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An optimized and validated ^{1}H NMR method for the quantification of α-pinene in essentials oils

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ABSTRACT

The authenticity and composition of commercial essential oils requires strict quality control. Due to the importance of α-pinene containing essential oils, a rapid and efficient method for quantification of this terpene in oils of eucalyptus, pink pepper and turpentine using ^{1}H NMR was developed and validated. All evaluated parameters (selectivity, linearity, accuracy/precision, repeatability, robustness, stability of analyte and internal standard in solutions) showed satisfactory results. The limit of detection (LOD) and limit of quantification (LOQ) were 0.1 and 2.5 mg respectively. These values indicated that α-pinene was detected in 35 mg samples containing at least 0.3% of this compound. In addition, a minimum of 8% of α-pinene in the sample was required for quantification. Furthermore, the standard deviations found in the ^{1}H NMR methodology were less than 1% and were lower than those obtained by gas chromatographic analysis. Statistical tests have shown that the results obtained by ^{1}H NMR methodology are similar to those obtained by GC-FID technique using external and internal standardization and normalization within 95% confidence. R&R values lower than 10% have shown that all the methods are appropriate and the ^{1}H NMR method is suitable for quantification of α-pinene in samples of essential oils since this method possessed the smallest R&R (1.81) value.

1. Introduction

Commercial essential oils used in the pharmaceutical, food and fragrance industries must be submitted to a strict quality control since their chemical composition may vary according to the source, storage conditions and adulteration [1,2]. Gas chromatography coupled to mass spectrometry (GC–MS) has been the most widely used technique for quality control of such samples. GC–MS allows the identification and quantification of the oil constituents in samples that have complex compositions. However, analyses are time consuming, usually taking more than one hour [1].

In recent years, quantitative nuclear magnetic resonance (qNMR) has been used for quality control of drugs [3,4], foods [1,5,6,7], beer [8], olive oil [9], and gasoline [10], for quantification of mycophenolic acid and citrinin produced by Penicillium sp [11], and of fatty acid in edible oils [12,13]. In addition, ^{1}H NMR has also been used to quantify co-products of chemical reactions and impurities in different types of samples [14,15].

However, the quantification of essential oils using NMR is seldom reported in the literature. The use of ^{13}C NMR for identification and quantification of the major constituents of essential oils is significantly more common than the use of ^{1}H NMR. To the best of our knowledge, only one report on the use of ^{1}H NMR to quantify the major components of the essential oil of Brickellia weronicaefolia has been published [16].

Ottavioli et al. [17] added dipropylene glycol (DPG) to essential oils for the development and validation of a method using ^{13}C NMR technique for subsequent qualitative and quantitative determination of DPG in perfume extracts. In addition, the ^{13}C NMR was associated with computer programs to identify and quantify eudesmane-type acids in essential oil of *Dittrichia viscosa* sp [18].

The content of ascaridole and isoascaridole, constituents of commercial samples of *Chenopodium ambrosioides* were determined by ^{13}C NMR analysis. The use of this technique was essential since ascaridole becomes isoascaridole after partial thermal isomerization and hence the amount of ascaridole is

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underestimated by gas chromatography [19].

The sesquiterpene furanodiene and its rearrangement products were quantitated using ^{13}C NMR, first in artificial mixtures and subsequently in essential oil of *Smyrnium olusatrum* [20]. In turn, the identification and quantification of the constituents of a mixture of limonene, linalool and pinene using ^{13}C NMR were efficiently performed (relative error of less than 5%) by Formacek and Kubeczka [21].

Gas chromatography with flame ionization detector (GC-FID) and ^{13}C NMR spectroscopy were used to develop a protocol for identifying and quantifying germacrene A, B, C and their corresponding elements in essential oils of *Cleistopholis patens* [22]. According to the results obtained, ^{13}C NMR was proven to be more efficient for quantifying germacrenes and other by-products. During GC-FID and GC–MS analyses germacrene A, B and C all suffered isomerization to β-, γ- and δ-elemene respectively yielding unreliable results.

NMR spectroscopy is a promising technique for quality control of essential oils since the commercial products are available in large quantities. Normally their prices are associated with the percentage of one or more major components, always present in high concentrations. So even being the NMR technique less sensitive than GC–MS, this would not impose a problem. For example, essential oils of lemongrass *Cymbopogon citratus* for phytotherapy purposes should contain at least 60% of citral [23] and essential oil of eucalyptus for medicinal purposes must contain at least 70% of cineole [1].

The main advantage of nuclear magnetic resonance spectroscopy for quantitative analysis (qNMR) is the fact that it is a quick and straightforward technique. Furthermore, for most applications, solubility in deuterated solvent is the only requirement for the sample to be analyzed [13]. Moreover, qNMR is a method that does not need construction of analytical curves and the use of reference standards identical to the analytes, making it more advantageous when compared to chromatographic and other spectroscopic techniques [24].

Some qNMR methods have been used in cases when chromatographic analysis was found to be ineffective [25]. This can occur when the compound to be quantified is unstable at high temperatures [19]. Furthermore, qNMR is a non-destructive technique, selective, able to detect simultaneously different components of a mixture (provided there is enough selectivity) and enables simultaneous qualitative and quantitative analysis [13,24–28]. However, factors such as the quality of shimming, the uniformity of the magnetic field, phase correction and manual integration of areas obtained in the spectra influence the accuracy/precision of qNMR [13,25].

α-Pinene is a widespread component of several commercial oils, being produced by plants from different species in small or large amounts [29–31]. It is also found in the resins of numerous plants such as juniper berries and eucalyptus but is most prevalent in coniferous pine trees [32]. Essential oils containing this component are endowed with bactericidal, fungicidal, insecticidal, pesticidal, anticarcinogenic, antioxidant, immunostimulant, anti-inflammatory, anti-convulsive, sedative, anti-stress, and hypoglycemic activities [33].

Despite the various reported medical properties of α-pinene, few methods for quantification of this compound have been developed.

Since essential oils from eucalyptus are priced at the international market according to their content of α-pinene, and other important commercial oils are also rich in this component, the aim of this work was to develop and validate a fast and efficient method for routine analysis for a large number of oil samples rich in α-pinene using ^{1}H NMR.

2. Experimental

2.1. Materials and reagents

Octamethylcyclotetrasiloxane (98%) lot 1451390V, α-pinene (98%) lot 05514JE-317, and octadecane analytical standard (98.5%) lot BCBM1032 were purchased from Sigma-Aldrich (Wisconsin, USA). Deuterated chloroform lot 13A-045 was purchased from Tedia Brazil and dichloromethane lot 1209154 from Vetec (Rio de Janeiro, Brazil).

Samples of *Eucalyptus* and *Corymbia* essentials oils from the species *E. saligna* (ES), hybrid *E. urophylla* $\times$ *E. grandis* (U $\times$ G), *E. tereticornis* (ET), *E. phoenicea* (EPE), *E. punctata* (EPN), *C. torelliana* (CTL), *C. maculate* (CMC), were available in the Chemistry Department, Universidade Federal de Viçosa, MG, Brazil. Sample of α-pinene (SOC) was provided by the company Socer Brazil Indústria e Comércio Ltda and pink pepper essential oils (*Schinus terebinthifolius)* (PR) was provided by the Chemistry Department, Universidade Federal do Espírito Santo, Alegre, ES, Brazil.

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

2.2. Solutions

2.2.1. NMR

A stock solution of α-pinene (98.0 mg mL^{-1}) and octamethylcyclotetrasiloxane (OMCTS) (2.2 mg mL^{-1}) were prepared to determine linearity, stability and robustness of the NMR method. For the study of repeatability (precision/accuracy) stock solutions of α-pinene (100.1 mg mL^{-1}) and (OMCTS) (2.3 mg mL^{-1}) were prepared. Stock solutions of α-pinene (100.4 mg mL^{-1}) and OMCTS (2.9 mg mL^{-1}) were prepared to determine limits of detection and quantification. For quantification, stock solutions of the internal standard OMCTS (concentrations from 2.1 to 2.3 mg mL^{-1}) were prepared. To prepare all solutions, the oils and reagents were weighed directly into the vials that were subsequently capped with a Teflon septum. Deuterated chloroform was added to the vials using a gas chromatographic syringe.

2.2.2. GC-FID

A dichloromethane solution of α-pinene (approximately 10.0 mg mL^{-1}) was prepared for the gas chromatography-external standard (GC-ES) and internal standard (GC-IS) experiments. A solution of octadecane (20.1 mg mL^{-1}) was prepared for the gas chromatography-internal standard (GC-IS) experiments. The methodology used to prepare all solutions was as described above.

2.3. Instrumentation

2.3.1. Nuclear magnetic resonance spectrometry (NMR)

All 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).

2.3.2. Gas chromatography-mass spectrometry (GC–MS)

The identification of the constituents of essential oils was performed by using a gas chromatograph (Shimadzu, model 17A) equipped with a fused silica capillary column (RTX-5, 30 m $\times$ 0.25 mm i.d, 0.25 μm film thickness) connected to a mass detector from Shimadzu, model PQ5050A.