A QUANTITATIVE ANALYSIS OF ASCORBIC ACID IN VITAMIN C RELATED PRODUCTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

MAHIRAH BINTI MAT

BACHELOR OF SCIENCE (Hons.) CHEMISTRY FACULTY OF APPLIED SCIENCES UNIVERSITI TEKNOLOGI MARA

JANUARY 2014

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	iii
TABLE OF CONTENTS	iv
LIST OF TABLES	vi
LIST OF FIGURES	viii
LIST OF ABBREVIATIONS	ix
ABSTRACT	Х
ABSTRAK	xi

CHAPTER 1 INTRODUCTION

1.1	Background of study	1
1.2	Problem statement	5
1.3	Objectives of study	6
1.4	Scope	6
1.5	Significance of study	7

CHAPTER 2 LITERATURE REVIEW

2.1	Ascor	bic acid	8
2.2	Recor	nmended Daily Allowance (RDA) for vitamin C	9
2.3	Antio	xidant	9
2.4	Method development		10
	2.4.1	HPLC	10
	2.4.2	UV-Visible detector	11
	2.4.3	Suitability of mobile phase	11
	2.4.4	Chromatographic separation	12
	2.4.5	Ion pair reagent	12
	2.4.6	Stability of standard	12
	2.4.7	Wavelength of ascorbic acid	13
2.5	Metho	od validation	14
	2.5.1	Limit of Detection (LOD) and Limit of Quantification (LOQ)	14
	2.5.2	Recovery	16
	2.5.3	Precision	17
	2.5.4	Reproducibility	17

CHAPTER 3 METHODOLOGY

3.1	Raw materials and equipments	18
	3.1.1 Reagents and chemicals	18
	3.1.2 Instruments	18
	3.1.3 Apparatus	18
3.2	Experimental work	19

	3.2.1 Condition of	HPLC	19
	3.2.2 Quantitative a	nalysis	19
	3.2.3 Setup of HPL	C	19
	3.2.4 HPLC analys	is of ascorbic acid extract	20
3.3	Analysis method		20
	3.3.1 Preparation of	f mobile phase	20
	3.3.2 Preparation of	f stock and standard solution	21
	3.3.3 Preparation of	f sample	21
	3.3.4 Preparation of	f sample for stability test	22
3.4	Preparation of sample	e for method validation	22
	3.4.1 Recovery		22
	3.4.2 Precision		22

CHAPTER 4 RESULTS AND DISCUSSIONS

4.1	Introduction	
4.2	Solvent system for HPLC analysis	23
4.3	Calibration curve of standard series of ascorbic acid	26
4.4	Analysis of ascorbic acid in tablet P	27
	4.4.1 Stability test for tablet P for wavelength 245 nm	29
	4.4.2 Stability test for tablet P for wavelength 270 nm	30
4.5	Analysis of ascorbic acid in tablet K	31
	4.5.1 Calibration curve of standard series of ascorbic acid	31
	4.5.2 Analysis of tablet K	33
	4.5.3 Stability test for tablet K for wavelength 245 nm	35
	4.5.4 Stability test for tablet K for wavelength 270 nm	36
4.6	Stability of ascorbic acid for both tablets	38
4.7	Method validation	39
	4.7.1 Limit of Detection (LOD) and Limit of Quantification (LOQ)	39
	4.7.2 Recovery	41
	4.7.3 Precision	44
СНА	APTER 5 CONCLUSION AND RECOMMENDATION	50

CITED REFERENCES	52
APPENDICES	55
CURRICULUM VITAE	85

LIST OF TABLES

Table	Caption	Page
2.1	Detector used for analysis of AA form previous studies	11
2.1	Wavelength used in analysis of A A by using HPLC from previous	14
2.2	studios	14
	studies	
4.1	Column used in analysis of AA by using HPLC from previous	24
	studies	
4.2	Mobile phase used for analysis of AA by using HPLC from previous	25
	Studies0	
4.3	Result for amount of AA in original tablet P	28
4.4	Result for stability test of tablet P for wavelength 245 nm	29
4.5	Result for stability test of tablet P for wavelength 270 nm	30
4.6	Result for amount of AA in original tablet K	35
4.7	Result for stability test of tablet K for wavelength 245 nm	36
4.8	Result for stability test of tablet K for wavelength 270 nm	37
4.9	Result for Limit of Quantification and Limit of Detection for	39
	wavelength 245 nm	
4.10	Result for Limit of Quantification and Limit of Detection for	40
	wavelength 270 nm	
4.11	Result for Limit of Quantification and Limit of Detection from	41
	previous studies	
4.12	Result for recovery method for wavelength 245 nm	41
4.13	Result for percentage of recovery method for wavelength 245 nm	42
4.14	Result for recovery method for wavelength 270 nm	42
4.15	Result for percentage of recovery method for wavelength 270 nm	43

ABSTRACT

QUANTITATIVE ANALYSIS OF ASCORBIC ACID IN VITAMIN C RELATED PRODUCTS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Antioxidant has gained interest among consumers and the scientific community regarding its significances in daily life. In this research, quantitative analysis of ascorbic acid in vitamin C related products by using High Performance Liquid Chromatography (HPLC) had been studied. The simple method with isocratic HPLC system had been developed for rapid determination of amount of ascorbic acid in vitamin C related products. A reversed phase High Performance Liquid Chromatography system with stationary phase of Phenomenex C₁₈ column (250 x 4.6 mm) was used for the separation at ambient temperature with 0.1% phosphoric acid as the mobile phase. The system was analyzed at the flow rate of 0.5 ml min⁻¹ at wavelengths of 245 nm and 270 nm with ultraviolet (UV) detector. Successful extraction of ascorbic in samples had been achieved and ascorbic acid was eluted at retention time between 8 minutes to 10 minutes. Both tablets were well separated and the peak was resolved completely. The amount of ascorbic acid for both samples almost similar to the one stated at the label of the bottle which was 1000 mg per tablet. For tablet P, the amount of ascorbic acid was 1021.50 mg for wavelength 245 nm and 990.00 mg for wavelength 270 nm. As for tablet K, the amount of ascorbic acid was 1125.82 mg for wavelength 245 nm and 1113.60 mg for wavelength 270 nm. The stability test had also been done by storing the samples to four storage conditions. The results showed that amount of ascorbic acid were stable for both samples after being stored for six hours. The optimized method was further validated according to The International Conference on the Harmonization of the Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines. The method showed good system suitability, linearity $(r^2 > 0.99)$, recovery (> 20%), precision (percentage of Relative standard deviation, %RSD) and sensitivity (limit of detection and limit of quantification), indicating that the proposed method could be used for quantitative analysis of ascorbic acid in vitamin C related products.