DEVELOPMENT AND VALIDATION OF A NEW ANALYTICAL REVERSE PHASE-HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF LESINURAD AND ALLOPURINOL IN PURE FORM AND MARKETED COMBINED DOSAGE FORM

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ABSTRACT

A streamlined, repeatable, and effective high-performance liquid chromatographic technique was developed for the simultaneous confirmation of Lesinurad and Allopurinol in pure form and showcased mixed pharmaceutical dosage forms. An isocratic mode column with a symmetry (C18) (150mm x 4.6mm, 5Âµm) and a flexible buffer consisting of Methanol: Phosphate (pH-3.8) (28:72% v/v) was previously used. Radiation at 252 nm was measured at a flow rate of 1 ml/min. Both Lesinurad and Allopurinol exhibited aid times of 1.794 and 3.440 minutes, respectively, and linearity extends of 10-30 and 10-50 parts per million, respectively. Linearity, accuracy, precision, satisfactoryness, limitation of disclosure, and management of quantification are all qualities that have been praised for the methodology. The limits of detection (LOD) and limits of quantification (LOQ) for Lesinurad were found to be 0.86Âµg/ml and 2.58Âµg/ml, respectively, whereas for Allopurinol the values were 1.28Âµg/ml and 3.84Âµg/ml. It was found that the produced method was accurate, precise, and applicable to the simultaneous confirmation of Lesinurad and Allopurinol in tablets.

Keyword: - *Lesinurad and Allopurinol, RP-HPLC, Validation, Accuracy, Robustness.*

1. INTRODUCTION

Allopurinol is a xanthine oxidase inhibitor specially used for the treatment of gout caused by high levels of uric acid in the body[1]. It is also used to prevent specific types of kidney stones and for high uric acid levels that can occur with chemotherapy [2].Allopurinol inhibits xanthine oxidase which is the enzyme that responsible for converting hypoxanthine to xanthine and xanthine to uric acid [3]. Stomach upset, nausea, diarrhea, or drowsiness are the possible side effects with the use of allopurinol.

Lesinurad is a non-nucleoside reverse transcriptase inhibitor and a novel uric acid transporter 1 inhibitor prescribed for the treatment of hyperuricemia associated with gout [4, 5]. It is prescribed for the patients that are not achieved target serum uric acid levels with xanthine oxidase inhibitor like allopurinol, oxypurinol and tisopurine [6].Lesinurad works by inhibiting urate anion transporter (URAT1) which is responsible for uric acid reabsorption in proximal tubule which leads to the increase of urate excretion in urine and reduce the concentration in plasma [7]. Kidney problems such as lower back pain, painful or difficult urination, nausea, vomiting, change in the amount of urine are the possible side effects associated with the use of Lesinurad. Allopurinol and Lesinurad are the combined medication used for the treatment of gout. In literature only

two HPLC assay methods were reported for the simultaneous analysis of Allopurinol and Lesinurad [8,9]. One Ultra-performance hydrophilic interaction liquid chromatography coupled with tandem mass spectrometry method was reported for the analysis of Allopurinol and Lesinurad in combination with oxypurinol in rat plasma [10]. Very few methods reported for the analysis of Lesinurad in single dosage form using UV spectrophotometry [11] or in combination with alpha lipoicacid and oxipurinol using HPLC. One HPLC method for the analysis of Lesinurad in formulations and one UHPLC-MS/MS method for the analysis of Lesinurad in rat plasma were reported. As the literature survey confirms that there is no stability indicating HPLC method reported for the simultaneous analysis of Allopurinol and Lesinurad in pharmaceutical formulations. Hence the present work aimed to develop a new stability indicating HPLC method for the separation and quantification of Allopurinol and Lesinurad.

2. MATERIAL AND METHODS

2.1 Instrumentation

The separation and quantification of Lesinurad and Allopurinol was carried in waters HPLC with auto sampler and PDA DETECTOR 996MODEL C18 column (150 mm x 4.6 mm, 5µ) equipped in isocratic LC-100 S-HPLC™. The samples were injected using Rheodyne manual inject port and data was analyzed.

2.2 Preparation of standard solutions

25 mg of standard drug Lesinurad was weighed accurately and was dissolved in 25 ml methanol. Lesinurad at a concentration of 1000 µg/ml was obtained and the obtained solution was filtered. Lesinurad standard solution at a concentration 100 µg/ml was prepared by diluting 10 ml from 1000 µg/ml to 100 ml. The same procedure was used for the preparation of Allopurinol standard solution separately. For preparing calibration curve dilutions, equal volume of known and fixed concentration of Lesinurad and Allopurinol were mixed separately. The combined solution of Lesinurad and Allopurinol having known concentrations were used for method development and validation study.

2.3 Method development

For development of the method for the identification and simultaneous quantification of Lesinurad and Allopurinol in pharmaceutical formulations, different method development trails were performed. In the method development, composition of mobile phase, pH of mobile phase, configuration of stationary phase, UV detector wavelength and mobile phase flow rate was studied. In each trail condition, the system suitability parameters like peak shape, peak response, number of theoretical plates, tail factor and resolution were checked and the conditions that produce best results were considered as optimized and further validated.

2.4.Method validation

The developed method for the simultaneous quantification of Lesinurad and Allopurinol was validated for the determination of range of analysis, sensitively, accuracy, precised, rugged and robust nature. The sensitivity of the developed method was determined by confirming detection and quantification limits.

3. RESULTS AND DISCUSSION

The present work was aimed to develop a simple, precise and accurate stability indicating HPLC method for the separation and quantification of Lesinurad and Allopurinol in pharmaceutical formulations. The optimized separation was achieved using isocratic elution at a flow rate of 1.0mL/min using mobile phase of methanol, and phosphate buffer (pH 3.8) in the ratio of 28:72 (v/v). C18 column (150 mm x 4.6 mm, 5µ) was used as stationary phase and UV detection was monitored at 252nm.

Table 1: System suitability results

Parameter	Lesinurad	Allopurinol
API Concentration	40 µg/ml	60 µg/ml
RT (min)	1.794	3.440
Area	558659	7856985
Height	7584	44658
USP tailing	1.10	1.13
USP Plate count	7659	8743

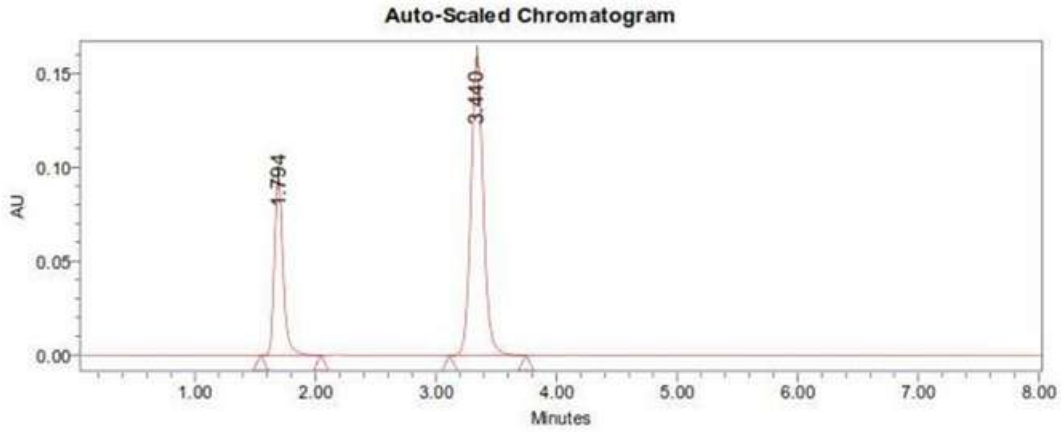

Fig. 1: Standard chromatogram in the optimized conditions

In the optimized conditions, Lesinurad and Allopurinol were well resolved and retained at a retention time of 1.794 min and 3.44 min respectively and clear base line was observed within run time of 8 min. The method obeys system suitability conditions (Table 1) for both Lesinurad and Allopurinol. Figure 1 shows the optimized chromatogram of Lesinurad and Allopurinol in the developed method.

Accurately correlated calibration range was observed in the concentration range of 10-30 µg/mL for Lesinurad and 10-50 µg/mL for Allopurinol. The regression equitation was found to be y=27337x + 11729 (R^2 = 0.999) and y = 261221x + 145629 (R^2 = 0.9995) for Lesinurad and Allopurinol respectively. The calibration curve was found to linear in the concentration studied for both Lesinurad and Allopurinol with a very high correlation coefficient of more than 0.999 for both the drugs. The results of linearity study were as given in table 2 and calibration curve is shown in figure 3 and 4 for Lesinurad and Allopurinol respectively.

Table 2: Linearity results

Lesinurad		Allopurinol	
Concentration in µg/ml	**Peak Area**	**Concentration in µg/ml**	**Peak Area**
10	292985	10	2828756
15	430752	20	5485784
20	565265	30	7999859
25	693487	40	10656542
30	821584	50	13085985

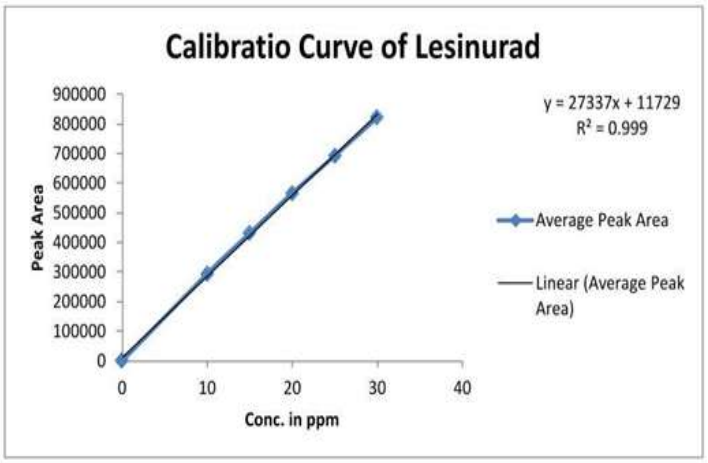

Fig.2: Linear calibration curve for Lesinuard

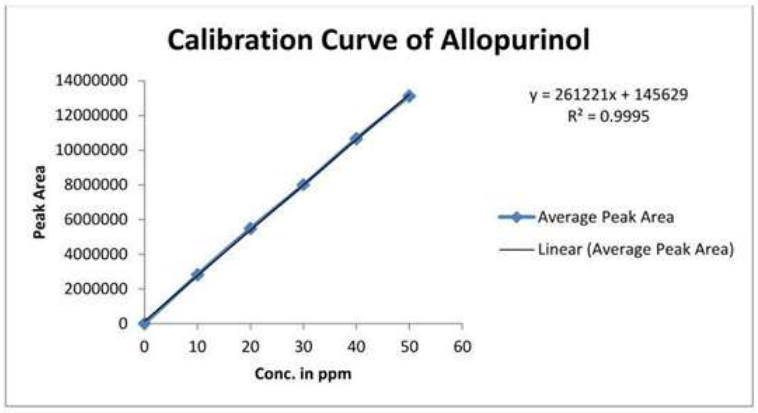

Fig.3: Linear calibration curve for Allopurinol

The repeatability and reproducibility were studied by intraday, interday precision and repeatability study. The standard solution at a concentration of 60 µg/mL of Allopurinol and 40 µg/mL of Lesinurad was analyzed six times in the same day for intraday precision, six times in three successive days for interday precision and six times for change in analyst for repeatability study. The % RSD in each study was calculated for both the drugs and was found to be 0.078, 0.149 in intraday precision, 0.35, 0.191in interday precision and 0.122, 0.219 in ruggedness study for Allopurinol and Lesinurad respectively. This confirms that the method developed was found to be precise for the simultaneous analysis of Allopurinol and Lesinurad.

The standard concentration of Allopurinol and Lesinurad were analyzed by change in analytical conditions *i.e.* mobile phase composition (±5%), mobile phase pH (±0.1) and detector wavelength (±5 nm). The % change was calculated in each changed condition for both the drugs and was found to be within the acceptable limit of less than 2 confirms that the method was found to be robust. The spiked recovery at 50%, 100% and 150% spiked levels at a target concentration of 30 µg/mL of Allopurinol and 20 µg/mL of Lesinurad were studied. The % Recovery and the % RSD of recovery in each spike level for 30 µg/mL was calculated (table 3 and 4) and was found to be within the acceptable limits for both Allopurinol and Lesinurad confirms that the method was found to be accurate. LOD and LOQ were found to be 0.86 µg/mL and 1.28 µg/mL; 2.58 and 3.84 µg/mL for both Lesinurad and Allopurinol respectively and was found to be within the acceptable limits.

Table 3: Accuracy results for Lesinurad

%Concentration (at Specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	286080.7	10.035	10	100.350%	100.291%
100%	561215	20.100	20	100.500%	
150%	833959.7	30.077	30	100.023%	

Table 4: Accuracy results for allopurinol

% concentration	Area	Amount added (ppm)	Amount found (ppm)	% Recovery	Mean Recovery
50%	408328	15	15.074	100.493	
100%	798306.3	30	30.003	100.01	100.163
150%	1189915	45	44.994	99.986	

4. CONCLUSION

In the present study, a simple, fast, accurate, and reliable HPLC method was developed and validated for the simultaneous analysis of Allopurinol and Lesinurad in pharmaceutical formulations as per ICH guidelines. The method obeys all the system suitability and other validation parameters. As there is no stability indicating HPLC methods reported for the simultaneous analysis of Allopurinol and Lesinurad, the method developed was found to be the reliable and convenient for the routine analysis and stability study of Allopurinol and Lesinurad in bulk drug and pharmaceutical formulations.

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