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Optimization of headspace solid-phase microextraction for analysis of β-caryophyllene in a nanoemulsion dosage form prepared with copaiba (*Copaifera multijuga* Hayne) oil

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ABSTRACT

Recent studies have shown the anti-inflammatory activity of Copaiba oils may be addressed to the high content of β-caryophyllene, the most common sesquiterpene detected, especially in the *Copaifera multijuga* Hayne species. In the present study, nanoemulsions were proposed as a delivery system for copaiba oil in view to treat locally inflamed skin. This article describes the optimization and validation of a stability-indicating SPME-GC method, for β-caryophyllene analysis in the nanoemulsions produced by high pressure homogenization. SPME methods are performed with PDMS (polydimethylsiloxane) fiber (100 μm). Three SPME parameters were evaluated by a three-level-three-factor Box–Behnken factorial design as potentially affecting the technique efficiency. According to the results obtained, the best conditions to extract β-caryophyllene were: (i) sampling temperature of 45 °C, (ii) sampling time of 20 min and (iii) no NaCl addition. Results coming from the forced degradation tests showed a reduction of β-caryophyllene peak area when both caryophyllene methanolic solution and nanoemulsions were exposed to acid hydrolysis, UV-A irradiation, oxidative (H_2O_2) and thermolitic (60 °C) conditions. Such reduction occurred in lower extent in the nanoemulsions, suggesting a protective effect of the formulation to β-caryophyllene content. Since no degradation products were detected in the same retention time of β-caryophyllene, the specificity of the method was demonstrated. The method was linear in the range of 0.14–0.68 μg mL^{-1} of β-caryophyllene ($r^2 > 0.999$), and was also validated for precision (R.S.D. ≤ 5.0%), accuracy (97.85–101.87%) and robustness. Finally, the method was applied to quantification of β-caryophyllene content in the developed formulations.

1. Introduction

Copaiba oil is obtained by tapping the trunk of trees of the *Copaifera* L. (Leguminoseae) species and is widely used in the Brazilian folk medicine, especially as anti-inflammatory, antiseptic and healing agent [1]. It is an oleoresin composed by a solution of diterpene acids in an essential oil having sesquiterpenes as mainly constituents, being β-caryophyllene (Fig. 1) the most common sesquiterpene detected [1–3].

The anti-inflammatory activity of Copaiba oils was confirmed by several studies [4–7] and according to Veiga Jr. and co-workers [7], this activity in *Copaifera multijuga* Hayne species may be addressed to the presence of high concentrations of β-caryophyllene (>50%), which has been described as an important anti-inflammatory compound [8,9].

However, the water-insoluble nature of copaiba oil impairs its use as a topical anti-inflammatory medicine. Moreover, the development of a topical dosage form may confer benefits with respect to oil stabilization. The use of nanoemulsions as colloidal carriers for the topical delivery of water insoluble drugs/molecules has gained popularity over the last years [10–13]. The large surface area and low surface tension of the oil nanodroplets adequately dispersed in the aqueous system may increase the skin permeation ability and enhance the topical effect due to a prolonged residence in the uppermost skin layers [14,15]. In this context, the development of a nanoemulsion from copaiba oil (*C. multijuga* Hayne) is a promising strategy to treat locally inflamed skin.

A literature survey reveals the existence of several studies of chemical composition of *Copaifera* species by gas chromatography with flame ionization detection (GC-FID) and/or gas

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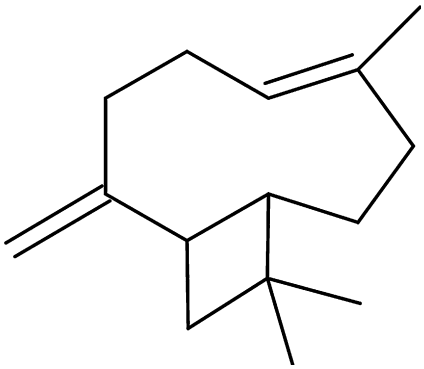

Fig. 1. Chemical structure of β-caryophyllene.

chromatography coupled with mass spectrometry (GC–MS) [1,2,16–18]. However, in a nanoemulsion sample matrix, the presence of water may adversely affect GC analysis (e.g. large vapor expansion volume, detection problems and chemical damage to the stationary phase) and the presence of surfactants impair the use of traditional liquid extraction with solvents due to emulsification. In addition, the use of high temperature in the sample preparation step can promote β-caryophyllene oxidation.

In this context, headspace solid-phase microextraction (HS-SPME) has received increasing attention as a rapid, simple, solvent free procedure that enables to extract analytes directly from a liquid sample matrix. This technique involves exposing a polymer-coated fiber to the headspace of sample matrix where analytes absorb onto the fiber, which is then inserted into a heated gas chromatograph injector where the analytes are desorbed and swept directly onto the column [19–21].

There is no report concerning the determination copaiba oil components by means of SPME as a sample preparation technique. In addition, there is only one reference regarding SPME-GC determination of an analyte in a nanoemulsion dosage form, using a method developed by our research group [21]. In the present study, a stability indicating SPME-GC method was optimized and validated for β-caryophyllene assay in nanoemulsion formulations prepared with copaiba oil (*C. multijuga* Hayne).

2. Materials and methods

2.1. Chemicals and reagents

This oleoresin from *C. multijuga* Hayne was collected in the Adolpho Ducke Forest Reserve of the Instituto Nacional de Pesquisas da Amazônia (INPA) at Manaus, Amazonas State (Brazil), and the exsiccate were deposited at the INPA herbarium. It was obtained by artificial exudation following the usual method [7,22–24]. Briefly, a metal auger was used to perforate the trunk about 1 m above soil level. After the oil ceased to drain, the hole was sealed in the trunk of the tree by introducing a 20 cm long PVC pipe into the auger hole and hermetically sealing the pipe with a PVC cap. After esterification with diazomethane, the oleoresin had its chemical composition characterized by gas chromatography coupled with mass spectrometry (GC–MS) using a method previously described [7]. The complete sesquiterpene and diterpene composition of this oil is disclosed in Table 1. β-Caryophyllene reference standard was purchased from Fluka Analytical Co. (St. Louis, MO, USA). Medium Chain Triglycerides (MCT) was kindly donated by Lipoid GmbH (Ludwigshafen, Germany). Span 80® and Tween 20® were obtained from Sigma–Aldrich (St. Louis, MO, USA). Ultrapure water was obtained from a Milli-Q® apparatus (Millipore, Billerica, USA). All other chemicals or reagents were of analytical grade.

Table 1
Components identified in the oleoresin of Copaiba oil (*Copaifera multijuga* Hayne).

Substances detected	IR	(%)
α-Cubebene	1345	0.68
α-Ylangene	1373	0.10
α-Copaene	1374	5.65
β-Elemene	1389	1.15
α-Gurjunene	1409	0.18
β-Caryophyllene	1417	29.82
Trans-α-bergamotene	1432	0.22
α-Humulene	1452	4.27
α-Amorphene	1483	4.48
Germacrene D	1484	15.86
β-Selinene	1489	0.20
α-Muurolene	1500	3.17
γ-Cadinene	1513	1.57
δ-Cadinene	1522	5.01
Caryophyllene oxide	1582	0.28
Globulol	1590	0.22
Viridiflorol	1592	0.38
Ledol	1602	0.21
α-Muurolol	1644	1.61
α-Cadinol	1652	2.10
Copalic acid		7.09
3β-Hydroxy-copalic acid		0.97
3β-Acetoxy-copalic acid		0.95
Pinifolic acid		4.22
Total		90.39

2.2. Preparation and characterization of nanoemulsions

Firstly, Span 80® (1%, w/w) was dispersed in the oil, which consisted of either copaiba oleoresin (10%, w/w) or a mixture of MCT:oleoresin 1:1 (10%, w/w). Tween 20® (2%, w/w) was dispersed in water (made up to 100%, w/v). Then, both oil and water phases were mixed under magnetic stirring (5 min, at room temperature) to obtain a coarse emulsion. Afterwards, the coarse emulsions (Emulsion #1 composed by only copaiba oil and Emulsion #2 composed by copaiba oil and MCT) were individually subjected to high-pressure homogenization (EmulsiFlex-C3®, Avestin, Canada) at 750 bars to get the final emulsion. The particle sizes and polydispersity indices ($n = 3$) were measured by photon correlation spectroscopy after adequate dilution of an aliquot of the samples in purified water (Zetasizer Nanoseries, Malvern Instruments, Worcestershire, UK). The zeta potential values were measured using the same instrument at 25 °C, after dilution of 10 μL in 10 mL of NaCl (1 mM) ultra-filtered (0.22 μm).

2.3. Instrumentations and chromatographic conditions

The samples were analyzed using a Shimadzu CG-FID 2010 (Kyoto, Japão), consisting of a split/splitless injector port and a flame ionization detector (FID). The injection was made in the split mode (20:1). The signal was recorded and processed with Labsolutions GC-Solution software. The GC system was equipped with a DB-5 column (30 m × 0.25 mm × 0.25 mm). The carrier gas was nitrogen (1.0 mL min^{-1}). The oven temperature was programmed from 60 to 160 °C at 40 °C min^{-1}, from 160 to 200 °C at 10 °C min^{-1}, and from 200 to 240 °C at 40 °C min^{-1}, finalizing the chromatographic run at 12 min. Injector and detector temperatures were set at 250 and 300 °C, respectively.

SPME devices and fiber assemblies (100 μm polydimethylsiloxane) were obtained from Supelco (Bellefonte, USA). Before their first use, SPME fibers were conditioned into GC injector at 150–300 °C for 30 min, according to the manufacturer's recommended procedure. Then, the analyte and a magnetic stirring bar were added to the vial, which was subsequently sealed with Parafilm®. The vial was placed in a thermostated bath. After this, the SPME fiber was inserted into the headspace for 20 min. After