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Technical Report

Comprehensive 2D GC with Rapid-Scanning Quadrupole Mass Spectrometry for the Analysis of Tea Tree Essential Oil

GC×GC–quadMS analysis of fresh and aged tea tree essential oil

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Abstract:

The present research is focused on the qualitative elucidation of the chemical profile of fresh tea tree essential oil, and an oxidized, aged (circa 1984) counterpart, by using comprehensive two-dimensional gas chromatography (GC×GC) in combination with a mass spectrometer. The rapid-scanning quadrupole MS system employed generated a sufficient number of spectra/s (20/s) for the reliable identification of the high-speed GC×GC peaks. Fresh TTO is characterized by a relatively low allergic capacity, while the oxidation products, generated from aged (after long storage periods) oils, have been proposed as potential allergens.

Keywords: comprehensive 2D gas chromatography, tea tree essential oil, mass spectrometry

1. Introduction

Tea tree essential oil (TTO) is produced on a commercial-scale by steam distillation of the foliage of the Australian tea tree (*Melaleuca alternifolia,* Myrtaceae). Overall, the tea tree essential oil's composition consists of about 50% monoterpene hydrocarbons and 50% oxygenated monoterpenes, although it can vary in relation to the climate, distillation conditions, leaf age, etc. TTO has a variety of usages, as a component of cosmetic products, a remedy for a series of infections or as an antiseptic. Despite freshly-distilled TTO is characterized by a relatively low allergic capacity, the oxidation products, formed during long storage periods, have been proposed as potential allergens. For such a reason, in 2008, the Scientific Committee on Consumer Products (SCCP) delivered an opinion on tea tree oil.

The composition of TTO is dramatically affected by the oxygen. In fact, the levels of α-terpinene, γ-terpinene, and terpinolene decrease, while the amounts of *p*-cymene can increase considerably. Other well-known oxidation products are ascaridole and 1,2,4-trihydroxymenthane, two potential allergens.

The SCCP opinion on tea tree oil concluded that it was difficult to measure and identify the degradation products, and reported that 1,2,4-trihydroxymenthane was not present at any detectable level during a 12 month in-use stability trial.

Even though GC–MS is a highly powerful tool, proper peak assignment is somewhat dependent on sample complexity. In fact, complete separation of essential oils carrying more than 100 compounds is a challenging task, and many trace-amount components are often not detected. Such problems can be dealt with comprehensive two-dimensional GC (GC×GC).

2. Experimental

2-1. Reagents and materials

The TTO was diluted (1:10, *v/v*) in ethanol prior to GC×GC–quadMS analysis.

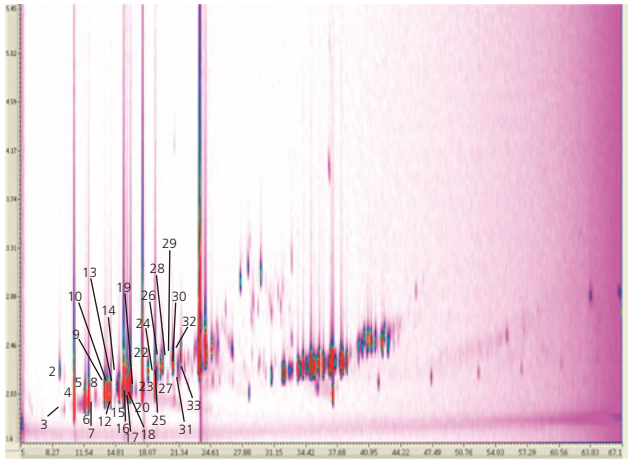

Fig. 1 GC×GC–quadMS chromatogram of fresh tea tree oil (*see* Table 1 for peak assignment).

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2-2. Instrumentation (Shimadzu)

- AOC-20i split-splitless auto-injector.
- GC-2010 gas chromatograph (GC2).
- Zoex dual-stage loop-type cryogenic modulator
- GCMS-QP2010 Ultra.
- D1 column : SLB-5ms 30 m × 0.25 mm ID × 0.25 µm d_f column [silphenylene polymer virtually equivalent in polarity to poly (5% diphenyl/95% methylsiloxane)] (Supelco, Milan, Italy)
- D2 column : Supelcowax-10 1 m × 0.1 mm ID × 0.1 µm d_f [100% poly (ethyleneglycol)]

2-3. Chromatographic method

GC×GC–quadMS analysis	
GC1 oven	: from 50°C to 280°C at 3°C/min
GC2 oven	: from 80°C to 280°C (10 min) at 3°C/min
Carrier gas	: Helium
Inlet pressure	: 133.3 kPa (constant linear velocity mode)
Injection	: 1 µL, split 1:100.
Modulation period	: 6 seconds.

GC–quadMS analysis	
GC	: from 50°C to 280°C at 3°C/min
Carrier gas	: Helium
Inlet pressure	: 37.1 kPa
linear velocity	: 32.4 cm/s
Injection	: 1 µL, split 1:50.

2-4. Software

- GCMSsolution version 4.0.

2-5. 2D Software

- ChromSquare version 2.0.

2-6. Detection

GC×GC–quadMS parameters	
MS ionization mode	: electron ionization
Scan speed	: 10,000 amu/sec
Mass range	: 40–400 *m/z*
Acquisition frequency	: 20 Hz
Ion source temperature	: 250°C
Interface temperature	: 280°C

GC–quadMS parameters	
Scan speed	: 1,666 amu/sec
Mass range	: 40–400 *m/z*
Acquisition frequency	: 4 Hz

3. Results and discussion

A GC×GC–quadMS method was developed for the qualitative comparison between a fresh and aged tea tree oil. A positive offset (30°C) was applied in GC2 in order to reduce wrap-around. The inlet pressure generated first and second dimension linear velocities of about 10.4 and 114 cm/s, respectively, and the MS sampling frequency (20 Hz) allowed a sufficient number of spectra per peak for reliable identification, while at least 2–3 modulations were attained for each 1D peak.

The GC×GC–quadMS chromatogram of the fresh TTO sample is shown in Fig. 1. The first part of the 2D chromatogram (defined by the compounds from 1 to 33) is characterized by low complexity, while the central part, illustrated in the expansion reported in Fig. 2, presents a higher complexity.

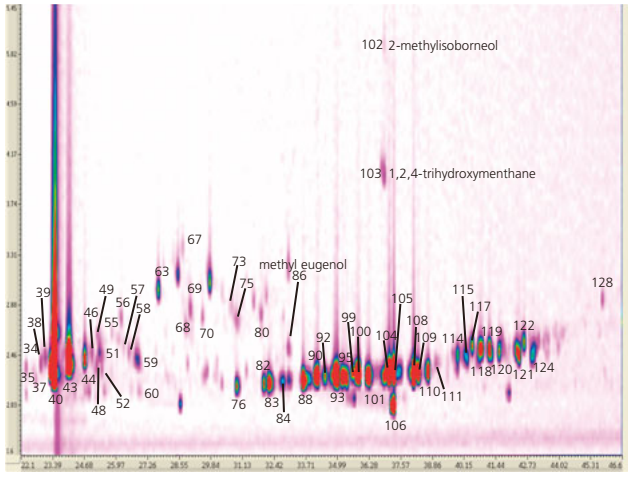

Fig. 2 GC×GC–quadMS chromatogram expansion of fresh tea tree oil (*see* Table1 for peak assignment).

Overall, 90 components were identified through mass spectral library searching (database matches with a spectral similarity lower than 80% were not considered) (Table 1). Initially, the application of an automatic library search filter, a function of the GC–MS software employed, deleted results with an MS similarity lower than 90% (*vs.* the experimental spectra), enabling the identification of 61 peaks. The remaining 29 compounds (apart from peak 102) were assigned with a library match in the 80–89% similarity range. In the conventional GC–MS experiment, 61 TTO constituents were identified with the above mentioned MS filters (Table 1).

A number of oxidation products were detected by using this GC×GC–quadMS approach. 1,2,4-Trihydroxymenthane (peak 103) was not identified in the conventional GC–quadMS analysis because: i) this compound was probably present in an amount below the LOD of the method [the *S/N* ratio (measured considering the highest modulated peak) was ~ 7 in the GC×GC–quadMS analysis], and ii) it was presumably hidden by a closely-eluting overloaded compound.

Peak 102 (2-methylisoborneol) exhibited a low S/N ratio and a relatively low library match (77%). The identity of this component was confirmed by comparing its 2D retention coordinates with the more abundant peak in the aged TTO. Methyl eugenol (peak 86) is a minor TTO constituent and suspected carcinogen. In the GC–quadMS experiment, it was identified with an 88% spectral similarity, while GC×GC–quadMS gave a notable improvement in signal intensity by approximately 4 fold.

The expansion of the GC×GC–quadMS chromatogram of the aged TTO sample is shown in Fig. 3. Overall, 108 compounds were identified (Table 1).

The qualitative profile, relative to the first part, was quite similar to that of the fresh sample, although the relative abundance of α-terpinene (peak 15), γ-terpinene (peak 20), and terpinolene (peak 23) are greatly reduced. Instead, *p*-cymene (peak 16) is approximately doubled in the aged essential oil. This result is in agreement with the fact that the distribution of monoterpene hydrocarbons in TTO drastically changes in the presence of O_2, high temperatures and light. Para-cymene is often used as an indicator of the extent of TTO oxidation, and it has been shown that 1,2,4-trihydroxymenthane increases proportionally to the content of *p*-cymene. Terpinen-4-ol (peak 40), the major TTO constituent, was present in higher amounts in the aged oil, while a similar peak area was observed for methyl eugenol in both samples. Moreover, the amount of 1,2,4-trihydroxymenthane was strikingly higher in the aged TTO. Trans-ascaridole glycol, another oxidation product (peak 63), was present in higher amounts in the aged product. Peak 65 (*cis*-ascaridole glycol) was also present in both samples, but was only reliably assigned in the aged sample. Finally, ascaridole epoxide (peak 55), contained in very low amounts in both samples, was identified with good library matches.

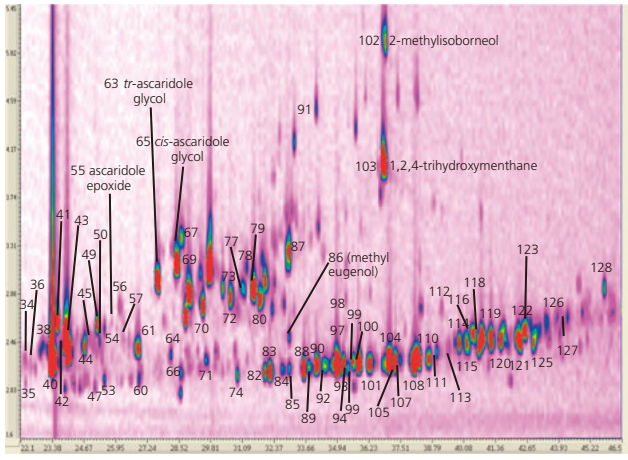

Fig. 3 GC×GC–quadMS chromatogram expansion of the aged tea tree oil (*see* Table 1 for peak assignment).

4. Conclusions

The results reported in the present technical report highlight the potential of GC×GC combined with rapid-scanning quadMS for the qualitative analysis of complex matrices. The GC×GC–quadMS method developed enabled high resolution separation and differentiation of the TTO samples.

Moreover, the method offered increased sensitivity (by a factor of ~ 5–10), allowing the detection of many trace-amount components.

A number of potential allergens were identified in the fresh and aged TTO samples. Notably these could not be detected using GC–quadMS, indicating that previous reports may have erroneously reported their absence.

Table 1 Compounds Identified in tea tree oil samples. MS% = spectral similarity (values reported in parenthesis are GC–quadMS results).

Peak/Compound	MS % fresh TTO	MS % aged TTO	Peak/Compound	MS % fresh TTO	MS % aged TTO
1. 6-methyl-3,5-heptadien-2-one	—	86	**20.** γ-terpinene	94 (89)	96 (95)
2. *cis*-3-hexen-1-ol	98 (94)	96 (95)	**21.** 1,2-diacetylethane	—	88
3. 3-methyloctane	94	—	**22.** *trans*-sabinene hydrate	93 (93)	92 (94)
4. nonane	85	98 (89)	**23.** terpinolene	96 (96)	98 (96)
5. α-thujene	98 (99)	96 (99)	**24.** *p*-cymenene	96 (95)	82 (81)
6. α-pinene	95 (97)	97 (98)	**25.** linalool	98 (98)	98 (97)
7. camphene	90 (97)	94 (97)	**26.** *cis*-sabinene hydrate	92 (96)	90 (94)
8. sabinene	97 (97)	91 (98)	**27.** verbenene	81	—
9. β-pinene	96 (97)	97 (97)	**28.** fenchyl alcohol	95 (97)	90 (97)
10. myrcene	98 (97)	97 (96)	**29.** *cis-p*-menth-2-ene-1-ol	95 (98)	96 (98)
11. 2,3,6-trimethyl-1,5-heptadiene	—	93	**30.** 1-terpineol	92 (96)	95 (98)
12. 2-undecene	81	—	**31.** 1,3-cyclohexadiene	90	—
13. *cis*-3-hexenyl acetate	98 (93)	96 (91)	**32.** *trans-p*-menth-2-ene-1-ol	96 (95)	90 (94)
14. α-phellandrene	98 (97)	97 (97)	**33.** *cis*-limonene oxide	84 (83)	81
15. α-terpinene	92 (96)	97 (97)	**34.** camphene hydrate	92 (95)	81 (81)
16. *p*-cymene	94 (98)	94 (81)	**35.** *cis*-pinene hydrate	80	80
17. limonene	98 (95)	98 (97)	**36.** carvone oxide	—	80
18. 1,8-cineole	97 (92)	98 (92)	**37.** *cis*-8-hydroxylinalool	83	—
19. *trans*-β-ocimene	95 (98)	92 (96)	**38.** γ-terpineol	93 (94)	95 (93)

39. borneol	89 (89)	—	**85.** longicyclene	—	87
40. terpinene-4-ol	90 (91)	94 (97)	**86.** methyl eugenol	94 (88)	92
41. *p*-cymen-8-ol	—	96 (94)	**87.** isocaucalol	—	81
42. cryptone	—	86	**88.** α-gurjunene	96 (96)	96 (96)
43. α-terpineol	96 (97)	98 (97)	**89.** γ-maaliene	—	91 (90)
44. *trans*-piperitol	95 (92)	94 (94)	**90.** β-caryophillene	97 (97)	97 (97)
45. dehydrosesquicineole	—	80	**91.** carvone hydrate	—	82
46. 1-*p*-Menthene-9-al	83	—	**92.** aromadendrene	93 (92)	92 (97)
47. 3,6-dimethyl-1,5-heptadiene	—	87	**93.** 9-epi-(E)-caryophyllene	91 (98)	91 (97)
48. santene	83	—	**94.** selina-5,11-diene	—	90 (92)
49. 4,6-dimethyl-2-octanone	80	83	**95.** germacrene D	90 (91)	—
50. dehydrolinalool	—	85	**96.** hydroxy citronellal	—	80
51. *trans*-geraniol	85 (90)	—	**97.** 6,7-epoxide citral	—	81
52. isoascaridole	88 (88)	—	**98.** valerena-4,7(11)-diene	—	91 (89)
53. 3-methyl-3-cyclohexen-1-ol	—	83	**99.** α-humulene	96 (97)	96 (96)
54. 6-methyl-5-hepten-2-one	—	86	**100.** allo-aromadendrene	97 (90)	97 (96)
55. limonene epoxide	84	80	**101.** γ-gurjunene	87	93 (87)
56. ascaridole epoxide	90	92	**102.** 2-methylisoborneol	77	85
57. β-sanatlol	81	80	**103.** 1,2,4-trihydroxymenthane	90	93 (93)
58. nerol	90 (86)	—	**104.** viridiflorene	97 (96)	97 (94)
59. piperitone	88 (92)	—	**105.** bicyclogermacrene	96 (97)	92 (89)
60. apo vertenex	80	—	**106.** bicycloelemene	95	—
61. carvenone	—	93 (95)	**107.** β-vetispirene	—	84
62. *trans,trans*-2,4-dodecadienal	—	85	**108.** γ-cadinene	95 (94)	97 (91)
63. *trans*-ascaridole glycol	90 (91)	91 (91)	**109.** zonarene	93 (91)	—
64. 5-hydroxy-isobornyl isobutanoate	—	82	**110.** *trans*-cadina-1(2),4-diene	97 (95)	92 (95)
65. *cis*-ascaridole glycol	—	90	**111.** α-calacorene	92 (97)	96 (99)
66. iso-thujyl acetate	—	85	**112.** 1(2H)-naphthalenone, 5-ethyl-3,4-dihydro-	—	80
67. carvacrol	85 (86)	96	**113.** caryophyllene oxide	—	80
68. 2,5-dimethyl-2,4-hexadiene	81	—	**114.** viridiflorol	93 (93)	92 (95)
69. 1,3-dioxolane, 2,2-dimethyl-4,5-di-1-propenyl-	84	85	**115.** palustrol	92	90
70. *cis*-linalool oxide	82	83	**116.** ledol	—	90 (91)
71. laciniata furanone E	—	80	**117.** isospathulenol	95 (96)	—
72. *p*-menth-6-en-2,3-diol	—	82	**118.** globulol	96 (92)	96 (93)
73. *cis*-piperitol	80 (81)	82	**119.** pogostol	86	88
74. β-cubebene	—	94 (98)	**120.** rosifoliol	83	84 (83)
75. diepoxide allocimene	84	—	**121.** 1-epi-cubenol	95 (97)	86 (85)
76. α-cubebene	94 (98)	—	**122.** spathulenol	90	93
77. menthone	—	80	**123.** bicyclo[7.2.0]undecan-3-ol <11,11-dimethyl-, 4,8-bis(methylene)->	—	86
78. dihydro-linalool acetate	—	82			
79. 4-methyl-dec-3-en-5-ol	80	82	**124.** cubenol	94 (97)	—
80. 1,4-cineole	80	83	**125.** torreyol	—	92
81. α-methylenedodecanal	—	80	**126.** hydroxycaryophyllene	—	90
82. isoledene	93 (99)	93 (98)	**127.** cedrol	—	80 (82)
83. α-copaene	94 (94)	94 (93)	**128.** 8-α-acetoxyelemol	83	82
84. β-elemene	95 (94)	93 (96)	**129.** flourensadiol	—	80

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