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Application of RP-HPLC for the Estimation of Allopurinol and Its Related Substances in Bulk and Tablet Dosage Form

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Authors' contributions

This work was carried out in collaboration among all authors. Author CJK' designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PP and SPM managed the analyses of the study. Authors KNRK and RRN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The main aim of the present study was to develop and validate a simple and cost- effective method for the estimation of allopurinol and its related substances by using RP-HPLC.
Study Design: Estimation of Allopurinol and its related substance in bulk and tablet dosage forms by RP-HPLC.
Place and Duration of Study: Chalapathi Drug Testing Laboratory, Chalapathi Institute of Pharmaceutical Sciences, Chalapathi Nagar, Lam, Guntur-522034 between October 2020 to January 2021.
Methodology: Method development was carried out by using Schimadzu, Prominence-i series LC 3D-Plus autosampler embedded with lab solutions software, equipped with PDA detector using YMC column (150 mm X 4.6 mm, 3 µm) and 0.1M Ammonium acetate buffer as a mobile phase in the ratio of 100% at a flow rate of 1.0 ml/min at a wavelength of 255nm. The developed method was validated according to ICH guidelines.

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Results: The linearity was observed in the range of 20-100 µg/ml with a regression (R^2) value of 0.999. Developed method was specific with no interactions and accurate with 100.11% for allopurinol and 99.54% for its related substance. The limit of detection for allopurinol was 2 µg/ml and for related substance was 0.0.1 µg/ml. The limit of quantification for allopurinol was 6 µg/ml and for related substance was 0.03 µg/ml respectively. The percentage relative standard deviation was found to be NMT 2 which indicates that the proposed method was precise and robust.
Conclusion: The developed method was simple, precise and accurate and can be successfully employed for the estimation of allopurinol in bulk and tablet dosage form.

Keywords: Allopurinol; related substances; PDA; validation; ICH guidelines.

1. INTRODUCTION

Allopurinol (1H-pyrazolo[3,4-d] pyrimidin-4-ol) is structural isomer of hypoxanthine which is xanthine oxidase inhibitor, commonly used in the treatment of chronic gout associated with pathological conditions like leukemia, inflammation and in cancer medications. The drug is particularly useful in patients with recurrent renal deposition of urates, proliferative disease and malignancies [1-7].

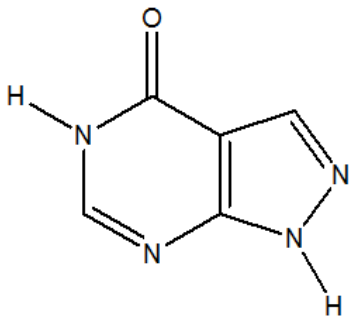

Fig. 1. Allopurinol

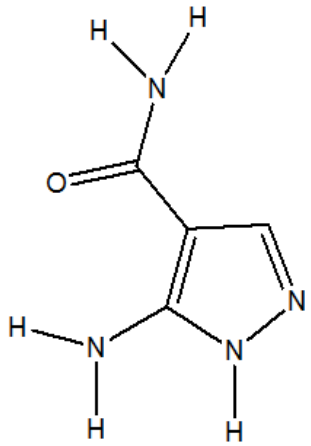

Fig. 2. Impurity-A

Few analytical methods were reported to establish the identity, purity, physical characteristic, and potency of Allopurinol and related substances. The development of analytical method and validation is vital in quality and purity checking of pharmaceuticals. When developed analytical procedure is not much effective, there is a need to develop newer analytical methods. The choice of analytical methodology is based on many considerations such as chemical properties of the analyte and its concentration, sample matrix, the speed and cost of analysis, type of measurement, that is, quantitative or qualitative, and the number of samples. Safety and efficacy of pharmaceutical product are fundamental aspects in drug therapy and these are dependent not only on the intrinsic toxicological properties of active ingredient but also on the impurities and degradation product that it may contain which could be present as a part of finished product. The impurity profile of drug is much important in case of manufacturing drugs of high purity [8-10]. One of the most important fields of activity in modern industrial pharmaceutical research is the estimation of impurity profiles of bulk drug substances. Separation, structure elucidation and quantitative determination of all impurities in drugs are all part of impurity profiling. The latter aspect should be highlighted, since even slight improvements in production technology, starting materials, purification and storage conditions can have a major effect on the impurity profile. Its role in pharmaceutical formulation research and development is also enormous. Impurity profiling is important for ensuring good accuracy, sensitivity, and stability over the life cycle of drugs from the standpoint of quality risk management [11-15].

2. MATERIAL AND METHODS

2.1 Materials

Allopurinol and impurity-A were obtained from Sigma Aldrich. Ausric-100 tablet containing

allopurinol 100 mg was procured from the local market. Reagents and solvents such as orthophosphoric acid, potassium dihydrogen orthophosphate, and HPLC water of analytical grade were procured from National scientific products.

2.2 Instrument

The chromatographic separation was carried out on HPLC Shimadzu 2030C 3D plus with using lab solutions software with photodiode array detector, YMC column (150 mm x 4.6 mm, 3 µm) with ambient temperature.

2.3 Method Development and Validation

2.3.1 Diluent preparation

Diluent was prepared by dissolving 0.3 gms of potassium dihydrogen phosphate in 100 ml of HPLC water and Ph was adjusted to 3 by using orthophosphoric acid.

2.3.2 Mobile phase preparation

Mobile phase was prepared by dissolving 1.7 gm of ammonium acetate in 100ml of water and the solution was filtered using a 0.45micron Millipore filter paper and was sonicated for 10 mins.

2.3.3 Preparation of Allopurinol standard solutions

About 10 mg of Allopurinol was accurately weighed and transferred into a 10 ml clean dry volumetric flask, added 3/4th volume with phosphate buffer, and made up with phosphate buffer. From the above stock solution, 0.1 ml was taken and transferred into another volumetric flask and made up with phosphate buffer (10 µg/ml).

2.3.4 Preparation of impurity-A standard solutions

About 1 mg of impurity-A was accurately weighed and transferred into a 10 ml clean dry volumetric flask, added 3/4th volume with methanol, and made up with methanol. From the stock solution 1 ml was transferred into another 10 ml volumetric flask and made up with methanol and from the above solution 0.1 ml was transferred into another 10 ml volumetric flask and made up with methanol (0.1 µg/ml).

2.3.5 Sample preparation for assay

Accurately 150 mg of tablet powder weighed and transferred into a 10 ml volumetric flask and volume was made up with phosphate buffer. The above 10 ml of the solution was further diluted with 100 ml with phosphate buffer. From the above solution, 6ml was taken and diluted to 100 ml with phosphate buffer. 20 µl of this solution was injected.

2.4 HPLC Method Development

Allopurinol and its related substances in the sample was analyzed by HPLC technique using the optimized conditions given below.

Optimized conditions for HPLC method development:

Column	: YMC (150mm X 4.6mm, 3µm)
Wavelength	: 255 nm
Flow rate	: 1.0 ml/min
Mobile phase	: Acetate buffer-100%
Run time	: 10 mins
Injection volume	: 20 µl

2.4.1 HPLC method validation

The proposed method was validated according to the ICH guidelines which include system suitability, specificity, linearity, accuracy, precision, limit of detection, limit of quantification and robustness. Under the validation study, the following parameters were studied.

System Suitability: HPLC system was optimized as per the chromatographic conditions. Standard solutions of 20 µl were injected six times into the chromatographic system. To ascertain the system suitability for the proposed method, the parameters such as retention time, the number of theoretical plates, resolution, tailing factor, and % RSD were calculated and compared with the standard specification of the system.

Specificity: The specificity of the method was determined by comparing the chromatograms of blank with standard and sample.

Linearity: Linearity was established by triplicate injections of solutions containing standard allopurinol and impurity-A. The linearity range maintained was 20 to 100 µg/ml for allopurinol and 0.1 to 0.5 µg/ml for impurity-A.

Accuracy: Accuracy was performed in triplicate for various concentrations of allopurinol and impurity-A to determine the accuracy of the proposed method. Amount equivalent to 50%, 100% and 150% of the standard amount was

injected into the HPLC system in accordance with the procedure. Accuracy was assessed as the percentage accuracy and mean % recovery.

Precision: Six replicate injections of a known concentration of allopurinol and impurity-A have been determined by injecting them into chromatographic system. The peak area of all injections was taken and the standard deviation, % relative standard deviation (% RSD), was calculated.

Limit of detection and Limit of Quantification: The Limit of detection LOD and limit of quantitation LOQ values were calculated from the calibration curves as per the protocol.

Robustness: The standard solution of allopurinol was injected by changing the chromatographic conditions like the flow rate of the mobile phase and wavelength.

% Assay: Separately blank, standard, and tablet solutions were injected and the areas for allopurinol and impurity-A were noted and the % assay was calculated.

3. RESULTS AND DISCUSSION

3.1 System Suitability

The system suitability parameters of allopurinol and impurity-A were within the acceptance limit and these are represented in Tables 1 and 2.

Table 1. System suitability parameters of allopurinol and impurity-A

Allopurinol			**Impurity-A**	
Injection No	**Retention time**	**Peak area**	**Retention time**	**Peak area**
1	7.176	1934687	2.977	120547
2	7.186	1944226	2.981	120254
3	7.168	1938752	2.977	122596
4	7.097	1945568	2.966	122547
5	7.080	1954457	2.956	121952
6	7.071	1945784	2.954	122456
Mean		**1943912**	**121725**	
Standard deviation		**6771.537**	**1055.627**	
%RSD		**0.35**	**0.87**	

Table 2. Data of system suitability

Parameters	**Imp-A**	**Allopurinol**
Retention time	2.977	7.176
Tailing factor	1.756	1.788
Theoretical plates (USP)	2154	3564
%RSD	0.87	0.35

Discussion: After system suitability studies results of allopurinol and impurity-A were observed that all the parameters were within the acceptable limit.

Acceptance Criteria: The % RSD should be NMT 2.0%

The number of theoretical plates (N) should be NLT 2000.

The Tailing factor (T) should be NMT 2.0.

Specificity: The blank solution does not interact with standard and sample so the method is specific, the specificity values are represented in Table 3. Blank, standard, sample chromatograms are represented in the Figs. 3,4,5.

Table 3. Specificity of allopurinol and impurity-A

Name	Allopurinol	Impurity-A
Blank	Not detected	Not detected
Standard	7.097	2.966
Sample	7.097	2.966

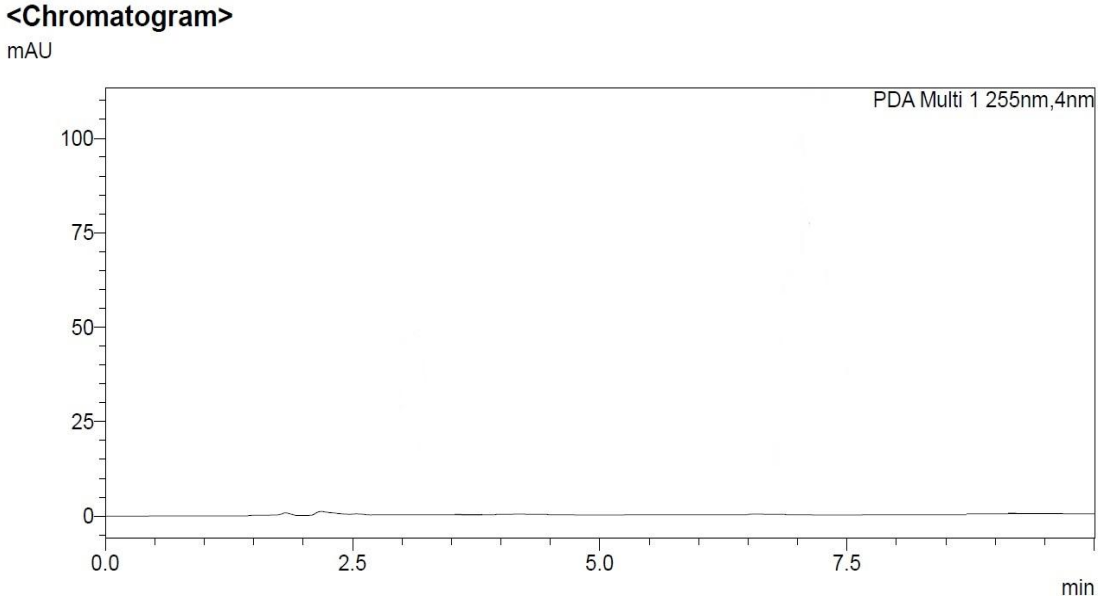

Fig. 3. Blank chromatogram

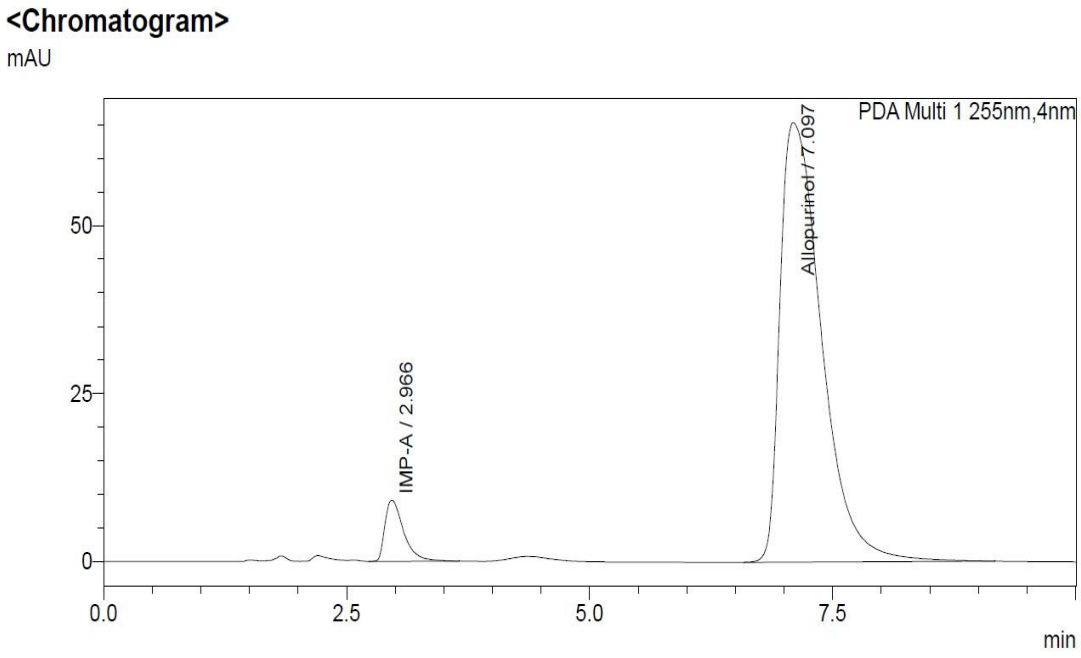

Fig. 4. Standard chromatogram

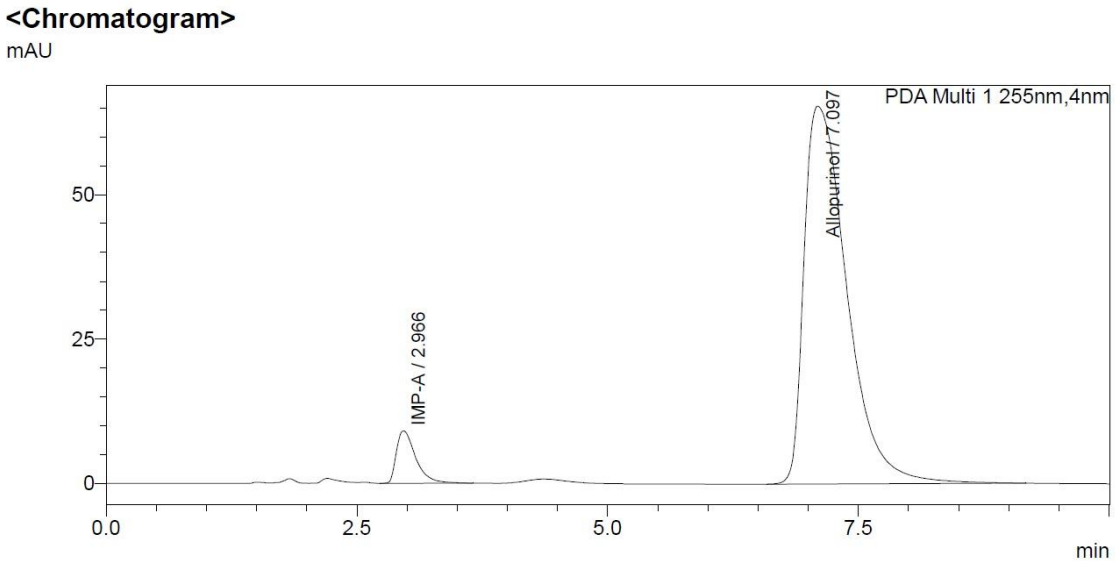

Fig. 5. Sample chromatogram

Discussion: There was no interaction of sample, standard with blank**,** So the method was specific.

Linearity: The method was linear with good correlation coefficient values and these are represented in Table 4 and linearity plots are represented in Figs. 6 and 7.

Table 4. Linearity data of allopurinol and impurity-A

Allopurinol		Impurity-A	
Concentration (µg/ml)	**Peak area**	**Concentration (µg/ml)**	**Peak area**
20	665293	0.1	38629
40	1261904	0.2	81570
60	1923036	0.3	120982
80	2609549	0.4	166685
100	3260995	0.5	209408
R^2= 0.9997		**R^2= 0.9994**	

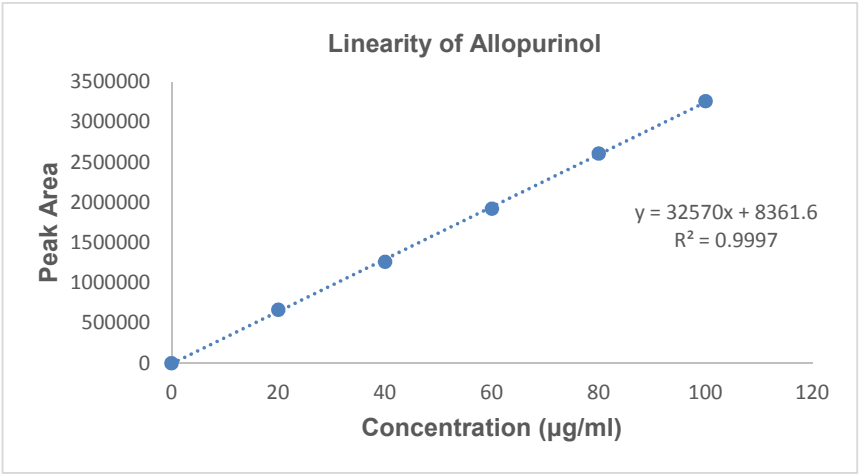

Fig. 6. Linearity plot of allopurinol

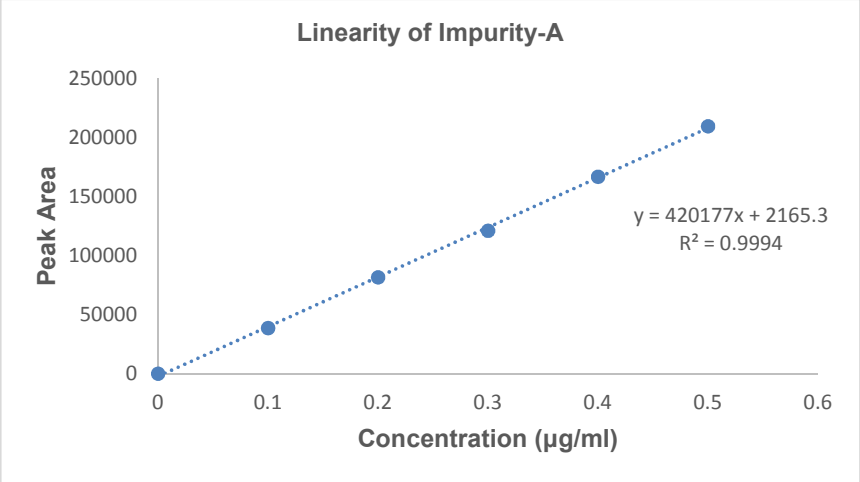

Fig. 7. Linearity plot of impurity-A

Discussion: Five linear concentrations of allopurinol and impurity-A (20-100 µg/ml & 1-5 µg/ml) were injected. Average areas were mentioned above and linearity equations obtained for Allopurinol was y =32570.x+ 8361.6 and impurity-A was y = 420177.x+2165.3. The correlation coefficient obtained was 0.999 for both allopurinol and impurity-A. correlation coefficient of allopurinol and impurity-A was found to be within the acceptable limit.

Acceptance criteria: The correlation coefficient (R^2) should be NLT 0.999

Accuracy: The method was accurate with a good % recovery and these results are represented in Tables 5 and 6.

Table 5. Accuracy data of allopurinol

%level	Standard peak area	Sample peak area	% recovery	Mean % recovery
	1943912	984250	101.03	
50%	1943912	974250	100.11	
	1943912	975250	99.99	
	1943912	1941357	99.82	
100%	1943912	1943760	99.86	**100.11%**
	1943912	1937576	99.47	
	1943912	2928185	100.18	
150%	1943912	2931124	100.32	
	1943912	2927894	100.21	

Table 6. Accuracy data of impurity-A

%level	Standard peak area	Sample peak area	% recovery	Mean % recovery
	121725	60525	99.21	
50%	121725	60745	99.68	
	121725	60568	99.17	
	121725	122252	100.39	**99.54%**
100%	121725	121524	99.70	
	121725	121789	99.85	
150%	121725	181596	99.22	
	121725	181895	99.41	
	121725	181569	99.25	

Discussion: Three levels of allopurinol and impurity-A accuracy samples were prepared. Triplicate injections were given for each level of accuracy and mean %recovery was obtained as 100.11% for allopurinol and 99.54% for impurity-A respectively. The % recovery for allopurinol and impurity-A were found to be within the acceptable limit.

Acceptance criteria: The mean % recovery at each level should be not less than 98% and not more than 102%.

Precision: The method was precise with %RSD NMT 2 for Intra assay and intermediate precision these results are represented in the Tables 7 and 8.

Table 7. Intra assay precision of allopurinol and impurity-A

S. No	Allopurinol	Impurity-A
1	1934687	120547
2	1944226	120254
3	1938752	122596
4	1945568	122547
5	1954457	121952
6	1945784	122456
Mean	**1943912**	**121725**
SD	**6771.537**	**1055.627**
% RSD	**0.35**	**0.87**

Discussion: The sample solution for six injections was given and the obtained areas were mentioned above table. Average area, standard deviation, and %RSD were calculated for allopurinol and impurity-A. %RSD obtained as 0.87%,0.37% respectively for Impurity-A and Allopurinol. The %RSD for Allopurinol and impurity-A peaks were found to be within the acceptable limit.

Acceptance criteria: The %RSD for the peak area should be NMT 2.0.

Table 8. Intermediate precision of allopurinol and impurity-A

S. No	Allopurinol	Impurity-A
1	1932454	121546
2	1942156	120853
3	1935687	122458
4	1942458	121572
5	1952596	121457
6	1944886	120540
Mean	**121404**	**1941706**
SD	**665.163**	**7089.434**
% RSD	**0.55**	**0.37**

Discussion: The sample solution for six injections was given and the obtained areas were mentioned above table. Average area, standard deviation, and %RSD were calculated for allopurinol and impurity-A. %RSD obtained as 0.55%,0.37% respectively for allopurinol and impurity-A. The %RSD for allopurinol and impurity-A peaks was found to be within the acceptable limit.

Acceptance criteria: The %RSD for the peak areas should be NMT 2.0.

LOD & LOQ: The lowest amount of the drug that can be detected and quantified is identified and represented in the Table 9.

Table 9. LOD and LOQ of allopurinol and impurity-A

	Allopurinol (µg/ml)	Impurity-A (µg/ml)
LOD	2.0	0.01
LOQ	6.0	0.03

Result: The LOD of allopurinol and impurity-a was found to be 2.0 and 0.01 µg/ml respectively. The LOQ of allopurinol and impurity-A was found to be 0.01 and 0.03 µg/ml respectively.

Discussion: The above results indicate the sensitivity of the method.

Robustness: The method was robust enough with % RSD values NMT 2 for various parameters such as a change in flow rate and wavelength and the results are given in Table 10.

Table 10. Robustness data of allopurinol and impurity-A

Drug	Flow rate (%RSD)		Wavelength (%RSD)	
	0.8 ml/min	**1.2 ml/min**	**250 nm**	**260 nm**
Allopurinol	0.42	0.32	0.41	0.32
Imp-A	0.35	0.51	0.35	0.55

Discussion: In robustness conditions like flow rate (0.8 ml/min), flow rate (1.2 ml/min), wavelength (250 nm), wavelength (260 nm) samples were injected in a duplicate manner. The %RSD was calculated and it was found to be NMT 2.

Acceptance criteria: The %RSD for the peak areas should be NMT 2.0.

% Assay: The % assay of allopurinol was found to be within the acceptable limit and results are given in the Table 11.

Table 11. Assay of allopurinol and impurity-A

Tablet sample	Label claim (mg)	% Assay
Allopurinol	100	100.04%

Discussion: % Assay was calculated for allopurinol tablet and it was found to be 100.04%.

Acceptance criteria: The % assay should be 98% - 102%.

4. CONCLUSION

A simple reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the cost-effective estimation of allopurinol and its related substances in the tablet dosage form. The developed method was validated according to ICH (Q2R1) guidelines. The linear response was observed in the range of 20-100 µg/ml for Allopurinol and 0.1 to 0.5 µg/ml for its related substances with regression of 0.9997 and 0.9994 respectively. The proposed method had adequate specificity for the estimation of allopurinol and related substances in the tablet dosage form. The percentage recoveries were found to be within limits of acceptance criteria between the ranges of 98 – 102%. Precision results were found to be within limits and the method was found to be robust with a %RSD limit of NMT 2.0. The results of the assay showed good agreement with the label claim. The method was validated statistically and was applied successfully for the estimation of allopurinol and its related substances.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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