SIMULTANEOUS ESTIMATION AND DEVELOPMENT OF ANALYTICAL METHOD VALIDATION FOR ESTIMATION OF ALLOPURINOL AND ACECLOFENAC IN BULK AND DOSAGE FORMS

ASHISH S MISHRA^{1*}, DEEPALI TOMAR², PEEYUSH KAUSHIK³ & HITESH MALHOTRA⁴

¹Faculty of Pharmacy, UPUMS, Saifai, Etawah, Uttar Pradesh, India

²Lala Birkha Ram College of Pharmacy, Golpura, Punchkula, Haryana, India

^{3,4}Chandigarh College of Pharmacy, Landran, Mohali, Punjab, India

ABSTRACT

This study was conducted with the objectives of performing simultaneous estimation and development of an unpretentious, straightforward, explicit, exact and specific UV spectrophotometric technique validation for assessment of Aceclofenac and Allopurinol in bulk as well as in dosage form. Various analytical performance parameters such as accuracy, linearity, and precision, limit of detection (LOD) and limit of quantification (LOQ) were resolved by ICH guidelines for method validation. The ideal conditions were established for the investigation or analysis of the drug. The results show that the λ_{max} of the Allopurinol and Aceclofenac were found to be 254 nm and 275 nm, respectively. The preferred method complies with Beer's law in the concentration range of 4-20 $\mu\text{g/ml}$ and show exceptional sensitivity with linearity. Moreover, the observed limit of detection and limit of quantification were in the range of 2-6 and 6-18 $\mu\text{g/ml}$, respectively. When the absorbance versus concentration graph was plotted on the calibration curves, a linear relationship was observed with the maximum observed coefficient correlation of 0.9994. The proposed method was interference free, accurate and simple technique, and it can be widely employed for monotonous quality control analysis.

KEYWORDS: Allopurinol, Aceclofenac, Spectroscopy, Precise & Calibration

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1. INTRODUCTION

Allopurinol is a xanthine oxidase compound inhibitor, with IUPAC named as 1, 5-Dihydro-4H-pyrazolo (3, 4-d) pyrimidin-4-one (figure 1). This is one of the best medications used to diminish urate levels, and is commonly utilized in the treatment of chronic gout. It is normally used to diminish uric acid levels in the blood and urine of individuals with high uric acid levels¹.

Aceclofenac, which is chemically known as [(2-{2, 6-dichlorophenyl} amino) phenyl]acetoxyacetic acid is an oral non-steroidal anti-inflammatory drug (NSAID) analog of Diclofenac. It is pharmacologically used as anti-inflammatory and as an analgesic, to cure arthritis and spondylitis. It acts fundamentally in the CNS, expanding the torment pain threshold by hindering both isoforms of cyclooxygenase, COX-I, COX-II, and COX-III enzymes engaged with synthesis of prostaglandin. It shows high penetrability to enter into synovial joints, wherein patients with osteoarthritis and related conditions, the loss of articular ligament in the region causes joint pain, tenderness, stiffness, crepitus and local inflammation²⁻⁷.

Literature survey reveals few strategies that have been utilized for the quantitative assurance of these two drugs.⁸⁻¹³ The goal of the work is to build up a novel yet straightforward UV spectrophotometric strategy for the simultaneous estimation of Allopurinol and Aceclofenac.

2. METHOD AND MATERIAL

2.1 Reagents and Chemicals

Aceclofenac and Allopurinol of working standard was provided by Jackson pharmaceuticals (p) Ltd, Amritsar, India. All other chemicals used in the analysis were AR grade.

2.2 Apparatus

A double beam spectrophotometer systronics (2202-2203) was used for the detection of absorbance; Shimadzu (BL 220 H) weighing balance and pH meter (Systronics μ pH system 361) were used for experimental purpose.

2.3 Experimental Part

2.3.1 Selection of Common Solvent

After evaluating the solubility of the two drugs in different solvents, 0.1N NaOH has been carefully chosen as common solvent for developing spectral characteristics.

2.3.2 Determination of Wavelength

The dilutions of the concentration of 10 μ g/ml were obtained for both Allopurinol and Aceclofenac solution. Both the solutions were scanned under UV range (200-400nm) in 10 mm cell against solvent blank.

2.3.3 Preparation of Stock Solutions

Preparation of Stock Solution of Allopurinol

10 mg of Allopurinol was precisely weighed into a volumetric flask of 10 ml and dissolved in a very small quantity of 0.1N NaOH, the volume was made up with the same. From above standard stock solution, further aliquots of 4, 8, 12, 16, 20 μ g/ml were prepared. The absorbance was taken using 0.1N HCl and phosphate buffer (pH 6.8) as blank.

Preparation of Stock Solution of Aceclofenac

10 mg of Aceclofenac was weighed correctly and poured in a 10 ml volumetric flask, and then small amount of methanol is added for its dissolution; followed by volume was made up with methanol. From above standard stock solution, further aliquots of 4, 8, 12, 16, 20 μ g/ml were prepared. The absorbance was taken using 0.1N HCl and phosphate buffer (pH 6.8) as blank.

2.3.4 Preparation of Sample Solutions

Pipette out 10ml of the stock solution of both Allopurinol and Aceclofenac in two separate volumetric flasks & then the volume was made up with phosphate buffer (pH 6.5). From the above standard solution, further aliquots were pipette into a volumetric flask (10 ml), and then diluted with sufficient quantity of phosphate buffer (pH 6.5). Blank solutions were also prepared similarly, except for the addition of the drug. Measured the absorbance of Allopurinol and Aceclofenac at 254 and 275 λ_{max} nm, respectively.

2.3.5 Preparation of Calibration Curve

Different fractions were taken out from both the standard stock solutions, and further diluted with phosphate buffer (pH 6.5) to provide a series of concentration (8-40 µg/ml). The λ_{max} of both the drugs in the phosphate buffer (pH 6.5) was observed by UV spectrum, in the range of 200-400nm. It was found that the peak at 254 nm (Allopurinol) and 275nm (Aceclofenac) showed maximum absorption, which has been used for further measurements. Absorbance measurement was performed at the determined wavelengths against the phosphate buffer (pH 6.5) as blank along with 0.1N HCl as co-solvent. By absorbance versus concentration plot, the calibration curves were made.

2.3.6 Simultaneous Estimation of Allopurinol and Aceclofenac

Preparation of Stock Solution

10 mg of Allopurinol was weighed accurately into a 10 ml of volumetric flask, and dissolved in small quantity of 0.1N methanolic NaOH, the volume was made up with 0.1N methanolic NaOH. From above standard stock solution, further aliquots were prepared. The absorbance was taken using 0.1N HCl/pH 6.8 phosphate buffer as blank at λ_1 254 and λ_2 275, respectively.

10 mg of Aceclofenac was weighed accurately into 10 ml of volumetric flask and dissolved in little quantity of 0.1N methanolic NaOH, the volume was made up with 0.1N methanolic NaOH. From above standard stock solution, further aliquots were prepared. The absorbance was taken using 0.1N HCl/pH 6.8 phosphate buffer as blank at λ_1 254 and λ_2 275, respectively.

Preparation of Mixture of Both the Drugs (Allopurinol and Aceclofenac)

10 mg each of mixture (Aceclofenac and Allopurinol) was weighed correctly into a volumetric flask of 10 ml and dissolved in small quantity of 0.1N methanolic NaOH, the volume was made up with 0.1N methanolic NaOH. From above standard stock solution, further aliquots were prepared. The absorbance was taken using 0.1N HCl/pH 6.8 phosphate buffer as blank at λ_1 254 and λ_2 275, respectively. The readings were taken in triplicate.

The concentration of each component was determined by using simultaneous equation method (Vierodt's method).

$$A_1 = E_1aC_1 + E_2aC_2 \text{ ----- (at } \lambda_1\text{--254 nm)}$$

$$A_2 = E_1bC_1 + E_2bC_2 \text{ ----- (at } \lambda_2\text{--275 nm)}$$

A_1 = absorbance value of the test solution at 254nm

A_2 = absorbance value of the test solution at 275nm

E_1a = absorptivity of Allopurinol at 254nm

E_1b = absorptivity of Allopurinol at 275nm

E_2a = absorptivity of Aceclofenac at 254nm

E_2b = absorptivity of Aceclofenac at 275nm

C_1 = concentration of Allopurinol in µg/ml

C_2 = concentration of Aceclofenac in µg/ml

2.4 Method Validation

The ICH guideline Q2 (R1) was considered for the validation of the proposed method. Numerous validation parameters for method validation like linearity and range; limit of Detection (LOD) and limit of Quantification (LOQ); Accuracy and Precision; and Robustness were calculated.

Linearity and Range

Linearity is the potential to produce experimental results that were directly proportional to the analyst concentration, in a specified range. Twenty replicated analyses were performed separately to access linearity, and absorbance versus concentration was plotted to obtain the graph of calibration. The least square regression method was employed for measurement of the linearity. The range of the proposed analytical method was given by the difference between the lower and the upper concentration levels of Allopurinol and Aceclofenac in standard solutions.

Accuracy

Accuracy is well-defined as the degree of how close the experimental results are, to the true value and the proportion of analyte recovered by performing an assay, from a known concentration of drug. The recovery studies were usually performed to establish the accuracy of the experiment. For which, a standard amount of drug was weighed correctly and transferred into a volumetric flask (50 ml) along with an appropriate amount of 0.1N HCl for dissolution, then the volume is made up with 0.1N HCl to 50ml. The 10ml of the above solution was pipetted out into another volumetric flask of 100 ml & the volume was made up of phosphate buffer (pH6.5). From the above standard solution, further aliquots of concentration 24 µg/ml were prepared in a volumetric flask of 10ml. Similarly, the Blank solution was also prepared without the addition of the drug. The absorbance for Allopurinol and Aceclofenac was taken at $\lambda_{\max}$ 254 and 275 nm, respectively.

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

The LOD is the minimum concentration of an analyte in a test sample that can be identified, but not quantified. The LOQ is the lowermost concentration of the standard curve that can be calculated with satisfactory accuracy and precision. The LOD and LOQ were estimated by considering the slope of the calibration curve and the standard deviation. The Following equations were used for the calculation;

$$\text{LOD}=3.3\sigma/S; \text{LOQ}=10\sigma/S$$

Robustness

The robustness is the ability of an analytical method to remain unaffected by small, but measurable variations in validation parameters of the method and indicates its reliability throughout its normal usage. The robustness was estimated by calculating the impact of minor variations of the most significant variables like

- Change in acid normality (0.01N HCl) & (1N HCl)
- Change in acid type (0.1N H₂SO₄ instead of 0.1N HCl)

% RSD was determined for concentration for 08µg/ml at pH values mentioned above in triplicate.

3. RESULTS & DISCUSSIONS

Simultaneous Estimation of Aceclofenac & Allopurinol

The concentration of each component was determined by using simultaneous equation method (Vierodt's method).

$$A_1 = E_1aC_1 + E_2aC_2 \text{ ----- (at } \lambda_1\text{--254 nm)}$$

$$A_2 = E_1bC_1 + E_2bC_2\text{----- (at } \lambda_2\text{--275 nm)}$$

[Table 1: about here]

*C1 and *C2 were calculated in pH6.8 phosphate buffer & C1 and C2 were calculated in 0.1N HCl.

*C1= 9.3 µg/ml, C1= 9.8 µg/ml,

*C2= 9.0 µg/ml, C2= 9.2 µg/ml,

Determination of Wavelength and Calibration Curve

The study of spectrum revealed that Aceclofenac shows a well-defined λ max at 275nm, whereas Allopurinol shows at 254nm. These two wavelengths were selected for the development of simultaneous equation. (See **figure 2 and 4** for **observed peak of λ_{max}** and **figure 3 and 5** for **calibration curves**).The optical characteristic parameters are shown in **table 1 and 2**.

Precision and Accuracy

The accuracy and precision have been summarized in **table 3 and 4**along with **table 5 and 6** respectively. The Assay percentage was found to be 99.04%. The outcomes of precision (measurements of inter and intraday) and accuracy exhibits excellent reproducibility with the percent relative standard deviation (%RSD) of less than 2.00%. Therefore, the results indicate that the method is highly precise and accurate.

Precision

Precision is defined as the degree of repeatability of the method under standard operating conditions. It was estimated for 3 concentration levels (12,16,20µg/ml) covering the entire linearity range by intermediate (inter-day) as well as repeatability (intra-day) studies and was reported as % RSD.

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

The LOD and LOQ for Allopurinol in 0.1 N HCl were found to be 3.375µg/ml and 10.22 µg/ml respectively, while the observed values in phosphate buffer is 2.175µg/ml and 6.59 µg/ml, respectively.

In case of Aceclofenac, the LOD and LOQ in 0.1 N HCl were obtained to be 6.16µg/ml and 18.66 µg/ml respectively while observed values in phosphate buffer is 3.60µg/ml and 11.16 µg/ml respectively.

Robustness

Data for robustness has been summarized and tabularized in **table 7 and 8**

This validation method was carried out, with the objective to provide reliable and accurate method for simultaneous estimation of Allopurinol and Aceclofenac in bulk as well as in formulation. Validation of analytical method defines the documented evidence, which provides the high degree of assurance of results meeting with predetermined specifications and quality attributes. The UV absorption spectrum of the sample (100µg/ml) was scanned and major peak was obtained at 254nm (Allopurinol) and 275nm (Aceclofenac) as λ_{max} due to higher absorbance value. The calibration curve indicated the

coefficient of correlation value of 0.9994, which indicates linear relationship between concentration and absorbance. The results of accuracy and precision were found to be within limits in accordance with ICH Q2 (R1) guidelines.

Slight deviation was observed during robustness with change in acid composition and change in acid itself RSD slightly exceeded in accordance with, ICH Q2 (R1) guidelines. The sample was found to be stable and all other parameters were well within acceptance level i.e. LOD and LOQ. The change in instrument, analyst and duration has not affected the method. The developed UV method was successful in accurate estimation of Allopurinol & Aceclofenac in formulation.

4. CONCLUSIONS

A UV-spectrophotometric method was proposed and validated. The proposed analytical method is easy, economical and experimentally prevents loss of time. Therefore, this will be appropriate for Allopurinol & Aceclofenac analysis of pharmaceutical formulations in bulk.

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APPENDIX

Figure 1: Structure of Allopurinol & Aceclofenac.

Figure 2: Observed Peak of $\lambda_{\max}$ of Aceclofenac.

Figure 3: Calibration Curves of Aceclofenac.

Figure 4: Observed Peak of λ_{max} of Allopurinol.

Figure 5: Calibration Curves of Allopurinol.

List of Tables

Table 1: Absorbance and Absorptivity Values for Simultaneous Estimation of Aceclofenac & Allopurinol

S. No.	Drug	Dilution	Parameter		Absorptivity(a) Value	
1	Allopurinol	0.1N HCl	E _{1a}	E _{2a}	0.074	0.011
		pH6.8 phoshphate buffer	E _{1a}	E _{2a}	0.030	0.094
2	Aceclofenac	0.1N HCl	E _{1b}	E _{2b}	0.003	0.044
		pH6.8 phosphate buffer	E _{1b}	E _{2b}	0.013	0.018
3	Mixture	0.1N HCl	A ₁	A ₂	0.790	0.472
		pH6.8 phosphate buffer	A ₁	A ₂	1.072	0.271
Every value is an average of 3 determinations						

Table 2: Optical Characteristic Parameters for Allopurinol in Phosphate Buffer (pH 6.5) and 0.1N HCl

Parameters for Allopurinol	Values (0.1 N HCl)	Values (pH6.5 Phosphate Buffer)
$\lambda_{\max}(\text{nm})$	254	
Linearity range($\mu\text{g/ml}$)	4-20	
Correlation coefficient (R^2)	0.9993	0.9994
Intercept	0.0131	0.0088
Slope	0.0481	0.0489
Equation	$Y=0.481x+0.0131$	$Y=0.0489x+0.0088$
LOD ($\mu\text{g/ml}$)	3.375	2.175
LOQ ($\mu\text{g/ml}$)	10.22	6.59

Table 3: Optical Characteristic Parameters for Aceclofenac in Phosphate Buffer (pH 6.5) and 0.1N HCl

Parameters for Aceclofenac	Values (0.1 N HCl)	Values (pH6.5 Phosphate Buffer)
$\lambda_{\max}(\text{nm})$	275	
Linearity range($\mu\text{g/ml}$)	4-20	
Correlation coefficient (R^2)	0.9991	0.9845
Intercept	0.006	0.0197
Slope	0.0487	0.0423
Equation	$Y=0.487x+0.006$	$Y=0.0423x+0.0197$
LOD ($\mu\text{g/ml}$)	6.16	3.60
LOQ ($\mu\text{g/ml}$)	18.66	11.16

Table 4: Results of Accuracy of Allopurinol

Conc. Taken (mg)	Accuracy in 0.1N HCl			Accuracy in Phosphate Buffer (pH6.5)		
	Conc. Observed (mg) $\pm$ SD	Standard Deviation (σ)	Relative Standard Deviation (RSD)	Conc. Observed (mg) $\pm$ SD	Standard Deviation (σ)	Relative Standard Deviation (RSD)
12	12.08 $\pm$ 0.007	0.007	0.057	12.92 $\pm$ 0.008	0.008	0.0619
Each value is an average of three determinants.						

Table 5: Results of Accuracy of Aceclofenac

Conc. Taken (mg)	Accuracy in 0.1N HCl			Accuracy in Phosphate Buffer (pH6.5)		
	Conc. Observed (mg) $\pm$ SD	Standard deviation (σ)	Relative standard deviation (RSD)	Conc. Observed (mg) $\pm$ SD	Standard deviation (σ)	Relative standard deviation (RSD)
12	12.23 $\pm$ 0.009	0.009	0.073	13.00 $\pm$ 0.043	0.043	0.330
Each value is an average of three determinants.						

Table 6: Results of Precision of Allopurinol

Conc. Taken (µg/ml)	Absorbance	Conc. Observed (µg/ml)	Standard Deviation (σ)	Relative Standard Deviation (RSD)	
12	0.595	12.38	0.020	0.120%	
16	0.781	16.75			
20	0.969	20.49			
Intermediate Precision Evening (Day1)					
Conc. Taken (µg/ml)	Absorbance	Conc. Observed (µg/ml)	Standard Deviation (σ)	Relative Standard Deviation (RSD)	Relative Standard Deviation between Morning and Evening
12	0.602	12.62	0.018	0.109%	0.114%
16	0.785	16.28			
20	0.973	20.48			
Intermediate Precision Morning Inter Day (Day II)					
Conc. Taken (µg/ml)	Absorbance	Conc. Observed (µg/ml)	Standard Deviation (σ)	Relative Standard Deviation (RSD)	
12	0.603	12.74	0.017	0.102 %	
16	0.785	16.55			
20	0.975	20.63			
Intermediate Precision Morning (Day Iii)					
Conc. Taken (µg/ml)	Absorbance	Conc. Observed (µg/ml)	Standard Deviation (σ)	Relative Standard Deviation (RSD)	Relative Standard Deviation for the Day I, II, III
12	0.331	16.64	0.04714	0.29%	0.43%
16	0.331	16.64			
20	0.332	16.69			
Each value is an average of three determinants.					

Table 7: Results of Precision of Aceclofenac

Conc. Taken (µg/ml)	Absorbance	Conc. Observed (µg/ml)	Standard Deviation (σ)	Relative Standard Deviation (RSD)	
12	0.573	12.26	0.030	0.183%	
16	0.783	16.54			
20	0.982	20.24			
Intermediate Precision Evening (Day1)					
Conc. Taken (µg/ml)	Absorbance	Conc. Observed (µg/ml)	Standard Deviation (σ)	Relative Standard Deviation (RSD)	Relative Standard Deviation between Morning and Evening
12	0.575	12.63	0.028	0.169%	0.176%
16	0.783	16.51			
20	0.984	20.38			
Intermediate Precision Morning Inter Day (Day ii)					
Conc. Taken (µg/ml)	Absorbance	Conc. Observed (µg/ml)	Standard Deviation (σ)		Relative Standard Deviation (RSD)
12	0.577	12.68	0.027		0.163%
16	0.784	16.54			
20	0.984	20.27			
Intermediate Precision Morning (Day iii)					
Conc. Taken (µg/ml)	Absorbance	Conc. Observed (µg/ml)	Standard Deviation (σ)	Relative Standard Deviation (RSD)	Relative Standard Deviation for the Day I, II, III
15	0.577	12.46			

15	0.785	16.64	0.025	0.151%	0.157%
15	0.986	20.35			
Each value is an average of three determinants.					

Table 8: Results of Robustness of Allopurinol

Conc. Taken (µg/ml)	Absorbance	Conc. Observed (µg/ml)	Actual Amount Present in µg/ml	Standard Deviation(σ)	Relative Standard Deviation (RSD)
08	0.523	8.60	08 µg/ml	0.007	0.076%
08	0.453	9.53			
08	0.399	9.23			
Readings for Robustness (0.1n hcl)					
Conc. Taken (µg/ml)	Absorbance	Conc. Observed (µg/ml)	Actual Amount Present in µg/ml	Standard Deviation (σ)	Relative Standard Deviation (RSD)
08	0.411	7.56	08 µg/ml	0.006%	0.072%
08	0.423	8.01			
08	0.398	9.32			
Readings For Robustness (Ph 6.5 Phosphate Buffer)					
Conc. Taken (µg/ml)	Absorbance	Conc. Observed (µg/ml)	Actual amount present in µg/ml	Standard deviation (σ)	Relative standard deviation (RSD)
08	0.413	7.35	08 µg/ml	0.005	0.062 %
08	0.424	7.98			
08	0.398	8.67			
Each value is average of three determinants.					

Table 9: Results of Robustness of Aceclofenac

Conc. Taken (µg/ml)	Absorbance	Conc. Observed (µg/ml)	Actual Amount Present in µg/ml	Standard Deviation(σ)	Relative Standard Deviation (RSD)
08	0.412	7.54	08 µg/ml	0.006	0.072%
08	0.423	8.03			
08	0.418	9.32			
Readings for Robustness (0.1n hcl)					
Conc. Taken (µg/ml)	Absorbance	Conc. Observed (µg/ml)	Actual Amount Present in µg/ml	Standard Deviation (σ)	Relative Standard Deviation (RSD)
08	0.410	7.84	08 µg/ml	0.005%	0.057%
08	0.427	8.97			
08	0.396	9.45			
Readings for Robustness (Ph 6.5 Phosphate Buffer)					
Conc. Taken (µg/ml)	Absorbance	Conc. Observed (µg/ml)	Actual amount Presenting µg/ml	Standard Deviation (σ)	Relative Standard Deviation (RSD)
08	0.345	8.79	08 µg/ml	0.019	0.230 %
08	0.348	7.54			
08	0.352	8.23			
Each value is average of three determinants.					