INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com ISSN 2230 – 8407
Research Article

DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF PHYTOMENADIONE IN INJECTION

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Article Received on: 18/01/12 Revised on: 20/02/12 Approved for publication: 09/03/12

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ABSRACT
The present manuscript describes simple, sensitive, accurate, precise and economical spectrophotometric method for the determination of phytomenadione in injection formulation. Method is based on the reaction of phytomenadione with 2, 4 dinitrophenyl hydrazine which produce deep yellow color. The yellow color chromogen shows wavelength of maximum absorbance at 350 nm. Linearity was obtained in the concentration range of 0.1 – 1.0 µg/ml. The method was successfully applied to pharmaceutical injection formulation because no interferences from formulation excipients were found. The suitability of this method for the quantitative determination of phytomenadione was proved by validation. The proposed method was found to be fast and cost effective and can be used for the routine quality control analysis of pharmaceutical formulations. The results of analysis have been validated statistically and by recovery studies.
KEYWORDS: Phytomenadione, Spectrophotometric method, 2,4 dinitrophenyl hydrazine, Injection, Validation, Recovery

INTRODUCTION

Chemically, phytomenadione is 1, 4 - naphthalenedione, 2-methyl-3-(3, 7, 11, 15-tetramethyl-2- hexadecenyl)-, [R-[R*, R*-(E)]]-.phylloquinone. Phytomenadione is a methyl napthoquinone derivative, has a key role in maintaining a normal blood clotting mechanism and preventing a hemorrhagic disease of the newborn[1]. Phytomenadione is official in British Pharmacopoeia (BP), United States Pharmacopoeia (USP), European Pharmacopoeia (EP) and Japanese Pharmacopoeia (JP). BP[2], USP[3], EP[4] and JP[5] describe liquid chromatographic method for its estimation. Literature survey reveals HPLC[6-10] methods for determination of phytomenadione in pharmaceutical formulations and biological fluids. The present communication describes simple and cost effective spectrophotometric method for the estimation of phytomenadione in injection.

MATERIALS AND METHODS

Apparatus

A shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

Reagents and Materials

Phytomenadione powder was kindly gifted by Lincoln Pharmaceuticals Ltd., Ahmedabad, India. The commercial fixed dose product was procured from the local market. Methanol and 2, 4 - dinitrophenyl hydrazine (AR Grade) were procured from S. D. Fine Chemicals Ltd., Mumbai, India.

Preparation of 2, 4 dinitrophenyl hydrazine reagent (0.1 %)

Accurately weighed portion of 2, 4 dinitrophenyl hydrazine (100 mg) was transferred to 100 ml volumetric flask and mixed with methanol (50 ml). The solution was sonicated for 15 min and diluted up to the mark with methanol.

Preparation of standard stock solution

The standard stock solution of phytomenadione was prepared by dissolving 10 mg of phytomenadione in 100 ml volumetric flask using methanol to obtain final concentration, 100 µg/ml.

Methodology

Standard stock solution of phytomenadione (1.0 ml) was transferred to 10 ml corning volumetric flask. To flask, 2 ml (0.1 %) reagent solution was added. After a thoroughly shaking the flasks were set aside for 10 minutes for the reaction to complete. The volumes of each flask were adjusted to 10 ml with methanol. The solution was scanned in the range of 300 to 700 nm against reagent blank, prepared similarly in which volume of standard solution was replaced by an equal volume of methanol. Maximum absorbance was obtained at 350 nm.

Preparation of calibration curve

Aliquots of 0.1 to 1 ml portion of standard stock solution were transferred to a series of 10 ml corning volumetric flasks. To each flask, 2 ml (0.1 %) 2, 4 dinitrophenyl hydrazine reagent was added. After a thoroughly shaking the flasks were set aside for 10 minutes for the reaction to complete. The volumes of each flask were adjusted to 10 ml with methanol. Again Aliquots of 1 ml portion of these solutions were transferred to a series of 10 ml corning volumetric flasks and dilute up to the mark with methanol. The absorbance of solution in each flask was measured at 350 nm against reagent blank and calibration curve was plotted.

Validation of the proposed method

The proposed method was validated for linearity, precision, accuracy, limits of detection and limits of quantification according to the International Conference on Harmonization (ICH) guidelines[11].

Analysis of phytomenadione from injection

An accurately measured injection solution (1.0 ml) containing 5.2 mg of phytomenadione (Brand A) or 1.0 mg of phytomenadione (Brand B) in 50 ml volumetric flask. The content was mixed with methanol (30 ml) and sonicated for 15 minutes. The solution was filtered through Whatman filter paper No.41 and the volume was made up to 50 ml with

distilled water. From this solution, aliquots containing required concentration of the drug were taken for analysis and the solutions were then analyzed as described under calibration curve procedure. The amount of drug was determined by referring to the calibration curve. The analysis procedure was repeated five times with pharmaceutical formulation.

RESULTS AND DISCUSSION

The present method involves the condensation reaction of phytomenadione with 2, 4 dinitrophenyl hydrazine reagent to produce yellow color chromogen having 350 nm as wavelength of maximum absorbance (Figure 1). Phytomenadione reacts positively with 2, 4 dinitrophenyl hydrazine reagent in methanol and producing deep yellow colored chromogen.

It was found that 2 ml (0.1 %) 2, 4 dinitrophenyl hydrazine reagent was sufficient for the development of maximum color intensity. Stability study of the developed chromogen was carried out by measuring the absorbance values at a time intervals of 30 minutes for 6 h and it was found to be stable for more than 4 h for the drug at room temperature.

The linearity was found in the concentration range of 0.1 to 1 µg/ml with high value of correlation coefficient (r^2>0.99) indicates linearity of the method. The reproducibility, repeatability and precision of the methods are very good as shown by the low values of standard deviation and percent relative standard deviation (% RSD). The % recovery values close to 100 % indicates accuracy of the method. The method was successfully applied to estimate phytomenadione from injection and the assay results are in good agreement with the label claim of the drug. The results of recovery studies and assay are given in Table 1 and Table 2, respectively. Summary of validation parameters for phytomenadione was given in Table 3.

CONCLUSION

The method described in this paper for the estimation of phytomenadione using 2, 4 - dinitrophenyl hydrazine reagent was found to be simple, sensitive, accurate, precise, rapid and economical and can be successfully employed for the routine analysis of phytomenadione in pharmaceutical injection dosage form.

ACKNOWLEDGMENT

The authors are thankful to Lincoln Pharmaceuticals Limited, Ahmedabad, India for providing gift sample of phytomenadione for research work. The authors are highly thankful to Center for Health Science Studies, Ganpat University, Kherva, Mehsana, Gujarat, India for providing all the facilities to carry out the work.

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TABLE 1: RESULTS OF RECOVERY STUDIES

Brand	Level	Amount of sample taken (µg/ml)	Amount of standard spiked (%)	% mean recovery ± S. D. (n = 3)
I	I	0.6	50	100.9 ± 0.68
	II	0.6	100	99.83 ± 0.86
	III	0.6	150	101.5 ± 1.29
II	I	0.6	50	101.7 ± 0.58
	II	0.6	100	101.9 ± 1.26
	III	0.6	150	100.0 ± 0.34

S. D. is standard deviation and n is the number of determinations.

TABLE 2: RESULTS OF ANALYSIS OF INJECTION FORMULATIONS

Brand	Label claim (mg/ml)	Amount found (mg)	% label claim ± S. D. (n = 3)
I	5.2	5.10	98.17 ± 0.84
II	1.0	1.01	101.73 ± 0.73

S. D. is standard deviation and n is the number of determinations.

TABLE 3: OPTICAL CHARACTERISTICS AND SUMMARY OF VALIDATION PARAMETERS

PARAMETERS	RESULTS	
Concentration range (µg/ml)	0.1 – 1.0	
Regression equation (y = a + bc)	y = 1.189x + 0.081	
Slope (b)	1.189	
Intercept (a)	0.081	
Correlation Coefficient (r^2)	0.9970	
Sandell's sensitivity (µg/cm^2/0.001 A.U.)	0.0013	
Accuracy (% recovery) (n = 3)	Brand - I	Brand - II
	100.7 ± 0.82	101.2 ± 1.00
Precision, Repeatability (% RSD, n = 6)	0.17	
Intermediate Precision		
Interday (n = 3) (% RSD)	0.35 – 0.48	
Intraday(n = 3) (% RSD)	0.26 - 0.45	
LOD (µg/ml)	0.028	
LOQ (µg/ml)	0.092	

RSD is relative standard deviation and n is the number of determinations.

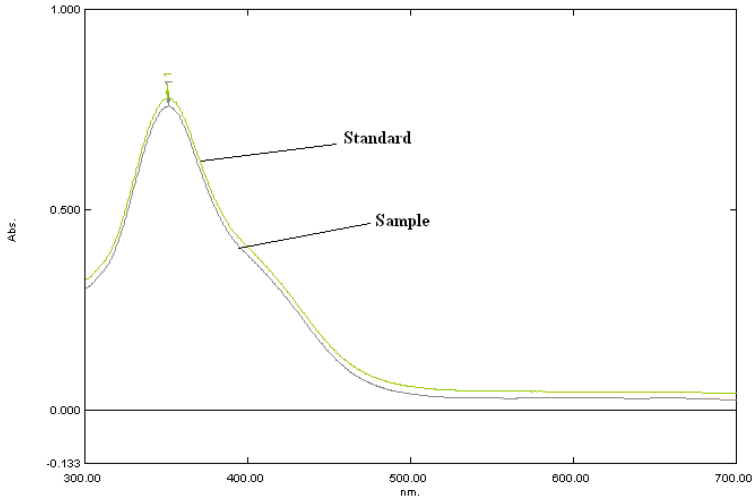

Figure 1: Spectra of phytomenadione standard and sample in methanol showing maximum absorbance at 350 nm

Source of support: Nil, Conflict of interest: None Declared