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Development and Validation of Stability Indicating LC-PDA Method for Simultaneous Assessment of Febuxostat and Ketorolac Tromethamine in Tablet Dosage Form

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A novel, rapid and highly selective stability indicating LC-PDA method was developed and validated for simultaneous assessment of febuxostat and ketorolac tromethamine in tablet dosage form. Agilent eclipus C18 (250 × 4.6 mm, 5 µm particle size) column and mobile phase-phosphate buffer (KH_2PO_4): acetonitrile (60:40 v/v, pH = 4 adjusted with orthophosphoric acid, 0.9 mL/min flow rate) were used for isocratic separation and eluent was monitored at 321 nm. Febuxostat and ketorolac tromethamine were retained at 7.933 and 3.797 min, respectively. The calibration plots were linear with correlation coefficients (r^2) of 0.9980 and 0.9984 for febuxostat and ketorolac tromethamine, respectively. The proposed method was validated as per ICH Q2 (R1) guidelines. Stability study was conducted under various stressed conditions and obtained degradation products did not interfere with the detection of febuxostat and ketorolac tromethamine. Hence, the proposed method was sensitive, reproducible, stability indicating and was successfully applied to the routine quantitative-qualitative analysis.

Keywords: Febuxostat, Ketorolac tromethamine, Stability study, Forced degradation, Liquid chromatographic method.

INTRODUCTION

Febuxostat (FB) is called xanthine oxidase (XO) inhibitors class of medications, used in the treatment of hyperuricemia and chronic gout. Gout is a type of arthritis in which uric acid, a naturally occurring substance in the body, builds up in the joints and causes sudden attacks of redness, swelling, pain and heat in one or more joints. It works by decreasing the amount of uric acid that is made in the body. Febuxostat is used to prevent gout attacks, but not to treat them once they occur [1].

Febuxostat (Fig. 1) is a novel non-purine urate lowering drug and it can selectively inhibit xanthine oxidase enzyme. Chemically it is known as a 2-[3-cyano-4-(2-methyl-propoxyl)-phenyl]-4-methyl-thiazole-5-carboxylic acid [2]. Febuxostat is a potent ligand and inhibitor of both the oxidized and reduced forms of xanthine oxidase. Treatment with febuxostat resulted in a significant reduction of serum urate (SUA) levels at all dosage. Febuxostat therapy is safe and well tolerated [3].

Ketorolac (KT) (Fig. 2) ((±)-5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid), a pyrrolizine carboxylic acid derivative structurally related to indomethacin. It is a potent and effective non-steroidal anti-inflammatory drug (NSAID),

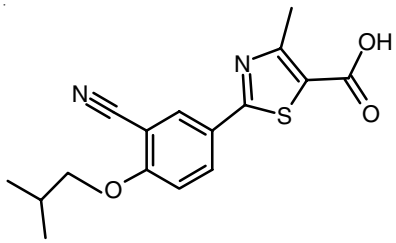

Fig. 1. Chemical structure of Febuxostat

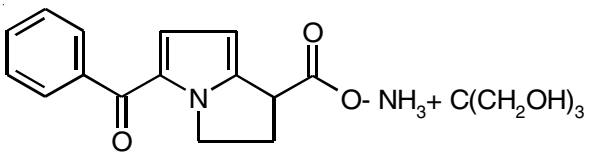

Fig. 2. Chemical structure of ketorolac tromethamine

used principally as analgesic in the short-term management of moderate to severe postoperative pain [4].

The assay of febuxostat is not official in IP, BP and USP. A survey of literature revealed that few methods have been reported for the estimation of febuxostat [5-8] and ketorolac tromethamine and for combination with other drugs [9-13] in bulk and pharmaceutical formulations. Few spectrophotometric

[14] and reverse phase-high performance liquid chromatography (RP-HPLC) [15] methods have been published for simultaneous estimation of febuxostat and ketorolac tromethamine. But there has not been reported any stability indicating liquid chromatography method for febuxostat and ketorolac tromethamine in combined pharmaceutical dosage form. Hence, an attempt has been made to assess the degradation behaviour of febuxostat and ketorolac tromethamine under hydrolytic, oxidative, photolytic and thermal stressed conditions by stability indicating RP-HPLC method and to validate the developed method as per ICH guidelines [16].

EXPERIMENTAL

Working standards of febuxostat was procured from Unimark Remedies, Vapi (Gujarat, India) and ketorolac tromethamine was procured from Dr. Reddy's Laboratories, Hyderabad (Telangana, India) as a gift sample. Acetonitrile (AR grade), potassium dihydrogen phosphate, orthophosphoric acid (OPA), sodium hydroxide (1 N NaOH), hydrochloric acid (1 N HCl), hydrogen peroxide (3 % H_2O_2) were purchased from Merck Specialist Pvt. Ltd. (Mumbai, India). Methanol (AR grade) was purchased from Ranchem RFCL Ltd. (New Delhi, India). Tablets with a label claim of 40 mg febuxostat and 15 mg ketorolac tromethamine were purchased from local pharmacy store.

Instrumentation and chromatographic conditions: The isocratic HPLC system SHIMADZU LC-2010 with auto sampler and photodiode array (PDA) detector was used for HPLC analysis. The analytical balance (BL-220H, Shimadzu Corporation, Japan) was used for weighing. Ll 610-Elico pH-meter was used for pH measurement. Sonication of mobile phase and other reagents was done on an ultra-sonicator (FS 4, Frantline, lndia). A UV chamber was used for photo degradation study.

Agilent eclipus C18 (250 × 4.6 mm, 5 µm particle size) column was used to optimize the chromatographic experimental conditions. An isocratic mobile phase (phosphate buffer (KH_2PO_4): acetonitrile (60:40 v/v), pH = 4 adjusted with orthophosphoric acid), flow rate of 0.9 mL/min and a temperature of 25 °C, were the final optimized chromatographic conditions for the method development. The mobile phase was filtered using a 0.45 µm micro porous filter and was degassed by sonication prior to use. A wavelength of 321 nm was chosen for the determination of both the active ingredients. 20 µL of sample volume was injected into the chromatographic system for the chromatogram development. Liquid chromatography solution software was used for data analysis.

Preparation of standard stock solutions: 40 mg of febuxostat and 15 mg of ketorolac tromethamine, each of which was accurately weighed and transferred into a 100 mL of clean and dry volumetric flask. Methanol was added as a diluent into it. Sonication was performed to dissolve the standard drug and filtered through 0.45 µm micro porous filter. Final volume was made upto the mark with diluent to prepare standard stock solution of 400 µg/mL of febuxostat and 150 µg/mL of ketorolac tromethamine. These solutions were further diluted with the diluent to give a series of working standards of required strength for both the drugs.

Preparation of working standards: Working standard of febuxostat and ketorolac tromethamine were prepared by taking 1.25, 1.88, 2.50, 3.13 and 3.75 mL of stock solution into a 10 mL of volumetric flask and volume was made upto the mark with diluent (methanol) to obtain final concentrations of 50, 75, 100, 125 and 150 µg/mL for febuxostat and 18.75, 28.13, 37.5, 46.88 and 56.62 µg/mL for ketorolac tromethamine, respectively. Solutions were filtered through a 0.45 µm membrane filter prior to injection.

Preparation of sample solution: Twenty tablets were accurately weighed and crushed into a fined powder. Powder equivalent to 40 mg of febuxostat and 15 mg of ketorolac tromethamine was accurately transferred into a 100 mL volumetric flask and diluent was added into it. Sonication was performed for 10 min to enable complete dissolution of febuxostat and ketorolac tromethamine. The solution was filtered through 0.45 µm micro porous filter and the residues were washed thoroughly with diluent. The filtrate and washings were combined in a 100 mL of volumetric flask and was diluted upto the mark with diluent. From filtered solution, 2.5 mL was pipette out into a 10 mL volumetric flask and volume was made upto the mark with diluent to give a final concentration 100 µg/mL of febuxostat and 37.50 µg/mL of ketorolac tromethamine. 20 µL of this solution was spiked into the system and the peak area was recorded from the respective chromatogram.

System suitability: System suitability parameters were evaluated to verify that the analytical system was working properly and would give accurate and precise results. Parameters such as tailing factor, resolution between febuxostat and ketorolac tromethamine and % RSD of theoretical area were calculated.

Method validation: Method validation was carried out as per ICH guidelines [16] for the following parameters: linearity, specificity, accuracy, precision, robustness, limit of quantitation (LOQ), limit of detection (LOD) and system suitability.

Linearity: Linearity study was carried out for both the drugs at five different concentration levels and calibration curves were constructed over the concentration range of 50-150 µg/mL for febuxostat and 18.75-56.25 µg/mL for ketorolac tromethamine, respectively.

Specificity: To check the specificity of the method, excipients such as lactose monohydrate, magnesium stearate, microcrystalline cellulose, hydroxyl propyl cellulose were spiked in a pre-weighed quantity of febuxostat (40 mg) and ketorolac tromethamine (15 mg). Final solutions were prepared in the range of 50-150 µg/mL and 18.75-56.25 µg/mL of febuxostat and ketorolac tromethamine, respectively. Then area was measured and a calculation was done to determine % interference.

Accuracy: Accuracy of the assay method was evaluated in triplicate at three concentration levels (50, 100 and 150 %) of targeted solutions and the percentage recoveries were calculated. The study was carried out in triplicate at 75, 100 and 125 mg/mL for febuxostat and 28.13, 37.50 and 46.88 µg/mL for ketorolac tromethamine.

Precision: Precision was performed by means of repeatability, intra-day and inter-day precision. Six replicates of standard mixture solution having 100 µg/mL of febuxostat and 37.5 µg/mL of ketorolac tromethamine were prepared and %

RSD was calculated for recorded chromatogram. The intra-day precision was evaluated at three concentration levels (75, 100 and 125 µg/mL for febuxostat and 28.13, 37.50 and 46.88 µg/mL for ketorolac tromethamine) (n = 3) against a qualified reference standard on the same day. The inter-day precision study was performed on three consecutive days at three different concentration levels (75, 100 and 125 mg/mL for febuxostat and 28.13, 37.50 and 46.88 µg/mL for ketorolac tromethamine) (n = 3). Standard deviation (SD) and % RSD was calculated.

Robustness: Robustness study was established by introducing small changes in the liquid chromatographic conditions such as percentage change of acetonitirile in the mobile phase (39.5 and 40.5) and flow rate (0.87 and 0.93 mL/min). Robustness of the method was studied using three replicates at a concentration level of 100 µg/mL febuxostat and 37.50 µg/mL ketorolac tromethamine. System suitability parameters *i.e.* mean peak area, tailing factor and resolution were calculated.

Limit of detection (LOD) and limit of quantitation (LOQ): Calibration curve was repeated for 6 times and the standard deviation (SD) of the intercepts was calculated. LOD and LOQ were calculated using formula LOD = (3.3XSD)/slope and LOQ = (10XSD)/slope.

Forced degradation study: Forced degradation study was intended to ensure the effective separation of febuxostat, ketorolac tromethamine and their degradation peaks from formulation ingredients at their retention times. It was carried out under various stressed conditions such as hydrolysis (acidic and basic), oxidative, thermal and photolytic to check degradation behaviour [17].

Standard drug stock solutions: Standard drug stock solutions were prepared as per the procedure given before.

Stock solution of standard febuxostat (400 µg/mL) and ketorolac tromethamine (150 µg/mL) were prepared separately in diluent and were diluted further for forced degradation studies to provide an indication of stability properties of both the drugs and specificity of the proposed method.

Acid degradation: 2.5 mL of standard stock solution of febuxostat and ketorolac tromethamine, each in separate 10 mL of amber volumetric flask was taken to perform forced degradation in acidic media. Then 1mL of 1 N HCl was added and the solutions of febuxostat and ketorolac tromethamine were kept separately for 2 h at room temperature for degradation. Degraded samples were then neutralized using 1 N NaOH before liquid chromatography analysis.

Alkali degradation: Alkali degradation was performed by taking 2.5 mL of standard stock solution of febuxostat and ketorolac tromethamine, each in separate 10 mL of amber volumetric flask. Then 1 mL of 1 N NaOH was added and the solutions of febuxostat and ketorolac tromethamine were kept separately for 30 min at room temperature for degradation. Degraded samples were then neutralized using 1 N HCl before liquid chromatography analysis.

Oxidative degradation: 3 mL of 3 % H_2O_2 was added into 2.5 mL of standard stock solution of febuxostat and ketorolac tromethamine, each in separate 10 mL of volumetric flasks and were kept for 8 h at room temperature for oxidative degradation.

Thermal degradation: 2.5 mL standard stock solution of febuxostat and ketorolac tromethamine was taken separately in 10 mL of volumetric flasks. To these, LC grade water was added upto the mark and volumetric flasks were kept in oven for 3 h at 105 °C temperature for thermal degradation.

Photo degradation: Standard stock solution of febuxostat and ketorolac tromethamine, 2.5 mL separately in volumetric flasks were exposed to UV radiation for 21 h in UV chamber for photo degradation.

For chromatographic analysis, degraded samples were diluted with diluent to obtain final concentration of 100 and 37.5 µg/mL of febuxostat and ketorolac tromethamine, respectively. Besides these, solutions of the same strength were also prepared separately without performing the degradation of both of the drugs. 20 µL volumes of the above solutions were injected into the HPLC system and analyzed under optimized chromatographic conditions. Chromatograms were recorded and % degradation of drug was calculated. Same procedure was repeated for drug product.

Assay of febuxostat and ketorolac tromethamine in marketed dosage form: A marketed formulation (40 mg of febuxostat and 15 mg of ketorolac tromethamine as an active pharmaceutical ingredient) was dissolved into a diluent to prepare a sample solution as per the procedure explained as above. 2.5 mL of above solution was pipetted out and diluted to a concentration of 100 µg/mL of febuxostat and 37.5 µg/mL of ketorolac tromethamine with diluent. Each of the solutions was prepared in triplicate. Chromatograms were recorded and the percent assay was calculated in terms of recovery studies.

RESULTS AND DISCUSSION

Method development and optimization: To develop an efficient and simple stability indicating chromatographic method for the assay of febuxostat and ketorolac tromethamine in combination, a number of liquid chromatographic trials were investigated to select the suitable and optimum conditions. Chromatographic parameters (detection wavelength, ideal mobile phase and their proportions, flow rate and column temperature) were carefully studied and were finally chosen based on the criteria of peak properties like height, area, retention time and peak symmetry. 321 nm wavelength was selected as a detection wavelength of febuxostat and ketorolac tromethamine as it has shown significance absorbance for both the drugs.

Optimized chromatographic conditions as mentioned above were used to get a symmetrical peak with good resolution and acceptable retention time.

Fig. 3 shows a typical chromatogram of placebo at the optimized conditions and Fig. 4 shows a typical HPLC chromatogram of the freshly prepared standard mixture of febuxostat (100 µg/mL) and ketorolac tromethamine (37.50 µg/mL).

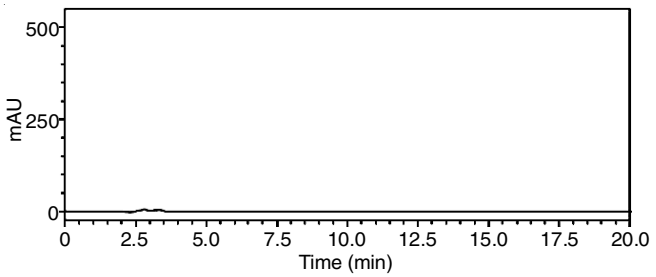

Fig. 3. Placebo chromatogram at the optimized conditions

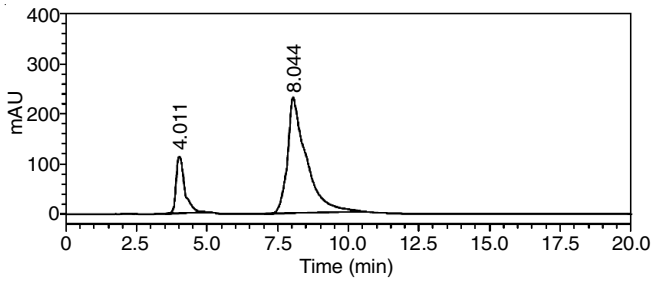

Fig. 4. Typical chromatogram of a standard mixture of 100 µg/mL of febuxostat (8.044 min) and 37.5 µg/mL of ketorolac tromethamine (4.011 min)

System suitability: Six replicates of the standard solutions of febuxostat (100 µg/mL) and ketorolac tromethamine (37.5 µg/mL) were injected for system suitability study and were analyzed for their retention time, peak area, theoretical plates, plates per meter, height equivalent to theoretical plate and peak asymmetry. The system suitability results revealed as % RSD which were less than 2.0 % for all the parameters. Table-1 showed that the proposed method meets the accepted requirements.

TABLE-1
SYSTEM SUITABILITY PARAMETERS

Parameters	Proposed method		Standard value
	Febuxostat	Ketorolac tromethamine	
Retention time (R_t)	7.933 min	3.797 min	–
Resolution (R_s)	–	7.203	< 2.0
Theoretical plates (N)	3086	2870	< 2000
Tailing factor (T)	1.491	1.730	> 2.0

Linearity: Calibration curves were constructed over the concentration range of 50-150 µg/mL and 18.37-56.25 µg/mL for febuxostat and ketorolac tromethamine, respectively. The peak area for each concentration was obtained by injecting 20 µL of the working standard solutions of both the drugs into the column. The linearity was assessed by the least square regression method. The regression line was linear with R^2 of 0.9982 and 0.9984 for febuxostat and ketorolac tromethamine, respectively. Linearity data is shown in Table-2.

Specificity: Percentage interference of febuxostat and ketorolac tromethamine (before and after addition of excipients) in the standard solutions of both the drugs was calculated to determine the specificity. It was within acceptance criteria as per ICH guidelines *i.e.* less than 1 %. Data shown in Table-3 indicates that the proposed method for assessment of febuxostat and ketorolac tromethamine is specific in nature.

TABLE-2
LINEARITY DATA OF FEBUXOSTAT AND KETOROLAC TROMETHAMINE

Concentration (µg/mL)		Peak area (n = 3)	
Febuxostat	Ketorolac tromethamine	Febuxostat	Ketorolac tromethamine
50	18.37	4254926	1101799
75	28.13	6692530	1834275
100	37.50	9177731	2462877
125	46.88	12010345	3037417
150	56.25	14059342	3692106

TABLE-3
SPECIFICITY DATA FOR FEBUXOSTAT AND KETOROLAC TROMETHAMINE

Drug	Conc. (µg/mL)	Area before addition of excipients	Area after addition of excipients	Interference (%)
Febuxostat	50	4256135	4255871	0.013
	75	6687304	66855425	0.028
	100	10081317	10075943	0.053
	125	12152390	12151495	0.007
	150	15226105	15217281	0.057
Ketorolac tromethamine	18.75	1102238	1102481	0.022
	28.13	1836285	1837417	0.061
	37.50	2461786	2460898	0.036
	46.88	3035714	3037112	0.046
	56.25	3692241	3691004	0.033

Accuracy: Percentage recovery for both the drugs was within acceptance criteria *i.e.* 98-102 % and % RSD not more than 2.0. Table-4 describes % recovery data that shows method is accurate.

Precision: Standard deviation and % RSD of febuxostat and ketorolac tromethamine was within acceptance criteria *i.e.* less than 1 that indicates the intended method is precised in nature. Repeatability, intraday precision and inter-day precision data are shown in Tables 5-7, respectively.

Robustness: Robustness study suggested that all the parameters-peak area, tailing factor, resolution have no significant influence on the determination. Results indicate that the

TABLE-5
REPEATABILITY DATA FOR FEBUXOSTAT AND KETOROLAC TROMETHAMINE

Drug	Target conc. (µg/mL) (n = 6)	Peak area (n = 6)	Mean conc. (µg/mL) ± SD	RSD (%)
Febuxostat	100	2463446	100.05 ± 0.92	0.92
Ketorolac tromethamine	37.5	10586680	37.73 ± 0.31	0.84

TABLE-4
ACCURACY DATA FOR FEBUXOSTAT AND KETOROLAC TROMETHAMINE

Drug	Level of recovery	Amount of spiked standard solution (µg/mL)	Total conc. (µg/mL)	Peak area of sample (n = 3)	Amount of drug found (µg/mL) (n = 3)	Mean % recovery ± SD (n = 3)
Febuxostat	50 % [50]	25	75	6696079	75.10	100.13 ± 0.21
	100 % [50]	50	100	10499367	99.80	99.80 ± 0.24
	150 % [50]	75	125	12165548	125.11	100.09 ± 0.10
Ketorolac tromethamine	50 % [18.75]	9.37	28.13	1830094	28.03	99.56 ± 1.31
	100 % [18.75]	18.75	37.50	2462284	37.50	100.77 ± 0.46
	150 % [18.75]	28.12	46.88	3036132	46.87	100.26 ± 0.35

TABLE-6
INTRA-DAY PRECISION DATA FOR FEBUXOSTAT AND KETOROLAC TROMETHAMINE

Drug	Target conc. (µg/mL) (n = 3)	Peak area (n = 3)	Mean conc. (µg/mL) ± SD	RSD (%)
Febuxostat	75	6681347	74.94 ± 0.06	0.08
	100	10526474	100.04 ± 0.39	0.39
	125	12165699	125.12 ± 0.59	0.47
Ketorolac tromethamine	28.13	1830094	28.03 ± 0.09	0.35
	37.50	2462951	37.54 ± 0.10	0.28
	46.88	3036132	46.88 ± 0.17	0.36

TABLE-7
INTER-DAY PRECISION DATA FOR FEBUXOSTAT AND KETOROLAC TROMETHAMINE

Drug	Target conc. (µg/mL) (n = 3)	Peak area (n = 3)	Mean conc. (µg/mL) ± SD	RSD (%)
Febuxostat	75	6683681	99.96 ± 0.15	0.15
	100	10509841	99.71 ± 0.85	0.85
	125	12172365	125.11 ± 0.82	0.66
Ketorolac tromethamine	28.13	1825760	27.97 ± 0.15	0.56
	37.50	2462284	37.50 ± 0.14	0.39
	46.88	3037799	46.90 ± 0.20	0.43

selected factors remained unaffected by small variation of these parameters. % RSD was less than 2, which demonstrates that the suggested method is robust. The variations imposed on the chromatographic method are summarized in Table-8.

Limit of detection and limit of quantification for febuxostat and ketorolac tromethamine: The proposed method can detect and quantify small amount of drugs precisely. Limit of detection and limit of quantification data presented in Table-9 recommended that the offered method is very sensitive in nature.

TABLE-9
LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ) FOR FEBUXOSTAT AND KETOROLAC TROMETHAMINE

Drug	LOD (µg/mL)	LOQ (µg/mL)
Febuxostat	0.246	0.741
Ketorolac tromethamine	0.062	0.186

Forced degradation study: Forced degradation study was carried out under various stressed conditions like acid hydrolysis (1 N HCl), base hydrolysis (1 N NaOH), oxidation (3 % H_2O_2), thermal and photolysis. Stressed sample solutions were analyzed against the freshly prepared standards and samples. Percentage degradation for the stressed standards and sample solutions were calculated and are summarized in Table-10. The degradation product is listed in Table-11.

Table-10 reveals that febuxostat and ketorolac tromethamine showed extensive degradation under acidic and basic hydrolytic conditions, whereas least degradation of febuxostat and ketorolac tromethamine showed under thermal and oxidative conditions, respectively. Resolution for both of the active ingredients was found to be greater than 2.0. There was no interference between the main active ingredients and any other stressed impurity peaks in the chromatogram.

Figs. 5-10 show the chromatographic profiles of the active ingredients and the degradation products after exposing the prepared sample solution to different stressed conditions - acid hydrolysis, alkali hydrolysis, oxidation, thermal and photolytic degradation, respectively.

Applicability of method to Marketed tablet formulation: The results revealed that the validated method gave satisfactory results with respect to the analysis of both drugs. It was applied to marketed tablet formulation as shown in Table-12.

This acceptable value indicated that the applicability of the proposed method for routine quality control of the tablet

TABLE-8
ROBUSTNESS DATA OF FEBUXOSTAT AND KETOROLAC TROMETHAMINE

Parameters varied	Drug name	System suitability parameters (n = 3)		
		Mean peak area ± % RSD	Tailing factor ± % RSD	Resolution ± % RSD
At flow rate 0.87 mL/min	Febuxostat	1052662 ± 0.17	1.470± 1.29	7.406 ± 0.25
	Ketorolac tromethamine	2461407 ± 0.08	1.767 ± 1.26	
At flow rate 0.93 mL/min	Febuxostat	1051940 ± 0.10	1.462 ± 1.38	7.478 ± 1.35
	Ketorolac tromethamine	2462826 ± 0.05	1.767 ± 1.30	
At mobile phase Buffer:Acetonitrile (39.5+60.5)	Febuxostat	1052688 ± 0.15	1.466 ± 1.02	7.743± 1.68
	Ketorolac tromethamine	2461642 ± 0.06	1.771 ± 0.95	
At mobile phase Buffer:Acetonitrile (40.5+59.5)	Febuxostat	1052900 ± 0.10	1.541 ± 1.68	7.843 ± 0.79
	Ketorolac tromethamine	2460995 ± 0.04	1.819 ± 0.84	
Proposed method	Febuxostat	10542754 ± 0.38	1.437 ± 1.54	7.273 ± 0.84
	Ketorolac tromethamine	2445568 ± 0.35	1.757 ± 1.80	

TABLE-10
FORCED DEGRADATION DATA OF FEBUXOSTAT AND KETOROLAC TROMETHAMINE (API AND TABLET SOLUTION)

Conditions of forced degradation	% Degradation of API		% Degradation of formulation	
	Febuxostat	Ketorolac tromethamine	Febuxostat	Ketorolac tromethamine
1 N HCl, reflux, 2 h	45.88	47.24	45.47	47.36
1 N NaOH, RT, 0.5 h	59.89	44.54	59.57	44.39
3 % w/v H_2O_2, 8 h	10.25	3.88	10.02	3.41
Thermal, 3 h at 105 °C	9.72	11.91	9.51	12.01
Photolytic, UV radiation for 21 h	10.07	14.42	10.19	14.22

TABLE-11
FORCED DEGRADATION PRODUCT OF FEBUXOSTAT AND KETOROLAC TROMETHAMINE TABLET SOLUTION

Conditions of forced degradation	Febuxostat		Ketorolac tromethamine	
	RT of drug	RT of degradation products	RT of drug	RT of degradation products
1 N HCl, reflux, 2 h	8.496	10.129, 13.020	4.368	5.253
1 N NaOH, RT, 0.5 h	8.225	10.651	3.837	5.118
3 % w/v H_2O_2, 8 h	7.942	9.354	3.893	5.487
Thermal, 3 h at 105 °C	7.970	10.034	3.970	5.325
Photolytic, UV radiation for 21 h	8.093	9.463	4.089	–

TABLE-12
PERCENTAGE ASSAY OF MARKETED TABLET FORMULATION

Drug	Concentration of dosage form	Concentration taken for assay (µg/mL)	Concentration found (µg/mL) ± SD (n = 3)	% Assay ± SD (n = 3)
Febuxostat	40 mg	100	100.39 ± 0.13	100.39 ± 0.13
Ketorolac tromethamine	15 mg	37	37.97 ± 0.34	100.24 ± 0.21

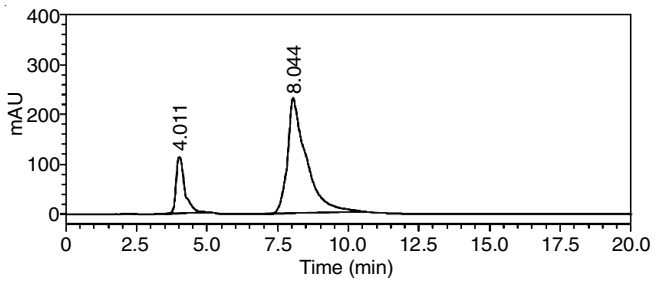

Fig. 5. HPLC chromatogram of sample under normal chromatographic condition - febuxostat 100 µg/mL and ketorolac tromethamine 37.5 µg/mL

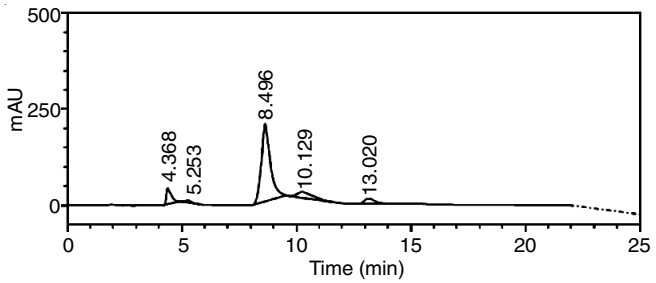

Fig. 6. HPLC chromatogram of acidic degradation (1 N HCl) of the tablet solution of febuxostat and ketorolac tromethamine after refluxing for 2 h

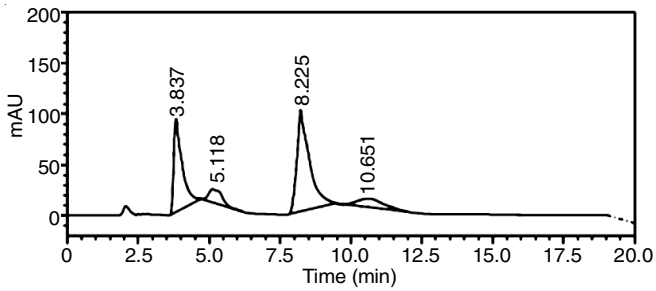

Fig. 7. HPLC chromatogram of alkali degradation (1 N NaOH) of the tablet solution of febuxostat and ketorolac tromethamine after refluxing for 0.5 h

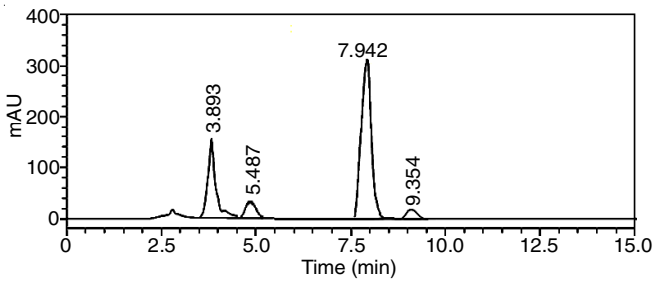

Fig. 8. HPLC chromatogram of oxidative degradation (3 % H_2O_2) of the tablet solution of febuxostat and ketorolac tromethamine after refluxing for 8 h

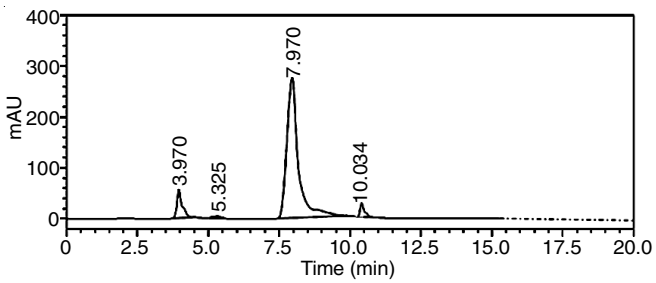

Fig. 9. HPLC chromatogram of thermal degradation of the tablet solution of febuxostat and ketorolac tromethamine after 3 h at 105 °C

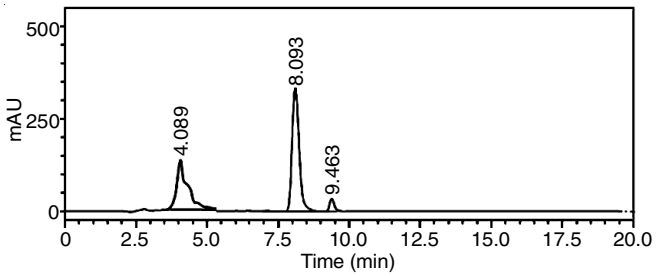

Fig. 10. HPLC chromatogram of photolytic degradation of the tablet solution of febuxostat and ketorolac tromethamine at UV radiation for 21 h

formulation without interference from the excipients. This was evidenced by the good labeled claim percentages as well as the absence of any placebo peaks in chromatogram.

Conclusion

In this study, the developed stability indicating liquid chromatographic assay method was found to be suitable for simultaneous assessment of febuxostat and ketorolac tromethamine in their pure and bulk forms. It was found to be simple, reliable, sensitive, economical and precise which could separate the drugs and their degraded products formed under various stressed conditions. The developed method was evaluated for validation parameters and the results were within the acceptance criteria as per ICH guidelines. Therefore, the

proposed liquid chromatographic method can be used for the quantitative quality control and routine analysis of the pharmaceutical dosage forms.

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