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## A New Validated Stability Indicating RP-HPLC Method for the Quantification of Allopurinol and Lesinurad in Bulk and Pharmaceutical Formulations

K. V. Ramanjaneyulu<sup>1,2,✉</sup>, K. Venkata Ramana<sup>2,3</sup>  
and M. Prasada Rao<sup>4</sup>

### ABSTRACT

The objective of this study was to develop and validate a method for simultaneous quantitative analysis of allopurinol and lesinurad in bulk drug and pharmaceutical formulations. An isocratic HPLC analysis method using a reverse phase Waters spherisorb ODS1 C18 column (250 mm × 4.6 mm, 5 μ) and a simple mobile phase without buffer was developed, optimized and fully validated. Analyses were carried out at a flow rate of 0.9 mL/min at 50 °C and monitored at 246 nm. This HPLC method exhibited good linearity, accuracy and selectivity. The recovery (accuracy) of both allopurinol and lesinurad from all matrices was greater than 98 %. The allopurinol and lesinurad peak detected in the samples of a forced degradation study and no interference of excipients or the degradation products formed during stress study. The method was rugged with good intra- and inter-day precision and sensitive. This stability indicating HPLC method was selective, accurate and precise for the simultaneous analysis of allopurinol and lesinurad in pharmaceutical formulations.

### KEYWORDS

Allopurinol, Lesinurad, HPLC analysis, Method development, Stress degradation.

### 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 tiopurine [6]. Lesinurad works by inhibiting urate anion transporter (URAT1) which is responsible for uric acid reabsorption in proximal tubule which leads

## Asian Journal of Organic & Medicinal Chemistry

Volume: 5

Year: 2020

Issue: 1

Month: January–March

pp: 51–55

DOI: <https://doi.org/10.14233/ajomc.2020.AJOMC-P244>

Received: 3 December 2019

Accepted: 8 February 2020

Published: 5 May 2020

#### Author affiliations:

<sup>1</sup>Department of Pharmaceutical Analysis, M.A.M College of Pharmacy, Kesanupalli, Narasaraopet-522601, India

<sup>2</sup>Department of Pharmaceutical Analysis, Acharya Nagarjuna University, Guntur, India

<sup>3</sup>A.S.N. College of Pharmacy, Tenali, India

<sup>4</sup>M.A.M. College of Pharmacy, Kesanupalli, Narasaraopet-522601, India

✉To whom correspondence to be addressed:

E-mail: ram.ramanji@gmail.com

Available online at: <http://ajomc.asianpubs.org>

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]. Few methods reported for the analysis of lesinurad in single dosage form using UV spectrophotometry [11] or in combination with alphalipoic acid [12] and oxipurinol [13] using HPLC. One HPLC method for the analysis of lesinurad in formulations [14] and one UHPLC-MS/MS method for the analysis of lesinurad in rat plasma [15] 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. The molecular structure of lesinurad and allopurinol are given in Fig. 1.

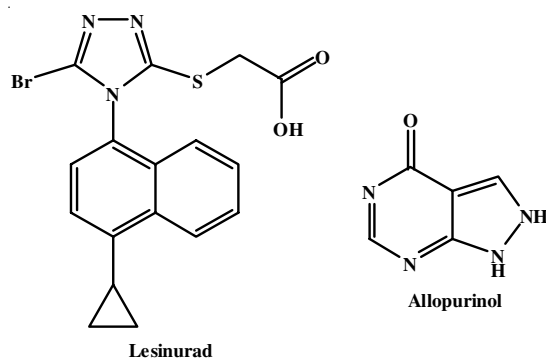


Fig. 1. Molecular structure of lesinurad and allopurinol

## EXPERIMENTAL

The separation and quantification of lesinurad and allopurinol was carried in waters spherisorb ODS1 C18 column (250 mm × 4.6 mm, 5 μ) equipped in isocratic LC-100 S-HPLC™ (Cyberlab- USA) LC 20AT pump for solvent delivery and variable wavelength programmable LC-100 UV-visible detector for detection. The samples were injected using Rheodyne manual inject port and data was analyzed by using WS-100 Workstation software (Cyberlab-USA). DENVER (SI-234) electronic balance and ultrasonic batch sonicator (1.5 L) in the study.

Lesinurad and allopurinol active pharmaceutical ingredients (APIs) were obtained from Klokter life sciences, Himachal Pradesh. The marketed formulation duzallo (Lesinurad-200 mg and Allopurinol-300) was purchased in local pharmacy. HPLC grade acetonitrile, methanol and water were purchased from Merck chemicals, Mumbai. The membrane filter papers (0.2 μ nylon) were purchased from millipore (India).

**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.

**Preparation of formulation solution:** The market formulation tablets of lesinurad and allopurinol with brand duzallo (lesinurad-200 mg and allopurinol-300) was powdered using sterile mortar and pestle. An amount of the tablet powder equivalent to 10 mg of lesinurad was weighed accurately and was dissolved in 10 mL methanol. Then it was filtered and was further diluted to get a concentration of 40 μg/mL of lesinurad. As per the label claim of the drugs in the formulation, sample solution having 60 μg/mL of Allopurinol. This solution is used for the determination of the applicability of the developed method for the analysis of lesinurad and allopurinol in pharmaceutical formulations.

**Method development:** The method development 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.

**Method validation:** The developed method for the simultaneous quantification of lesinurad and allopurinol was validated for the determination of range of analysis, sensitivity, accuracy, precise, rugged and robust nature. The sensitivity of the developed method was determined by confirming detection and quantification limits.

**Force degradation studies:** Forced degradation study was carried for the standard drugs lesinurad and allopurinol in the developed method to evaluate the effectiveness of the method for the separation and identification of known and unknown impurities in the drug. About 50 mg of standard drug was mixed with 50 mL of 0.1 N HCl for acid hydrolysis study, 50 mL of 0.1 N NaOH in base hydrolysis study and 50 mL of 3 % hydrogen peroxide solution for oxidative degradation study. These conditions were carried separately for both the drugs and the solutions were incubated 24 h and then neutralized separately. The equal volume of selected concentration of both the drugs were mixed and then neutralized. The neutralized solutions were analyzed in the developed method condition. In photolytic and thermal degradation conditions, standard drug was kept under UV light at 254 nm and oven at 60 °C for 24 h, respectively. Then the standard drug was diluted to 300 μg/mL and was analyzed in the developed method condition.

The % degradation, number of degradation products formed in the degradation study and the % effectiveness of the method for the separation of degradation products was evaluated.

**Formulation analysis:** The formulation solution prepared from the formulation tablets of lesinurad and allopurinol was analyzed in the developed method. The % assay of lesinurad and allopurinol in the developed method was calculated in the developed method.

## RESULTS AND DISCUSSION

The present work is 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 0.9 mL/min using mobile phase of methanol, acetonitrile and water in the ratio of 60:25:15 (v/v) at pH 6.1. Waters spherisorb ODS1 C18 column (250 mm × 4.6 mm, 5 μ) was used as stationary phase and UV detection was monitored at 246 nm.

In optimized conditions, lesinurad and allopurinol were well resolved and retained at a retention time of 6.1 min and 2.8 min, respectively and clear base line was observed with in run time of 10 min. The method obeys system suitability conditions (Table-1) for both lesinurad and allopurinol. Fig. 2 shows the optimized chromatogram of lesinurad and allopurinol in the developed method.

Parameter	Lesinurad	Allopurinol
Api concentration	40 μg/mL	60 μg/mL
Retention time (min)	6.1	2.8
Area	232160	481409
Resolution	15.09	—
Theoretical plates	13483	2842
Tailing factor	0.72	0.60

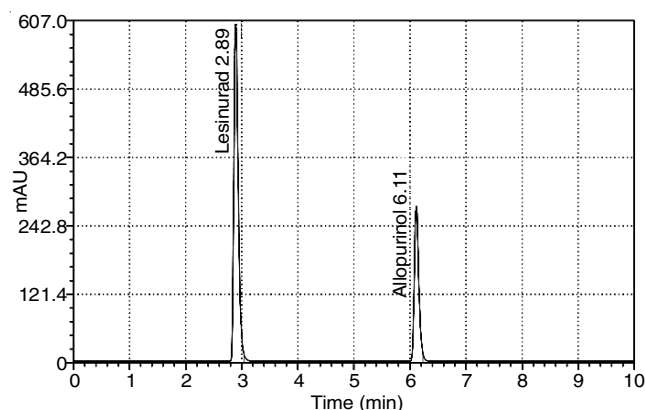


Fig. 2. Standard chromatogram in the optimized conditions

Accurately correlated calibration range was observed in the concentration range of 10-60 μg/mL for lesinurad and 15-90 μg/mL for allopurinol. The regression equation was found to be  $y = 7661.6x + 19830$  ( $R^2 = 0.9994$ ) and  $y = 4149.1x + 64473$  ( $R^2 = 0.9995$ ) for allopurinol and lesinurad, respectively. The calibration curve was found to linear in the concentration studied for both lesinurad and allopurinol with a high correlation

coefficient of more than 0.999 for both the drugs. The results of linearity study were given in Table-2.

Lesinurad		Allopurinol	
Concentration (μg/mL)	Peak area	Concentration (μg/mL)	Peak area
10	106833	15	131804
20	144908	30	257549
30	190287	45	356754
40	232160	60	481409
50	270302	75	597481
60	313659	90	707380

The repeatability and reproducibility were studied by intra-day, inter-day precision and ruggedness 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 inter-day precision and six times for change in analyst for ruggedness study. The % RSD in each study was calculated for both the drugs and was found to be 0.279, 0.562 in intraday precision, 0.581, 1.091 in inter-day precision and 0.593, 0.849 in ruggedness study for allopurinol and lesinurad, respectively. This confirms that the method developed was found to be precise and rugged 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 ( $\pm 5\%$ ), mobile phase pH ( $\pm 0.1$ ) and detector wavelength ( $\pm 5$  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 (Tables 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.

In the stress degradation study, the % degradation of allopurinol was found to be 8.61 (acidic), 10.67 (basic), 4.72 (peroxide), 5.38 (thermal) and 7.28 (UV light) whereas the % degradation of lesinurad was found to be 7.76 (acidic), 8.78 (basic), 3.34 (peroxide), 6.46 (thermal) and 5.28 (UV light). Less % degradation was observed for both the drugs in peroxide conditions whereas the % degradation was found to be high in acidic and base conditions. In the stress degradation studies, both the standard drugs were retained in the same retention time compared with unstressed conditions and the additional degradation products formed were effectively separated and retained in the developed method. Hence the method can be used for the identification of known or unknown impurities formed during the stress study. Hence the method was considered as stability indicating method. The stress degradation chromatograms are given in Figs. 3-7.

The formulation assay was found to be 99.39 % for allopurinol and 98.21 % for lesinurad in the developed method. In

TABLE-3  
LINEARITY RESULTS FOR ALLOPURINOL

Recovery level (%)	Concentration ( $\mu\text{g/mL}$ )			Peak area	Concentration obtained ( $\mu\text{g/mL}$ )	Recovery (%)	RSD of recovery (%)
	Target	Spiked	Total				
50	30	15	45	354636	44.733	99.406	0.145
	30	15	45	353613	44.604	99.119	
	30	15	45	354046	44.658	99.241	
100	30	30	60	481697	60.036	100.060	0.242
	30	30	60	480952	59.943	99.905	
	30	30	60	483236	60.228	100.379	
150	30	46	75	591687	74.273	99.030	0.432
	30	46	75	590752	74.155	98.874	
	30	46	75	586879	73.669	98.225	

TABLE-4  
LINEARITY RESULTS FOR LESINURAD

Recovery level (%)	Concentration ( $\mu\text{g/mL}$ )			Peak area	Concentration obtained ( $\mu\text{g/mL}$ )	Recovery (%)	RSD of recovery (%)
	Target	Spiked	Total				
50	20	10	30	189567	29.886	99.622	0.828
	20	10	30	188723	29.753	99.178	
	20	10	30	186545	29.410	98.033	
100	20	20	40	235823	40.631	101.578	0.493
	20	20	40	233937	40.306	100.765	
	20	20	40	236049	40.670	101.675	
150	20	30	50	267319	49.448	98.896	0.150
	20	30	50	268075	49.588	99.176	
	20	30	50	267935	49.562	99.124	

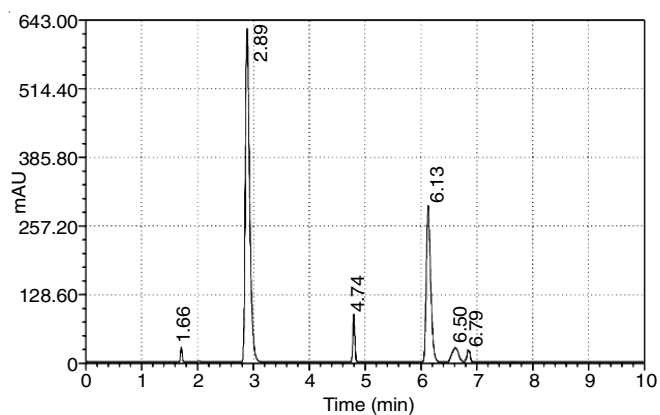


Fig. 3. Acid degradation chromatogram

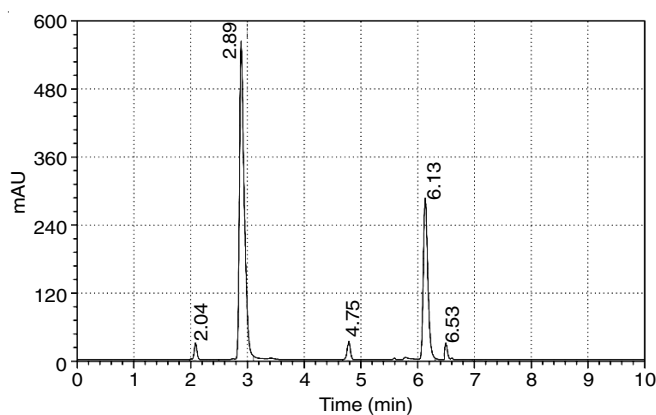


Fig. 5. Peroxide degradation chromatogram

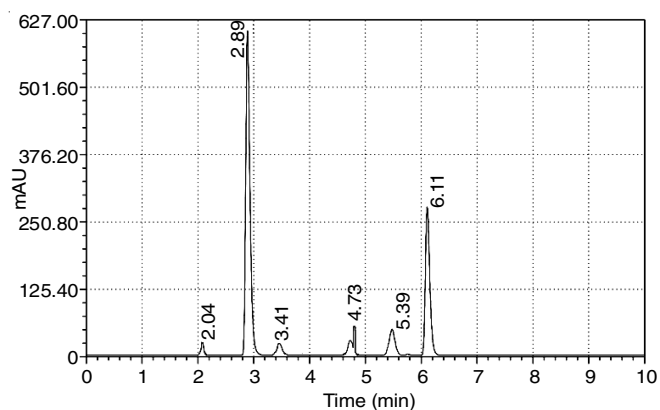


Fig. 4. Base degradation chromatogram

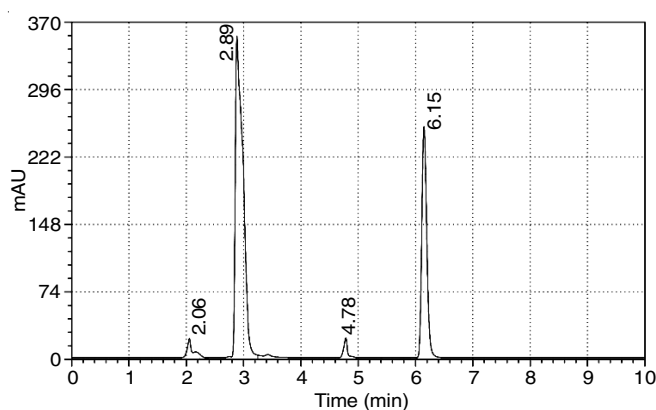


Fig. 6. Thermal degradation chromatogram

the formulation chromatogram, both the drugs allopurinol and lesinurad were well retained and the retention time was found

to be similar to the standard. There is no detection of formulation excipients and clear base line was observed (Fig. 8).

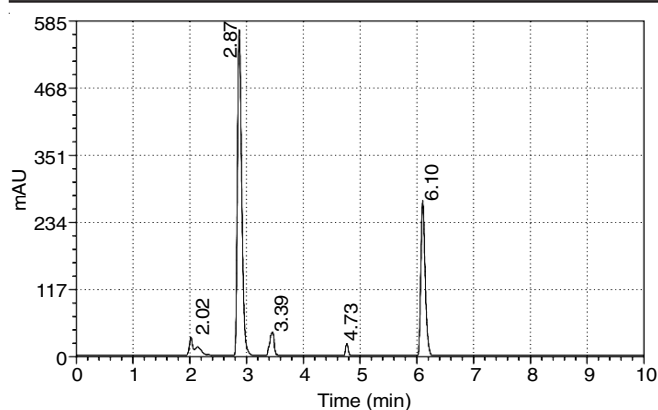


Fig. 7. UV light degradation chromatogram

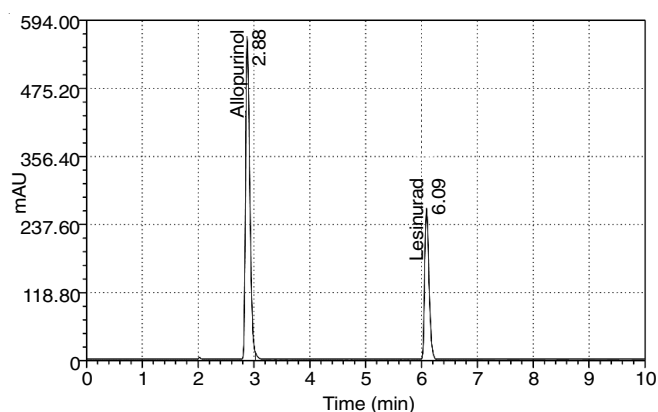


Fig. 8. Chromatogram of the formulation excipients

The developed method conditions were compared with the methods available in literature. The method developed by Dastiagiramma *et al.* [8] was found to be having highly acidic pH in the mobile phase and the resolution of allopurinol and lesinurad was found to be very less. The calibration range also found to be narrow that facilitates less applicability of the method. The simultaneous analysis method reported by Rama Rao *et al.* [9] also found to having high acidic pH, less sensitive. The stress degradation behaviour of drugs and the effective separation of degradation products was not reported previously. The method developed in this study was found to be simple with pure solvent mixture without any salt buffers and no pH modification in the mobile phase and all other validation parameters were found to be with in the acceptable limits. The method also effectively separates the degradation products formed during the stress study. Hence, the method developed was found to be the most suitable and reliable method for the simultaneous analysis and stability study of allopurinol and lesinurad in pharmaceutical formulations.

## 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. The method can effectively separate the degradation products formed during the stress degradation study. 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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