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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.

Keywords

Nerolidol; Sesquiterpene; Plasma; GC–MS; Quantification

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]

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¹In memoriam.

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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 (IC₅₀) 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, 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 $\mu\text{g/mL}$ 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 $1000 \times g$ 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 $1000 \times g$ 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 $\mu\text{g/mL}$. 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 $\mu\text{g/mL}$) 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 $\mu\text{g/mL}$ 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.

2.5. Animals

BALB/c mice were bred and housed in specific pathogen-free facilities of the Instituto de Ciências Biomédicas II (USP, Brazil). Experiments were performed in adult males, between 4 and 5 weeks of age. All animals were fed a standard diet and all procedures were in accordance with national regulations on animal experimentation adopted by the Brazilian Society of Animal Science Laboratory (SBCAL) and authorized by the Ethics Committee in Animal Experimentation (ECAE, ICB, USP), process no. 140/2009.

2.6. Quantification of nerolidol in mice plasma

Three male BALB/c mice, ranging from 4 to 5 weeks old and weighing 20 ± 2 g received a single oral dose of 1000 mg/kg of nerolidol. The oral administration was done by gavage technique. Blood samples were taken at 30 min, 1, 2, 3, 4, 5, 6, 8, and 12 h after oral dosing. Plasma was separated from blood cells by centrifugation at $3000 \times g$ for 10 min at 4°C and stored at -20°C for 72 h before being processed as indicated in Section 2.3. A one-way analysis of variance (ANOVA) and post-test comparison (Bonferroni *post hoc*) were used for statistical analysis. The Mann–Whitney test was used for comparison of different time points. GraphPad Prism[®]5 (GraphPad Software, California, USA) and Origin[®] v8.5 (OriginLab, Northampton, USA) software were used for data analysis.

3. Results and discussion

3.1. Optimization of the GC–MS conditions

Despite the broad use of nerolidol, most of the reported analytical techniques focus on detection and semi-quantification of this sesquiterpene from natural products [14,18]. For example, quantitative determination of nerolidol in tea bags by GC–MS has been previously reported using a headspace solid phase micro extraction and gas chromatography–flame ionization detection method [13]. The present study aimed to optimize a simple and robust GC–MS method to quantify nerolidol in plasma for preliminary quantification and assessment of biological interferences of nerolidol in mouse plasma after oral dosing to aid future malaria efficacy studies. The GC–MS chromatograms of *cis*-nerolidol, *trans*-nerolidol, and IS are shown in Fig. 1. The retention time of *cis*-nerolidol, *trans*-nerolidol, and IS were 5.87, 5.98, and 6.61 min, respectively. The total run time for our method was only 7 min, which is shorter than those reported previously [13]. Farnesol was selected as the internal standard (IS) for its similarity in structure and molecular weight to nerolidol, having similar retention time and solubility in the extraction solvent. Nerolidol and farnesol were monitored by selected ion monitoring (SIM) mode at m/z 161 and m/z 93, the dominant and characteristic ions as reported previously [16,17] (Fig. 2).

3.2. Analytical performance

3.2.1. Linearity and sensitivity—The LOD, LLOQ, and linearity for nerolidol were evaluated using the optimized GC–MS method. The correlation coefficient (r) for all calibration curves was >0.99 indicating good correlation between the concentration and the metabolite:internal standard ratio of MS signals within the tested ranges (Table 1). Calibration curves were linear in the concentration range of 0.010–5 $\mu\text{g/mL}$ nerolidol in mouse plasma. To determine 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 0.0017 µg/mL while the LLOQ was 0.0035 µg/mL under the analytical conditions described above.

3.2.2. Matrix effect, precision, and accuracy—The matrix effect and the possibility of ionization suppression or enhancement were evaluated. Matrix interference on nerolidol quantification in mouse plasma was determined using QC samples at low (0.09 µg/mL), medium (2 µg/mL), and high (4 µg/mL) concentrations (Table 2). As indicated in Table 2, the highest matrix effect was observed at 4 µg/mL of nerolidol (84.1%) as compared to an authentic standard solution of nerolidol in the absence of plasma. The precision (% of relative standard deviation, %RSD) was below 10.3% (Table 2), showing no significant ion suppression or enhancement due to the sample matrix (mouse plasma).

Intra-day precision (% RSD values) ranged between 6.7 and 12.2% (Table 3) and the accuracy ranged from 0.4 to 13.9%. Inter-day variation presented similar values to intra-day variation and ranged between 7.6 and 11.6%. The accuracy ranged from 0.2 to 9.3% (Table 3). The % RSD values were, in general, below 14%; thus, the method is reproducible and was successfully applied to nerolidol quantification in plasma from orally treated mice.

3.3. Quantification of nerolidol in mice plasma

The concentration of nerolidol in mouse plasma following a single oral dose of 1000 mg/kg reached the maximum concentration of $\sim 0.27 \pm 0.07$ µg/mL within 30 min after administration and remained constant for up to 3 h after administration, reaching a maximum concentration of $\sim 0.35 \pm 0.05$ µg/mL after 6 h of administration (Fig. 3). The concentration of nerolidol in plasma after 6 h was twice the *in vitro* *P. falciparum* IC₅₀ value (0.169 µg/mL) [10]. Moreover, the maximum concentration detected was ~ 1460 times lower than the concentration required to induce 50% hemolysis (~ 511 µg/mL) [15]. After 12 h of oral administration, the concentration of nerolidol in plasma decreased to near zero. To our knowledge, this is the first report of nerolidol quantification in mouse plasma following oral dosing of 1000 mg/kg.

4. Conclusion

The present study aimed to develop a simple and fast GC–MS method for preliminary quantification and assessment of biological interferences of nerolidol in mouse plasma after oral dosing. The developed method is rapid, simple, and shows satisfactory analytical performance. The optimized GC–MS was successfully applied to the quantification of nerolidol in mouse plasma. Our quantitative assessment of nerolidol in mouse plasma will aid future efficacy and safety studies of nerolidol as a potential antimalarial. Moreover, our results revealed that the maximum concentration in mouse plasma after oral dosing was below the hemolytic concentration supporting its safety at the dose tested of 1000 mg/kg. On the other hand, the developed method is suitable for monitoring absorption of nerolidol from therapeutic formulations, cosmetics, and food.

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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.

Fig. 2.
Mass spectra of nerolidol and the IS (farnesol). (A) *cis*-Nerolidol and (B) *trans*-nerolidol at m/z 189 and (C) IS at m/z 197.

Fig. 3. Nerolidol detected over time in plasma of animals treated with a single oral dose of 1000 mg/kg ($n = 3$).

Table 1

Linear regression parameters of calibration curves of nerolidol in mouse plasma.

	Mean $\pm$ SD ($n = 3$)
Slope	4.9 ± 0.2
Intercept	1.1 ± 0.6
Correlation coefficient (r)	0.994 ± 0.002

Table 2Matrix effect of mouse plasma on nerolidol quantification ($n = 6$).

Concentration added ($\mu\text{g/mL}$)	Concentration detected (mean $\pm$ SD, $\mu\text{g/mL}$)	% related to standard solution	RSD (%)
0.09	0.093 ± 0.006	103.1	7.2
2	1.8 ± 7.4	92.6	3.7
4	3.36 ± 0.04	84.1	10.3

Table 3
Intra-day and inter-day precision and accuracy of nerolidol quantification in mouse plasma.

Concentration added ($\mu\text{g/mL}$)	Intra-day ($n = 3$)			Inter-day ($n = 6$)		
	Concentration detected (mean $\pm$ SD, $\mu\text{g/mL}$)	Precision (% RSD)	Accuracy (% RSD)	Concentration detected (mean $\pm$ SD, $\mu\text{g/mL}$)	Precision (% RSD)	Accuracy (% RSD)
0.09	0.08 $\pm$ 0.01	12.2	8.9	0.09 $\pm$ 0.006	7.6	0.2
2	2.27 $\pm$ 0.06	8.6	13.9	2.0 $\pm$ 0.2	11.6	1.1
4	3.98 $\pm$ 0.26	6.7	0.4	4.4 $\pm$ 0.4	10.2	9.3