/ml solution of Diclofenac Potassium.

For preparation of 150% solution:

Accurately weighed quantity of 60 mg of Febuxostat and 150mg of Diclofenac Potassium sample was taken into 100ml clean dry volumetric flask, mobile phase was added and sonicated to dissolve it completely and volume was made up to the mark with the same mobile phase. 1ml of above solution was pipetted out into 10ml volumetric flask and made up to the mark with mobile phase to get a concentration of 60µg/ml solution of Febuxostat and 150µg/ml solution of Diclofenac Potassium. The accuracy studies (% recovery) of Febuxostat and Diclofenac Potassium were determined **Table 4** and **5**.

TABLE 4: RECOVERY STUDY DATA OF FEBUXOSTAT

Sample name	Amount added (µg/ml)	Amount found (µg/ml)	%Recovery	Statistical Analysis
S_1:50%	20	19.94	99.7	Mean-99.75
S_2:50%	20	19.96	99.8	S.D-0.05
S_3:50%	20	19.95	99.75	%RSD-0.05
S_4:100%	40	39.88	99.7	Mean-99.63
S_5:100%	40	39.82	99.55	S.D-0.07
S_6:100%	40	39.86	99.65	%RSD=0.07
S_7:150%	60	59.77	99.61	Mean-99.58
S_8:150%	60	59.71	99.51	S.D-0.06
S_9 :150%	60	59.78	99.63	%RSD-0.06

TABLE 5: RECOVERY STUDY DATA OF DICLOFENAC POTASSIUM

Sample name	Amount added (µg/ml)	Amount found (µg/ml)	%Recovery	Statistical Analysis
S_1:50%	50	49.96	99.92	Mean-99.56
S_2:50%	50	49.67	99.34	S.D-0.31
S_3:50%	50	49.72	99.44	%RSD-0.31
S_4:100%	100	99.55	99.55	Mean-99.53
S_5:100%	100	99.53	99.53	S.D-0.02
S_6:100%	100	99.51	99.51	%RSD=0.02
S_7:150%	150	148.87	99.24	Mean-99.74
S_8:150%	150	149.65	99.76	S.D-0.49
S_9 :150%	150	150.35	100.23	%RSD-0.49

Method precision (Repeatability):

Tablet powder equivalent to 40 mg of Febuxostat and 100mg of Diclofenac Potassium sample was taken into 100ml clean dry volumetric flask, mobile phase was added and sonicated to dissolve it completely and volume was made up to the mark with the same mobile phase. 1ml of above solution was pipetted out into 10ml volumetric flask and made up to the mark with mobile phase to get a concentration of 40µg/ml solution of Febuxostat and 100µg/ml solution of Diclofenac Potassium. A homogenous sample of a single batch analysed six times and was checked whether the method is giving consistent results. The %RSD for the area of six replicate injections was calculated as mentioned in **Table 6**.

TABLE 6: METHOD PRECISION DATA FOR FEBUXOSTAT AND DICLOFENAC POTASSIUM

Febuxostat				Diclofenac Potassium		
S.No.	Conc. (µg/ml)	Rt	Peak Area	Conc. (µg/ml)	Rt	Peak Area
1	40	2.305	2064216	100	4.108	4674525
2	40	2.301	2051462	100	4.11	4648952
3	40	2.305	2044963	100	4.105	4715478
4	40	2.304	2064784	100	4.109	4625881
5	40	2.304	2068885	100	4.108	4687954
6	40	2.301	2042761	100	4.111	4658445
Average		2.30333333	2056178.5	**Average**	4.109	4668539.17
SD		0.0018619	11208.68679	**SD**	0.00207	31372.8816
%RSD		**0.08**	**0.55**	**%RSD**	**0.05**	**0.67**

System precision:

The system precision was carried out to ensure that the analytical system is working properly. The standard preparation concentration of 40µg/ml solution of Febuxostat and 100µg/ml solution of Diclofenac Potassium was injected six times into the HPLC and the %RSD for the area of six replicate injections was calculated as mentioned in **Table 7**.

TABLE 7: SYSTEM PRECISION DATA FOR FEBUXOSTAT AND DICLOFENAC POTASSIUM

Febuxostat				Diclofenac Potassium		
S.No.	Conc. (µg/ml)	Rt	Peak Area	Conc. (µg/ml)	Rt	Peak Area
1	40	2.302	2049324	100	4.11	4669871
2	40	2.301	2040560	100	4.105	4644548
3	40	2.301	2058082	100	4.103	4678518
4	40	2.301	2053916	100	4.111	4680893
5	40	2.302	2056718	100	4.106	4685284
6	40	2.305	2072274	100	4.112	4721423
Average		2.3020	2055145.667	**Average**	4.10783	4680089.5
SD		0.00154919	10513.03613	**SD**	0.00366	24924.5752
%RSD		**0.07**	**0.51**	**%RSD**	**0.09**	**0.53**

Intermediate precision/ruggedness:

The intermediate precision (also known as Ruggedness) of the method was evaluated by performing precision on different days by different analysts. Tablet powder equivalent to 40 mg of Febuxostat and 100mg of Diclofenac Potassium sample was taken into 100ml clean dry volumetric flask, mobile phase was added and sonicated to dissolve it completely and volume was made up to the mark with the mobile phase. 1ml of above solution was pipetted out into 10ml volumetric flask and made up to the mark with mobile phase to get a concentration of 40µg/ml of Febuxostat and 100µg/ml of Diclofenac Potassium. The sample solution was injected for six times and the area for all six injections was measured in HPLC. The %RSD for the area of six replicate injections was calculated as mentioned in **Table 8**.

TABLE 8: RUGGEDNESS DATA FOR FEBUXOSTAT AND DICLOFENAC POTASSIUM BY DIFFERENT ANALYST

Febuxostat				Diclofenac Potassium		
S.No.	Conc. (µg/ml)	Rt	Peak Area	Conc. (µg/ml)	Rt	Peak Area
1	40	2.304	2059865	100	4.112	4612178
2	40	2.301	2051248	100	4.108	4605215
3	40	2.305	2070854	100	4.109	4586542
4	40	2.305	2059962	100	4.111	4655475
5	40	2.304	2064154	100	4.105	4596842
6	40	2.305	2042452	100	4.108	4584568
Average		2.304	2058089	**Average**	4.109	4606803
SD		0.00155	9977.042	**SD**	0.00248	26094.06
%RSD		**0.07**	**0.48**	**%RSD**	**0.06**	**0.57**

Limit of Detection (LOD) and Limit of Quantification (LOQ):

Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated as 3.3×SD/S and 10×SD/S respectively as per ICH guidelines, Where SD is the standard deviation of the response (Y-intercept) and S is the slope of the calibration curve. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD of Febuxostat and Diclofenac Potassium was calculated and shown in **Table 9**. The LOQ is the smallest concentration of the analyte which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ of Febuxostat and Diclofenac Potassium was calculated and shown in **Table 9**.

Robustness:

As part of the Robustness, deliberate change in the flow rate and buffer solution of ±10% was made to evaluate the impact on the method. The results reveal that the method is robust. The results are summarized in **Table 10** and **11**.

TABLE 9: SUMMARY OF VALIDATION PARAMETER FOR FEBUXOSTAT AND DICLOFENAC POTASSIUM

Parameters	RP-HPLC method	
	Febuxostat	Diclofenac Potassium
Concentration range (µg/ml)	10-60	25-150
Slope	51165	46819
Intercept	4619	6820
Correlation coefficient	0.9999	0.9999
LOD (µg/ml)	0.52	1.27
LOQ (µg/ml)	1.57	3.87
Method Precision (% RSD, n=6)	0.55	0.67
System precision (% RSD, n=6)	0.51	0.53
Ruggedness (% RSD, n=6)	0.48	0.57
% Accuracy	99.58-99.75	99.53-99.74

TABLE 10: SUMMARY OF ROBUSTNESS (CHANGE IN FLOW RATE) FOR FEBUXOSTAT AND DICLOFENAC POTASSIUM

Drug	Flow rate (ml/min)	Retention Time (Mins)	Robustness			
			Average peak area (n=3)	% RSD	USP Plate Count	Asymmetry
Febuxostat	0.9	2.852	2544380	0.1	2978	1.27
	1.0	2.303	2047754	0.01	2777	1.23
	1.1	1.934	1709715	0.01	2569	1.21
Diclofenac Potassium	0.9	5.093	5781120	0.06	4020	0.91
	1.0	4.105	4655523	0.09	3713	0.94
	1.1	3.431	3891115	0.05	3385	0.94

TABLE 11: SUMMARY OF ROBUSTNESS (CHANGE IN BUFFER SOLUTION) FOR FEBUXOSTAT AND DICLOFENAC POTASSIUM

Drug	Change in the buffer solution	Retention Time (Mins)	Robustness			
			Average peak area (n=3)	% RSD	USP Plate Count	Asymmetry
Febuxostat	10% less	2.291	1934218	0.07	2790	1.22
	Actual	2.303	2047754	0.01	2777	1.23
	10% more	2.292	2049778	0.11	2806	1.23
Diclofenac Potassium	10% less	3.852	4346816	0.007	2462	0.79
	Actual	4.105	4655523	0.09	3713	0.94
	10% more	3.903	4673593	0.006	2055	0.84

System Suitability:

The column efficiency, resolution and peak asymmetry were calculated for Febuxostat and Diclofenac Potassium. The values obtained, demonstrated the suitability of the system for the analysis of this drug combinations **Table 12**.

TABLE 12: SYSTEM SUITABILITY TEST PARAMETERS FOR FEBUXOSTAT AND DICLOFENAC POTASSIUM

Parameter	Febuxostat	Diclofenac Potassium
Retention Time (Mins)	2.303	4.105
Theoretical plates	2777	3713
Tailing factor	1.23	0.94
Resolution		7.52

Forced Degradation study of Febuxostat and Diclofenac Potassium:

Degradation study of Febuxostat and Diclofenac Potassium in 0.1N HCl at 70°C for 4 hours in reflux condition: Febuxostat and Diclofenac Potassium peak was observed at retention time 2.325 min and 4.080 min respectively **Figure 7**. The % drug degradation observed of Febuxostat and Diclofenac Potassium was 23.49 % and 16.78 % respectively **Table 13**. From this it is observed that Febuxostat and Diclofenac Potassium showed maximum degradation in acid hydrolysis degradation condition.

TABLE 13: FORCED DEGRADATION DATA OF FEBUXOSTAT & DICLOFENAC POTASSIUM IN DIFFERENT CONDITIONS

Degradation condition	Peak Area		Concentration(µg/ml)		% Potency		% Degradation	
	Febuxostat	Diclofenac Potassium	Febuxostat	Diclofenac Potassium	Febuxostat	Diclofenac Potassium	Febuxostat	Diclofenac Potassium
Acidic/0.1N HCl/70°C/ Reflux/4hr/Solution	2047754	4655523	40	100	99.67	99.69	23.49	16.78
	1562826	3872260	30.57	83.17	76.18	82.91		
Alkaline/ 0.1N NaOH/ 70°C/Reflux/4hr/ Solution	2047754	4655523	40	100	99.67	99.69	6.74	4.58
	1909186	4442270	37.29	95.41	92.93	95.11		
Thermal/60C/24 hr/ Solid	2047754	4655523	40	100	99.67	99.69	11.40	5.34
	1814949	4406672	35.45	94.65	88.27	94.35		
Photo/1.2 million lux hrs fluore –scent light /200w/m2 of UV/7days	2047754	4655523	40	100	99.67	99.69	4.43	13.09
	1958538	4044579	38.25	86.87	95.24	86.60		

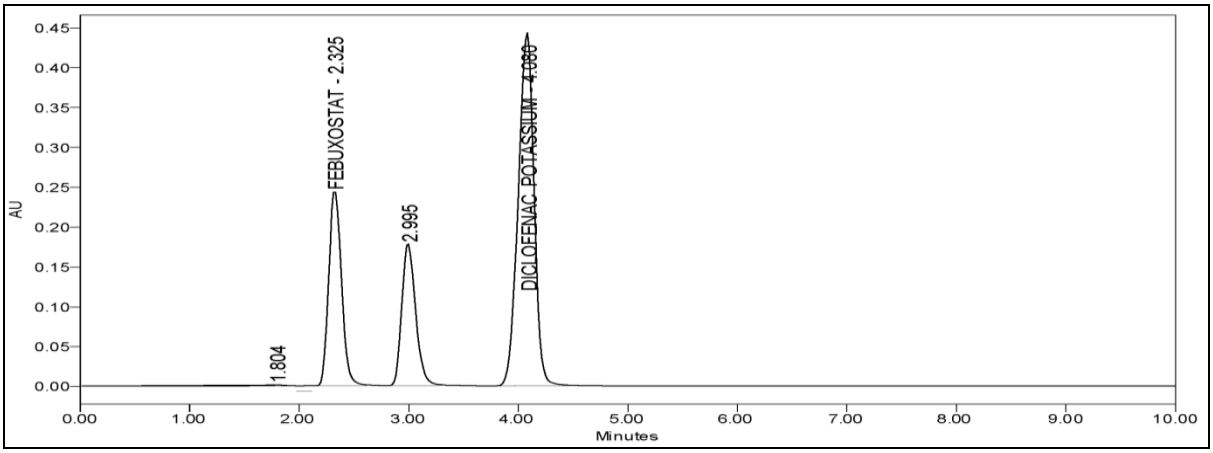

FIG. 7: ACID HYDROLYSIS OF FEBUXOSTAT AND DICLOFENAC POTASSIUM

Degradation study of Febuxostat and Diclofenac Potassium in 0.1N NaOH at 70°C for 4 hours in reflux condition: Febuxostat and Diclofenac Potassium peak was observed at retention time 2.308 min and 4.099 min respectively Figure 8. The % drug degradation observed of Febuxostat and Diclofenac Potassium was 6.74 % and 4.58 % respectively **Table 13**. From this it is observed that Febuxostat and Diclofenac Potassium showed minimum degradation in base hydrolysis degradation condition.

Thermal Degradation study of Febuxostat and Diclofenac Potassium at 60°C for about 24 hrs: Thermal degradation of Febuxostat and Diclofenac Potassium at 60°C for about 24 hrs in hot air oven was carried out and the peak was observed at retention time 2.303 min and 4.089 min respectively. There was no degradation peak found

in thermal degradation chromatogram. % Degradation of Febuxostat and Diclofenac Potassium was found to be 11.40 % and 5.34 % respectively **Figure 9** and **Table 13**.

Photolytic Degradation study of Febuxostat and Diclofenac Potassium: Febuxostat and Diclofenac Potassium were exposed to energy of 1.2 million lux hrs fluorescent light and 200 w/m^2 of UV for about 7 days was performed and the peak was observed at retention time 2.307 min and 4.503 min respectively. The % degradation of Febuxostat and Diclofenac Potassium was found to be 4.43 % and 13.09 % respectively **Figure 10** and **Table 13**.

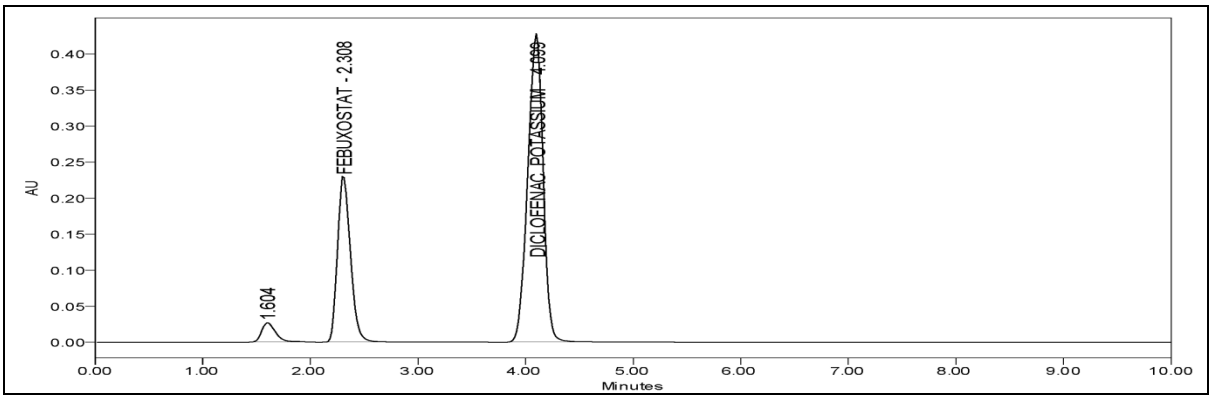

FIG. 8: BASE HYDROLYSIS OF FEBUXOSTAT AND DICLOFENAC POTASSIUM

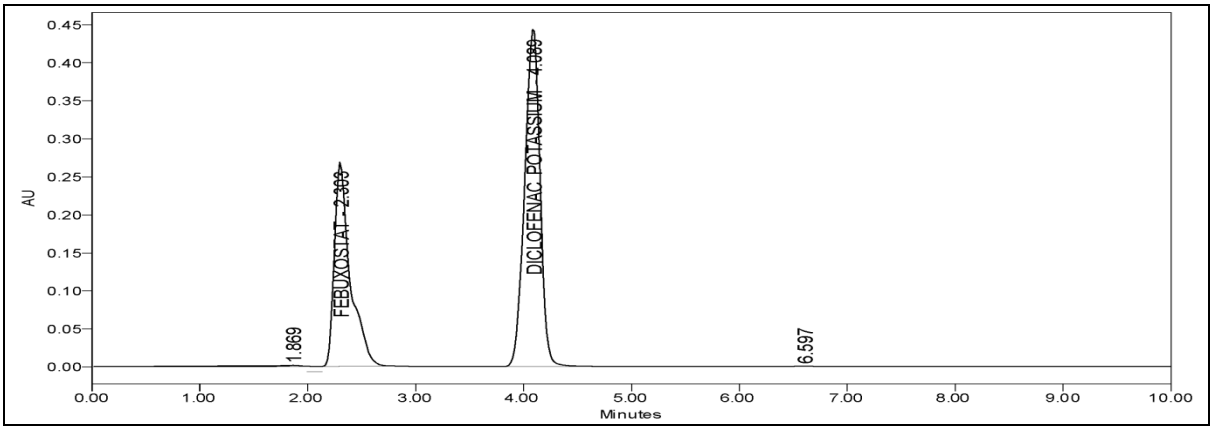

FIG. 9: THERMAL DEGRADATION OF FEBUXOSTAT AND DICLOFENAC POTASSIUM

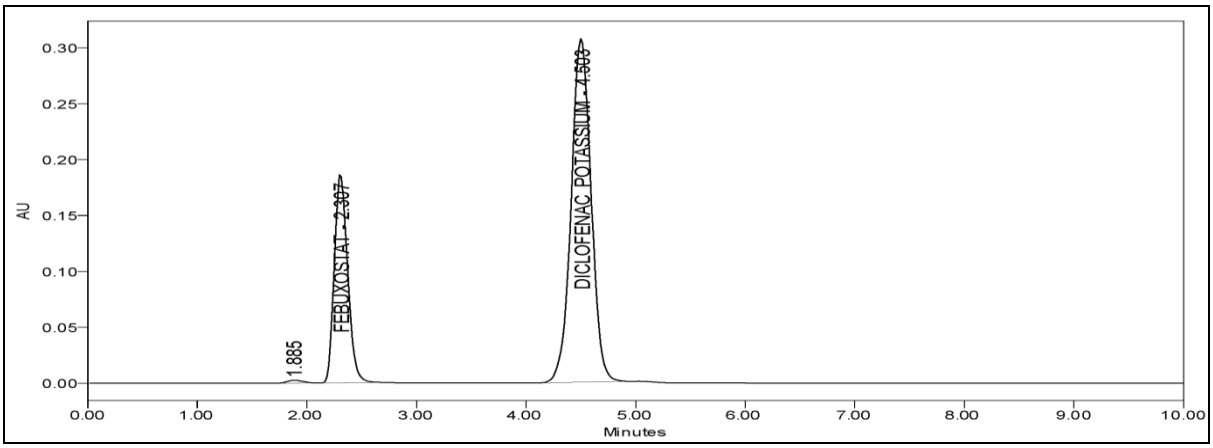

FIG. 10: PHOTO STABILITY OF FEBUXOSTAT AND DICLOFENAC POTASSIUM

RESULTS AND DISCUSSION:

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for Febuxostat and Diclofenac Potassium were obtained with a mobile phase containing a mixture of 0.01M Ammonium di-hydrogen phosphate buffer (pH adjusted to 5 with orthophosphoric acid) and Acetonitrile (60:40, v/v) was delivered at a flow rate of 1 ml/min to get better reproducibility and repeatability. Quantification was achieved with PDA detection at 287nm based on peak area. The

retention time of Febuxostat and Diclofenac Potassium was found to be 2.303 and 4.105min respectively **Figure 3**.

Linear correlation was obtained between peak area versus concentrations of Febuxostat and Diclofenac Potassium in the concentration ranges of 10-60 µg/ml and 25-150 µg/ml with correlation coefficients r^2=0.9999 and r^2=0.9999 and mean accuracies are 99.58-99.75% and 99.53-99.74% for Febuxostat and Diclofenac Potassium, which indicates accuracy of the proposed method. The % RSD values of accuracy for Febuxostat and Diclofenac Potassium were found to be < 2 %. The % RSD values of method precision are 0.55% and 0.67% for Febuxostat and Diclofenac Potassium respectively and % RSD values of system precision are 0.51% and 0.53% for Febuxostat and Diclofenac Potassium respectively.

The % RSD values of ruggedness are 0.48% and 0.57% for Febuxostat and Diclofenac Potassium respectively, reveal that the proposed method is precise. LOD values for Febuxostat and Diclofenac Potassium were found to be 0.52µg/ml and 1.27µg/ml, respectively and LOQ values for Febuxostat and Diclofenac Potassium were found to be 1.57µg/ml and 3.87µg/ml, respectively **Table 9**. The results reveal that the method is robust enough **Table 10** and **11**. Degradation study of Febuxostat and Diclofenac Potassium in 0.1N HCl at 70°C for 4 hours in reflux condition was performed and the peak was observed at retention time 2.325 min and 4.080 min respectively Figure 7. The % drug degradation observed of Febuxostat and Diclofenac Potassium was 23.49 % and 16.78 % respectively **Table 13**. From this it is observed that Febuxostat and Diclofenac Potassium showed maximum degradation in acid hydrolysis degradation condition.

Degradation study of Febuxostat and Diclofenac Potassium in 0.1N NaOH at 70°C for 4 hours in reflux condition was performed and the peak was observed at retention time 2.308 min and 4.099 min respectively Figure 8. The % drug degradation observed of Febuxostat and Diclofenac Potassium was 6.74 % and 4.58 % respectively **Table 13**. From this it is observed that Febuxostat and Diclofenac Potassium showed minimum degradation in base hydrolysis degradation condition. Thermal degradation of Febuxostat and Diclofenac Potassium at 60°C for about 24 hrs in hot air oven was carried out and the peak was observed at retention time 2.303 min and 4.089 min respectively.

There was no degradation peak found in thermal degradation chromatogram. % Degradation of Febuxostat and Diclofenac Potassium was found to be 11.40 % and 5.34 % respectively **Figure 9** and **Table 13**. Photolytic Degradation study of Febuxostat and Diclofenac Potassium was performed and the peak was observed at retention time 2.307 min and 4.503 min respectively. The % degradation of Febuxostat and Diclofenac Potassium was found to be 4.43 % and 13.09 % respectively Figure 10 and Table 13. These data show that the proposed method is sensitive for the determination of Febuxostat and Diclofenac Potassium. The results of system suitability testing are given in **Table 12**.

CONCLUSION: Stability indicating RP-HPLC method for simultaneous estimation of Febuxostat & Diclofenac Potassium in their combine dosage form was established and validated as per the ICH guidelines. The forced degradation study confirmed that there was no merging between peaks of active ingredients and any other degradation products as well as other additives. Hence the specificity of the proposed method was established. The linearity of developed method was achieved in the range of 10-60 µg/ml for Febuxostat (r^2=0.9999) and 25-150 µg/ml for Diclofenac Potassium (r^2=0.9999).

The percentage recovery of drug was achieved in the range of 98-102 % which was within the acceptance criteria. The percentage RSD was NMT 2 % which proved the precision of the developed method. Different degradation products were found for drug product in acidic, alkaline, thermal and photolytic force degradation. Peak of degraded products were not interfering with the main drug peak of Febuxostat & Diclofenac Potassium. Thus, these degradation products have not been identified. The developed method is simple, sensitive, rapid, linear, precise, rugged, accurate, specific, and robust. Hence it can be used for the routine analysis of Febuxostat & Diclofenac Potassium in their bulk and combine dosage form in quality control laboratory and stability studies.

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