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HPLC Determination of Phytomenadione in Drop

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ABSTRACT

The simple, sensitive, accurate, precise and economical HPLC method have been developed for the determination of phytomenadione in injection formulation. Method is RP-HPLC method and is based on determination of phytomenadione with mobile phase (Ethanol:Water) at 254 nm. Linearity was obtained in the concentration range of 5 – 150µg/ml. The method was successfully applied to pharmaceutical formulations because no interferences from injection excipients were found. The suitability of the method for the quantitative determination of phytomenadione was proved by validation. The proposed method were found to be simple, sensitive, accurate, precise, rapid and economical for the routine quality control application in pharmaceutical formulations.

Keywords: Phytomenadione, HPLC method, Injection, Validation

1. INTRODUCTION

Chemically phytomenadione (Figure 1) is 1,4-Naphthalenedione,2-methyl-3-(3,7,11,15-tetramethyl-2- hexadecenyl)-,[R-[R*,R*-(E)]]-. Phylloquinone [84-80-0]. Phytomenadione is a methyl napthoquinone derivative, has a key role in maintaining a normal blood clotting mechanism and preventing a haemorrhagic disease of the newborn. Phytomenadione is official in British Pharmacopoeia (B.P), United States Pharmacopoeia (U.S.P), European Pharmacopoeia (E.P) and Japanese Pharmacopoeia (J.P). BP[2], USP[3], EP[4], JP[5], describes liquid chromatographic method for its estimation. A deep literature survey reveals HPLC[6-10] method for determination of phytomenadione in pharmaceutical formulations and biological fluids. The present communication describes simple and cost effective HPLC method for the estimation of phytomenadione in pharmaceutical dosage form.

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2. MATERIALS AND METHODS

2.1 Apparatus

The HPLC system (Dionex, Ultimate 3000), consisted of a system controller, on-line degasser, low-pressure gradient flow control valve, solvent delivery module, auto injector, column oven, UV – VIS detector and Chromeleon software (version 6.8). A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

2.2 Materials and Reagents

Phytomenadione standard was used from Lincoln Pharmaceutical Limited (Gujarat, India) having purity of 100%. The Phytomenadione drops containing 50 µg/0.25 ml label claim of Phytomenadione and placebo for Phytomenadione drops from Lincoln Pharmaceutical Limited (Gujarat, India). A nylon 0.22 µm – 47 mm membrane filter (Gelman Laboratory, Mumbai, India). The water for RP-HPLC was prepared by triple glass distillation and filtered through a nylon 0.22 µm – 47 mm membrane filter. Ethanol (99%) from Lincoln Pharmaceutical Limited (Gujarat, India). HPLC grade acetonitrile (Rankem, India).

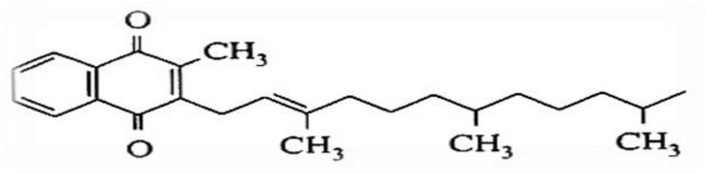

Fig 1: Chemical structure of Phytomenadione

2.3 Preparation of Standard and Sample Solutions

2.3.1 Preparation of Mobile Phase

About 970 ml Ethanol (99%) and 30 ml HPLC grade water was taken in 1000 ml glass beaker and mixed it well and kept in sonicator for 5 minutes.

2.3.2 Preparation of a Standard Stock Solution

Phytomenadione standard (50 mg) was accurately weighed and transferred into 50 ml volumetric flask, made up the volume with mobile phase and mixed well by continuous shaking. 5 ml of this solution was taken and transferred it into 50 ml volumetric flask and made up the volume with mobile phase, mixed it well by continuous shaking and then place it in a sonicator for 5 minutes. The final solution has a concentration of 100 µg/ml. Solution was filtered through Whatman filter paper.

2.3.3 Preparation of a Sample Solution

Phytomenadione drops (5 ml) was accurately measured and transferred it into 10 ml volumetric flask, made the volume with mobile phase, mixed it well by continuous shaking and then place it in a Sonicator for 5 minutes. The final solution has a concentration of 100 µg/ml. Solution was filtered through Whatman filter paper.

2.3.4 Placebo Preparation

Phytomenadione drops placebo (5 ml) was taken and transferred it into 10 ml volumetric flask, made the volume with mobile phase, mixed it well by continuous shaking and then place it in a Sonicator for 5 minutes. Solution was filtered through Whatman filter paper.

2.4 Chromatographic Condition

Stationary phase: Oyster BDS C_{18} column (250 mm x 4.6 mm i.d., 5 µm particle size) used at ambient temperature
Mobile Phase: Water : Ethanol (3: 97) (v/v)
Flow rate: 1.5 ml/min
Injection volume: 20 µl
Detection: At 254 nm with UV-Visible detector

2.5 Determination of the analytical wavelengths

Standard solution of Phytomenadione was injected under the chromatographic condition described above. Detection was carried out at different wavelength; best response was achieved at 254 nm with UV detector. So drug was detected at this analytical wavelength.

2.6 Development of the Methods

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak for Phytomenadione was obtained with a mobile phase Water: Ethanol (3:97) (v/v) at a flow rate of 1.5 ml/min to get better reproducibility and repeatability. Quantification was carried out at 254 nm based on peak area. System suitability test parameters for Phytomenadione for the proposed method are reported in Table No 1.

Table No. 1: System Suitability data for Proposed Methods

Parameters	**Phytomenadione ± %RSD (n = 6)**
Retention time (min)	10.79 ± 0.49
Tailing factor	1.503 ± 0.93
Theoretical plates	5842 ± 0.41

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

2.7 Validation of the proposed method[11]

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

2.7.1 System Suitability

System suitability was performed and calculated at the start of study of each validation parameter. System Suitability values were from the first injection of six replicates of standard. Relative standard deviation (%RSD) was calculated from six replicate injections of standard.

2.7.2 Calibration Curve (linearity)

Calibration curve was constructed by plotting peak areas Vs concentrations of Phytomenadione. The calibration curve was plotted over the concentration range 5-150 µg/ml for Phytomenadione. Accurately measured (0.5, 1, 2.5 and 5 ml) standard working solution (100 µg/ml) of Phytomenadione and transferred them in to a series of 10 ml of volumetric flasks and diluted to the mark with mobile phase. For 150 µg/ml of Phytomenadione standard (7.5 mg) was accurately weighed and transferred in to 50 ml volumetric flask, made up the volume with mobile phase. Aliquots (20 µl) of each solution were injected under the operating chromatographic conditions described above.

2.7.3 Method Precision (% repeatability)

Precision of the instrument was checked by repeatedly injecting six sample solutions of Phytomenadione (100 µg/ml) under the same chromatographic condition and measurements of peak area, retention time and tailing factor. Relative standard deviation (%RSD) or coefficient of variation should not be more than 2 %.

2.7.4 Intermediate Precision (reproducibility)

Intraday and Interday precision of the proposed method was determined by analyzing standard solutions of Phytomenadione at 3 different concentrations (50, 100 and 150 µg/ml) 3 times on the same day and on 3 different days. The results are reported in terms of relative standard deviation (%RSD).

2.7.5 Accuracy (% recovery study)

Accuracy of the method was determined by calculating recoveries of Phytomenadione by the standard addition method. Known amounts of standard solution of Phytomenadione were added at 50%, 100% and 150% levels to placebo to attain different working concentrations of Phytomenadione. The amounts phytomenadione was estimated by applying obtained values to the regression equation of the calibration curve. Each sample was prepared in triplicate at each level and injected. The chromatograms were recorded and from the peak area of drug, % recovery was calculated from regression equation of the calibration curve.

2.7.6 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.

2.8 Analysis of Phytomenadione from Drop

Sample: Phytomenadione drops.
Strength: 50 µg/0.25 ml.
Manufacturer: Lincoln Pharmaceuticals Ltd. (Gujarat, India).

2.9 Procedure

The response of the sample solution was measured at 254 nm under the chromatographic condition mentioned above for the quantification of Phytomenadione. The amounts of Phytomenadione present in sample solution were determined by applying values of the peak area to the regression equations of the calibration graph. The spectrum for standard and sample were shown in Figure 2 & Figure 3 respectively.

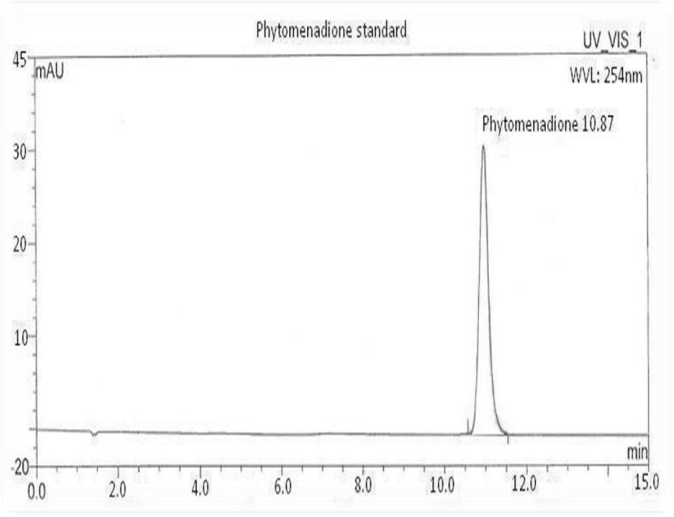

Figure 2: Spectrum for Phytomenadione standard

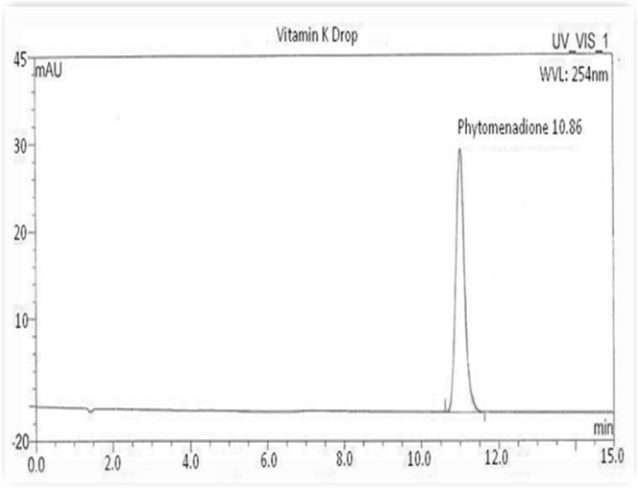

Figure 3: Spectrum for Phytomenadione drop

3. RESULTS AND DISCUSSION

A satisfactory separation and good peak for Phytomenadione was obtained with a mobile phase Water: Ethanol (3:97) (v/v) at a flow rate of 1.5 ml/min to get better reproducibility and repeatability. Quantification was carried out at 254 nm based on peak area. System suitability test parameters for Phytomenadione for the proposed method are reported in Table No 1. The correlation coefficient value should not be less than 0.9950 over the working range. Linear correlation was obtained between peak areas versus concentrations in the ranges 5 – 150 µg/ml for Phytomenadione. Regression parameters are mentioned in Table 2. The correlation coefficient values are 0.9980. The areas obtained are directly proportional to the concentration of analyte in the sample. The method can therefore be considered to be linear in the range considered. Based on the linearity results, the working range of the method can be established as 5-150 µg/ml for phytomenadione. The recovery experiment was performed by the standard addition method. The recoveries obtained were 100.6 ± 0.83% for Phytomenadione. The low value of standard deviation indicates that the proposed method is accurate. Results of recovery studies are shown in Table No 2. The %RSD values for Phytomenadione were found to be 0.23%, respectively (Table – 5.4). The %RSD values were found to be <2 %, which indicates that the proposed method is repeatable. The robustness of the method was established by introducing small changes in column oven temperature. Set column temperature at (25°, 30° and 35°C) and analyze the sample (n=6). The %RSD should not be more than 2%. Robustness study data by changing column oven temperature are shown in Table No 2. The data indicated that there was no significant difference between the results obtained under normal temperature conditions and varied temperature condition. Therefore, the method is said to be robust. The proposed method were found to be simple, sensitive, rapid, accurate, precise and

Table No. 2. Analysis of Phytomenadione by proposed method

Sample No.	**Label claim**	**Amount found**	**% Label claim**
	Phytomenadione (µg/0.25 ml)	**Phytomenadione (µg/0.25 ml)**	**Phytomenadione**
1	50	49.56	99.12
2	50	48.83	97.66
3	50	49.43	98.85
4	50	50.27	100.54
5	50	49.59	99.19
6	50	49.68	99.35
Mean		49.56	99.11
SD		0.46	0.92
%RSD		0.93	0.93

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

Table No 3: Regression Analysis Data And Summary Of Validation For Praposed Method

Parameters		**Results**
Specificity		Complies
Solution Stability		The solutions were stable at least up to 24 hours at room temperature
Linearity and Range		Correlation coefficient: 0.9980 Range: 5-150 µg/ml
Regression equation y=mx+c		y = 3771.x + 1635
Limit of Detection:		1.51 µg/ml
Limit of Quantification:		4.58 µg/ml
Method precision (n=6) (Repeatability) (%RSD)		0.230
Intermediate precision	Intraday (n=3) (%RSD)	0.210 – 0.680
	Interday (n=3) (%RSD)	0.210 – 0.440
Mean Recovery (Accuracy) ± %RSD		100.6 ± 0.830
Robustness		Complies
System suitability		Complies
% Label claim (n=6)		99.11

economic for the routine analysis of phytomenadione in pharmaceutical formulations. LOD and LOQ for Phytomenadione are found to be 1.51 µg/ml and 4.58 µg/ml respectively indicates sensitivity of the proposed method. The method were successfully used to determine the amounts of phytomenadione presents in drop. The results obtained are in good agreement with the corresponding labeled amount (Table No 3). By observing the validation parameters, the methods were found to be sensitive, accurate and precise (Table No 2). Hence the methods can be employed for the routine analysis of phytomenadione in injection formulations.

4. CONCLUSION

From the results obtained, it is obvious that the proposed method is applicable for the determination of Phytomenadione without interference and with good sensitivity. The results obtained indicate that the proposed method for the estimation of Phytomenadione is simple, specific, accurate, precise, robust, sensitive and suitable for intended use. These merits suggest the use of the proposed method in routine and quality control analysis of Phytomenadione without interference from commonly encountered excipients and additives.

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REFERENCES

1. Maryadele J. O' Neil. The Merck Index: An Encyclopedia of chemicals, drugs and biologicals, 14th ed. New Jersey: Published by Merck Research Laboratories, Division of Merck and Co., Inc. Whitehouse station (2006) pp. 1271.
2. British Pharmacopoeia, Vol. III, Medicines and Healthcare Products Regulatory Agency, Stationary Office, London: (2010) pp. 3006.
3. The United States Pharmacopoeia, USP 32, NF 27, Vol. 3, The United States Pharmacopoeial Convention, Inc, Rockville, MD (2009) pp. 3302.
4. European Pharmacopoeia, Volume 2, European Directorate for the Quality of Medicines and Healthcare (EDQM), Strasbourg, France. (2008) pp. 422.
5. The Japanese Pharmacopoeia, Society of Japanese Pharmacopoeia, 15th edition, Shibuya, Tokyo, Japan. (2006) pp. 993.
6. Langenberg JP, Tjaden UR. Improved method for the determination of vitamin K1 epoxyde in human plasma with electrofluorometric reaction detection. J Chromatogr. 1984; 289: 377-385.
7. Langenberg JP, Tjaden UR. Determination of vitamin K1 in human plasma by RP-HPLC using fluorimetric detection after post column electrochemical reduction: comparison with ultraviolet single and dual electrochemical detection. J Chromatogr. 1984; 305(1): 61-72.
8. Wakabayashi H, Onodera K, Yamato S, Shimada K. Simultaneous determination of vitamin K analogs in human serum by sensitive and selective high-performance liquid chromatography with electrochemical detection. Nutrition. 2003; 19(7-8): 661-665.
9. Haroon Y, Bacon DS, Sadwoski JA. Liquid chromatography detection of vitamin K1 in plasma with fluorimetric detection Clin Chem. 1986; 32: 1925-29.
10. Gong BY, Ho JW. Simultaneous separation and detection of ten common fat soluble vitamins in milk. J Liq Chromatogr Rel Technol. 1997; 20: 2389-2397.
11. Validation of Analytical Procedures, Methodology, ICH harmonized tripartite guidelines. 1996.