DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD OF ACENOCOUMAROL IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A simple, accurate and precise UV spectrophotometric method has been developed for the quantitative estimation of Acenocoumarol in bulk and tablet dosage form. The λmax was found to be 283 nm. Beer's law was obeyed in the concentration range of 3-18 μg/ml. The regression equation was $y = 0.064 x + 0.013$ with value of R^2 as 0.996. The method showed good linearity, accuracy and reproducibility. Accuracy was determined using standard addition method and mean percent recovery was found to be 99.66%. Percent relative standard deviation values for the intra-day and inter-day precision were found to be 0.29 and 0.82 respectively. The limit of detection and limit of quantitation values were found to be 0.64 and 1.94 respectively. Assay of Acenocoumarol in tablet formulation was performed and percent purity of tablet was found to be 101.1%.

Keywords: Acenocoumarol, Anticoagulant, UV spectrophotometry, Validation.

INTRODUCTION

Acenocoumarol[1] (previously known as nicoumalone) is an oral anticoagulant that functions as a vitamin K antagonist. Chemically it is 4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-Coumarin (Fig.1). It is administered in the management of thromboembolic disorders.

Fig. 1: The structure of Acenocoumarol

Acenocoumarol is official in Indian[2] and British[3] pharmacopoeia. Both the pharmacopoeias explain the assay of acenocoumarol API by aqueous acid base titration while that of the tablet formulation by Spectrophotometric method (λmax at 306 nm and specific absorbance at 521). A few analytical methods have been reported for its quantitative estimation in pharmaceutical formulations, which include spectrophotometric[4] (involving formation of chromogens in presence of an oxidizing agent), HPLC[5-9], LC-MS[10] and GC-MS[11] methods. But to the best of our knowledge, a simple UV spectrophotometric method for estimation of Acenocoumarol for routine laboratory purpose is not yet reported. Hence, the authors have made an attempt to develop and validate a simple and economical UV spectrophotometric method for the estimation of Acenocoumarol in the bulk and in pharmaceutical formulations.

MATERIALS

A Shimadzu UV/VIS double beam spectrophotometer (model 1800) with 1 cm matched quartz cells was used for all spectral measurements. All the chemicals used were of analytical grade. The pharmaceutical grade Acenocoumarol was gifted by Century Pharmaceuticals Ltd, Vadodara. The tablet samples of Acenocoumarol (Acitrom, Abbott Healthcare Pvt. Ltd.) were procured from local market.

MATERIALS AND METHODS

Preparation of standard solution

100 mg of pure drug was weighed accurately and dissolved in 100 ml of ethanol (1000 μg/ml) with the help of sonication. This solution was further diluted with the same solvent to obtain a solution of concentration 100 μg/ml. This solution was scanned in the range of 400-200 nm against solvent ethanol as blank.

Preparation of sample solution for assay

20 tablets containing label claim of 4 mg were weighed accurately and average weight of tablet was determined. The tablets were triturated and the weight of the powder equivalent to 10 mg of acenocoumarol was dissolved in 70 ml of ethanol and sonicated for about 20 minutes. Volume was then made up to 100 ml using ethanol and filtered with the help of Whatman filter paper no. 41 to separate the insoluble matter.

Validation of the Developed Method

The developed method was validated[12] as per ICH guidelines for the following parameters.

Linearity and Range

The standard solutions were prepared by dilution of the stock solution with ethanol in the range of 3-18 μg/ml. The Absorbances were measured at 283 nm and plotted against the corresponding concentration to obtain the calibration graph (Fig.3).

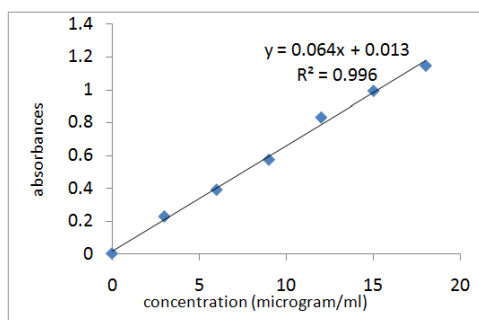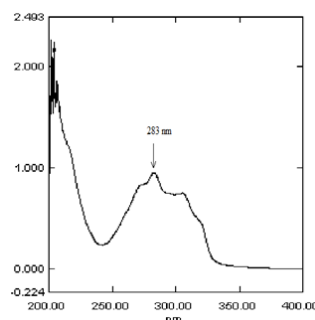**Fig. 2: UV spectrum of Acenocoumarol**

Fig. 3: Calibration graph of Acenocoumarol in ethanol

Precision

Intra and inter-day precision was performed by measuring the absorbance of standard solution at three different times during the single day and on three subsequent days respectively. The percentage relative standard deviation (% RSD) was calculated.

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

6 sets of known concentrations (3-18µg/ml) were prepared. Calibration curves were plotted for each set. LOD and LOQ were calculated using the formulae as LOD= 3.3 (SD)/S and LOQ= 10 (SD)/S, where S is average value of slopes of calibration plots and SD is calculated using values of y intercepts of regression equations.

Accuracy

Recovery studies were carried out by applying the method to drug sample to which known amount of Acenocoumarol corresponding to 80, 100, 120% of label claim was added (standard addition method).Total three determinations at each level were performed and the results were expressed as % RSD.

RESULTS AND DISCUSSION

The standard solution of acenocoumarol, when scanned in UV range, showed the λmax at 283 nm. Linear relationships between drug concentrations were obtained over the range of 3-18µg/ml. The regression equation was y = 0.064 χ + 0.013 with value of R^2 as 0.996. Assay of Acenocoumarol tablet formulation was successfully performed and the percentage purity of tablet was found to be 101.1%. No interference from the excipients of tablet formulation was found. Percent relative standard deviation values for the intra-day and inter-day precision were found to be 0.29 and 0.82 respectively. The limit of detection and limit of quantitation values were found to be 0.64µg/ml and 1.94µg/ml respectively. Results of the accuracy studies indicated good recovery of the drug. The % recovery values were found to be 99.8 %, 99.46 % and 99.72 % at 80, 100 and120 % levels. Table 1 shows summary of validation parameters.

Table 1: Summary of validation parameters

Parameter	Description
Linearity	Range- 3-18µg/ml
Intra-day precision	SD = 0.0026
	% RSD = 0.29
Inter-day precision	SD = 0.0074
	% RSD = 0.82
LOD	0.64 µg/ml
LOQ	1.94 µg/ml
Accuracy	Mean % Recovery = 99.60

CONCLUSION

A simple, accurate and precise UV Spectrophotometric method for estimation of acenocoumarol is developed. The method is suitable for routine estimation of acenocoumarol in pharmaceutical laboratories.

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