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Application of a multidimensional gas chromatography system with simultaneous mass spectrometric and flame ionization detection to the analysis of sandalwood oil

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ABSTRACT

The production and trade of Indian sandalwood oil is strictly regulated, due to the impoverishment of the plantations; for such a reason, Australian sandalwood oil has been evaluated as a possible substitute of the Indian type. International directives report, for both the genuine essential oils, specific ranges for the sesquiterpene alcohols (santalols). In the present investigation, a multidimensional gas chromatographic system (MDGC), equipped with simultaneous flame ionization and mass spectrometric detection (FID/MS), has been successfully applied to the analysis of a series of sandalwood oils of different origin. A detailed description of the system utilized is reported. Three santalol isomers, (*Z*)-α-*trans*-bergamotol, (*E,E*)-farnesol, (*Z*)-nuciferol, epi-α-bisabolol and (*Z*)-lanceol have been quantified. LoD (MS) and LoQ (FID) values were determined for (*E,E*)-farnesol, used as representative of the oxygenated sesquiterpenic group, showing levels equal to 0.002% and 0.003%, respectively. A great advantage of the instrumental configuration herein discussed, is represented by the fact that identification and quantitation of target analytes are carried out in one step, without the need to perform two separate analyses.

1. Introduction

Sandalwood essential oil is obtained from trees belonging to the genus *Santalum*, namely *Santalum album* L. and *Santalum spicatum* (R. Br.) A. DC., commonly known as Indian and West Australian sandalwood oil, respectively. The Indian type is considered the most valuable, for its organoleptic and technological characteristics, which make it a unique ingredient in perfumery. Sandalwood oil is obtained by means of distillation of the heartwood of 20-year-old trees, a fact that has caused an impoverishment of natural sources, not compensated by sufficient plant regeneration. Furthermore, smuggling has deteriorated the situation of this endangered tree, obliging the Indian government to issue specific regulations for oil production and exportation. Being India the world's biggest producer of sandalwood oil, the global production of this oil has dramatically diminished in the last years, causing: (i) an increase of the commercialization of low quality products; (ii) a price enhancement; (iii) the need for a substitute *Santalum* species.

Indian and Australian sandalwood oils have been chromatographically investigated, with the conclusion that the two sandalwood oils are qualitatively similar, and quantitatively different, with a consequently dissimilar olfactive impression [1]. Synthetic chemical mixtures, such as Sandalore® and Ebanol®, produced by an international flavour and fragrance supplier, resemble sandalwood oil fragrance very much; in many cases these synthetic products are used to rectify oils of low quality.

According to the International Standard, *S. album* L. essential oil must present a primary free alcohol content, expressed as santalol, not lower than 90%, with, in particular, (*Z*)-α-santalol falling in the 41–55% range, and (*Z*)-β-santalol falling in the 16–24% range [2,3]; Howes et al. suggested 43% and 18% as lower limits for these alcohols [4].

Different techniques have been exploited for the assessment of genuineness of essential oils, such as gas chromatography coupled to combustion-isotope ratio mass spectrometry [5,6] or GC–Fourier transform infrared spectroscopy [7]. Multidimensional gas chromatography, characterized by the combination of different stationary-phase types, has proved to be an effective tool

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Table 1
List of the samples analyzed.

Sample	Species	Type
S1	*Santalum album* L.	East Indian
S2	*Santalum album* L.	Indonesian
S3	*Santalum album* L.	Indian
S4	*Santalum album* L.	Indian
S5	*Santalum spicatum* (R. Br.) A. DC.	Australian
S6	*Santalum austrocaledonicum* Vieill.	New Caledonian

in essential oil analysis [8]. Among multidimensional techniques, heart-cutting GC has become very popular since the early years of its introduction [9]. The "heart-cuts" consist of portions of eluate that are selected and transferred from the first to the second dimension, with columns located inside the same or two distinct GC ovens. There are several possible column combinations, *i.e.*, apolar–chiral [10–12], a prime choice for the determination of enantiomeric distributions, apolar–polar [13] or ionic liquid–apolar, recently utilized for the determination of aromatics in gasoline [14]. Recently, a Deans-switch, twin-oven MDGC system, equipped with an FID detector in the first dimension, and a quadrupole (q) mass spectrometer in the second dimension, was successfully employed in the analysis of mandarin essential oil [15]. The aim of the investigation was to compare the performance of conventional enantio-GC, with that of enantio-MDGC–MS, and to evaluate the extent of peak overlap using the former methodology.

In the present study, an MDGC system, with simultaneous FID and MS detection, has been applied to the analysis of the volatile constituents present in a variety of sandalwood oils. The compliance with legal authenticity requirements, and the quantification of farnesol have been ascertained. Among the sesquiterpene alcohols present in sandalwood oil, farnesol falls into the list of compounds regulated by the E.U. [16]. The determination of farnesol is generally recognized as a difficult task, both for quantitative and qualitative aims [17]. Farnesol, generally present in one or two of its isomeric forms, namely (*2Z,6Z*)-, (*2Z,6E*)-, (*2E,6E*)- and (*2E,6Z*)-farnesol, is embedded in the sesquiterpene zone, therefore making quantification quite complicated. For example, considering sandalwood oil, (*E,E*)-farnesol overlaps with (*Z*)-β-santalol on a non-polar capillary, and with (*Z*)-α-santalol on a polar one.

2. Experimental

2.1. Samples

Commercial samples of sandalwood essential oil were kindly provided by different international producers and were: one from East India, one from Indonesia, two from India, one from Australia and one from New Caledonia. Classification and description of samples are reported in Table 1. 4,4′-dibromobiphenyl was used as internal standard (I.S.) and was supplied by Sigma–Aldrich (Milan, Italy). An aliquot of standard corresponding to 5% (*v/v*) was added to each oil prior to analysis. For calibration, (*E,E*)-farnesol, supplied by Sigma–Aldrich, was used as representative of the oxygenated sesquiterpenic group, due to the unavailability of other standard compounds on the market.

2.2. GC–qMS analysis

The GC–qMS application was carried out on a Shimadzu GCMS-QP2010 Plus instrument (Kyoto, Japan), equipped with an AOC-20 s auto sampler and an AOC-20i auto injector. An SLB-5 ms 30 m × 0.25 mm I.D. × 0.25 μm d_f column was used [silphenylene polymer, virtually equivalent in polarity to poly(5% diphenyl/95% methylsiloxane)] (Supelco, Milan, Italy), and temperature programmed as follows: 50–300 °C at 3 °C/min. Split/splitless injector (280 °C); injection mode: split, 1:200 ratio; injection volume, 1.0 μL; inlet pressure, 50.9 kPa; carrier gas, He; constant pressure, initial linear velocity, 35 cm/s.

MS conditions: scan range 40–400 amu, scan speed 2000 amu/s, ion source temp. 200 °C, and interface temperature 250 °C.

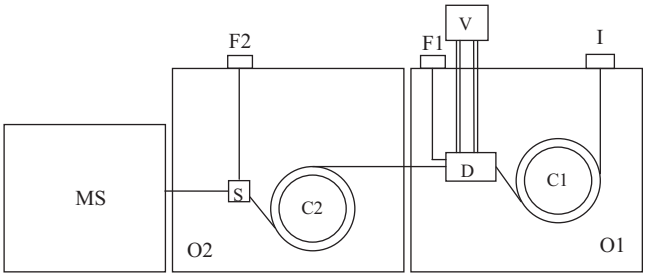

Fig. 1. A scheme of the MDGC system. I, injector; C1, first column; O1, first oven; D, Deans switching device; F1, first-dimension FID; V, Valve; C2, second column; O2, second oven; S, splitter; F2, second-dimension FID.

2.3. MDGC–qMS analysis

The MDGC–qMS (Shimadzu) system consisted of two GC ovens (GC-2010), GC-1 and GC-2, the latter coupled to a quadrupole mass spectrometer detector (QP-2010). GC-1 was equipped with a split/splitless injector, and an FID (FID 1) detector. A scheme of the equipment is shown in Fig. 1. The primary apolar column end was connected to the Deans switching device, located in GC-1. The connection of the Deans switch with FID 1 was performed by means of stainless steel tubing. GC-2 was equipped with a polar column, having one end connected to the Deans switch and the other end connected to a T union (SGE, Australia) which divided the eluate between the MS detector and the GC-2 FID (FID 2), by means of two uncoated deactivated columns. The dimensions of the latter were 35 cm × 0.10 mm I.D. and 30 cm × 0.05 mm I.D. for the FID 2 and MS, respectively. The split ratio between the MS and FID 2 was constantly ~1:8 throughout the analysis. A scheme of the transfer device employed has been described elsewhere [18]. An APC (advanced pressure control) supplied helium at constant pressure (160 kPa) to the interface.

GC-1: carrier gas was helium. Injector pressure (constant) was 230 kPa at 300 °C, operated in the split mode (split ratio 1:50); initial average gas velocity ~20 cm/s. An SLB-5 ms 30 m × 0.25 mm I.D. × 0.25 μm d_f column was used (Supelco). Oven program: 80–280 °C at 3 °C/min. Detector: FID 1 temperature (sampling frequency: 20 Hz): 300 °C; detector flows were air: 400 ml/min; H_2: 40 ml/min. Transfer line: 180 °C.

GC-2: a Supelcowax (100% polyethylene glycol) 30 m × 0.25 mm I.D. × 0.25 μm d_f capillary was employed (Supelco); initial average gas velocity ~30 cm/s. Oven program: 80 °C for 10 min, to 280 °C at 3 °C/min.

MS detector: mass range 40–400 amu, scan speed: 2000 amu/s. Ion source temperature: 200 °C, interface temperature: 250 °C.

FID 2 conditions were the same as used for FID 1. No make-up gas flow was used in both the FID systems. The instrument was provided with reference spectral libraries and data were handled by means of GCMSsolution ver. 2.5 software (Shimadzu).

3. Results and discussion

Sandalwood oil can be considered as a complex sample and, thus, a monodimensional GC technique could fail in the satisfactory elucidation of its quali–quantitative composition. The main consequence of insufficient resolving power is analyte overlapping, something to be avoided because it can cause the production of

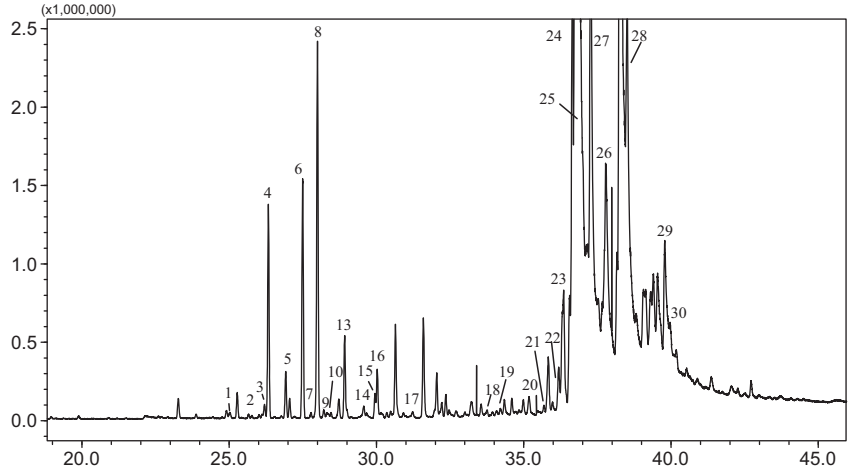

Fig. 2. Monodimensional GC–qMS profile of sandalwood oil (S1). See Table 2 for peak identification.

impure spectra and unreliable, if not impossible, quantification. In a preliminary application, one of the samples (S1) was subjected to GC–qMS analysis (Fig. 2). Peak assignment was carried out by means of three commercial databases, namely FFNSC 1.3 [19], Adams [20] and Hochmuth [21], as reported in Table 2. The libraries utilized were provided with linear retention index (LRI) information; through the use of the GC–MS software, it was possible to apply a twin-filtered search procedure, combining a minimum similarity limit and an LRI range [22,23]. The results attained highlight the limits of a monodimensional GC process in the analysis of such a complex sample. In fact, the similarity score exceeded 90% only for twelve compounds, and in some cases (*e.g.*, (*Z*)-*β*-santalol), neither the spectral match, nor the similarity of retention index values were enough to establish peak identity. Most of the compounds reported in Table 2 can be considered as only "tentatively identified", due to the low similarity values attained.

After the non-exceptional GC–qMS result, a multidimensional GC process was taken into consideration. In particular, attention was first devoted to comprehensive two-dimensional GC (GC × GC), a multidimensional method characterized by continuous/sequential cutting, focusing and reinjection of primary-column fractions onto a second fast column [24]. Hence, in GC × GC the entire initial sample is subjected to two different separation steps. GC × GC has been used successfully in the analysis of sandalwood oil (West Australian): Shellie et al. used GC × GC–FID and GC × GC–MS (a time-of-flight system was employed) for the characterization of eight important constituents, namely (*Z*)-*α*-santalol, epi-*α*-bisabolol, (*Z*)-*α*-*trans*-bergamotol, epi-*β*-santalol, (*Z*)-*β*-santalol,

Table 2
Peaks identified in sample S1 by using monodimensional GC–qMS.

Peak no.	Compound	Similarity (%)	MS library	LRI_{exp}	LRI_{lib}
1	7-Epi-sesquithujene	84	Adams	1392	1390
2	*α*-Funebrene	86	FFNSC	1407	1403
3	*α*-Cedrene	86	FFNSC	1421	1414
4	*α*-Santalene	97	FFNSC	1424	1418
5	*α*-*trans*-bergamotene	96	FFNSC	1439	1432
6	Epi-*β*-santalene	96	FFNSC	1453	1446
7	Sesquisabinene	87	FFNSC	1460	1455
8	*β*-Santalene	97	FFNSC	1465	1459
9	*β*-Acoradiene	91	FFNSC	1471	1471
10	10-Epi-*β*-acoradiene	88	FFNSC	1473	1471
11	*α*-Acoradiene	85	Hochmuth	1475	1466
12	*γ*-Curcumene	95	Adams	1483	1481
13	Ar-curcumene	95	Adams	1488	1479
14	**β-Curcumene**	82	Adams	1505	1514
15	*β*-Bisabolene	95	FFNSC	1515	1508
16	**β-Curcumene**	97	Adams	1517	1514
17	(*E*)-*α*-bisabolene	83	FFNSC	1548	1540
18	Helifolen-12-al	85	Adams	1614	1619
19	12-Isoitalicenol	82	Hochmuth	1627	1636
20	*β*-Himachalol	88	Adams	1648	1652
21	Bulnesol	81	Adams	1667	1670
22	(*Z*)-*α*-santalol	88	Adams	1681	1674
23	*α*-Bisabolol	85	Adams	1686	1685
24	*α*-*trans*-bergamotol	81	FFNSC	1694	1688
25	Epi-*β*-santalol	88	Adams	1698	1702
26	***β*-Santalol**	93	FFNSC	1726	1716
27	***β*-Santalol**	96	Adams	1741	1738
28	(*E*)-nuciferol	92	Adams	1747	1754
29	***α*-Santalol acetate**	86	Adams	1784	1777
30	***α*-Santalol acetate**	84	Hochmuth	1789	1779

LRI_{exp}: experimental LRI values measured on an SLB-5 ms column.
LRI_{lib}: LRI values for the correspondent compound reported in the MS library.
Compounds recognized two times due to the low spectral similarity and same LRI values are indicated in bold.

(*E,E*)-farnesol, (*Z*)-nuciferol, and (*Z*)-lanceol [25]. Though there are no doubts on the exceptional separation-power capabilities of GC × GC; however, it is also true that cryogenic transfer devices require considerable amounts of cooling gas (Shellie et al. used CO_2) and, hence, operational costs are rather high. In classical MDGC, on the contrary, cryogenic gases are normally not employed for chromatography band transfer, while each heart-cut is generally subjected to the high resolving power of a conventional column (typically 25–30 m × 0.25 I.D.). Considering the specific sample-type herein investigated, only a limited zone of the first-dimension chromatogram (oxygenated sesquiterpene region) required a further separation. Consequently, the method that appeared most adequate was heart-cutting MDGC with MS/FID simultaneous detection. The great advantage of such an instrumental configuration is represented by the fact that identification and quantification of target analytes are carried out in one step. In fact, the possibility to achieve quantitative analysis through the use of a mass spectrometer has been recently debated, leading to the conclusion that GC–FID analysis is the most reliable method for such an objective [26,27]. Furthermore, the possibility to achieve a variable split ratio between the two detectors, by changing the dimensions of two uncoated columns, is useful to direct a higher amount of eluate to the FID for quantitative purposes, and only a small amount to the MS for identification.

Table 3 reports the Australian Standard (AS2112-2003) for *S. spicatum*, with the ranges for the santalol fraction. Despite the Australian normative, the ISO 3518:2002(E), relative to *S. album*, reports the ranges only for (*Z*)-α- and (*Z*)-β-santalol (Table 3), although it mentions α-*trans*-bergamotol and epi-β-santalol as components of a typical chromatogram.

Table 3
Australian and International Standard for sandalwood oil.

Constituent	AS 2112-2003		ISO 3518:2002(E)	
	% min	% max	% min	% max
(*Z*)-α-santalol	15.0	25.0	41.0	55.0
Epi-α-bisabolol	2.0	12.5		
(*Z*)-β-santalol	5.0	20.0	16.0	24.0
Epi-β-santalol	0.5	3.5		
(*Z*)-α-*trans*-bergamotol	2.0	10.0		
(*E,E*)-farnesol	2.5	15.0		
(*Z*)-nuciferol	2.0	15.0		
(*Z*)-lanceol	2.0	10.0		

Limits of detection (LoD) relative to the MS detector and limits of quantification (LoQ) relative to the FID detector were measured [using (*E,E*)-farnesol solutions], in order to establish the most suitable second-dimension outlet split ratio. Since a signal to noise ratio higher than ten is needed for accurate quantification, a higher amount of eluate must be directed toward the FID, whereas, a substiantially lower amount of eluate can be directed to the MS detector, where a signal to noise ratio of three is sufficient; moreover, it must be emphasized that a sensitivity increase can be achieved by operating in the SIM mode. An FID:MS split ratio of *circa* 8:1 was achieved by means of the 35 cm × 0.10 mm I.D. and 30 cm × 0.05 mm I.D. columns, linked to the FID and MS, respectively. Under such operational conditions, (*E,E*)-farnesol LoD and LoQ values corresponding to 0.002 and 0.003 (%, *v/v*) were attained, respectively. Negligible retention time differences were observed (~0.03 s) between the FID and MS traces. A four-point calibration curve was constructed considering (*E,E*)-farnesol concentration (%)

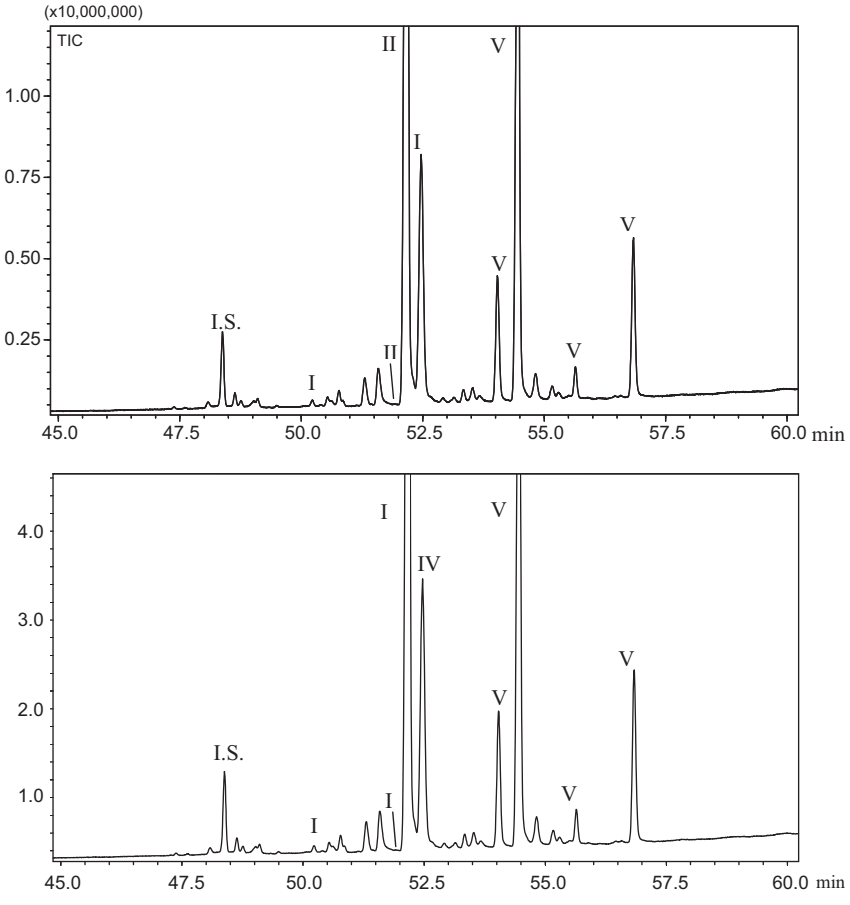

Fig. 3. MDGC chromatograms for sample S5. Top: MS chromatogram. Bottom: FID chromatogram. Peak identification: (I) epi-α-bisabolol; (II) (*E,E*)-farnesol; (III) (*Z*)-α-santalol; (IV) (*Z*)-α-*trans*-bergamotol; (V) epi-β-santalol; (VI) (*Z*)-β-santalol; (VII) (*Z*)-lanceol; (VIII) (*Z*)-nuciferol.

Table 4
Percent (*v/v*) chemical composition of the sesquiterpene alcoholic fraction. All the values are the mean of five replicates.

	Constituent	%S1	%S2	%S3	%S4	%S5	%S6
I	Epi-α-bisabolol	0.3	0.2	0.5	0.8	5.9	0.4
II	(*E,E*)-farnesol	*tr*	0.3	0.2	3.9	11.5	0.7
III	(*Z*)-α-santalol	46.7	44.2	40.9	4.1	21.3	44.9
IV	(*Z*)-α-*trans*-bergamotol	9.3	7.3	6.4	2.3	4.3	9.2
V	Epi-β-santalol	3.7	3.6	2.9	0.7	1.8	3.1
VI	(*Z*)-β-santalol	20.9	19.7	17.6	1.3	8	19.4
VII	(*Z*)-lanceol	2.1	1.3	1.9	0.5	2	3.9
VIII	(*Z*)-nuciferol	4.9	3.8	0.8	1.1	8	1.7
	(*Z*)-α-santalol + (*Z*)-β-santalol + (*Z*)-α-*trans*-bergamotol	76.9	71.2	64.9	7.7	33.6	73.5
	(*Z*)-α-santalol/(*Z*)-lanceol	22.2	34.0	21.5	8.2	2.7	26.4

* Trace amount.

versus (*E,E*)-farnesol area/I.S. area ratio, using serial dilutions of the stock standard solution with 4,4′-dibromobiphenyl as the internal standard; the regression coefficients was 0.9996.

MDGC–qMS/FID analyses were performed on the samples listed in Table 1. Two heart-cuts were performed, one for the oxygenated sesquiterpenes (35.05–37.96 min) and the other for the internal standard (44.60–45.10 min). Compared to GC–qMS analysis, a completely different result (sample S5 is illustrated in Fig. 3) was obtained. In fact, it was possible to unravel the sesquiterpenic zone and to improve the resolution of the compounds of interest through heart-cutting. For example, (*E,E*)-farnesol is now nicely separated both from (*Z*)-β-santalol, with which it coelutes on a single apolar column, and from (*Z*)-α-santalol, with which it coelutes on a polar column. Table 4 reports quantitative data (% composition, in accordance with the measure unit reported in the normative) for all the samples analyzed, as the mean values of five replicates. The data were derived presuming that the response factors of all the sesquiterpenic alcohols studied was the same as that of (*E,E*)-farnesol. The results obtained are herein discussed considering the ranges prescribed by the normative and reported in Table 3: samples S1, S2, S3 (Indian type) presented values in agreement with the International Standard for *S. album*. On the contrary, sample S4 (Indian type), to which attention will be devoted in this study, did not abide the ISO normative, presenting values of (*Z*)-α- and (*Z*)-β-santalol of 4.1% and 1.3%, against the accepted ranges of 41.0–55.0% and 16.0–24.0%, respectively [2,3]. Furthermore, samples S1–S3 showed similar concentrations for the other components, while this condition did not occur for sample S4. It can be affirmed that the latter sample showed an atypical composition. Sample S5 (Australian type) was in compliance with the Australian Standard. Finally, sample S6 (Austrocaledonicum) was characterized by values that fell within the ranges prescribed by the International Standard for *S. album*, hence close to the Indian-type range, in agreement with a previous work on the same type of sandalwood oil [28]. Consequently, *Santalum austrocaledonicum* (and also Indonesian *S. album*) can be considered as an alternative source for sandalwood oil.

From a sensorial viewpoint, the three most important sandalwood compounds are (*Z*)-α- and (*Z*)-β-santalol, as well as (*Z*)-α-*trans*-bergamotol. According to Hammerschmidt et al. [3], the sum of these alcohols must be 70–80% in *S. album,* up to 65% in *S. austrocaledonicum*, and up to 25% in *S. spicatum*. Considering such ranges and the results reported in Table 4 it can be affirmed that: samples S1 (76.9%) and S2 (71.2%) fell within the *S. album* range, sample S3 presented a slightly lower value (64.9%), while the sum of the three alcohols in sample S4 was very low (7.7%). Moreover, the results for sample S5 (33.6%) and S6 (73.5%) were slightly higher, than the values reported for *S. spicatum* and *S. austrocaledonicum*, respectively. In particular, the result for *S. austrocaledonicum* fell inside the *S. album* range. Furthermore, the same authors provided another indicator of genuineness [3], namely the ratio (*Z*)-α-santalol/(*Z*)-lanceol, with approximate values of 25, 5 and 10 for *S. album, S. austrocaledonicum,* and *S. spicatum*, respectively. Again, considering such ratio values and the results attained in the present study, (Table 4) it can be affirmed that: samples S1 (22.2) and S3 (21.5) presented values in good agreement, while S2 was characterized by a slightly higher result (34.0); samples S4 and S5, with ratio values of 8.2 and 2.7, respectively, differed completely (each *circa* three times lower); sample S6 (26.4) was characterized by a ratio result that was much higher than that reported for *S. austrocaledonicum*, though very similar again to the *S. album* value.

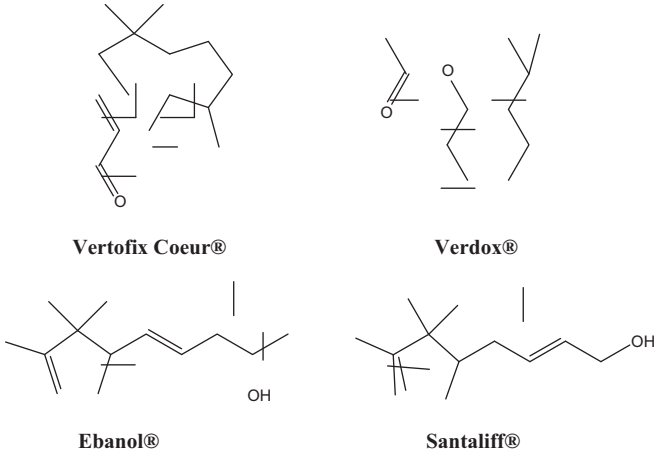

Fig. 4. Structures of commercially available chemicals which resemble the fragrance of sandalwood oil and found in sample S4.

As previously mentioned in the introductory section, it is common to find adulterated sandalwood oils on the market, which have been subjected to chemical manipulation. One of the samples analyzed in this study (S4) fell into such a category: a series of synthetic chemicals were identified using GC–qMS. The structures of these chemicals are reported in Fig. 4, and they are: 2-tert-butylcyclohexyl acetate (Verdox®), 2-methyl-4-(2,2,3-trimethyl-3-cyclopentenyl)-2-buten-1-ol (Santaliff®), 1-(2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulen-5-yl)-ethanone (Vertofix coeur®) and 3-methyl-5-(2,2,3-trimethyl-3-cyclopentenyl)-2-ol (Ebanol®) [29]. The first three are produced by IFF, while the last by Givaudan.

4. Concluding remarks

The present research has shown the advantages of using a multidimensional GC system when the sample under investigation is highly complex. The MDGC–qMS/FID method developed enabled the high-resolution separation of the santalol fraction, which characterizes sandalwood oil. The resolving-power limitation of monodimensional GC was avoided, enabling reliable identification and quantitation of target analytes. The quality of a series of commercial samples was assessed on the basis of the ranges reported

by the International and Australian Standards. One of the samples under investigation demonstrated to be a "fake" oil, despite the quality certificate reported on the label. It can be affirmed that heart-cutting MDGC is the multidimensional method of choice when a limited number of first-dimension chromatography bands requires separation on a full-length conventional capillary.

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