International Journal of Chemistry and Pharmaceutical Sciences
ISSN: 2321-3132 | CODEN (USA): IJCPNH
Available online at:http://www.pharmaresearchlibrary.com/ijcps

Analytical Method Development and Validation for the Simultaneous Estimation of Lesinurad and Allopurinol by RP-HPLC Method

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ABSTRACT

On the basis of experimental results, the proposed method is suitable for the quantitative determination of Lesinurad and Allopurinol in pharmaceutical dosage form. The method provides great sensitivity, adequate linearity and repeatability. The estimation of Lesinurad and Allopurinol was done by RP-HPLC. The Phosphate buffer was pH 2.5 and the mobile phase was optimized which consists of Acetonitrile: Phosphate buffer mixed in the ratio of 80:20 % v/ v. A Symmetry C18 (4.6 x 150mm, 5µm, Make XTerra) column used as stationary phase. The detection was carried out using UV detector at 274 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Lesinurad and Allopurinol were found to be from 25-125 µg/ml. Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 97-102% of Lesinurad and Allopurinol LOD and LOQ was found to be within limit. The proposed method is precise, simple and accurate to determine the amount of Lesinurad and Allopurinol in formulation. High percentage of recovery shows that the method is free from the interference of excipients used in the formulation. So the method can be useful in the routine quality control of these drugs.
Keywords: Symmetry C18, Lesinurad and Allopurinol, RP-HPLC.

ARTICLE HISTORY: Received 11 March 2020, **Accepted** 10 May 2020, **Available Online**27July 2020

Citation: Gampa Vijaya Kumar, *et al.* Analytical Method Development and Validation for the Simultaneous Estimation of Lesinurad and Allopurinol by RP-HPLC Method, 8(7), 2020: 135-139.

CONTENTS:

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JOURNAL QR-CODE

1. Introduction

Lesinurad is used in combination with a xanthine oxidase inhibitor, such as allopurinol or febuxostat, for treating hyperuricemia (high levels of uric acid in the blood serum) associated with gout. It is approved only for patients who have not achieved target uric acid levels with a xanthine oxidase inhibitor alone.

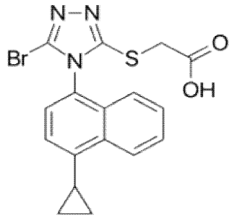

Fig 1: Chemical structure of Lesinurad

Allopurinol, sold under the brand name Zyloprim among others, is a medication used to decrease high blood uric acid levels.It is specifically used to prevent gout, prevent specific types of kidney stones and for the high uric acid levels that can occur with chemotherapy. It is taken by mouth or injected into a vein.

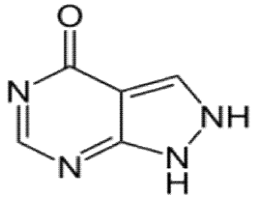

Fig 2: Chemical structure of Allopurinol

2. Materials and Methods

Instrumentation:

HPLC Auto Sampler : Shimadzu Model number SPD20A, Software LC Solutions, Detector: Photo diode array detector, Thermosil C18 Column (4.0×1.25mm, 5µ), Sonicator: Model number SE60US Enertech , U.V double beam spectrophotometer: PG Instrument Model number T60 Software UV Win5, pH meter: ADWAModel number AD102U, Digital Weighing machine:a Model number ER200A .

Chemicals:

Lesinurad and Allopurinol, KH_2PO_4, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid, K_2HPO_4

Optimized Chromatographic conditions:

Mode of operation : Isocratic
Column : Symmetry C18 (4.6 x 150mm, 5µm,
Buffer pH : 2.5
Mobile phase : 20% buffer 80% acetonitrile
Flow rate : 0.8 ml per min
Wavelength : 274 nm
Temperature : ambient.
Run time : 7min.

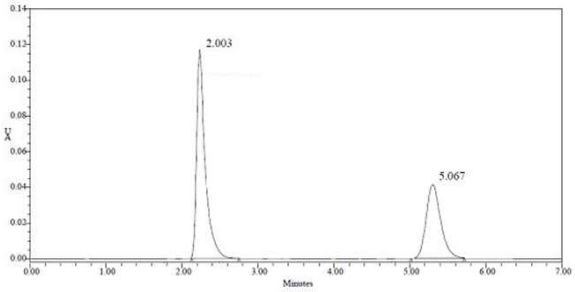

Fig 3: Optimized Chromatogram

Standard Solution Preparation

Accurately weighed amount of 50mg Lesinurad and 50 mg Allopurinol were taken to a 100 ml clean and dry volumetric flask. This was then diluted with 70 ml of diluent and was sonicated. The volume was made to100 ml with the same solvent. This was taken as stock solution. Further, 1.5 ml of above stock solution was diluted to 10ml with the diluent to get final concentration of 75µg/ml.

Sample Solution Preparation

Weight equivalent to 50 mg of Lesinurad and Allopurinol sample were weighed this was taken into a 100 ml clean dry volumetric flask and about 70ml of diluent was added and sonicated to dissolve it completely and volume made up to the mark with the same solvent. This was taken as stock solution. Further, 1.5 ml of above stock solution was diluted to 10ml with diluent to get final concentration of 75µg/ml.

Method Validation

- Linearity
- Accuracy
- Precision
- Intermediate Precision
- Limit of Detection
- Limit of Quantification
- Robustness
- System suitability testing

3. Results and Discussion

Table1: Results of system suitability parameters for Lesinurad and Allopurinol

S. No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Lesinurad	2.003	920101	116666	1.5	1.6	2711.8
2	Allopurinol	5.067	552058	41531	11.0	1.3	3428.2

Table 2: Results of method precision for Lesinurad

S. No	Sample area	Standard area	Percentage purity
1	983375	971536	101.04

2	985049	973007	101.03
3	982956	975717	100.54
4	985219	978909	100.44
5	994145	981422	101.09
Average	983234	976311	100.84
%RSD	49.5	48.2	0.304

Table 3: Results of method precision for Allopurinol

S. No	Sample area	Standard area	Percentage purity
1	592403	577531	101.36
2	592352	580381	101.85
3	592357	577723	102.32
4	592323	582190	101.44
5	596525	583378	101.09
Average	592325	582755	101.24
%RSD	29.5	28.7	0.46

Table 4: Results of Intermediate precision for Lesinurad

S. No	Sample area	Standard area	Percentage purity
1	979556	984395	99.30
2	982467	984039	99.64
3	979717	983976	99.36
4	978909	984278	99.28
5	981432	973915	100.57
Average	985321	984824	99.63
%RSD	48.2	48.5	0.54

Table 5: Results of Intermediate precision for Allopurinol

S. No	Sample area	Standard area	Percentage purity
1	583416	593403	99.12
2	583657	594352	99.01
3	584731	593357	99.52
4	583594	592673	99.61
5	597649	593671	99.12
Average	596537	592542	99.27
%RSD	29.3	29.2	0.27

Table 6: Results of Accuracy

Sample concentration	Sample set no	Sample area		Assay		% Recovery	
		Lesi	Allo	Lesi	Allo	Lesi	Allo
50%	1	460064	276931	24.9	25.0	99.8	100
	2	460124	276694	24.6	24.9	99.6	99.6
	3	460216	276891	24.8	24.9	99.8	99.6
	Avg					99.7%	99.7%
100%	1	923429	554156	49.9	50.0	99.8	100
	2	923654	554897	49.8	49.9	99.6	99.8
	3	923742	556371	49.8	49.9	99.6	99.8
	Avg					99.6%	99.8%
150%	1	1387901	828113	74.8	75.0	99.8	100
	2	1385360	828794	74.9	74.9	99.8	99.8
	3	1386984	828349	74.6	74.8	99.6	99.8
	Avg					99.7%	99.8%

Table 7: Area of different concentration of Lesinurad and Allopurinol

Concentration (µg/ml)	Peak area of Lesinurad	Peak area of Allopurinol
25	296800	179891

50	653819	387781
75	983775	599708
100	1342535	799619
125	1694286	1019614

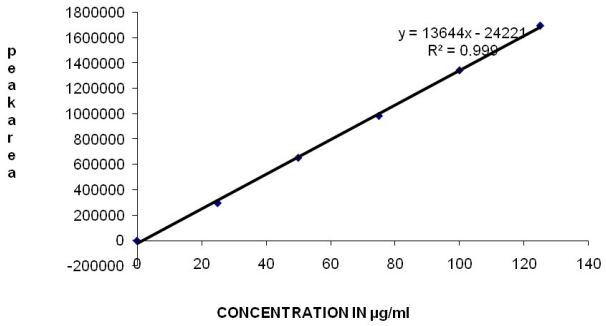

Fig 4: Calibration graph for Lesinurad

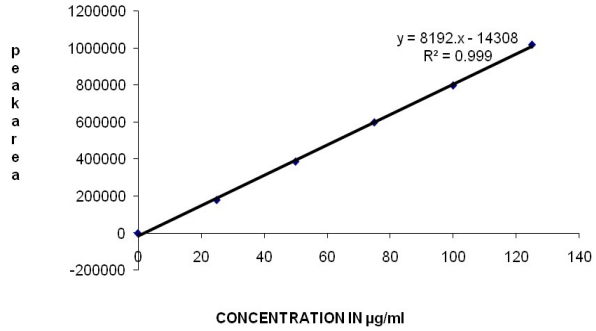

Fig 5: Calibration graph for Allopurinol

Table 8: Analytical performance parameters of Lesinurad and Allopurinol

Parameters	Lesinurad	Allopurinol
Slope (m)	13644	8192
Intercept (c)	24221	14308
Correlation coefficient (R^2)	0.999	0.999

Table 9: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Lesinurad	56	176	3.14
Allopurinol	56	154	2.75

Table 10: Results of LOQ

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Lesinurad	56	563	10.05
Allopurinol	56	558	9.96

Table 11: Results for effect of variation in flow

S. No	Peak area for Less flow (0.7 ml/min)		Peak area for More flow (0.9 ml/min)	
	Lesinurad	Allopurinol	Lesinurad	Allopurinol
1	983465	575351	971563	592641
2	985134	580381	973021	592352
3	983467	587724	975674	595471
4	985217	583190	978974	594416
5	994245	584468	984542	583453
Mean	986306	582223	976755	591667
%RSD	0.45	0.80	0.53	0.80

Table 12: Results for effect of variation in mobile phase composition

S. No	Peak area for Less organic (70%)		Peak area for More organic (90%)	
	Lesinurad	Allopurinol	Lesinurad	Allopurinol
1	984565	574371	981565	593761
2	986134	585481	983527	592462
3	984268	587627	985489	594491
4	986216	585362	987954	596316
5	995247	585448	994672	587353
Mean	987286	583658	986641	592877
%RSD	0.45	0.90	0.51	0.57

4. Conclusion

The RP-HPLC method developed and validated allows a simple and fast quantitative determination of Lesinurad and Allopurinol by RP-HPLC method. All the validation parameters were found to be within the limits according to ICH guidelines. The proposed method was found to be specific for the drugs of interest irrespective of the excipients present and the method was found to be simple, accurate, precise, rugged and robust.

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