PHARMA SCIENCE MONITOR
AN INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES

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

Krupa D. Shah*, Ujjwal Sahoo, Ashim kumar Sen, Dhanya B Sen, Arti Zanwar and A.K. Seth

Department of pharmacy Sumandeep Vidyapeeth University, Piparia, Waghodia, Vadodara-391760, Gujarat, India.

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 291 nm. Beer's law was obeyed in the concentration range of 1-21 µg/ml. The regression equation was y = 0.0541 x + 0.1014 with value of R^2 as 0.9994. The method showed good linearity, accuracy, LOD, LOQ and reproducibility. The result of analysis has been validated statistically. Hence the proposed method can be used for the reliable Quantification of Acenocumarol in tablet formulation.

Keywords: Acenocoumarol, Anticoagulant, UV spectrophotometer, 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.

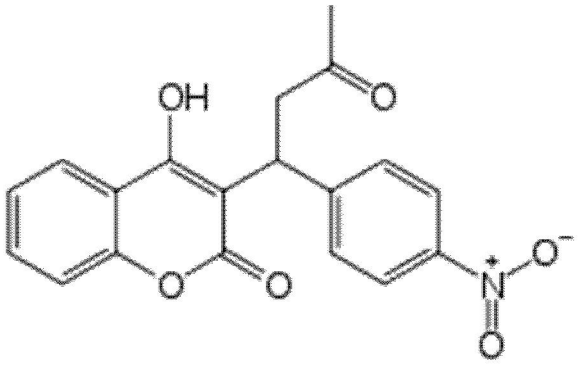

Fig. 1: the structure of Acenocoumarol

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.

EXPERIMENTAL SECTION

Material and Method:

UV method was developed for Acenocumarol in bulk and pharmaceutical formulation in 0.1N NaoH.

Reagent and Chemical:

Acenocumarol: - Pure drug and 0.1N NaoH as solve

Stock Solution:

Preparation of standard solution:-

100 mg of pure drug was weighed accurately and dissolved in 100 ml of 0.1N NaoH (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.

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 0.1N NaoH and sonicated for about 10 minutes. Volume was then made up to 100 ml using 0.1N NaoH and filtered with the help of what man filter paper to separate the insoluble matter. From the filtrate take1.0 ml of the filtrate and dilute up to 10ml with the same solvent. And measured the absorbance at 291nm with the help of the UV Spectroscopic method.

Instrument:

All the experiments were carried out on SIMADZU UV-VIS 1700 spectrophotometer using 1cm matched quartz cuvettes.

Determination of λmax.

An absorption maximum or λmax is the wavelength at which maximum absorption takes place.

1. **λmax of Acenocoumarol in 0.1 N NaoH:**

The solution of Acenocumarol were suitably diluted with 0.1N NaoH and subjected for determination of λmax given in figure 2.

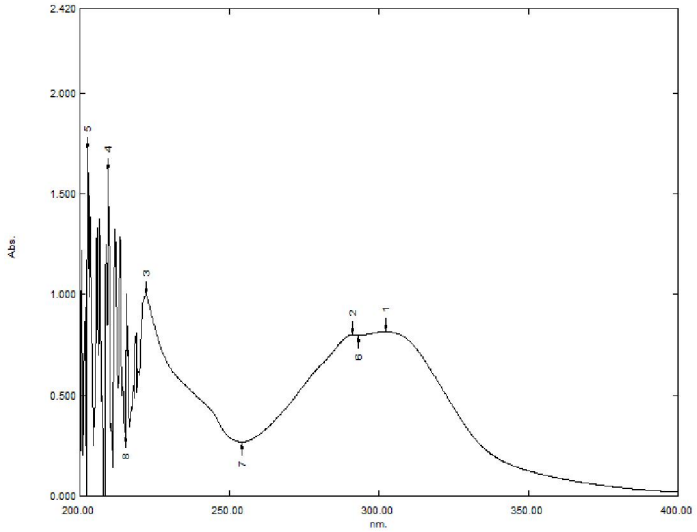

Figure 2: Acenocoumarol with diluents

Validation of the Developed Method

The developed method was validated 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 1-21 µg/ml. The Absorbances were measured at 291 nm and plotted against the corresponding concentration to obtain the calibration graph (Fig.3).

Calibration curve of samples:

From the working standard 0.3, 0.6, 0.9, 1.2 and 1.5 ml of drug solution were placed in 5different 10ml volumetric flasks and volume was made up to the mark with 0.1N NaoH and their absorbance was measured against corresponding reagent blank at 291nm and result are recorded in table no. 1 and figure no. 3.

TABLE 1: ABSORBANCE OF DIFFERENT CONC. OF ACENOCUMAROL OBEYING BEER'S LAW

Sr. No	Concentration of drug taken (100µg/ml)	Concentration Range (µg/ml) in 10 ml	Absorbance At 291nm
1	0.3 ml	3 µg	0.260
2	0.6 ml	6 µg	0.425
3	0.9 ml	9 µg	0.599
4	1.2 ml	12 µg	0.745
5	1.5 ml	15 µg	0.911

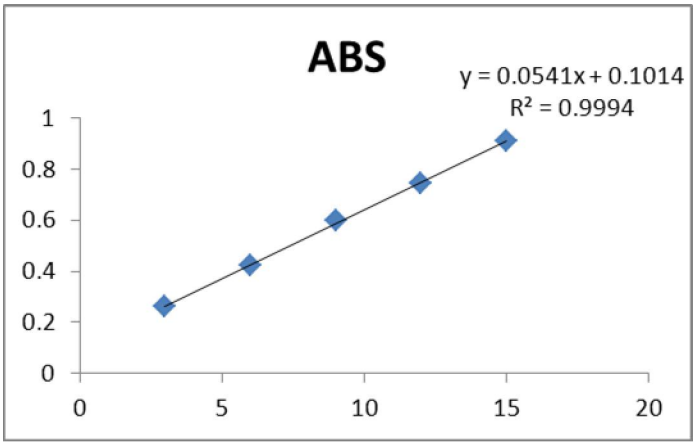

Fig. 3: Calibration graph of Acenocoumarol in 0.1N NaoH

Precision

Method precision was established by analyzing six separate samples at 100% of the Working concentration. Percent of result was calculated against claimed label. The % RSD of assay result of six separate from a single batch was found to be 0.138% (limit NMT2.0%) which indicates that the method is precise to analyze the tablet

TABLE 2: PRECISION DATA FOR ACENOCUMAROL AT 291 NM

No	Parameter (n=6)	Acenocumarol
1	Mean	0.589
2	Standard deviation	0.000816
3	%RSD	0.138%

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

6 sets of known concentrations (1-21µg/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.

TABLE: 3 LOD & LOQ

No	Parameter	Acenocumarol
1	LOD	0.049
2	LOQ	0.151

Accuracy

Recovery studies were carried out by applying the method to drug sample to which known amount of Acenocoumarol corresponding to 50, 100, 150% of label claim was added (standard addition method).Total three determinations at each level were performed and the results were expressed as % RSD. The percent recovery at each level should be within 98.0% to 102.0%. The percent recovery was calculated for nine determinations and found to be within limit. Thus it has been concluded that the method was accurate for analyze the tablet.

TABLE: 4 DETERMINATION OF ACCURACY

Level of Recovery	Amt of drug added (mg)	Amt of drug recovered (mg)	%Recovery	Mean (%)	%RSD
0	0	0	-	-	-
	10	10.05	100.6		
50%	10	10.05	100.6	100.4	0.287
	10	10.01	100.1		
	15	15.03	100.2		
100%	15	15.03	100.2	100.08	0.196
	15	14.98	99.86		
	20	20.03	100.15		
150%	20	19.99	99.95	100.08	0.115
	20	20.03	100.15		

Robustness:

Robustness of this method was determined by analyzing the Acenocumarol tablet in different Equipment in different day and by different analyst. From the above mentioned data it observed that the method is robust enough to analyse Acenocumarol tablet.

TABLE: 5 DATA FOR ROBUSTNESS TEST

Sr No.	Variable Parameter	Assay Result
1	Analyst – 1 Analyst – 2	99.6% 100.46%
2	Day – 1 Day – 2	99.73% 100.25%

RESULT & DISCUSSION

In the method Acenocumarol was estimated by using ultraviolet spectroscopic method. The method obeys beer's lamberts law in the concentration range of 1-21 µg/ml and its wavelength of detection was 291nm. Finally recovery studies were undertaken. The quantitative parameters for determination of Acenocumarol in bulk and pharmaceutical dosage form are listed in table 6.

TABLE 6: THE QUANTITATIVE PARAMETERS FOR DETERMINATION OF ACENOCUMAROL

Parameter	Result
λmax (nm)	291
Beer's law limits (µg/ml)	1-21
Regression equation (y=bc+a) Slope (b) Intercept (a)	Y=0.0541x+0.1014 0.0541 0.1014
Correlation coefficient (r)	0.9994
Accuracy (% recovery)	100.4-100.8
Precision (%)	0.138%
LOD (µg/ml)	0.049
LOQ (µg/ml)	0.151
%Drug found in tablet formulation	99.6

CONCLUSION

From the above data it was observed that all validation parameter (like system suitability, method precision, accuracy, specificity, linearity, robustness, LOD, LOQ) meet the predetermined acceptance criteria. Thus it has been concluded that the method is validated for the analysis of assay of Acenocumarol in tablet

ACKNOWLEDGEMENT

Authors thank Century Pharmaceuticals Ltd., Vadodara for providing gift sample of Acenocoumarol. We acknowledge the facilities provided by the Prof. Mr. Ujjwal Sahoo, Mr. Ashim kumar Sen, Mrs. Arti Zanwar, and Principal Dr. A. K. Seth, Sumandeep Vidhthyapeeth University, piparia, Vadodara to carry out the research work.

REFERENCES

1. Supriya NM, Madhuri AN, Sughandha VM. Development and Validation of Spectrophotometer method of Acenocumarol in Bulk and Tablet dosage form. Int J Pharma Pharma Sci 2012; 4(4):288-289
2. Indian Pharmacopoeia. Vol. III. New Delhi: Controller of Publication. 2007; 826-27.
3. British Pharmacopoeia. Vol. I. United Kingdom: publication of MHRA. 2007; 43-44.
4. Ankita SM, Gurupadayya BM, Gopinath B, Manohara YN and Akhilesh Chandra. Spectrophotometric Estimation of Acenocoumarol in Tablets. Indian J. Pharm. Educ. Res. 2008; 42(4): 310-13
5. Rentsch KM, Gutteckamseler U, Buhrer R. Sensitive stereo specific determination of Acenocoumarol and Phenprocoumon in plasma by High performance liquid chromatography. J. Chromatogr. B. Biomed. Sci. 2000; 742: 131- 42.
6. Deorsi D, Gagliardi L, Turchetto L. HPLC determination of Warfarin and Acenocoumarol in raw material and pharmaceuticals. J. Chromatogr. Sci. 1998; 17: 891-95.
7. Devries JX, Schmitz-kummer E. Direct column liquid chromatographic enatiomer separation of the Coumarin anticoagulants Phenprocoumaron, Warfarin, Acenocoumarol and metabolites on a α-1 acid glycoprotein chiral stationary phase. J. Chromatogr. Sci. 1993; 644: 315-20.
8. Wong LT, Solomonraj G. Quantitation of Acenocoumarol in plasma by reverse phase High performance liquid chromatography. J. Chromatogr. B. Biomed. Sci. 1979; 163: 103-8.

9. Ceresole R, Rosasco MA, Forastieri CC, Segall AI. HPLC Determination of Acenocoumarol and its Major Thermal Degradation Product. J. Liq. Chromatogr. & Rel. Tech. 2008; 31: 179–87
10. Devriesic JX, Kyumber KA. Thermospray and particle beam liquid chromatographic-mass spectrometric analysis of Coumarin anticoagulants. J. Chromatogr. Biomed. 1991; 562: 31-38.
11. Maurer HH, Arlt JW. Detection of 4- hydroxyl coumarin anticoagulants and their metabolites in urine as part of a systemic toxicological analysis procedure for acidic drugs and poisons by gas chromatography – mass spectrometry after extractive methylation. J. Chromatogr. B. Biomed. Sci. 1998; 714: 181-95.
12. Ravichandran V, Shalini S, Sundram KM and Harish Rajak. Validation of analytical methods- strategies and importance. Int. J pharma Pharma Sci 2010; 2(3):18-22.

For Correspondence:
Krupa Shah
Email: shahkrupa26@yahoo.in