Analytical Method Development and Validation of Allopurinol in Bulk and it's Dosage Form by Using RP-HPLC

Ms. Komal Kendre, Dr. Ashok Bhosale, Mr. Vikram Veer, Dr. Amit Kasabe, Ms. Pranali Pinjari, Almisba Shaikh

Research Scholar, PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune

Principal, PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune

Assitant Professor , PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune

Assitant Professor , PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune

Research Scholar, PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune

Research Scholar, PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune

ABSTRACT

Development of a simple, accurate, time-tested, high-performance liquid chromatography method for determination of Allopurinol in bulk and Pharmaceutical Formulation. HPLC system used was JASCO system equipped with model PU 4180 RHPLC pump, Rheodyne sample injection port (20 µl), JASCO UV-4075 UV-VIS detector. Separation was carried out on HiQSil C18 (250 mm × 4.6 mm, 5 µm) column using mixture of acetonitrile and ammonium acetate buffer (20 mM) in the ratio of 55:45, v/v as mobile phase at flow rate of 1.0 mL/min. Samples were injected using Rheodyne injector with 20 µL loop, Detection was carried out at 254nm. The retention time was found to be 3.05 min. The HPLC linear regression analysis results for calibration plots demonstrated a good relationship with (r^2 = 0.9992). The method has been validated for its accuracy, Recovery, Robustness and documented. The LOD and LOQ were found to be 1.52 & 2.55 µg/m/ respectively for API and 1.78 & 2.78 µg/ml respectively for tablet. The proposed HPLC method has been proven to be effective for the determination of Allopurinol in Bulk as well as pharmaceutical dosage forms.

KEYWORDS : RP-HPLC, Allopurinol, API, TABLET

INTRODUCTION

Allopurinol is a structural isomer of hypoxanthine and a purine analog. (a purine that occurs naturally in the body) and is a xanthine oxidase inhibitor.That means it stops xanthine oxidase enzyme to function properly. Xanthine oxidase. Converts oxypurines (hypoxanthine and xanthine) to uric acid. It is prevalent in many organs like the kidneys, blood plasma, the liver, the stomach, the heart, and the brain. Xanthine oxidase turns hypoxanthine into xanthine, which is then converted into uric acid[1-4]. Foods that have been broken down and excreted by cells produce uric acid, which is then excreted to the kidneys.Xanthine oxidase production was up but at a decreased level. The can be raised by hypoxanthine, xanthine, or by having less efficient kidneys the blood's level of uric acid[5-6]. Blood uric acid levels are too high. causes the gout-related pain and swelling when it accumulates around joints. Increases in uric acid are also linked to organ damage and failure. Allopurinol used in the treatment of medical conditions associated with high uric acid levels, such as gout and cancer prevention lysis syndrome Allopurinol is effective for treating both primary hyperuricemia of gout and secondary hyperuricemia related to haematological disorders or antineoplastic therapy. Its molecular weight is 136.11. It is chemically named as 1,5-dihydropyrazolo[3,4-d]pyrimidine-4-one.[7-12]

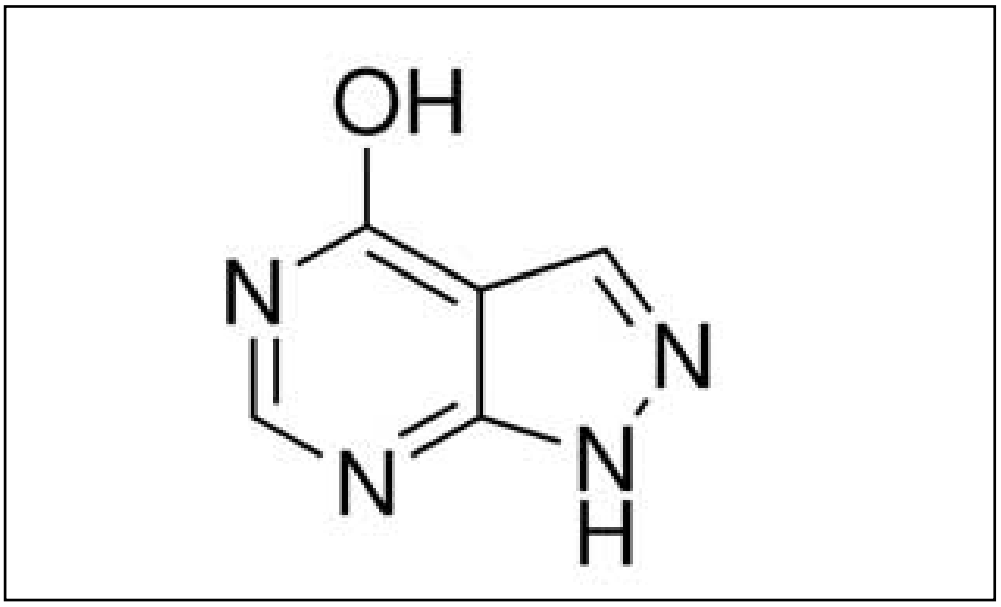

Figure 1: Structure of Allopurinol

Analytical method development

Allopurinol API and Tablet

Determination of Lambda maximum

Preparation of stock solution of Allopurinol API and Tablet

Allopurinol API and Tablet (100 mg) in a 100mL volumetric flask and 25 mL of ACN to it and it was vortexed (Eltek) for 2 minutes. This was the main stock accounting for concentrations of 1000 µg/mL. A diluted solution was used to scan in UV-Spectrophotometer in the range of 200-400nm, taking ACN as blank.

The lambda maximum for Allopurinol API was found to be 254nm.

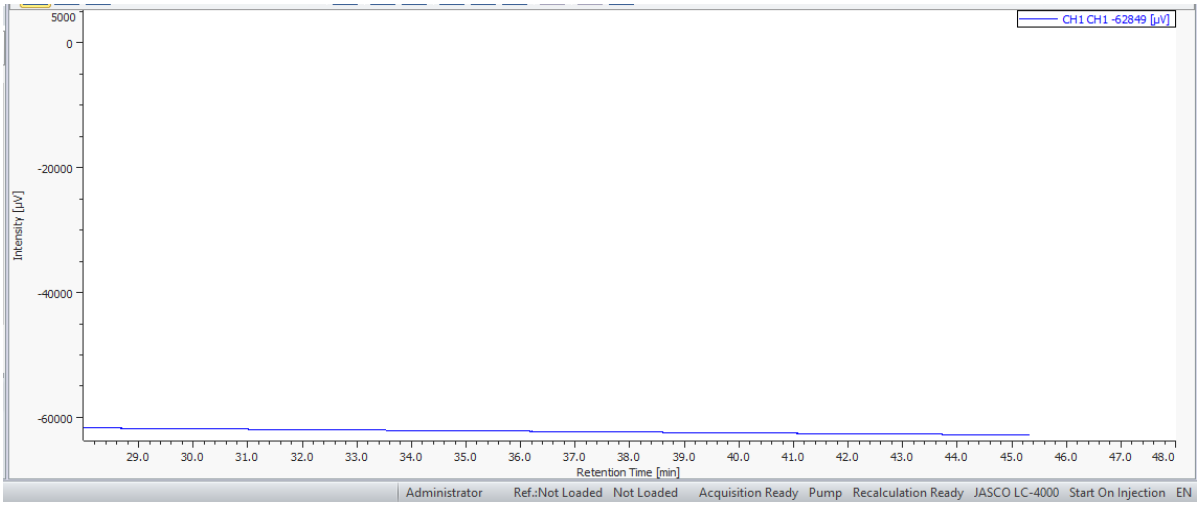

Figure 2: HPLC chromatogram of blank.

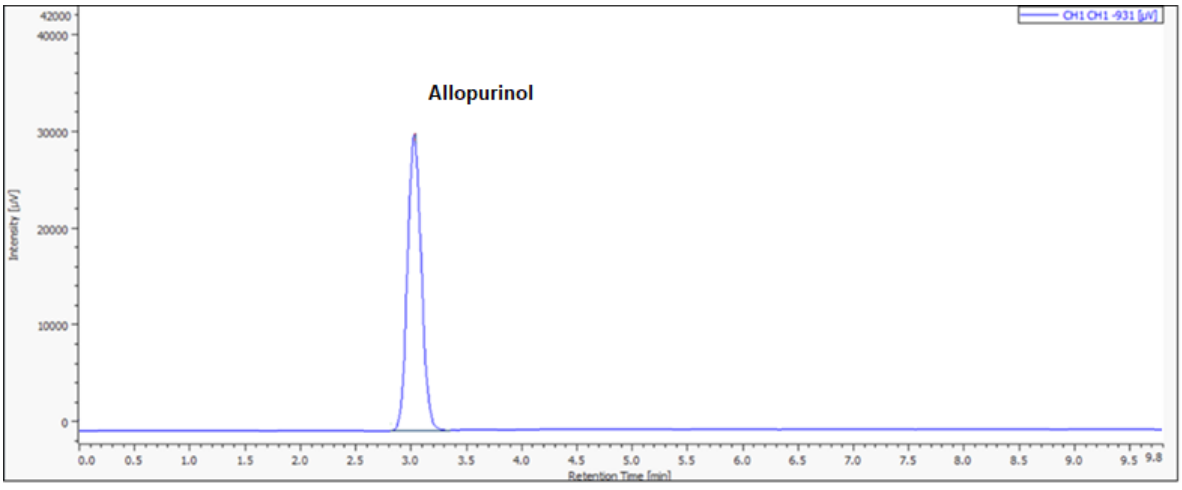

Figure 3 : HPLC chromatogram of standard Allopurinol API

The retention time was found to be 3.05 min with distinct peak.

MATERIALS AND METHODS

Materials

Allopurinol API and Tablet was procured from Solanki enterprises (Pune, India) Chemicals utilized for method development are of HPLC grade includes ACN, Ammonium acetate buffer (20 mM) were purchased from Merck (India) Ltd.

Preparation of mobile phase

The preparation of mobile phase was done by mixing mixture of acetonitrile and ammonium acetate buffer (20 mM) in the ratio of 55:45, v/v. Removal of gases was carried out in ultrasonic water bath for 15 minutes. Filtered the solution through 0.45µ filter.

Diluent preparation

Mobile phase used as diluents.

Preparation of standard stock solution

100mg of Allopurinol API standard was transferred into 100ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase and performed the subsequent dilutions.

Preparation of test solution

100mg equivalent of Allopurinol API and Tablet was transferred into 100ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase and performed the subsequent dilutions.

Selection of analytical wavelength

It is the characteristic of a compound which helps to provide the electronic structure of the compound or analyte. The structural analysis of Allopurinol API and Tablet was carried out under UV ranging from 200-400nm using the standard solution.

Method Validation[13-22]

Linearity

The linearity of the developed method was studied over the concentration ranges between 10-60µg/ml. The aliquots of 10, 20, 30, 40, 50 and 60µg/ml were prepared by diluting standard stock solution of 1, 2, 3, 4, 5 and 6 ml with mobile phase. The obtained concentrations were injected into the chromatographic system. Calibration curve of Allopurinol API was constructed by plotting peak area versus used concentration of Allopurinol API. To assure the concentration range studied is linear the regression equation and correlation coefficient were evaluated.

Accuracy

Accuracy was carried out by % recovery studies at three different concentration levels. To the pre-analysed sample solution of Allopurinol API and Tablet, a known amount of standard drug powder of Allopurinol API and Tablet was added to 80, 100, 120% level.

Precision method

By studying the changes in the inter-day and intra-day determined the precision of the method. In the intra-day studies, six repeated injections of standard solution were made and % RSD were calculated. In the inter-day variation studies, six repeated injections of standard solution were made for six consecutive days and %RSD were calculated.

Limit of Detection and Limit of Quantitation

Based on the standard deviation of response of the calibration curve the LOD and LOQ of the drug was determined separately.

Robustness

Robustness of the method was tested by small but deliberate variations of flow rate, mobile phase composition and wavelength.

RESULTS AND DISCUSSION

Selection of wavelength maxima

The solution of Allopurinol API was scanned between ranges 200- 400nm. UV spectra of the drug show maximum absorbance at 254nm.

Method development

The proposed chromatographic method was found to be suitable for effective separation of Allopurinol API and Tablet with good resolution, peak shape given in the figure. The mobile phase composed of mixture of acetonitrile and ammonium acetate buffer (20 mM) in the ratio of 55:45 v/v, at a flow rate of 1.0 ml/min was selected as it gave well resolved peaks of standard Allopurinol API and Tablet. The optimum wavelength 254nm selected for detection and quantitation.

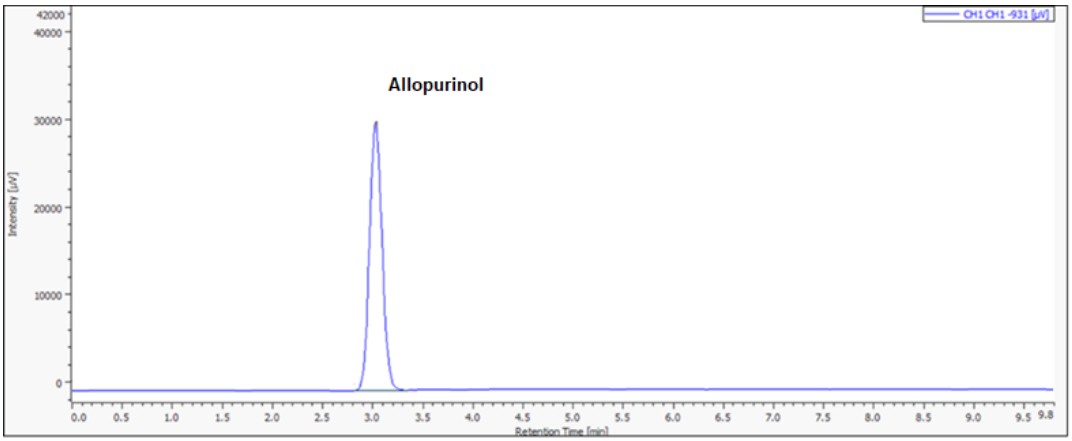

Figure 4 : HPLC Chromatogram with resolved peak of Allopurinol pure drug

Figure 5 : HPLC Chromatogram with resolved peak of Allopurinol Tablet

Sr. No.	Parameter	Conditions used for analysis
1	Column	HiQSil C18 (250 mm × 4.6 mm, 5 µm)
2	Mobile phase	Acetonitrile and ammonium acetate buffer (20 mM)) in the ratio of 55:45, v/v
3	Flow rate	1.0 mL/min

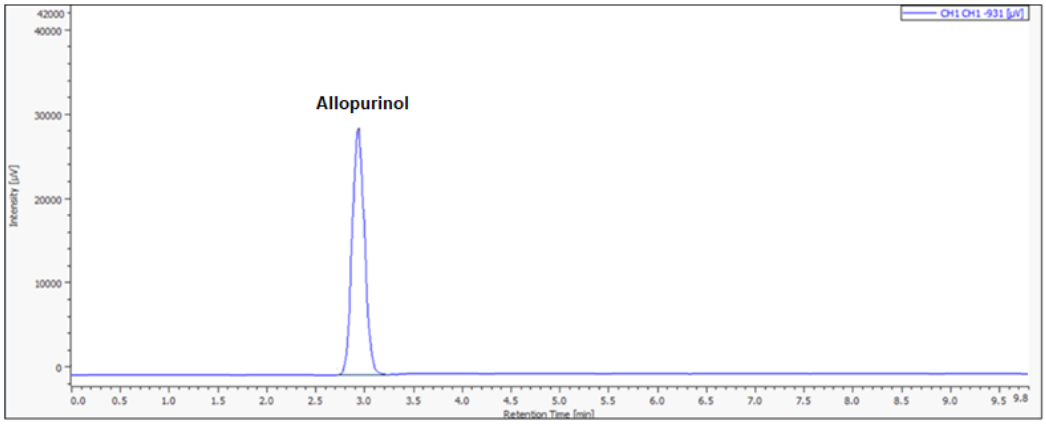

Table no. 1 : Optimized Chromatographic Conditions

Method validation

Linearity

The calibration curves were found be linear for the concentration range of 10-60ppm. The standard working curve equation for drug was found to be y = 9304x + 9301.1 with correlation coefficient value r^2 = 0.9992. The results of linearity are given in Table and Figure.

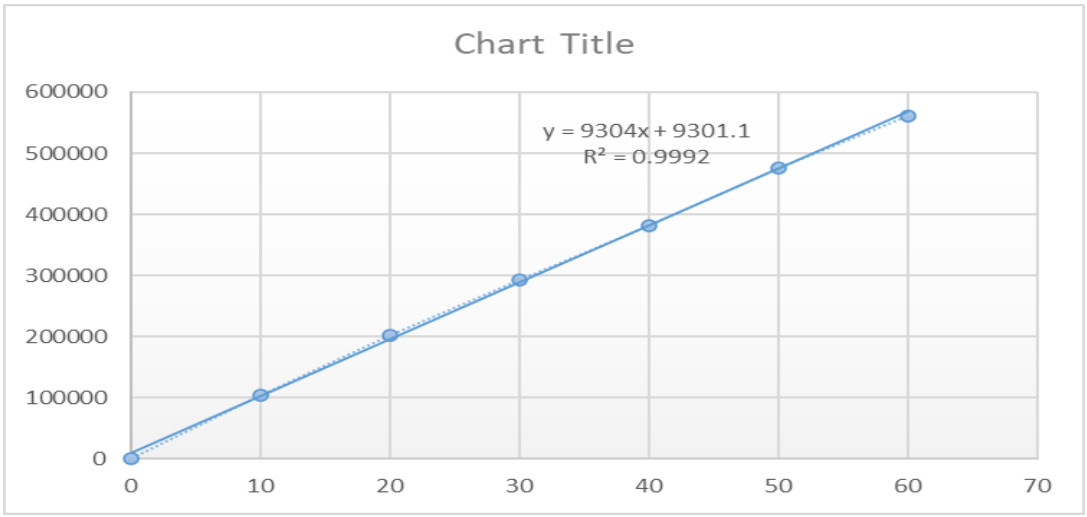

Figure 6 : Linearity curve of standard Allopurinol API

Concentration µg/mL	**Area**
10	104562
20	202775
30	293123
40	381478
50	475489
60	561521

Table- 2: Linearity data of Allopurinol API

Recovery studies

The mean % recovery at 80, 100, 120 % of the test concentration along with its statistical validation for drug Allopurinol API and Tablet given in Table. The % recovery at 80, 100, and 120 % is given below. It was confirmed that the developed method was accurate as the percent recovery was in the range of 100%.

Level (%)	Drug Conc (mg)	Amount recovered (mg)	% Recovery
80%	8	8.1	101.2
100%	10	10.04	100.4
120%	12	11.8	98.33

Table-3: Recovery data of Allopurinol API

Level(%)	Drug conc (mg)	Amount recovered (mg)	% Recovery
80%	8	8.2	102.5
100%	10	10.15	101.5
120%	12	12.11	100.9

Table-4: Recovery data of Allopurinol Tablet

Precision

The repeatability of sample application and measurement of peak area were expressed in terms of % RSD and was found to be less than 2.0%. The % RSD of intra-day precision is given below. The results of precision studies are shown in Table.

Conc µg/mL	Area	AVG	%RSD
10	125462	125502.333	0.04507605
	125567		
	125478		
20	184256	186077	0.88351421
	187452		
	186523		
30	245458	245865.333	0.18445195
	245784		
	246354		

Table- 5: Precision study (intra- day) of Allopurinol API

Conc µg/mL	Area	AVG	%RSD
10	125364	125402.333	0.05225673
	125478		
	125365		
20	185421	185590.667	0.50013497
	186592		
	184759		
30	245632	246450	0.49230598
	247844		
	245874		

Table- 6: Precision study (intra- day) of Allopurinol Tablet

Conc µg/mL	Area	AVG	%RSD
10	125463	125620	0.17112147
	125865		
	125532		
20	184526	184970.667	0.31570784
	184754		
	185632		
30	245156	246298.667	0.56289403
	245899		
	247841		

Table- 7: Precision study (inter-day) of Allopurinol API

Conc µg/mL	Area	AVG	%RSD
10	125478	125298	0.27907057
	125521		
	124895		
20	184756	182949	0.14237934
	178452		
	185639		
30	235878	235436.667	0.24467624
	235647		
	234785		

Table- 8: Precision study (inter-day) of Allopurinol Tablet

Limit of Detection (LOD) and Limit of Quantification (LOQ)

This data showed that the sensitivity of method to determine the drug Allopurinol API and Tablet. The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 1.52 & 2.55 µg/m/ respectively for API and 1.78 & 2.78 µg/m/ respectively for tablet.

Robustness

Robustness of method was measured by multiple injections of a homogenous sample containing Allopurinol API and Tablet by changing flow rate 0.9 mL/min and 1.1 mL/min, mobile phase composition ammonium acetate buffer (20 mM) in the ratio of 54:46 and 56:44 v/v, wavelength i.e., 253nm and 255nm. The method was found to be robust in the range of deliberate changes made.

Flow rate mL/min	**Conc µg/mL**	**Area**	**AVG**	**%RSD**
0.9	20	187454	185874.667	0.76517091
0.9		185475		
0.9		184695		
1.1	20	185478	185684.667	0.31866782
1.1		186352		
1.1		185224		

Table-9: Robustness study with change in flow rate of Allopurinol API

Flow rate mL/min	**Conc µg/mL**	**Area**	**AVG**	**%RSD**
0.9	20	184755	187388.333	1.31858649
0.9		189656		

0.9		187754		
1.1	20	186320	185837.667	0.22813348
1.1		185524		
1.1		185669		

Table-10: Robustness study with change in flow rate of Allopurinol Tablet

Mobile phase (ACN: 01% OPA)	**Conc µg/mL**	**Area**	**AVG**	**%RSD**
54:46	20	187474	186234.667	0.57698876
54:46		185667		
54:46		185563		
56:44	20	180254	183800.333	1.6711687
56:44		185524		
56:44		185623		

Table-11: Robustness study with change in concentration of mobile phase of Allopurinol API

Mobile phase (ACN: 01% OPA)	**Conc µg/mL**	**Area**	**AVG**	**%RSD**
54:46	20	180263	182761.333	1.25197629
54:46		184755		
54:46		183266		
56:44	20	185996	185438.333	0.34110935

56:44		184751		
56:44		185568		

Table-12: Robustness study with change in concentration of mobile phase of Allopurinol Tablet

Wavelength nm	**Conc µg/mL**	**Area**	**AVG**	**%RSD**
253	20	187549	186221.667	0.62014868
253		185447		
253		185669		
255	20	182563	184521.333	0.9192206
255		185475		
255		185526		

Table-13: Robustness study with change in Wavelength of Allopurinol API.

Wavelength nm	**Conc µg/mL**	**Area**	**AVG**	**%RSD**
253	20	180254	180461	1.22701005
253		175466		
253		185663		
255	20	182052	182648.333	1.49886542
255		180258		
255		185635		

Table-14: Robustness study with change in Wavelength of Allopurinol tablet

CONCLUSION

A HPLC method developed has been validated as per ICH guidelines in terms of accuracy, precision, linearity, robustness, limit of detection and limit of quantitation, for the determination of Allopurinol API and Allopurinol tablet. A good linear relationship was observed in concentration ranges of 10 and 60 µg/ml. The correlation coefficients was 0.9992. The inter day and intraday precision results were good enough to say that the method developed is precise and reproducible. Accuracy studies revealed that mean recoveries after spiking experiments were between 100.6 and 101 %, an indicative of accurate method. Accordingly it can be concluded that the developed method is accurate, precise, linear, and robust.

ACKNOWLEDGEMENTS

The author wishes to express gratitude to the Solanki Enterprises for providing sample of Allopurinol API and Aster Lab for permission and facilities to carry out the research work.

REFERENCES

1. Ammar AA, Samy AM, Marzouk MA, Ahmed MK. Formulation, characterization and biopharmaceutical evaluation of Allopurinol tablets. International Journal of Biopharmaceutics 2011; 2(2):63-71.

2. Derek K, Da-peng wang TL. Formulation development of Allopurinol suppositories and injectables. Drug development and Industrial pharmacy 1999; 25(11):1205-1208.

3. Clark's analysis of drugs and poisons in pharmaceutical body fluids and post mortem materials. 3rdEdn. London: Pharmaceutical press;2004, 601-603

4. Analytical procedures and methods validation (2000): Chemistry, manufacturing, and controls federal register (Notices). PP. 776-7

5. Cameron, J.S; Moro, F and Simmonds, H.A. (1993) "Gout, uric acid and purine metabolism in paediatic nephrology".7(1):105-118

6. Dalbeth, N and Stamp, L (2007). Allopurinol Dosing in Renal Impairment: Walking the Tightrope between Adequate Urate Lowering and Adverse Events 20 (5): 391–5.

7. International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human use, Validation of analytical procedures (1996): definition and terminology ,Geneva 2005; 3-10.

8. Lindholm J, Development and Validation of HPLC Method for Analytical and Preparative Purpose, Acta Universities Upsaliensis Uppsala, 2004; 13-14.

9. Analytical procedures and methods validation (2000): Chemistry, manufacturing, and controls federal register (Notices). PP. 776-7

10. Pacher, P; Nivorozhkin, A and Szabo, C. (2006). "Therapeutic affected of xanthine oxidase inhibitors: Renaissance half century the discovery of allopurinol". 58(1):87-114

11. World Health Organization (2007) "Quality assurance of pharmaceutical". p (5)

12. Kasture A V, Mahadil K.R, Wadodhkar S G, More A H, Pharmaceutical Analysis vol-2 instrumental method,Nirali Prakashan page no.6-9, 60-65.

13. Willard H H,Merritt L L, Dean J A,Settle F A,Instrumental method of analysis,New Delhi,cbs publishers and distributors pg no:513-538.

14. McMurry J (2011). Organic chemistry: with biological applications (2nd ed.). Belmont, CA: Brooks/Cole. pp. 395.

15. Hostettmann K, Marston A, Hostettmann M (1998). Preparative Chromatography Techniques Applications in Natural Product Isolation (Second ed.). Berlin, Heidelberg: Springer Berlin Heidelberg. p. 50.

16. Gerberding SJ, Byers CH. Preparative ion-exchange chromatography of proteins from dairy whey. J Chromatogr A. 1998;808:141–51. [PubMed]

17. Donald PL, Lampman GM, Kritz GS, Engel RG. Introduction to organic laboratory techniques. 4th ed. Thomson Brooks/Cole; 2006. pp. 797–817.

18. Harwood LM, Moody CJ. Experimental organic chemistry : Principles and Practice. Oxford :Blacwell Science; 1989:180–5.

19. Das M, Dasgupta D. Pseudo-affinity column chromatography based rapid purification procedure for T7 RNA polymerase. Prep Biochem Biotechnol.1998;28:339–48.

20. Karlsson E, Ryden L, Brewer J Protein purification. Principles, High Resolution Methods, and Applications. Ion exchange chromatography. 2nd ed. New York: Wiley; 1998; 456

21. Amercham Biosciences. Ion Exchange chromatohgraphy, Principles and methods, Amercham Pharmacia. Biotech SE. 2002;751

22. Walls D, Loughran ST. Protein chromatography: Methods and protocols, methods in molecular biology. 2011;681