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QUANTIFICATION OF WARFARIN SODIUM CLATHRATE USING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY FROM BULK DRUG AND PHARMACEUTICAL DOSAGE

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ABSTRACT

This paper describes a simple, accurate and precise HPTLC method for determination and validation of Warfarin Sodium Clathrate in tablet dosage form. During optimization of Mobile phase different solvents viz. n-Hexane, methanol and acetic acid were tried, however peak shape of Warfarin Sodium Clathrate was not proper. A mobile phase of Toluene : Acetone : Methanol (7 : 2 : 1 v/v) was developed for the densitometric determination of the drug product. Aluminum plate coated with the Silica Gel 60 F254 was used as stationary phase. UV spectra were taken in the range of 200 nm to 400 nm. Densitometric evaluation of the separated band of Warfarin Sodium Clathrate was performed at 306 nm. The Rf value of Warfarin Sodium Clathrate was 0.65. Developed method was linear over the range of 12.50 µg/ml to 37.50 µg/ml. Precision of the method was evaluated by calculating RSD for peak response by inter-day and intraday analysis. Accuracy was determined in terms of percentage recovery at three concentration levels for Warfarin Sodium Clathrate. The results for Warfarin Sodium Clathrate at three concentration levels are 101.00%, 101.10% and 100.60%. There was no any interference of methanol being used as a diluent and placebo at the Rf values of Warfarin Sodium Clathrate. Standard spectrum and sample spectrum of Warfarin Sodium Clathrate were overlain to confirm Rf values. ICH guidelines were referred for validation of an optimized method.

Keywords:

Warfarin Sodium Clathrate, Anticoagulants, High Performance thin layer chromatography, tablet dosage.

INTRODUCTION

Warfarin Sodium Clathrate belongs to the class of medications called anticoagulants commonly known as blood thinners. Vitamin K antagonist, is used for the prevention and treatment of venous thrombosis and its extension and the prevention and treatment of the thromboembolic complications associated with atrial fibrillation[1] . The most widely used drugs are warfarin sodium and acenocoumarol[2]. Warfarin **Fig.1** is chemically 4-hydroxy-3-(3-oxo-1-phenylbutyl)chromen-2-one with molecular formula of C19H16O4 has also been used to prevent recurrent transient ischemic attacks and to reduce the risk of recurrent myocardial infarction[1] . Vitamin K antagonists exert their anticoagulant effect by interfering with the cyclic conversion of vitamin K, thus, inhibiting the carboxylation of glutamate residues to gamma-carboxyglutamate at the N-terminal regions of the vitamin K-dependent coagulation factors (FIX, FVII, FX and FII) and inhibitors (protein C and protein S). The process of gamma-carboxylation is mediated by carboxylase with vitamin K functioning as co-factor. As a result of the antagonizing effect of oral anticoagulants, vitamin K- dependent coagulation factors are synthesized as a-carboxy forms, which do not bind phospholipid surfaces leading to defective coagulation. The dose – response effect of vitamin K antagonists is highly variable and their anticoagulant dosage must be closely monitored to prevent over- or under anticoagulation. The effect of warfarin in patients on oral anticoagulants is usually assessed by the prothrombin time test with results expressed as International Normalized Ratio (INR)[2].

From the literature review many analytical methods3,4,5,7,8 have been reported for the determination of Warfarin Sodium such as spectrophotometry, HPLC. There is no reported HPTLC method for the determination of Warfarin Sodium in tablet dosage form. The objective of this work is report a simple, precise, accurate and cost effective HPTLC method for estimation of Warfarin Sodium is quantified at 306 nm.

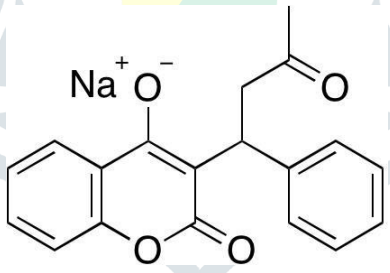

Figure 1 Chemical structure of Warfarin Sodium

MATERIALS & METHODS

Chemical, Reagents and Solutions

IP standard of Warfarin Sodium Clathrate was purchased from Indian Pharmacopoeia Commission (Ghaziabad, Uttar Pradesh). Fixed dose tablet WARF-5 (Cipla) containing 5 mg Warfarin Sodium Clathrate was purchased from local market, Miraroad (Mumbai), Maharashtra, India. Methanol (HPLC grade), Toluene, Acetone used were AR grade, purchased from Merck Chemicals. All dilutions were performed in standard volumetric flask.

Standard Preparation

Stock standard solution (0.25 mg/ml) of warfarin sodium clathrate was prepared by accurately weighing 25.00 mg of IP reference standard into 100 ml volumetric flask. About 60 ml of diluent was added to the flask, sonicated for 15 minutes, cool to room temperature and make up volume with diluent.

Reference standard solution (25µg/ml) of warfarin sodium clathrate was prepared by accurately transferring 5.0 ml of the stock solution into 50 ml volumetric flask and diluted to volume with diluent.

Sample Preparation

Twenty tablets were taken and average weight was calculated. The content of warfarin sodium clathrate was weighed equivalent to 5 mg and it was taken into the 100 ml volumetric flask. About 60 ml of methanol was added to the flask, sonicated for 20 minutes with intermittent shaking, cool to room temperature and made up the volume with diluent.

Filtered the above sample solution through 0.45µ PVDF. Sample solution (25 µg/ml) of warfarin sodium clathrate was prepared by accurately transferring 5.0 ml filtered solution into 10 ml volumetric flask and diluted to volume with diluent.

Instrumentation and Chromatographic Conditions

Chromatography was performed on 10 cm × 10 cm and 20 cm × 10 cm Aluminium-backed TLC plates coated with silica gel 60 F254 from Merck. Before use the plates were washed with methanol and activated at 120°C for 20 min. Reference standard and sample solutions (20 µl) were applied to the plates as bands 8 mm long, 10 mm from the bottom, and 11.9 mm apart by CAMAG Linomat 5 sample applicator. Linear ascending development with Toluene : Acetone : Methanol (7 : 2 : 1 v/v) as mobile phase was performed in previously saturated twin trough chromatographic chambers (10 cm × 10 cm and 20 cm × 10 cm; CAMAG). Migration distance was 80 mm (development time 15 min). Densitometric scanning with a TLC Scanner 4 equipped with vision CATS software was performed at 306 nm.

RESULTS AND DISCUSSION

Validated an efficient method for analysis of warfarin sodium clathrate, preliminary tests were performed with the objective of selecting appropriate optimum conditions. Conditions such as detection wavelength, ideal mobile phase components and their proportions, and the concentration of standard solutions were exhaustively studied.

The mobile phase was selected on the basis of polarity. A mobile phase was selected that would give dense and compact bands with appropriate Rf value **Fig.2** and good peak symmetry **Fig.3**. UV spectra were taken in the range of 200 nm to 400 nm and 306 nm was chosen as working wavelength **Fig.4**. Complete resolution of the peaks with clear baseline separation was achieved.

Figure 2 Chromplate of warfarin sodium clathrate.

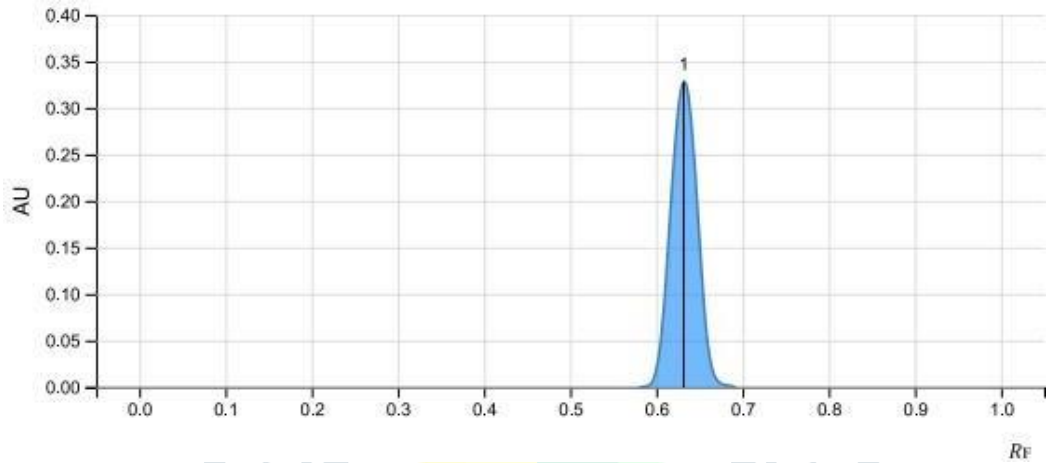

Figure 3 Chromatogram of warfarin sodium clathrate standard.

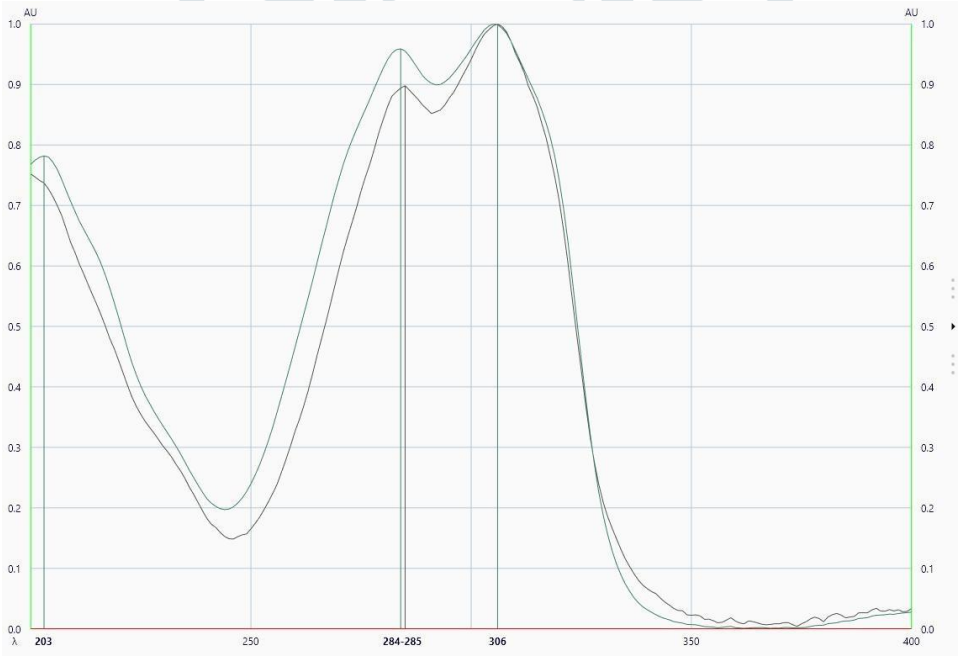

Figure 4 Overlay Spectra of Standard and Sample.

Precision: The precision of the method was checked by repeatedly (n = 6) injecting 25.00 µg/ml sample solutions of warfarin sodium clathrate and the percentage RSD were calculated. From the data obtained, the developed HPTLC method was found to be precise.

Limit of Detection and Limit of Quantification: The limit of detection and the limit of quantification of the drug was calculated using the following equations as per ICH guidelines[6].

LOD =
3.3 x σ x
S LOQ
= 10 x σ
x S

Where σ = the standard deviation of the response,
S = the standard deviation of y-intercept of regression lines.

LOD and LOQ for warfarin sodium clathrate were found to be 1 µg/ml and 3 µg/ml respectively. These data show that the method is sensitive for the determination of warfarin sodium clathrate.

Linearity: Calibration curves were constructed by plotting peak areas versus concentrations of warfarin sodium clathrate and the regression equations were calculated. Accurately measured standard stock solution of warfarin sodium clathrate (0.5, 0.8, 0.9, 1.0, 1.1, 1.2, 1.5 ml) were transferred in a series of 10 ml volumetric flasks and diluted to the mark with diluent. Of each solution, 20 µl was injected under operating chromatographic conditions. Each solution was applied three times. A calibration curve was plotted over a concentration range 12.50 – 37.50 µg/ml. The mean regression equation of calibration plots relating the warfarin sodium standard peak-area ratio to warfarin sodium concentration was y = 0.0004x
+ 0.002, correlation coefficient 0.9997 **Fig.5**.

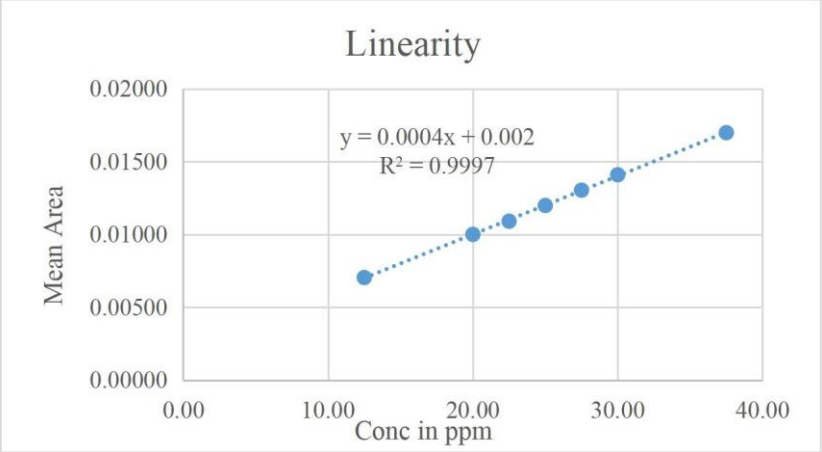

Figure 5 Linearity of warfarin sodium clathrate.

Accuracy: The recovery experiment was carried out by a standard addition method. Accuracy, as relative standard deviation (RSD), were calculated for low (L) (12.50 µg/ml), medium (M) (25.00 µg/ml), and high (H) (37.50 µg/ml) quality-control levels for three replicates each in the same analytical run, with three bands of each solution. Accuracy was calculated from the test results as the amount [%] of the analyte recovered by the assay. Accuracy was between 98.00 % to 102.00 % (as percentage recovery). RSD for accuracy did not exceed 2.0 % at any level. Table 1

Robustness: The robustness of the method was demonstrated by variation in injection volume and variation in scanning wavelength. % assay for working standard and sample solution were calculated for the injection volume of 18 µl and 22 µl and at 304 nm and 308 nm, which was found to be in the prescribed range.

Filter Paper Study: The filter interference was studied by calculating % assay of the sample filtered using 0.45µ Nylon filter against centrifuged sample. % assay was found to be 98.8 % and 99.7 for centrifuged sample and 0.45µ Nylon filtered sample respectively. The filter saturation study was done for initial sample filtrate and after discarding initial 1 ml, 2 ml and 3 ml of the filtrate. % assay was found to be well within the limit for initial sample filtrate and after discarding initial 1 ml, 2 ml and 3 ml of the filtrate.

Table 1
Summary of validation parameters for the proposed HPTLC method

Parameter	**Warfarin sodium clathrate**
LOD	1 µg/ml
LOQ	3 µg/ml
Precision:	
1) Inter Day	100.4 %
2) Intra Day	99.6 %
% Accuracy	
1) Low Level	101.00 %
2) Medium Level	101.10 %
3) High Level	100.60 %

CONCLUSION

The results of the analysis of pharmaceutical dosage form by the proposed method is highly reproducible, reliable, and are in good agreement with the label claim of the drug. The additives usually present in the pharmaceutical formulations of the assayed samples did not interfere with warfarin sodium clathrate.

It can be said that the proposed method is precise, sensitive, and accurate, so that this can be used as standard pharmacopoeial method for the determination of warfarin sodium clathrate in tablet doses form using the HPTLC. The advantages of the proposed method involve a simple procedure for sample preparation and relatively short time of analysis.

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