SIMULTANEOUS ESTIMATION PHARMACEUTICAL DOASGE FORM BY RP-HPLC Through SSAY METHOD

DR.R.SUTHAKARAN
Professor and Principal
Department of Pharm. Chemistry and Analysis
Vijaya College of Pharmacy,Hyderabad

SYED GHOUSE
Assistant Professor
Department of Pharmaceutical Analysis and Quality Assurance
VIJAYA college of Pharmacy
Hyderabad

Abstract

High performance liquid chromatography is a very sensitive analytical technique most widely used for quantitative and qualitative analysis of pharmaceuticals. 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.

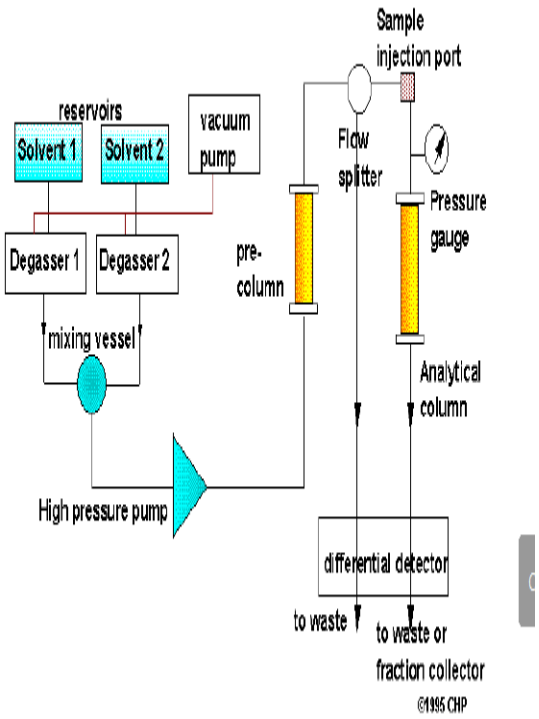
Introduction

A new simple ,Rapid selective , precise and accurate gradient reversed phase high performance liquid chromatographic method (RP-HPLC)method has been developed And validated for simultaneous estimation of allopurinol and alphalipoic acid in bulk and tablet dosage form. Chromatographic analysis was performed on a c-18 column (250×4.6×5 µ)at ambient temperature .the column used was as BDS in Isocratic mode, with mobile phase containing tetrabutylammoniumhydroxide buffer and acetonitrile(70:30v/v) Adjusted to Ph 6.6 with dilute orthophosphoric acid solution . The flow rate was 0.8 Ml/min and effluents were monitored at 230nm. The retention times of allopurionol and alpha lipoicacid were found to be 2.33 min and 6.32 min, respectively. A new spectrophotometric method for the determination of allopurinol drug was investigated. The proposed method was based on the reaction of the intended drug with catechol and Fe(II) to form a blue soluble complex which was measured at λmax 580 nm.

Types of Analytical Procedures to be Validated:

The discussion of the validation of analytical procedures is directed to the four most common 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 orother selected component(s) in the drug product.



Block diagram of HPLC

S.No.	Parameter	Acceptance Criteria
1	USP Plate Count	NLT 2000
2	USP Resolution	NLT 2
3	USP Tailing Factor	NMT 2
4	Linearity	Corelation coefficient NLT 0.999
5	Accuracy	% Recovery 98-102%
6	Precision	%RSD NMT 2
7	Robustness	Should pass SST parameters
8	LOD	S/N Ratio should be 3
9	LOQ	S/N Ratio should be 10

Validation Parameters and their Acceptance criteria

PREPARATION OF BUFFER AND MOBILE PHASE:

PREPARATION OF THE ALLOPURINOL & LESINURAD STANDARD & SAMPLE SOLUTION:

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:

LINEARITY:

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)

Preparation of Level – I:

0.25ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent.

Preparation of Level – II:

0.5ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent.

Preparation of Level – III:

0.75ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent.

Preparation of Level – IV:

1ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent

Preparation of Level – V:

1.25ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent.

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.

DEGRADATION STUDIES

Preparation of stock:

Accurately weigh and transfer 300 mg of Allopurinol and 150 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)

Hydrolytic degradation under acidic condition

Pipette 0.75 ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials.

Hydrolytic degradation under alkaline condition

Pipette 0.75 ml of above solution into a 10ml volumetric and add 3ml of 0.1N NaOH was added in 10ml of volumetric flask. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials.

Oxidative degradation

Pipette 0.75 ml above stock solution into a 10ml volumetric flask and 1ml of 30% w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room

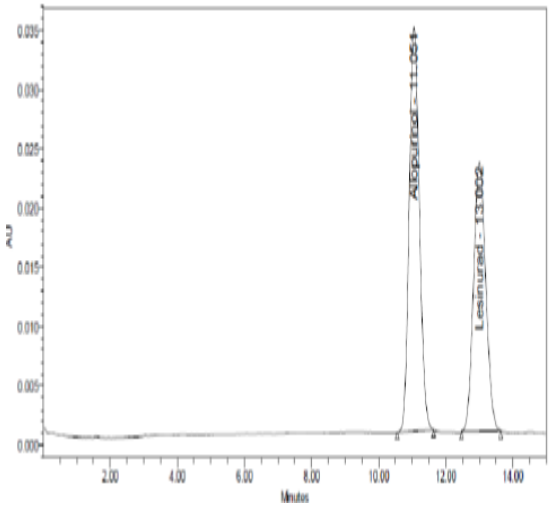
temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

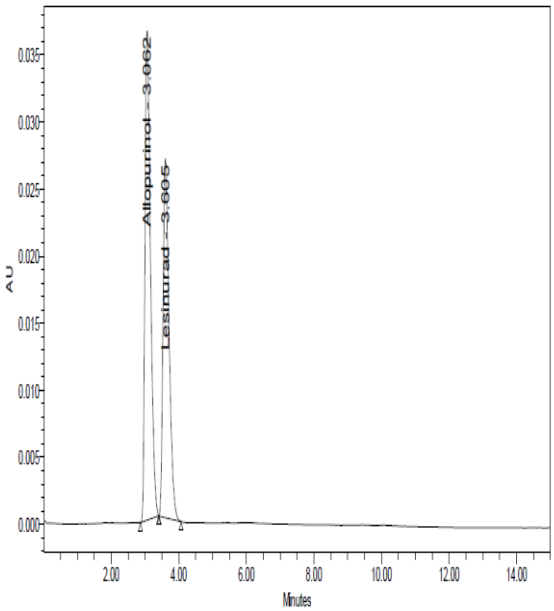
Thermal induced degradation

Allopurinol and Lesinurad sample was taken in petridish and kept in Hot air oven at 1100 C for 3 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analyzed.

RESULTS AND DISCUSSION

Variation of mobile phase organic composition





Results for variation in flow for Lesinurad

S. No	Flow Rate (ml/min)	System Suitability Results		
		USP Resolution	USP Plate Count	USP Tailing
1	1.35	2.68	3407.85	1.23
2	1.5	2.66	3302.43	1.23
3	1.65	2.66	3345.08	1.20

Results for variation in mobile phase composition for Allopurinol

S. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6196.70	1.12
2	*Actual	3022.90	1.30
3	10% more	4608.01	1.49

Results for variation in mobile phase composition for Lesinurad

S. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results		
		USP Resolution	USP Plate Count	USP Tailing
1	10% less	3.16	6196.70	1.12
2	*Actual	2.66	3302.43	1.23
3	10% more	2.11	5906.60	1.46

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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