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A validated ^{1}H NMR method for quantitative analysis of α-bisabolol in essential oils of *Eremanthus erythropappus*

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ABSTRACT

α-Bisabolol is a natural terpene produced by *Eremanthus erythropappus* and is widely used in cosmetics and pharmaceuticals due to its anti-inflammatory, antibacterial and antimycotic properties. Due to these applications, a control of composition and authenticity of commercial oils rich in this terpene is required, resulting in a demand for new methodologies for quality control. In this work a rapid and efficient method for quantification of α-bisabolol in the essential oil of *E. erythropappus* (candeia) using ^{1}H NMR was developed, validated and compared to gas chromatography (GC) method. The quantification of α-bisabolol by ^{1}H NMR was successfully achieved for most of the essential oil samples of *E. erythropappus* evaluated, except for those with a more complex composition. To circumvent this limitation a 2D NMR COSY contour map was used. This method proved to be a fast and efficient alternative, providing results with standard deviations SD < 0.3%. All evaluated parameters (selectivity, linearity, accuracy/precision, repeatability, robustness and stability of analyte and internal standard in solution) gave satisfactory results. Using the ^{1}H NMR signals at 5.36 and 5.13 ppm, the limit of detection (LOD) and limit of quantification (LOQ) were 0.26 and 2.59 mg, respectively. The results obtained by the ^{1}H NMR method presented SD = 0.59%, smaller than the value found for GC (SD = 1.18%). Tukey tests have shown that the results obtained by ^{1}H NMR and COSY methodology are similar to the obtained by the traditional GC-FID technique using external and internal standardization and normalization with 95% confidence.

1. Introduction

The quality of essential oil has been widely evaluated by gas chromatography coupled to mass spectrometry (GC-MS) and gas chromatography equipped with a flame ionization detector (GC-FID) [1,2]. Typically, analyses by these techniques takes around 90 min and this is a limiting factor in situations where time is of the essence. The quantification of the major constituents of commercial essential oils is vital because they are extracted from different sources and their price varies with their content [3,4]. The essential oils are composed by one or more major components present in high concentrations, as for example, citral [5] and 1,8-cineole [3] in *Cymbopogon citratus* and *Eucalyptus* which should be in at least 60% and 70% respectively for medicinal purposes.

The quality control of drugs [6], alcoholic beverages and fermented foods, coffee, tea, fruits, vegetables, olive oils, milk and dairy products, honey, fish [7,8], meat [7–9] and gasoline [10] has been evaluated by quantitative hydrogen nuclear magnetic resonance (qNMR). In addition, NMR enables the determination of the ratio of diasteroisomers and enantiomers using different displacement reagents in a sample, and the simultaneous quantification of analytes, impurities, and residual solvents [6].

The extraction of carbohydrates using solid phase extraction (SPE) followed by simultaneous determination of the alkaloids codotubulosine A and B from Radix Codonopsis plants was accomplished by using ^{1}H NMR [11].

Affinity capillary electrophoresis (ACE) and nuclear magnetic resonance spectroscopy (NMR) were used in the study of the complexation of risperidone and 9-hydroxyrisperidone with seven cyclodextrins in pH 2.5 and 7.4. The stoichiometry of the complexes, the binding constants of cyclodextrins that allow greater complexation, and the proposal for a mechanism dependent on pH were established by ^{1}H NMR. The influence of pH on

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thermodynamic of complexation was evaluated by ACE [12].

In the literature only reports using ^{1}H NMR to determine the active components of Brickellia weronicaefolia [13], *Eucalyptus*, *Corymbia*, *Schinus terebinthifolius* and turpentine essential oils [14] were encountered. However, the use of ^{13}C NMR for this purpose is far more explored in the literature.

^{13}C NMR experiments were used to quantify dipropylene glycol in perfume extracts [15], and eudesmane-type acids in the essential oil of *Dittrichia Viscosa* sp. [16]. In addition, the combination of GC-FID and ^{13}C NMR techniques were used for to determine the content of ascaridole and isoascaridole in *Chenopodium ambrosioides* essential oil [17], and of germacrenes and elemenes in *Cleistopholis patens* essential oils [18].

The main condition for using NMR quantification is that at least one of the signals of the analyte does not overlap with any other signal from the sample. However, the separation of the signals can be achieved by adjusting parameters and conditions such as solvent, pH, concentration of sample, temperature and addition of lanthanide shift reagents and auxiliary reagents such as cyclodextrins [6].

Unlike chromatography, NMR is a quick and non-destructive method, and it does not require calibration curves [19,20]. The NMR method allows simultaneous quantitation of many components using one internal standard and a single spectrum [19,21]. Moreover, it is not necessary to employ a high purity internal standard [22] that it is similar to the analyte of interest for accurate quantification. Also, NMR can be used for quantification of compounds that degrade at elevated temperatures which cannot be determined by GC [17,23].

However, NMR has low sensitivity which has demanded the use of high magnetic field spectrometers. In addition, the accuracy/precision of the qNMR is influenced by the quality of the shimming, the stability of the magnetic field [6], by the phase and baseline adjustments and manual integration of signals [19]. In mixtures containing many components, it is common the occurrence of overlapping signals in the ^{1}H NMR spectrum leading to overestimation of the analytes. In these situations, the COSY technique allows the assignment of overlapping signals [6,24], and can be used as a quantitative tool providing satisfactory results [24,25].

α-Bisabolol is a monocyclic sesquiterpene alcohol found in considerable amounts in chamomile (*Matricaria chamomilla*), sage (*Salvia runcinata*), *Myoporum grassifolium*, *candeia* (*Eremanthus erythropappus*) [26] and recently in *negramina* (Siparuna guianensis) [27]. Due to the anti-carcinogenic, anti-inflammatory, antimycotic and antibacterial properties, α-bisabolol is considered an important ingredient found in the formulation of various cosmetics and pharmaceuticals [26].

The interest in the development of a fast, simple and accurate method for quality control of essential oils rich in α-bisabol arises from the need of the cosmetics and pharmaceuticals industries to obtain feedstock of high quality for the development of their numerous products.

2. Experimental

2.1. Materials and reagents

Octamethylcyclotetrasiloxane (98%) lot 1451390V, octadecane analytical standard (98.5%) lot BCBM 1032, and α-bisabolol (95.5%) lot BCD7168V were purchased from Sigma-Aldrich (Wisconsin, USA). Deuterated chloroform lot 13A-045 and dichloromethane lot 1209154 were purchased from Tedia Brazil and Vetec (Rio de Janeiro, Brazil) respectively.

All the samples used are from *E. erythropappus* essentials oils. The samples E1, E2, E3, E4, and E5 were available in the Chemistry Department, Universidade Federal de Viçosa, MG, Brazil. E6 and E7 were provided by the company Atlântica Óleos Essenciais (Caeté, MG, Brazil). E8 and E9 were provided by the company Citróleo (Torrinha, SP, Brazil). Samples E10 and E11 were provided by the Distillery Bauru (Catanduva, SP, Brazil) and by the Chemistry Department, Universidade Federal do Espírito Santo, Alegre, ES, Brazil, respectively.

All weighings were carried out with an analytical balance from Shimadzu (capacity 82/220 g and accuracy 0.1 mg/0.01 mg). The volumes were transferred using chromatographic syringes of 500 μL and 5.00 mL. Caped vials of 10.0 mL and NMR tubes with 5 mm outer diameter were employed in all experiments.

2.2. NMR method

2.2.1. Solutions

2.2.1.1. ^{1}H NMR. A stock solution of α-bisabolol (105.0 mg mL^{-1}) and octamethylcyclotetrasiloxane (OMCTS) (1.9 mg mL^{-1}) were prepared to determine linearity, repeatability (precision/accuracy), and stabilities of α-bisabolol and OMCTS in solution and robustness of the NMR method. Stock solutions of α-bisabolol (103.7 mg mL^{-1}) and OMCTS (2.3 mg mL^{-1}) were prepared to determine limits of detection and quantification. For quantification, stock solutions of the internal standard OMCTS (concentrations from 1.7 to 2.9 mg mL^{-1}) were prepared.

To prepare all solutions, the oils and reagents were weighed directly into the vials and capped with a Teflon septum. Deuterated chloroform was added to the vials using chromatographic syringe.

2.2.1.2. COSY. Stock solutions of α-bisabolol (197.9 mg mL^{-1}) and OMCTS (3.5 mg mL^{-1}) were prepared for construction of a calibration curve used in the COSY method. The stock solution of OMCTS was used also for quantification of all samples by COSY method. All solutions were prepared as described in Section 2.2.1.1.

2.2.2. Instrumentation

The analyses were carried out in a Varian Mercury 300 MHz Spectrometer equipped with a 5 mm ^{1}H-^{13}C dual probe head and multinuclear broad band observer (BBO).

The determination of reproducibility was performed in a Bruker 400 MHz, model Avance III with auto sample, 5 mm BBI probe head with ATMA, unit generating of field gradient and unit of temperature control.

2.2.3. Experimental conditions

2.2.3.1. ^{1}H NMR. The pulse width 45° (pw45) was determined for all samples using the calibration sequence implemented in VnmrJ4.2 program of Agilent Technologies. The relaxation time was determined by inversion recovery experiment for all the hydrogens of the oil samples, α-bisabolol, and octamethylcyclotetrasiloxane. The longest relaxation time obtained for the validation and for measurement of the sample was 7 s. The experiments were performed at controlled probe temperature of 25 °C, using a number of scans (nt) of 16, a number of points in the FID of 32,768 (np); 45° pulse width (pw), spinning off; spectral width of 4800.8 Hz; acquisition time (at) of 4.550 s; delay time (d1) of 5 times $T1$ between pulses was used to ensure fully relaxation of hydrogens [28–30], according to established by Malz and Jancke [21] d1 $\geq$ (7/3) x longest $T1$, and pulse power attenuation (tpwr) of 57 dB. The shimming has been done automatically for all experiments and the peak width at half height of the chloroform was always smaller than 0.7 Hz.

To evaluate the robustness of the method, the ^{1}H NMR spectra