UNIVERSITI TEKNOLOGI MARA

DEVELOPMENT OF HPLC ASSAY METHOD FOR GAMMA-LINOLENIC ACID

NOOR IZZATI FILZA BINTI SALIHOUDIN

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ABSTRACT

Reverse phase high performance liquid chromatography (RP-HPLC) method using UV detector was developed for analysis of gamma-linolenic acid (GLA). Zorbax 300SB C-18 (5 μm, 250 x 4.6 mm internal diameter) column was the stationary phase and 90% (v/v) acetonitrile and 10% (v/v) deionized water were employed as the mobile phases in an isocratic manner. The detection was done by using UV detector at 211 nm wavelength with the flow rate of 0.8 ml/min. Calibration curves for GLA were linear with average correlation coefficient 0.995. The coefficient of variation for interday analysis were all less than 1% except for day 2. This study was able to have a great reduction in retention time in comparison to the previous research finding.

CHAPTER 1

INTRODUCTION

Chromatography is a method of separation of mixture of chemical compounds that involves the interaction between two phases of stationary phase and mobile phase. The differential rates of migration as the mixture moves over adsorptive materials provide separation. Repeated sorption and desorption acts that take place during the movement of the sample over the stationary bed determine the rates. The smaller the affinity a molecule has for the stationary phase, the shorter the time spent in a column.

Gas chromatography (GC) has been the method of choice for half a century to analyse fatty acids due to the properties of GC which is sensitive, rapid and precise as well as give good reproducible analysis (Azmir & Azhari, 2009). However, the main pitfall of high temperature in operation GC may alter the configuration and stability of fatty acids has made high performance liquid chromatography (HPLC) become alternative method to identify and analyse fatty acids in a sample (Bravi *et al.*, 2006).

HPLC is a chromatographic technique used to separate a mixture of compound by placement of a small volume of liquid sample into a tiny porous packed of particles in a column and is transported along the column by means of liquid mobile phase that is delivered by high force from a pump system.