Talanta 189 (2018) 404–410

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Fast authentication of tea tree oil through spectroscopy

D. Gallart-Mateu[a], C.D. Largo-Arango[a], T. Larkman[b], S. Garrigues[a,*], M. de la Guardia[a]

[a] Department of Analytical Chemistry, University of Valencia, Jeroni Munoz Building, 50th Dr. Moliner St., 46100 Burjassot, Valencia, Spain
[b] Australian Tea Tree Industry Association Ltd., PO Box 903, Casino, NSW 2470, Australia

ARTICLE INFO

Keywords:
Tea tree oil authentication
Melaleuca alternifolia
Discriminant analysis
Near infrared spectroscopy
Attenuated total reflection middle infrared spectroscopy
Green analytical chemistry

ABSTRACT

Two new procedures, based on infrared spectroscopy in the near infrared (NIR) and mid infrared (MIR), have been developed for the authentication of tea tree oil (TTO) commercial samples. Infrared measurements were made on untreated samples by transmission NIR and attenuated total reflectance (ATR) followed by partial least square discriminant analysis (PLS-DA). These methods offer a fast and low cost alternative to enantiomeric two-dimensional gas chromatography coupled to mass detection usually employed to discriminate between authentic and non-authentic samples. In these studies, a set of 267 samples, including authentic and non-authentic labelled tea tree oil samples, were used to build the models based on the wavenumber range, data pre-processing and latent variables number selection. Infrared methods can be discriminant for authentic and non-authentic TTO samples with a 98% certainty for both ATR and NIR methodologies, employing 92 and 142 external samples respectively. Developed PLS-DA infrared based methodologies and the reference methodology have been evaluated and compared from a Green Analytical point of view.

1. Introduction

Essential oils have been employed for a long time and are used in increasing quantities for many applications from cosmetic to health care. Therefore, high quantities of natural essential oils extracted from plants are produced and consumed around the world [1,2]. For many industries these oils are considered as an added value for marketed products. However, as a consequence of the associated cost of natural essential oils, there are a large and ever increasing number of cases of adulteration, mainly through use of diluted solutions of pure oils or their replacement by synthetic terpenes or the substitution of the main essential oil compounds from other natural sources, thus creating economic problems and health risks.

From a legislative point of view, to assess the quality of essential oils, standards levels have been developed defining the chemical composition of each essential oil; these emphasise the importance of authentication of essential oils used in products to avoid fraudulent commercialisation [3].

Tea tree (*Melaleuca alternifolia*), an Australia native plant, produces an essential oil with high commercial value for industry, especially for its use in both pharmaceutical and cosmetic applications, as an ingredient in deodorants, body lotions, ointments and herbal remedies [4,5]. This wide applicability of tea tree essential oil (TTO) is related to its antiseptic, cytotoxic, antibacterial, antifungal, anti-inflammatory, antioxidant, anticancer and antiviral activity [6]. The main constituents of TTO are terpenoids, monoterpenes and sesquiterpenes, and their associated oxygenated analogues [7]. This chemical composition and the proportion of basic terpenes are correlated to the plant chemotype, with the terpinen-4-ol rich chemotype most suited to producing commercial TTO.

Usually, the composition of TTO is regulated by the international standard ISO 4730:2017, which specifies the concentration ranges for the 15 most relevant terpenes found in samples and their physical properties [8]. However, there are many cases of adulteration reported in TTO. These adulterations can be performed by dilution of pure oil, addition of synthetic materials and volatile compounds arising from other sources [9] or by adding a cheaper essential oil [10]. This is an important problem for the quality control of essential oils, due to the modification in the composition producing unsatisfactory products that do not maintain the characteristics and properties of original products.

From an analytical point of view, chromatography techniques, especially gas chromatography [11] coupled to mass [12–14] or flame ionization detection [4,12,13,15–17], are the most commonly employed methods in the analysis and detection of adulteration of TTO. These are accepted as an analytical tool for quality control analysis. However the natural composition of TTO can vary depending on the species grown, the growing conditions and the essential oil extraction methods employed. This natural variation of compounds presents a

* Corresponding author.
E-mail address: Salvador.Garrigues@uv.es (S. Garrigues).

https://doi.org/10.1016/j.talanta.2018.07.023
Received 27 March 2018; Accepted 10 July 2018

challenge especially in the presence of adulterants and so poses a high level of challenge in the identification and analysis of specific chemical compounds as quality control markers. In this sense, the chiral analysis of selected isomeric terpenic compounds, such as (+)-terpinene-4-ol/(-)-terpinene-4-ol, (+)-α-terpineol/(-)-α-terpineol or (+)-limonene/(-)-limonene [10], and the enantiomeric chromatography techniques are, nowadays, one of the most commonly used methods for authentication of TTO [18]. In recent years the evaluation of TTO has been performed by techniques such as heart-cutting multidimensional gas chromatography (HC-MDGC) and enantioselective heart-cutting multidimensional gas chromatography (Enantio-HC-MDGC), chiral gas chromatography (C-GC-MS) or enantio comprehensive gas chromatography (Enantio-GC × GC) [7,9,10,19–23], allowing the identification of TTO components, possible adulterations and changes in the composition due to adverse reactions performed by atmospheric oxygen, light and temperature [19].

Nevertheless, other analytical methodologies have been applied in essential oil authentication. On one hand, techniques such as isotope ratio mass spectrometry detection coupled gas chromatography (GC-C-IRMS) [24,25] or NRM [26] have been employed in the characterization and quality assessment of mandarin, savory, oregano or citrus essential oils, where synthetic adulterants derived from fossil sources have been used. On the other hand, vibrational spectroscopy has been used to determine the terpene content in camellia, eucalyptus, cinnamon or TTO [23,27–30], employing partial least square calibration as chemometric tool for the treatment of spectra.

The main objective of this work has been to develop and evaluate new analytical methodologies for the fast authentication of TTO. These methods will be based on the use of infrared spectrometry, in particular near infrared (NIR) and attenuated total reflectance Fourier transform at mid infrared (ATR-FTIR). The aforementioned methodologies will try to perform the authentication of TTO samples offering a direct, fast, easy, cheap and green alternative to the general use of chromatography while reducing solvent consumption and minimizing waste generation.

2. Material and methods

2.1. Apparatus and samples

For MIR spectra acquisition, a Tensor 27 MIR spectrometer from Bruker (Karlsruhe, Germany) equipped with a DLaTGS detector was used. Samples were measured using a DuraSampl*IR II* attenuated total reflection module for liquids with a nine reflection diamond/ZnSe DuraDisk plate from Smiths Detection Inc. (Warrington, UK).

A multipurpose analyser (MPA) Fourier transform NIR spectrometer from Bruker (Karlsruhe, Germany), equipped with a NIR source, a quartz beam splitter and InGaAs detector, was used for NIR transmission measurements using cylinders of 8 mm external diameter and 6 mm optical pass as measurement cells. For both spectra measurements, instrument control and data acquisition, OPUS 6.5 software from Bruker was employed.

A set of 267 samples were provided by the Australian Tea Tree Industry Association (ATTIA), including authentic and non-authentic TTO samples, collected around the world, were employed in these studies for both calibration and prediction of their composition using infrared spectra.

2.2. Reference methodology

Enantioselective gas chromatography mass spectrometry (Enantio-GC-MS) was used as reference methodology according to the procedure proposed by Wong et al. [20]. An Agilent 7890A GC System coupled to an Agilent 5975c inert XL EI/CI MSD triple axis single quadrupole mass spectrometer (Palo Alto, CA, USA), was employed for the characterization of samples based on the isomeric ratios (+)-α-terpineol/(-)-α-terpineol and (+)-terpinen-4-ol/(-)-terpinen-4-ol. The analyte separation was carried out using an Astec Chiraldex B-PM (2,3,6-tri-O-methyl derivative β-cyclodextrin) capillary column (30 m × 0.25 mm I.D. × 0.12 μm) provided by Sigma-Aldrich (St. Louis, USA). A volume of 0.2 μL of 0.5% diluted sample was directly injected into the chromatograph with a split ratio 100:1 at 230 °C employing a constant 1.0 mL min^{-1} helium flow as carrier gas. Separation was performed using the following oven program: an initial 40 °C temperature increased at a rate of 3 °C min^{-1} until 150 °C, and then increased to 180 °C employing a 10 °C min^{-1} rate, holding this temperature for 10 min. Electron impact ionization (EI) at 70 eV and selected ion monitoring mode was used to acquire the analyte signals, acquiring in the scan mode from 40 to 300 *m/z*, and in selected ion monitoring (SIM) at *m/z* 68. The transfer line and source temperatures were 260 °C and 230 °C, respectively. Samples, diluted with hexane (0.5% v/v) were measured under these conditions.

2.3. ATR-FTIR based methodology

Samples were measured by direct ATR-FTIR. A drop of sample, approximately 40 mg, was deposited on the crystal of the ATR module. Spectra were obtained in the range between 4000 and 600 cm^{-1} by addition of 50 scans per spectrum and with a resolution of 4 cm^{-1}. The background spectrum was performed by measuring the clean ATR crystal before each sample measurement. Each sample was measured three times and the averaged spectrum was employed to develop the partial least square discriminant analysis models (PLS-DA) and to predict the authenticity of unknown samples with as low as possible number of latent variables.

2.4. NIR based methodology

To obtain transmission NIR spectra of samples, 1 mL clear sepcap glass vials with 6 mm optical path were filled directly with tea tree oil samples, acquiring the spectra in the range from 14,000 to 4000 cm^{-1} by co-adding 50 scans per spectrum at a resolution of 4 cm^{-1}. The background spectrum was performed by measuring an empty glass vial employing the same instrumental conditions as samples. Each sample was measured three times after rotating the vial position and the averaged spectrum was employed to develop the partial least square discriminant analysis (PLS-DA) models and to predict the authenticity of samples, as indicated in the case of ATR-FTIR measurements.

2.5. Spectral data treatment

Chemometric data treatment was performed by partial least squares regression discriminant analysis by using Matlab 2013b software from Mathworks (Natick, MA, USA) and the PLS Toolbox 6.2 from Eigenvector Research Inc. (Wenatchee, WA, USA).

Spectral data were employed to develop the chemometric models to predict the authenticity of unknown samples. Different spectral ranges and data pre-processing were evaluated to select the most adequate prediction model.

3. Results and discussion

3.1. ATR-FTIR

3.1.1. ATR-FTIR spectra of TTO

Fig. 1 shows the ATR-FTIR spectra of TTO samples without any data pre-treatment in the range between 4000 and 600 cm^{-1}. The absorption band at 3450 cm^{-1} corresponds to the O-H vibration, while strong bands at 2961, 2921 and 2876 cm^{-1} arise from the C-H and -CH_2- as well as from the asymmetric -$CH(CH_3)$ stretching vibration and -$CH(CH_2)$-, symmetric and asymmetric stretching vibrations.

The weak bands in the range between 1690 and 1580 cm^{-1}, are due the alkene functional group (C=C) [4], while the strong bands located at