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Short communication

Quantification of nerolidol in mouse plasma using gas chromatography–mass spectrometry

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ABSTRACT

Nerolidol is a naturally occurring sesquiterpene found in the essential oils of many types of flowers and plants. It is frequently used in cosmetics, as a food flavoring agent, and in cleaning products. In addition, nerolidol is used as a skin penetration enhancer for transdermal delivery of therapeutic drugs. However, nerolidol is hemolytic at low concentrations. A simple and fast GC–MS method was developed for preliminary quantification and assessment of biological interferences of nerolidol in mouse plasma after oral dosing. Calibration curves were linear in the concentration range of 0.010–5 μg/mL nerolidol in mouse plasma with correlation coefficients (r) greater than 0.99. Limits of detection and quantification were 0.0017 and 0.0035 μg/mL, respectively. The optimized method was successfully applied to the quantification of nerolidol in mouse plasma.

1. Introduction

Nerolidol (3,7,11-trimethyl-1,6,10-dodecatrien-3-ol) is a sesquiterpene alcohol found in essential oils from several plants [1]. It is frequently used in cosmetics (*e.g.*, shampoos and perfumes) and in non-cosmetic products (detergents and cleansers). Nerolidol is approved by the U.S. Food and Drug Administration as a food flavoring agent [2]. Many medicinal benefits of nerolidol have been identified including anti-tumor [3,4] and anti-bacterial properties [1]. Generally, terpenes are considered potent skin permeation enhancers with low toxicity and nerolidol has been tested as a skin permeation enhancer for transdermal delivery of therapeutic drugs [5,6]. In addition, nerolidol has demonstrated activity against several parasites including *Leishmania* [7], *Trypanosoma* [8,9], *Plasmodium* [10], *Schistosoma* [11] and *Babesia* [12]. *Leishmania amazonensis*-infected BALB/c mice treated with nerolidol significant reduction of lesion sizes was observed [7].

The analytical technique that is often used for nerolidol detection is gas chromatography–flame ionization detection (GC–FID) or gas chromatography–mass spectrometry (GC–MS) [13]. Although GC alone is the most commonly used method for detection of terpene olefins, the retention time or retention index alone is often not sufficient for positive confirmation of a particular terpene. Sesquiterpene olefins that possess boiling points ranging from ~250 to 280 °C, are ideal candidates for GC–MS analysis and it is generally considered sufficient for positive identification of a terpene when the sample is run in conjunction with an authentic standard [14].

Detection of nerolidol is usually related to studies of essential oils from plants and, to our knowledge, quantification of nerolidol in mouse plasma has not been reported. We previously reported that nerolidol has demonstrated activity against *in vitro Plasmodium falciparum* with a maximum inhibitory concentration (IC_{50}) of 760 nM (0.169 μg/mL) [10]. The aim of this present study was developing a simple and fast GC–MS method for preliminary quantification and assessment of biological interferences of nerolidol in mouse plasma after oral dosing to aid future malaria efficacy studies. In addition, nerolidol is hemolytic at low concentrations [15]; therefore, having a robust platform to quantify nerolidol levels in plasma after administration of a therapeutic dose is of great value. The analytical performance of the method was evaluated and successfully applied to the quantification of nerolidol in mouse plasma.

2. Materials and methods

2.1. Chemicals and reagents

Cis-/*trans*-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol (*cis*-/*trans*-nerolidol, 1:3, w/w) was obtained from Sigma–Aldrich (St. Louis,

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MO, USA). The 3,7,11-trimethyldodeca-2,6,10-trien-1-ol (farnesol) obtained from Sigma–Aldrich (St. Louis, MO, USA) was used as the internal standard (IS). Acetonitrile, methanol, and *n*-hexane (HPLC grade) were purchased from Fisher Scientific (Pittsburgh, PA, USA).

2.2. Instrumentation and GC–MS conditions

Separations and analyses were performed using a Trace GC gas chromatography system in tandem with a mass spectrometer Y2K ion trap (MS) PolarisQ System (Finnigan, ThermoQUEST Inc., San Jose, CA, USA). Data acquisition and analyses were performed using Xcalibur V1.4. A TR-5MS capillary column (30 m × 0.25 mm × 0.25 μm, Thermo Scientific, USA) was used for this study. The temperature of the GC injector was 220 °C, equipped with a splitless liner. The GC column temperature was set to 60 °C for 1 min and programmed to ramp up to 220 °C at a rate of 40 °C per min, at which point this temperature was maintained for 2 min and then cooled to initial conditions. The total run time was 7 min. The transfer line was maintained at 280 °C and helium flow was 1 mL min^{-1}. For mass spectrometry detection, ionization was carried out by electronic impact (EI) with a voltage of 70 eV and full scan mode in the *m*/*z* range of 20–300 with an ion source temperature of 200 °C in positive ion mode. For quantification of nerolidol and farnesol (IS) characteristic ions for terpenes at *m*/*z* 93 and 161 were monitored by selected ion monitoring (SIM) mode as described previously for qualitative analysis [16,17]. The nerolidol standard used was a mixture of *cis*- and *trans*-nerolidol (1:3, w/w). *cis*-Nerolidol peak was used to evaluate the limit of detection (LOD) and lower limit of quantification (LLOQ). In order to quantify nerolidol in plasma from treated mice, the MS signals of both isomers were added to calculate the metabolite:internal standard ratio to construct the calibration curve and to report the total concentration of nerolidol in mouse plasma.

2.3. Sample preparation

Ten μL of *n*-hexane and 10 μL of an IS stock solution of 2 μg/mL in *n*-hexane were added to a glass tube containing 0.1 mL of plasma at room temperature. Then, 200 μL acetonitrile were added to precipitate proteins and samples were vigorously mixed for 3 min with a vortex followed by centrifugation at 1000 × *g* for 10 min at 4 °C. Supernatants were transferred to a new glass tube and 100 μL of *n*-hexane were added. Samples were mixed with a vortex for 5 min and centrifuged at 1000 × *g* for 10 min at 4 °C. The *n*-hexane layer was transferred to a new glass tube and 5 μL were injected for GC–MS analysis.

2.4. Evaluation of the analytical performance

The analytical performance of the method was based on the guidelines of the United States FDA for method validation [15].

2.4.1. Linearity

Calibration curves consisted of different concentrations of nerolidol added to pooled plasma from mice at a final concentration ranging from 0.010 to 5 μg/mL. A blank sample (plasma from an animal that did not receive nerolidol and processed without IS) and a zero sample (plasma from an animal that did not receive nerolidol and processed with IS) were also analyzed. Ten μL of IS solution (2 μg/mL) were added to samples and processed as indicated in Section 2.3. The correlation between the nerolidol concentrations and the nerolidol:internal standard area ratio of MS signals detected by SIM were obtained with Origin® v8.5 software. Dynamic range (linearity) was determined by linear regression and was considered acceptable when linear coefficient correlation of Pearson® was equal or higher than 0.98. To determine the LOD and LLOQ, different concentrations of *cis*-nerolidol were added to pooled plasma from mice at a final concentration ranging from 0.05 to 50 ng/mL. LOD was defined as three times the signal-to-noise ratio and LLOQ was defined as 10 times the signal-to-noise ratio of *cis*-nerolidol. The detected signal of the IS was four times the signal-to-noise.

2.4.2. Matrix effect, precision and recovery

One replica of QC samples spike with nerolidol before extraction at 0.09, 2, and 4 μg/mL were processed as described in Section 2.3 and analyzed on six different days to determine inter- and intra-day precision and accuracy. Assessment of matrix effect on nerolidol quantification was calculated by ratio between peak area values obtained in the test of intra-day precision and the peak area values of reference standard solutions of the same concentration without plasma.

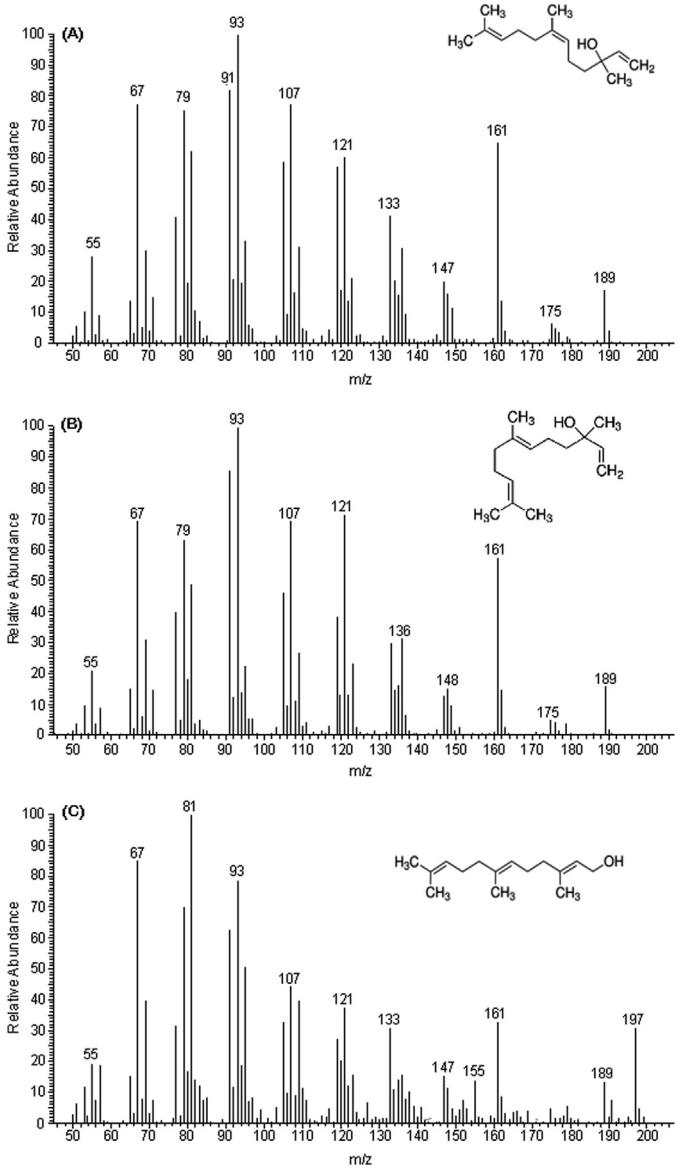

Fig. 1. GC–MS chromatograms of (A) plasma from an animal that did not receive nerolidol and was processed without IS (blank), (B) plasma from an animal that did not receive nerolidol and was processed with IS, and (C) plasma from an animal that received a single oral dose of 1000 mg/kg of nerolidol three hours before blood sample collection and was processed with IS.