

**UNIVERSITI TEKNOLOGI MARA**

**DEVELOPMENT OF HPLC ASSAY METHOD  
FOR GAMMA-LINOLENIC ACID**

**NOOR IZZATI FILZA BINTI SALIHOUDIN**

**Dissertation submitted in partial fulfilment of the requirements for  
the degree of Bachelor of Pharmacy (Hons)**

**Faculty of Pharmacy**

**2013**

## **ACKNOWLEDGEMENTS**

My deepest gratitude goes to Pn Nor Amlizan Ramli and Dr Hannis Fadzillah Mohsin as my supervisor and co-supervisor respectively who had guided me endlessly by giving their advices and opinions upon completion of this research project. A very special thankfulness to Mr Mohd Rahimi and Mr Mohd Rozaideen for their helpful hands and kind hearts in teaching me the technical aspects of handling high performance liquid chromatography (HPLC ) system. I also wish to express my appreciation for the unconditional love, care and support from my mother and family. Last but not least, to all friends who were also being very supportive and positive whom I met in the HPLC laboratory upon conducting this project. Thank you.

# TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENTS .....	ii
TABLE OF CONTENTS .....	iii
LIST OF TABLES .....	v
LIST OF FIGURES .....	vi
LIST OF ABBREVIATION .....	vii
ABSTRACT .....	viii
CHAPTER 1 .....	1
INTRODUCTION .....	1
CHAPTER 2 .....	4
LITERATURE REVIEW .....	4
2.1 Definition of GLA .....	4
2.1.1 Essential Fatty Acids .....	4
2.1.2 Sources of GLA .....	4
2.1.3 Chemical Structure of GLA .....	5
2.2 The Metabolism of GLA in Human Body .....	6
2.3 Benefits of GLA .....	9
2.4 Principles of HPLC .....	10
2.5 Types of HPLC .....	11
2.6 HPLC Instrumentation .....	13
2.7 Advantages of HPLC .....	14
2.8 HPLC Assay Method for Essential Fatty Acids .....	16
CHAPTER 3 .....	18
METHODOLOGY .....	18
3.1 Chemicals and Reagents .....	18
3.2 Instrumentation and Chromatographic Conditions .....	18
3.3 Preparation of Standard Solutions .....	19
CHAPTER 4 .....	20
RESULTS .....	20
4.1 HPLC Method Development for GLA .....	20
4.2 Linearity .....	21
4.3 Accuracy and Precision .....	23

## **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  $\mu\text{m}$ , 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.