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Colorimetric Estimation of Acenocoumarol in Bulk and Pharmaceutical Formulations

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Two simple spectrophotometric methods for the analysis of acenocoumarol in pure form and in pharmaceutical formulation have been developed. **Method A** is based on diazotization of amino group of reduced acenocoumarol with nitrous acid followed by its coupling with N-(1-naphthyl)ethylenediamine-dihydrochloride (BMR) reagent in presence of ammonium sulphamate to form a blue colour chromogen and maximum absorbance at 565 nm. Beer's law is obeyed in the concentration range of 4-14 µg/mL. **Method B** is based on amino group of reduced acenocoumarol forms a condensation product with Gibb's reagent (2,6-dichloroquinone-4-chlorimide) and the maximum absorbance at 544 nm. Beer's law is obeyed in the concentration range of 4-20 µg/mL. The methods have been statistically evaluated and are found to be precise and accurate. The results obtained are reproducible with coefficient of variation less than 1 %.

Key Words: Acenocoumarol, Spectrophotometric, N-(1-naphthyl)-ethylene diaminedihydrochloride reagent, Gibb's reagent.

INTRODUCTION

Acenocoumarol[1] is chemically (RS)-4-hydroxy-3-(1,4-nitrophenyl-3-oxo-butyl)coumarin and used as anticoagulant agent for the management of thromboembolic disorders. It is official in Indian and British Pharmacopoeia[1,2]. A few analytical methods have been reported for its quantitative estimation in pharmaceutical formulations, which include UV method[2], biological fluid using HPLC[3-6], LC-MS[7] and GC-MS[8] methods. In view of the above fact, some simple analytical methods are in need for its quantitative estimation. The objectives of present work were to develop simple visible spectrophotometric methods that can be used for the routine analysis of the formulations containing the drug. The aromatic nitro group present in acenocoumarol was reduced to amine group using zinc dust and hydrochloric acid before treating the N-(1-naphthyl)ethylene diaminedihydrochloride reagent (BMR) and Gibb's reagent.

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EXPERIMENTAL

A Shimadzu model 1700 double beam UV-Visible spectrophotometer with a pair of 1 cm matched quartz cells was used to measure absorbance of the resulting solutions. Gift sample of acenocoumarol was obtained from Sarabhai Piramal Pvt. Ltd, Baroda. All chemicals used are of analytical grade from S.D. Fine Chemical, Mumbai, hydrochloric acid (0.5 M in distilled water), $NaNO_2$ (0.1 % w/v in distilled water), ammonium sulphamate (0.2 % w/v in distilled water), BMR (0.2 % w/v in distilled water), Gibb's reagent (0.5 % w/v in distilled water).

Standard and sample solution of acenocoumarol: About 100 mg of acenocoumarol (bulk or formulation) bulk was weighed accurately, dissolved in 20 mL of methanol in a 100 mL volumetric flask, reduce the drug solution using 1.2 g zinc and 10 mL of hydrochloric acid (4 N). After standing for 1 h to room temperature the solution was filtered through cotton wool and the residue was washed with 0.1 M sodium hydroxide solution and make up 100 mL (1 mg/mL). The final concentration of drug was brought to 100 µg/mL with 0.1 M sodium hydroxide solution.

Assay procedure: Method A: Aliquots of reduced acenocoumarol ranging from 0.4-1.4 mL (1 mL = 100 µg) were transferred into a series of 10 mL volumetric flasks. After 10 min, to each flask 0.5 mL of hydrochloric acid (0.5 M), 1 mL of sodium nitrite, 1 mL of ammonium sulphamate (0.2 %), 1.5 mL of BMR reagent (0.2 %). After 15 min, make up the volume to 10 mL using distilled water. The absorbance of the blue coloured chromogen was measured at 565 nm against reagent blank. The coloured species was stable for more than 1 h. The amount of acenocoumarol present in the sample was computed from calibration curve.

Method B: Aliquots of acenocoumarol ranging from 0.4-2.0 mL (1 mL = 1000 µg) were transferred into a series of 10 mL volumetric flasks. To each flask 0.5 mL of Gibb's reagent (0.3 %) and 1 mL of sodium nitrite were added and set a side for 10 min for complete colour development. The volumes were made up to the mark with distilled water. The absorbance of the purple coloured chromogen was measured at 544 nm against reagent blank. The coloured species was stable for more than 2 h.

RESULTS AND DISCUSSION

The proposed methods were based on the reaction of reduced acenocoumarol with nitrous acid followed by reaction with ammonium sulphamate and finally coupling with BMR reagent to give blue colour in **method A** and with Gibb's reagent to give purple colour in alkaline medium in **method B**. The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity are presented in

Table-1. The regression analysis using method of least squares was made for the slope (b), intercept (a) and correlation (r) obtained from different concentrations and the results are summarized in Table-1. The percent relative standard deviation and range of error (0.05 and 0.01 level of confidence limits) calculated from the eight measurements, three-fourth of the upper Beer's law limits of acenocoumarol are given in Table-1. The results showed that these methods have reasonable precision.

TABLE-1
OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY OF PROPOSED METHOD

Parameter	**Method A**	**Method B**
λ_{max} (nm)	565	544
Beer's law limit (μg/mL) (C)	4-14	4-20
Molar absorptivity (L mol^{-1} cm^{-1})	2.5705×10^4	2.0053×10^4
Sandell's sensitivity (μg/cm^2 0.001absorption units)	0.0137	0.0173
Coefficient of correlation	0.9999	0.9999
Regression equation (Y*)	Y = 0.0736x + 0.0057	Y = 0.0577x + 0.0077
Intercept (a)	0.0057	0.0077
Slope (b)	0.0736	0.0557
% RSD**	0.361	0.337
Range of errors		
Confidence limits with 0.05 level	0.001759	0.001922
Confidence limits with 0.01 level	0.002603	0.002844

*Y = bC + a; where C is the concentration of acenocoumarol in μg/mL and Y is the absorbance. **For eight measurements.

The optimum conditions for colour development for **method A** and **B**, have been established by varying the parameters one at a time and keeping the other parameters fixed and observing the effects of product on the absorbance of the coloured species and incorporated in the procedures. To evaluate the validity and accuracy of the methods, known amounts of pure drug were added to the previously analyzed pharmaceutical preparations and the mixtures were analyzed by the proposed methods. The percent recoveries are given in Table-2. Interference studies revealed that the additives like antioxidants, preservatives and solubilizers that are usually present in tablets did not interfere at their regularly added levels.

TABLE-2
ESTIMATION OF ACENOCOUMAROL IN PHARMACEUTICAL FORMULATIONS

Formulations	Labelled amount (mg)	Recovery by proposed method (%)	
		Method A	**Method B**
Tablet 1	1	99.89	99.95
Tablet 2	4	99.93	99.94

Thus, the proposed methods are simple and sensitive with reasonable precision and accuracy. These can be used for the routine determination of acenocoumarol in quality control analysis.

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