

Indirect Determination of Vitamin K₃ Using 2,4,6-tris(2'-Pyridyl)-S-Triazine by Spectrophotometric and Flow Injection Techniques

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Summary: Vitamin K₃ (menadione sodium hydrogen sulphite) was indirectly determined by the reduction of iron (III) to iron(II) and an intense blue color formation by the reaction of iron(II) and 2,4,6-(2'-pyridyl)-s-triazine (TPTZ). The color development was monitored spectrophotometrically at 595 nm with linear calibration range with 2-10 µg/ml of vitamin K₃. The method was used for the determination of vitamin K₃ in a pharmaceutical preparation. A single lined flow injection manifold was also examined. A large excess of iron(III) containing different concentrations of vitamin K₃ was run, on the line and a constant amount of TPTZ was injected through the injector. The peak height was proportional to concentration between 2-10 µg/ml vitamin K₃.

Introduction

Vitamin K refers to a group of substances, wide spread in nature, having similar biological activity. These compounds are derived from the parent compound 2-methyl-1, 4-naphthoquinone. Vitamin K is essential in human physiology due to its involvement in blood clotting system, through synthesis of prothrombin and other clotting agents. A poor vitamin K status is associated with an increased risk of osteoporetic fracture [1].

Synthetic derivatives with similar activity to natural vitamin K include menadione (menaphthone) or vitamin K₃. Water soluble derivatives of menadione such as, menadione sodium hydrogen sulphite have been reported [2] (Fig-1). These compounds yield menadione (menaphthone) after decomposition with microorganisms [3].

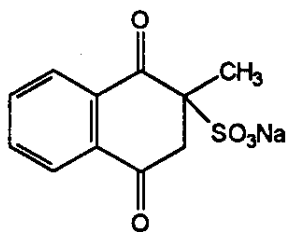


Fig. 1: Menadione sodium hydrogen sulfite (MSB)

The analytical methods for the determination of vitamin K₃ are based on titrimetric in oxidation reduction reaction [2], spectrophotometric [4-5], electro-analytical [6] and chromatographic techniques [7-9]. A large number of methods are reported using, chromatographic techniques with

particular reference to high performance liquid chromatography [9-13]. However, the spectrophotometric methods are simple and involve less expensive equipment. The spectrophotometric methods for vitamin K are based on natural absorbance in the ultraviolet region or after derivatization as phenyl hydrazones [4, 14]. The determination of sulphide, sulphite and isoniazid based on the reduction of Iron (III) to Iron (II) followed by color development with an appropriate chromogenic reagent have been reported [15-16]

In the present work the method developed is based on indirect spectrophotometric determination of vitamin K₃ (menadione sodium hydrogen sulphite) by quantitative reduction of iron (III) to iron (II) in slightly acidic medium and subsequent formation of intense blue colored complex of iron (II) with 2,4,6-tris(2'-pyridyl)-S -triazine (TPTZ).

Results and Discussion

The reagent TPTZ has been reported as spectrophotometric reagent for iron (II). The method is based on indirect determination of vitamin K₃ by the reduction of iron (III) to iron (II) using vitamin K₃ and subsequent formation of intense blue color of iron (II) TPTZ complex with molar absorptivity of $2.2 \times 10^4 \text{ l.mole}^{-1} \text{ cm}^{-1}$. The reaction was examined spectrophotometrically. The effect of pH, reagent concentration, concentration of iron (II), time of measurement and heating time on the color reaction were examined following spectrophotometric determinations. It was observed that iron (III) added in excess tends to precipitate above pH 5 and pH 5

was considered as optimal with maximum color development (Fig. 2). The concentration of reagent and Iron (III) was varied between 0.5 to 3 ml of 0.1% reagent solution and 0.2 to 4 ml iron(III) containing 1 mg /ml. Same responses were obtained in all the concentrations tested however, the amount of 2 ml and 0.5 ml of reagent and iron(III), respectively, per determination of each was used. The color development takes five minutes and the measurement was made when stable absorbance was observed (Fig. 3). Using the conditions linear calibration curve was obtained with 2-10 $\mu\text{g/ml}$ vitamin K_3 with the coefficient of correlation (r) 0.9995 (Fig. 4). Four test solutions of vitamin K_3 prepared were analyzed and relative % error was obtained within ± 0.5 -4.50 The reproducibility of the analysis of 5 $\mu\text{g/ml}$ of vitamin K_3 ($n=4$) and relative standard deviation (RSD) was calculated 1.5%. The method was applied for the determination of vitamin K_3 in ampules. The amount found was 10.0 mg/ampule. The amount of vitamin K_3 reported by the manufacturer is 10 mg/ampule. The use of flow injection for the analysis of vitamin K_3 was next examined. Single lined manifold was only considered. The options of injecting vitamin K_3 or reagent TPTZ were considered. The drug vitamin K_3 is inexpensive, therefore for the determination of vitamin K_3 in pharmaceutical products, vitamin K_3 and iron (III) with buffer were run on peristaltic pump together and the reagent TPTZ was injected. Average peak height of at least three determinations was measured and linear calibration curve was obtained with the final concentration of 2-10 μg injected (Fig. 4).

Experimental

The reagent 2,4,6-*tris* (2'-pyridyl) -*S*-triazine (TPTZ) (E. Merck) in ethanol, and vitamin K_3 (Menadione sodium hydrogen sulphite) were used. Iron (III) solution containing 1 mg/ml was prepared from iron(III) chloride (E.Merck). A required amount of iron (III) was dissolved in water and was added hydrochloric acid (1.0 ml, 37%) followed by hydrogen peroxide (0.5 ml, 24%) to oxidize any iron (II) present in it. The contents were heated to remove an excess of hydrogen peroxide and volume was adjusted to 100 ml using distilled water.

Spectrophotometric Determination

Iron (III) solution (0.5 ml) containing (1 mg/ml) was transferred to a 10 ml volumetric flask and was added reagent TPTZ solution (2 ml, 0.1%, w/v) followed by sodium acetate - acetic acid buffer

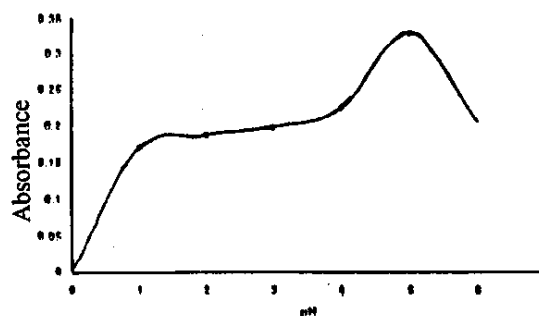


Fig. 2: Effect of pH on the absorbance of iron (II) TPTZ after reduction with vitamin K_3

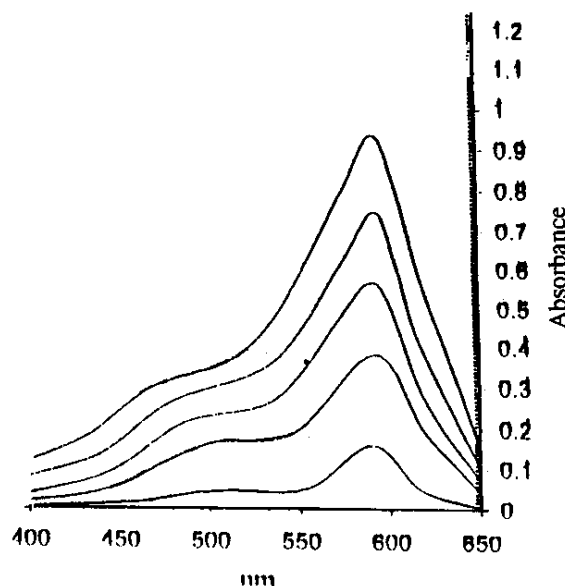


Fig. 3: Absorption spectra for iron(II) TPTZ complex obtained with 2,4,6,8,10 $\mu\text{g/ml}$ vitamin K_3

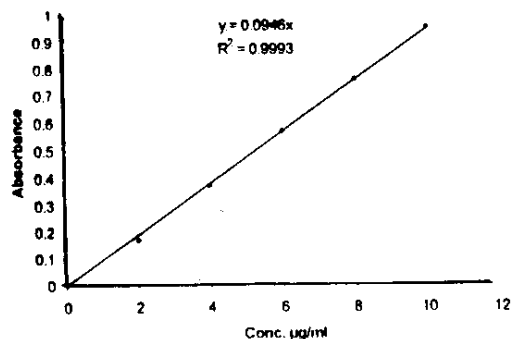


Fig. 4: Calibration curve of vitamin K_3 at 595 nm

pH 5 (1 ml), in an aqueous solution to adjust the final concentration of 2.0 to 10 µg/ml vitamin K₃ and the volume was adjusted to the mark with water. A reagent blank was prepared without using vitamin K₃ solution following the above procedure. The absorbance for each of the standard was recorded against the reagent blank on Hitachi Model 220 spectrophotometer at 595 nm.

Analysis of Vitamin K₃ in Pharmaceutical Preparation

Six ampules (SPIC Shanghai, China) containing 10 mg each were thoroughly mixed and the sample solution 1 ml (10 mg/ml) was adjusted to 50 ml with water. The solution 0.2 ml. was used and the above procedure was followed. The amount of vitamin K₃ was calculated from the calibration plot.

Flow Injection Procedure

A single lined manifold with Gillsons M 312 Mini plus Peristaltic Pump, coupled with spectronic 21 spectrophotometer, Rheodyne 7125 injector and a Hitachi Model 561 recorder were used. The length of reaction coil was 3 meters (Fig. 5). Vitamin K₃ solutions containing 20 to 100 µg were transferred to a 10 ml volumetric flask and was added iron (III) solution (0.5 ml) containing 1 mg/ml, sodium acetate, acetic acid buffer pH 5 (1.0 ml) and volume was adjusted to 10 ml. The solution was run on FIA

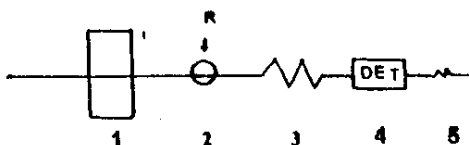


Fig. 5: Single lined FIA manifold (1) peristaltic pump, (2) injector (3) reaction coil, (4) detector (5) drain

system and 20 µl of TPTZ (0.2% in ethanol) was injected. The spectrophotometer was fixed at 595 nm.

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