SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF ACENOCOUMAROL IN BULK AND ITS PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Four simple, sensitive, accurate and precise visible spectrophotometric methods (A, B, C and D) have been developed for the quantitative estimation of acenocoumarol in bulk drug and pharmaceutical formulations (tablets). Method A is based on the reaction of reduced acenocoumarol with aromatic aldehyde, paradimethylamino cinnamaldehyde (PDACA) producing colored Schiff's bases at the λ_{max} 527.5 nm and. Method B is based on the reaction of reduced acenocoumarol with aromatic aldehyde, paradimethylaminobenzaldehyde (PDAB) in acidic condition producing colored Schiff's bases having λ_{max} at 441.5 nm. Method C is based on the oxidation followed by coupling reaction of reduced acenocoumarol with 3-methyl-2-benzothiazolinone hydrazone (MBTH) in presence of ceric ammonium sulphate to form green colored chromogens at λ_{max} 585.0 nm. Method D is based on the reaction of reduced acenocoumarol with Folin's (1, 2-naphthoquinone-4-sulphonate) reagent to form brown colored chromogen with λ_{max} at 480.0 nm. Beer's law is obeyed in the concentration range of 2-10 µg/mL for Method A, 5-25 µg/mL for method B, 20-100 µg/mL for Method C and 2-12 µg/mL for Method D. The reduction of acenocoumarol is carried out with zinc dust and hydrochloric acid at room temperature in methanol. The results obtained with proposed methods are in good agreement with labeled amounts when marketed pharmaceutical formulations are analyzed. The results of analysis have been validated statistically and by recovery studies

Key words: Acenocoumarol, Spectrophotometric, PDACA, PDAB, MBTH, Folin's reagent.

INTRODUCTION

Acenocoumarol[1] is chemically (RS)-4-hydroxy-3-(1,4-nitrophenyl-3-oxobutyl)

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coumarin and used as anticoagulant agent for the management of thromboembolic disorders. It is official in Indian and British Pharmacopoeia. 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 required for its quantitative estimation.

Four simple and sensitive visible spectrophotometric methods (A, B, C and D) have been developed for the quantitative estimation of acenocoumarol after converting it into its reduced form (RS)-4-hydroxy-3-(1,4-aminophenyl-3-oxobutyl) coumarin**.**

Method A is based on the reaction of reduced acenocoumarol with aromatic aldehyde, paradimethylamino cinnamaldehyde (PDACA) producing colored Schiff's bases at the λ_{max} 527.5 nm and Beer's law is obeyed in the concentration range of 2-10 µg/mL - Method B is based on the reaction of reduced acenocoumarol with aromatic aldehyde, paradimethylaminobenzaldehyde (PDAB) in acidic condition producing colored Schiff's bases having λ_{max} at 441.5 nm and Beer's law is obeyed in the concentration range 5-25 µg/mL. Method C is based on the oxidation followed by coupling reaction of reduced acenocoumarol with 3-methyl-2-benzothiazolinone hydrazone (MBTH) in presence of ceric ammonium sulphate to form green colored chromogens at λ_{max} 585.0 nm and Beer's law is obeyed in the concentration range 20-100 µg/mL. Method D is based on the reaction of reduced acenocoumarol with Folin's (1, 2-naphthoquinone-4-sulphonate) reagent to form brown colored chromogens with λ_{max} at 480.0 nm and Beer's law is obeyed in the concentration range 2-12 µg/mL. Spectrophotometric parameters are established for the standardization of the methods including statistical analysis of data. These methods have been successfully extended to the pharmaceutical dosage forms (tablets) containing acenocoumarol.

EXPERIMENTAL

Instruments

A Shimadzu model 1700 double beam UV/VIS spectrophotometer with 1 cm matched quartz cells was used to measure absorbance of the resulting solutions.

Chemicals and reagents

All chemicals used were of analytical grade and obtained from s.d. fine-chem., Mumbai.

   (i)   Double distilled water,

  (ii)   PDACA (prepared by adding 30 mL of 95% ethanol, 180 mL of 1-butanol and 30 mL of HCl to 1 g of p-dimethyl amino cinnamaldehyde,

 (iii)   Methanolic PDAB (0.5% w/v in methanol),

 (iv)   Methanol,

  (v)   Hydrochloric acid (0.1 M in water),

 (vi)   3-Methyl-2- benzothiazolinone hydrazone (MBTH) (0.2% in water),

(vii)   Sulphuric acid (1 M in water),

viii)   Ceric ammonium sulphate (1% w/v in 1 M H_2SO_4),

 (ix)   Folin's reagent (1% w/v in water).

Bulk drug of acenocoumarol was obtained from Sarabhai Piramal Pvt. Ltd., Baroda. Commercial tablets of acenocoumarol were procured from local market.

Preparation of standard drug solution

100 mg of bulk drug was weighed accurately, dissolved in 20 mL of methanol in a 100 mL volumetric flask and the drug solution was reduced by using 1.2 g zinc and 10 mL of 4 N HCl. After standing for 1 h at room temperature, the solution was filtered through cotton wool and the residue was washed with 0.1 M sodium hydroxide solution and made up 100 mL (1 mg/mL). The final concentration of drug was brought to 100 μg/mL with 0.1 M sodium hydroxide solution.

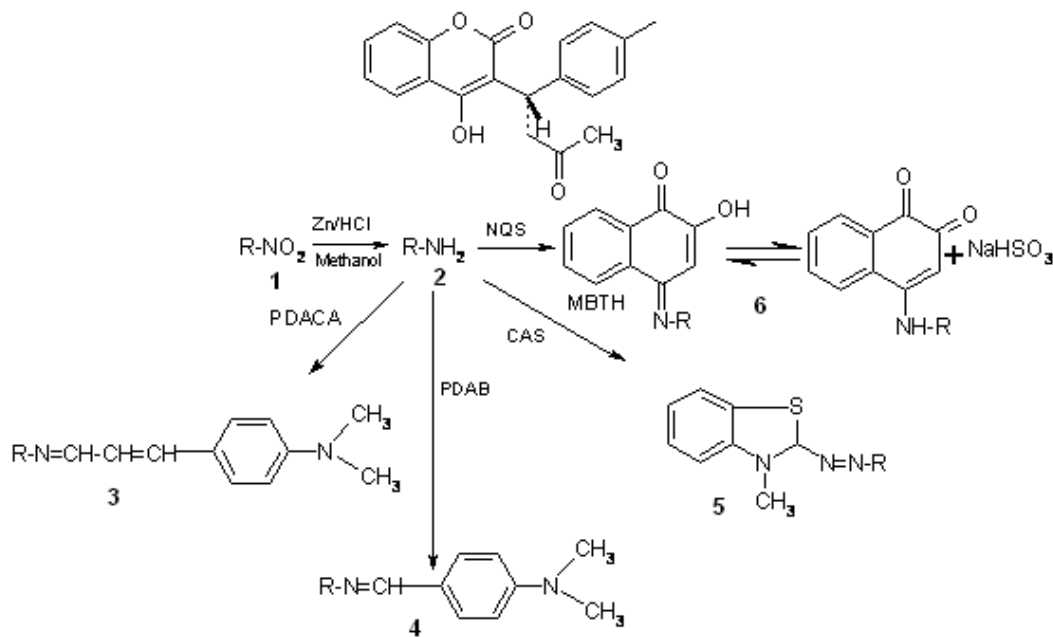## Preparation of sample drug solution

For sample solution, tablets of acenocoumarol were accurately weighed and average weight per tablet was determined. The tablets were powdered and powders equivalent to 100 mg of drug was taken into a 100 mL volumetric flask and solutions were prepare by the same method as for standard drug solution.

Assay

Method A

Aliquots of reduced acenocoumarol ranging from 0.2-1.0 mL (1 mL = 100 μg/mL) were transferred into a series of 10 mL volumetric flasks. To each flask, 1.0 mL of alcoholic PDACA were added and after 15 min. at room temperature, the volumes were

made up to the mark with methanol. The absorbance of the red colored chromogen was measured at 527.5 nm against reagent blank. The amount of acenocoumarol present in the sample was computed from calibration curve.

Scheme 1

Method B

Aliquots of reduced acenocoumarol ranging from 0.5-2.5 mL (1 mL = 100 µg/mL) were transferred into a series of 10 mL volumetric flasks. To each flask 2.0 mL of methanolic PDAB and 1.5 mL of HCl were added. After 15 min. at room temperature, the colour development was observed. The volumes were made up to the mark with methanol. The absorbance of the yellow colored chromogen was measured at 441.5 nm against reagent blank. The amount of acenocoumarol present in the sample was computed from calibration curve.

Method C

Aliquots of acenocoumarol ranging from 0.2-1.0 mL (1 mL = 1000 µg/mL) were transferred into a series of 10 mL volumetric flasks. To each flask 1.0 mL of ceric ammonium sulphate and 1.0 mL of MBTH were added and flasks were set aside for 15

min. for complete color development. The volumes were made up to the mark with water. The absorbance of the green colored chromogen was measured at 585.0 nm against reagent blank. The amount of acenocoumarol present in the sample was computed from calibration curve.

Method D

Aliquots of reduced acenocoumarol ranging from 0.2-1.2 mL (1 mL = 100 µg/mL) were transferred into a series of 10 mL volumetric flasks. To each flask 0.5 mL of Folin's reagent (1, 2-naphthoquinone-4-sulphonate) was added and kept at a room temperature for 20 min and made up to volume with distilled water. The absorbance of the red colored chromogen was measured at 480.0 nm against reagent blank. The amount of acenocoumarol present in the sample was computed from calibration curve.

RESULTS AND DISCUSSION

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, ¾ of the upper Beer's law limits of acenocoumarol are also given in Table 1.

Table 1. Optical characteristics and precision of the proposed method

	Method A	Method B	Method C	Method D
λ_{max} (nm)	527.5	441.5	585	480
Beer's law limits (µg/mL)	4-20	5-25	20-100	2-12
Molar absorptivity (L/mol/cm)	4.2815×10^4	1.5547×10^4	3.716×10^3	3.3568×10^4
Sandell's sensitivity (µg/cm^2 0.001 absorbance unit)	0.00851	0.02173	0.09174	0.002577
Regression equation (Y* = a + bC)				
Slope (b)	0.1191	0.0433	0.0103	0.0932
Intercept (a)	0.0036	0.0032	0.0126	0.0062
Correlation coefficient (r)	0.9997	0.9998	0.9998	0.9995

	Method A	Method B	Method C	Method D
% RSD **	0.3448	0.3755	0.3225	0.2982
Range of errors**				
Confidence limits with 0.05 level	0.002096	0.002072	0.001701	0.001895
Confidence limits with 0.01 level	0.003101	0.003065	0.002517	0.002804

*Y= bC + a where C is the concentration of acenocoumarol in µg/mL and Y is the absorbance at the respective $\lambda_{max.}$

** For eight measurements

The optimum conditions for color development for method A, B, C and D 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 colored 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 solubilisers that are usually present in parenterals did not interfere at their regularly added levels.

Table 2. Evaluation of acenocoumarol in pharmaceutical preparation

Formulation*	Labeled amount (mg/tab)	Amount obtained by proposed method		Percentage recovery**± s.d
Tablet (Acitrom)	4	A	3.91	99.86 ± 0.024
		B	3.90	99.92 ± 0.025
		C	3.88	99.91 ± 0.031
		D	3.95	99. 89 ± 0.020

* Tablets from Sarabhai Piramal Pvt. Ltd., Baroda.

** Average ± s.d. of eight determinations

CONCLUSION

The proposed methods are applicable for the assay of drug (acenocoumarol) and

have the advantage of wider range under Beer's law limits. The decreasing order of sensitivity and λ_{max} among proposed methods are D > A > B > C and C > A > D > B, respectively, so the proposed visible spectrophotometric methods are found to be simple, sensitive, selective, accurate, precise, and economical and can be used in the determination of acenocoumarol in bulk drug and its pharmaceutical preparations in a routine manner.

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