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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 ($\lambda_{max}$) | 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].

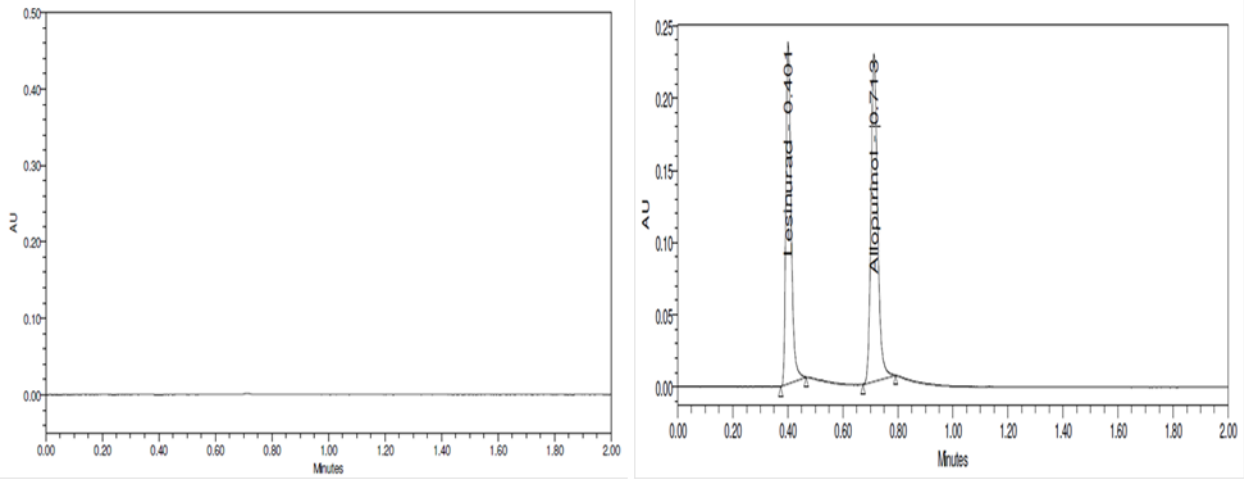
## RESULTS AND DISCUSSION

**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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