

UNIVERSITI TEKNOLOGI MARA

**DEVELOPMENT AND VALIDATION OF A
GRADIENT HPLC METHOD FOR THE
DETERMINATION OF INSULIN IN RAT
PLASMA: APPLICATION TO
PHARMACOKINETIC STUDY**

EDA FRIDA BINTI SALLEH

**FACULTY OF PHARMACY
MARA UNIVERSITY OF TECHNOLOGY (UiTM)**

JANUARY 2012

ACKNOWLEDGEMENTS

First of all, I would like to express my gratitude to Allah swt for giving me courage and perseverance throughout completion of this research. Secondly, I would like to express my deepest appreciation to my supervisor, Dr. Khuriah Abdul Hamid for her guidance, assistance, comments and advice throughout the whole research. It was indeed a valuable experience and great opportunity to work under her supervision. I also take this opportunity to express my sincere thanks especially to Mr. Tommy Julianto and Mr. Ahmad Mustafa for kindly giving the permission to conduct my study in their laboratory and to all the lecturers of Faculty of Pharmacy, UiTM for their guidance and valuable advice throughout my study.

This study would not have been possible without the kind and ongoing assistance of the postgraduate students and lab assistance especially to Mohd Hafiz, Madam Liza, Miss Noormeliza, Madam Masnira, and Mohd Nur Asmadi, so my grateful thanks also go to them. I would also like to thank my parents for their support, understanding and continuous positive advice while completing this research.

TABLE OF CONTENTS

	Page
TITLE PAGE	
ACKNOWLEDGEMENTS	ii
TABLE OF CONTENTS	iii
LIST OF ABBREVIATIONS	v
LIST OF TABLES AND FIGURES	vii
ABSTRACT	viii
CHAPTER ONE: INTRODUCTION	
1.1 Background	1
1.2 Problem Statement	3
1.3 Objectives	3
CHAPTER TWO: LITERATURE REVIEW	
2.1 Intestinal Drug Absorption	4
2.2 Peptide Drugs	5
2.3 Insulin	6
2.4 Method Validation	9
2.4.1 Linearity	9
2.4.2 Recovery, Accuracy and Precision	10
2.4.3 Limit of Detection (LOD) and Limit of Quantitation (LOQ)	12
2.4.4 Stability	13
2.5 High-Performance Liquid Chromatography (HPLC)	14

CHAPTER THREE: MATERIALS AND METHODS

3.1	Materials	15
3.2	Chromatographic System and Conditions	15
3.3	Preparation of Standard Solutions	16
3.4	Sample Preparation	16
3.5	Method Validation for Determination of Insulin	16
	3.5.1 Linearity	17
	3.5.2 Accuracy and Precision	17
	3.5.3 Limit of Detection and Limit of Quantitation	17
	3.5.4 Stability	18
3.6	<i>In vivo</i> Intestinal Absorption of Insulin	18

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1	Method Validation for Determination of Insulin in Rat	20
	4.1.1 Linearity	20
	4.1.2 Accuracy and Precision	21
	4.1.3 Limit of Detection (LOD) and Limit of Quantitation (LOQ)	23
	4.1.4 Stability	24
	4.1.5 Retention Time	26
4.2	Assessment of Pharmacokinetic Profiles of Insulin	27

CONCLUSION	30
-------------------	----

BIBLIOGRAPHY	33
---------------------	----

ABSTRACT

A simple, specific and sensitive reversed-phase high performance liquid chromatography method was developed to determine insulin in rat plasma. The method involves direct injection of rat plasma sample after deproteinization using acetonitrile - propanol (1:1). The mobile phase comprises a mixture of water and acetonitrile containing 0.1% v/v trifluoroacetic acid (TFA) with gradient analysis by increasing the proportion of mobile phase of water from 25% v/v to 40% v/v within 20 minutes. A Phenomenon C18 column (5 μ m 4.6 x 150 mm) was used for the chromatographic separation which was delivered at a flow rate of 0.6 mL/min at 210 nm wavelength. Under these conditions, the method was validated with respect to linearity, recovery, specificity, accuracy, precision, and stability. The method was proven to be linear over the concentration range of 0.39 - 50.00 μ g/ml with mean correlation coefficient of 0.9994. The mean extraction recovery was 97.4%, while the precision of within-day and between-day measurements was a <9%. The limit of detection (LOD) and limit of quantitation (LOQ) of the method were 0.13 μ g/ml and 0.39 μ g/ml, respectively. The short run time, specificity, sensitivity and reproducibility of this method were found to be satisfactory and thus suitable for routine determination of insulin in rat.