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Journal of Chromatography A, 1 (2000) 000–000

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Characterisation of lavender essential oils by using gas chromatography–mass spectrometry with correlation of linear retention indices and comparison with comprehensive two-dimensional gas chromatography

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Abstract

Nine samples of lavender essential oil were analysed by GC–MS using low-polarity and polar capillary columns. Linear retention indices (LRI) were calculated for each component detected. Characterisation of the individual components making up the oils was performed with the use of an mass spectrometry (MS) library developed in-house. The MS library was designed to incorporate the chromatographic data in the form of linear retention indices. The MS search routine used linear retention indices as a post-search filter and identification of the ''unknowns'' was made more reliable as this approach provided two independent parameters on which the identification was based. Around 70% of the total number of components in each sample were reliably characterised. A total of 85 components were identified. Semi-quantitative analysis of the same nine samples was performed by gas chromatography (GC) with flame ionisation detection (FID). The identified components accounted for more than 95% of each oil. By comparing the GC–MS results with the results from the GC×GC–FID analysis of a lavender essential oil, many more components could be found within the two-dimensional separation space.

Keywords: Lavender; Essential oils; Linear retention indices; Comprehensive two-dimensional gas chromatography

1. Introduction

Lavandula essential oils are obtained from the flowering tips of the plants *Lavandula angustifolia* (lavender), *Lavandula hybridia* (lavandin) and *Lavandula latifolia* (spike lavender). These oils have a popular and easily recognisable fragrance. Pure *L. angustifolia* essential oils are used in aromatherapy, and are thought to have calmative, anti-flatulence, and anti-colic properties [1]. The primary use of lavender oils however is as raw ingredients in industrial perfume and fragrance materials, with the bulk of this market filled by lavandin oils [2]. Lavender essential oil is characterised by high levels of linalool, and linalyl acetate, moderate levels of lavandulyl acetate, terpinen-4-ol and lavandulol. The amount of 1,8-cineole and camphor often varies between very low to moderate [2]. This is a somewhat simplified description; indeed lavender oil typically contains many more than 100 individual components (many minor ones often unidentified

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0021-9673/02/$ – see front matter
PII: S0021-9673(02)00653-2

and/or not quantitated), each contributing to the chemical and sensory properties of the oils.

For many years, gas chromatography–mass spectrometry (GC–MS) has been the benchmark technique for the qualitative analysis of flavour and fragrance volatiles, and commercially available MS libraries contain many hundreds of mass spectra of such compounds. MS libraries allow tentative identification of essential oil components. Thus GC and GC–MS have been used extensively for the characterisation of lavender essential oils (as reviewed by Boelens [3]). The analysis of living lavender flowers using solid-phase microextraction and GC–MS has also been described [4]. Identification of individual components of essential oils however is not always possible using MS data alone. Differences in mass spectra may be observed if the spectra were obtained using a quadrupole MS, as opposed to using an ion trap MS [5]. Often different spectra are reported in a library for a single compound, with different common names, or systematic name, corresponding to an individual component sometimes apparent. The spectral similarity of a great number of essential oil components causes difficulty in obtaining positive identification of individual components; mass spectra for sesquiterpenes are often identical or nearly identical [6]. More than 230 naturally occurring sesquiterpenes have a molecular mass of 204 [7].

Chromatographic retention data can support MS data, providing an independent parameter on which to base compound identity. The reproducibility and reliability of retention indices allows assignment of identity to unknown components with greater confidence. Both retention indices and MS data of essential oil components are reported in compilations such as Adams [8], Jennings and Shibamoto [9], and Davies [10], and in a number of more recent publications [11–13]. An MS library incorporating the use of linear retention indices (LRI) as part of an interactive library search has also been described [14]. Called FFC (flavour and fragrance compounds), the library was used to characterise citrus essential oils, and more recently used to characterise several varieties of Australian tea tree oil [15].

The GC–MS analyses and the GC–flame ionisation detection (FID) quantitative analyses in the present investigation were all performed using two independent columns. The sample complexity demanded two independent analyses, on dissimilar stationary phase columns. Adequate resolution of many individual components was not possible in a single analysis. Comprehensive two-dimensional gas chromatography (GC×GC) provides a substantial increase in peak capacity by serially coupling two capillary columns. The principles, modulation processes and applications of GC×GC have been reviewed recently [16,17]. Cryogenic modulation was used in the present investigation to achieve the GC×GC result, allowing effective modulation of effluent from the first column [18]. Separation of many unresolved components from the first column is achieved in the second column. The application of GC×GC to the analysis of essential oils has been reported [19–23], with two studies focussing on lavender essential oil [19,20]. By using a low-polarity column–polar column combination, and by using suitable operating conditions, the two-dimensional separation achieves an increase in peak capacity of the order of 7–12 times.

The present study describes the use of high-resolution GC–MS with LRI to characterise a range of lavender essential oils obtained by steam distillation from a number of different lavender cultivars used for the production of essential oils in Australia. Results are also compared to those obtained from GC×GC analysis of a similar sample. Future opportunities of this technique are discussed.

2. Experimental

2.1. GC–MS analysis

For all analyses, a Shimadzu QP5050A GC–MS, fitted with a Shimadzu AOC-20i auto sampler, and Shimadzu Class-5000 Chromatography Workstation software (Shimadzu, Italy) was used.

All analyses were carried out by using two different stationary phase columns. Column 1 was an Rtx-5MS (0.25 μm film thickness) fused-silica capillary column. The column dimensions were 30 m× 0.25 mm. Column 2 was an Rtx-WAX (0.25 μm film thickness) fused-silica capillary column. The column dimensions were 30 m×0.25 mm. Both columns were from Restek (Milan, Italy).

The GC was operated under temperature pro-

grammed conditions from 45 °C (6 min) to 250 °C at 3 K min^{-1}. The GC was equipped with a split/splitless injector; an injection volume of 0.5 μl was employed using the auto sampler, and a split ratio of ca. 70:1 was used. The carrier gas was helium, and the column head pressure was 24.9 kPa. The GC was operated in constant pressure mode.

The MS scan parameters included a mass range of 40–400 $m.z^{-1}$, a scan interval of 0.5 s, a scan speed of 1000 $amu.s^{-1}$, and a detector voltage of 1.5 kV.

2.2. GC–FID analysis

Quantitative results were obtained using a Shimadzu GC17A ver.3 gas chromatograph (Shimadzu, Milan, Italy) equipped with a FID detector and Shimadzu Class 4.3 Chromatography Workstation software.

The same two columns as described for the GC–MS analyses were used.

The GC was operated under temperature program conditions from 45 °C (6 min) to 250 °C at 3 K min^{-1}. The GC was equipped with a split/splitless injector; an injection volume of 1.0 μl was employed using manual injection, and a split ratio of ca. 100:1 was used. The carrier gas was helium, and the column head pressure was 100 kPa. The GC was operated in constant pressure mode.

2.3. GC×GC analysis

GC×GC analysis was performed using an Agilent Technologies 6890 model gas chromatograph (Agilent Technologies, Burwood, Australia) equipped with a FID detector (operated at 100 Hz data acquisition frequency) 7683 series auto sampler, and Chemstation software.

The GC was retrofitted with an Everest model longitudinally modulated cryogenic system (Chromatography Concepts, Doncaster, Australia). A modulation frequency of 0.2 Hz (5 s cycle) was applied and the thermostatically controlled cryogenic trap was maintained at ca. 0 °C for the duration of the analysis.

The column set for GC×GC analysis consisted of two columns, which were serially coupled by a zero-dead-volume fitting. The primary column was a BPX5 (0.25 μm film thickness) fused-silica capillary column. The column dimensions were 30 m×0.25 mm. The secondary column was a BP20 (0.10 μm film thickness) fused-silica capillary column. The column dimensions were 1.0 m×0.10 mm. Both columns were from SGE International (Ringwood, Australia).

The GC was operated under temperature programmed conditions from 60 °C to 210 °C at 2 K min^{-1}, then to 260 °C at 20 K min^{-1}. The GC was equipped with a split/splitless injector; an injection volume of 1.0 μl was employed using the auto sampler, and a split ratio of ca. 100:1 was used. The carrier gas was hydrogen, and the column head pressure was 52 kPa. The GC was operated in constant pressure mode.

2.4. Samples

A total of nine different lavender essential oils were analysed. All samples were provided by Australian Botanical Products (Hallam, Australia). Prior to injection all samples were diluted 1:10 (v/v) with *n*-hexane. A C_9 to C_{30} *n*-paraffin hydrocarbons mixture diluted in *n*-hexane was prepared for determination of linear retention indices. Authentic reference standards of borneol, bornyl acetate, camphor, 1,8-cineole, geranyl acetate, hexyl butyrate, limonene, linalool, linalyl acetate, nerol, neryl acetate, *cis*-3-ocimene, 3-octanone, α-pinene, α-terpinene and γ-terpinene for confirmation of components identified in the GC×GC analysis were provided by Australian Botanical Products.

3. Results and discussion

Qualitative characterisation of nine different lavender essential oil samples was performed using GC–MS. Some of the difficulties faced whilst characterising the individual components are demonstrated in Table 1 which is the library report for an authentic α-terpinene standard using the conditions described in the experimental section above. Thus comparison of library spectra alone as the method of identification of unknown compounds is not reliable. The first nine library matches to the experimental spectrum acquired for the α-terpinene standard are listed as given with all matches reported to have

Table 1
Library match data from the first nine library matches to the experimental spectrum of α-terpinene

Hit #	Quality (%)	Compound name systematic name; common name; (library ref #)	Retention index [5]
1	95	3,7,7-trimethyl bicyclo[4.1.0]hept-2-ene; 2-carene (117923–NIST98 GCMS library)	1001
2	95	3,7,7-trimethyl bicyclo[4.1.0]hept-4-ene; 4-carene (40327–NIST98 GCMS library)	[a,b]
3	95	3,7,7-trimethyl bicyclo[4.1.0]hept-2-ene; 2-carene (40304–NIST98 GCMS library)	1001
4	95	1-methyl-4-(1-methylethylidene)-cyclohexene; terpinolene (121080–NIST98 GCMS library)	1088
5	94	1-methyl-4-(1-methylethyl)-1,3-cyclohexadiene; **α-terpinene** (54660–NIST98 GCMS library)	1018
6	91	1,7,7-trimethyl bicyclo[2.2.1]hept-2-ene; (117922–NIST98 GCMS library)	[a]
7	91	1,7,7-trimethyl bicyclo[2.2.1]hept-2-ene; (117924–NIST98 GCMS library)	[a]
8	91%	1-methyl-4-(1-methylethylidene)-cyclohexene; terpinolene (121079–NIST98 GCMS library)	1088
9	90%	1-methyl-4-(1-methylethyl)-1,3-cyclohexadiene; **α-terpinene** (121075–NIST98 GCMS library)	1018

[a] No reliable RI data.
[b] 4-carene is not expected in lavender oil [3].

match quality ≥90% with respect to the experimental spectrum. The similarity of the ten spectra is apparent from visual comparison of library spectra (not shown herein). Examples of different spectra reported for the same compound within the library is also noted (hits 1&3; 4&8; 5&9; 6&7). Table 1 also shows that whilst these compounds have similar mass fragmentation patterns, their retention indices are quite different. Thus components may be reliably differentiated based on RI data. The FFC library uses a two-step library matching routine, which is built into the Shimadzu GC–MS software. The first step is a spectrum comparison, which inevitably offers a range of incorrect responses. The second step is a post-search filter using retention indices (with a match window of ±5 index units); any match offered in the first step whose retention index is not consistent with the retention index of the unknown component is rejected. By using calculated retention indices and the interactive search filter built into the FFC library, the only possible match offered for the above example would be α-terpinene, which is of course *correct identification*.

For the calculation of LRI shown in Table 2, a C_9–C_{30} *n*-paraffin hydrocarbon series was chromatographed independently to the lavender oil samples. Van den Dool and Kratz's equation [24] was used to calculate linear retention indices by linear interpolation. A cubic spline interpolation has also been suggested for the calculation of temperature programmed retention indices [25]. Some debate has arisen over the reproducibility of retention indices, Tóth reported random variation in calculated retention indices of a range of volatiles over a 2-month period using a temperature program of 4 K min^{-1} (using an older type GC and argon as the carrier gas) [26]. Poor reproducibility of LRI was not experienced during the present investigation (nor is it the general experience of this group). Provided a low temperature program rate is used, the difference in the programmed retention index, and the true isothermal retention index generally does not exceed 1% for terpenoid compounds [9]. Jennings determined retention indices using low temperature program rates of 2 K min^{-1} [9]. Adams similarly used a low temperature programmed rate of 3 K min^{-1} [8]. The correlation between the calculated retention indices and literature retention indices in Table 2 (for

Table 2
GC data for essential oil components identified in lavender

	Compound	Calc. LRI	Ref. RI	Sample								
				1	2	3	4	5	6	7	8	9
1	Tricyclene	923	926	0.03	0.02	0.02	0.03	0.04	0.02		0.02	0.02
2	α-Thujene	930 (1020)	931 (1024)	0.02	0.03	0.05	0.09	0.17	0.09	0.14	0.07	0.07
3	α-Pinene	935 (1015)	939 (1020)	0.52	0.08	0.25	0.63	0.33	0.18	0.73	0.13	0.41
4	Camphene	950 (1052)	985 (1063)	0.54	0.16	0.09	0.45	0.30	0.02	0.08	0.08	0.37
5	Thuja-2,4 (10)-diene	956	957	0.02		0.03	0.02	0.02	0.03	0.03	0.03	
6	Sabinene	976	976	0.19		0.06	0.24	0.04	0.04	0.39	0.06	0.13
7	β-Pinene	978	980	0.63	0.03	0.61	0.62	0.07	0.30	1.21	0.26	0.49
8	Octen-3-ol	988 (1442)	986 (1428)	0.82	0.26	0.59	0.59	1.14	0.03	1.08	0.09	0.24
9	3-octanone	994 (1242)	986 (1205)	0.96	2.61	3.49	0.65	0.95	1.84	0.59	2.69	0.33
10	Myrcene	995 (1157)	991 (1160)	0.37	0.26	0.33	0.53	0.66	1.22	0.66	0.78	0.55
11	3-octanol	1004 (1387)	993 (1398)	0.09	0.40	0.88		0.25	0.53	0.04		0.04
12	α-phellandrene	1009	1005		0.02		0.05	0.07	0.08	0.11		
13	δ-3-carene	1012	1011		0.05	0.07	0.28	0.08		0.30	0.08	0.12
14	1,4-Cineole	1023	1016	0.02	0.04		0.04	0.05	0.03	0.09	0.02	0.04
15	*o*-cymene	1026 (1254)	1022 (1266)	0.03	0.04	0.12	0.08	0.09	0.10	0.07	0.05	0.03
16	*p*-cymene	1028 (1185)	1026 (1197)	0.10	0.11	0.37	0.42	0.45	0.32	0.43	0.38	0.19
17	Limonene	1031 (1193)	1031 (1206)	1.76	0.18	0.29	3.37	0.68	0.64	3.92	2.35	1.89
18	1,8-cineole	1036 (1227)	1033 (1229)	10.87	0.59	0.10	11.64	1.18	0.54	20.28	0.35	5.56
19	(Z)-β-ocimene	1043	1040	2.78	0.95	4.77	3.15	6.17	3.12	2.41	3.91	1.94
20	(E)-β-ocimene	1053	1050	1.04	0.92	0.61	0.96	0.75	2.36	0.57	1.35	0.34
21	γ-terpinene	1062	1062	0.05	0.04	0.07	0.12	0.20	0.08	0.22	0.21	0.19
22	*trans*-sabinene hydrate	1075 (1426)	1068 (1430)	0.20	0.05		0.16	0.08	0.05	0.17	0.04	0.10
23	*cis*-linalool oxide	1079	1074	1.09	0.79	0.95	0.94	0.52	0.50	1.00	0.52	0.34
24	Terpinolene	1088	1088	0.18	0.03	0.05	0.14	0.19	0.22	0.07	0.02	0.25
25	*trans*-linalool oxide	1088 (1460)	1088 (1460)	0.99	0.64	0.74	0.77	0.38	0.35	0.92	0.39	0.26
26	Perillene	1102 (1540)	1099 (1531)	0.04	0.02	0.04	0.08	0.04	0.08	0.05	0.05	
27	Linalool	1112	1098	42.68	57.48	34.37	33.74	38.89	23.03	40.37	25.22	26.73
28	Endo-fenchol	1114 (1371)	1112	0.34	0.27	0.54	0.20	0.21	0.12	0.33	0.20	0.11
29	octen-3-yl acetate	1120	1110	0.19	0.37	2.09	0.16	4.16	1.00	0.23	2.01	0.47
30	*cis*-*p*-menth-2-en-1-ol	1126	1121	0.02	0.02		0.03	0.04	0.03		0.02	
31	Norborneol acetate	1132	1127	0.04	0.08	0.42	0.06	0.09	0.27	0.06	0.51	
32	α-Campholenal	1135	1125	0.06			0.06	0.04		0.08	0.02	0.06
33	*trans*-pinocarveol	1146	1139	0.11	0.01	0.34	0.11	0.03		0.16	0.04	0.05
34	Camphor	1152 (1482)	1143 (1508)	5.08	0.34	0.35	2.57	0.36	0.09	0.58	0.09	7.10
35	Hexyl-iso butyrate	1156	1150	0.19	0.09	0.10	0.19	0.11	0.08	0.24	0.05	0.16
36	Isoborneol	1159	1156					0.06	0.04	0.11	0.06	
37	Sabina ketone	1160	1165	0.10	0.03	0.05	0.10	0.05	0.07		0.02	0.02
38	*cis*-chrysanthenol	1162	1162	0.02	0.02	0.06	0.04		0.02			0.02
39	3-thujanol	1167	1166					0.03		0.03	0.01	
40	Borneol	1172	1165	10.98	0.71	0.65	14.04	0.79	0.30	0.91	0.44	0.45
41	Lavandulol	1175 (1662)	1166 (1682)	0.14	3.27	0.72	0.10	0.32	0.16	0.86	0.27	0.05
42	terpinen-4-ol	1184 (1581)	1177 (1592)	0.27	1.93	1.67	3.01	8.07	3.21	4.17	0.11	1.87
43	*m*-cymen-8-ol	1187	1180	0.02	0.02	0.03	0.12	0.18	0.21	0.09	0.11	0.07
44	*p*-cymen-8-ol	1190	1183	0.14	0.12	0.27	0.24	0.19	0.19	0.16	0.12	
45	Neoisomenthol	1193	1188	0.41	0.12	0.27	1.10	0.54	0.47	2.26	0.33	4.25
46	α-Terpineol	1198 (1667)	1189 (1680)	0.90	1.01	1.59	1.57	3.02	6.02	1.72	3.28	0.12
47	Hexyl butyrate	1199 (1406)	1191 (1407)	0.22	0.35	0.35	0.56	0.45	0.47	1.72	0.39	0.12
48	Myrtenol	1204	1194	0.17	0.04	0.12	0.14		0.14	0.21	0.07	0.09
49	*Cis*-carveol	1228	1229	0.09	0.02	0.04	0.13	0.09	0.07	0.11	0.05	0.02
50	Dihydro carveol	1234	1226		0.05	0.05	0.51	0.07		0.10	0.04	0.03
51	Isobornyl formate	1237	1233	0.36	0.13	0.19	0.16	0.44	0.10	0.15	0.52	0.13

Table 2. Continued

	Compound	Calc. LRI	Ref. RI	Sample								
				1	2	3	4	5	6	7	8	9
52	Hexyl-2-methyl butyrate	1243	1234	0.07	0.03	0.08	0.26	0.06	0.04	0.30	0.07	0.05
53	Cumin aldehyde	1248	1239	0.32	0.05	0.04	0.49	0.32	0.29	0.53	0.22	0.12
54	Carvone	1254	1242	0.13	0.03	0.18	0.19	0.12	0.02	0.16	0.07	0.03
55	Linalyl acetate	1264 (1548)	1257 (1548)	8.18	15.74	27.24	6.48	17.05	35.39	4.01	35.25	33.26
56	Dihydro linalool acetate	1286	1275	0.11	0.05	0.13	0.21	0.14	0.07	0.16	0.13	0.02
57	Bornyl acetate	1293	1285	0.09	0.04	0.04	0.14	0.32	0.04	0.08	0.12	0.03
58	Lavandulyl acetate	1298 (1594)	1289 (1597)	0.70	3.96	2.72	1.07	1.48	6.16	0.65	6.00	2.73
59	Carvacrol	1301	1298	0.11	0.03	0.15	0.18	0.17	0.13	0.16	0.13	
60	Hexyl tiglate	1337	1331	0.11	0.05	0.10	0.13	0.09	0.06	0.21	0.05	0.10
61	Neo-isopulegol	1347	1340	0.03	0.03	0.09	0.04	0.04	0.04	0.05	0.04	0.04
62	Neryl acetate	1371	1356	0.07	0.22	0.45	0.11	0.60	1.23	0.14	0.73	0.21
63	α-Copaene	1380	1372	0.02	0.03	0.03		0.05	0.03		0.03	
64	Daucene	1384	1380	0.05		0.02	0.03			0.05	0.01	0.10
65	Geranyl acetate	1390 (1740)	1383 (1750)	0.19	0.39	0.62	0.35	1.15	2.37	0.32	1.31	0.48
66	β-Bourbonene	1394	1384	0.04	0.04	0.04	0.08	0.04	0.02	0.07	0.03	0.09
67	α-Cedrene	1410	1409	0.06	0.01	0.02	0.06	0.02	0.02	0.07	0.02	0.09
68	α-*cis* bergamotene	1420	1415	0.03	0.03	0.09	0.02	0.05		0.03	0.05	0.03
69	E-caryophyllene	1426	1418	0.90	2.20	2.83	0.45	1.62	1.04	1.09	1.88	1.49
70	Lavandulyl isobutyrate	1435	1423	0.06			0.04		0.02	0.03	0.02	0.08
71	α-*trans*-bergamotene	1440	1436	0.10			0.07	0.13	0.02	0.12	0.12	0.15
72	(E)-β-farnesene	1461 (1653)	1458 (1668)	0.27	1.09	0.29	1.69	0.74	0.17	0.62	0.60	1.21
73	Germacrene D	1464		0.54	0.60	0.37	0.22	0.16	0.24	0.46	0.35	0.94
74	Bicyclogermacrene	1488 (1680)	1480 (1681)	0.03	0.03	0.09	0.02	0.05		0.03	0.05	0.04
75	α-Bulnesene	1491	1494	0.05	0.02	0.02	0.05			0.07	0.01	0.10
76	Lavandulyl isovalerate	1504	1505	0.48			0.55	0.03		0.49	0.03	0.73
77	*trans*-γ-cadinene	1515	1510	0.13	0.03	0.05	0.09	0.15		0.13	0.04	0.39
78	δ-Cadinene	1522		0.03	0.13	0.24	0.03	0.12	0.08	0.04	0.13	0.38
79	Spathulenol	1586	1576	0.02	0.01	0.06		0.04		0.02		0.02
80	Globulol	1589	1583	0.02	0.02	0.05	0.03	0.02		0.02	0.02	
81	Epicubenol	1638	1627	0.02	0.01	0.03				0.02		
82	α-Muurolol	1652	1645	0.19	0.04	0.02	0.16	0.47		0.20		0.48
83	α-Cadinol	1662	1653	0.02	0.02	0.42	0.05	0.03		0.03	0.09	0.02
84	Bisabolol oxide B	1666	1655	0.04	0.01	0.04	0.08	0.02		0.07	0.02	0.02
85	α-bisabolol	1698	1683	0.37		0.03	0.70	0.02		0.71		

For calculated and reference retention indices of the components, the numbers in parenthesis represent the retention indices derived from a polar (polyethylene glycol) capillary column, whilst all other retention indices are derived from a non-polar (Rtx-5MS 5% phenyl equivalent) capillary column. Relative amounts of the 85 identified components in the nine lavender essential oil samples. All figures represent % abundance (area percent, not inclusive of solvent peak).

the same stationary phase) confirms that the conditions used were satisfactory for this class of sample.

A typical GC–MS total ion count chromatogram for a lavender essential oil sample is shown in Fig. 1. The numbered peaks refer to the identities of some of the components listed in Table 2 (some minor components not labeled). More than 95% of the total mass of each sample has been identified using GC–MS, LRI and the FFC library. 72, 67, 60, 69, 63, 63, 67, 62 and 77% of the components in samples one to nine, respectively were characterised. For a number of reasons, assignment of around 30 or more components in each oil could not be made. These minor components should not be overlooked as they also contribute to the overall qualities of an essential oil. The main reason that these oil components were not characterised is that sufficiently accurate mass spectra could not be obtained for the components. Many of the components which remain uncharacterised eluted in the sesquiterpene region of the chromatogram, where there is a high number of poorly

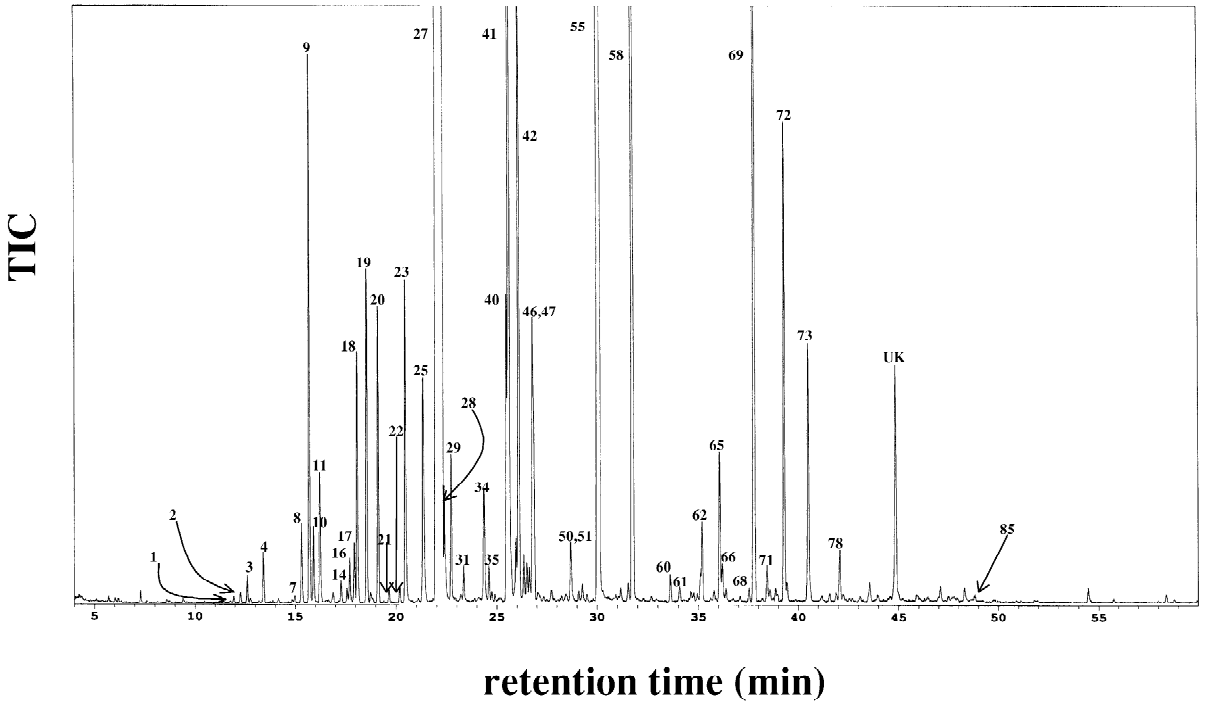

Fig. 1. Typical GC–MS TIC chromatogram for lavender essential oil. The numbers refer to those in Table 2. A Rtx-5MS column was used. UK, unknown.

resolved, structurally related compounds. In this case background correction is imprecise and hence spectral quality is degraded. Identification of individual components was especially difficult in the case where a minor components' retention time was very similar to that of a more prominent component. Complete characterisation of essential oils is the aim of future investigations and may be possible using the superior resolution provided by comprehensive two-dimensional gas chromatography, coupled with time-of-flight mass spectrometry (GC×GC–TOF-MS).

Semi-quantitative data for the nine samples analysed are also reported in Table 2. Quantitation was performed using FID. Although GC–MS is commonly used to obtain quantitative sample information, many operators (including this group) prefer FID data to TIC response data for this task, in which case, the well-characterised FID response is used, rather than relying on the MS total ion count response data. MS response factors for different analytes often vary significantly and cannot always provide accurate quantitative results for multi-component samples. Acceptable ranges for the major components of *L. angustifolia* essential oil according to ISO Standard 3515 are as follows: 1,8-cineole, 0–15%; limonene, 0–0.5%; *trans*-β-ocimene, 2–6%; *cis*-β-ocimene, 4–10%; 3-octanone, 0–2%; camphor, 0–0.5%; linalool, 25–38%; linalyl acetate, 25–45%; terpinen-4-ol, 2–6%; lavandulol minimum, 0.3%; lavandulyl acetate minimum, 2.0%; α-terpineol, 0–1% [2]. Thus samples 2, 3 and 6 fit closest to the ISO standard, and sample 9 is also close but has a higher percentage of camphor. Most of the samples contain higher levels of limonene and α-terpineol than is stated in the ISO standard.

3.1. Comparison of GC–MS with GC×GC–FID

The 2D-separation space contour plot for the GC×GC analysis of a *L. angustifolia* essential oil is presented in Fig. 2B and is contrasted with the single column GC result presented in the more familiar manner in Fig. 2A. Many components which were

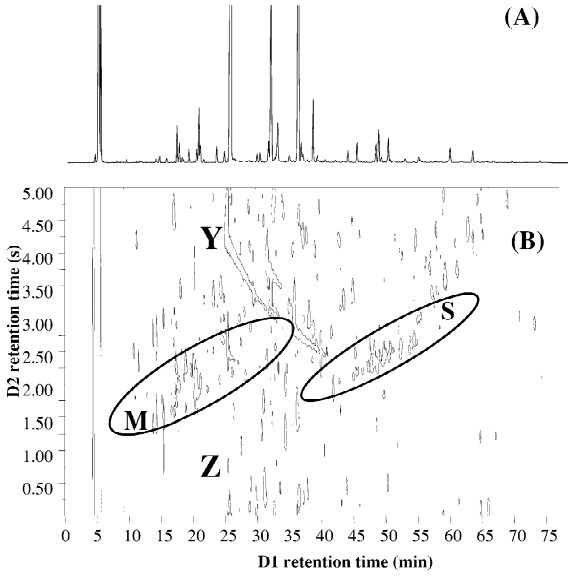

Fig. 2. (A) Reconstructed gas chromatographic trace for a lavender essential oil, and (B) the two-dimensional separation space for the GC×GC analysis of the same sample. **Z** is a minor component which is completely unresolved from major component **Y** in the first dimension. M, monoterpene hydrocarbons; S, sesquiterpene hydrocarbons.

not apparent in the one-dimensional GC analyses are immediately revealed using GC×GC. At least 203 individual component contour peaks were counted in this 2D diagram. The intensity of components represented here ranges between ~3 pA (height of the tallest pulse for the minor components), to >4000 pA (for the most abundant component linalool, **Y**). For simplicity only one contour level is shown at 12 pA (~3 pA above the baseline response), however more contour levels can be plotted which would provide information about the relative heights of individual peaks. The superior resolution that GC× GC offers over single column methods can be further appreciated by comparing the responses for the components marked **Y** and **Z.** The component **Z,** which has a maximum peak height of 10 pA would be difficult (if not impossible) to detect buried beneath the signal of the major component **Y** (4054 pA) in a single column analysis. Using GC–MS to analyse components **Y** and **Z**, spectral de-convolution may be useful, however if these two compounds produce similar MS fragmentation patterns, then the smaller component might (and most probably will) still be missed.

The major components in the sample are represented in Fig. 3 by drawing a single contour line at 250 pA response level. With this smaller data set it is relatively easy to make tentative identification of several components. The excellent retention time reproducibility of peaks produced by cryogenic modulation has been reported [21,27] and the identities of components can be confirmed by the comparison of the first dimension (D1) and second dimension (D2) retention times obtained from the analysis of authentic reference standards.

The ability of GC×GC to produce structured chromatograms has been well documented in the literature and is also apparent in the analysis of essential oils. Within a typical 2D contour plot for an essential oil will be observed a monoterpene hydrocarbon region, and a similar region at higher $^{1}t_{R}$ (D1 retention time) comprising the sesquiterpene hydrocarbons. The oxygenated derivatives of both of these groups are generally found to elute closely after the main group in the first dimension, but owing to their wide range of component polarity these are found to spread throughout a wider region of the 2D-plane. This investigation has revealed further evidence of chromatogram structure for essential oils. The components identified as alcohols are found in the region marked (A) in Fig. 3. Likewise a series of terpene

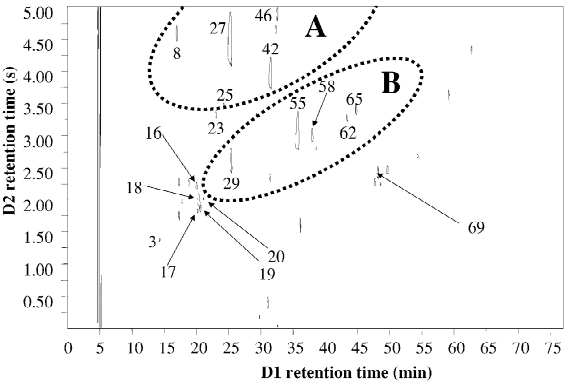

Fig. 3. Two-dimensional separation space showing only the major components present in Fig. 2. Tentative identification is given of some components (refer to Table 2), and groups of structurally related compounds are illustrated by the enclosed regions (A) and (B).

acetates is located in the region marked (B). Some of the major components in this sample are shown to have extended tailing which is an interesting observation, not apparent in the GC–MS results. Beens suggested the use of GC×GC as a diagnostic tool of injector performance and observed similar tailing effects [28]. We concur that this is indeed an effect of the injector, and it is possible deactivation of the injector may reduce this observation. It is also apparent that this effect reduces the quantitative measurement of affected components. In a single column analysis such behaviour would result in an increased background/baseline response. In GC–MS this increased background response would cause interferences in the experimental spectra of other components and cause difficulty in acquiring accurate spectra for these components. This problem may be partly alleviated by using background correction, however ''highly structured'' backgrounds may be less reliably corrected for.

Future opportunities for the use of GC×GC–MS will be important in characterising essential oils, as this will provide three independent parameters on which to base the identity of sample components. It should be noted however, that the typical peak width of an individual GC×GC pulsed peak is to the order of 80–200 ms, and time-of-flight (TOF)-MS is the only technology presently available with sufficiently fast data acquisition capability to accurately detect these peaks. The improved resolution of GC×GC makes the task of obtaining accurate spectra for each component in the sample more attainable. There is high potential for GC×GC–FID and GC×GC–MS to operate side by side as two complimentary techniques. In the past, truly complex samples could only be satisfactorily analysed using GC–MS, relying on spectral differences of overlapped compounds to de-convolute the individual peaks. The superior resolution capability of GC×GC removes much of the need for spectral de-convolution. GC×GC–MS will be initially required to characterise most samples, but compounds have very specific co-ordinates within the 2D-separation plane and assignment of component identity in well-known samples is possible using retention data alone. GC–FID can not always be used as an alternative to GC–MS, but GC×GC–FID *can* provide an alternative to GC×GC–MS for routine analysis, potentially reducing operating costs. A further benefit is that the FID response can be used to acquire more reliable quantitative data.

4. Conclusion

The essential oils industry depends on reliable techniques for characterising essential oils to ensure product quality. For essential oils to be used as pharmaceutical products or as food products, reliable characterisation techniques are especially important. By incorporating LRI data into the MS library and using these two independent parameters to make assignments, identification of the components in the samples investigated was greatly simplified. Automation of library search algorithms to incorporate linear retention indices as part of the match criteria would further assist in the GC–MS analysis of essential oils. This study has shown that GC×GC analysis reveals a clearer indication of the true molecular complexity of essential oils. GC×GC further aids interpretation of the number of components of the oil and is useful to gauge the numbers of components that may co-elute in the primary column. The GC–MS and GC×GC procedures described here can also provide an opportunity for differentiation between different plant cultivars, by analysing the essential oils obtained from them. Especially with the use of GC×GC, the detection of subtle differences in closely related oil samples should be more facile, since it may be based on a 2D pictorial representation of the oil volatile components. Since there are many different cultivars of lavender (of varying capability to produce high quality essential oils), such procedures would be of use to commercial lavender farmers and plant suppliers. Time-of-flight mass spectrometry (TOF-MS), with its capability of fast data acquisition is ideally suited to the detection of GC×GC peaks, and it is expected that GC×GC–TOF-MS will play an important future role in analysis of this class of samples.

Acknowledgements

The authors would like to acknowledge the assistance provided by Shimadzu Italy to conduct this

investigation. R.S. acknowledges support to conduct this project under the UROP program at RMIT. We thank Australian Botanical Products for their assistance in sourcing samples for analysis. The authors wish to express their thanks to Mr Peter Dawes, SGE International for continuing to support our research work on GC×GC. Agilent Technologies is acknowledged for the support of facilities development at RMIT. The technical support of Mr Paul Morrison is always greatly appreciated.

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