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Performance evaluation of a versatile multidimensional chromatographic preparative system based on three-dimensional gas chromatography and liquid chromatography–two-dimensional gas chromatography for the collection of volatile constituents

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ABSTRACT

The present research deals with the multi-collection of the most important sesquiterpene alcohols belonging to sandalwood essential oil, as reported by the international regulations: (*Z*)-α-santalol, (*Z*)-α-*trans* bergamotol, (*Z*)-β-santalol, *epi*-(*Z*)-β-santalol, α-bisabolol, (*Z*)-lanceol, and (*Z*)-nuciferol. A versatile multidimensional preparative system, based on the hyphenation of liquid and gas chromatography techniques, was operated in the LC–GC–GC-prep or GC–GC–GC-prep configuration, depending on the concentration to be collected from the sample, without any hardware or software modification. The system was equipped with a silica LC column in combination with polyethylene glycol-poly(5% diphenyl/95% dimethylsiloxane)-medium polarity ionic liquid or β-cyclodextrin based GC stationary phases. The GC–GC–GC-prep configuration was exploited for the collection of four components, by using a conventional split/splitless injector, while the LC–GC–GC-prep approach was applied for three low abundant components (<5%), in order to increase the quantity collected within a single run, by the LC injection of a high sample amount. All target compounds, whose determination is hampered by the unavailability of commercial standards, were collected at milligram levels and with a high degree of purity (>87%).

1. Introduction

Plants belonging to the genus Santalum (Santalaceae) are evergreen parasitic trees consisting of about 25 species that are distributed in India, Indonesia, Malaysia and Australia [1]. Sandalwood oil is obtained by distillation of the heartwood of 20-year-old trees, and this caused, during the last years, a lowering of natural sources, not compensated by sufficient plant regeneration. This oil is widely used in aromatherapy and it is employed by industry as an ingredient of perfumes and cosmetics. Furthermore, several biological activities have been reported, including the antidepressant, anti-inflammatory, antifungal, antiviral [2], anticarcinogenic [3] and antitumorous [4,5] ones.

The volatile composition of *Santalum album* (*S. album* L.) essential oil, contains >90% sesquiterpene alcohols. According to the International Standard Organization (ISO 2002), *S. album* L. essential oil must have a free alcohol content, expressed as santalols, not lower than 90%; in particular, (*Z*)-α-santalol must fall within the 41–55% range, while (*Z*)-β-santalol within the 16–24% range [6]. These two compounds together are responsible for the chemoprotective effects and neuroleptic properties of the oil, as observed in *in vitro* and *in vivo* assays [7–9]. In addition to (*Z*)-α- and (*Z*)-β-santalol, the Australian regulation [10] on Sandalwood oil also regulates the content of (*Z*)-α-*trans* bergamotol, *epi*-β-santalol, α-bisabolol, (*E*,*E*)-farnesol, (*Z*)-lanceol and (*Z*)-nuciferol. Except for

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(*Z*)-α-santalol, (*Z*)-β-santalol and (*E*,*E*)-farnesol, the determination of those components of interest is hampered by the unavailability of commercial standards on the market.

Although several synthetic procedures have been exploited for the production of such components, these ways are not cost-effective and, hence, sandalwood essential oil remains the only viable source of these sesquiterpene alcohols [11–16]. The widespread used fractional distillation may be unrewarding procedure, since these components have the tendency to be distilled at the same temperature as a mixture [17]. In similar situations, preparative gas chromatography (prep-GC) may be regarded as a valid tool for the isolation of compounds of interest in a pure form, provided that a proper stationary phase is selected, to attain baseline separation of such components or, whenever this is not achievable, exploiting the coupling of more (different) stationary phases, in the heart-cut mode [18].

Several prep-GC applications have been carried out in the last decade, using either one or two separation columns (multidimensional GC, MDGC or GC–GC) for the collection of volatile constituents, like isomers from complex mixtures [19,20], pure substances from the flavor and fragrance [21–23] and environmental field [24,25], and pheromones [26]. In most cases a prep-GC analysis precedes ^{1}H NMR [20,24,27,28] or a compound-specific radiocarbon analysis [20,29].

Recently, a new system was developed for the collection of pure volatile components from complex matrices, consisting of the hyphenation of three GC separation dimensions (GC–GC–GC). Such a system was successfully used for the collection of discrete amounts of components of interest [30], or unknown molecules for further identification [31], in a reduced collection time if compared with conventional GC-prep applications and with a high degree of purity. In these applications, the collection yield was increased by injecting a higher-than-optimum volume of an undiluted sample (2–3 μL in splitless mode) onto the first GC widebore column. Overloading the column obviously led to a decrease in the separation efficiency (reduced by 40–50%) with respect to the optimal injection conditions, however it allowed for mg-amounts of the desired compounds to be collected.

On the other hand, the separation and collection of sufficient quantities of very-low or trace level components from a sample matrix is more challenging, since the required amounts to be loaded would overwhelm the injection capability of a split/splitless GC device. A viable option would be the use of a large-volume injection technique, however such approach could lead to column damage and/or contamination, following the introduction of non-volatile sample components as well as of high amounts of the more abundant volatile ones. In such situations, the use of either an additional front-end (micro)packed GC column [32] or an LC dimension can be exploited to attain an increased sample capacity, while at the same time affording a class-type separation of the sample components [33].

In this work, we demonstrated the feasibility of using a versatile multidimensional preparative system, for the multicollection of some of the most important sesquiterpene alcohols contained in sandalwood essential oil as reported by the international regulations, and namely: (*Z*)-α-santalol, (*Z*)-β-santalol, (*Z*)-α-*trans* bergamotol, *epi*-(*Z*)-β-santalol, α-bisabolol, (*Z*)-lanceol, and (*Z*)-nuciferol. The system was operated in GC–GC–GC-prep configuration, for the collection of pure amounts of (*Z*)-α-santalol, (*Z*)-β-santalol, (*Z*)-α-*trans* bergamotol, and *epi*-(*Z*)-β-santalol, by a device placed at the outlet of the third column. For the collection of α-bisabolol, (*Z*)-lanceol, and (*Z*)-nuciferol, which are very low-concentrated components (<5%) scarcely amenable to prep-GC, LC–GC–GC-prep was exploited in on-line mode, taking advantage from the much higher loading capacity of an LC packed column at the injection site.

2. Material and methods

2.1. Samples and sample preparation

Sandalwood essential oil (*S. album* L., Indonesian), GC-grade *n*-hexane, LC-grade dichloromethane, *tert*-butyl methyl ether (MTBE), *n*-hexane and nootkatone were kindly provided by Sigma–Aldrich/Supelco (Bellefonte, USA).

A stock solution, containing 20 mg/mL of nootkatone was prepared in dichloromethane and used for recovery and calibration purposes. The essential oil was diluted 1:2 (*v*/*v*) in dichloromethane prior to injection.

2.2. LC pre-separation

The LC pre-separation of the sandalwood essential oil was performed on an LC system (Shimadzu, Kyoto, Japan) consisting of a CBM-20A communication bus module, two LC-20AD dual-plunger parallel-flow pumps, a DGU-20A online degasser, an SPD-20A UV detector, a CTO-20A column oven and a SIL-20AC autosampler. Twenty microliters of the sandalwood oil solution were injected onto a 250 mm × 4.6 mm ID × 5 μm d_p amino column (SUPELCOSIL™ LC-NH_2, Supelco, Milan, Italy). Mobile phases consisted of (A) *n*-hexane and (B) methyl tert-butyl ether, under the following stepwise gradient conditions: 0–3 min, 0% B, 3–15 min, 3% B, 15–25 min, 10% B, 25–35 min, 30% B, 35–45 min, 100% B. Flow-rate was 1 mL/min (reduced to 0.35 mL/min during the transfer steps). Data were acquired by the LCsolution software ver 1.25 (Shimadzu, Kyoto, Japan).

2.3. LC–GC interface

The LC–GC interface was based on a dual side-port syringe, controlled by means of a AOC-5000 autosampler (Shimadzu, Kyoto, Japan). Chromatography band transfer was achieved, in the stop-flow mode, through a modified 25-μL syringe. The lower part of the syringe was connected, *via* two transfer lines, to the LC detector exit and to waste. A teflon plug was located at the end of the syringe plunger, the latter was characterized by an external diameter smaller than the barrel internal one, thus enabling the mobile phase to flow into the syringe. In the waste mode, the plunger plug was located below both lines and the effluent was directed to waste. In the cut position, the plunger plug was located between the upper and lower lines and the effluent flowed to a large volume injector (LVI) [34].

2.4. Multidimensional prep GC analysis

The preparative MDGC instrument consisted of three GC 2010 systems (GC1, GC2, and GC3) connected by means of three Deans-switch transfer devices, namely TD1 (between GC1 and GC2), TD2 (between GC2 and GC3) and TD3 (between GC3 and the collection station). The Deans switch elements were connected to three advanced pressure control systems (APC1, APC2, and APC3), which supplied He as a carrier gas. The system configuration has been previously described elsewhere [30]. GC1 was equipped with a split/splitless injector, an Optic 3 large volume injector (LVI) (ATAS GL International, Eindhoven, The Netherlands) and a flame ionization detector (FID1). The LVI temperature-program and flow-rate were optimized as described in our previous work [33]. During the transfer step (4 min) and for the first 0.50 min of the GC analysis run time, the split mode was used (flow rate was 332 mL/min at 35 °C), followed by a 1 min run in the splitless mode. After this period, the split mode was again applied (at 126 mL/min) while heating the injector to 280 °C (at 15 °C/s). When a conventional split/splitless injector was used, the temperature was maintained at 280 °C.