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Research Article

DEVELOPMENT AND VALIDATION OF REVERSED-PHASE HPLC ISOCRATIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF LESINURAD AND ALLOPURINOL

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ABSTRACT

An isocratic reversed-phase liquid chromatograpic assay method was developed for the quantitative determination of Lesinurad and Allopurinol. A Inetsil C-18, 5 µm column with a mobile phase containing Acetonitrile: Methanol: 0.1% Triethylamine buffer (pH-adjusted to 3 using o-phosphoric acid) 25:35:40 (v/v/v). The flow rate was 1.0 mL/min and effluents were monitored at 250 nm. The retention times of oxycodone and naltrexone were 5.57 min and 2.60min, respectively. The proposed method was validated with respect to linearity, accuracy, precision, and robustness.

KEYWORDS: *RP-HPLC, Lesinurad, Allopurinol, Method validation.*

INTRODUCTION

Lesinurad (Fig. 1), a newer drug to treat hyperuricemia associated with refractory gout that functions by targeting the urate-anion exchanger transporter (URAT1), was approved by the US Food and Drug Administration (USFDA) in December 2015, for combination therapy with a xanthine oxidase inhibitor [1-5].

It was also approved by the European Medicines Agency's Committee for Medicinal Products for Human Use for this clinical indication throughout the European Union in February 2016. URAT1, a transmembrane protein that serves as a highly urate-specific and organic anion exchanger, is localized to the luminal membrane of the proximal tubular epithelial cells. All or nearly all uric acid is freely filtered at the glomerulus and most of the filtered urate is reabsorbed in the proximal tubule through URAT1. Lesinurad functions as a selective uric acid reabsorption inhibitor by inhibiting URAT1 and organic anion transporter 4 (OAT4), and so increases the urinary excretion of uric acid [6, 7].

Allopurinol (ALP), is 1,5-Dihydro-4H-Pyrazolo[3,4-d] pyrimidin-4-one (Fig. 1). It is an official drug in British (BP) and United States (USP) Pharmacopoeias which is used for treatment of gout and hyperuricaemia. It is a xanthine oxidase inhibitor, which prevents the oxidation of hypoxanthine to xanthine and xanthine to uric acid. Thus results in the reduction of urate and uric acid concentrations in plasma and urine [8-11].

Very few spectrophotometric and chromatographic assays were reported for Lesinurad (LES) and allopurinol (ALO) and detection so far. Therefore, the developed RP-HPLC method has the advantage of being more selective and sensitive than the published one [12-17].

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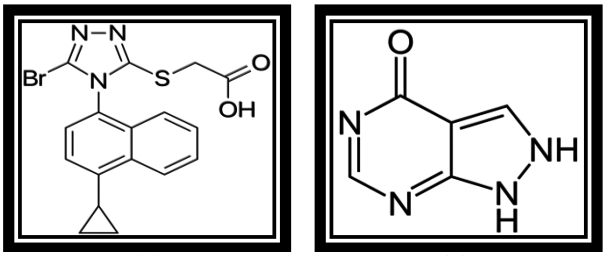
The proposed methods have been optimized and validated as per the International Conference on Harmonization (ICH) guidelines ICH, and were found to comply with the acceptance criteria [18-27].



(A) (B)

Fig. 1: Chemical Structures of A) Lesinurad (LES) B) Allopurinol (ALO)

MATERIALS AND METHODS

The Liquid chromatographic system consisted of Shimadzu-Model LC20AT, Spin chrome software HPLC with variable wavelength programmable UV/VIS detector and Rheodyne injector with 20 µL fixed loop. The analytes were monitored at 250nm. Chromatographic analysis was performed on Inertsil C-18 column having 250 × 4.6 mm i.d. and 5 µm particle size. All drugs and chemicals were weighed on Shimadzu electronic balance (AX 200, Shimadzu Corp., Japan).

1. Chemicals and Reagents:

Analytically pure samples of Lesinurad (LES) and allopurinol (ALO) were obtained as a gift samples from Alembic Pharmaceuticals Ltd (Baroda, India) HPLC grade methanol obtained from E. Merck Ltd., Mumbai, India while analytical reagent grade acetonitirle, methanol, triethyl amine (pH-adjusted to 3 using o-phosphoric acid) obtained from Astron Chemicals, India.

2. Chromatographic conditions:

A Inertsil C-18 (250×4.6 mm i.d) chromatographic column equilibrated with mobile phase Acetonitrile: Methanol: 0.1%

Triethylamine buffer (pH-adjusted to 3 using o-phosphoric acid) 25:35:40 (v/v/v) was used. Mobile phase flow rate was maintained at 1 mL min-1 and effluents were monitored at 250 nm. The sample was injected using a 20 µL fixed loop, and the total run time was 10 min.

3. Preparation of standard stock solutions:

Lesinurad (LES) and allopurinol (ALO) were weighed (25 mg each) and transferred to two separate 25 ml volumetric flasks and dissolved in methanol. Volumes were made up to the mark with methanol to yield a solution containing 1000 µg mL^{-1} of Lesinurad (LES) and allopurinol (ALO), respectively. Aliquots from the stock solutions of Lesinurad (LES) and allopurinol (ALO) were appropriately diluted with mobile phase to obtain working standard of 100 µg mL^{-1}.

4. Method Validation:

The developed method was validated for various parameters like linearity and range, accuracy, precision, robustness, system suitability, specificity, LOQ and LOD.

4.1. Linearity and Range:

Appropriate aliquots of Lesinurad (LES) and allopurinol (ALO) working standard solutions were taken in different 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentration range of 5-25 µg/ml for LES and 2-10 µg/ml for ALO. The solutions were injected using a 20 µL fixed loop system and chromatograms were recorded. Calibration curves were constructed by plotting average peak area versus concentrations and regression equations were computed for both drugs.

4.2. Precision:

The intra-day and inter-day precision studies were carried out by estimating the corresponding responses 3 times on the same day and on 3 different days for three different concentrations and the results were reported in terms of relative standard deviation. The instrumental precision studies were carried out by 3 different concentrations and results are reported in terms of relative standard deviation.

4.3. Accuracy:

The accuracy of the method was determined by calculating recoveries of Lesinurad (LES) and allopurinol (ALO) by method of standard additions. Known amount of Lesinurad (LES) and allopurinol (ALO) were added to a pre quantified sample solution and the amount of Lesinurad (LES) and allopurinol (ALO) were estimated by measuring the peak areas and by fitting these values to the straight-line equation of calibration curve.

4.4. Detection limit and Quantitation limit:

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated using the following equation as per ICH guidelines.

$$LOD = 3.3 \times s / S; \quad LOQ = 10 \times s / S;$$

Where,

s = is the standard deviation of y-intercepts of regression lines.

S = is the slope of the calibration curve.

4.5. Solution stability:

Stability of sample and standard solution were stable up to 48 h at room temperature.

4.6. Specificity:

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities and degradation products. Commonly used excipients (starch, microcrystalline cellulose and magnesium stearate) were spiked into a pre weighed quantity of drugs. The chromatogram was taken by appropriate dilutions and the quantities of drugs were determined.

4.7. Robustness:

Robustness of the method was studied by deliberately changing the experimental conditions such as flow rate and percentage of organic phase.

RESULTS AND DISCUSSION

1. Optimization of mobile phase:

Various mixtures containing methanol, water, ACN and aqueous buffer were tried as mobile phases in the initial stage of method development. Finally, the system containing mixture of Acetonitrile: Methanol: 0.1%Triethylamine buffer (pH-adjusted to 3 using ortho-phosphoric acid) 25:35:40 (v/v) was found to be satisfactory and gave two well-resolved peaks for Lesinurad (LES) and allopurinol (ALO). The retention time for Lesinurad (LES) and allopurinol (ALO) were 5.57 min and 2.60 min, respectively (Fig.2). The resolution between Lesinurad (LES) and allopurinol (ALO) was found to be 2.0, which indicates good separation of both the compounds. Column efficiency parameters were reported in table 1. The mobile phase flow rate was maintained at 1 mL/min. Overlaid UV spectra of both the drugs showed that Lesinurad (LES) and allopurinol (ALO) absorbed appreciably at 250 nm, so detection was carried out at 250 nm. Blank and standard chromatograms were depicted in Figure.2 &3.

2. Validation of the Proposed Methods:

The developed method was validated for various parameters including linearity and range, accuracy, precision, robustness, system suitability, specificity, LOQ and LOD.

2.1. Linearity and Range:

The calibration curve for LES was found to be linear in the range of 5–25 µg/ml with a correlation coefficient of 0.9998. The calibration curve for ALO was found to be linear in the range of 2-10 µg/ml with a correlation coefficient of 0.9997. The regression analysis of calibration curves are reported in table 2.

2.2. Precision:

Instrument precision was determined by performing injection repeatability test and the RSD values for Lesinurad (LES) and allopurinol (ALO) were found to be 0.64% and 0.11%, respectively. The intra-day and inter-day precision studies were carried out and the low RSD values indicate that the method is precise.

2.3. Accuracy:

The accuracy of the method was determined by calculating recoveries of Lesinurad (LES) and allopurinol (ALO) by method of standard addition. The recoveries were found to be 97.85–100.83% and 99.38–100.37% for Lesinurad (LES) and allopurinol (ALO), respectively (table 3). The high values indicate that the method is accurate.

2.4. Limit of detection and limit of quantification:

The LOD and LOQ were measured by using an equation. The detection limits for Lesinurad (LES) and allopurinol (ALO) were 0.316 µg/ml and 0.022µg/ml, respectively, while quantitation limits were 0.959 µg/ml and 0.065 µg/ml, respectively. The above data shows that a nano gram quantity of both drugs can be accurately and precisely determined.

2.5. Stability of standard and sample solutions:

Stability of standard and sample solution of Lesinurad (LES) and allopurinol (ALO) were evaluated at room temperature for 48 h. The relative standard deviation was found to be below 2.0%. It showed that both standard and sample solution were stable up to 48 h at room temperature.

2.6. Specificity:

The specificity study was carried out to check the interference from the excipients used in the formulations by preparing synthetic mixture containing both the drugs and excipients. The chromatogram showed peaks for both the drugs without any interfering peak and the recoveries of both the drugs were above 98%.

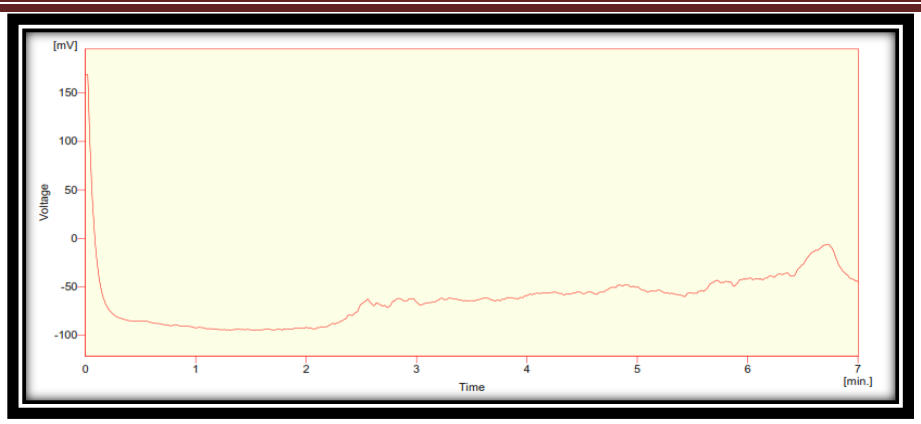


Fig. 2: Blank chromatogram of Lesinurad (LES) and Allopurinol (ALO)

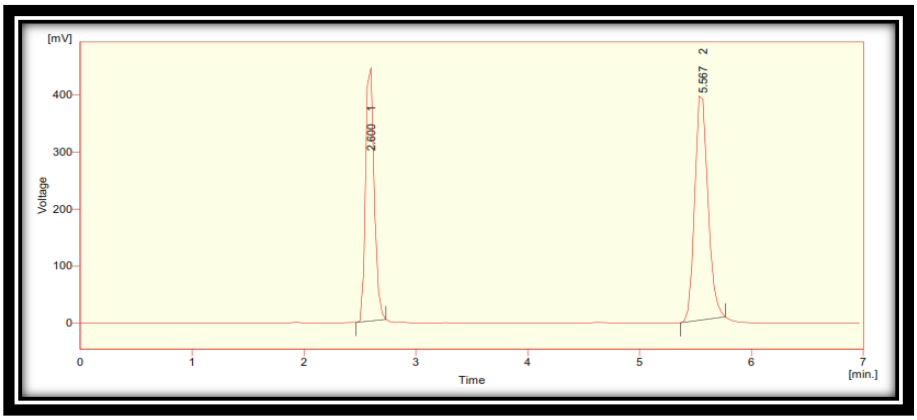


Fig. 3: RP-HPLC chromatogram of Lesinurad (LES) (RT 2.60 min) and Allopurinol (ALO) (RT 5.57 min)

Table No. 1: System suitability results of Lesinurad (LES) and Allopurinol (ALO)

Retention Time	Lesinurad (LES)	5.57 min
	Allopurinol (ALO)	2.60 min
Peak Area	Lesinurad (LES)	1081625
	Allopurinol (ALO)	891011
Theoretical plates	Lesinurad (LES)	85697
	Allopurinol (ALO)	95771
Tailing Factor	Lesinurad (LES)	1.07
	Allopurinol (ALO)	1.02
Resolution	Lesinurad (LES)	-
	Allopurinol (ALO)	3.6

Table No. 2: Calibration curve data

Parameters	Lesinurad (LES)	Allopurinol (ALO)
Range	5-25 (µg/mL)	2-10 (µg/mL)
Slope	72737	150167
Correlation coefficient	0.9998	0.9997

Table No. 3: Recovery data

Level of % recovery	Mean %recovery		%RSD	
	Lesinurad (LES)	Allopurinol (ALO)	Lesinurad (LES)	Allopurinol (ALO)
80	99.98	100.37	1.45	0.29
100	100.93	99.38	1.77	1.41
120	97.85	99.92	1.79	0.84

CONCLUSION

This developed and validated method for simultaneous analysis of oxycodone and naltrexone in pharmaceutical preparations is very simple, rapid, accurate and precise. Moreover, it has advantages of short run time and the possibility of analysis of a large number of samples, both of which significantly reduce the analysis time per sample. Hence, this method can be conveniently used for routine quality control analysis of Lesinurad (LES) and allopurinol (ALO) in their pharmaceutical formulations.

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