UNIVERSITI TEKNOLOGI MARA

DEVELOPMENT OF HPLC ASSAY METHOD FOR VITAMIN E DERIVATIVES

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ABSTRACT

Reverse phase high performance liquid chromatography (HPLC) method using UV detection was developed for the analysis of vitamin E derivatives namely α- and γ-tocotrienol. Zorbax 300SB C-18 (5 μm, 250 x 4.6 mm internal diameter) column was used as a stationary phase. Analytes were monitored by UV detection at 295 nm using 0.5:99.5% water in methanol elution. Analysis was run at a flow rate of 1.5 ml/min. The retention time for α-tocotrienol was slightly longer than γ-tocotrienol. Calibration curves for α- and γ-tocotrienol were linear over concentration range of 10-100 μg/mL with correlation coefficients 0.999 and 0.997, respectively. the coefficient of variation for within-day analysis of α- and γ-tocotrienol were all less than 1%. The recovery of α-tocotrienols and γ-tocotrienol from sodium carboxymethylcellulose (CMC) was in the range of 86.691 to 94.582% and from 91.330 to 100.714% respectively. Assay method for tocotrienols using UV detector was successfully developed with reduced retention time in comparison to previous research findings.

CHAPTER 1

INTRODUCTION

Chromatography is a technique used to separate and analyze components of a mixture (Betts, 2009). In liquid chromatography, a liquid is used to carry a mixture across a bed of material. The liquid is called mobile phase because it moves through a bed of material. The bed of material, on the other hand, is called stationary phase. As the mobile phase carries the mixture across the stationary phase, some of the components of the mixture retain at the stationary phase longer than others. Therefore, the components travel at different rates across the stationary phase, and exit the stationary phase at different times. The process thus separates various components of the mixture.

High performance liquid chromatography (HPLC) uses pressure to force mixtures to move through the stationary phase (Betts, 2009). A simple HPLC would include a solvent reservoir to hold the liquid mobile phase, a pump to pressurize the liquid mobile phase, an injector to allow injection of a small volume of the sample mixture under high pressure, a column containing the bed of stationary phase, a detector to detect the presence of components as they exit the column, and some means to record the detector signal.