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DESIGN AND DEVELOPMENT OF NOVEL RP-HPLC METHOD FOR THE ESTIMATION OF ANTI-ARTHRITIC DRUGS.

M. Jyothi*, D. Lahari, K. Girija, B. Charan Kumar, A. Naveena, M. Yogendra Varma.

Department of Pharmaceutical Analysis, Sun Institute of Pharmaceutical Education and Research, Kakupalli, Nellore-524346, AP, Indi

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ABSTRACT

The present study is focused on the design and development of a new simultaneous RP-HPLC technique for the estimation of Febuxostat and Lesinurad in synthetic mixtures, as well as the validation of the approach as it is implemented. The determination of Febuxostat and Lesinurad was accomplished by the use of RP-HPLC. The assay for Febuxostat and Lesinurad was carried out using a synthetic combination, and the percent assay results were determined to be 99.93 and 99.95, respectively, demonstrating that the approach is suitable for routine testing. In addition, the linearity of Febuxostat and Lesinurad was shown to be linear, with correlation coefficients ranging between 0.999 and 0.999, demonstrating that the approach is capable of creating high sensitivity. This approach may be utilised for regular analysis since the sample recoveries in all formulations were in excellent agreement with their corresponding amounts. It may be used for normal laboratory analysis and is ideal for quality control of raw materials, formulations, dissolution studies, and bioequivalence studies for the same formulation. It can also be used for bioequivalence studies for the same formulation.

Keywords: Febuxostat, Lesinurad, Linearity, RPHPLC.

INTRODUCTION

Analytical chemistry is the science that seeks ever improved means of measuring the chemical composition of natural and artificial materials. A chromatographic separation involves the placing of a sample onto a liquid or solid stationary phase and passing a liquid or gaseous mobile phase through or over it, a process known as elution. High performance liquid chromatography is a very sensitive analytical technique most widely used for quantitative and qualitative analysis of pharmaceuticals. The principle advantage of HPLC compared to classical column chromatography is improved resolution of the separated substance, faster separation times and the increased accuracy, precision and sensitivity [1].

HPLC involves a solid stationary phase, normally packed inside a stainless- steel column, and a liquid mobile phase. Separation of the components of a solution results from difference in the relative distribution ratios of the solutes between the two phases. The Reverse phase chromatography retention of a compound is determined by its polarity and experimental conditions, mobile phase, column and temperature [1].

Febuxostat is a newly introduced nonpurine xanthine oxidase inhibitor used for the treatment of gout. Chronic febuxostat therapy has been associated with minor serum aminotransferase elevations, but has yet to be linked to cases of clinically apparent acute liver injury [2].

Febuxostat is an orally available, non-purine inhibitor of xanthine oxidase with uric acid lowering activity. Lesinurad is an oral uric acid transporter 1 (URAT1) inhibitor indicated for the treatment of hyperuricemia associated with gout. It reduces serum uric acid concentration through the inhibition of URAT1, an enzyme responsible for reuptake of uric acid from the renal tubule, and OAT4, another uric acid transporter associated with diuretic-induced hyperuricemia [3,4]. So the current work aims for design and development of novel

Corresponding Author:- **M. Jyothi** Email:- mjyothipharmacy@gmail.com

simultaneous RP-HPLC method for the estimation of Febuxostat and Lesinurad in synthetic mixture and validation of the developed method. [2].

MATERIALS AND METHODS

Chemicals and Reagents

All the reagents in this assay along with triple distilled water were of analytical grade. Drugs were obtained as gift samples and the formulations were bought on the market.

Wave length selection:

UV spectrum of 10 µg/ml Febuxostat and 10 µg/ml Lesinurad in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 255 nm. At this wavelength both the drugs show good absorbance.

HPLC Method Development

Mobile Phase Optimization:

Initially the mobile phase tried was methanol: Ortho phosphoric acid buffer and Methanol: phosphate buffer, Acetonitrile: methanol with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to Phosphate buffer pH 3.0: Methanol in proportion 70: 30 v/v respectively [5].

Optimization of Column:

The method was performed with various columns like C18 column Phenomenex column, YMC, and Inertsil ODS column. Inertsil ODS (150 x 4.6, 5µm) was found to be ideal as it gave good peak shape and resolution at 1.5 ml/min flow [5].

Preparation of mobile phase:

Accurately measured 700 ml (70%) of above potassium hydrogen orthophosphare Buffer and 300 ml (30%) of Acetonitrile were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Assay:

Preparation of Synthetic Mixture of Standard Solution (SM):

Accurately weigh and transfer 300 mg of Febuxostat and 200 mg of Lesinurad working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.75 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent [5].

Procedure:

Inject 20 µL of the SM solution into the chromatographic system and measure the areas for Febuxostat and Lesinurad peaks and calculate the %Assay by using the formulae.

Calculation:

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Where,

AT = average area counts of sample preparation.
AS = average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P = Percentage purity of working standard
LC = Label Claim mg/ml.

Determination of Linearity

Accurately weigh and transfer 300 mg of Febuxostat and 200 mg of Lesinurad working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). 0.25ml, 0.5ml, 0.75ml, 1ml, 1.25ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration and calculate the correlation coefficient [5].

Table 1: Results of Assay for Febuxostat and Lesinurad

	Claim (mg)	% Assay
Febuxostat	300	99.95
Lesinurad	200	99.98

Table 2: Results of system suitability parameters

S.No	Name	RT(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Febuxostat	4.974	463741	34149	2.71	1.31	3119.41
2	Lesinurad	6.006	373269	23648		1.19	3419.52

Table 3. Area of different concentration of Febuxostat and Lesinurad

S. No	Febuxostat		Lesinurad	
	Conc (µg/ml)	Area	Conc (µg/ml)	Area
1	75	163131	50	123690
2	150	324880	100	258148
3	225	484985	150	374267
4	300	622091	200	500740
5	375	774840	250	622360

Table 4: Analytical performance parameters of Febuxostat and Lesinurad

Parameters	Febuxostat	Lesinurad
Slope (m)	2026.3	2480.1
Intercept (c)	17789	3859.9
Correlation coefficient (R^2)	0.998	0.995

RESULTS AND DISCUSSION

Optimization of the analytical parameters

Peak obtained with broad in shape in trial 1 and trial 2. The separation of two analytical peaks was not proper, the mobile phase ratio has been changed for next trial. In Trial 4 and 5 the separation of two analytical peaks was not proper, So the mobile phase ratio has been changed for next trial. In trial 6 the separation of two analytical peaks is occurred but fronting occurs in peak.

Assay of the selected drugs

Sample solution injected as described under experimental work. The corresponding chromatograms and results are shown in table 1.

Determination of system suitability

It was found from below data in table 2 that all the system suitability parameters for developed method were within the limit.

Validation of the analytical method

The linearity range was found to lie from 75µg/ml to 375µg/ml of Febuxostat, 50µg/ml to 250µg/ml of Lesinurad and chromatograms are shown below. Correlation coefficient (R^2) should not be less than 0.999. The correlation coefficient obtained was 0.999 which is in the acceptance limit.

CONCLUSION

The suggested HPLC approach for simultaneous quantification of Febuxostat and Lesinurad in a synthetic combination was found to be exact, specific, accurate, quick, and cost-effective for the estimation of both drugs. This approach may be utilised for regular analysis since the sample recoveries in all formulations were in excellent agreement with their corresponding amounts. It may be used for normal laboratory analysis and is ideal for quality control of raw materials, formulations, dissolution studies, and bioequivalence studies for the same formulation. It can also be used for bioequivalence studies for the same formulation.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest

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