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RESEARCH ARTICLE

A VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF FEBUXOSTAT AND KETOROLAC TROMETHAMINE IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple, fast, precise, selective and accurate RP-HPLC method was developed and validated for the simultaneous determination of Febuxostat and Ketorolac from bulk and formulations. Chromatographic separation was achieved isocratically on a Waters C_{18} column (250×4.6 mm, 5 µ particle size) using a mobile phase, Methanol and Ammonium acetate buffer (adjusted to pH 6.0 with 1% orthophosphoric acid) in the ratio of 60:40. The flow rate was 1 ml/min and effluent was detected at 321nm. The retention time of Febuxostat and Ketorolac were 2.62 min and 3.96 min. respectively. Linearity was observed in the concentration range of 5-30µg/ml and 10-60 µg/ml for Febuxostat and Ketorolac respectively with correlation coefficient 0.999 for both the drugs. Percent recoveries obtained for both the drugs were 99.94-99.22% and 99.44-101.06%, respectively. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness. The method developed can be used for the routine analysis of Febuxostat and Ketorolac from their combined dosage form.

Key words: RP-HPLC Method; UV-VIS detection; Febuxostat and Keterolac; Tablet dosage forms.

INTRODUCTION

Febuxostat chemically is 2- [3- cyano-4- (2-methlypropoxy) phenyl] - 4- methlythiazole- 5 -carboxylic acid[1] (**Figure 1**). It is a non purine selective inhibitor of xanthine oxidase that is indicated for use in the treatment of hyperuricemia and gout. In contrast to allopurinol, febuxostat inhibits both oxidized and reduced forms of xanthine oxidase and has minimal effects on other enzymes of purine and pyrimidine metabolism 3, 4. A study comparing febuxostat to allopurinol found that more individuals treated with febuxostat had decreased levels of uric acid, but there was no difference in the amount of initial gout flares or the surface area of gout tophi[2].

Ketorolac tromethamine

Systematic (IUPAC) name: (±)-5-benzoyl-2, 3- dihydro-1H-pyrrolizine-1-carboxylic acid, 2- amino-2 (hydroxymethyl)-1, 3-propanediol[1, 3] (**Figure 2**).

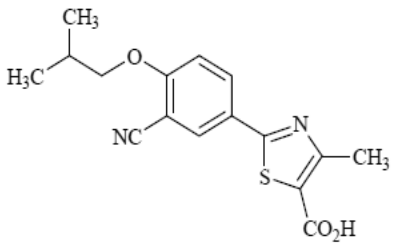

Figure 1: Chemical structure of Febuxostat

Ketorolac tromethamine (Brand name **Toradol and Acular**) is a non-steroidal anti-inflammatory drug (NSAID) in the family of heterocyclic acetic acid derivative, often used as an analgesic, antipyretic and anti-inflammatory.

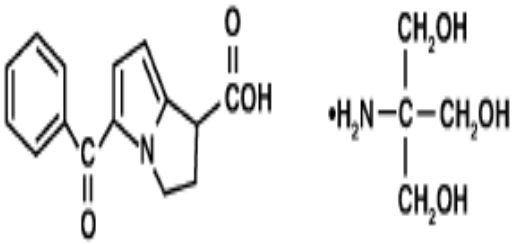

Figure 2: Chemical structure of Keterolac

Ketorolac acts by inhibiting the bodily synthesis of prostagladins. Both Febuxostat and Ketorolac have been analyzed by various techniques either alone or in combination with other drugs. Several methods have been applied to determine Febuxostat involving spectrophotometry[4], HPLC [5,] LC/MS[6].

Literature review revealed different analytical methods such as UV Method[7], HPLC[8-11] and HPTLC[12-13] for the quantitative determination of Ketorolac. The present work deals with analytical evaluation of ketorolac in tablets by HPLC[14] using UV-VIS Detector. The main objective of the work was to develop simple, fast, sensitive and accurate method.

In this paper we reported a sensitive liquid chromatographic method with wide linearity range, good resolution where running time is less than 10 mins. The method has been validated as per the ICH guidelines [15-16] and can be successfully applied to pharmaceutical formulations.

MATERIALS AND METHODS

Reagents and chemicals

Methanol HPLC grade was procured from E.Merck Ltd, Mumbai. Methanol, orthophosphoric acid, Ammonium acetate buffer AR grade were procured from S.D. fine chemicals, Hyderabad. Water HPLC grade was prepared using Millipore purification system. Febuxostat and Keterolac reference standards procured from Dr.Reddy's laboratories, Hyderabad.

Instrumentation

The HPLC system consists of water Empower 2487 having photodiode array detector system, which was connected with the help of Empower-2 software for data integration and processing. Inertsil ODS-3V (250 X 4.6 mm) 5µ column was used for the analysis.

HPLC conditions

The contents of the mobile phase were Methanol and Ammonium acetate buffer (adjusted to pH 6.0 with 1% orthophosphoric acid) in the ratio of 60:40. These were filtered through 0.45µ membrane filter and degassed by sonication before use. The flow rate of mobile phase was optimized to 1.0 ml / min. The run time was set at 10 min and column temperature was maintained at ambient. The volume of injection was 10µl, and the eluent was detected at 321nm. Each of standard and test preparations was injected into the column and the responses recorded (Figure 3 and 4).

METHOD

The RP-HPLC Method of Febuxostat and Ketorolac were achieved by isocratic elution technique with UV –VIS Detector. FBT and KTC were determined at 321nm respectively with the concentration range of 5-30µg/ml for FBT and 10-60µg/ml for KTC respectively.fig.03 &04. For analysis of tablet formulation, the tablet powder equivalent to 25 mg was taken, dissolved in 25 ml volumetric flask and made up to 25ml with Methanol. The solution was sonicated for 15min, centrifuged at 100 rpm for 15 min and filtered through Whatmann filter paper No.41. From clear solution, further dilutions were made to get 10 µg/ml of FBT and KTC theoretically.

Preparation of standard stock solution

Standard stock solutions Febuxostat and Ketorolac of strength 1mg/ml were prepared using dichloromethane. Appropriate amounts of these stock solutions were then further diluted to get the required concentrations of standard stock solutions.

Table 1: It shows system suitability parameters

Parameters	Febuxostat	Keterolac
Theoretical plates	34075.88	15192.87
Asymmetry Factor	1.05	1.15
HETP (cm)	0.00075	0.00162
Resolution*		4.63

System suitability studies

The resolution, number of theoretical plates, retention time and peak asymmetry were calculated for the working standard solutions and is as shown in Table 1.

The values obtained demonstrated the suitability of the system for the analysis of these drugs in combination. The typical chromatogram of standard solution is as shown in Figure 3.

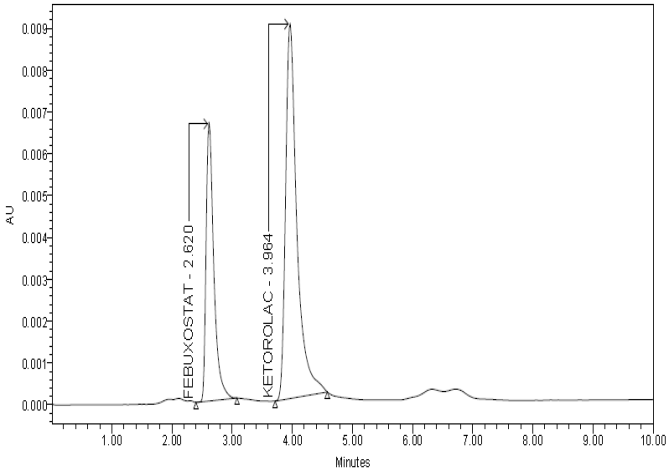

Figure 3: Typical chromatogram of Febuxostat and Keterolac

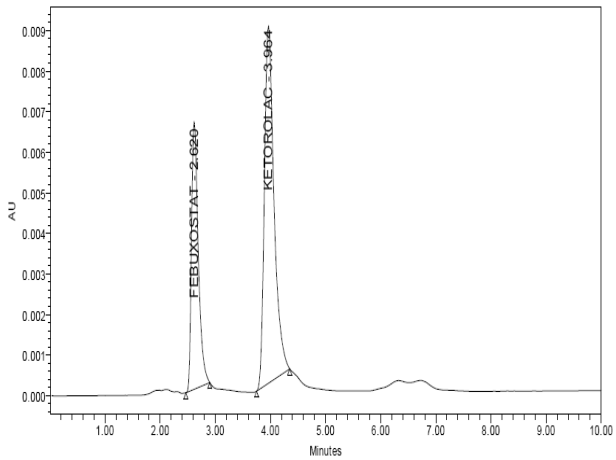

Figure 4: Typical chromatograms for recovery studies

ASSAY

Preparation of sample solutions

Twenty tablets were weighed and powdered. Powder equivalent to 10 mg of Febuxostat was weighed and transferred to 10 ml volumetric flask. Keterolac about 8 ml was added and sonicated for 10 min, volume was made up with the same solvent. This solution was then filtered through membrane filter paper. Further dilutions were made in dichloromethane to get concentrations in Beers law range. The retention times of Febuxostat and Ketorolac were found to be 2.62 ± 0.02 and 3.96 ± 0.03 respectively. The assay was calculated from the equation of regression line for each drug. The percentage assay of individual drug was calculated and presented in Table 2.

Table 2: It shows assay of tablet

Drug	Amount Present (mg/tab)	Amount Found ((mg/tab)	% Label Claim
Febuxostat Furic® Tablets	40	39.23	98.075
Keterolac	15	14.97	99.08

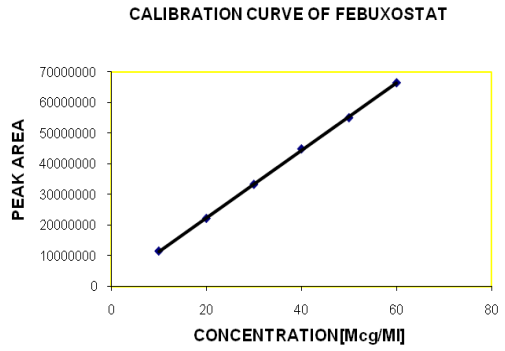

Figure 3: Calibration Graph of Febuxostat

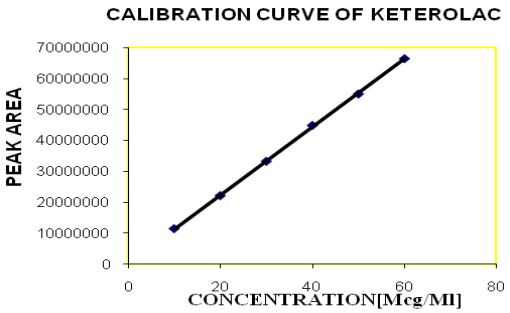

Figure 4: Calibration Graph of Keterolac

For recovery studies, to the reanalyzed formulation, solutions of raw material containing different concentrations were added and the amount of drug recovered was calculated.

The procedure was repeated as per the analysis of formulation. The amount of drug recovered was calculated by using slope and intercept values from the calibration graph. Finally the method was validated as per ICH guide lines for precision, accuracy, specificity, linearity, reproducibility, limit of detection and limit of quantification.

METHOD VALIDATION

As per ICH guidelines, the method validation parameters checked were specificity, linearity, precision, accuracy, limit of detection, limit of quantitation and robustness.

Specificity

A blank solution (mobile phase) was injected and the chromatogram showed no inferring peaks at retention time of the two drugs. The chromatogram of Febuxostat and Ketorolac extracted from the tablet were compared with those acquired from Febuxostat and Ketorolac standards, correlation was good (in terms of tR and area) indicates specificity of method. Common tablet excipients like starch, lactose, magnesium stearate were dispersed in dichloromethane, filtered and injected. There was no interference found.

Linearity and range

Aliquots of standard stock solutions of Febuxostat and Ketorolac were taken in 10 ml volumetric flasks and diluted with dichloromethane to get final concentrations in range of 5-30µg/ml for Febuxostat and 10-60µg/ml for Ketorolac. Triplicate injections were made five times for each concentration for each drug separately and chromato graphed under the conditions as described above. The plots of peak area versus respective concentrations of Febuxostat and Ketorolac were found to be linear in the concentration range of 5-30µg/ml and 10-60µg/ml respectively. The linear regression equations of the lines are:

For Febuxostat -y = 48266x + 491236, (r^2 = 0.9996)

For Ketorolac -y = 82864x + 163170, (r^2 = 0.9993)

Precision

Precision study was performed to find out intra-day and inter-day variations. The percent relative standard deviation for intra-day precision was 0.288% for Febuxostat and 0.232% for and Ketorolac and inter-day precision was 1.137% for Febuxostat and 1.323% for Ketorolac. Both the values were well within the limit of 2% as per ICH guidelines.

Accuracy

The accuracy was determined by recovery studies. The recovery studies were performed by standard addition method, at 80%, 100%, 120% level. Percent recovered was calculated using regression equation. For both the drugs, recovery was performed in same way and in triplicate. The percentage recovery were calculated and presented in Table 3.

Limit of detection and limit of quantitation

The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value.

$$LOD = \frac{3.3 \times \text{Standard deviation of y intercept}}{\text{Slope of calibration curve}}$$

Febuxostat - 1.13µg/ml
Keterolac - 0.23µg/ml

The LOQ is the lowest amount of analyte in the sample that can be quantitatively determined with suitable precision and accuracy.

$$LOQ = \frac{10 \times \text{Standard deviation of y intercept}}{\text{Slope of calibration curve}}$$

Febuxostat - 3.03µg/ml
Keterolac - 0.87µg/ml

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Robustness

Robustness of the method was determined by making slight deliberate changes in chromatographic conditions like 1% change in ratio of mobile phase constituents, ± 1nm change in detection wavelength and 0.05% change in flow rate. It was observed that there were no marked changes in the chromatogram. It suggests that the developed method is robust.

RESULTS AND DISCUSSION

The proposed method was found to be simple and sensitive with linearity in the concentration range of 5 to 30µg/ml for Febuxostat and 10 to 60µg/ml for Ketorolac. The method was found to be accurate and precise as indicated by results of recovery studies and %RSD not more than 2%. LOD and LOQ for Febuxostat were found to be 0.23µg/ml and 0.87µg/ml respectively and for Ketorolac were 1.13µg/ml and 3.03µg/ml respectively. The proposed method was found to be specific as there is no interference from common tablet excipients like lactose, starch etc.

Table 3: Recovery Studies

Drug	Sample No.	Amount % (µg/ml)	Amount added (µg/ml)	% Recovery*	S.D	% R.S.D
FBT	1	15.0235	5.00	97.94		
	2	15.0235	10.00	98.71	0.6433	0.6533
	3	15.0235	15.00	99.22		
KTC	1	30.0431	10.00	100.66		
	2	30.0431	20.00	99.44	0.8438	0.8406
	3	30.0431	30.00	101.06		

CONCLUSION

The results of the analysis of pharmaceutical dosage forms by the proposed methods are highly reproducible, reliable, and are in good agreement with the label claims of the drug. The additives usually present in the pharmaceutical formulations of the assayed samples did not interfere with Febuxostat and Ketorolac. It may be said that the proposed methods are precise, sensitive, and accurate, so that these can be used as standard pharmacopoeial methods for the simultaneous determination of Febuxostat and Ketorolac in tablets using the HPLC systems. The advantages of the proposed method involve a simple procedure for sample preparation and relatively short time of analysis. Apart from this, it can be used for assays of Febuxostat and Ketorolac in biological fluids or in pharmacokinetic investigations.

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DECLARATION OF INTEREST:

The authors have no conflicts of interest.

REFERENCE:

1. Available from: http//www.rx list.com/Febuxostat/Ketorolac.
2. The Merck Index, 14th ed., Whitehouse Station, NJ: Merck Research Laboratories Division of Merck and Co., Inc.; 2006, p. 491, 4781.
3. The Merck Index, 14th ed., Whitehouse Station, NJ: Merck Research Laboratories Division of Merck and Co., Inc.; 2006, p. 4781
4. H. H. Siddiqui, Abdul M. Ansari, Tariq Mehmood "A Simple Uv Spectrophotometric Method for the Determination of Febuxostat in Bulk and Pharmaceutical Formulations" IJPSR, 2011; Vol. 2(10).
5. ZHANG Cong,WANG Shao-jie,MA Rong-li,"Determination of content of febuxostat and its related substances by HPLC" Journal of Shenyang Pharmaceutical University,2010 27 (08).
6. ZHOU Ying, DING Li, LIU Xiao-xue "Study on Urinary Excretion Characteristics of Febuxostat in Healthy Chinese Volunteers" Chinese pharmaceutical journal-2011vol.46.
7. Loretta Franceschi, Sara D'aronco and Mario FurlanutA Simple and Sensisitive HPLC Method to Monitor Serum and Synovial Fluid Concentrations of Ketorolac in Reumathologic Patients" Journal of Bioanalysis & Biomedicine- (2010), Volume 2(6): 121-124.
8. Wu AT, Massey IJ "Simultaneous Determination of ketorolac and its hydroxylated metabolite in plasma by high-performance liquid chromatography," Journal of Chromatography, vol. 534, pp. 241–246, 1990.
9. Harshal P. Mehta, Rajesh Y. Chaudhari, Vijay R.Patil "Simultanious Spectrophotometric Estimation of Ofloxacin and Ketorolac Tromethamine in Ophthalmic Dosage Form. International Journal of ChemTech Research, Vol.1, No.2, 189-194, April-June 2009
10. R. S. Chaudhary, S. S. Gangwal, K. C. Jindal, and S. Khanna, "Reversed-phase high-performance liquid chromatography of ketorolac and its application to bioequivalent studies in human serum," Journal of Chromatography B, vol. 614, no. 1, pp. 180–184, 1993
11. K. R. Ing-Lorenzini, J. A. Desmeules, M. Besson, J.-L. Veuthey, P. Dayer, and Y. Daali, "Two-dimensional liquid chromatography-ion trap mass spectrometry for the simultaneous determination of ketorolac enantiomers and paracetamol in human plasma. Application to a pharmacokinetic study," Journal of Chromatography A, 2009, vol. II.
12. Ch.S.G. Teja, D.V.N.S. Radhika, K. Kavitha "Revalidation and analytical evaluation of ketorolac Tromethamine by HPTLC using reflectance scanning densitometry" IJRPC 2011, 1(2)
13. Sejal P.Gandhi, Mohit G.Dewani, Tejas C. Borole "Development and Validation of Stability Indicating HPTLC Method for Determination of Ofloxacin and Ketorolac Tromethamine in Combination" Journal of Advanced Scientific Research, 2011, 2(3).
14. R.I. Snyder, J.J. Kirkland, J.L Glajch, Practical HPLC Method development, Published By John Wiley and Son, Inc, New York, 2ndEdn., 1997, pp.21-57.
15. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedures: Methodology, ICH Steering Committee, Geneva, Switzerland, 1996.
16. ICH Validation of analytical procedures: text and methodology Q2 (R1), International Conference on Harmonization, 2005.

