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NAVIGATING GENETIC POLYMORPHISMS OF *CYP2C8* USING DENATURING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (DHPLC)

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Candidate's Declaration

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated or acknowledged as reference work. This thesis has not been submitted to any other academic institution or non-academic institution for any other degree or qualification.

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ABSTRACT

Cytochrome P450 2C8 (CYP2C8), which was reported to be polymorphic, plays an important role in the metabolism of several therapeutically important drugs and endogenous substances. The large inter - subjects pharmacokinetics variations were therefore under the great influence of genetic polymorphism of *CYP2C8*. However, the inability to detect the genetic variation despite well known inter - subjects pharmacokinetics variabilities of CYP2C8, suggested that new variants may exist in the population or the current detection methods are not sensitive enough to detect new variants. The ability to identify the genetic variations is important to help predict the different responses in different individuals and maximise clinical drug safety. The aim of the study was to develop and validate a higher throughput screening method for detection of *CYP2C8* polymorphisms. The method would then be used to determine the genetic variations in healthy volunteers and cardiovascular patients in Malaysia. Whole blood was obtained and DNA was extracted from 200 subjects; 100 of healthy volunteers and 100 of cardiovascular patients. Each DNA sample was screened for variations using the PCR and DHPLC method developed. The method was compared with other existing method (allele specific PCR) and validated by direct sequencing. Six variants were detected using the method developed. Two of the variants were found to be novel. In comparison with healthy volunteers and cardiovascular patients (CVS), the frequency of IVS9-24 (C35322T) was lower in healthy volunteers which was 18% compared to 48.5% in CVS patients with a P value of <0.001. Meanwhile in exon 8 of *CYP2C8,* variant Ala410 (C33468T) was only detected in CVS patients with a frequency of 3%. In conclusion, we had successfully developed a higher throughput and automated method for the population study of *CYP2C8* in Malaysia. The presence of significantly higher frequencies of variant IVS -24 among the CVS patients suggests possible risk factor to CVS. However, further studies are required to confirm this finding.

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CHAPTER 3: MATERIALS AND METHODS