Journal of Chromatography A, 1217 (2010) 6422–6427

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Evaluation of tea tree oil quality and ascaridole: A deep study by means of chiral and multi heart-cuts multidimensional gas chromatography system coupled to mass spectrometry detection

Danilo Sciarrone[a], Carla Ragonese[a], Caterina Carnovale[a], Anna Piperno[a], Paola Dugo[a], Giovanni Dugo[a], Luigi Mondello[a,b,*]

[a] Dipartimento Farmaco-chimico, Facoltà di Farmacia, Università di Messina, viale Annunziata, 98168 Messina, Italy
[b] Università Campus Bio-medico di Roma, Via Alvaro del Portillo, 21, 00128 Roma, Italy

ARTICLE INFO

Article history:
Received 14 June 2010
Received in revised form 4 August 2010
Accepted 6 August 2010
Available online 13 August 2010

Keywords:
Ascaridole
Deans switch
Chiral chromatography
ISO/FDIS 4730:2004
MDGC/MS
Multidimensional chromatography
Preparative GC
Tea tree oil

ABSTRACT

The natural-like assessment of essential oils is a demanding task due to the growing trend toward adulterations. Usually chiral chromatography was used for this purpose due to the capability of assessing stereospecificity which is directly related to the enzymatic pathways of each plant species. On the other hand, the quality of an essential oil involves also the evaluation of its oxidative state, mainly connected with the age and storage conditions. In fact, some modifications in the chemical profile of the oil can occur if not properly preserved. Alterations of the components due to oxidative reactions lead to the formation of peroxides, endoperoxides and epoxides, such as ascaridole and 1,2,4-trihydroxymenthane, usually present in very low amount, formed by the oxidation of terpinen-4-ol and α-terpinene, respectively. Therefore, in the present research, the quality of Australian Tea Tree oil (*Melaleuca alternifolia* (Maiden & Betche) Cheel, Myrtaceae) was investigated by means of a multi heart-cut multidimensional gas chromatographic system coupled to a mass spectrometer detector and by conventional enantio-GC. The MDGC system allowed the complete separation of the compounds of interest transferred from the first column to a second dimension based on a different separation mechanism. The MS detector at the end of the second column provided the identification of the peaks with high similarity values because of their high purities after the multidimensional separation. Method validation was carried out, in order to use this procedure for routine application, monitoring the repeatability of 1D retention times and 2D peak areas, LoD and LoQ. Finally, enantiomeric ratios for chiral compounds were established to support quality data obtained.

1. Introduction

Melaleuca is a genus of plants in the family Myrtaceae. The plants are generally found in open forest, woodland or shrub land, particularly along watercourses and the edges of swamps. Tea tree essential oil (TTO) is a complex mixture of compounds obtained by steam distillation from the leaves and twigs of *Melaleuca alternifolia* (Maiden & Betche) Cheel, Myrtaceae with a fresh camphoraceous fragrance. Sometimes, however, other essential oils from *Leptospermum* spp. and other *Melaleuca* spp. may be summarised under this name like, for instance, cajuput oil obtained from *Melaleuca leucadendra* (L.) L. and niaouli oil obtained from *Melaleuca viridiflora* Sol. ex Gaertn., which are typical of the northeast coast of New South Wales, Australia. The main components of TTO are terpene hydrocarbons, mainly monoterpenes, sesquiterpenes and their associated alcohols. The oil has beneficial medical (including antiseptic and antifungal action), as well as cosmetic properties. Monoterpenes and sesquiterpenes have been evaluated for their various bioactivities, including antimicrobial [1] and antioxidant activities [2]. Terpinen-4-ol, γ-terpinene, α-terpinene, 1,8-cineole, *p*-cymene, α-terpineol, α-pinene, terpinolene, limonene and sabinene account for 80–90% of the oil. Terpinen-4-ol has been found to suppresses inflammatory mediator production by activated human monocytes [3]. TTO also exhibited strong cytotoxicity towards human lung cancer cell line (A549), human breast cancer cell line (MCF-7) and human prostate cancer cell line (PC-3) [4]. From about 100 terpenes found in TTO more than 60 individual substances have been identified by means of gas chromatography coupled to mass spectrometry detection. The natural content of the individual terpenes may vary consider-

* Corresponding author at: Dipartimento Farmaco-chimico, Facoltà di Farmacia, Università di Messina, viale Annunziata, 98168 Messina, Italy.
Tel.: +39 090 6766536; fax: +39 090 358220.
E-mail address: lmondello@unime.it (L. Mondello).

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doi:10.1016/j.chroma.2010.08.019

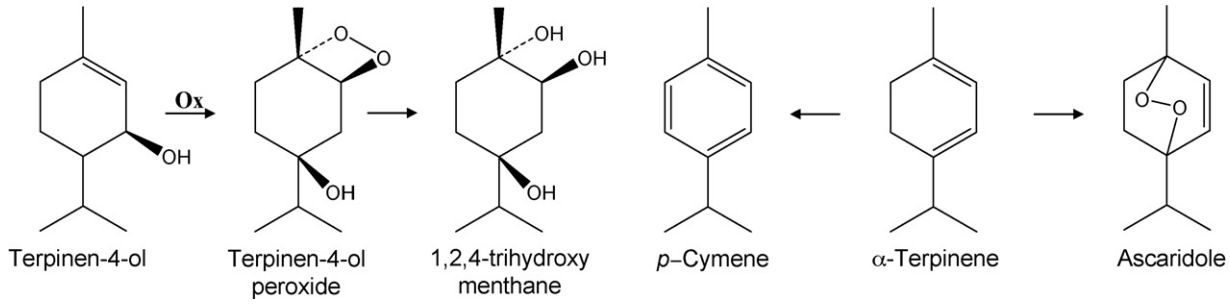

Fig. 1. Terpinen-4-ol (a) and α-terpinene (b) oxidation process pathways.

ably depending on the *M. alternifolia* population used, the climate, the leaf maceration, the age of the leaves and the duration of distillation. Enantio-GC is commonly adopted for the genuineness assessment of essential oils since the enantiomeric ratios of chiral compounds are peculiar of each plant chemotype and family [5]. In this concern, this technique can easily demonstrate adulteration of genuine oils as for example for the detection of spiking with cheaper distilled oil. The composition of tea tree oil changes along with aging particularly in presence of atmospheric oxygen but also depending on the storage conditions, i.e. if the oil is exposed to light and high temperatures. The oil is usually used diluted, as adverse reactions can occur with the use of pure tea tree oil, due to the presence of sensitizers compounds. In fact, alterations of some components due to oxidative reactions lead to the formation of peroxides, endoperoxides and epoxides: moreover, *p*-cymene concentration can rise to levels approaching its upper limit while α-terpinene and terpinolene decrease. Two pathways are involved in this reactions (Fig. 1), one based on the oxydation of the π bond of terpinen-4-ol leading to its peroxide form and then to 1,2,4-trihydroxy menthane (a), the other involving oxidation of the α-terpinene, γ-terpinene and terpinolene to their benzene analogue, *p*-cymene, or to the endoperoxide ascaridole (b). The two pathways are well known in terpene chemistry [6]. As a consequence, a range for a list of compounds has been proposed to evaluate the quality of the oil (Table 2).

Recently TTO has been determined by means of HPTLC in cosmeceutical formulations [7] even if GC methods are often preferred for the determination of the essential oils. The gas chromatographic approach offers a significant improvement in sensitivity, thus GC and GC/MS methods for tea tree oil have been reported [8,9]. Owing to the widespread use of GC in routine essential oils analysis, it is necessary that good GC methods are developed and that these are thoroughly validated. The complexity of the matrices sometimes is a limitation for monodimensional analytical approaches because of the limited peak capacity of a single column driving to coelutions. This hinders peak identification and quantification even using mass spectrometry detection because of the non-specific fragmentation of this class of compounds. Based on the SCCP opinion [10], listed compounds have to be investigated for the quality assessment of tea tree oil, while some further investigation is required for oxidation products that could lead to allergic problems for which a range of concentration in the oil have not been evaluated. The latter could be important for the quality assessment of the oil as raw material for cosmetic purposes in the industrial field. In the last decades multidimensional techniques have been recognized as the best approach due to their higher capability for the separation of complex matrices. Comprehensive two-dimensional chromatography has been extensively applied for food analysis [11–14]. Comprehensive chromatography has been exploited for enantioselective analysis of TTO [15,16] but the high cost per analysis and the lack of skilful operators have limited the use of this powerful technique for routine purpose. Heart-cutting MDGC technique based on Deans switch device [17,18], which involves the transfer of one or more unresolved fraction from a first to a second dimension, has already proven its utility in essential oils field for separations that require very high efficiencies [19,20]. This technique could be considered the most suitable approach for this purpose due to the user-friendly instrumentation nowadays available and the lower costs per analysis, in comparison with comprehensive techniques employing cryogenic focusing gas and interfaces. MDGC finds particular application in essential oil analysis, i.e. for chiral separation, due to the complex nature of the materials, and the need for highly efficient separation for specific analysis goals. Heart-cut GC has been used after stir bar sorptive extraction for the enantioselective analysis of TTO by Mosandl and co-workers in 2002 [21]. The same multidimensional instrumentation used in this study coupled to mass spectrometry has been recently employed in the essential oils field for enantiomeric ratio assessment by our group [22,23], as well as in the identification and quantification of allergens in flavour and fragrance matrices [24]. The aim of this study was to develop a rapid and accurate multidimensional method for the determination of the compounds reported in ISO/FDIS 4730:2004 for the quality assessment of pure tea tree oil. Moreover, the presence of the two possible allergenic agents ascaridole and 1,2,4-trihydroxymenthane was investigated. While the standard of 1,2,4-trihydroxymenthane was available, ascaridole, due to his peroxide nature could not be purchased from suppliers since its shipment is strictly prohibited to prevent terrorism actions.

2. Experimental

2.1. Materials

Pure standard of α-pinene, sabinene, α-terpinene, p-cymene, limonene, 1,8-cineole, γ-terpinene, terpinolene, terpinen-4-ol, α-terpineol, aromadendrene, ledene, caryophyllene, farnesol and globulol were kindly provided by Sigma–Aldrich (Supelco, Milan, Italy) while 1,2,4-trihydroxymenthane was kindly provided by University of Tasmania (ACROSS center). For ascaridole, due to the unavailability of the standard, a synthesis with a final yield of 37% was carried out followed by a purification step by means of preparative GC. Calibration curves for each chemical class, for 1,2,4-trihydroxymenthane and ascaridole, with regression factors always higher then 0.9992, were built using nonane as internal standard (IS) at a fixed concentration of 3% (w/v). γ-Terpinene was used for monoterpenes, α-terpineol for oxygenated monoterpenes, caryophyllene for sesquiterpenes and farnesol for oxygenated sesquiterpenes. All the solutions were prepared in ethanol (GC grade) and injected at different concentrations (from 0.1 to 50%, w/v) considering their original purity. The identification of single components in tea tree oil has been achieved by means both of mass spectra comparison with a LRI filter of ±5 units and by means of injection of pure standards.

2.2. Synthesis of ascaridole

Ascaridole was obtained by addition of O_2 to α-terpinene (1 g) using methylene blue (100 mg) as photosensitizer in dichloromethane. During irradiation with three 500-W halogen lamps, the reaction mixture was kept at room temperature and oxygen was continuously insufflated in the solution. A Freidrichs condenser cooled with ethylene glycol (2 °C) was used to avoid solvent evaporation. The consumption of α-terpinene and the formation of ascaridole were monitored by thin layer chromatography using cyclohexane/ethyl acetate 7:3 as eluent. After 3 h, solvent was evaporated at reduced pressure with an EZ-2 evaporator (Genevac, UK). The residue was then washed with hexane to remove the photosensitizer because of its insolubility in this solvent and purified by means of preparative gas chromatography. The presence of ascaridole was confirmed by ^{1}H NMR and GC/MS. Yield 37%.

2.3. Preparative gas chromatography

A Shimadzu GC-2010 gas chromatograph, equipped with a split/splitless injector and an FID detector was employed, coupled to a fractionation system View Prep Station VPS278 (Atas GL International B.V., Veldhoven, The Netherlands). A wide bore non-polar column, 30 m × 0.53 mm i.d., 5.0 μm f.t. (Supelco), was connected to a T-union; the effluent was splitted by means of two retention gaps, 1 and 2.5 m each, 0.25 i.d. respectively going partly to the FID and through a heated transfer line (250 °C) to the VPS278. The latter is composed of an inert valve located in a heated interface, able to distribute the compounds eluted from the column, according to the fractionation times that was selected through a software, the *View Prep Station Control*, in seven micro tubes (5 °C), six used to collect and one for waste. To facilitate the collection an additional gas flow (He) was used while a back gas flowed against this flow in order to avoid cross contamination inside the tubes.

2.4. Enantio-GC-FID

A Megadex DETTBS-β column (diethyl-*tert*-butyl-silyl β-cyclodextrin) 25 m × 0.25 mm i.d. × 0.25 μm d_f (Mega, Legnano, Italy) was used; temperature program: 50–200 °C at 2.0 °C/min; split/splitless injector (220 °C); injection mode: split, 1:10 ratio; injection volume: 1.0 μl; inlet pressure: 96.6 kPa; carrier gas: He; constant gas linear velocity: 35.0 cm/s. Detector FID (220 °C). H_2: 40.0 ml/min. Air: 400.0 ml/min, sampling rate: 80 ms (12.5 Hz).

2.5. MDGC/MS chromatographic conditions

The MDGC/MS system consists of two GC-2010 gas chromatographs (GC-1 and GC-2), a MS-QP2010 quadrupole mass spectrometer and an AOC-20i autosampler (Shimadzu Corporation, Kyoto, Japan). GC 1 presents a split/splitless injector and a flame ionization detector, while GC-2 presents a split/splitless injector, flame ionization detector and a rapid scanning quadrupole mass spectrometer. The MDGC transfer device, located in the first GC oven, is connected to an automatic pressure control (APC) unit which supplies carrier gas, at constant pressure. APC pressure-balances are able to redirect the flow exiting from the first column to the FID ("stand-by" condition) or to the second column situated in GC 2 ("cut" condition). Fig. 2 reports two schemes of the transfer system device in the stand-by (Fig. 2a) and cut position (Fig. 2b).

GC-1: Column – SLB-5MS 30 m × 0.25 mm i.d. × 0.25 μm f.t. [silphenylene polymer, virtually equivalent in polarity to poly(5% diphenyl/95% methylsiloxane)] (Supelco, Milan, Italy). Column head pressure – 200 kPa, helium (constant mode), auxiliary pressure (APC) – 130 kPa, helium. Oven temperature program – from 50 to 280 °C at 3 °C/min, transfer line – 180 °C, detector FID (310 °C). H_2: 40.0 ml/min. Air: 400.0 ml/min, sampling rate: 80 ms (12.5 Hz).

GC-2: Column – Omegawax (100% polyethylene glycol) 30 m × 0.25 mm i.d. × 0.25 μm f.t. (Supelco, Milan, Italy), temperature program – 50 °C for 10 min to 280 °C at 3 °C/min, detector – MS, ion source: 200 °C, interface temp.: 220 °C, interval scan: 40–400 *m*/*z*, scan speed: 5000 amu/s (12.5 Hz).

Table 1
Enantiomeric ratios for chiral compounds in tea tree oil.

Component	+	–
β-Pinene	60.7	39.3
Sabinene	60.2	39.8
α-Phellandrene	37.3	62.7
Linalool	65.1	34.9
Terpinen-4-ol	66.2	33.8
α-Terpineol	76.8	23.2

2.6. Sample

A sample of Australian tea tree oil was kindly provided by Essential Oils and Tea Tree Research department of Wollongbar Primary Industries Institute (Wollongbar, Australia). The oil was dil. 1:10 in ethanol and added with 3% (w/v) of internal standard before analysis. All the solutions were stored at 4 °C.

3. Results and discussion

Due to the growing trend toward adulterations, the natural-like assessment of essential oils is a demanding task. Chiral chromatography was used for this purpose due to the capability of assessing stereospecificity which is directly related to the enzymatic pathways of each plant species. Table 1 reports the enantiomeric ratios of β-pinene, sabinene, α-phellandrene, linalool, terpinen-4-ol and α-terpineol. Small amount of α-thujene prevented the possibility to calculate the enantiomeric ratio due to the lower than detection limit amount of the first eluting enantiomer, while α-pinene was not baseline separated due to the poor selectivity of the cyclodextrin adopted in this study. Due to the oxidation state of the oil the assessment of limonene enantiomeric ratios was not achievable because of the coelution of the (+) enantiomer with an increased amount of *p*-cymene. Concerning the other chiral compounds, the results were in agreement with literature data [31]. According to the opinion on Tea Tree oil of the Scientific Committee on Consumer Products (SCCP) of December 2008, some modifications in the chemical profile of the oil can occur if not properly stored [10]. Requirements were imposed in the Australian Standard AS 2782-2009 [25], in the International Standard ISO 4730 (ISO 4730, 1996 and ISO/FDIS 4730, 2004) [26] (see Table 2) and in a former issue

Table 2
Chromatographic profile set by ISO/FDIS 4730:2004.

Component	Min (%)	Max (%)
α-Pinene	1	6
Sabinene	Trace	3.5
α-Terpinene	5	13
p-Cymene	0.5	8
Limonene	0.5	1.5
1,8-Cineole	Trace	15
γ-Terpinene	10	28
Terpinolene	1.5	5
Terpinen-4-ol	30	48
α-Terpineol	1.5	8
Aromadendrene	Trace	3
Ledene	Trace	3
δ-Cadinene	Trace	3
Globulol	Trace	1
Viridiflorol	Trace	1

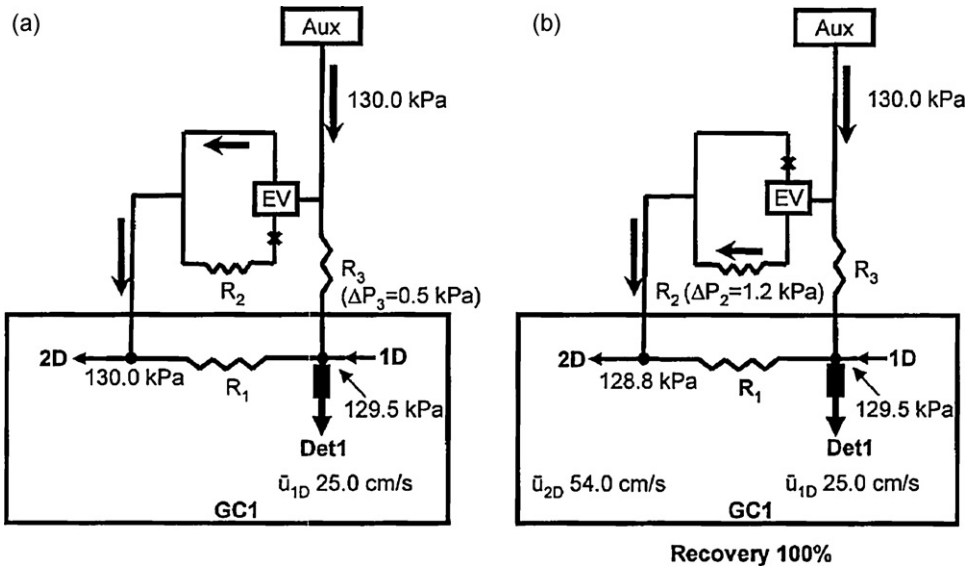

Fig. 2. Schemes of the MDGC interface in the stand-by (a) and cutting (b) modes.

of the German Drugs Code (DAC) with respect to the level of individual ingredients evaluating some compound range to allow the assessment of genuineness and quality of the oil. Moreover, due to the increase of allergic contact dermatitis presented by tea tree oil users, which restricted its use only to the diluted oil, even if in this conditions it can also cause irritation, the interest of many researchers has been focused onto the quantification of allergenic constituents, specially in raw materials. Allergic reactions may be due to the various ingredients naturally present in the oil or to oxidation products that are formed by exposure of the oil to light and/or air leading to the formation of peroxides, endoperoxides (ascaridole) and epoxides. As a further oxidation product 1,2,4-trihydroxymenthane was identified as a product of terpinen-4-ol conversion. The complexity of the oil makes the quantification of target compounds a hard task specially for compounds such as sesquiterpenes and oxidation products, i.e. ascaridole and 1,2,4-trihydroxymenthane, usually present in very low amounts. In this concern, allergenic compounds from essential oils have been investigated in cosmetic products by means of GC/MS [27]. The presence of unresolved components compromises their accurate measurement, thus an increased resolving power is desirable and single capillary column analysis is unlikely to offer the required separation of closely eluting compounds. A way to enhance resolving power is through multidimensional gas chromatography using two separate conventional capillary columns. In this work an MDGC/MS system, based on a Deans switch device [17,18], coupled with mass spectrometry detection in the second dimension, was used in multi heart-cut mode, providing the complete separation of all the compounds of interest for the quality assessment of the oil. The same system has been widely described in previous papers of our

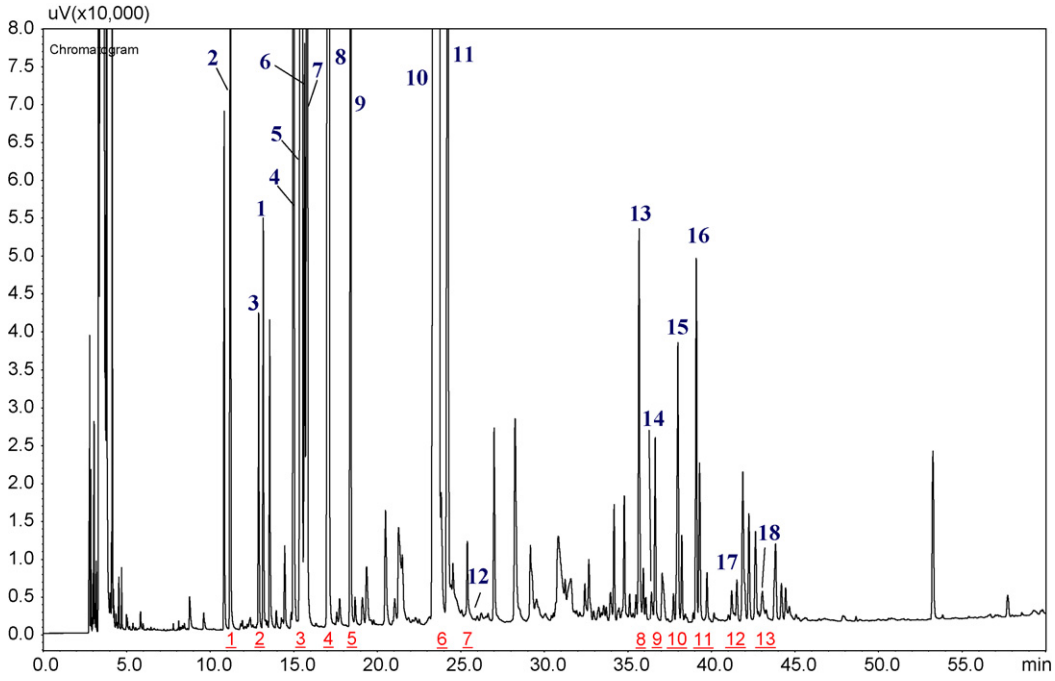

Fig. 3. Conventional GC-FID analysis of the tea tree oil (for peaks assignment refer to Table 3). The number of each heart-cut is indicated.

Table 3
Comparison of experimental and reference LRI, relative amount results for monodimensional GC (conv) and MDGC analysis, relative standard deviation for the retention times in the first dimension and for the areas in the second dimension and ‰ LoD and LoQ.

ID	Component	LRI[a]	LRI[b]	Amount(%) Conv	Amount(%) MDGC	1D t_RRSD%	2D areaRSD%	LoD‰	LoQ‰
1	IS	899	900	2.98	3.02	0.041	2.01	0.005	0.015
2	α-Pinene	930	933	2.15	2.23	0.068	3.08	0.005	0.015
3	Sabinene	968	972	0.57	0.58	0.055	2.09	0.005	0.015
4	α-Terpinene	1017	1018	2.94	2.91	0.050	3.06	0.005	0.015
5	p-Cymene	1026	1025	13.67	14.36	0.050	2.02	0.005	0.015
6	Limonene	1030	1030	4.56	1.12	0.056	3.02	0.005	0.015
7	1,8-Cineole	1030	1026	4.01	6.43	0.061	3.01	0.005	0.015
8	γ-Terpinene	1055	1058	10.63	10.73	0.070	3.00	0.005	0.015
9	Terpinolene	1083	1086	2.10	2.15	0.061	2.00	0.005	0.015
10	Terpinen-4-ol	1183	1180	38.75	38.61	0.054	1.05	0.010	0.015
11	α-Terpineol	1192	1195	2.56	2.50	0.027	3.05	0.010	0.030
12	Ascaridole	–	1306	n.d.	0.20	0.070	2.06	0.010	0.030
13	Aromadendrene	1435	1438	0.96	0.91	0.065	2.05	0.005	0.015
14	1,2,4-trihydroxy menthane	–	1488	n.d.	0.01	0.058	3.01	0.010	0.030
15	Ledene	1375	1374	0.77	0.75	0.065	3.00	0.005	0.015
16	δ-Cadinene	1523	1518	1.08	0.83	0.070	2.06	0.005	0.015
17	Globulol	–	1590	n.d.	n.d.	0.000	0.00	0.010	0.030
18	Viridiflorol	1591	1594	0.60	0.38	0.072	2.08	0.010	0.030

[a] Experimental LRI.
[b] Wiley, FFNSC 1.3 GC–MS library.

group regarding the analysis of allergens in perfumes [14], chiral compounds in *Pistacia lentiscus* L. [23] and mandarin essential oil [22] and for the simultaneous investigation of oxygenated compounds and benzene, toluene, ethyl benzene and xylenes (BTEX) in gasoline [28]. Thirteen heart-cuts were selected from the first non-polar column to be transferred to the polar column (Fig. 3) via software, using a cut-time method, in a following cut application. The identification of the components to be heart-cutted in tea tree oil has been achieved by means both of mass spectra comparison with a LRI filter of ±5 units and by means of injection of pure standards. With respect to monodimensional separation the 2D chromatogram (Fig. 4) showed an increased resolution for the target compounds. By using both a non-polar and a polar column in conventional monodimensional GC, due to the complexity of the matrix, many compounds of interest were completely or partially coeluted with interfering compounds as confirmed by the differences in the relative amount between the mono and multidimensional analysis reported in Table 3. Limits of detection (LoD, y_D) and the quantitation limit (LoQ, y_Q) were estimated, according to Eurachem guidelines, as signals based on the mean value (y_b) and the standard deviation (s_b) of the blank signal as follows:

$$y_D = \bar{y}_b + 3s_b \qquad y_Q = \bar{y}_b + 10s_b$$

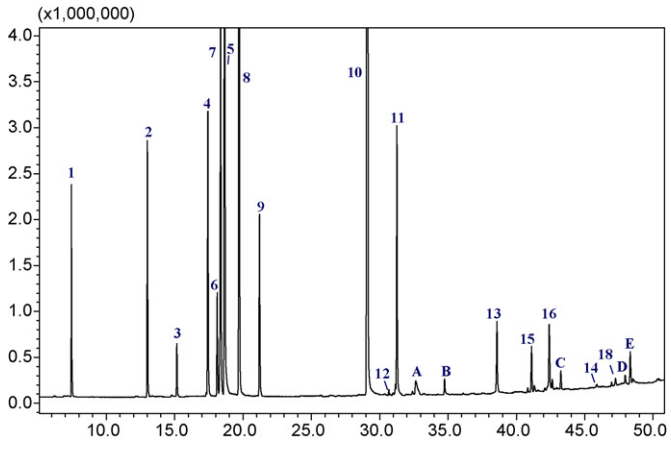

Fig. 4. 2D-GC/MS chromatogram of the 13 heart-cuts from the first dimension. Peak A: *trans*-piperitol, peak B: p-cymen-8-ol, peak C: *trans*-calamenene, peak D: cubeban-11-ol, peak E: spathulenol.

For y_b and s_b determination, 10 blank measurements were performed; the concentration values of detection limit (LoD) and quantitation limit (LoQ) were obtained by projection of the corresponding signals y_D and y_Q through a calibration plot $y = f(x)$ onto the concentration axis. The results obtained are reported in Table 3. In the case of non-polar column (Fig. 3) the coelution of p-cymene (peak 5), limonene (peak 6) and 1,8-cineole (peak 7) is very well known but, by transferring all of them in a single heart-cut in the 2D column, it was possible to achieve a baseline separation for all of them allowing an accurate quantification. In order to calculate percentage amounts in the whole oil, calibration curves for each chemical class were built using C9 as internal standard with regression factors always higher than 0.9992. γ-Terpinene was used for monoterpenes, α-terpineol for oxygenated monoterpenes, caryophyllene for sesquiterpenes, farnesol for oxygenated sesquiterpenes and 1,2,4-trihydroxymenthane for its quantification. To achieve quantification of ascaridole, due to the unavailability of the standard, a synthesis with a final yield of 37% was carried out. To obtain pure ascaridole, preparative gas chromatography was performed to remove trace amounts of limonene, 1,8-cineole, 1,4-cineole and p-cymene. To allow an accurate weighing of ascaridole, one of the seven glass collection tubes of the preparative instrument was brought to constant weight before and after the collection. The identification of ascaridole was confirmed by means of ^{1}H NMR in accordance with literature data [29] and by GC/MS analysis, exploiting FFNSC 1.3 (Wiley) mass spectra database.

Comparing the conventional results with those obtained by means of MDGC/MS it has to be pointed out that, while for p-cymene and 1,8-cineole the underestimation was respectively 5% and 10%, for limonene the quantification was four times higher than the real value obtained by MDGC (4.56% vs. 1.12%). Terpinen-4-ol (peak 10) partially coeluted in the first dimension with a handsome amount of p-cymen-8-ol, identified by means of monodimensional GC/MS (89% mass spectra similarity) and comparing the experimental with the reference linear retention index on the apolar column (LRI_{exp} 1190/LRI_{ref} 1189), since this value was compatible with the LRI of terpinen-4-ol (LRI_{exp} 1183/LRI_{ref} 1180). Furthermore, a confirmation of the identification was the presence of p-cymen-8-ol in the 2D chromatogram, completely separated, at about 35 min (peak B), with a mass spectra similarity of 97%. α-Terpineol (peak 11) coeluted with *trans*-piperitol: also in this case confirmed by the presence of the compound in the 2D chro-

matogram (peak A, mass spectra similarity of 92%). It must be added that, in both these last cases, considering the low amount of coeluting compounds, the quantification of terpinen-4-ol and α-terpinene was practically not affected due to the higher amount of the latter. On the other hand sesquiterpenes in lower concentrations were greatly affected by coelutions, i.e. δ-cadinene (LRI_{exp} 1523/LRI_{ref} 1518 peak 16,) coeluted with *trans*-calamenene (LRI_{exp} 1530/LRI_{ref} 1527, peak C), which lead to an overestimate of 30% (1.08% vs. 0.83%). Globulol (LRI_{ref} 1590) was not identified in monodimensional GC/MS but, considering the possible presence at trace level, an heart-cut was performed anyway, with a time window corresponding to its LRI ±5 (the time corresponding to the LRI 1585 and 1595 was retro calculated from the LRI formula). The absence of this compound was confirmed by the 2D chromatogram where spathulenol (LRI_{exp} 1588/LRI_{ref} 1586, peak E) appeared, probably transferred in the same heart-cut. The last heart-cut, relative to viridiflorol (LRI_{exp} 1591/LRI_{ref} 1594, peak 18) showed the coelution with cubeban-11-ol (LRI_{ref} 1599, peak D) justifying the difference between 1D and 2D quantification (0.60% vs. 0.38%). All the coelutors were in agreement with the chemical composition of TTO reported in literature [9,30]. Concerning the quantification of 1,2,4-trihydroxymenthane and ascaridole, it was clear that the monodimensional separation power was not satisfactory to achieve the separation and quantification of the compounds present in lower concentrations. The repeatability of the method for 10 replicates was calculated in terms of first dimension retention times and second dimension areas showing values lower than 0.07% and 3.08%, respectively (Table 3). The repeatability of the retention times in the first dimension was the goal that allows multi heart-cuts, thanks to the constant back pressure at the end of the first dimension column due to the use of the three restrictors configuration of the transfer system [24]. LoD and LoQ relative amounts calculated were largely below the limits reported in the normative for all the compounds. As expected, the data obtained from the multidimensional application have to be considered the most accurate, thanks to the higher resolution power as previously proven. The amount of terpinen-4-ol in the analyzed oil is in the range proposed by the ISO/FDIS 4730:2004: the conversion to the oxidation product 1,2,4-trihydroxymenthane appears to be negligible, which was confirmed by the trace amount of this allergenic compound in the 2D separation. A certain extent of oxidation of the oil was observed considering the conversion of α-terpinene to p-cymene. α-terpinene levels decreased from 5 to 2.9%, while p-cymene levels increased to about twice the maximum level reported by the regulation (14.4%). Ascaridole, the endoperoxide product of α-terpinene oxidation, was found in an amount (0.2%) that, considering the total coelution with other compounds in the monodimensional application, should be very difficult to identify and to quantify using conventional GC-FID or GC/MS.

4. Concluding remarks

A deep study for the quality assessment of the tea tree oil was carried out enclosing chiral information to confirm the natural-like and multidimensional GC for the evaluation of the oxidation state of the oil. The MDGC/MS system described has demonstrated the capability to provide accurate quantification of different chemicals within such a complex matrix as the essential oils, thus allowing the quality assessment with regards to the international regulations. After the first column pre-separation, the MS detector in the second dimension was able to identify the compounds of interest as well the possible coeluents with high similarity matches due to the high spectrum purities. The heart-cuts prevented the possible over/underestimation of the relative amount; as a matter of fact, the presence of other peaks in the 2D chromatogram was a confirmation of the coelutions with interfering compounds in the mono dimensional application. Moreover, the multi heart-cut capability of the system described provide the possibility to achieve the simultaneous investigation on practically an unlimited number of fractions due to the high reproducibility of the retention times in the first dimension chromatogram. LoD and LoQ relative amounts obtained were largely below the limits of the regulations. Finally, the instrumentation is user-friendly, equipped with dedicated software for MDGC operation, and can be used by inexperienced operators in routine analysis.

Acknowledgments

The project was funded by the Italian Ministry for the University and Research (MUR) with a PNR 2005–2007 Project n. RBIP06SXMR “Sviluppo di metodologie innovative per l’analisi di prodotti agroalimentari”. The authors gratefully acknowledge Shimadzu and Sigma–Aldrich/Supelco Corporations for the continuous support.

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