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UV SPECTROSCOPIC METHOD DEVELOPMENT AND VALIDATION OF ALLOPURINOL IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Allopurinol, 1*H*-pyrazolo [3,4-d]pyrimidin-4(2H)-one is a drug used primarily to treat hyperuricemia and its complications, including chronic gout. A simple, rapid, accurate, specific and highly sensitive UV spectroscopic methods were developed and validated for the estimation of Allopurinol in bulk and pharmaceutical dosage form. The absorption maxima was found to be 250.6nm for the method A(Zero order), 238nm for method B(first order derivative) and for method C(Area under curve) was measured from 245-255nm. The solvent used was methanol for the preparation of stock solution and distilled water was used for the further dilutions. The methods were found to be linear in the range of 5-35µg/ml and the correlation coefficient values were found to be 0.999, 0.999, 0.9985 respectively. The developed methods were validated in terms of linearity, accuracy, precision in accordance with the ICH guidelines. The proposed methods can be used for the estimation of Allopurinol in bulk and Pharmaceutical dosage forms.

KEYWORDS: Allopurinol, Area under curve, First order derivative spectroscopy, Validation.

ABSTRACT

Allopurinol is a drug used primarily to treat hyperuricemia and its complications, including chronic gout. It is administered orally and is an inhibitor of the enzyme xanthine oxidase which is responsible for the successive oxidation of hypoxanthine and xanthine, resulting in the production of uric acid, the product of human purine metabolism. Its IUPAC name is 1*H*-

pyrazolo [3, 4-d]pyrimidin-4(2H)-one. Molecular formula is $C_5H_4N_4O$ and the molecular weight is 136.112 g/mol[1]. It is slightly soluble in N, N Dimethyl formamide, very slightly soluble in water and practically insoluble in ethanol and diethyl ether. Allopurinol is almost completely metabolized to oxypurinol within two hours of oral administration and it is slowly excreted by the kidneys over 18–30 hours.[2]

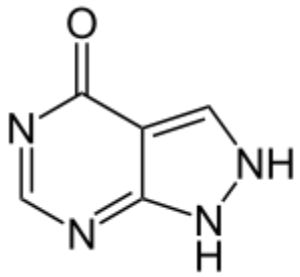

Figure 1: Structure of Allopurinol

Several methods were developed for the estimation of allopurinol by HPLC, visible and UV spectroscopy [3-13] individually or in combination. So our aim is to develop and validate a new simple, rapid, accurate, specific and highly sensitive UV spectroscopic methods for the estimation of allopurinol in bulk and pharmaceutical dosage form by using mobile phase which was more economical when compared to other methods.

MATERIALS AND METHODS

The instrument used in the present study was double beam UV-VIS spectrophotometer (Evolution 220, Thermo Scientific, Japan) connected to computer loaded with spectra manager software Thermo Insight was employed with spectral bandwidth of 1nm and wavelength accuracy of ± 0.3 nm with a pair of 10 mm matched quartz cells. All weights were taken on electronic balance (Schimadzu, Japan). Methanol (AR Grade, Merck, India) and double distilled water were used for the study. Working standard Allopurinol was obtained as a gift sample from Chandra laboratories, Hyderabad, India. Zyloric tablets was taken for study which contains Allopurinol 100mg was purchased from local market.

METHOD DEVELOPMENT

Selection of Solvent

Selection of solvent for the drug Allopurinol was done by testing its solubility in various solvents, which includes distilled water, methanol, ethanol, 0.1N HCl, 0.1N NaOH. Methanol was chosen as solvent for developing the method (for preparation of stock solution methanol is used and further dilutions are made with distilled water).

Determination of λmax

Preparation of Stock Solution

50mg of working standard Allopurinol was accurately weighed and transferred to 50ml volumetric flask. Then 20ml of Methanol was added to dissolve the drug by shaking the flask for few seconds. Then the final volume was made upto the mark with methanol to get the concentration of 1000µg/ml.

Preparation of Working Standard Solution

From the above standard stock solution, 1ml was pipetted out into a 10mL volumetric flask and the volume was made up to the mark with distilled water to get a concentration of 100µg/ml.

Preparation of Calibration Curve

Method A: Zero Order Spectroscopic Method

From the working standard solution, 0.5ml, 1.0ml, 1.5ml,2.0ml, 2.5ml, 3.0ml, 3.5ml were pipetted into 10ml volumetric flasks and volume was made upto the mark with distilled water to produce the concentrations ranging from 5-35µg/ml respectively. The analytical wavelength was selected by scanning 10µg/ml in the wavelength range of 400-200nm using distilled water as a blank and the wavelength corresponding to maximum absorbance (λmax) was found to be 250.6nm and the corresponding UV spectrum was shown in the figure 2 and the overlay spectrum for zero order was shown in the figure 3.Then, the calibration curve was plotted in the concentration range of 5-35µg/ml at 250.6nm by taking concentration on X-axis and absorbance on Y-axis. The correlation coefficient (r^2) was found to be 0.999.The calibration curve of allopurinol was shown in figure 4.

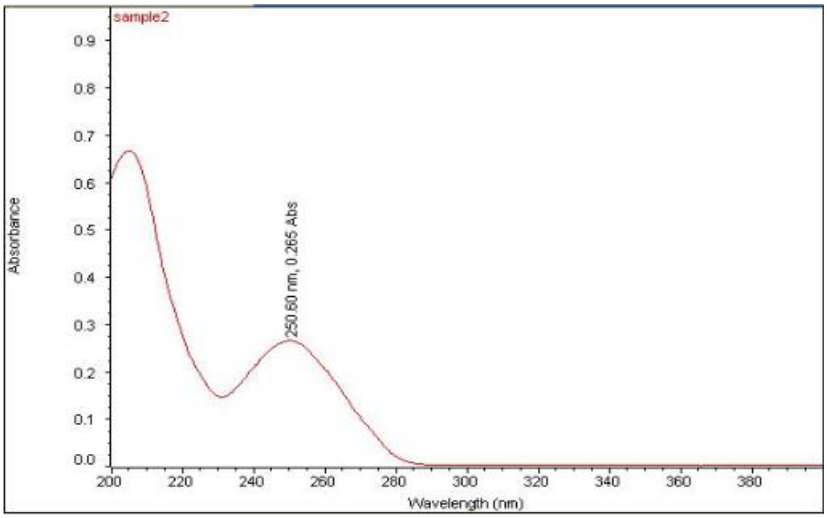

Figure 2: UV spectrum of Aceclofenac.

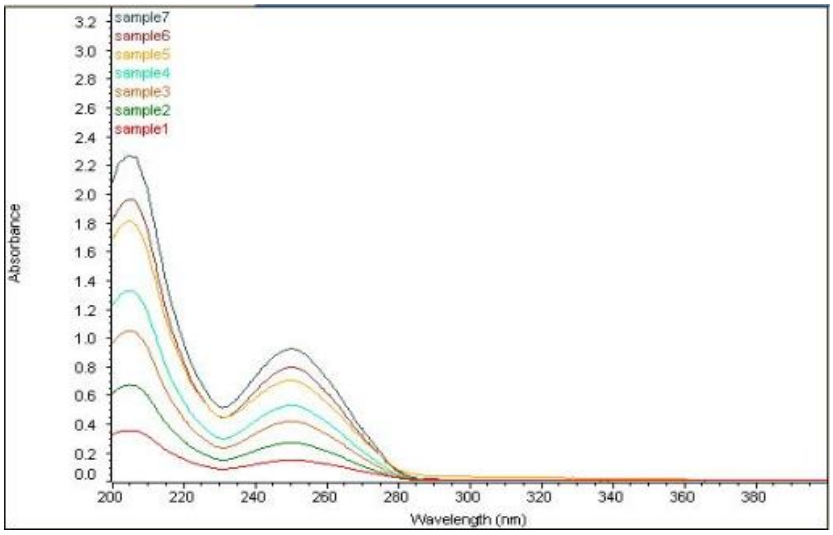

Figure 3: Zero Order Spectrum (overlay) of Allopurinol.

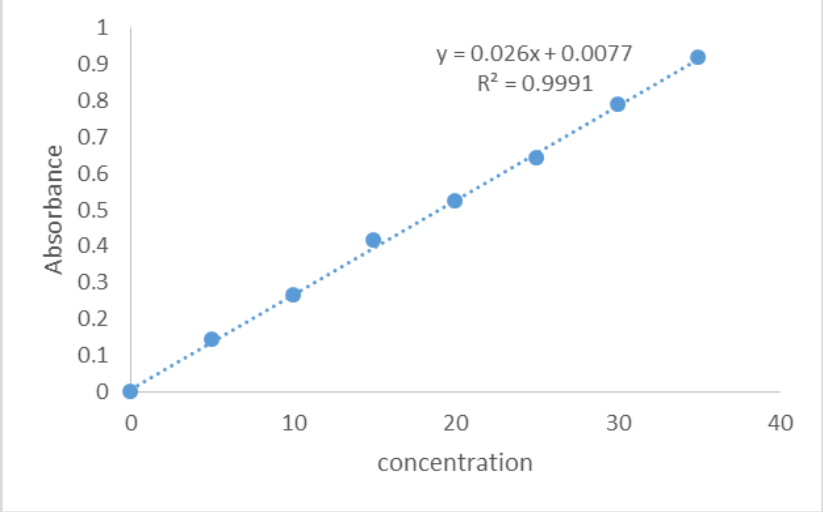

Figure 4: Calibration curve of Allopurinol (zero order).

Method B: First Order Derivative Spectroscopic Method

For the selection of analytical wavelength solution of 10µg/ml was scanned in the spectrum mode in the wavelength of 200-400nm and the absorption spectra thus obtained was derivatized in the first order. First order derivative spectrum showed a sharp peak at λ_{max} at 238nm and the corresponding spectrum was given in the figure 5. The amplitude of absorbance was measured for all solutions in the concentration range of 5-35µg/ml at 238nm and was plotted against concentration for getting the calibration curve and the regression equation was calculated. The calibration curve for the first order derivative spectra was shown in the figure 6.

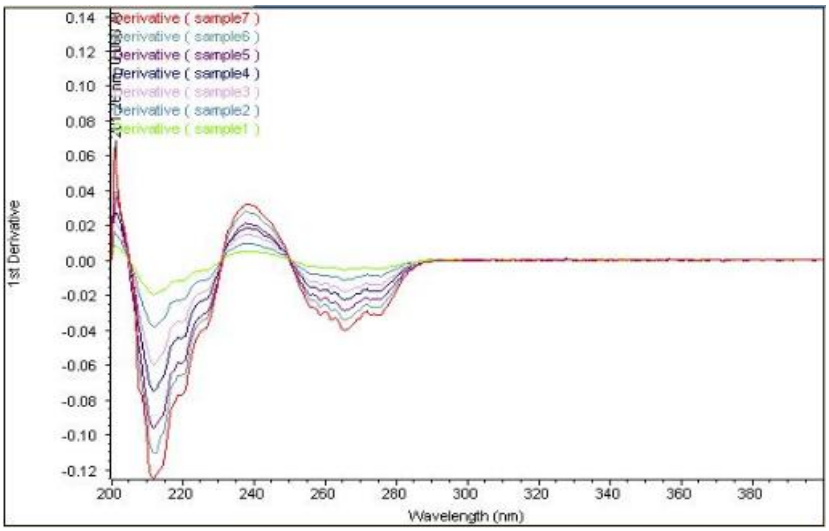

Figure 5: First order derivative spectrum of Allopurinol.

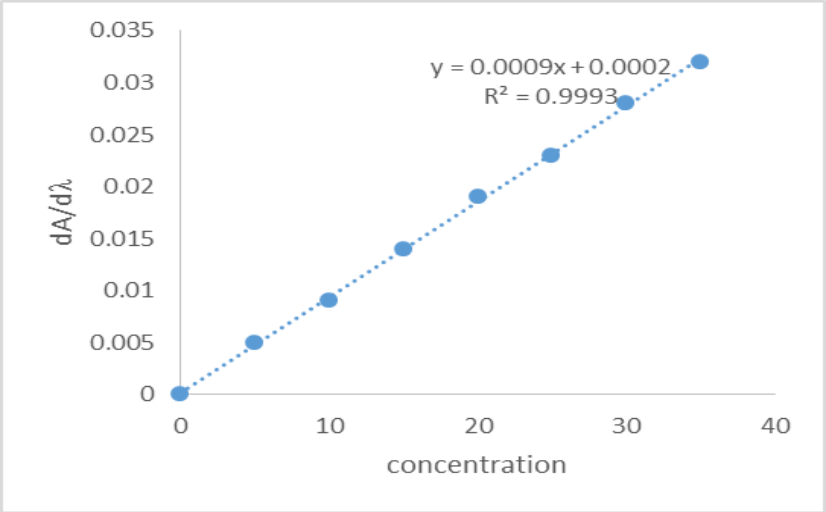

Figure 6: Calibration curve of Allopurinol (first order).

Method C: AUC Method

It involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths λ_1 and λ_2. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. From the spectrum of the drug, AUC in the wavelength range of 245-255nm was selected for the analysis and the corresponding spectrum was given in the figure 7. The calibration curve was plotted against concentration v/s $\alpha+\beta$ and was shown in the figure 8.

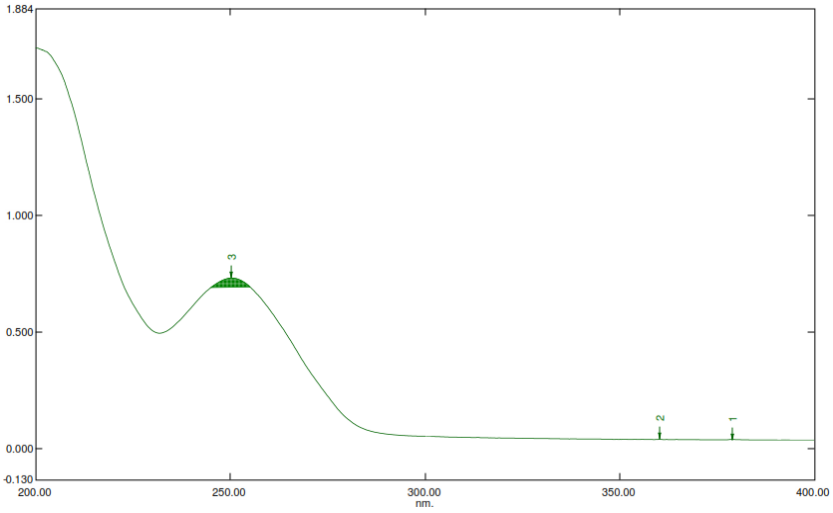

Figure 7: Area under curve spectrum of Allopurinol

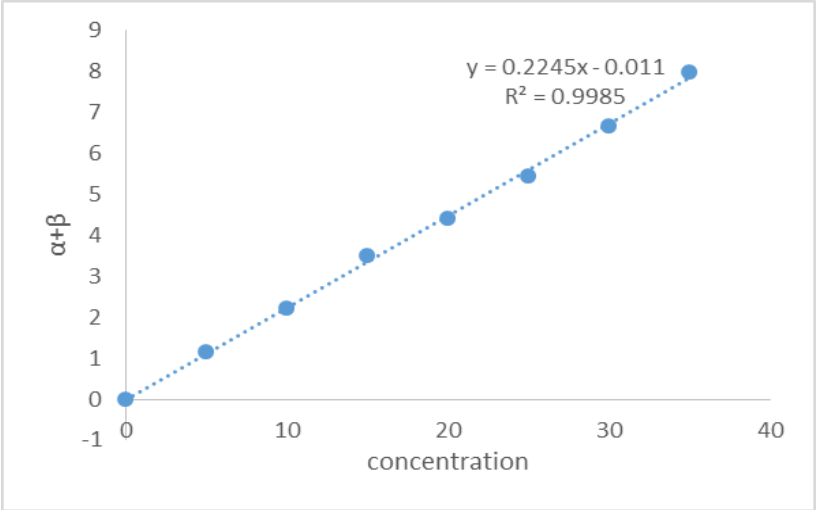

Figure 8: Calibration curve of Allopurinol (AUC method).

Estimation of Allopurinol in Tablet Formulation

For estimation of Allopurinol in tablet formulation by this method 10 tablets of marketed brand of Zyloric each containing 100mg of allopurinol was weighed and triturated to fine powder. Amount of powder equivalent to 50 mg drug was taken and dissolved in 20 ml of methanol and made up to the mark with methanol in 50 ml volumetric flask (1000 µg/ml). It was filtered through whatmann filter paper no. 41. From that stock solution further dilution was made with distilled water to get required concentration. The concentration of Allopurinol was determined by measuring the absorbance of sample solution at 250.6nm. The assay procedure was repeated six times (n=6).The result of marketed formulation analysis was given in the table 1.

Table 1: Assay of the Marketed Formulation.

Analysis method	Label claim (mg)	Amount found(mg)	% Recovery
A	100 mg	99.43	99.43
B	100mg	100.4	100.4
C	100mg	99.89	99.89

Validation of the Proposed Method

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. The method was validated for different parameters like Linearity, Accuracy and Precision.

Linearity

The linearity of the proposed UV spectroscopic methods were evaluated by plotting absorbances against concentrations of the analyte. Beers law was obeyed for all the three methods in the concentration range of 5-35 µg/ml. The correlation coefficient values were found to be 0.9991, 0.9993, 0.9985 respectively. All the results were given in the table 2.

Table 2: Linearity studies of Allopurinol by proposed methods.

S. No.	Parameter	Method A	Method B	Method C
1	Linearity(µg/ml)	5-35	5-35	5-35
2	Slope	0.026	0.0009	0.2245
3	Intercept	0.0077	0.0002	0.011
4	Correlation coeffecient	0.9991	0.9993	0.9985

Precision

The precision of the method was expressed in terms of % relative standard deviation (% RSD). To check the intra-day and inter-day variation of the methods, solutions containing 15, 20, 25µg/ml concentration of Allopurinol were subjected to the proposed spectroscopic methods of analysis **.**The % RSD values were found to be less than 2 for intraday and inter day precision, the precision results showed good reproducibility. The results are expressed in Table 3, 4, 5 respectively for the three methods.

Table 3: Precision studies of Allopurinol for Zero order method.

Concentration taken(µg/ml)	Intra -day precision		Inter day precision	
	Mean±SD	%RSD	Mean±SD	%RSD
15	0.436333±0.001528	0.35	0.428667±0.002517	0.251
20	0.526333±0.002517	0.478	0.531223±0.002646	0.498
25	0.713333±0.003055	0.428	0.70567±0.003512	0.497

Table 4: Precision studies of Allopurinol for First order method.

Concentration taken(µg/ml)	Intra -day precision		Inter day precision	
	Mean±SD	%RSD	Mean±SD	%RSD
15	0.014167±0.000153	1.08	0.0137±0.0002	1.4
20	0.019033±0.000153	0.803	0.018833±0.00208	1.1
25	0.023033 ±0.000153	0.664	0.0227±0.0002	0.881

Table 5: Precision studies of Allopurinol for Area Under Curve method.

Concentration taken(µg/ml)	Intra -day precision		Inter day precision	
	Mean±SD	%RSD	Mean±SD	%RSD
15	3.4916±0.005205	0.149	3.453±0.018248	0.528
20	4.406067±0.003325	0.075	4.403667±0.003786	0.0859
25	5.427±0.00859	0.128	5.4283±0.007917	0.145

Accuracy

Accuracy for the methods was established at 80, 100, 120% levels by the addition of standard drug of Allopurinol to the pre-quantified sample solution. Each dilution was observed six times and the percentage recovery of the drug was measured and the results were given in the table no.6.

Table 6: Recovery studies of Allopurinol by proposed methods.

Concentration taken(µg/ml)	Spiked level (%)	Amount added(mg)	Amount found(mg)			%Recovery		
			A	B	C	A	B	C
10	80	8	17.92	18.09	17.97	99.55	100.5	99.83
10	100	10	19.89	20.09	19.98	99.45	100.45	99.9
10	120	12	21.86	22.07	21.94	99.36	100.31	99.72

Table 7: Summary of Optical characteristics and Validation parameters.

S. No	Parameter	Method A	Method B	Method C
1	Absorption maxima(λ_{max})	250.60	238	245-255
2	Beer's limit(µg/ml)	5-35	5-35	5-35
3	Linearity indicated by correlation coefficient	0.9991	0.9993	0.9985
4	Regression Equation	y=0.026x+0.0077	y=0.0009x+ 0.0002	y= 0.2245x - 0.011
5	Accuracy indicated by %Recovery	99.36-99.55	100.31-100.5	99.72-99.9
6	Precision Intraday(%RSD) (n=6) Interday (% RSD) (n=6)	 0.35-0.478 0.251-0.498	 0.664-1.08 0.881-1.4	 0.075-0.149 0.0859-0.528

RESULTS AND DISCUSSION

The methods discussed in the present work provided a convenient and accurate way for the analysis of Allopurinol in bulk and in pharmaceutical dosage form. The absorbance maxima of Allopurinol was found to be 250.6 nm for the method A, the absorption maxima of first

order derivative spectra was found to be 238nm for method B and for method C the area under curve in the range of 245-255nm was selected for the analysis. Linearity for all the three methods was observed in the concentration range of 5-35µg/ml as shown in the table 2. The assay of the three methods were found to be within the range of 98-102% as shown in the table 1. The developed method was validated in terms of linearity, accuracy, precision in accordance with the ICH guidelines. In both the intra-day and inter-day precision study the %RSD was found to be less than 2.0 indicating the good precision of the method as shown in the table3,4,5 repectively for the three methods. The validation of proposed methods were further confirmed by recovery studies, the %recovery values vary from 98- 102% as shown in the table 6. Optical characteristics and validation parameters of Allopurinol were given in the table 7. Based on results obtained, it was found that the proposed methods were found to be accurate, precise and reproducible and can be employed for routine quality control analysis of Allopurinol in tablet dosage form.

CONCLUSION

The proposed methods were found to be simple, sensitive, accurate and precise and showed no interference from the common additives and excipients. The developed method was validated in terms of linearity, accuracy, precision in accordance with the ICH guidelines. Hence the proposed methods can be routinely used for the estimation of Allopurinol in bulk and pharmaceutical dosage forms.

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