UNIVERSITY TEKNOLOGY MARA

CARBAMAZEPINE METABOLISM BY CYP3A4: *IN VITRO* STUDIES USING NMR SPECTROSCOPY

KHALIDAH BINTI MARUAN

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

Faculty of Pharmacy

NOVEMBER 2008

ACKNOLEDGEMENTS

First of all, I would like to thank God for His blessing I was able to complete this final year project and thesis writing successfully without any major problems. I am deeply indebted to many who rose the occasion and provided extra time, at short notice, to help in the completion of this project. In this regard I am particularly thankful to Dr. Syed Adnan Ali Shah for his kindness, supervision, guidance and especially for his comments, suggestions and encouragements throughout this project.

Special thanks to Prof. Ass. Dr. Teh Ley Kek and Mr. Tommy Julianto who assisted my friends and me. Their supervisory task drove us to be more dedicated throughout the completion of this project.

I am also extremely grateful to many of the staffs of Pharmacogenomic and Institute of Chemistry and Herbal Remedies (iKUS) laboratory especially Miss Ainul Zahiah Abd Malik for providing me with the beneficial knowledge about the equipments in the laboratory and the exact way to use the equipments.

And finally, ventures such as this exact a heavy toll from the families of mine. I thank them for their tolerance of my absences, physically and emotionally. I am blessed and strengthened by their unconditional support and love, and for their sharing with us the belief that my efforts are worthwhile and useful.

TABLE OF CONTENTS

ABSTRACT

The main objective of conducting this study is to identify the metabolites of carbamazepine metabolised by CYP3A4 using various concentrations of carbamazepine and CYP3A4 enzymes at different pH condition and investigate the metabolism through spectroscopic technique. A number of processes involved in order to achieve this objective. First process was enzymatic reaction of carbamazepine and followed by extraction and evaporation in order to isolate pure metabolites. The metabolites were further subjected to Thin Layer Chromatography technique for purification and structural characterization by ^{1}H-NMR. Investigation through ^{1}H-NMR identified the metabolic changes in carbamazepine nucleus and the resulting metabolites formed had been identified as 10,11-dihydro-10-hydroxycarbamazepine and 10,11-dihydrocarbamazepine. Therefore, with the identification of metabolites formed, this study has accomplished its objective as stated above.

CHAPTER ONE

INTRODUCTION

Carbamazepine is one of the most widely prescribed drugs to treat epilepsy. However, a variety of idiosyncratic adverse reactions such as cutaneous, haematological, immunological, renal and hepatic disorders were reported which may result from the formation of chemically reactive metabolites (Pearce et al., 2002).

Numerous researches in the past had been done in order to identify the metabolites that are formed as a result of carbamazepine metabolism. Recent studies have shown that many researches done involving the role of CYP3A4 in metabolizing carbamazepine but not all the studies focused on the enzymatic reaction of different concentration of carbamazepine at different pH.

The approach of conducting this study is to identify the metabolites of carbamazepine metabolized by CYP3A4 by using different concentration of carbamazepine in different pH condition. Thus, with that aim, the primary objectives of this study was to identify and analyze the metabolites of carbamazepine catalyzed by CYP3A4 enzyme at different pH. The structures of metabolites were investigated through ^{1}H-NMR.