Application of Gas-Liquid Chromatography to the Analysis of Essential Oils

GLC fingerprint chromatograms of five essential oils

MJ Milchard, R Clery, R Esdale, L Gates, F Judge, N Moss, DA Moyler, A Sherlock, B Starr, J Webb and EJ Newman,
The Royal Society of Chemistry (RSC), London

As a part of the study, samples of five oils together with the NC-hydrocarbon mixture were distributed to members of the essential oils sub-committee (of the analytical methods committee, RSC) with instructions to prepare a standard fingerprint chromatogram for each oil using a methyl polysiloxane non-polar capillary column. Details of the NC-hydrocarbon mixture and its application are given in Part XVII. [1] Each member was asked to submit the chromatograms of the oils and one of the NC-hydrocarbon mixtures was run at the beginning and end of the series. The latter was to ensure that the characteristics of the column did not change during the exercise. A total of 30 chromatograms together with the retention times and percentage relative peak areas (Aarea %), values from the flame ionization detector (FID) for each detected peak, were received. The identities of the components of interest, usually those accounting for more than 1% of the total peak area, were confirmed by two different laboratories using capillary gas chromatography/mass spectrometry (GC/MS) with identities determined by comparison with in-house and commercial mass spectral libraries.[2] The relative retention index (RRI) of each peak of interest was calculated relative to the n-alkane series of hydrocarbons run under identical conditions and used to cross-check the peak identities. The g-pack values were calculated for each column used.[1]

It was noted that 1,8-cineole and limonene were not completely resolved on the methyl polysiloxane non-polar column at certain relative concentrations. In these cases, the oils were examined on a polar column and the relative proportions were used to determine their contents on the non-polar column.

Results

The results for each essential oil are presented in the form of an annotated chromatogram and a table of identified components with their relative retention indices and area percentage concentrations. The published chromatograms are representative of those obtained by individual sub-committee members. Only those results obtained on columns that had g-pack values within the accepted range were used for the calculation of RRI and component concentration.

Observation of the contents of the low and high boiling n-alkane hydrocarbons in the mixture helped indicate if discrimination had taken place in the split injector, which could lead to erroneous quantitative results. No discrimination was observed in any of the results received.

The agreement between the results of the analysis of the NC-hydrocarbon mixture run at the beginning and end of the series of essential oils was very good within individual laboratories. This demonstrates the stability and reproducibility of modern instruments.

As instrument variability for the NC mixture was low, it was concluded that the observed variation in the RRI for the same component in different oils was due to the loading of that component on the column, as higher loading leads to a later peak apex, and hence a greater RRI. If the observed RRI differed significantly from the reported value, it was checked by diluting the oil and rerunning the sample.

The physical dimensions of the columns used by the laboratories ranged 15–60 m in length, 0.2–0.32 mm in internal diameter and 0.25–1.0 µm in film thickness. It was also noted that the number of peaks obtained on the same oil varied between laboratories, as a result of different detection and data handling systems. Carrier gases used were helium, hydrogen and nitrogen. In spite of these variations, the agreement between laboratories was very good. This demonstrates the robustness of the procedure and shows that, provided the column meets the

At a Glance

This report presents GLC fingerprint chromatograms of five authentic essential oils that were obtained in a collaborative study using the recommended procedure given in Part XVII of this series (this paper represents part XX).[1] The samples examined were oils of spearmint (US native), spearmint (US Scotch), spearmint (Chinese 60%), spearmint (Chinese 80%), and tea tree. They were selected in consultation with the UK essential oil trade through the British Essential Oil Association (BEOA).

requirements of the g-pack concept, any laboratory should be able to obtain reproducible fingerprints of essential oils on non-polar columns with their existing equipment.

Some high percentage standard deviations (%SD) for certain compounds present in the oils at less than 2% relative peak area (RPA) were observed. This was related neither to the functional group, nor to known labile materials. These variations were likely to be due to "system" variables such as concentration, split ratio and in particular electronic integration parameters between the different laboratories. This should be taken into consideration when investigating low levels of significant compounds in essential oils. Although detection limits are often accepted as being at or below 0.01% RPA the accuracy of relative quantitation below 1% RPA should be considered only as a guide. If accurate quantitation below this level is required, then alternative methods should be considered such as those using internal standards or system calibration.

During the course of its studies on the fingerprinting of essential oils, the sub-committee noticed the effect of temperature on the relative elution order of certain compounds. Two recent publications have highlighted this so-called crossover phenomenon both on polar (polyethylene glycol) columns and, more relevant to this present study, on non-polar (methyl polysiloxane) columns.[3,4] Examples include the crossover of several monoterpene hydrocarbons common to many essential oils. This phenomenon emphasizes the importance of closely following the given analytical conditions, including column performance, in order to obtain reproducible fingerprints.

The physical constants of oil of spearmint given in the ISO 3033 **T-1**

	Relative density at 20°C	Refractive index at 20°C	Optical rotation at 20°C
Part 1 Native type	0.921–0.938	1.484–1.491	-59 to -48
Part 2 Chinese 60%	0.918–0.938	1.484–1.491	-60 to -48
Part 2 Chinese 80 %	0.942–0.955	1.488–1.496	-55 to -50
Part 3 Indian type	0.921–0.938	1.484–1.491	-59 to -48
Part 4 Scotch variety	0.921–0.938	1.484–1.491	-59 to -48

Sample analysis of spearmint oil of two US origins (Native and Scotch) **T-2**

Peak identity	Native USA		Scotch USA	
	RRI	Area%	RRI	Area%
1. α-pinene	930	0.5	930	0.6
2. sabinene	968	< 0.05	968	< 0.05
3. β-pinene	971	0.6	971	0.7
4. 3-octanol	979	1.0	979	2.2
5. myrcene	982	2.6	982	0.8
6. 1,8-cineole	1018	1.6	1020	1.1
7. limonene	1022	10.0	1023	17.0
8. γ-terpinene	1050	0.5	1050	< 0.05
9. *trans*-sabinene hydrate	1054	1.3	1054	< 0.05
10. menthone	1132	< 0.05	1132	0.9
13. terpinen-4-ol	1163	1.0	1164	0.2
14. *cis*-dihydrocarvone	1172	1.5	1172	1.7
15. *trans*-dihydrocarvone	1180	0.9	1180	0.4
16. *trans*-carveol	1193	0.4	1201	0.3
17. carvone	1224	66.2	1225	68.3
18. piperitone	1230	0.2	1231	0.2
21. β-bourbonene	1384	1.9	1384	1.0
22. β-caryophyllene	1418	1.6	1417	0.7
23. (Z)-β-farnesene	1447	1.1	1442	0.3
24. germacrene D	1480	1.4	1480	0.3
		94.3		96.7

Conclusion

This collaborative exercise shows that by following the analytical conditions given and by ensuring that the g-pack value of the chromatographic column is within the specified limits, it is possible to obtain reproducible fingerprint chromatograms on essential oils.

Oil of spearmint: The herbs of the genus *Mentha*, members of the Lamiaceae family, are small plants that are grown commercially for essential oil distillation in the United States, China and India. However, many species and hybrids of this culinary herb are grown all over the world in most temperate climates. Typically, for oil production, the herb is cut just before flowering and allowed to wilt before being used for steam distillation. If cut too early, the herb gives out a "green odor" oil with a lower yield and higher *cis*-3-hexenol content; cut too late, and the oil yield suffers.

The oil of *M. spicata* L. grown in the United States is known as "Native." It is a mobile, pale yellow colored oil with a fresh, minty odor that is not particularly substantive; it has relatively good water solubility, hence it does not have a low flavor threshold. A different hybrid, *M. gracilis* Sole (syn. *M. cardiaca* Gerarde ex Baker), known

Sample analysis of oil of spearmint of two Chinese grades of 60% and 80% carvone contents **T-3**

Peak identity	Chinese 60%		Chinese 80%	
	RRI	Area%	RRI	Area%
1. α-pinene	930	0.6	930	< 0.05
2. sabinene	968	0.3	968	< 0.05
3. β-pinene	971	0.6	971	< 0.05
4. 3-octanol	978	0.3	978	0.1
5. myrcene	982	0.4	982	< 0.05
6. 1,8-cineole	1022	0.3*	1021	< 0.05*
7. limonene	1022	17.0*	1021	2.6*
8. γ-terpinene	1050	< 0.05	1050	< 0.05
9. *trans*-sabinene hydrate	1054	< 0.05	1054	< 0.05
10. menthone	1133	1.3	1133	1.2
11. isomenthone	1144	0.2	1144	0.2
12. menthol	1161	0.7	1161	0.7
13. terpinen-4-ol	1163	0.1	1163	< 0.05
14. *cis*-dihydrocarvone	1172	2.6	1172	2.5
15. *trans*-dihydrocarvone	1179	4.2	1180	3.7
16. *trans*-carveol	1202	0.8	1204	0.9
17. carvone	1224	63.0	1225	79.6
18. piperitone	1231	0.1	1233	0.1
19. menthyl acetate	1279	0.3	1279	0.3
20. dihydrocarvyl acetate	1310	1.9	1310	1.4
21. β-bourbonene	1384	0.8	1384	1.1
22. β-caryophyllene	1417	1.2	1418	2.1
23. (Z)-β-farnesene	1447	< 0.05	1147	< 0.05
24. germacrene D	1481	0.1	1481	0.3
		96.8		96.8

* calculated by proportion by analysis on a polar column.

locally as "Scotch" is also grown in the United States for oil production.

Although some *M. spicata* is grown in China, most of the essential oil production is from *M. gracilis* Sole, sometimes known (incorrectly) as *M. viridis* L. *crispa* Benth.[5] When distilled, this oil contains about 55% carvone. It is fractionally distilled to give the commercial grades of 60% and 80% minimum carvone content. The spearmint oil produced in India, *M. spicata*, is now being offered at 60% and 80%, but has a different odor profile because it is obtained from a different cultivar to that grown in China, and is usually more expensive.

With L(-) carvone being produced from D(+) limonene ex *Citrus sinensis* (L.) Osbeck produced in Brazil, Mexico, the United States, Israel and now China, attention to analytical detail is recommended when examining GLC profiles, particularly in the case of the 80% oil.

In addition to its global use in confectionary, chewing gum and dentifrice, oil of spearmint is used as a modifier for a milder, sweeter flavor note in many peppermint based products such as "doublemint" brands.

There are four international standards for this oil—ISO 3033:2005 Parts 1–4. There are also specifications in the British Pharmacopoeia, European Pharmacopoeia and the Food Chemicals Codex (FCC). The physical constants given in the ISO 3033 are listed in **T-1**. In addition, **T-2** and **T-3** describe sample analyses of two US origins (Native and Scotch), and two Chinese grades of 60% (see **F-1**) and 80% carvone contents, respectively. All samples tested were within these physical constant ranges.

Oil of tea tree: *Melaleuca alternifolia* (Maiden et Betche) Cheel, a member of the Myrtaceae family, is the main source of oil of tea tree. Other *Melaleuca* species, rich in terpinen-4-ol, such as *M. linariifolia* Smith and *M. dissitiflora* F. Muell., are also used for oil production. *Melaleuca alternifolia* is a medium sized shrub or small tree grown commercially for oil production in Australia; it is now being grown in the Guangxi province of China. The leaves and terminal branchlets are used for distillation in the main growing area of the Australian Gold Coast around Ballina.

Oil of tea tree is a mobile, colorless liquid with a powerful fresh, medicinal odor that is moderately substantive. Oils that have aged or that have been stored in clear containers exposed to UV light have a higher p-cymene content (formed by oxidation of γ-terpinene), which detracts from the clean, fresh odor of the oil.

Apart from being used in medicated shampoos and herbal toiletry fragrances, the oil is used as a modifier for oil of eucalyptus in Australian pharmaceutical products, as well as in toothpaste. Similarly, there are documented references to its use as a skin disinfectant and local anesthetic, and it is one of the few antimicrobial agents reputed to combat MRSA and finds some use in hospitals in Australia.

There is an international standard for this oil—ISO 4730:2004. There are also specifications in the British Pharmacopoeia and European Pharmacopoeia. The physical constants given in the ISO 4730:2004 are as follows:

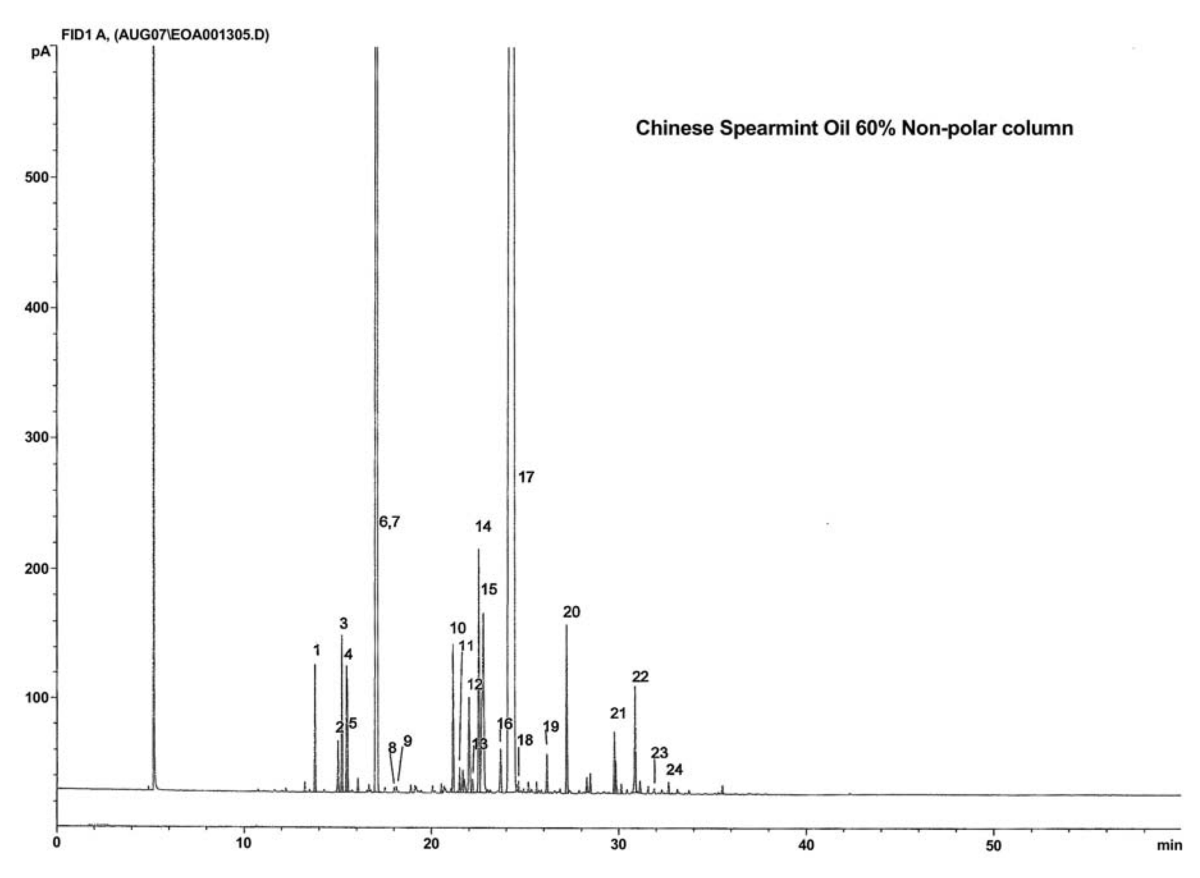
Relative density at 20°C	0.885 to 0.906
Refractive index at 20°C	1.475 to 1.482
Optical rotation at 20°C	+5° to +15°

The sample analyzed (see **F-2** and **T-4**) by the subcommittee was of Australian origin, and conformed to these figures.

Oil of patchouli: Following further detailed analysis by the sub-committee it has been confirmed that two

Oil of spearmint 60% Chinese

F-1



Sample analysis of oil of tea tree of Australian origin

T-4

Peak Identity	RRI	Area%
1. α-thujene	919	0.8
2. α-pinene	927	2.3
3. sabinene	968	0.2
4. β-pinene	971	0.7
5. myrcene	982	0.8
6. α-phellandrene	998	0.4
7. α-terpinene	1011	9.3
8. p-cymene	1014	2.4
9. β-phellandrene	1022	0.7*
10. limonene	1022	0.9*
11. 1,8-cineole	1022	2.4*
12. γ-terpinene	1051	20.3
13. terpinolene	1079	3.4
14. terpinen-4-ol	1168	41.5
15. α-terpineol	1175	3.0
16. β-caryophyllene	1418	0.4
17. aromadendrene	1444	1.2
18. viridiflorene	1495	1.5
19. δ-cadinene	1518	1.1
		93.3

* calculated by proportion by analysis on a polar column

peaks previously reported in the analysis of this oil (Part XIX) were incorrectly named.[6,7]

The corrected identifications are highlighted in **T-5;** also see **F-3**.

Acknowledgments

The subcommittee gratefully acknowledges the staff time and samples of essential oils provided by the companies involved in this study.

Address correspondence to The Secretary, Analytical Methods Committee, Analytical Division, The Royal Society of Chemistry, Piccadilly, London W1J OBA, United Kingdom; michael.milchard@virgin.net.

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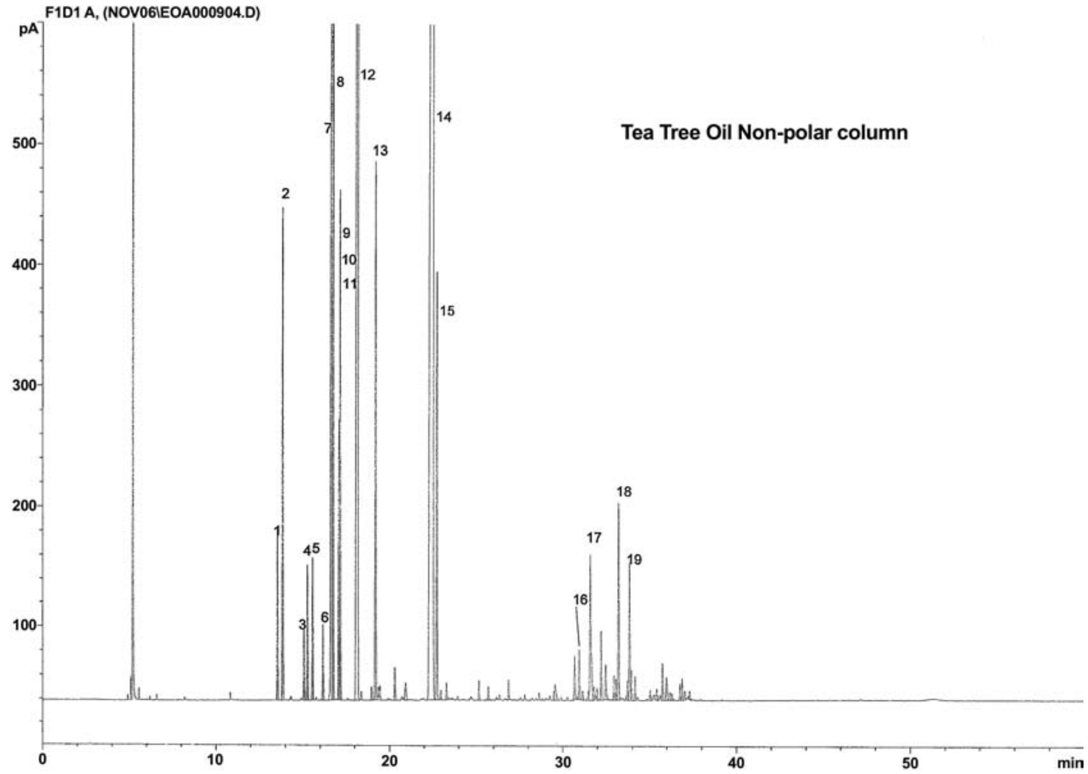
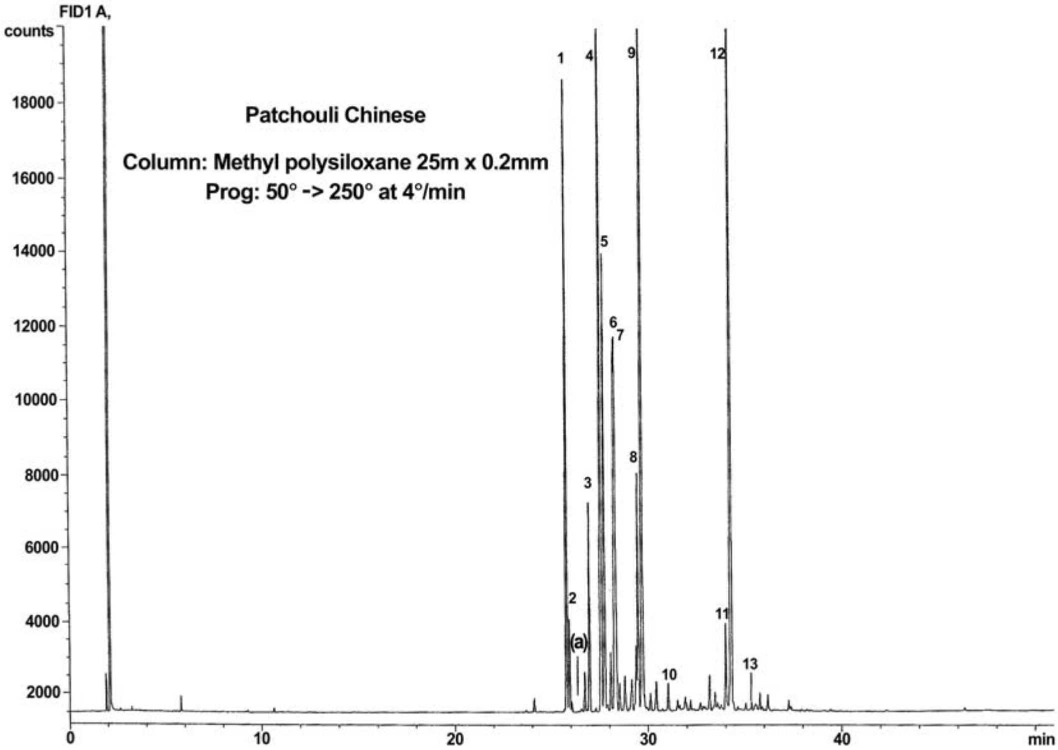
Oil of tea tree Australian

F-2



Oil of patchouli Chinese

F-3



(a) indicates the position of α-gurjunene if present

Sample analysis of oil of Chinese patchouli

T-5

Peak identity	Chinese		Indonesian 30%		Indonesian 35%	
	RRI	Area %	RRI	Area %	RRI	Area %
1. β-patchoulene	1386	9.3	1384	2.3	1384	2.1
2. β-elemene	1388	1.3	1387	0.9	1388	0.7
3. β-caryophyllene	1420	3.1	1420	3.8	1420	3.1
4. α-guaiene	1441	15.3	1441	14.6	1441	13.8
5. **seychellene**	1445	6.7	1445	6.3	1445	6.7
6. α-patchoulene	1463	5.9	1462	5.1*	1461	5.1*
7. allo-aromadendrene	1464	5.0	1462	2.4*	1461	2.3*
8. **aciphyllene◊ + γ-patchoulene**	1498	3.9	1498	3.7	1498	3.4
9. δ-guaiene (bulnesene)	1508	20.7	1508	18.8	1507	16.7
10. nor-patchoulenol	1546	0.4	1546	0.6	1546	0.6
11. pogostol	1646	1.5	1647	2.2	1649	2.4
12. patchouli alcohol	1656	17.5	1657	28.2	1657	32.7
13. pogostone	1679	0.1	1679	1.1	1680	1.0
		90.7		90.0		90.6

*calculated by proportion by analysis on a polar column

◊ tentative identification based on mass spectrometry and retention index without a reference standard

7. Charles Cornwell, private communication refuting information found in Application of Gas-Liquid Chromatography to the Analysis of Essential Oils, *Perfum Flavor*, 29(5), 28-36 (2004)

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