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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT TO DETERMINE INHIBITION OF CYP2D6 BY *ANDROGRAPHIS PANICULATA*

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ABSTRACT

This paper describes the development of an HPLC method for the determination of Bufuralol and 1-Hydroxybufuralol. Separations were achieved by using a Luna CN C18 column with Fluorescent detection (excitation 252nm and emission 302nm) for quantification. The mobile phase consists of 70% 10m*M* Ammonium Acetate (pH 3) and 30% Methanol. The method was validated for linearity in three days assay. The peak of Bufuralol was observed at 7.1 minutes and 1-Hydroxybufuralol at 3.2 minutes. This method produced narrower and taller peaks for both samples. Besides, this method was used to detect andrographolide, isoandrographolide, neoandrographolide and 14-deoxy-11,12-didehydroandrographolide in commercial product and plant materials of *Andrographis paniculata* (Hempedu bumi). Andrographolide is believed to prevent free radical production and the peak was observed at 2.4 minutes, while Isoandrographolide was reported to possess cell differentiating inducing activity and for this compound the peak was observed at 2.8 minutes. Neoandrographolide was reported to possess anti-hepatotoxicity and the peak was observed at 2.1 minutes. On the other hand, 14-deoxy-11,12-didehydroandrographolide's peak was observed at 4.0 minutes. This compound shows marked hypotensive effects. In this method, the *Andrographis paniculata* components were eluted faster than the previous researches. However, the effect of these components on Bufuralol metabolism by CYP2D6 enzyme cannot be concluded as the enzyme did not metabolized the substrate under *in vitro* condition.

CHAPTER 1

INTRODUCTION

Herbal and natural products of folk medicine have been used for centuries in every culture throughout the world. Hempedu bumi or *Andrographis paniculata* is one of the examples. In the system of medicine, it has been used as a treatment of gastric disorders, infectious diseases and common cold. Pharmacological research has demonstrated that *Andrographis paniculata* possesses anti-inflammatory, anti-allergic, anti-diabetic, anti-hypertension and recent study has found anti-HIV and inhibitory effect on platelet aggregation of the plant. (Li & Fitzloff, 2004).

All these therapeutic effects have made *Andrographis paniculata* as a choice for alternative treatment in system of medicine. However, in depth study on the specific catabolism path way of this herb has not been conducted. Patient that consumes the plant preparations is believed to have an indicated illness and take the herb as a supplement besides other prescribed drug. The herb action on specific enzyme has not been studied in detail and the effect on metabolism of other drugs which is metabolized by the same enzyme cannot be concluded.