ASSAY METHOD FOR SIMULTANEOUS ESTIMATION OF LESINURAD AND ALLOPURINOL IN ITS BULK AND PHARMACEUTICAL DOASGE FORM BY RP-HPLC METHOD

Janat beula
Associate professor
TRR College of pharmacy
Meerpet Hyderabad

K. Chaitanya Prasad
Assistant Professor
Department Of Pharmaceutical Chemistry
Samskruthi College Of Pharmacy
,Hyderabad

Abstract

HPLC is based on the mechanism of adsorption, partition, ion exchange or size exclusion, depending on the type of stationary phase used. HPLC involves a solid stationary phase, normally packed inside a stainless- steel column, and a liquid mobile phase. Separation of the components of a solution results from difference in the relative distribution ratios of the solutes between the two phases.

Introduction

Fundamentals of Separation/ system suitability parameters:

Column efficiency (N):

Column efficiency is called as number of theoretical plates. It measures that the band spreading number of theoretical plate is higher. If it is higher it indicates good column and system performance[4].It should be more than 2000.Column performance can be defined on terms of values of N.$N = 16(t_R/w)$ 2 or 3500 L (cm)/ dp (μm) Plate height, H = N /L where L = length

Types of Analytical Procedures to be Validated:

The discussion of the validation of analytical procedures is directed to the four mostcommon types of analytical procedures:

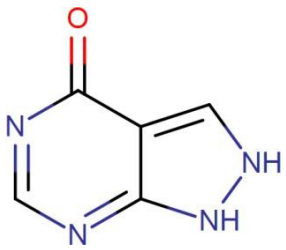
- Identification tests.
- Quantitative tests for impurities' content.
- Limit tests for the control of impurities.
- Quantitative tests of the active moiety in samples of drug substance or drug product orotherselected component(s) in the drug product.

DRUG PROFILE

1. ALLOPURINOLDescription:

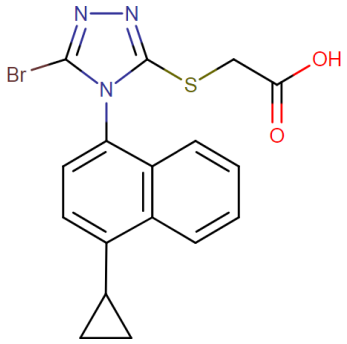
A Xanthine Oxidase Inhibitor That Decreases Uric Acid Production.

Structure:



Synonyms:

1, 5-Dihydro-4h-Pyrazolo (3, 4-D) Pyrimidin-4-One
1, 5-Dihydro-4h-Pyrazolo (3, 4-D) Pyrimidine-4-One1h-Pyrazolo (3, 4-D) Pyrimidin-4-Ol
4-Hpp
4-Hydroxy-1h-Pyrazolo (3, 4-D) Pyrimidine
CAS Number: 315-30-0
Weight: Average: 136.1115
Chemical Formula: C5h4n4o



Iupac Name: 1h, 2h, 4h-Pyrazolo [3, 4-D] Pyrimidin-4-One

Indication:

For The Treatment Of Hyperuricemia Associated With Primary Or Secondary Gout. Also Indicated For The Treatment Of Primary Or Secondary Uric Acid Nephropathy, With Or Without The Symptoms Of Gout, As Well As Chemotherapy-Induced Hyperuricemia And Recurrent Renal Calculi.

Structured Indications:

Hyperuricemia

Calcium Oxalate Calculi Renal Calculi

Metabolism: Hepatic AllopurinolOxypurinol

Route of Elimination:

Approximately 20% Of The Ingested Allopurinol Is Excreted In The Feces.

Half Life: 1-3 Hours

Toxicity: Ld50=214 Mg/Kg (In Mice)

Affected Organisms: Humans And Other Mammals

2. LESINURADDescription:

Lesinurad Is An Oral Uric Acid Transporter 1 (Urat1) Inhibitor Indicated For The TreatmentOf Hyperuricemia Associated With Gout.

{[5-Bromo-4-(4-Cyclopropylnaphthalen-1-Yl)-4h-1,2,4-Triazol-3-Yl]Sulfanyl}Acetic Acid

CAS Number: 878672-00-5

Weight: Average:404.28 **Monoisotopic:** 402.999011 **Chemical Formula:** C17h14brn3o2s

Iupac Name: 2-{[5-Bromo-4-(4-Cyclopropylnaphthalen-1-Yl)-4h-1,2,4-Triazol-3-Yl]Sulfanyl}Acetic Acid

EXPERIMENTAL METHOD

Table 1: Instruments used

SL. NO	INSTRUMENT	MODEL
1	HPLC	WATERS, software: Empower, 2695 separation module.2487 UV detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000+
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

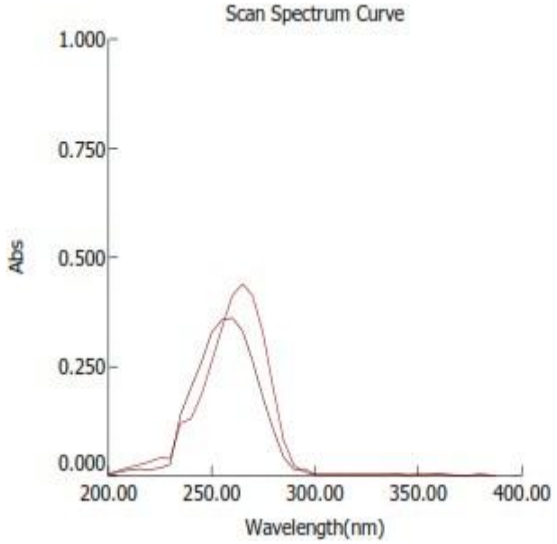
Table 2: Chemicals used

SL. NO	CHEMICAL	BRAND
1	Allopurinol	Supplied by Pharmatrain
2	Lesinurad	Supplied by Pharmatrain
3	KH2PO4	FINAR chemical LTD
4	Water and Methanol for HPLC	Standard solutions Ltd
5	Acetonitrile for HPLC	Standard solutions Ltd

6	Water HPLC	MERCK
7	Ortho phosphoric acid	MERCK

Wave length selection:

UV spectrum of 10 µg/ml Allopurinol and 10 µg/ml Lesinurad in diluents (mobile phasecomposition). From the UV spectrum wavelength selected as 255 nm.



HPLC METHOD DEVELOPMENT

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used : Waters HPLC with auto sampler and PDA detector.Temperature :Ambient (25□ C)
Mode of separation : Isocratic mode
Column : Inertsil ODS (150 x 4.6, 5µm)
Buffer :Phosphate buffer pH 3
Mobile phase :Phosphate buffer pH 3: Acetonitrile (70: 30)Flow rate: 1.5ml per min
Wavelength : 255 nm
Injection volume : 20 □l
Run time : 15 min.

PREPARATION OF BUFFER AND MOBILE PHASE:

Standard Solution Preparation:

Accurately weigh and transfer 300 mg of Allopurinol and 200 mg of Lesinurad working standard into a 100 ml VF add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.75 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 300 mg of Allopurinol and 200 mg of Lesinurad sample into a 100 ml volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.75 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

METHOD VALIDATION SUMMARY:

INTERMEDIATE PRECISION/RUGGEDNESS:

Preparation of stock solution:

Accurately weigh and transfer 300 mg of Allopurinol and 200 mg of Lesinurad working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.75 ml of the above stock

solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

ACCURACY:

Preparation of Standard stock solution:

Accurately weigh and transfer 300 mg of Allopurinol and 200 mg of Lesinurad working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.75 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh and transfer 150 mg of Allopurinol and 100 mg of Lesinurad working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.75 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weigh and transfer 300 mg of Allopurinol and 200 mg of Lesinurad working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.75 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 150% solution (With respect to target Assay concentration):

Accurately weigh and transfer 450 mg of Allopurinol and 300 mg of Lesinurad working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.75 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

ROBUSTNESS:

A. The flow rate was varied at 1.35 ml/min to 1.65ml/min. Standard solution 225 ppm of Allopurinol & 150 ppm of Lesinurad was prepared and analysed using the varied flow rates along with method flow rate.

B. The Organic composition in the Mobile phase was varied from ±10%.

Standard solution 225 ppm of Allopurinol & 150 ppm of Lesinurad was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

RESULTS AND DISCUSSION

ASSAY:

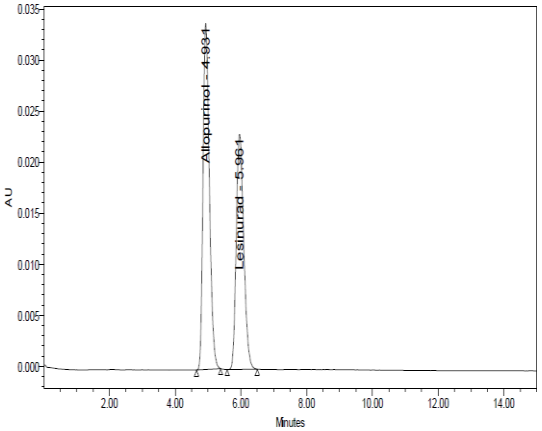


Figure 1: Chromatogram for Standard

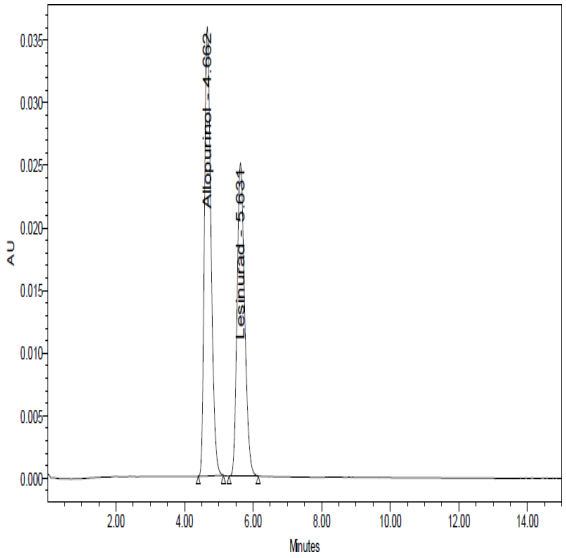


Figure 2: Chromatogram for Sample

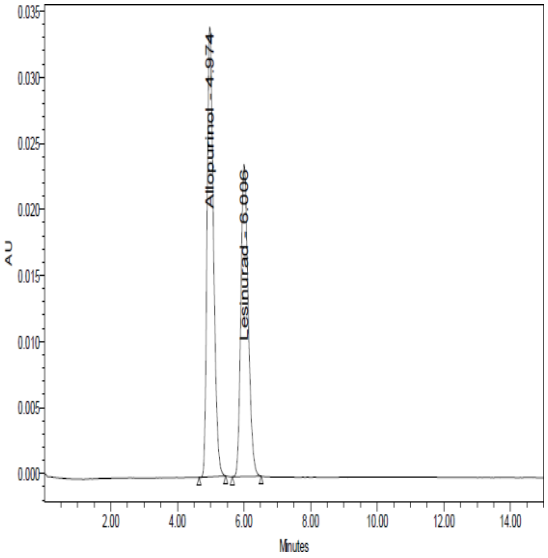


Figure 3: Chromatogram for system suitability

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 1: Results of Assay for Allopurinol and Lesinurad

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

SYSTEM SUITABILITY:

VALIDATION PARAMETERS:

LINEARITY:

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.

Table 2: Area of different concentration of Allopurinol and Lesinurad

S. No	Allopurinol		Lesinurad	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	75	163126	50	123687
2	150	324879	100	258151
3	225	484999	150	374272
4	300	622089	200	500737
5	375	774838	250	622363

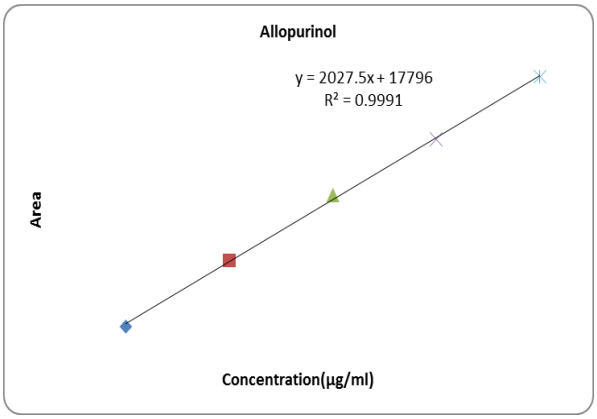


Figure 4: Calibration graph for Allopurinol

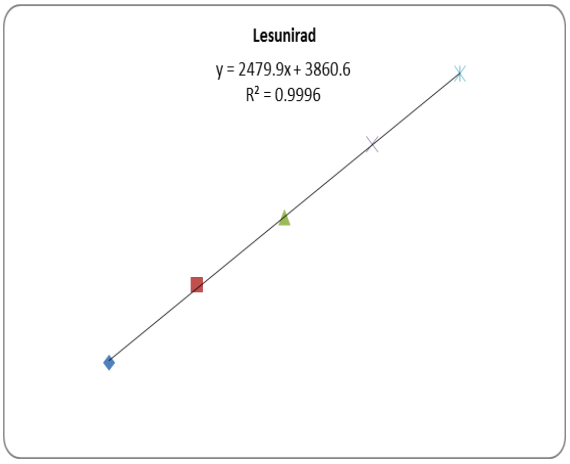


Figure 5: Calibration graph for Lesinurad

Table 3: Analytical performance parameters of Allopurinol and Lesinurad

Parameters	Allopurinol	Lesinurad
Slope (m)	2027.5	2479.9
Intercept (c)	17796	3860.6
Correlation coefficient (R^2)	0.999	0.999

PRECISION:

Precision of the method was carried out for both sample solutions as described under experimental work. The corresponding chromatograms and results are shown below.

Table 4: Results of Precision for Allopurinol and Lesinurad

Injection	Area for Allopurinol	Area for Lesinurad
Injection-1	469199	378542
Injection-2	466480	370422
Injection-3	463505	377395
Injection-4	465113	375692
Injection-5	463129	375700
Injection-6	460972	372893
Average	464733.0	375107.3
Standard Deviation	2876.4	2985.9
%RSD	0.6	0.8

Table 5: Results of Intermediate precision for Allopurinol and Lesinurad

Injection	Area for Allopurinol	Area for Lesinurad
Injection-1	466111	372909
Injection-2	463354	378218
Injection-3	467721	375833
Injection-4	463219	376144
Injection-5	469297	379868
Injection-6	462378	377714

Average	465346.7	376781.0
Standard Deviation	2797.8	2398.4
%RSD	0.6	0.6

SPECIFICITY:

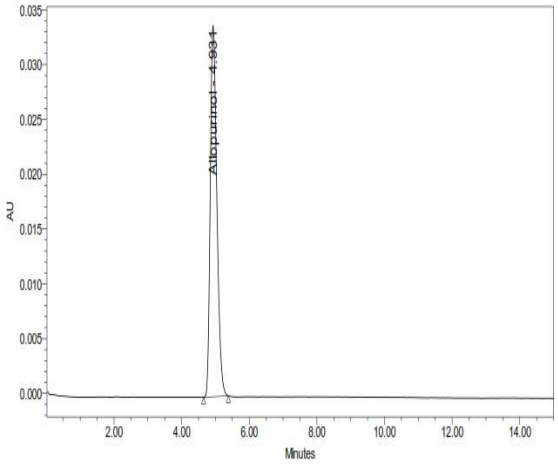


Figure 6: Chromatogram for Allopurinol

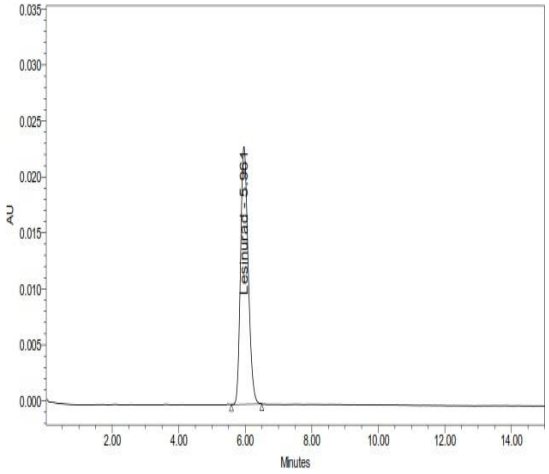
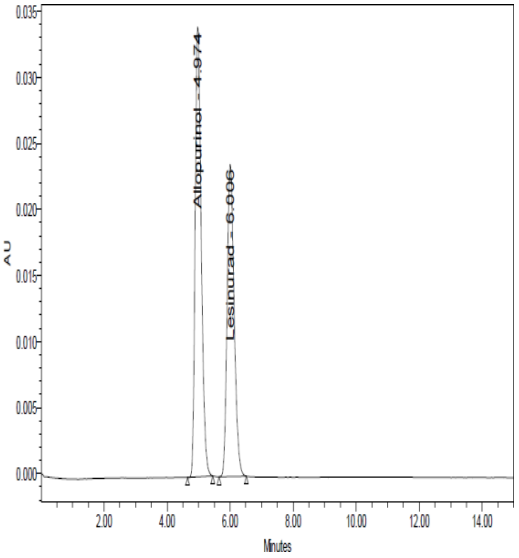


Figure 7: Chromatogram for Lesinurad



ACCURACY:

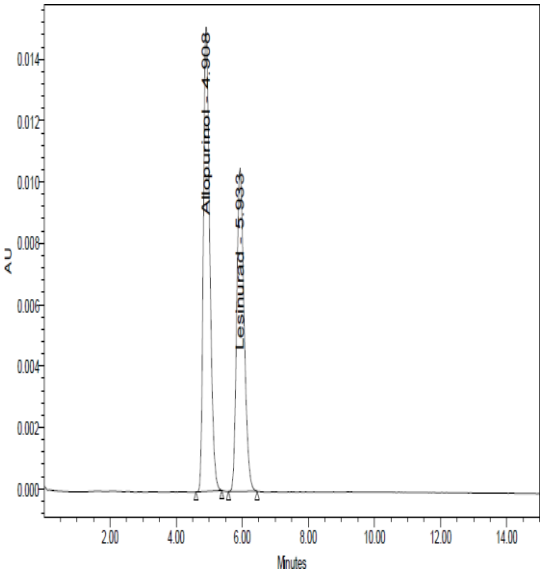


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Figure 8: Chromatogram for Accuracy 50%-1

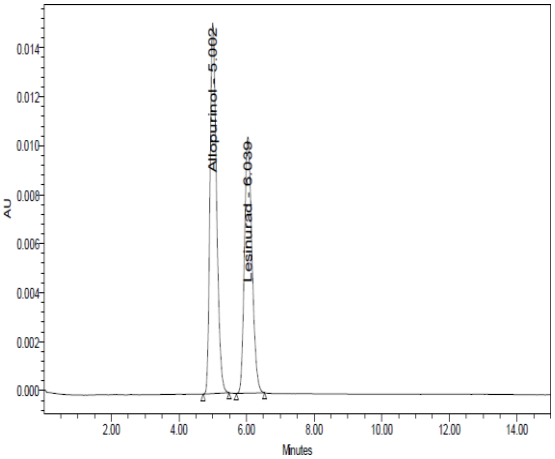


Figure 9: Chromatogram for Accuracy 50%-2

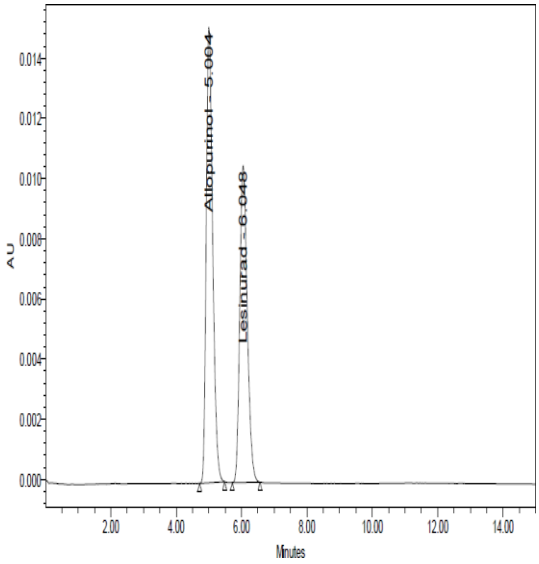


Figure10: Chromatogram for Accuracy 50%-3

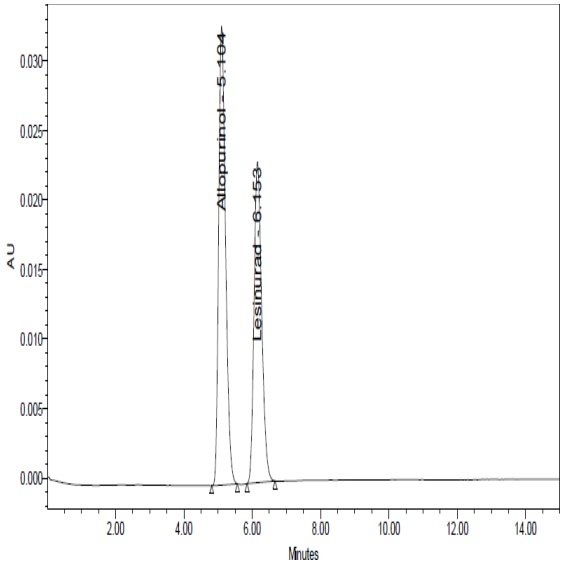


Figure 11: Chromatogram for Accuracy 100%-1

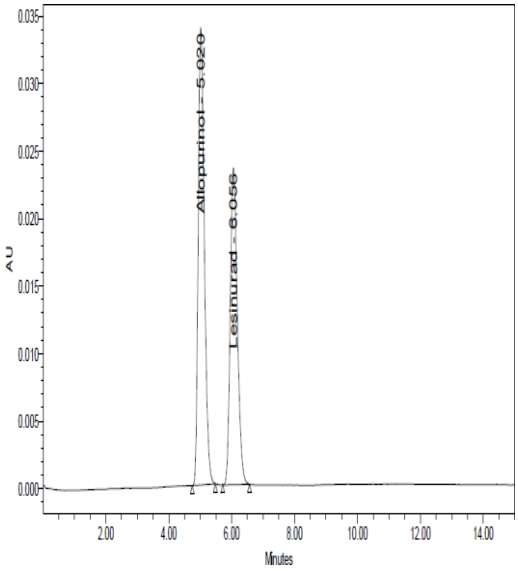
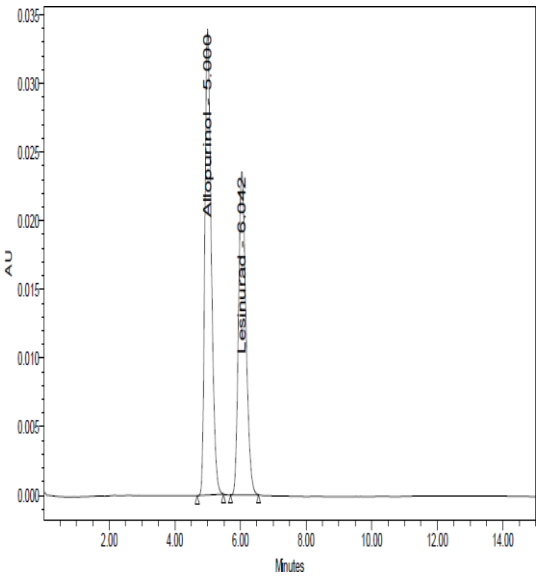


Figure 12: Chromatogram for Accuracy 100%-2



Chromatogram for Accuracy 150%-2

Table 6: Accuracy (recovery) data for Allopurinol

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	233775.3	150	150.42	100.28	

100%	462242.7	300	297.42	99.14	99.60
150%	695121.3	450	447.25	99.39	

*Average of three determinations

Table 7: Accuracy (recovery) data for Lesinurad

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	188250.7	100	100.19	100.19	100.15
100%	374491	200	199.32	99.66	
150%	567073.3	300	301.81	100.60	

*Average of three determinations

LIMIT OF DETECTION FOR ALLOPURINOL AND LESINURAD

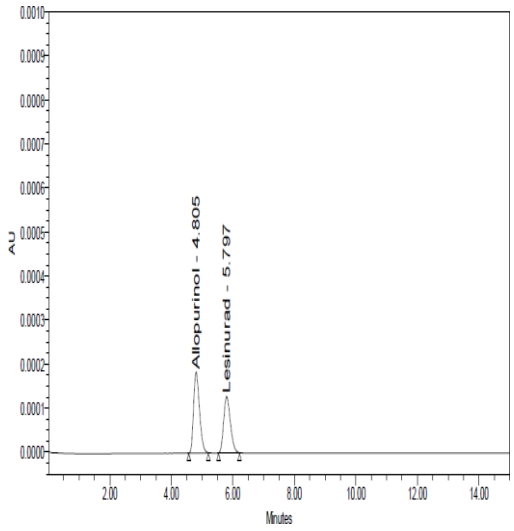


Figure 13: Chromatogram of Allopurinol, Lesinurad showing LOD

Table 8: Results of LOD

Drug name	line noise (µV)	l obtained (µV)	S/N ratio
Allopurinol	43	132	3.07
Lesinurad	43	127	2.95

- Signal to noise ratio shall be 3 for LOD solution
- The result obtained is within the limit.

LIMIT OF QUANTIFICATION FOR ALLOPURINOL AND LESINURAD

Figure 14: Chromatogram of Allopurinol, Lesinurad showing LOQ

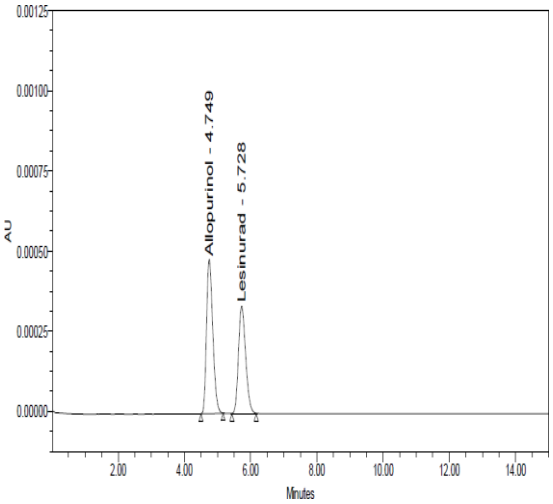


Table 9: Results of LOQ

Drug name	line noise (µV)	l obtained (µV)	S/N ratio
Allopurinol	43	434	10.09
Lesinurad	43	427	9.93

ROBUSTNESS:
Variation in flow

Variation of mobile phase organic

DEGRADATION STUDIES:

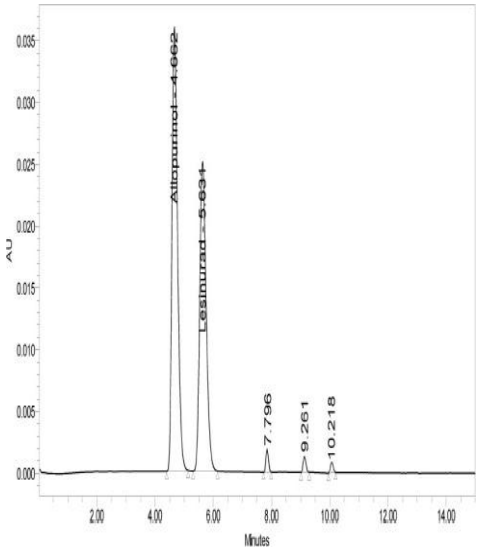


Figure 10: Chromatogram showing Acid degradation

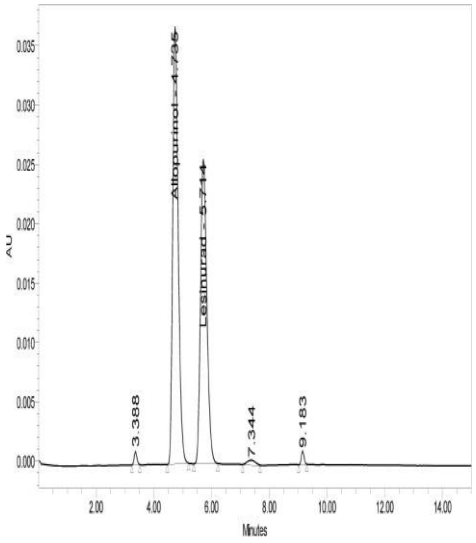


Figure 11: Chromatogram showing Base degradation

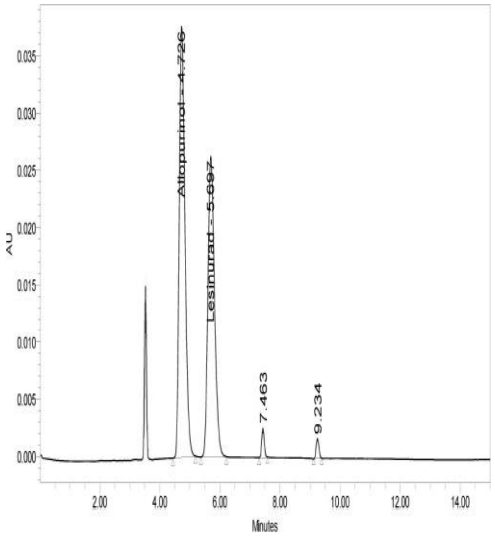


Figure 12: Chromatogram showing Peroxide degradation

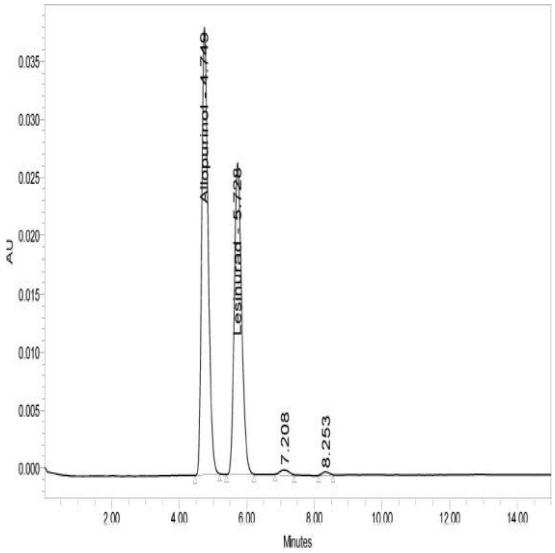


Figure 13: Chromatogram showing Thermal degradation

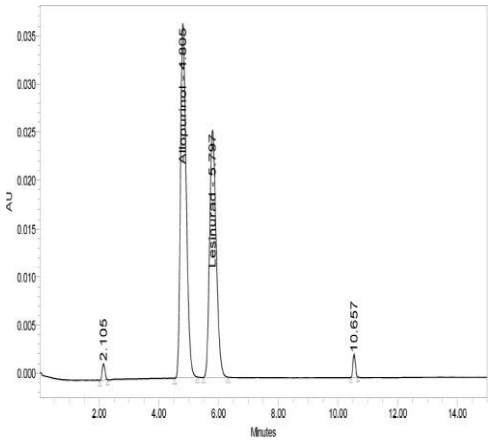


Figure 14: Chromatogram showing Photo degradation

Table 15: Results for Stability of Allopurinol and Lesinurad

Sample Name	Allopurinol		Lesinurad	
	Area	% Degraded	Area	% Degraded
Standard	465326.7		375025.0	
Acid	446578	4.03	359788	4.06
Base	453567	2.53	362545	3.33

Peroxide	439786	5.49	343876	8.31
Thermal	448788	3.55	349675	6.76
Photo	437675	5.94	351989	6.14

SUMMARY AND CONCLUSION

The estimation of Allopurinol and Lesinurad was done by RP-HPLC. The assay of Allopurinol and Lesinurad was performed with tablets and the % assay was found to be 99.93 and 99.95 which shows that the method is useful for routine analysis.

The linearity of Allopurinol and Lesinurad was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.6 and 0.8 for Allopurinol and Lesinurad which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.6 and 0.6 for Allopurinol and Lesinurad which shows that the method is repeatable when performed in different days also.

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