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ORIGINAL ARTICLE

Development and Validation of a Novel Method for the Simultaneous Estimation of Ketorolac and Febuxostat in bulk and tablet formulation by RP-HPLC

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ABSTRACT

An efficient, rapid, precise, selective, and accurate RP-HPLC technique was developed and validated for the simultaneous quantification of Febuxostat and Ketorolac from bulk substances and formulations. Isocratic chromatographic separation was performed on a Waters PhenomenexC_{18} column (150x3.9 µm i.d; particle size 4 µm) with a mobile phase composed of phosphate buffer in water pH-5.8 adjusted with O-Phosphoric Acid: Methanol in the isocratic mode (40:60 v/v). The flow rate was 1.2 ml/min, and the effluent was seen at 321 nm. The retention times for Febuxostat and Ketorolac were 1.923 minutes and 3.104 minutes, respectively. Linearity was detected in the concentration ranges of 20-60 µg/mL for Febuxostat and 5-15 µg/mL for Ketorolac, both exhibiting a correlation value of 0.999. The percent recoveries for the two medicines were 100.16-101.49% and 99.35-101.75%, respectively. The technique was verified in accordance with ICH requirements for specificity, linearity, accuracy, precision, and robustness. The outcomes conformed to the acceptance requirements. The suggested techniques were deemed adequate and may be used for the regular analysis of Febuxostat and Ketorolac in their formulations.

Keywords: *RP-HPLC Method; UV-VIS detection; Febuxostat and Ketorolac; Tablet dosage forms.*

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INTRODUCTION

Ketorolac is a nonsteroidal anti-inflammatory medication (NSAID) having analgesic and antipyretic characteristics, used for the treatment of osteoarthritis and the management of acute pain. It is an analgesic that acts peripherally. The S-form of ketorolac is responsible for its biological action. Ketorolac lacks sedative or anxiolytic effects. Ketorolac is a nonsteroidal anti-inflammatory medication (NSAID) chemically associated with indomethacin and tolmetin. Ketorolac is a racemic combination of the [-]S- and [+]R- enantiomers, with the S-form exhibiting analgesic properties. The anti-inflammatory effects are attributed to the inhibition of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), resulting in reduced prostaglandin synthesis and diminished production of prostaglandin and thromboxane precursors from arachidonic acid. The consequent decrease in prostaglandin production and activity may be somewhat accountable for several unfavorable and therapeutic effects of these drugs[1,2].

Febuxostat is an inhibitor of xanthine oxidase (XO) suggested for the long-term control of hyperuricemia in individuals with gout. An inhibitor of xanthine oxidase. The principal component of Febuxostat is 2-[3-cyano-4-(2-methylpropoxy)phenyl]-4-methylthiazole-5-carboxylic acid (Fig. 1B). It received approval

from the European Medicines Agency in 2008 and the FDA for the therapy of hyperuricemia in patients with gout. Febuxostat is a selective non-purine inhibitor of xanthine oxidase. It functions by non-competitively inhibiting the molybdenum pterin center, the active site of xanthine oxidase. Xanthine oxidase is required to sequentially oxidize both hypoxanthine and xanthine to uric acid. Consequently, Febuxostat inhibits xanthine oxidase, hence reducing uric acid synthesis. Febuxostat inhibits both the oxidized and reduced forms of xanthine oxidase, making it difficult to displace from the molybdenum pterin site[3,4].

This study concentrates on the establishment of a straightforward and accurate analytical procedure using RP-HPLC for tablet dosage formulations of Febuxostat (40 mg) and Ketorolac (10 mg).

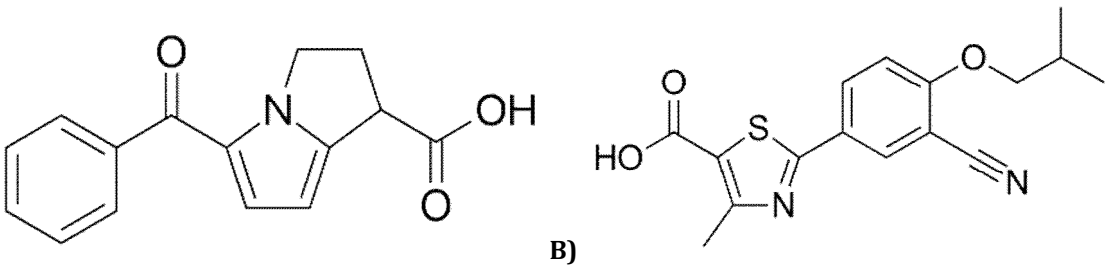

Fig. 1: Chemical structures of A) Ketorolac and B) Febuxostat.

MATERIALS AND METHODS

Chemicals and Reagents

A pure sample of Ketorolac (Active Pharmaceutical Ingredient), 99% purity, with a molecular weight of 255.26 g/mol, was obtained from Mylan Laboratories Pvt. Ltd, Hyderabad, India. A pure sample of Febuxostat, 99% purity, with a molecular weight of 316.37 g/mol, was acquired from Sun Pharmaceuticals Private Limited, Chennai, India.

Instruments

The list of instruments used in the research was given in Table 1.

Table 1: Instruments used

UV-Visible Spectrophotometer	Nicolet evolution 100
HPLC software	Spin chrome (LC SOLUTIONS)
Ultra sonicator	Citizen, Digital Ultrasonic Cleaner
pH meter	Global digital
Electronic balance	Shimadzu
Syringe	Hamilton
HPLC Column	A PhenomenexC_{18} column (150x3.9 µm i.d; particle size 4 µm)

Preparation of Standard Drug Solution

10 mg of Ketorolac and Febuxostat RS were weighed and dissolved in 70 ml and 7 ml of the mobile phase, respectively, and transferred to 100 ml and 10 ml volumetric flasks, followed by sonication for 20 minutes to achieve a concentration of 1000 ppm. Subsequently, 1.0 ml and 0.4 ml of the aforementioned stock solution of Ketorolac and Febuxostat were pipetted into a 10 ml volumetric flask with the mobile phase to obtain concentrations of 40 µg/mL for Febuxostat and 10 µg/mL for Ketorolac.

Preparation of the Sample Solution

Ten tablets of Febuxostat and Ketorolac were weighed and then pulverized. A sample of powdered tablets, corresponding to a mixture with concentrations of 1000 µg/mL of Febuxostat and 100 µg/mL of Ketorolac active ingredients, was combined with 7 mL and 70 mL of Phosphate buffer: HPLC Grade Methanol at pH 5.8, in 10 mL and 100 mL volumetric flasks. The mixture was allowed to stand for one hour with intermittent sonication to ensure complete drug solubility, then filtered through a 0.45 µm membrane filter. Methanol was added to achieve a total volume of 10 mL, resulting in a stock solution of 40 µg/mL of Febuxostat and 10 µg/mL of Ketorolac. A 10 mL aliquot of this stock solution was transferred to a volumetric flask and diluted with a suitable diluent to obtain concentrations of 40 µg/mL of Febuxostat and 10 µg/mL of Ketorolac.

RESULTS AND DISCUSSION

Analytical Method Development

The aim of this experiment was to enhance the assay technique for the quantification of Febuxostat and Ketorolac, informed by a literature review. The experiments described above outline the optimization process, with the resultant optimum conditions shown in Table 2 (Figure 2).

Table 2: Optimized chromatographic conditions

Parameter	Content
Mobile Phase	Phosphate buffer in water pH-5.8 adjusted with O-Phosphoric Acid: Methanol in the isocratic mode (40:60 v/v)
Column	A PhenomenexC_{18} column (150x3.9 µm i.d; particle size 4 µm)
Run time	8 minutes
Detection and Wavelength	PDA Detector, 321 nm
Flow Rate	1.2 ml/min
Temperature	Ambient
Injection Volume	20 µl
Retention times	1.9302 minutes for Febuxostat and 3.104 minutes for Ketorolac

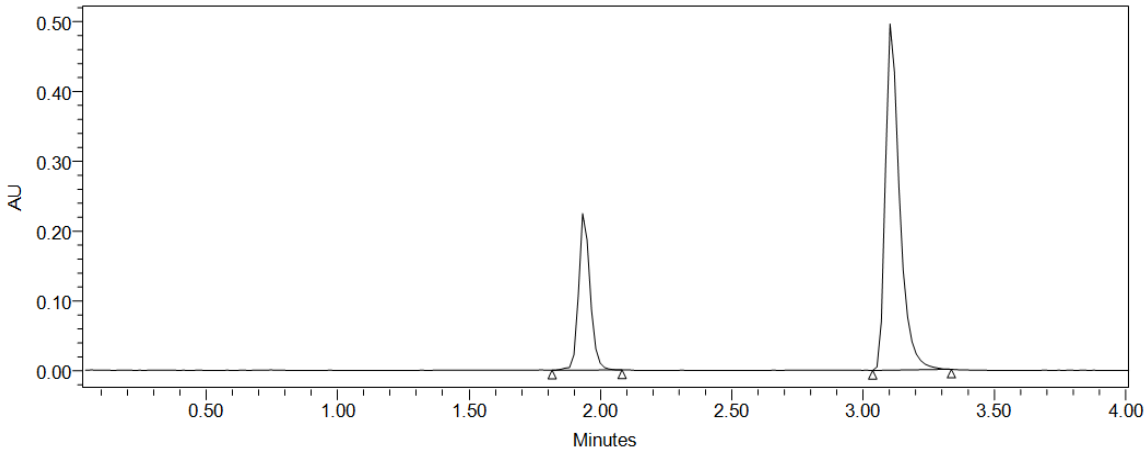

Figure 2: Optimized Chromatogram of Febuxostat and Ketorolac.

Method validation

Precision

Separately from the peak area that was acquired by the actual determination of six replicas of a fixed quantity of the medication and formulation, the accuracy of the procedure was determined [5-8]. After establishing the HPLC systems, the chromatographic conditions were described as described above. Additionally, the system was equilibrated using a working standard solution that included 40 µg/mL of Febuxostat and 10 µg/ml of Ketorolac. This was accomplished by injecting the solution six times and recording the response peak areas, as shown in Table 3.

Table 3: Precision of Febuxostat and Ketorolac.

Injection Number	Name of the Drug & Concentration (40µg/ml)	Retentiontime in minutes	PeakArea	Name of the Drug & Concentration (10 µg/ml)	Retentiontime in minutes	PeakArea
1	Febuxostat	1.929	202642	Ketorolac	3.107	2341636
2	Febuxostat	1.932	201775	Ketorolac	3.104	2330078
3	Febuxostat	1.928	202796	Ketorolac	3.114	2327547
4	Febuxostat	1.929	205616	Ketorolac	3.114	2334985
5	Febuxostat	1.931	208228	Ketorolac	3.114	2335565
Mean		1.928	204263		3.1106	2333974
Standard Deviation			2691.25			5447.47
% RSD			1.27			0.34

Accuracy

Different quantities of bulk samples of Febuxostat and Ketorolac that were within the linearity limits were collected and analysed using the suggested technique [9-11]. This was done in order to evaluate the correctness of the proposed approach. Both Febuxostat and Ketorolac was found to have percentage test values of 100.56% and 99.24%, respectively at the time of the analysis.

Linearity

As shown in Tables 4 and 5, aliquots of the primary standard Febuxostat and Ketorolac stock solution were taken from a variety of 10 ml volumetric flasks and diluted up to the mark with the mobile phase. This was done in order to ensure that the final concentrations of Febuxostat and Ketorolac were within the range of 20 to 60 µg/mL and 5 to 15 µg/mL, respectively, for linearity. Every single one of these drug solutions, which had a volume of twenty microliters, was injected into the column three times, and the peak areas and retention periods were recorded [12-15]. The PDA detector was used to conduct the evaluation at a wavelength of 321 nm. A calibration curve graph was created by graphing the peak area against the concentration of Febuxostat, as shown in Figures 3 and 4 (Table 4).

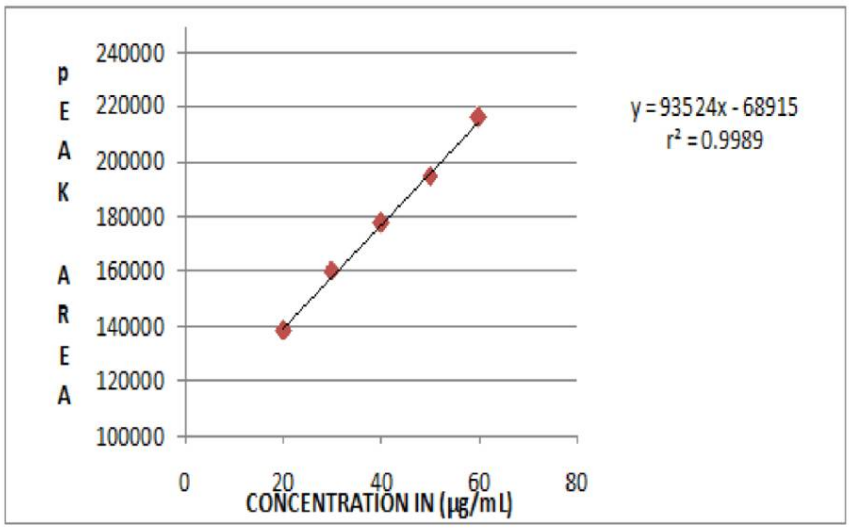

Figure 3: Standard Calibration Curve of Febuxostat

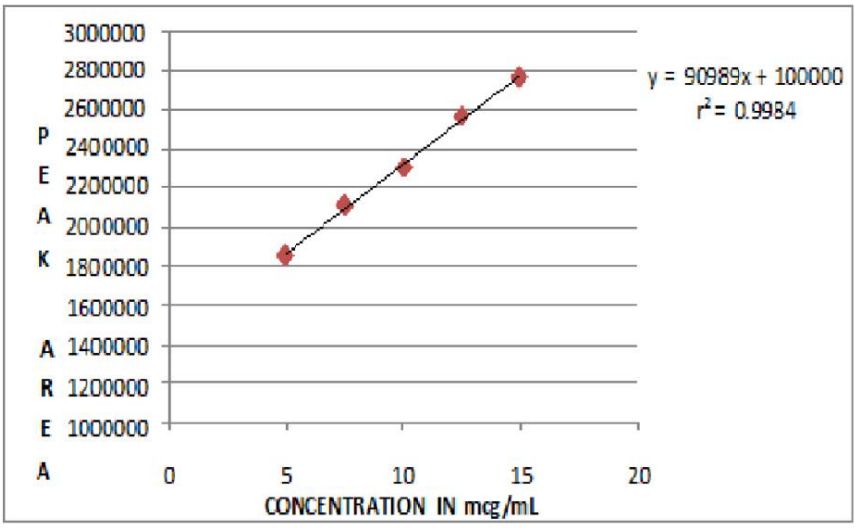

Figure 4: Standard Calibration Curve of Ketorolac

Table. 4: Calibration data of Febuxostat and Ketorolac

Febuxostat		Ketorolac	
Concentration of (µg/mL)	**Peak Area**	**Concentration (µg/mL)**	**Peak Area**
20	138235	5.0	1857074
30	159787	7.5	2098365
40	177674	10.0	2294617
50	194552	12.5	2558295
60	215995	15.0	2764564

Analysis of Formulations

A pharmaceutical formulation that included Febuxostat and Ketorolac was examined using the suggested technique in order to determine whether or not the method is suitable for the assay of the pharmaceutical formulation. A comparison between the suggested approach and the reference method revealed that there was not a significant difference in terms of precision and accuracy between the two.

Recovery Studies

In order to conduct recovery experiments, the formulations were first analyzed for the active ingredients at concentrations of 50%, 100% (40 µg/mL of Febuxostat and 10 µg/mL of Ketorolac), and 150% of the working standard solution. This was done by using the suggested technique. There were three separate administrations of each concentration, and the peak areas were recorded separately. After incorporating the specified quantity of the pure drug from the working standard solution into each of the three formulations that had been previously evaluated, the total drug content was re-evaluated using the proposed method (each concentration was injected three times). This was done to ensure that the concentration of the active ingredient remained within the linearity limits. Figures 5 through 7 demonstrate the chromatograms, which may be found in Table 5.

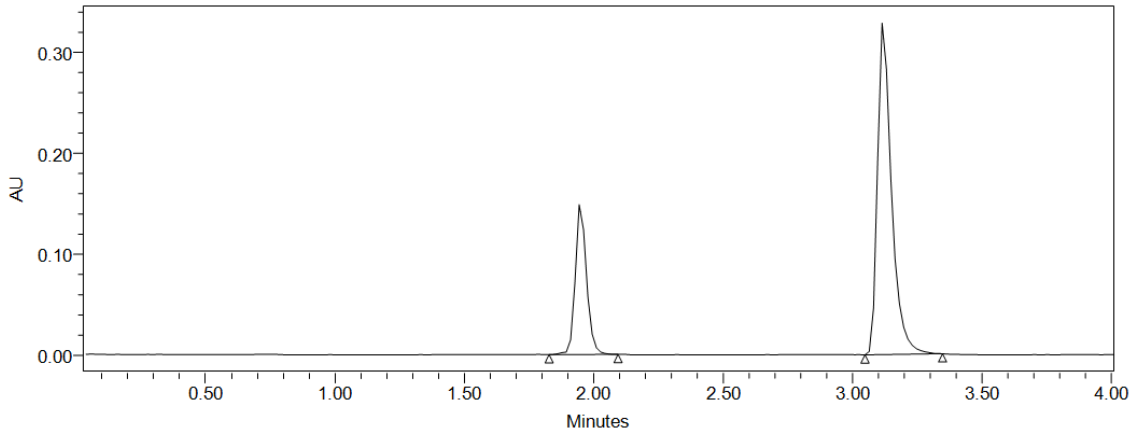

Figure 5: Chromatograms illustrating Recovery studies (50%)

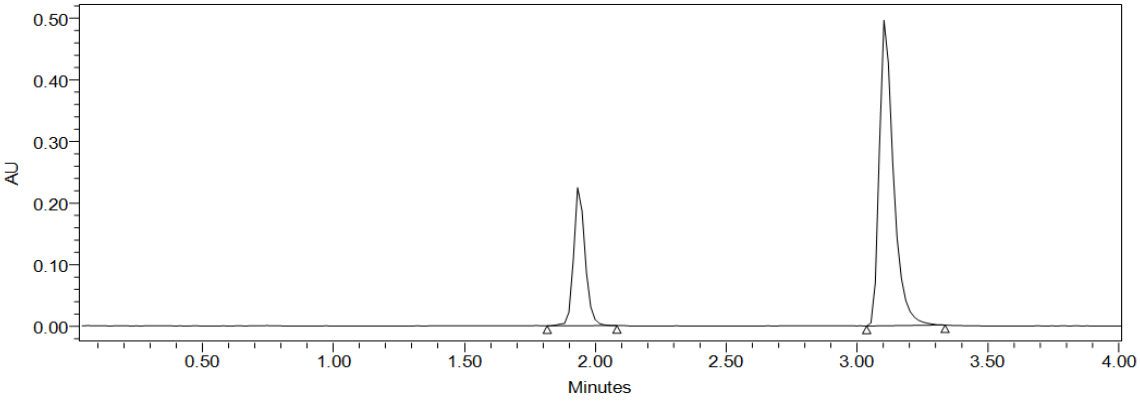

Figure 6: Chromatograms illustrating Recovery studies (100%)

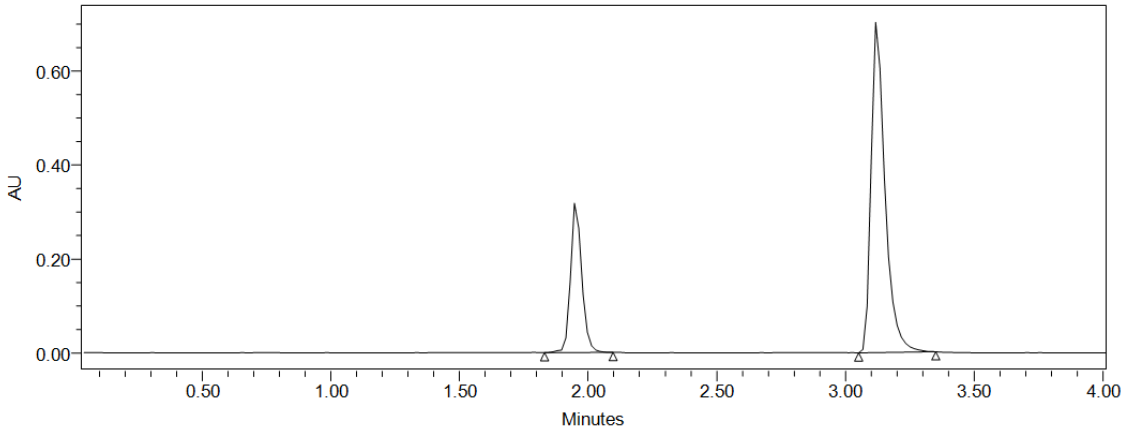

Figure 7: Chromatograms illustrating Recovery studies (150%)

Table 5: Recovery data for Febuxostat and Keterolac

Febuxostat					
%Concentration (at specification Level)	**Area**	**Amount Added (mg)**	**Amount recovered (mg)**	**% Recovery**	**Mean Recovery**
50%	1164141	5.0	5.06	101.14%	101.00%
100%	2331832	10.0	10.21	101.58%	
150%	3451985	15.0	15.16	100.62%	
Keterolac					
50%	93794	5.0	5.14	101.13 %	100.84%
100%	188174	10.0	9.74	101.65 %	
150%	275564	15.0	14.63	99.78%	

Robustness

Effect of variation of flow rate

Research was carried out to assess the impact of flow rate fluctuation. A standard solution was generated according to the test procedure and injected into the HPLC system at flow rates of 0.8 mL/min and 1.2 mL/min. The system suitability characteristics were assessed and determined to be within the acceptable limits for flow rates of 0.8 mL/min and 1.2 mL/min. Febuxostat (FEB) and ketorolac (KET) were distinguished from all other peaks, and their retention durations were consistent with those recorded for the mobile phase at a flow rate of 1.0 mL/min. The Tailing Factor for FEB and KET standards must not exceed 2.0 for flow variation.

Limit of detection (LOD) and limit of quantification

The method's detection limit was assessed by introducing reference solutions onto the HPLC column. The signal-to-noise (S/N) technique quantifies the peak-to-peak noise around the analyte retention time. Subsequently, the concentration of the analyte that would provide a signal corresponding to a certain noise-to-signal ratio was also determined. A signal-to-noise ratio (S/N) of 3 is often accepted for calculating the limit of detection (LOD), whereas a S/N ratio of 10 is used for measuring the limit of quantification (LOQ).

The Limit of Quantification (LOQ) may be established by a signal-to-noise ratio of 10:1 or estimated by multiplying the Limit of Detection (LOD) by 3. This technique is often used in analytical approaches that demonstrate baseline noise [16-18]. The limit of detection (LOD) was determined to be 0.04 µg/mL for Febuxostat and 0.12 µg/mL for Ketorolac. The limit of quantification (LOQ) was determined to be 0.16 µg/mL for Febuxostat and 0.42 µg/mL for Ketorolac. Figures 8 and 9 [Table 6] provide chromatograms that depict the LOD and LOQ with the peak locations.

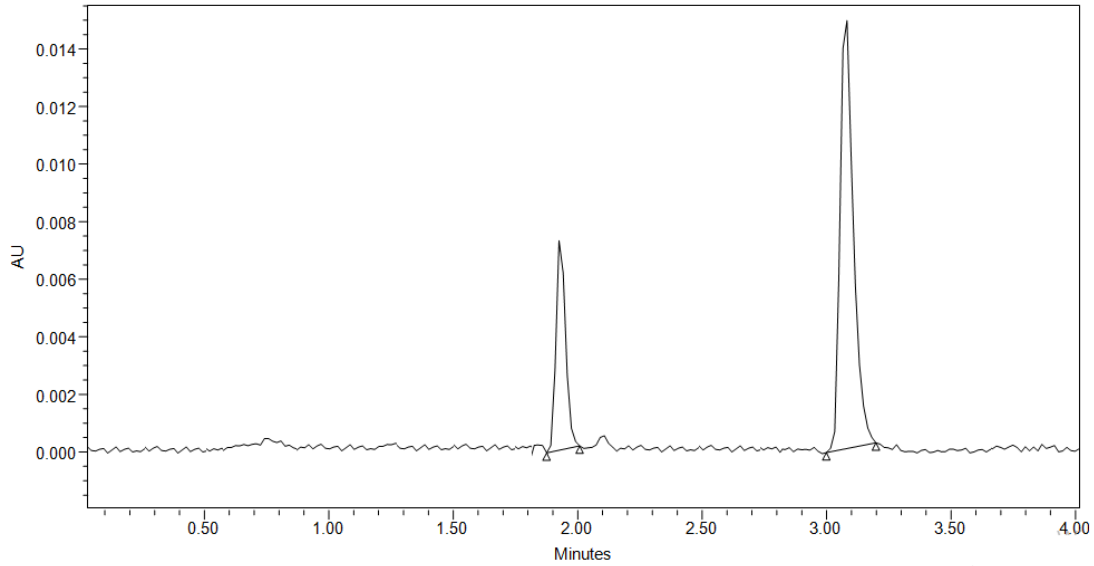

Figure 8: Chromatograms illustrating LOD of Febuxostat and Ketorolac

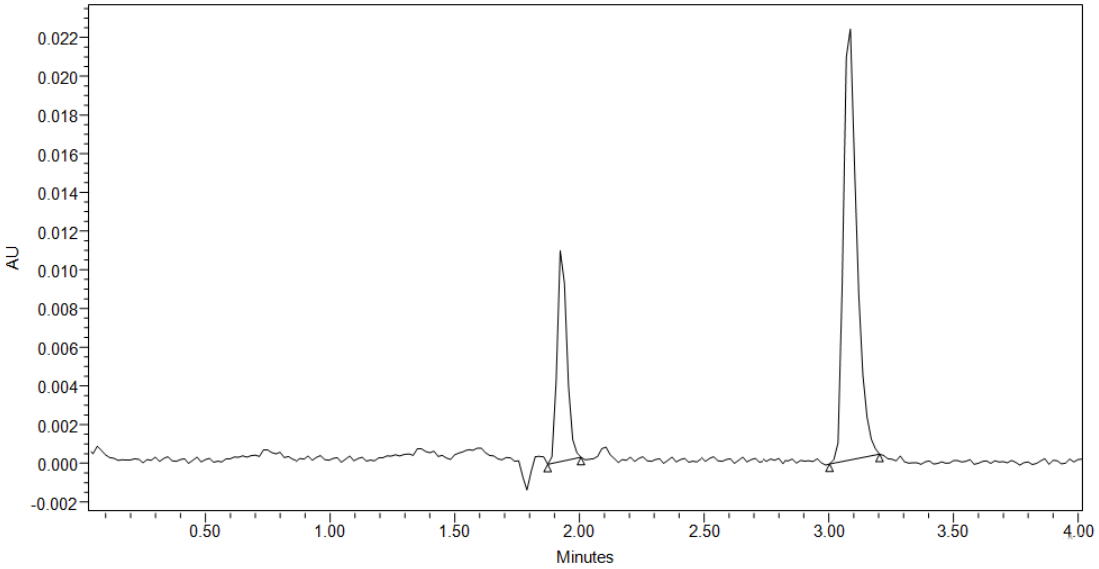

Figure 9: Chromatograms illustrating LOQ of Febuxostat and Ketorolac.

Table 6: Summary of Validation data of Febuxostat and Ketorolac

Parameters	Results	
	Febuxostat	Ketorolac
Wave Length (λ max)	321 nm	321 nm
Linearity range(µg/mL)	20-60	5-15
Limit of detection (µg /ml)	0.04	0.12
Coefficient of determination	0.9991 ± 0.01	0.9991± 0.01
% Recovery (n = 3)	101.00	100.84
Limit of quantification (µg /ml)	0.16	0.42
LOD	3.02	2.98
Precision (RSD [%])	1.32	0.23
LOQ	9.97	9.98

CONCLUSION

Prior to the initiation of this study, there were only a limited number of documented techniques for the RP-HPLC determination of Febuxostat and Ketorolac in tablet dosage forms in the literature. The author has devised a sensitive, accurate, and exact RP-HPLC method for the quantification of Febuxostat and Ketorolac in bulk substances and pharmaceutical formulations. The typical chromatogram for Febuxostat

and Ketorolac, shown in Figures 6.1-6.3, indicates retention periods of 1.923 minutes for Febuxostat and 3.104 minutes for Ketorolac. A combination of Disodium hydrogen phosphate dehydrates and Potassium Dihydrogen Phosphate in HPLC-grade water, with pH adjusted to 5.2 using 1% Ortho-Phosphoric Acid: Methanol (20:80 v/v), was identified as the optimal solvent for elution, yielding well-defined peaks devoid of tailing, in accordance with ICH guidelines. The HPLC approach required little time for standard and sample preparations, eliminating the need for cumbersome extraction procedures. A strong linear correlation (r = 0.9998) was noted between the concentration ranges of 20-60 µg/mL for Febuxostat and 5-15 µg/mL for Ketorolac, respectively.

The minimal standard deviations indicate the method's great accuracy. The test results for Febuxostat and Ketorolac were determined to be 99.24% and 100.56%, respectively. The recovery trials indicated that around 101.00% of Febuxostat and 100.84% of Ketorolac were recovered, demonstrating the method's great accuracy.

The lack of supplementary peaks in the chromatogram signified the absence of interference from the standard excipients used in the tablets. The new RP-HPLC technique is proved to be simple, linear, accurate, sensitive, and repeatable. Consequently, the established approach may be readily used for the regular quality control of bulk and pharmaceutical formulations of Febuxostat and Ketorolac, requiring little analytical time.

The findings indicate that the suggested approach demonstrates high precision and accuracy. The investigation of pharmaceutical formulations shown that the suggested methodologies are appropriate for their evaluation, exhibiting little influence from typical additives included in these formulations.

The aforementioned suggested procedure eliminates the need for any preparatory treatment and is straightforward, sensitive, and dependable. This method is applicable for the regular quantification of Febuxostat and Ketorolac in bulk materials as well as in pharmaceutical formulations. The current processes represent the RP-HPLC approach characterized by high precision, accuracy, and sensitivity for the simultaneous quantification of Febuxostat and Ketorolac in both pure and pharmaceutical formulations.

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