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Simultaneous Estimation of allopurinol and aceclofenac in bulk drug and pharmaceutical formulation by using UV-visible Spectroscopy method

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ABSTRACT

The present manuscript describes sensitive, rapid, accurate, and precise spectrophotometric method for simultaneous estimation of allopurinol and aceclofenac in pharmaceutical dosage form. Simultaneous equation method used at 250nm & 273nm as λ_{max} of allopurinol and aceclofenac respectively in 0.1N HCl. Both the drugs follow beer-lamber's law on the concentration range 2-12µg/ml and 2-24µg/ml respectively for allopurinol & aceclofenac. The mean percentage recovery is 100.01% and 99.4% for allopurinol and aceclofenac respectively by simultaneous equation. The method has been validated as per ICH guidelines. The proposed method effectively applied to bulk drug and pharmaceutical formulation. The accuracy and reproducibility are close to 100% with acceptance range of %R.S.D. The method was successfully applied to pharmaceutical dosage form because no interference was found from excipient.

Keywords: Simultaneous equation, Validation, Allopurinol, Aceclofenac, Methanol.

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INTRODUCTION

Allopurinol is a structural similar of the natural purine base, hypoxanthine for treatment of hyperuricaemia and gout associated with various biological problems[1]. Chemical structure of allopurinol is 1H-pyrazolo [3, 4-d] pyrimidin-4-ol and prevent the production of uric acid by inhibit the function of enzyme xanthine oxidase [1, 2, 4].

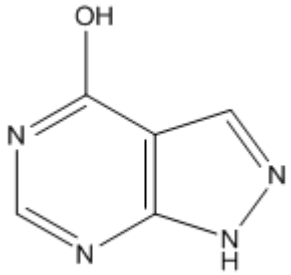

Figure 1: Chemical structure of Allopurinol

Allopurinol also increases the level of ribotides and inhibits the purine biosynthesis[3]. Allopurinol is rapidly absorbed from the upper gastrointestinal tract and it has plasma half-life of about 1 to 2 hours[4]. It is relatively insoluble in water and freely soluble in alkaline aqueous solutions [5, 6].

Aceclofenac is a non steroidal anti-inflammatory drug having analgesic activity. Chemically Aceclofenac is 2-[2, 6-Dichlorophenyl) amino] benzoic acetic acid carboxymethyl ester and used in various pain condition like rheumatoid arthritis, osteoarthritis and ankylosing spondylatis[7, 8]. The structural formulae of Aceclofenac (I) and its main degradation product is Diclofenac (II). Diclofenac also has analgesic and anti inflammatory activity[9].

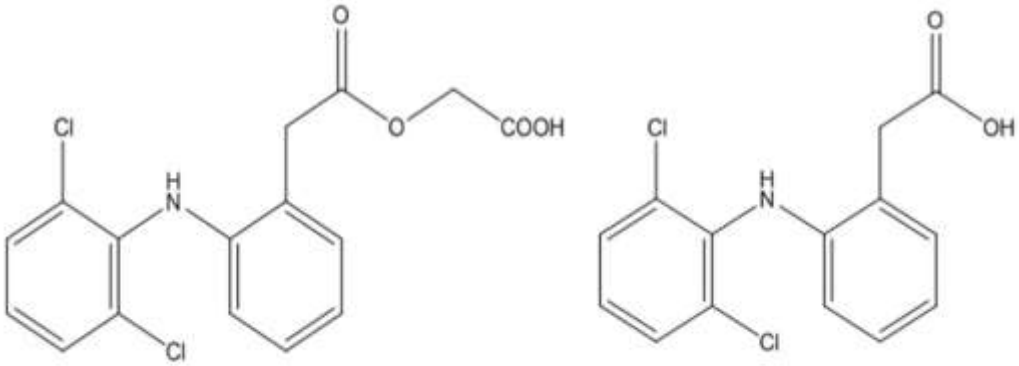

Figure 2: Chemical structure of Aceclofenc and Diclofenac

The mechanism of action of aceclofenac is inhibition of prostaglandin synthesis and also inhibits interleukin (IL)–1β and tumour necrosis factor (TNF**)** [10]. In vitro data, it indicates Aceclofenac is selectivity for COX2 inhibator[11]. Aceclofenac has greater patient compliance and tolerated as compared to other NASID due to their lesser gastrointestinal and cardiovascular side effects[12].

Aceclofenac is observed statistically superior to diclofenac in terms of epigastric discomfort, dyspepsia and abdominal pain and efficacy similar to naproxen and piroxicam in patients with osteoarthritis[11]. It is rapidly absorbed after oral dose. The half life of drug is approximately 4 hrs and dosing frequency 2-3 times daily with dose range 100-200 mg[13].

Several analytical techniques reported for estimation of allopurinol such as Titration[14], Derivatization spectrophotometric[19] by using catechol and Fe (II) to form a blue soluble complex which was measured at λ_{max} 580 nm., but this method needs large amounts of chemicals and many steps to prepare standard solutions before measurement[3], High performance liquid chromatography [15], Capillary electrophoresis [16], Flow injection [17] and Differential-pulse Polarography[18].

Several analytical techniques like titrimetry[20], colorimetric and spectrofluorimetry[21], densitometry[22], high performance liquid chromatography[23], reverse phase high performance liquid chromatography[24], spectrophotometry[25] and stripping volumetry[26] have been reported for quantitative analysis of aceclofenac.

A deep literature review state that combination of these two drugs are not official in any pharmacopeia and no official method report for simultaneous estimation of allopurinol and acelofenac. In the present investigation an attempt has been made to develop and validate simultaneous UV- spectrophotometric method for allopurinol and aceclofenac by simultaneous equation method in bulk drug and pharmaceutical formulation.

MATERIALS AND METHOD

Apparatus

A double beam UV/Visible spectrophotometer (shimadzu model 1800, Japan), attached to the computer software UV-Probe system software (UV Probe version 2.10), with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was employed. Auto pH meter LT 71/ L710 (Labtronics laboratory instrument) was used in the study

Reagents and Chemicals

Kindly gifted reference standard of allopurinol (Jackson pharmaceutical, Amritsar) and aceclofenac (Kwality pharmaceutical, Amritsar) were used without further purification for the study. The Methanol (AR Grade, S. D. Fine Chemicals Ltd., Mumbai, India) and Whatman filter paper no. 41 (Millipore, USA) were used in the study.

Preparation of Standard Solution

Accurately weighed 10 mg of allopurinol and aceclofenac standard powder were transferred in two separate 100 ml volumetric flasks, dissolved in 10 ml of methanol and volumes were made up

to mark with 0.1N HCl. Aliquots were prepared by using respective media for UV analysis and standard plots of respective drug.

Determination of Analytical wavelength

The standard solution of allopurinol and aceclofenac were scanned separately in the UV range of 200-400nm. Data were recorded at an interval of 1nm.

Preparation of combination of both the drugs

The mixture of both the drugs were prepared by dissolving 20 mg of allopurinol and 20 mg of aceclofenac in 20 ml of methanol and then volume was made up to 100 ml with 0.1N HCl. Absorbance was measured at both the wavelength (250 nm and 273 nm) by using 0.1N HCl as blank. The readings were taken in triplicate.

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

$$A_1 = E_{1a}C_1 + E_{2a}C_2 \text{ ---------- (at – 250 nm)}$$

$$A_2 = E_{1b}C_1 + E_{2b}C_2 \text{---------- (at – 273 nm)}$$

A_1= absorbance value of the sample solution at 250 nm

A_2= absorbance value of the sample solution at 273 nm

E_{1a}= absorptivity of Allopurinol at 250 nm

E_{1b}= absorptivity of Allopurinol at 273 nm

E_{2a}= absorptivity of Aceclofenac at 250 nm

E_{2b}= absorptivity of Aceclofenac at 273 nm

C_1= concentration of Allopurinol in µg/ml

C_2= concentration of Aceclofenac in µg/ml

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[27, 28].

Linearity

Aliquots from the standard stock solutions of allopurinol and aceclofenac were used to prepare different sets of dilutions. Aliquots of different concentration for Allopurinol were used upto the highest concentration, till linearity was observed and absorbance was recorded at 250 nm. Same procedure as mentioned above was repeated for Aceclofenac except that the wavelength used was 273 nm.

Method precision (repeatability)

The precision was checked by repeated scanning and measurement of absorbance of solutions (n = 6) for allopurinol and aceclofenac with different concentration without changing the parameter of the proposed spectrophotometry method.

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of allopurinol and aceclofenac. The result was reported in terms of relative standard deviation (% RSD).

Accuracy (recovery study)

Accuracy was investigated by using different concentration combination of both the drugs in 3 replicates at both the wavelength. Accuracy was reported as percent recovery by the assay of known added amount of both the drugs in the sample solution.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response

And, S = slope of the calibration curve.

Analysis of combination of Allopurinol and Aceclofenac

Twenty tablets formula were weighed and the average weight was calculated. The tablet powder equivalent to 100 mg of allopurinol and 100 mg of aceclofenac were weighed and transferred to 100 ml volumetric flask. Methanol (30 ml) was added and sonicated for 20 min, and volume was made up to the mark with 0.1 N HCl. The solution was filtered through Whatman filter paper no. 41 and filtrate was suitably diluted with 0.1 N HCl to achieve a final concentration of 100µg/ml of allopurinol and 100µg/ml of aceclofenac. The absorbance of final solution was recorded at selected wavelengths for determination of allopurinol and aceclofenac. The analysis procedure was repeated three times with tablet excipient.

RESULTS AND DISCUSSION

The standard solution of allopurinol and aceclofenac were prepared separately in 0.1 N HCl and the solution were scanned the entire UV range to determine absorption maximum. Overlain spectra

show 250nm as the λ_{max} of allopurinol and 273nm as the λ_{max} of aceclofenac (Figure 3 & 4). Allopurinol solution was found to follow Beer's lambert law in concentration range of 2-12µg/ml with correlation of coefficient was found to be 0.999 (Figure 5). Similar aceclofenac solution was found to follow Beer's lambert law in concentration range of 2-24µg/ml with correlation of coefficient was found to be 0.999 (Figure 6).

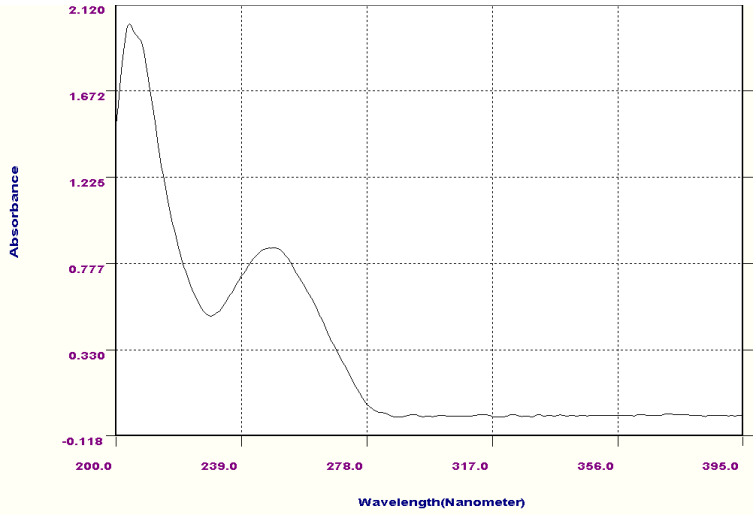

Figure 3: Absorption Spectra of Allopurinol

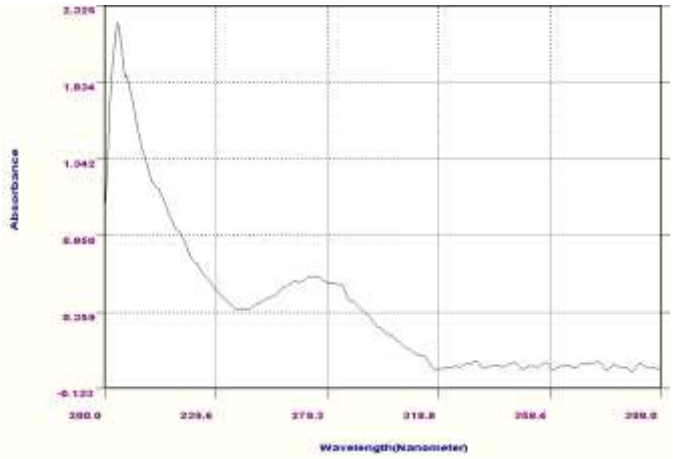

Figure 4: Absorption Spectra of Aceclofenac

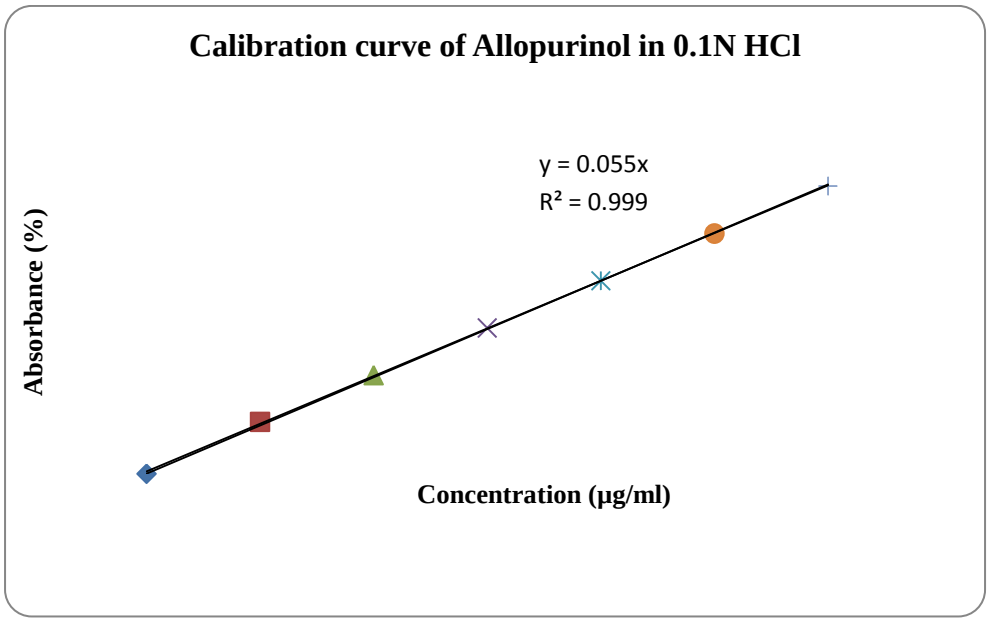

Figure 5: Calibration graph of Allopurinol

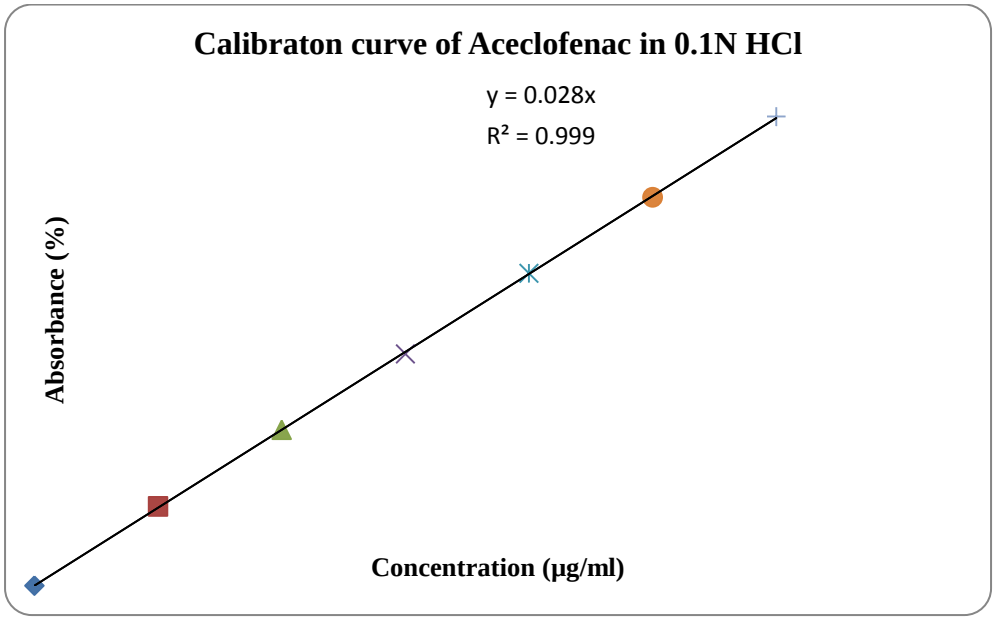

Figure 6: Calibration graph of Aceclofenac

The absorptivity value of the drugs is the ratio of absorbance at selected wavelengths with concentration of drugs in µg/ml. A set of two simultaneous equations were framed using these absorptivity values.

$$A_1 = 48.5C_1 + 16.3C_2 \text{ ---------- (at – 250 nm)}$$

$$A_2 = 14.2C_1 + 25.7\ C_2\text{----------} \text{ (at – 273 nm)}$$

Where, A1 and A2 are absorbance values of the sample solution at 250 nm and 273 nm respectively. 48.5 and 14.2 are absorptivities of allopurinol at 250 nm and 273 nm, respectively.

16.3 and 25.7 are the absorptivities of aceclofenac at 250 nm and 273 nm, respectively. C_1 is the concentration of allopurinol and C_2 is the concentration of aceclofenac in µg/ml.

The proposed method was found to be accurate, precise and economic for the routine simultaneous estimation of two drugs. The linearity range for allopurinol and aceclofenac were found to be 10-50µg/ml and 10-90µg/ml. Regression analysis data and summary of all validation parameters are given in Table 1.Precision was calculated as repeatability (% RSD) and intra and inter day variation (% RSD) for both the drugs. The LOD and LOQ were found to be 0.15 and 0.48 µg/ml, respectively for allopurinol and 0.14 and 0.42 µg/ml, respectively for aceclofenac indicates sensitivity of the proposed method.

Table 1: Regression analysis data and summary of validation parameters

Parameters	ALL	ACE
Wavelength (nm)	250	273
Beer's Law Limit (µg/ml)	2-12µg/ml	2-24µg/ml
Regression equation	y = 0.055x	y = 0.028x
Slope (m)	0.055	0.028
Correlation Coefficient (r2)	0.999	0.999
Repeatability (%RSD[a], n=6)	0.28	0.53
Interday (n=3) (% RSD)	0.20-0.52%	0.40-0.87%
Intraday(n=3) (% RSD)	0.83-1.52%	0.99-1.65%
LOD[b]	0.159	0.14
LOQ[c]	0.48	0.42

RSD[a] = Relative standard deviation. LOD[b] = Limit of detection. LOQ[c] = Limit of quantification

Accuracy was determined by calculating the recovery study at 3 different concentration levels. Known amounts of standard solutions of allopurinol and aceclofenac were added at 100%, 120% and 80 % level to pre quantified sample solutions and the mixture were analyzed by the proposed method. The mean percentage recovery values were close to the taken theoretical concentration and their R.S.D. values were within the acceptable range as per ICH guidelines i.e. less than 2. This indicates that the method has high accuracy shown in Table 2. The simultaneous method successfully applied to the tablet dosage formula with excellent results which shown in Table 3.

Table 2: Recovery data

Concentration of drug taken (mg)		%Recovery of drug* ± S.D		% R.S.D.	
Allopurinol	Aceclofenac	Allopurinol	Aceclofenac	Allopurinol	Aceclofenac
100	100	99.4±0.176	99.4 ± 0.020	0.44	0.20
120	80	99.18 ± 0.521	96.7 ± 0.132	1.26	1.36
80	120	100.3 ± 0.453	104.3 ± 0.06	0.90	0.59

***Every reading is average of 3 reading.**

Table 3: Analysis of combination

Concentration of drug taken (mg)		Excipients added	%Recovery of drug*± S.D.		% R.S.D.	
Allopurinol 100	Aceclofenac 100	Mannitol HPMC K100 Dicalcium phosphate Microcrystalline cellulose Carboxymethyl cellulose Lactose	Allopurinol 100.10 ± 0.76	Aceclofenac 99.57 ± 0.20	Allopurinol 0.75	Aceclofenac 0.20

***Every reading is average of 3 reading.**

CONCLUSION

The developed method was validated as per ICH guidelines. The standard deviation and % R.S.D values are low indicates high degree of accuracy and precision in method for estimation of allopurinol and aceclofenac. The method utilizes easily available and cheap solvent for analysis of allopurinol and aceclofenac hence the method was economic with highly reproducibility and recovery. The common excipients and other additives do not interfere in the analysis of allopurinol and aceclofenac, therefore it can be conveniently adopted for routine quality control analysis of the drugs in combined pharmaceutical formulation.

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