LONG-TERM STABILITY OF PHYTONADIONE (VITAMIN K_1) IN AQUEOUS DISPERSIONS INJECTION

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Introduction

Phytonadione an antihemorrhagic agent is a co-factor for the post-ribosomal γ carboxylation of glutamyl residues in clotting factors II,VII,IX and X (1). Several high-performance liquid chromatographic (HPLC) methods have been published for determining concentrations of phytonadione in biological media (2). Instability of phytonadione in hyperalimentation fluids has been reported by Leon Longe (3). Phytonadione decomposes upon exposure to light and the aqueous dispersions for injection may be sterilized by filtration (4). Thus, the stability studies of phytonadione injection is of importance. The aim of this work was to study the long-term chemical stability of fabricated phytonadione injection at different temperatures, using a developed USP HPLC method.

Experimental methods

Materials

Phytonadione (F. Hoffmann-La Roche, Switzerland) obtained as a gift . All chemicals were of analytical or HPLC grade and were used as received. One commercially available brand (10 mg mL^{-1}) of phytonadione ampoule obtained from a local pharmacy.

Methods

Preparation of phytonadione injection

The PEG 400 and polysorbate 20 and benzyl alcohol were mixed. Phytonadione slowly was added to above mixture. While stirring, acetate buffer pH 4.5 was added and the dispersed solution was filtered through 0.22 μ membrane filter and filled in the amber ampoules (1mg mL^{-1}) under aseptic condition.

High-performance liquid chromatographic (HPLC) determination of phytonadione in ampoule

The HPLC (LKB Bromma, Sweden) consisted of a model pump set at constant flow rate of 1.5 mL min^{-1} ,a variable UV detector (LKB Bromma 2141 Programmable Multiwavelength) set at 248 nm , a Bondapak C_{18} 4.0 x 250 mm reversed-phase column 10μm, and an automatic integration system (LKB Bromma 2221). The solvent system was methanol- water (98:2).

Standard preparation

An accurately weighed quantity of phytonadione reference standard as well as fabricated and brand samples were dissolved in suitable solvent (absolute ethanol- water 95:5). The resulting solution was filtered through a 0.45μ filter paper and 20μl was injected into the chromatograph. Peak heights were used for quantitation. All standard curves were linear over the concentration range of 0.05-0.1 mg mL^{-1}.

Stability study

A stability test (Arrhenius method) has been conducted by storing phytonadione fabricated and brand ampoules at 40, 50 and 60°C. The content of phytonadione was tested after 1,2 and 3 months. The assay of phytonadione for stability followed the same procedure as we have described above.

Results and discussion

Results obtained from the analysis of phytonadione showed that the decomposition rate of this compound is first-order and "LogC" versus "t" yields a straight line. The degradation rates of phytonadione (fabricated and brand injections) at different storage conditions (40, 50 and 60°C) are shown in Figure 1. Obviously, the potency of phytonadione in fabricated and brand samples decreased impressively, at 60°C.Thus,the potency of phytonadione in brand sample was reduced from almost 100% (at 25°C) to 77.8% after 3 months storage at 60°C. Furthermore, considering the same condition the phytonadione content of fabricated injection dropped down from approximately 100% (at 25°C) to 79.3% after 3 months storage at 60°C. The logarithms of the specific rates of degradation plotted against the reciprocols of the absolute temperatures (Arrhenius plot) are depicted in Figures 2,and the resulting line is extrapolated to room temperature. The results of this study suggest that, by considering the standard error and its effects on calculating the rate constants and expiration date, the room temperature shelf-life of fabricated and brand injectable formulations are 1649 and 1846 days, respectively.

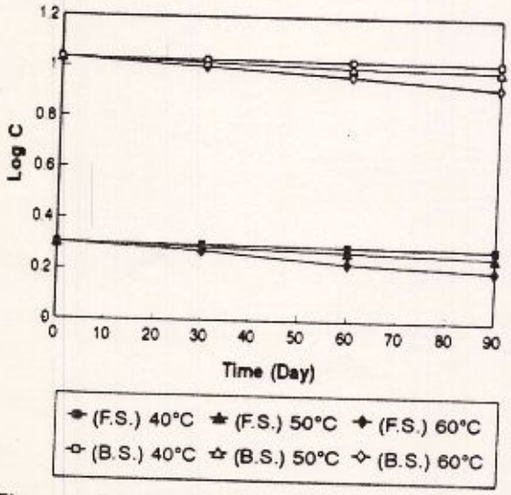

Figure 1 Degradation rates of phytonadione at three different storage conditions (F.S.= fabricated sample, B.S.=brand sample).

Conclusion

This study has demonstrated that fabricated as well as brand phytonadione injections revealed a stable characteristics when stored under stress conditions.

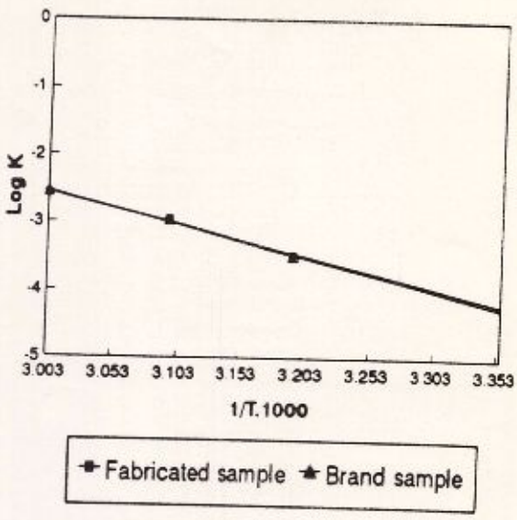

Figure 2 Arrhenius plot for predicting stability of injectable formulation of phytonadione.

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