International Journal of Current Research and Techniques (IJCRT)
E-ISSN: 2349-3194 (Online) | P-ISSN: 2348-4446 (Print)
UGC Approved Journal No: 47722 [No: 2616]
DOI: https://doi.org/10.5281/ZENODO.15598316

Stability Indicating RP-HPLC Method Development and Validation for the Simultaneous Estimation of Allopurinol and Lesinurad in Bulk and its Dosage Form

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Manuscript Received: Jun 03, 2025; Revised: Jun 04, 2025; Published: Jun 05, 2025

Abstract: For the simultaneous estimation of Allopurinol and Lesinurad in bulk and combined pharmaceutical dosage forms, a novel, precise, and stability-indicating reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated. An Inertsil ODS C18 column with an isocratic mobile phase of phosphate buffer (pH 3.0) and acetonitrile in a 70:30 v/v ratio was used to optimize the chromatographic separation. The detection was carried out at a wavelength of 255 nm, with the flow rate maintained at 1.5 ml/min. With correlation coefficients (R2) greater than 0.999 for both drugs, the method was validated in accordance with ICH Q2(R1) guidelines and demonstrated excellent linearity over the concentration ranges of 75–375 g/ml for Allopurinol and 50–250 g/ml for Lesinurad. Studies of precision revealed %RSD values below 2%, confirming intermediate precision and repeatability. Recovery studies provided evidence of accuracy, with results ranging from 100.60 percent to 99.14 percent. Additionally, the method was able to withstand deliberate changes in flow rate and mobile phase composition. The method's ability to indicate stability was confirmed by forced degradation tests conducted in thermal, photolytic, acidic, basic, oxidative, and thermal conditions. The validated method is suitable for routine quality control, assay, and stability analysis of Allopurinol and Lesinurad in pharmaceutical preparations due to its simplicity, sensitivity, and dependability.

Keywords: RP-HPLC, Allopurinol, Lesinurad, Method Validation, Stability-Indicating, ICH Guidelines.

1. Introduction:

Gout and other metabolic disorders are linked to hyperuricemia, which is characterized by elevated serum uric acid levels. One of the most common treatments for chronic gout is allopurinol, a xanthine oxidase inhibitor. By inhibiting the transformation of hypoxanthine into xanthine and then uric acid, it reduces uric acid production. Lesinurad, a newer uricosuric, works in conjunction with Allopurinol therapy to increase uric acid excretion by inhibiting the URAT1 transporter in the renal tubules. In patients with refractory or severe gout, the rational therapeutic strategy of combining Allopurinol and Lesinurad, which targets both the production and elimination pathways of uric acid, provides improved control.

The development of a straightforward, sensitive, and trustworthy analytical method for the simultaneous estimation of Allopurinol and Lesinurad is crucial considering the growing clinical reliance on fixed-dose combinations. Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) is still one of the most reliable and widely used methods for drug analysis in pharmaceutical formulations and bulk quantities. The guidelines issued by the International Council for Harmonization (ICH) emphasize the necessity of stability-indicating techniques that can precisely distinguish between the intact drug and its degradation products under a variety of stress conditions.

A novel, rapid, and stability-indicating RP-HPLC method for the simultaneous quantification of Allopurinol and Lesinurad is the goal of this study. Various combinations of columns, mobile phases, and pH conditions were used in several trials. An Inertsil ODS column with a mobile phase of phosphate buffer (pH 3.0) and acetonitrile in the ratio of 70:30 v/v was used to finalize the method's optimization. Under isocratic conditions, the separation was achieved with a detection wavelength of 255 nm and a flow rate of 1.5 ml/min. In terms of parameters like system suitability, linearity, precision, accuracy, robustness, limit of detection (LOD), and limit of quantification (LOQ), the method was validated in accordance with ICH guidelines. With correlation coefficients (R2) greater than 0.999, the method demonstrated excellent linearity over a concentration range of 75–375 g/ml for Allopurinol and 50–250 g/ml for Lesinurad.

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The method's reproducibility was confirmed by the fact that the precision and ruggedness %RSD values were well within the acceptable ranges. Under acidic, alkaline, oxidative, thermal, and photolytic stress conditions, stability studies were carried out. The method demonstrated its stability-indicating capability by successfully separating the active pharmaceutical ingredients from their degradation products. As a result, this RP-HPLC method provides a tried-and-true analytical strategy for simultaneously estimating Allopurinol and Lesinurad in bulk and dosage forms. This makes it suitable for routine quality control and stability testing in the pharmaceutical industry.

2. Aim

In accordance with ICH guidelines, to develop and validate a straightforward, precise, stability-indicating Reverse Phase High-Performance Liquid Chromatographic (RP-HPLC) method for the simultaneous estimation of Allopurinol and Lesinurad in pharmaceutical dosage forms.

3. Objectives

1. To create a RP-HPLC method that can simultaneously separate and quantify Lesinurad and Allopurinol with the highest resolution and peak symmetry.
2. To get the best separation possible by optimizing chromatographic parameters like pH, flow rate, mobile phase composition, detection wavelength, and column selection.
3. To ensure that the developed method meets the requirements of ICH Q2(R1) for the following parameters:
   - System suitability
   - Linearity and range
   - Accuracy and precision (intra-day and inter-day)
   - Specificity
   - Limit of Detection (LOD) and Limit of Quantification (LOQ)
   - Robustness
4. To demonstrate the method's ability to indicate stability by carrying out forced degradation studies under a variety of stress conditions—acidic, alkaline, oxidative, thermal, and photolytic.
5. To use the tested method for routine quality control and analysis of bulk drugs containing Allopurinol and Lesinurad and their combined pharmaceutical dosage form.

4. Materials and Methods

Materials

Chemicals and Reagents:

- Allopurinol and Lesinurad (working standards) were supplied by Pharmatrain.
- Potassium dihydrogen phosphate (KH_2PO_4) – FINAR Chemicals
- Methanol, Acetonitrile, and HPLC Water – Standard Solutions Ltd. and MERCK
- Ortho Phosphoric Acid – MERCK

Table 1: Instruments Used

S.NO	Instrument	Model/Details
1	HPLC System	Waters 2695 with 2487 UV detector (Empower software)
2	UV-Visible Spectrophotometer	LabIndia UV 3000+
3	pH Meter	Adwa AD 1020
4	Weighing Balance	Afcoset ER-200A
5	Glassware	Borosil (Pipettes, Burettes, Beakers)

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Chromatographic Conditions

- **Column:** Inertsil ODS C18 (150 mm × 4.6 mm, 5 µm)
- **Mobile Phase:** Phosphate buffer (pH 3.0): Acetonitrile (70:30 v/v)
- **Flow Rate:** 1.5 ml/min
- **Detection Wavelength:** 255 nm
- **Injection Volume:** 20 µL
- **Run Time:** 15 minutes
- **Mode:** Isocratic

Temperature: Ambient (25°C)

Preparation of Buffer and Mobile Phase

Phosphate Buffer (pH 3.0):
Using ortho-phosphoric acid, 6.8 grams of KH2PO4 were dissolved in 1000 milliliters of HPLC-grade water and the pH was adjusted to 3.0. An ultrasonic bath was used to degas the solution before it was filtered through a 0.45 m membrane.

Mobile Phase:
A membrane filter of 0.45 m was used to mix 700 ml of the prepared buffer and 300 ml of acetonitrile.

Diluent:
For both the standard and sample solutions, the mobile phase served as the diluent.

Standard Solution Preparation

A 100 ml volumetric flask containing 300 mg of Allopurinol and 200 mg of Lesinurad were accurately weighed, dissolved in 7 ml of diluent, sonicated, and the volume was increased with diluent. Working standard solutions were obtained by further diluting 0.75 milliliters to 10 milliliters from this.

Sample Solution Preparation

The dosage form's equivalent of 300 mg of Allopurinol and 200 mg of Lesinurad were dissolved, sonicated, and mixed with diluent in a 100 ml volumetric flask. For analysis, 0.75 ml of this was diluted to 10 ml.

Method Validation

Following ICH Q2(R1) guidelines, the method was validated for system suitability, linearity, precision, accuracy, specificity, robustness, and stability-indicating properties.

- **System Suitability:** Resolution ≥ 2, Tailing factor ≤ 2, Theoretical plates ≥ 2000
- **Linearity Range:** 75–375 µg/ml for Allopurinol; 50–250 µg/ml for Lesinurad
- **Precision:** Evaluated as %RSD of peak areas for six replicates
- **Accuracy:** Recovery studies at 50%, 100%, and 150% of target concentration
- **Robustness:** Assessed by varying flow rate and mobile phase composition ±10%
- **LOD & LOQ:** Calculated based on signal-to-noise ratios (S/N = 3 for LOD, S/N = 10 for LOQ)

Forced Degradation: Carried out in thermal, photolytic, acidic, alkaline, oxidative, and alkaline conditions

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5. Results

The proposed RP-HPLC method for the simultaneous estimation of Allopurinol and Lesinurad was successfully developed and validated as per ICH guidelines. In terms of specificity, precision, linearity, accuracy, robustness, and stability, the method performed exceptionally well.

1. System Suitability

Prior to sample analysis, system suitability parameters were evaluated. Lesinurad and Allopurinol had retention times of 4.974 and 6.006 minutes, respectively. The resolution between both peaks was 2.69, indicating proper separation. The theoretical plates were 3122.36 for Allopurinol and 3422.48 for Lesinurad, and tailing factors were within acceptable limits (<2), confirming the efficiency and symmetry of peaks.

Table 2: System Suitability Parameters

Parameter	Allopurinol	Lesinurad
Retention Time (min)	4.974	6.006
Area (µV·s)	463731	373273
Height (µV)	34151	23654
USP Plate Count	3122.36	3422.48
USP Tailing Factor	1.29	1.22
USP Resolution	–	2.69

Standard and sample solution injected as described under experimental work. The corresponding chromatograms and results are shown below.

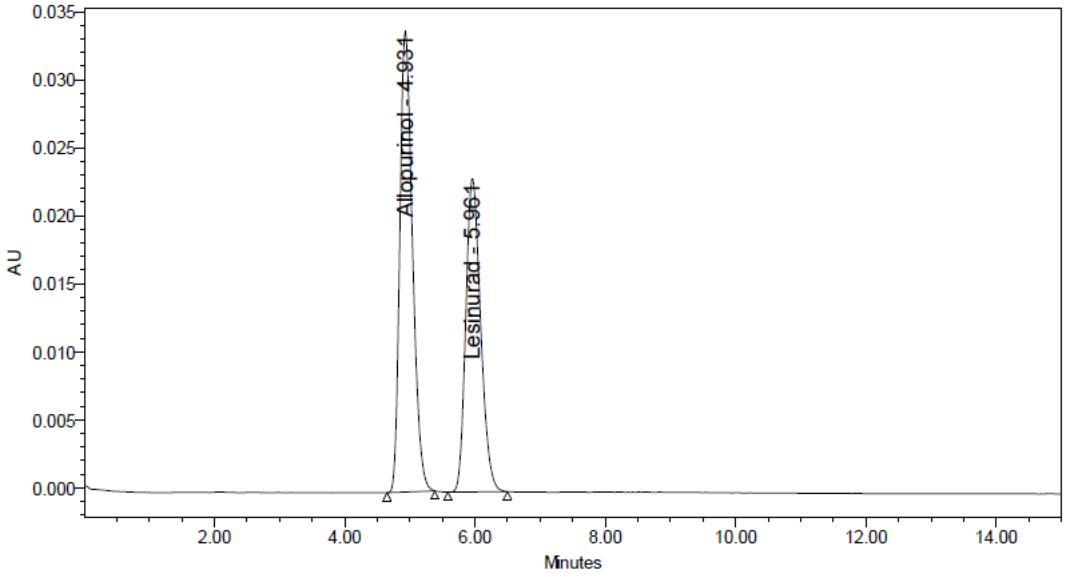

Figure 1: Chromatogram for Standard

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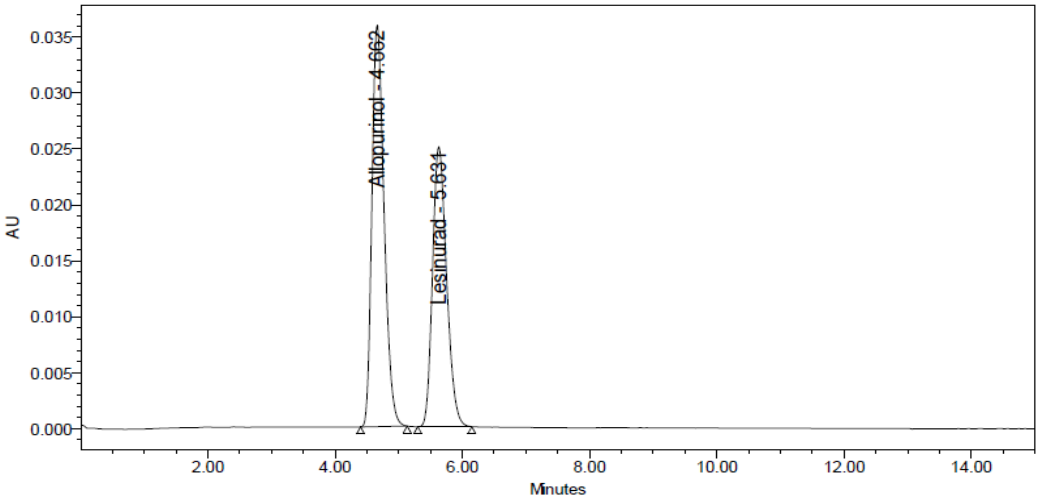

Figure 2: Chromatogram for Sample

Assay Results: (For Allopurinol)

$$\frac{465928.7}{465326.7} * \frac{300}{100} * \frac{3}{10} * \frac{100}{693} * \frac{10}{3} * \frac{693}{300} * \frac{99.8}{100} * 100 = 99.93\%$$

Assay Results: (For Lesinurad)

$$\frac{375589}{375025} * \frac{200}{100} * \frac{3}{10} * \frac{100}{693} * \frac{10}{3} * \frac{693}{200} * \frac{99.8}{100} * 100 = 99.95\%$$

Table 3: Results of Assay for Allopurinol and Lesinurad

	Label Claim (mg)	% Assay
Allopurinol	300	99.93
Lesinurad	200	99.95

System Suitability:

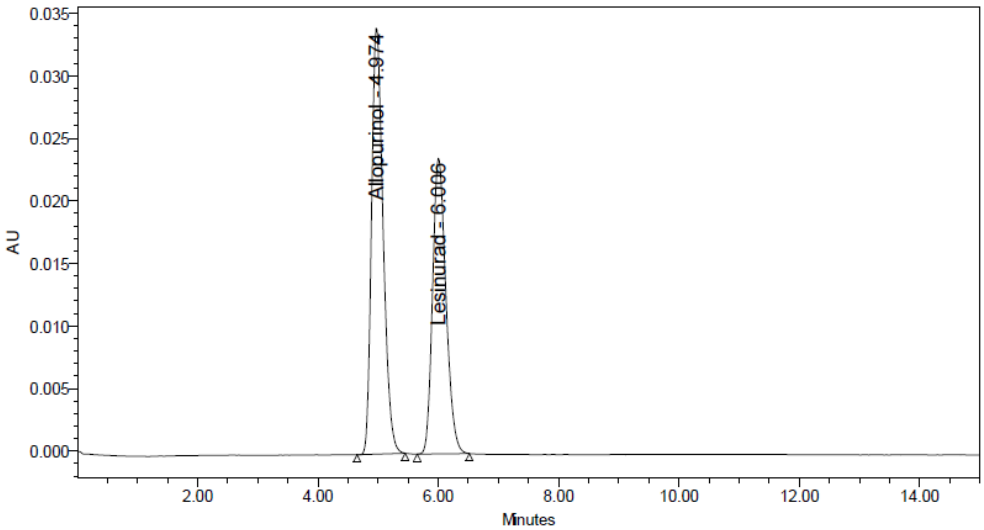

Figure 3: Chromatogram for system suitability

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2. Linearity

The method showed excellent linearity in the range of:

- **75–375 µg/ml** for Allopurinol
- **50–250 µg/ml** for Lesinurad

Allopurinol and Lesinurad had correlation coefficients (R2) of 0.9991 and 0.9990, respectively, indicating a strong linear relationship between peak area and concentration. The linearity range was found to lie from 75µg/ml to 375µg/ml of Allopurinol, 50µg/ml to 250µg/ml of Lesinurad and chromatograms are shown below.

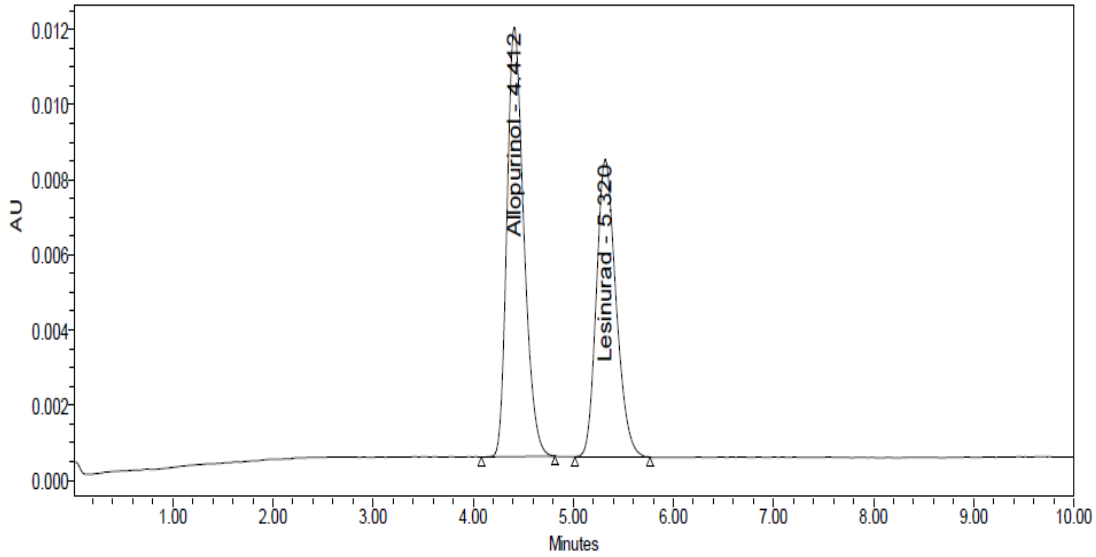

Figure 4: Chromatogram for linearity-1

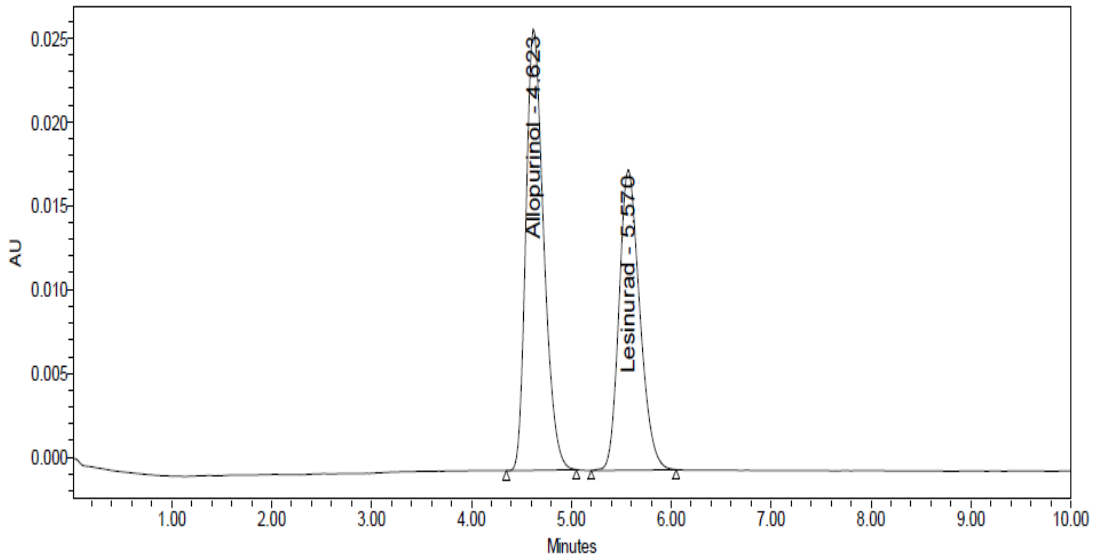

Figure 5: Chromatogram for linearity-2

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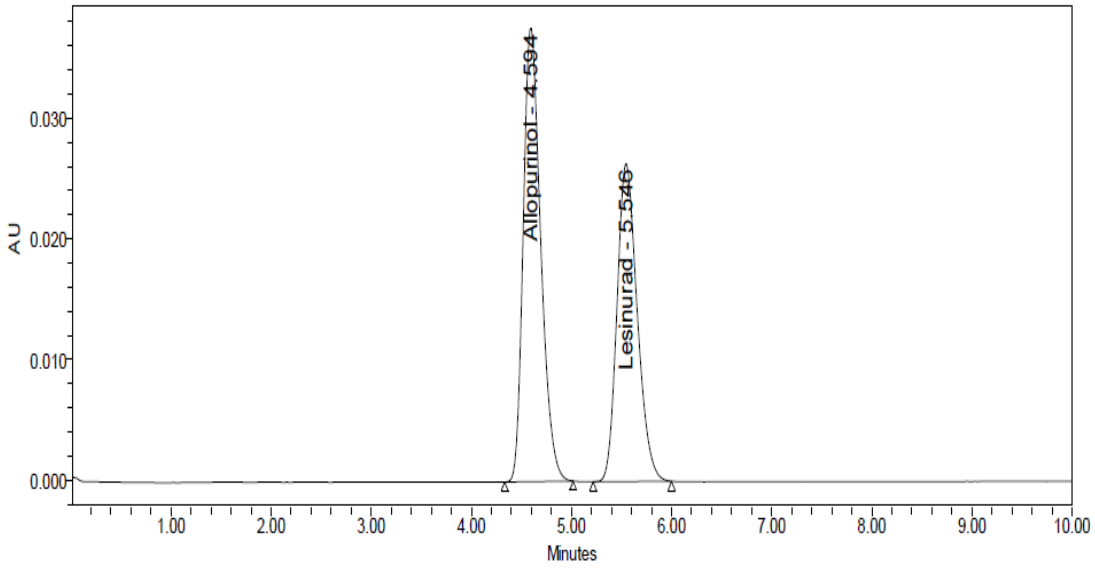

Figure 6: Chromatogram for linearity-3

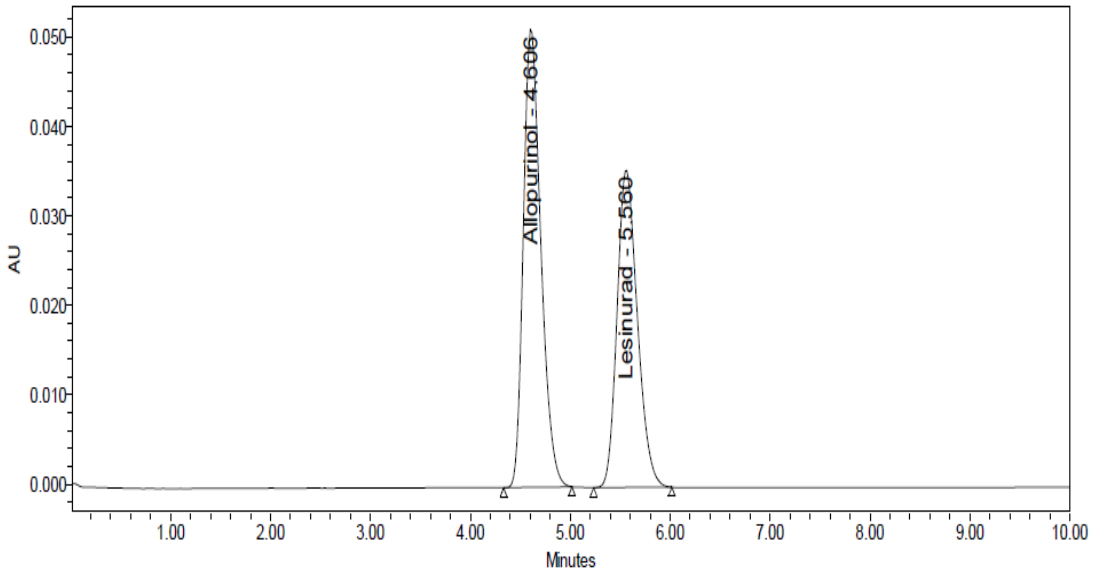

Figure 7: Chromatogram for linearity-4

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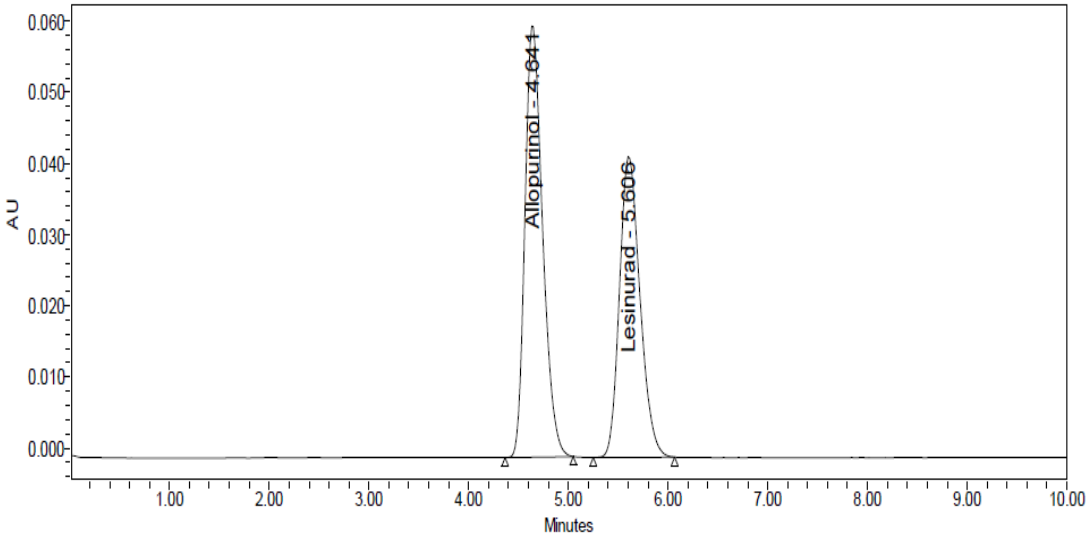

Figure 8: Chromatogram for linearity-5

Table 3: Linearity Data

Concentration (µg/ml)	Allopurinol Area	Lesinurad Area
75 / 50	163126	123687
150 / 100	324879	258151
225 / 150	484999	374272
300 / 200	622089	500737
375 / 250	774838	622363

Regression Equations:

- **Allopurinol: $y = 2027.5x + 17796$ ($R^2 = 0.9991$)**
- **Lesinurad: $y = 2479.9x + 3860.6$ ($R^2 = 0.9990$)**

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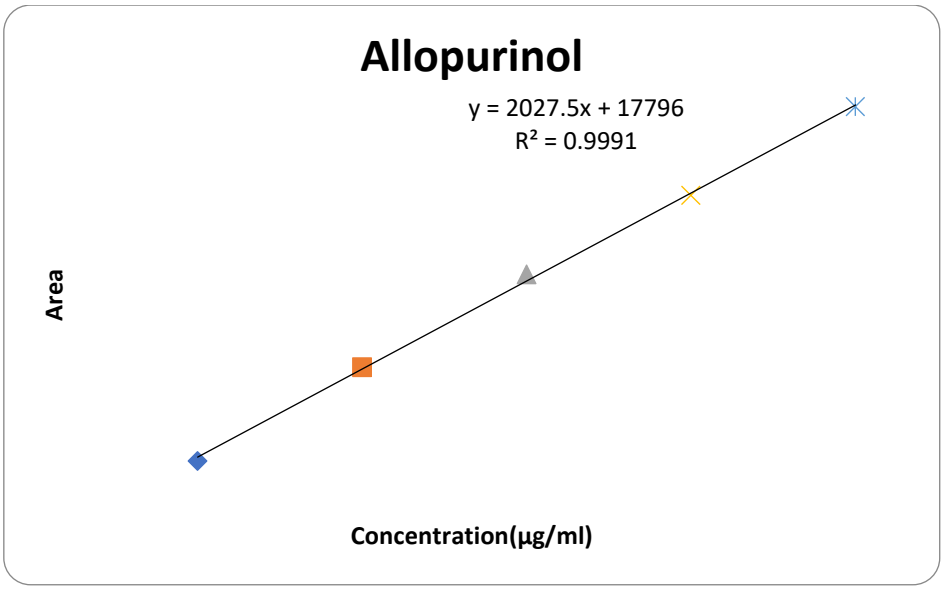

Figure 9: Calibration graph for Allopurinol

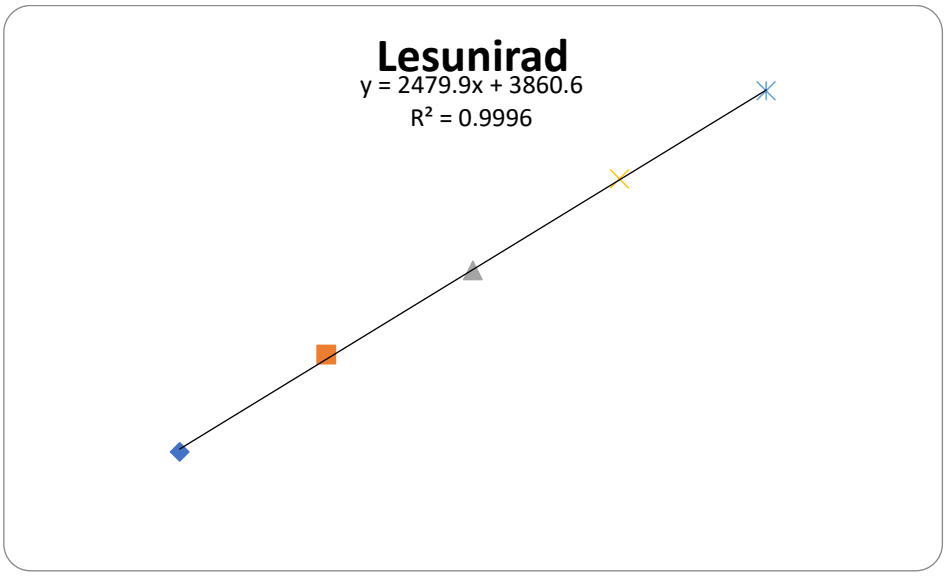

Figure 10: Calibration graph for Lesinurad

3. Precision

Repeatability was evaluated using six injections of standard solutions. Lesinurad and Allopurinol had %RSDs of 0.6 and 0.8 percent, respectively. For both drugs, intermediate precision (ruggedness) on various days resulted in %RSD values of 0.6%, which are within acceptable limits (NMT 2%).

Table 4: Precision (Repeatability)

Injection No.	Allopurinol Area	Lesinurad Area
1	469199	378542
2	466480	370422
3	463505	377395

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4	465113	375692
5	463129	375700
6	460972	372893
Mean	464733.0	375107.3
%RSD	0.6	0.8

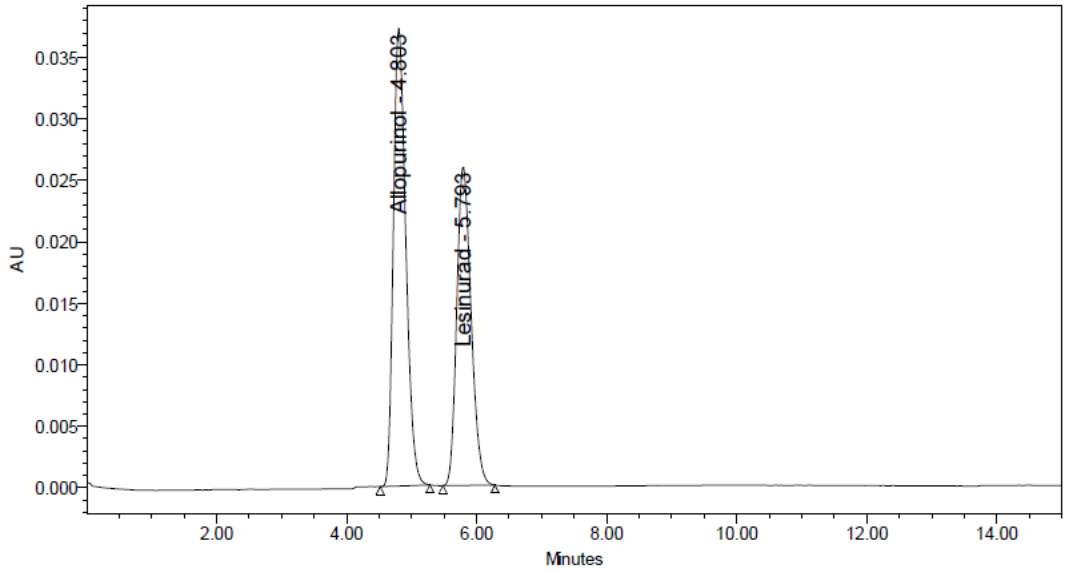

Figure 11: Chromatogram for Precision -1

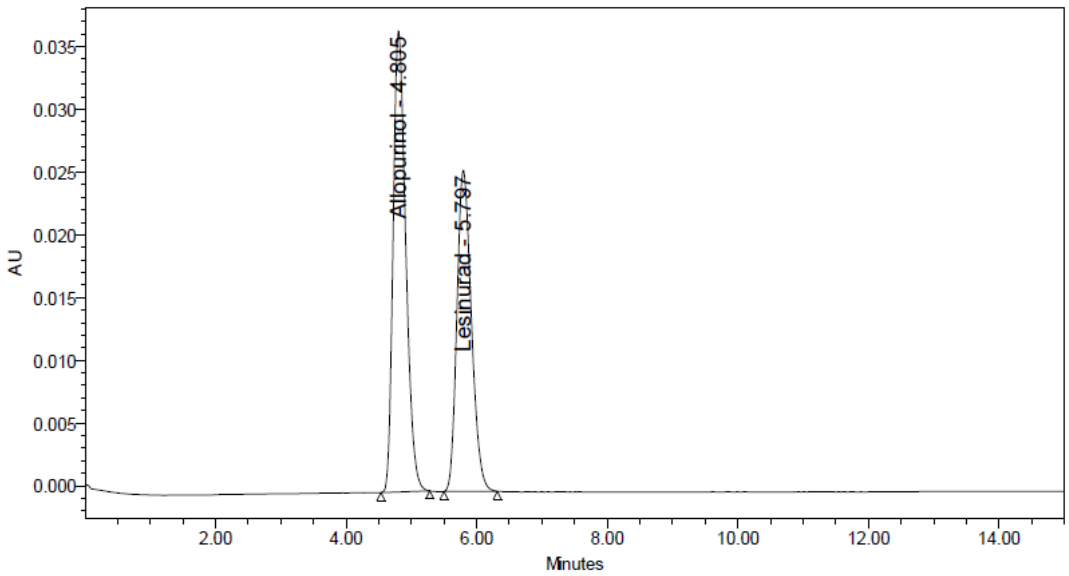

Figure 12: Chromatogram for Precision -2

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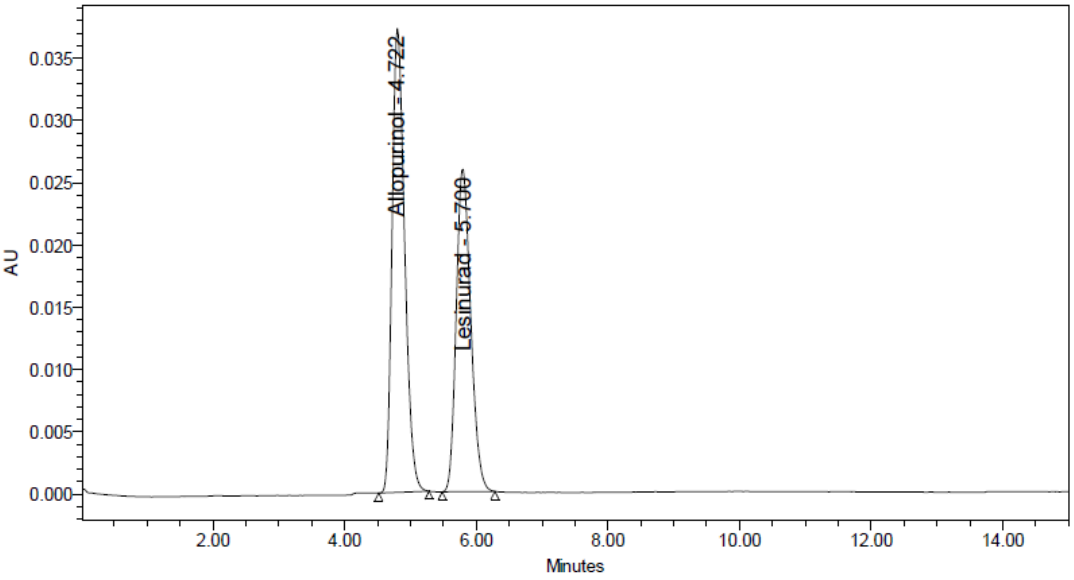

Figure 13: Chromatogram for Precision -3

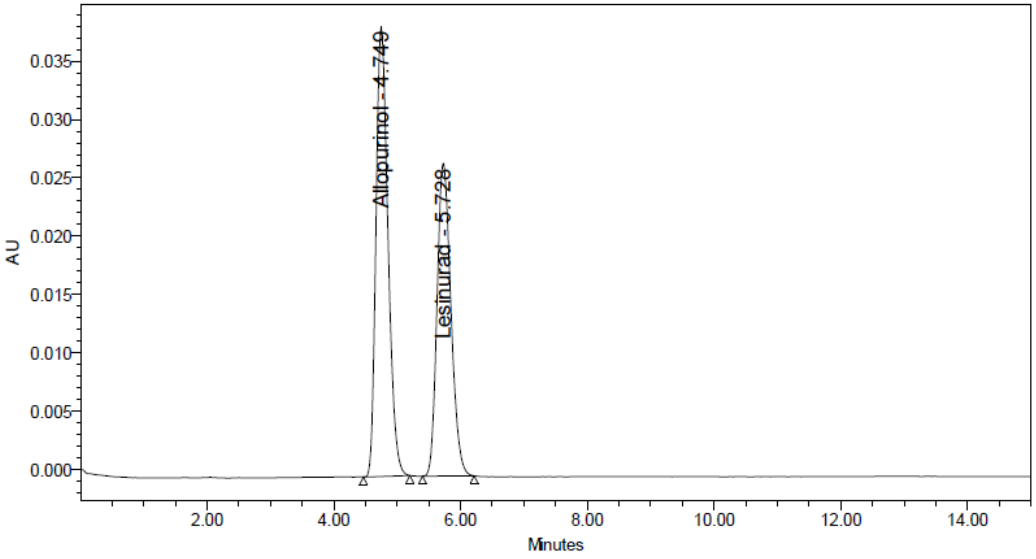

Figure 14: Chromatogram for Precision -4

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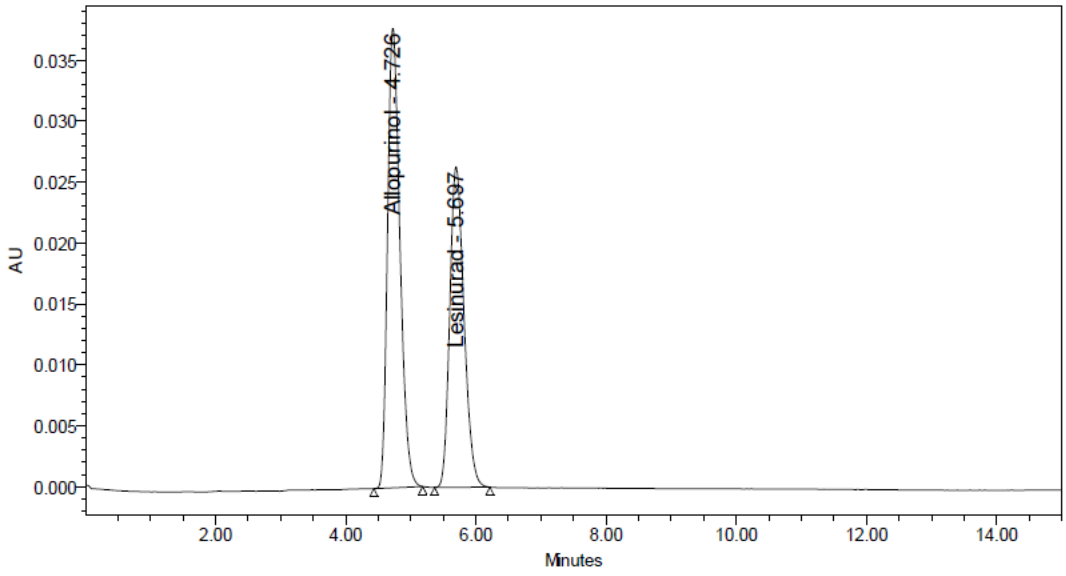

Figure 15: Chromatogram for Precision -5

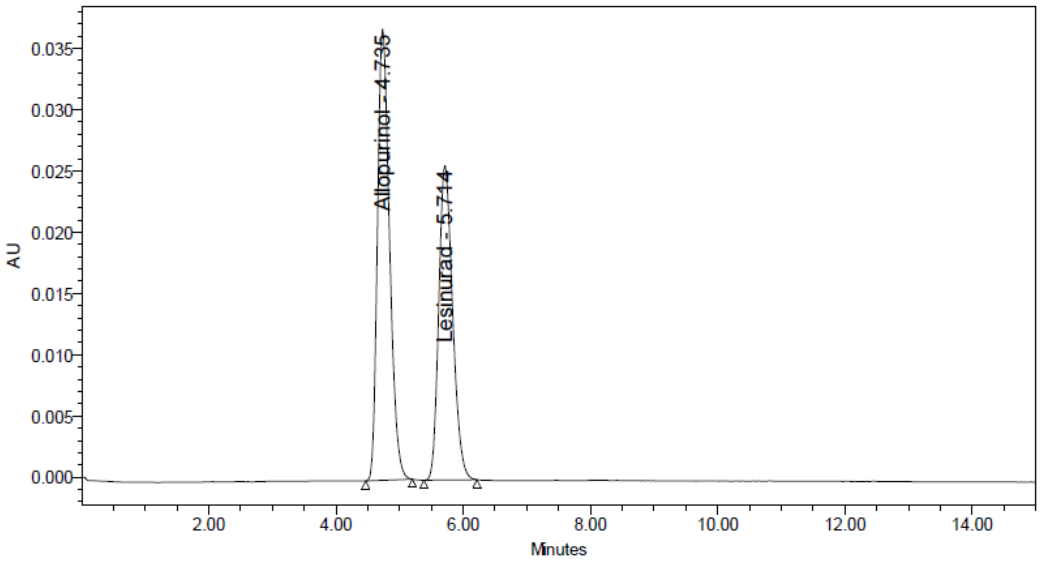

Figure 16: Chromatogram for Precision -6

4. Accuracy

Recovery studies were used to measure accuracy at three levels: 50%, 100%, and 150%. The mean recovery was found to be:

- **Allopurinol:** 99.60% to 100.28%
- **Lesinurad:** 99.66% to 100.60%

This indicates that the quantitative analysis method is highly accurate.

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Table 5: Accuracy (Recovery Studies)

Allopurinol

% Level	Amount Added (mg)	Amount Found (mg)	% Recovery
50%	150	150.42	100.28
100%	300	297.42	99.14
150%	450	447.25	99.39
Mean Recovery	–	–	**99.60**

Lesinurad

% Level	Amount Added (mg)	Amount Found (mg)	% Recovery
50%	100	100.19	100.19
100%	200	199.32	99.66
150%	300	301.81	100.60
Mean Recovery	–	–	**100.15**

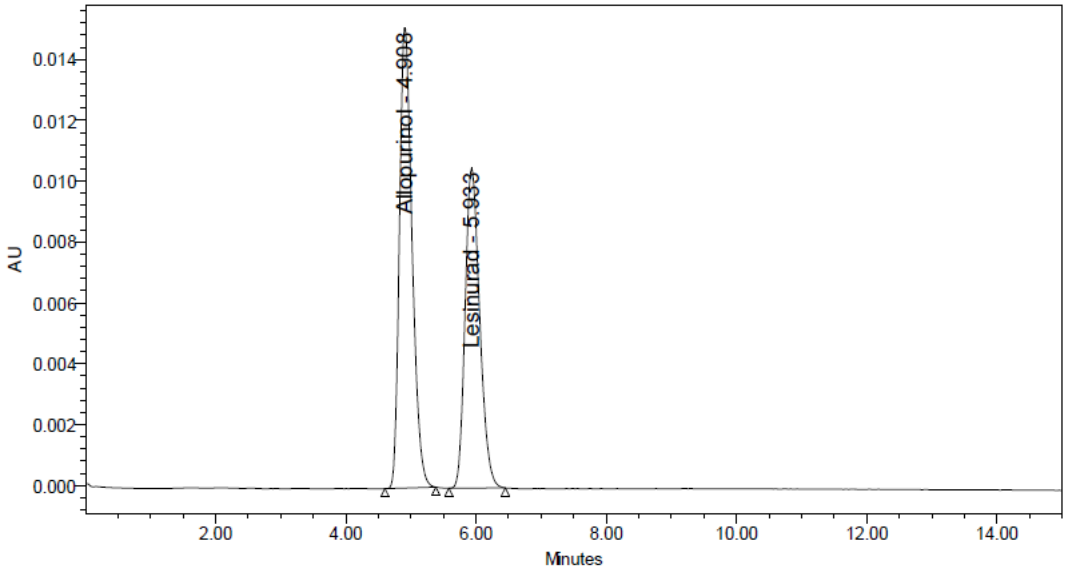

Figure 17: Chromatogram for Accuracy 50%-1

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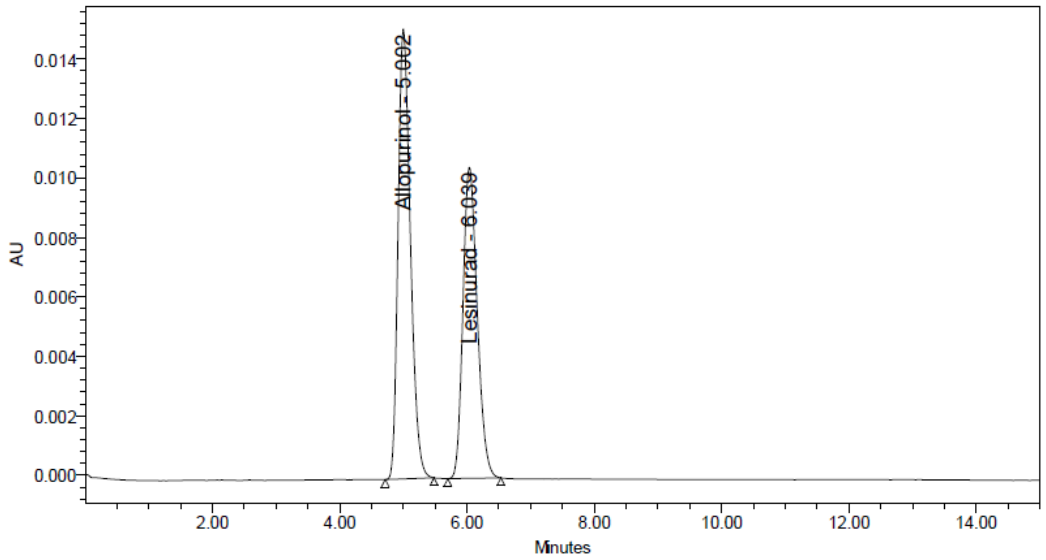

Figure 18: Chromatogram for Accuracy 50%-2

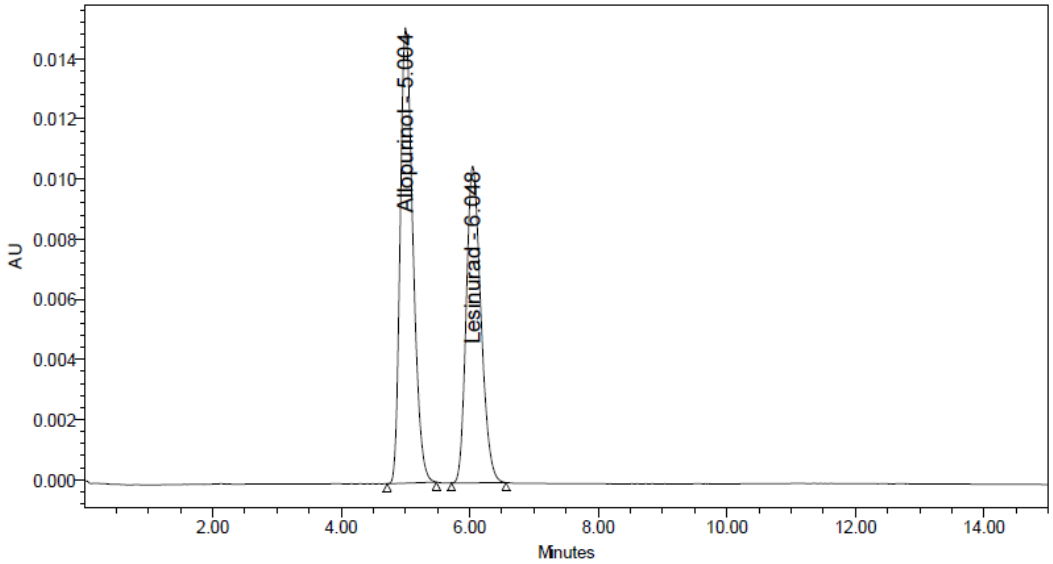

Figure 19: Chromatogram for Accuracy 50%-3

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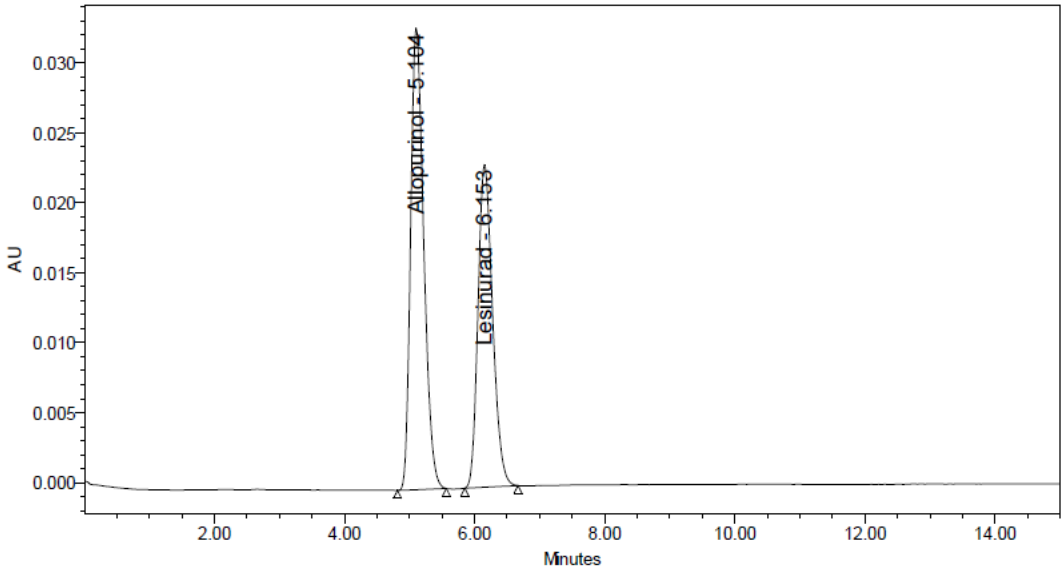

Figure 20: Chromatogram for Accuracy 100%-1

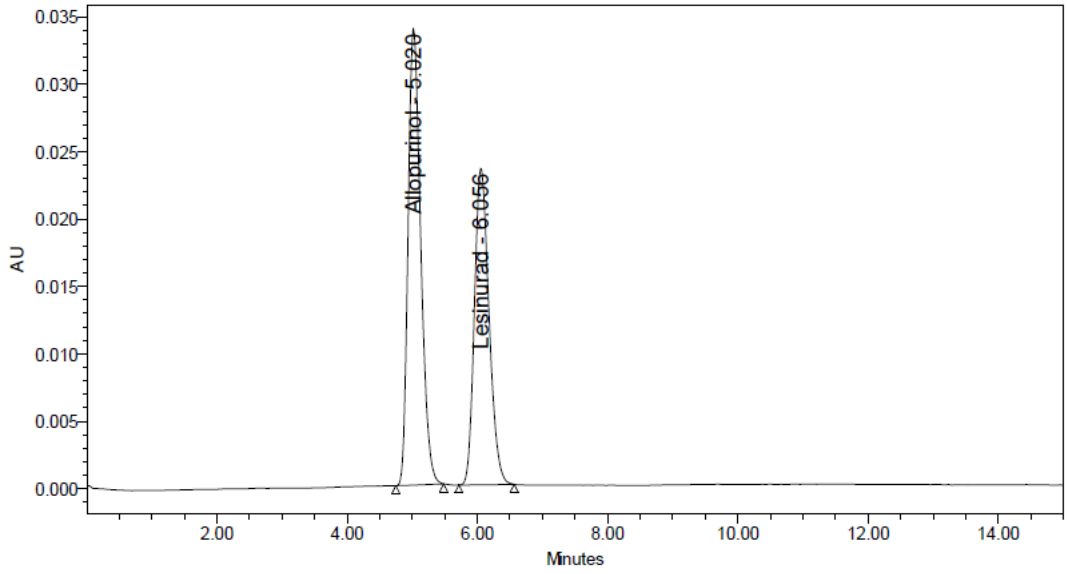

Figure 21: Chromatogram for Accuracy 100%-2

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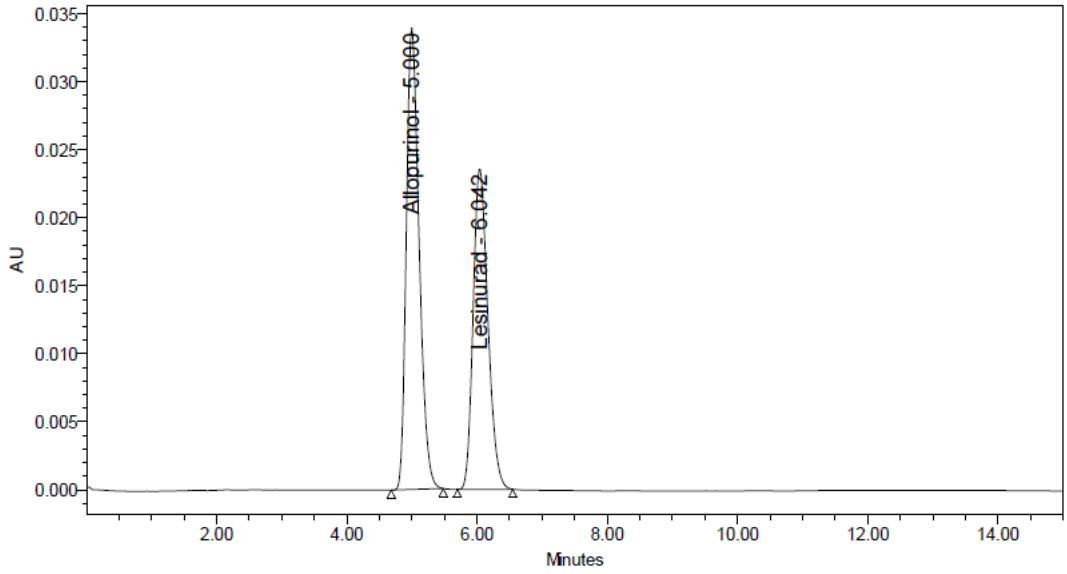

Figure 22: Chromatogram for Accuracy 100%-3

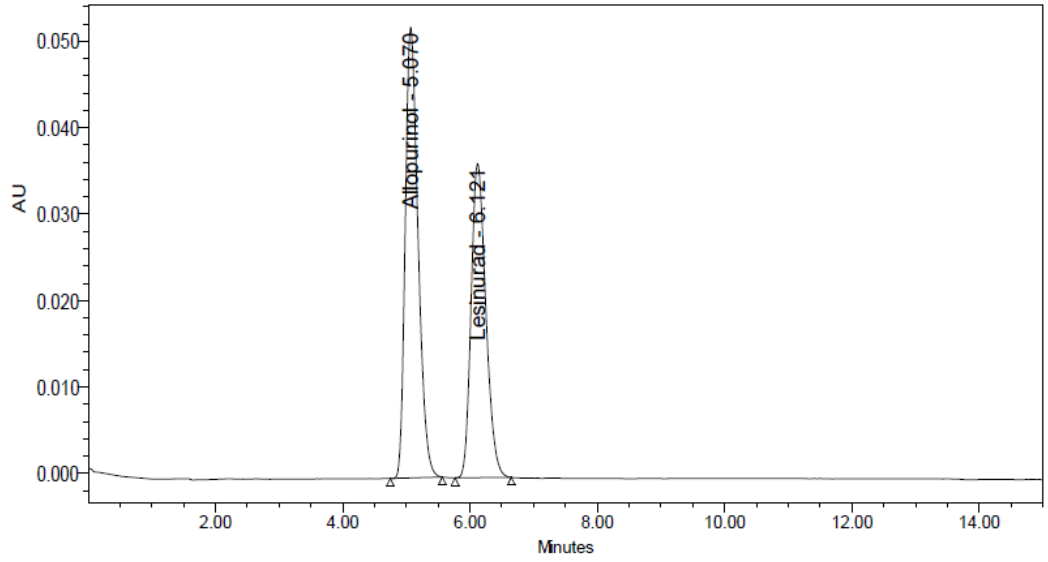

Figure 23: Chromatogram for Accuracy 150%-1

5. Specificity

The specificity of the method was confirmed as no interference was observed at the retention times of Allopurinol and Lesinurad from blank or excipient peaks, confirming that the method is selective for the analytes.

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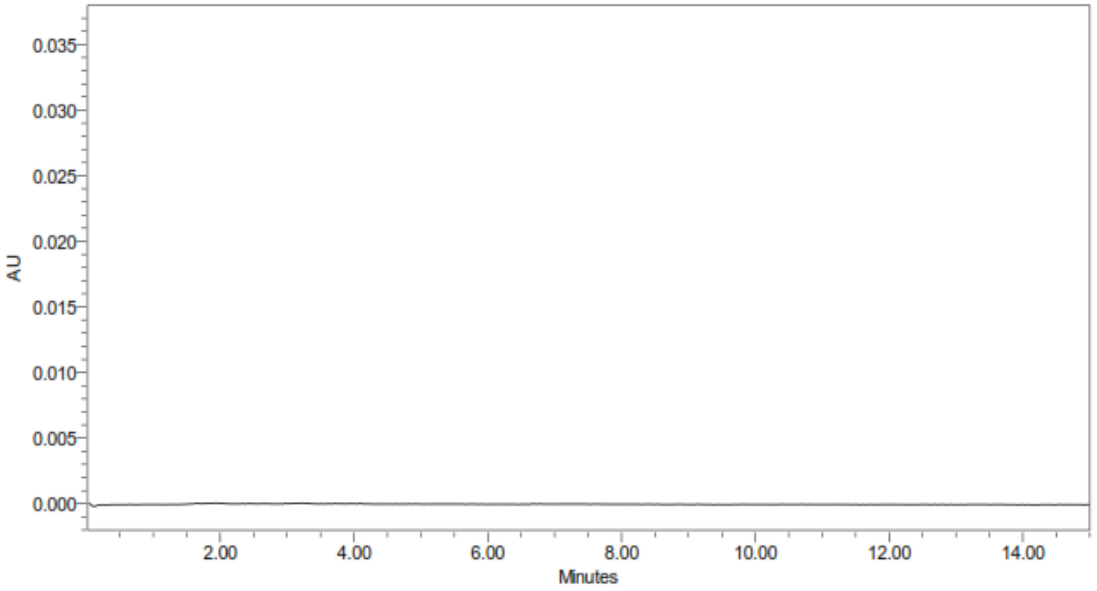

Figure 17: Chromatogram for Blank

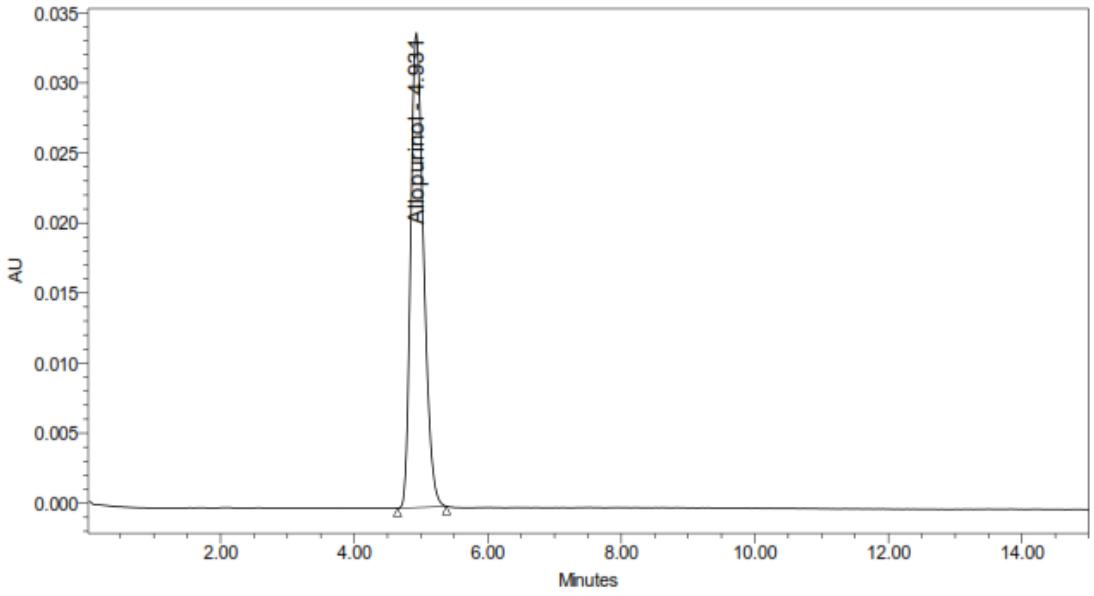

Figure 26: Chromatogram for Allopurinol

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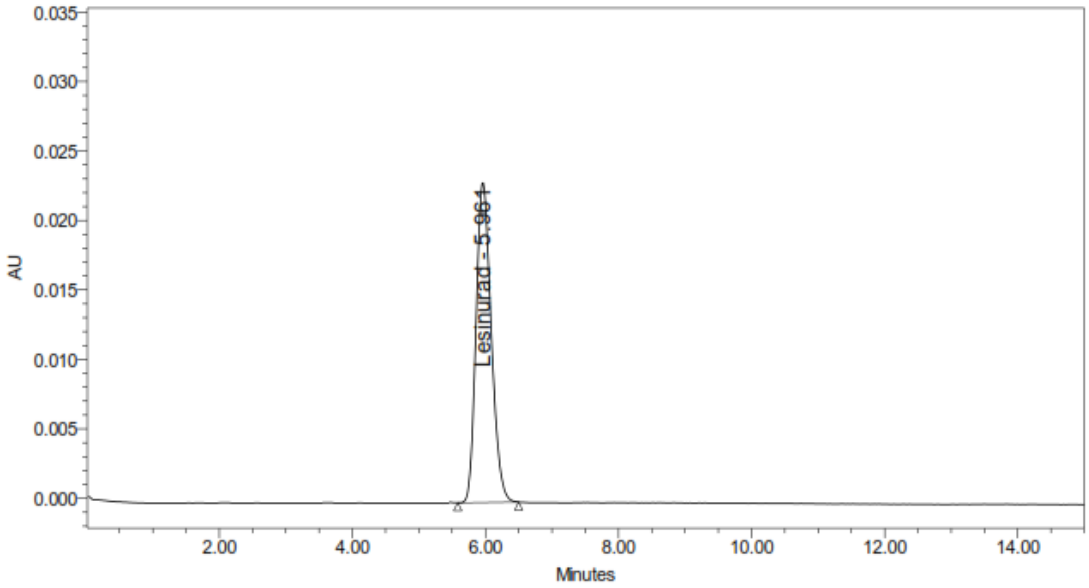

Figure 27: Chromatogram for Lesinurad

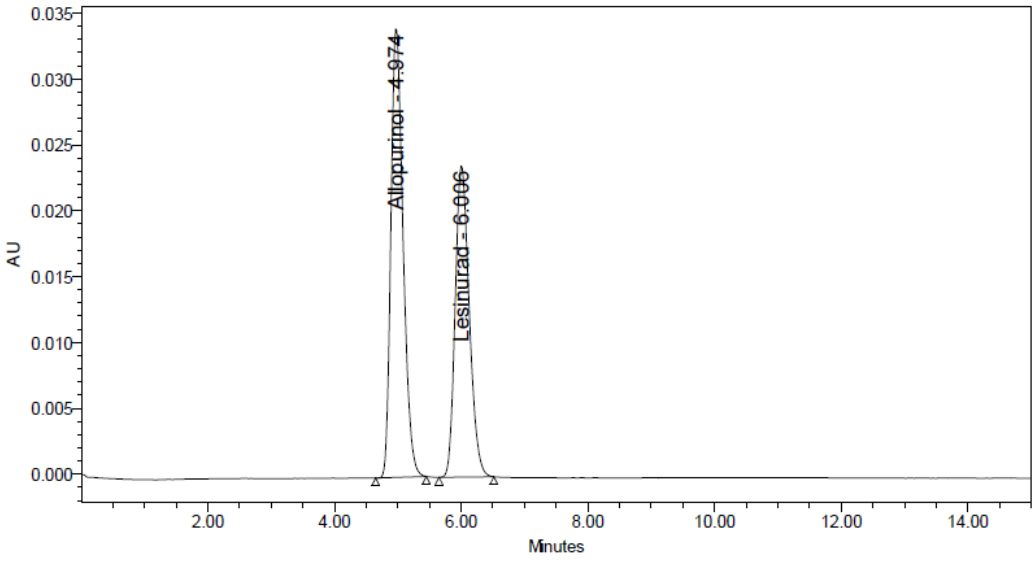

Figure 27: Chromatogram for Standard

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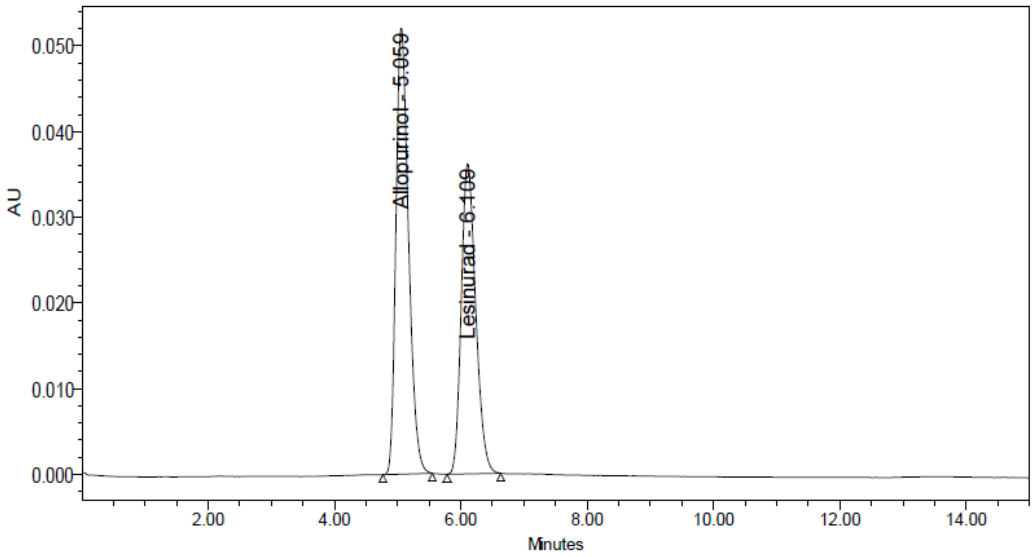

Figure 24: Chromatogram for Accuracy 150%-2

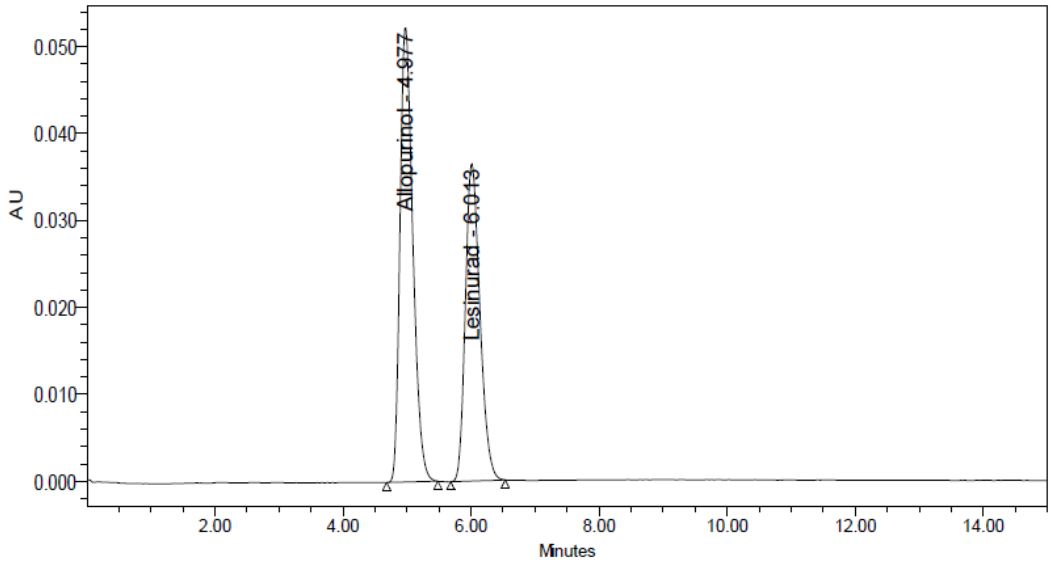

Figure 25: Chromatogram for Accuracy 150%-3

6. LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) were determined based on the signal-to-noise ratio:

- **Allopurinol:** LOD = 0.09 µg/ml, LOQ = 0.27 µg/ml
- **Lesinurad:** LOD = 0.06 µg/ml, LOQ = 0.18 µg/ml

These results indicate the high sensitivity of the developed method.

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Table 6: LOD and LOQ

Drug	LOD (µg/ml)	LOQ (µg/ml)
Allopurinol	0.09	0.27
Lesinurad	0.06	0.18

7. Robustness

Retention time, resolution, and peak shape were unaffected by deliberate variations in mobile phase composition and flow rate (10%). The method's robustness was confirmed by the fact that all system suitability parameters remained within acceptable limits.

8. Assay

The assay results for the tablet formulation showed:

- **Allopurinol**: 99.93% of label claim
- **Lesinurad**: 99.95% of label claim

This confirms the suitability of the method for routine analysis of pharmaceutical formulations.

9. Stability-Indicating Nature

Forced degradation studies under acidic, basic, oxidative, thermal, and photolytic conditions showed clear separation of degradation products from the main peaks. The method's stability-indicating degradation ranged from 2.5% to 8.3%.

Table 7: Forced Degradation Studies

Stress Condition	Allopurinol % Degraded	Lesinurad % Degraded
Acidic	4.03	4.06
Alkaline	2.53	3.33
Oxidative (H_2O_2)	5.49	8.31
Thermal	3.55	6.76
Photolytic	5.94	6.14

6. Discussion

For the simultaneous estimation of Allopurinol and Lesinurad in bulk and dosage forms, the developed RP-HPLC method proved to be a straightforward, precise, robust analytical tool. It was necessary to optimize chromatographic parameters, such as pH and the composition of the mobile phase, to attain sufficient resolution and symmetric peak shapes. The use of a phosphate buffer (pH 3.0) and acetonitrile (70:30 v/v) with an Inertsil ODS C18 column provided optimal results under isocratic conditions.

Method dependability was ensured by system suitability parameters like resolution (>2), tailing factor (2), and theoretical plates (>2000). Linearity was observed over a wide concentration range for both drugs, with correlation coefficients close to 1. Precision studies demonstrated that the method is repeatable, with %RSD values well

below the 2% threshold. The method's reliability for quantitative analysis was confirmed by recovery studies at three levels of accuracy.

Robustness testing indicated that minor changes in chromatographic conditions did not significantly affect method performance. By clearly separating degradation products from the main peaks under a variety of stress conditions, forced degradation studies also demonstrated the stability-indicating nature of the method. As a result, Allopurinol and Lesinurad formulation stability and quality control studies can benefit from this approach.

7. Conclusion

The present study successfully established a novel, accurate, and robust RP-HPLC method for the simultaneous estimation of Allopurinol and Lesinurad in both bulk drug and pharmaceutical dosage forms. Method development involved extensive trials with various mobile phases and chromatographic conditions. Under isocratic conditions, an Inertsil ODS C18 column with a mobile phase of phosphate buffer (pH 3.0) and acetonitrile (70:30 v/v) was used to achieve optimal separation. Both analytes showed clear and well-resolved peaks at the 255 nm detection wavelength.

The developed method was validated as per ICH Q2(R1) guidelines and showed excellent system suitability, linearity, accuracy, precision, robustness, and sensitivity. Correlation coefficients greater than 0.999 demonstrated linearity across a broad range, demonstrating the method's dependability. Accuracy was confirmed through recovery studies at multiple concentration levels, yielding results within the acceptable range of 99–101%.

Precision and ruggedness had %RSD values below 2%, indicating reproducibility. Importantly, the method was able to distinguish the active drug peaks from their degradation products under a variety of stress conditions, including acidic, basic, oxidative, thermal, and photolytic environments, demonstrating that it was stability-indicating. As a result, the proposed RP-HPLC method is very suitable for routine quality control, assay, and stability testing of Allopurinol and Lesinurad in pharmaceutical industries. It is easy to use, specific, and inexpensive.

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