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THE RP-UPLC METHOD FOR SIMULTANEOUS QUANTIFICATION OF LESINURAD AND ALLOPURINOL

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Abstract:
In order to develop a newer or improved analytical method, the analyst has to set some goals. The method should be precise to the drug under study. It is necessary to determine the analyte at trace levels accurately. The UPLC techniques have now become extremely reliable and indispensable. Lesinurad is an oral uric acid transporter 1 (URAT1) inhibitor indicated for the treatment of hyperuricemia associated with gout. Allopurinol is a xanthine oxidase enzyme inhibitor that is considered to be one of the most effective drugs used to decrease urate levels and is frequently used in the treatment of chronic gout. Run time was selected to be 2 min because the analysis gave peaks around 0.401 and 0.718 ±0.02 min of Lesinurad and Allopurionol. The percent recovery was found to be in between 98.0 to 102.0%. The analytical method was found to be linear over the range 50-300 µg/mL of Lesinurad and Allopurionol of the target concentration.

Keywords: *RP-UPLC, Lesinurad, Allopurionol, Precision, Accuracy*

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INTRODUCTION:

Ultra Performance Liquid Chromatography spectroscopic detection is a powerful hyphenated technique for the analysis of drugs. Its sensitivity, accuracy and short analysis time make it ideal for determination of many drugs in dosage forms. Further, with the development of more sophisticated instrumentation, efficient column materials, sensitive detectors and moderate pricing, the UPLC techniques have now become extremely reliable and indispensable. In view of these advantages, the author has chosen to develop UPLC methods in this investigation for determination of some of selected drugs[1].

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. Lesinurad inhibits the activity of uric acid transporter 1 (URAT1) and organic anion transporter 4 (OAT4). URAT1 is a major transporter enzyme responsible for reuptake of uric acid from the renal tubules; inhibition of URAT1 function thereby increases excretion of uric acid[2].

Allopurinol is a xanthine oxidase enzyme inhibitor that is considered to be one of the most effective drugs used to decrease urate levels and is frequently used in the treatment of chronic gout. It was initially approved by the FDA in 1966 12 and is now formulated by several manufacturers[3]. Allopurinol and its active metabolite inhibit xanthine oxidase, the enzyme that converts hypoxanthine to xanthine and xanthine to uric acid. Inhibition of this enzyme is responsible for the effects of allopurinol[4].

MATERIALS AND METHODS:

The reference standard samples of Lesinurad and Allopurionol were obtained from Suven life sciences Ltd. Acetonitrile and Orthophosphoric acid used were of HPLC grade, while Sodium hydroxide, hydrogen peroxide was of GR grade (Merck Ltd. Mumbai, India). Milli-Q water was used throughout the analysis. The chromatographic system consisted of a Waters Acquity H-Class UPLC (Model 2695) chromatograph equipped with HSS C18 100 X 2.1 mm; 1.7µ column, LC-20AD pumps and an SPD-20A photo diode array (PDA) detector. Samples were injected into the system through a Rheodyne 7725 injector valve via a 0.3 µL loop. The output signal was monitored and integrated by Empower-2 software. Solubility of the compound was enhanced by sonication on an ultrasonicator (PCI Analytics PCI81). All the weighings in the experiments were done with a Sartorius balance (model CPA225D). PVDF membrane filters used for filtration were purchased from Merck Millipore.

Preparation of the standard solution of Lesinurad and Allopurionol: Weighed and transferred 50 mg of Lesinurad and 50 mg of Allopurionol working Standards into a 25 mL clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 min and make up to the final volume with diluents. (2000 ppm of Lesinurad and 2000 ppm of Allopurionol). 5 mL from the above two stock solutions was taken into a 50 mL volumetric flask and made up to 50mL. (200 ppm of Lesinurad and 200 ppm of Allopurionol)[5].

Optimization of chromatographic conditions and method development: Under the below mentioned Table 1, the optimized conditions, the retention times obtained for Lesinurad and Allopurionol were 0.401 and 0.718 min respectively.

Table 1: Optimized chromatographic conditions of the proposed method

S. No.	Parameter	Value
1	Mobile phase	Buffer: Methanol (50:50 %)
2	Diluent	Water: methanol (50:50%)
3	Stationary phase	HSS C18 (100 x 2.1mm; 1.7µ)
4	Flow rate	0.3 mL/min
5	Column temperature	30ºC
6	Volume of injection	0.3 µL
7	Detection wavelength (λ_{max})	250 nm
8	Run time (min)	2 min

System suitability: System suitability was assessed by analyzing the mixed standard drug solution (200 ppm of Lesinurad and 200 ppm of Allopurionol) and calculating the chromatographic parameters such as resolution, theoretical plates, and tailing factor[6].

Specificity: Specificity is the extent to which the procedure applies to the analyte of interest and is checked by examining the formulation samples for any interfering peaks. The specificity of the method was evaluated with regard to interference due to presence of excipients. The excipients used in formulation did not interfere with the drug peaks and thus the method is specific[6].

Linearity: To establish linearity, a stock solution containing 200 µg/mL of Lesinurad and 200 µg/mL of Allopurionol were prepared using diluent and further diluted to yield solutions in the concentration range of 50-300 µg/mL of Lesinurad and Allopurionol[7]. The solutions were prepared and analyzed in triplicate. The experiment was repeated thrice by preparing different solution and analyzed by injecting 0.3 µL in UPLC.

Accuracy: Accuracy for Lesinurad and Allopurionol was conducted by spiking the drug to the placebo powder at three different levels of the test concentration (i.e. 50%, 100%, and 150%) and each level three times. The mean % Recovery and % RSD values were calculated[8].

Precision: To ascertain the effectiveness of method system suitability tests were carried out on freshly prepared standard stock solution containing 200 µg/mL of Lesinurad and 200 µg/mL of Allopurionol. 3 µL of solution was injected into the optimized chromatographic system. For system suitability 6 replicates of working standard samples were injected and the peak response of sample was calculated[9].

Limit of detection (LOD) and Limit of Quantification (LOQ): LOD and LOQ values were calculated from the average standard deviation and slope from the calibration curve as per ICH guideline.

Robustness: Robustness study was done by applying small deliberate changes in the chromatographic conditions and studying the system suitability parameters of both the drugs. The conditions selected for testing were the flow rate, column oven temperature and composition of the mobile phase. The study was conducted on a mixed standard solution containing 200 µg/mL of Lesinurad and 200 µg/mL of Allopurionol[10].

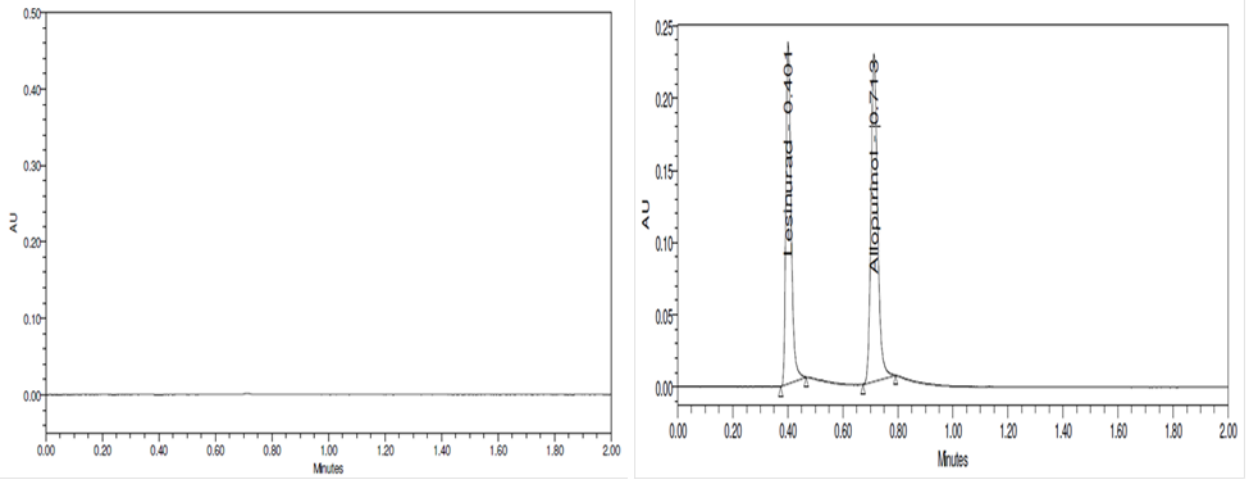
RESULTS AND DISCUSSION

System suitability: It was represented in Table 2.

Table 2: System suitability values for the present method

	Parameter	Lesinurad	Allopurionol
1.	Retention time (min)	0.401	0.713
2.	Peak area	399100	414135
3.	Resolution	-	8.0
4.	Theoretical Plates	2266	4630
5.	Tailing Factor	1.34	1.19

Specificity: The UPLC chromatograms recorded for the drug matrix (mixture of the drug and the excipients) showed almost no interfering peaks within retention time ranges. Figure 1 a and Figure 1 b show the representative chromatograms for standard and the formulation. The figures show that the selected drugs were clearly separated. Thus the proposed UPLC method is selective.



(A) (B)

Figure 1: (a) A Typical Chromatogram of Placebo (b) A Typical Chromatogram of Lesinurad and Allopurionol in mixed standard solution

Linearity: Linearity data for Lesinurad and Allopurionol are given in the tables 3 and 4 respectively.

Table 3: Linearity of Lesinurad

Concentration of Lesinurad (µg/mL)	Peak Area	Mean Area	RSD
50	102574	104883	3.1
	108647		
	103427		
100	203523	203742	0.2
	204235		
	203468		
150	306311	302739	1.0
	300286		
	301619		
200	406341	405135	0.7
	406964		
	402101		
250	502500	506022	0.7
	509158		
	506408		
300	604272	601863	0.9
	605509		
	595808		

Table 4: Linearity data of Allopurionol

Concentration of Allopurionol(µg/mL)	Peak Area	Mean Area	RSD
50	107547	104385	2.7
	102267		
	103340		
100	205074	206685	1.2
	209437		
	205544		
150	302315	304840	0.7
	305528		
	306676		
200	413255	412511	0.2
	411844		
	412435		
250	514016	512825	0.4
	510198		
	514260		
300	619536	614785	0.7
	613951		
	610869		

Accuracy: The results are present in Table 5 and 6**.** The %Recovery value was found to be in between 98.0 % to 102.0 %.

Table 5: Recovery of Lesinurad

Accuracy Level	Peak area difference	Amount added (µg/mL)	Amount Found (µg/mL)	% Recovery	Mean % Assay
50%	197343	100	99.096	99.10	99.54
	199369	100	100.11	100.11	
	197969	100	99.408	99.41	
100%	39306	200	197.034	98.52	98.89
	396766	200	198.509	99.25	
	395346	200	197.802	98.90	
150%	590325	300	295.000	98.33	98.64
	593034	300	296.350	98.78	
	593094	300	296.300	98.79	

Table 6: Recovery of Allopurionol

Accuracy Level	Peak area difference	Amount added (µg/mL)	Amount Found (µg/mL)	% Recovery	Mean % Assay
50%	201664	100	99.636	99.64	99.35
	200402	100	99.020	99.02	
	201175	100	99.398	99.40	
100%	405843	200	199.382	99.69	99.38
	403820	200	198.394	99.20	
	404085	200	198.523	99.26	
150%	614534	300	301.332	100.44	100.47
	616682	300	302.381	100.79	
	612940	300	300.553	100.18	

Precision: The inter-day precisions were determined by analyzing a mixed solution containing 200 µg/mL of Lesinurad and 200 µg/mL of Allopurionol. The intermediate precision was determined on two consecutive days different instrument. The results are depicted in the table 7.

Table 7: Inter-day precision data

S.No	Injection	Day-1		Day-2	
		Lesinurad	Allopurionol	Lesinurad	Allopurionol
1.	Injection-1	396221	414529	383998	388337
2.	Injection-2	394095	414641	388174	390913
3.	Injection-3	401185	414080	387697	390005
4.	Injection-4	401245	412821	390885	389344
5.	Injection-5	400888	416724	385685	384136
6.	Injection-6	400964	412017	390959	386056
Mean		399100	414135	387900	388132
SD		3129.2	1632.8	2775.5	2570.2
% RSD		0.8	0.4	0.7	0.7

LOD and LOQ: The LOD and LOQ values for Lesinurad were found to be 0.12 and 0.37. The LOD and LOQ values for Allopurionol were found to be 0.27 and 0.81.

Robustness: The results remained unaffected by small variations in these conditions. The results were represented in tables 8 and 9.

Table 8: Robustness data of Lesinurad

Chromatographic conditions	Lesinurad			
	% assay	Theoretical Plates	Asymmetry	Retention time
Water: Methanol (60:40% v/v)	99.85	2217	1.37	0.408
Water: Methanol (40:60% v/v)	99.06	2194	1.36	0.399
0.2 mL/min	99.79	2199	1.39	0.481
0.4 mL/min	98.77	2034	1.44	0.345
28°C	101.02	2120	1.36	0.401
32°C	98.78	2093	1.37	0.403

Table 9: Robustness data of Allopurionol

Chromatographic conditions	Allopurionol				
	% assay	Theoretical Plates	Asymmetry	Resolution	Retention time
Water: Methanol (60:40% v/v)	99.78	3886	1.15	9.7	0.858
Water: Methanol (40:60% v/v)	99.02	4520	1.25	6.6	0.636
0.2 mL/min	99.57	4100	1.21	8.1	0.859
0.4 mL/min	100.05	3980	1.26	7.5	0.611
28°C	100.07	4450	1.20	7.3	0.682
32°C	99.87	4143	1.17	8.6	0.767

CONCLUSION:

The present analytical method was developed by studying different parameters. The column used for the study was HSS C18 100 X 2.1 mm 1.7µ column because it gave good separation and peak shapes. Ideal λ max for both the drugs was found to be at 250 nm as the peak purity was good. Injection volume was selected to be 1µL which gave a good peak area. The flow rate was fixed at 0.3 mL/min for giving satisfactory retention times. A mixture of buffer and

methanol (50:50% v/v) was found to be ideal for the proposed study as it resulted in good resolution of the drugs. Run time was selected to be 2 min because the analysis gave peaks around 0.401 and 0.718 ±0.02 min of Lesinurad and Allopurionol respectively. The percent recovery was found to be in between 98.0 to 102.0%. The analytical method was found to be linear over the range 50-300 µg/mL of Lesinurad and Allopurionol of the target concentration. The analytical method passed both the robustness and ruggedness tests. In both the cases, relative standard deviation was below 2.0.

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