International Journal of Chemistry; Vol. 6, No. 3; 2014
ISSN 1916-9698 E-ISSN 1916-9701
Published by Canadian Center of Science and Education

Determination of Safrole in Ethanol Extract of Nutmeg (*Myristica fragrans* Houtt) Using Reversed-Phase High Performance Liquid Chromatography

Febrina Amelia Saputri[1], Mutakin[1], Keri Lestari[2] & Jutti Levita[1]

[1] Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang Km. 21, Jatinangor, West Java, Indonesia

[2] Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang Km. 21, Jatinangor, West Java, Indonesia

Correspondence: Jutti Levita, Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang Km 21, Jatinangor, West Java, Indonesia. E-mail: jutti.levita@unpad.ac.id

Received: February 17, 2014 Accepted: April 27, 2014 Online Published: June 28, 2014

doi:10.5539/ijc.v6n3p14 URL: http://dx.doi.org/10.5539/ijc.v6n3p14

Abstract

Dehydrodiisoeugenol (DDIE), myristicin, and safrole are chemical compounds contained in fruit and seed of nutmeg (*Myristica fragrans* Houtt). DDIE shows antidiabetic activity on PPARγ receptor, while myristicin is hallucinogenic agent. Of the three compounds, safrole is the most toxic substance due to its carcinogenic activity. In this work, we developed an analytical method to determine safrole in ethanol extract of nutmeg. Reversed-Phase High Performance Liquid Chromatography (Dionex Ultimate 3000) using C-18 LiChroCART 250-4, LiChrospher 100 RP 18e (5 µm) 250 mm column as stationary phase, was selected as the method of analysis. A mixture of methanol:water (73:27) at flow rate 1 mL/min was used as mobile phase. Detection was done at 282 nm. Using such conditions, retention time for safrole was 10.45 minutes. The recovery was 101.421%, while the value of CV was 0.838%. LOD and LOQ were 0.668 µg/mL and 2.023 µg/mL, respectively. Mean concentration of safrole in the nutmeg seed extract was 10.979%.

Keywords: HPLC, safrole, validation

1. Introduction

Dehydrodiisoeugenol (DDIE), myristicin, and safrole are chemical compounds contained in fruit and seed of nutmeg (*Myristica fragrans* Houtt). DDIE shows antidiabetic activity on PPARγ receptor, while myristicin is hallucinogenic agent. Of the three compounds, safrole is the most toxic substance due to its carcinogenic activity (Lestari, 2010; Li & Yang, 2012). Safrole and isosafrole (0.5%) has been shown to increase the occurance rate of malignant tumours in mice (Benedetti, Malnoe, & Broillet, 1977). The major toxicity of safrole is caused by its metabolite character. Safrole is oxidized into 1-hydroxysafrole in human body, which is carcinogenic (Peele Jr. & Oswald, 1978). The maximum dose of safrole as stated by UK and French governments is 1 mg/day (European Commission, 2002).

Currently Indonesian pharmaceutical industry is developing a formula of nutmeg extract as antidiabetic drug, based on its DDIE activity which inhibits PPARγ receptor, therefore a rapid and accurate method for the quantification of safrole in nutmeg extract is interesting to be developed. At present, methods of analysis of safrole are HPLC and GC (Archer, 1988; France, Association Francaise de Normalisation, 1986; Choong & Lin, 2001). AOAC described a procedure to determine safrole and isosafrole in soft drinks, where both compounds have to be distilled with steam, extracted with organic solvent, e.g $CHCl_3$, and then injected into GC column for separating and analyzing steps. HPLC using small particles with a high-pressure pump system and sensitive detector. Advantage of HPLC is to provide high-resolution, efficient and fast separation (Skoog, Holler, & Nieman, 1992; Willard, Merrit Jr., Dean, & Settle Jr., 1988; Nagore, Vinod, Pankaj, & Tushar, 2013; Chan, Herman, Lee, & Zhang, 2004). The aim of this study was to determine the concentration of safrole in ethanol extract of nutmeg.