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Development and validation of spectrophotometric methods for determination of phytomenadione in injection

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ABSTRACT

Three simple and sensitive spectrophotometric methods have been developed for determination of phytomenadione in injection. Method A is simple UV spectrophotometric method based on determination of phytomenadione in methanol at 249 nm. Linearity was obtained in the concentration range of 2 – 14 ug/ml. Method B is first order derivative spectrophotometric method and involves estimation of phytomenadione in methanol using the first - order derivative technique at 259 nm as maxima and 276 nm as minima. Calibration curve was prepared by plotting the absorbance difference between maxima and minima versus concentration. Linearity was obtained in the concentration range of 2-14 µg/ml. Method C is area under curve (AUC) method. The method involved calculation of integrated value of absorbance with respect to the wavelength between two selected wavelength 241 nm and 280 nm, respectively. Linearity was obtained in concentration range of 2- 14 µg/ml. These methods were successfully applied to pharmaceutical formulations because no interferences from injection excipients were found. The suitability of these methods for the quantitative determination of phytomenadione was proved by validation. The proposed methods were found to be simple and cost effective and can be employed for the routine quality control application of phytomenadione in pharmaceutical formulations.

Keywords: Phytomenadione, Spectrophotometric, First order derivative, Area under curve, Injection, Validation

1. 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 methods for the estimation of phytomenadione in injection.

2. 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 2.0 system software. A Sartorius CP224S analytical balance (Goettingen, 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 (AR Grade) was procured from S. D. Fine Chemicals Ltd., Mumbai, India.

Preparation of diluent, standard and sample solutions

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.

Preparation of sample solution

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 using proposed methods.

Development of the methods

Method A: UV spectrophotometric method

Method A is simple UV spectrophotometric method. In this method, the UV spectrum of phytomenadione in methanol was obtained which exhibits absorption maxima (λ max) at 249 nm. Aliquots of standard stock solution (0.2 – 1.4 ml) were transferred into a series of 10 ml volumetric flask and diluted up to mark with methanol. The absorbances of the resulting solutions were measured at 249 nm against methanol as blank. Calibration curve was prepared by plotting absorbance versus concentration. The calibration curve was linear in concentration range of 2 – 14 µg/ml.

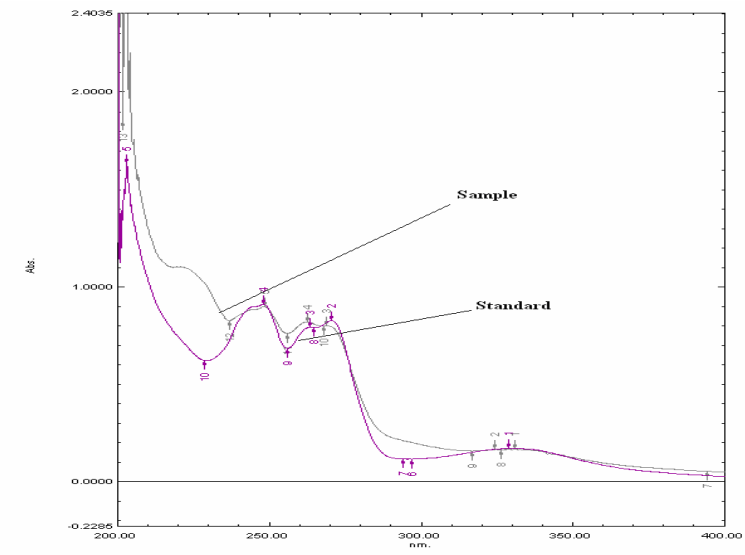

Figure 1: Zero order UV spectra of phytomenadione in methanol

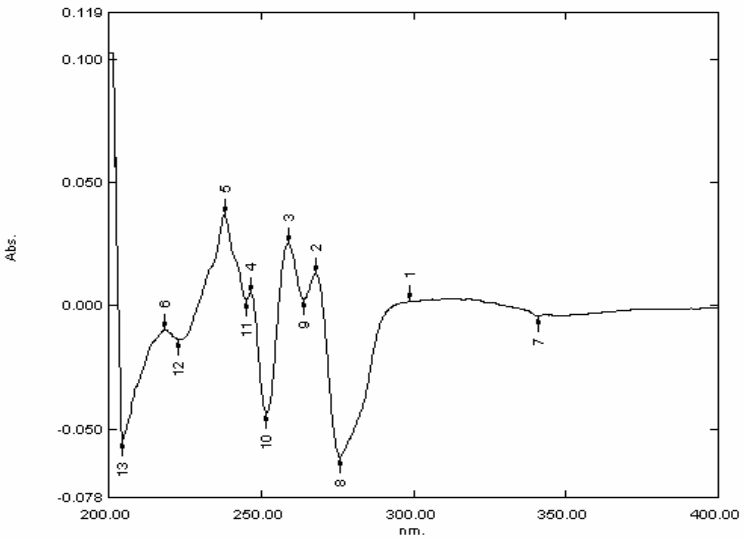

Figure 2: First order derivative spectra of phytomenadione in methanol

Method B: First order derivative spectroscopic method

Method B is the first – order derivative spectrophotometric method. In this method, the zero order UV spectrum of phytomenadione in methanol was obtained and derivatized to 1st order (n = 1). Maxima occur at 276 nm and minima at 259 nm. Aliquot of standard stock solution of phytomenadione (0.2 – 1.4 ml) were transferred into series of 10 ml volumetric flask. These solutions were diluted with methanol up to the mark and first derivative spectra were obtained which shows absorbance maxima at 259 nm and minima at 276 nm. A calibration curve was prepared by plotting the absorbance difference between maxima and minima versus concentration. The calibration curve was linear in concentration range of 2 – 14 µg/ml.

Method C: Area under curve (AUC) method

The AUC method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelength 241 nm and 280 nm. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. The wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration. Aliquot of standard stock solution of phytomenadione (0.2 – 1.4 ml) were transferred into series of 10 ml volumetric flask. These solutions were diluted with methanol up to the mark and spectra were obtained which shows area between 241 nm and 280 nm. A calibration curve was prepared by plotting the area versus concentration. The calibration curve was linear in concentration range of 2 – 14 µg/ml.

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines. [11]

Calibration curve (linearity)

Calibration curves for phytomenadione were plotted over a concentration range of 2 – 14 µg/ml for all the methods. Accurately measured standard stock solutions of phytomenadione (0.2 – 1.4 ml) were transferred to a series of 10 ml volumetric flasks and diluted up to the mark with methanol. The absorbances of the resulting solutions were measured at 249 nm and was plotted versus concentration to obtain calibration curve and regression equation was calculated (Method A). First derivative curves of these solutions (Method B) were obtained, which shows maxima and minima at 259 and 276 nm, respectively. The calibration curve of amplitude against concentration of the drug showed linearity for method B. Area of the zero order spectra's were calculated and the calibration curve of area against concentration was plotted for method C.

Table No. 1. . Regression analysis data and summary of validation parameters

Parameters	Simple UV method	1st derivative method	Area under Curve method
Absorption Maxima	249 nm	276	280
Absorption minima	-	259	241
Beer's Law Limit (µg/ml)	2 - 14	2 - 14	2 - 14
Regression equation (y = a + bc) Slope (b) Intercept (a)	y = 0.083x+0.023 0.083 0.023	y = 0.004x - 0.003 0.004 0.003	0.690X-0.472 0.690 0.472
Correlation Coefficient (r^2)	0.9965	0.9980	0.9973
Repeatability (% RSD[a], n[b] = 6)	0.77	1.25	0.11
Precision(% RSD) (n=3) Interday Intraday	1.12 - 1.89 0.35 - 1.60	0.83 - 1.88 0.76 - 1.90	0.46 - 1.18 0.59 - 1.62
LOD[c] (µg/ml)	0.55	0.41	0.60
LOQ[d] (µg/ml)	1.81	1.35	1.98

[a] LOD = Limit of detection [b] LOQ = Limit of quantitation

[c] RSD = Relative standard deviation [d] n = number of determinations

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solution (*n* = 6) for phytomenadione (10 µg/ml for method A, B and C) without changing the parameter of the method. The repeatability was expressed in terms of percentage relative standard deviation (% RSD).

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of Phytomenadione (4, 8 and 10 µg/ml for method A, B and C).

The result was reported in terms of relative standard deviation (% RSD).

Table No. 2. Recovery data for proposed methods

Method	Level	Amount of sample taken (µg/ml)	Amount of standard added (%)	% Mean recovery ± S. D. (n = 3)
A	I	4	50	98.54 ± 1.74
	II	4	100	99.23 ± 0.54
	III	4	150	100.4 ± 1.35
B	I	4	50	101.1 ± 1..68
	II	4	100	98.61 ± 0.97
	III	4	150	100.6 ± 1.09
C	I	4	50	99.14 ± 0.94
	II	4	100	98.45 ± 1.291
	III	4	150	99.87 ± 0.79

Method A is the simple and direct UV spectrophotometric method, Method B is the first derivative method and Method C is Area under Curve method. n is number of determination and S. D. is standard deviation.

Accuracy (% recovery)

The accuracy of the method was determined by calculating recovery of phytomenadione by the standard addition method. Known amounts of standard solutions of phytomenadione was added at 50%, 100% and 150 % level to prequantified sample solutions of phytomenadione (4 µg/ml for method A, B and C). The amount of phytomenadione was estimated by applying obtained values to the respective regression line equations. The experiment was repeated for three times.

Limit of detection and limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines[11].

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Table No. 3. Recovery data for proposed methods

Injection	Label claim (mg/ml)	Parameter	% amount found (n = 3)		
			Method A	Method B	Method C
Brand I	5.2	Mean	99.15	101.9	98.73
		S. D.	1.27	1.34	0.82
Brand II	2.0	Mean	99.60	98.48	99.55
		S .D.	1.08	1.65	0.46

n is number of determination and S.D. is standard deviation.

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 respective calibration curve procedures (Method A, B and C). The amount of drug was determined by referring to the calibration curve. The analysis procedure was repeated five times with pharmaceutical formulation.

3. RESULTS AND DISCUSSION

Method A is simple UV spectrophotometric method. In this method the simple UV spectrum of phytomenadione in methanol was obtained which exhibits absorption maxima (λ max) at 249 nm (Figure 1). The calibration curve was linear in concentration range of 2 – 14 µg/ml. Method B is the 1st derivative spectrophotometric method. Maxima occur at 259 nm and minima at 276 nm (Figure 2). The calibration curve was linear in

concentration range of 2 – 14 µg/ml. Method C is the Area under Curve (AUC) method. In this method the simple UV spectrum of phytomenadione in methanol was obtained and area between two selected wavelengths was measured. Area measured between 241 nm and 280 nm. The calibration curve was linear in concentration range of 2 – 14 µg/ml.

The proposed methods were found to be simple, sensitive, rapid, accurate, precise and economic for the routine analysis of phytomenadione in pharmaceutical formulations. The linearity ranges was found to be 2-14 µg/ml for all the methods. Characteristic parameters for regression equation and correlation are given in Table 1. Precision was calculated as repeatability (% RSD) and intra and inter day variation (% RSD) for phytomenadione. Accuracy was determined by calculating the recovery, and the mean was determined (Table 2). The LOD and LOQ for phytomenadione were found to be 0.55 and 1.81, 0.41 and 1.35, 0.60 and 1.98 µg/ml for method A, B and C, respectively indicates sensitivity of the proposed methods. The methods were successfully used to determine the amounts of phytomenadione presents in injection. The results obtained are in good agreement with the corresponding labeled amount (Table 3). By observing the validation parameters, the methods were found to be sensitive, accurate and precise. Hence the methods can be employed for the routine analysis of phytomenadione in injection formulations.

4. CONCLUSION

The methods described in this manuscript for the estimation of phytomenadione were found to be simple, sensitive, accurate, precise, rapid and economical and can be successfully employed for the routine analysis of Phytomenadione in pharmaceutical dosage form.

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