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METHOD DEVELOPMENT TO ASSAY THE INHIBITION OF CYP2D6 BY *MORINDA CITRIFOLIA* L. USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Recently, there are many studies that show the interaction between CYP2D6 enzyme and herbs as the use of herbs as alternative and/or complementary therapy is on the rise. In this research, the interaction of CYP2D6 and *Morinda citrifolia* L. was being investigated. But first of all the the sensitive and selective method to analyze bufuralol and its main metabolite, 1'-hidroxybufuralol which were used as a probe had been developed using high-performance liquid chromatographic (HPLC). The chromatogram of both compounds were achieved using Agilent 1200 Series Automatic Injector system, Luna 5u CN 100A (5 µm, 150 x 4.6 mm i.d) column with fluorescence detection set at excitation wavelength 252 nm and emission wavelength 302 nm. The mobile phase consisting of methanol HPLC grade and ammonium acetate (CH_3COONH_4) buffer, pH 3 (30:70, v/v) have been used at a flow rate of 1.0 ml/min. The method was highly specific where other coformulated compounds did not interfere. The results showed good separation of the peaks which were at 7.11 minutes for bufuralol and 3.37 minutes for 1'-hydroxybufuralol. The method was validated for its linearity, accuracy, precision, sensitivity and selectivity. An experimental design was used during validation to evaluate method robustness. The calibration curves in plasma were linear over the range of 2.5–100 ng/ml for 1'-hydroxybufuralol with detection limit of 2.5 ng/ml. The coefficient variation (CV) of the results of within-day precision and accuracy of the drug were <7%. There was no significant difference between inter-day and intra-day studies for 1'-hydroxybufuralol which confirmed the reproducibility of the assay method. The validated method then had been applied for the incubation of bufuralol and CYP2D6.

Keywords: CYP2D6, herb, *Morinda citrifolia* L., bufuralol, 1'-hydroxybufuralol, HPLC, chromatogram.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Recently, there are many studies that show the interaction between bufuralol and herbs as the use of herbs as alternative and/or complementary therapy is on the rise and gaining increasing popularity. When herbs and drugs are taken together, there is a high potential of occurrence for both pharmacokinetic and pharmacodynamic herb-drug interactions that can disturb the drug efficacy and safety (Rossler *et al.*, 2007). As herbal preparations consist of multiple, often unidentified, biological active or inactive constituents, the chance that an interaction for a single drug might be higher towards a complex herbal product compared to another single drug. It is proved that herbs can interfere in the metabolism of drugs in many different ways (Desmeules *et al.*, 1991). To avoid interaction between herbs and drugs, it is necessarily to clearly define the inhibitory potential of different herbs (Iwata *et al.*, 2004).

Studies show drug metabolism is the main factor governing the efficacy, duration of action and toxicity of drugs. However, it is a highly variable process, regulated by one or more genes and modified by environmental factors (Lennard, 1985). Bufuralol, a non-selective β-adrenoreceptor antagonist is metabolized by CYP2D6