

UNIVERSITI TEKNOLOGI MARA

**DEVELOPMENT OF HPLC ASSAY METHOD
FOR VITAMIN E DERIVATIVES**

NUR AZEILA LAILA BINTI ABD. RAHIM

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

Faculty of Pharmacy

November 2009

ACKNOWLEDGEMENTS

First and foremost, a special mention of appreciation must go to AP Dr. Wong Tin Wui and Puan Nor Amlizan Ramli as my supervisor and co-supervisor. They have help me a lot in conducting this study by giving me advices and guidances regarding the project. Thanks for giving me the opportunity to learn and run this project. My special thanks also go to Mr. Tengku Azlan who had helped me a lot in handling high performance liquid chromatography (HPLC) system and also for sharing valuable information with me. I also wish to express my gratitude to my beloved parents who always care about me and give their endless encouragement to me in order to complete this project. A special thanks also to all of my friends who offering their ears to all my gripes and grumbles and for their support and also ideas. Last but not least, to all of post-graduate students under supervision of AP Dr. Wong Tin Wui for all their help. Thank you.

TABLE OF CONTENTS

	Page
TITLE PAGE	
APPROVAL FORM	
ACKNOWLEDGEMENTS	ii
TABLE OF CONTENTS	iii
LIST OF TABLES	v
LIST OF FIGURES	vi
LIST OF ABBREVIATIONS	vii
ABSTRACT	viii
CHAPTER 1 (INTRODUCTION)	1
CHAPTER 2 (LITERATURE REVIEW)	3
2.1 HPLC Instrumentation	3
2.2 Principles of HPLC	4
2.3 Tocotrienol	8
2.3.1 Chemical Properties	8
2.3.2 Benefits of Tocotrienol	9
2.4 HPLC Assay Method for Vitamin E Derivatives	12
2.5 Carboxymethylcellulose (CMC)	15
CHAPTER 3 (METHODOLOGY)	16
3.1 Chemicals and Reagents	16
3.2 Instrumentation and Chromatographic Conditions	16
3.3 Preparation of Standard Solutions	17
3.4 Preparation of Carboxymethylcellulose (CMC) Solution	18
3.5 Sample Pre-treatment for HPLC Analysis	18
CHAPTER 4 (RESULTS)	19
4.1 HPLC Method Development for Tocotrienols	19
4.2 Linearity	21
4.3 Accuracy and Precision	22
4.4 Drug Content Study	25
4.5 Recovery	26
CHAPTER 5 (DISCUSSION)	28
5.1 Mobile Phase and Flow Rate Selection	29
5.2 Linearity	30
5.3 Accuracy and Precision	30
5.4 Drug Content Study	30
5.5 Recovery	31

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 μ m, 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 μ g/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.