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

GLC fingerprint chromatograms of six essential oils

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As a part of a collaborative study, samples of the six oils together with the NC-hydrocarbon mixture were distributed to members of the subcommittee (of the analytical methods committee, RSC, London) with instructions to prepare a standard fingerprint chromatogram of each of the oils 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 mixture run at the beginning and at the end of the series. The latter was to ensure that the characteristics of the column did not change during the exercise.

A total of 36 chromatograms were received, along with the retention times and percentage relative peak areas (area %) and values from the flame ionization detector (FID) for each detected peak. 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 RRI and area percentage concentrations. The published chromatograms are representative of those obtained by individual subcommittee members. For oils (of different geographical origins) displaying similar compositions, only one chromatogram is presented. 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 any 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 at the 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 re-running the sample.

The physical dimensions of the columns used by the laboratories varied 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 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.

At a Glance

This report presents GLC fingerprint chromatograms of six essential oils that were obtained in a collaborative study using the recommended procedure given in Part XVII of this series (this paper represents part XXI).[1] The samples examined were oils of citronella (Chinese), citronella (Vietnamese), citronella (Sri Lankan), lemongrass (Cochin), lemongrass (Guatemalan) and *Litsea cubeba* (Chinese). They were selected in consultation with the UK essential oil trade through the British Essential Oil Association (BEOA).

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 neither related to the functional group, nor to known labile materials. These variations were likely 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 as a guide. If accurate quantitation below this level is required, then alternative methods, such as those using internal standards or system calibration, should be considered.

During the course of its studies on the fingerprinting of essential oils, the subcommittee 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.

Conclusion

This collaborative exercise demonstrated that, by following the analytical conditions given and 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. In addition, it also indicated the presence of isocitrals in high citral content essential oils, which has not been previously reported.

Oil of Citronella

The plants of citronella, *Cymbopogon winterianus* Jowitt and *C. nardus* (L.) Rendle, belong to the Poaceae family; they are medium-sized grasses that are cultivated

Sample analysis of oil of citronella of Sri Lankan origin **T-1**

Peak lidentity	Sri Lankan	
	RRI	Area%
1. tricyclene	916	1.3
2. α-pinene	928	2.3
3. camphene	942	8.8
4. myrcene	981	0.9
5. limonene	1022	9.3*
6. 1,8-cineole	1022	0.5*
7. (Z)-β-ocimene	1027	1.8
8. (E)-β-ocimene	1038	1.1
9. terpinolene	1080	0.8
10. linalool	1084	0.7
11. citronellal	1134	4.5
12. neoisopulegol	1147	1.2
13. borneol	1151	5.9
14. terpinen-4-ol	1162	1.3
15. α-terpineol	1172	1.3
16. citronellol	1210	3.5
17. neral	1216	0.3
18. geraniol	1239	17.7
19. geranial	1245	0.4
20. bornyl acetate	1272	0.5
21. citronellyl acetate	1335	0.9
22. geranyl acetate	1361	3.1
23. methyl eugenol	1371	1.1
24. β-elemene	1388	1.0
25. β-caryophyllene	1418	1.9
26. *trans*-α-bergamotene	1436	0.9
27. (E)-methyl isoeugenol	1466	8.8
28. germacrene D	1480	1.6
29. α-farnesene isomer †	1484	4.2
30. δ-cadinene	1516	1.1
31. elemol	1538	1.6
32. geranyl butyrate	1540	1.1
		91.4

*calculated by proportion by analysis on a polar column

† The correct configuration of the double bond system could not be determined by mass spectrometry. From published retention index data (terpenoid library list: *www.massfinder.com*), one of the isomers could be (3Z,6E)-α-farnesene.

Sample analysis of oil of citronella of Chinese and Vietnamese origins **T-2**

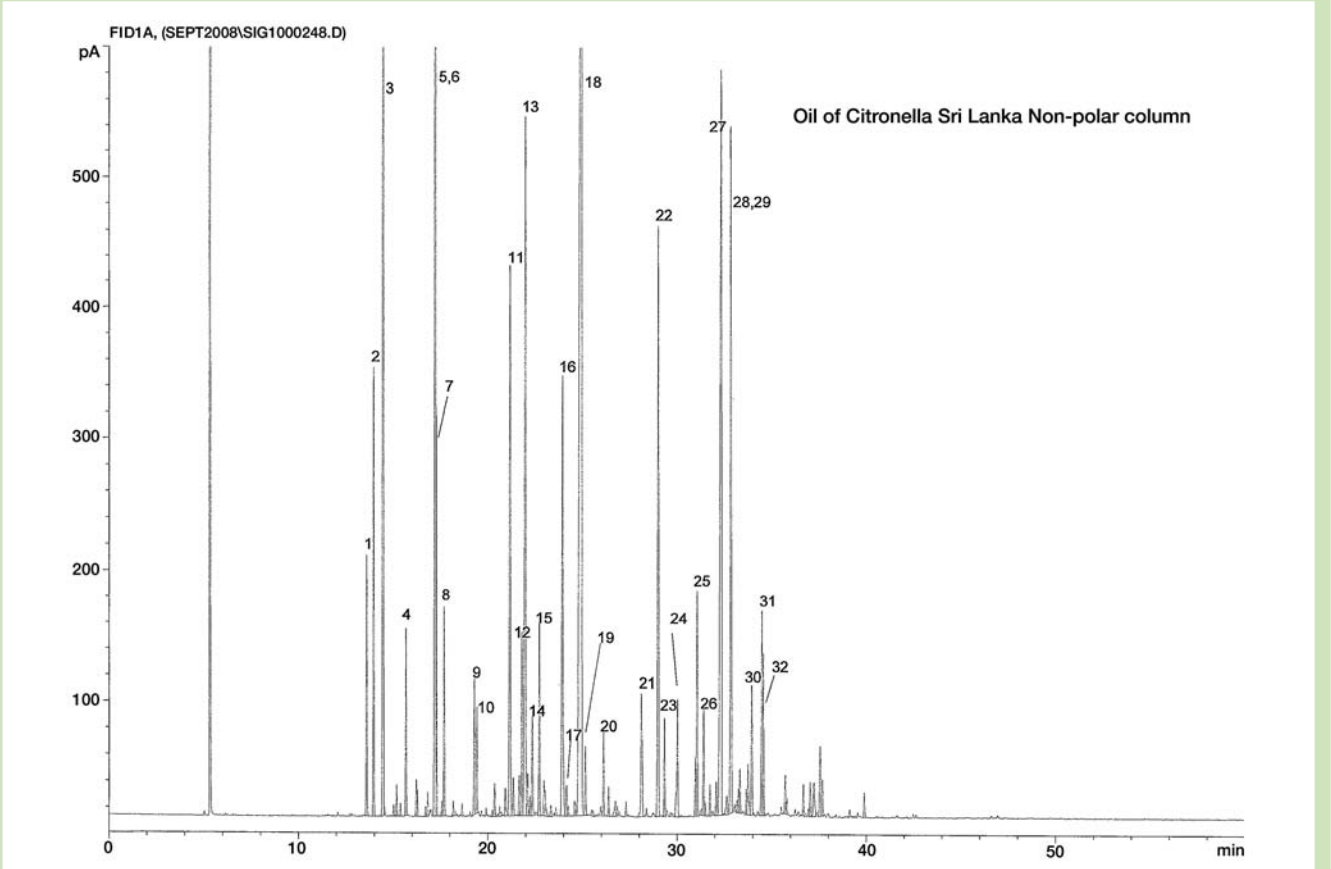
Peak identity	Chinese		Vietnamese	
	RRI	Area%	RRI	Area%
1. α-pinene	930	0.3	930	< 0.05
2. limonene	1021	3.7	1021	3.1
3. linalool	1083	0.8	1084	0.9
4. isopulegol	1129	1.1	1128	1.0
5. citronellal	1135	36.8	1136	33.8
6. citronellol	1212	10.9	1212	11.3
7. neral	1216	0.4	1216	0.3
8. geraniol	1239	21.1	1240	21.6
9. geranial	1245	0.5	1246	0.4
10. eugenol	1331	1.0	1331	1.0
11. citronellyl acetate	1336	2.9	1336	2.9
12. geranyl acetate	1361	3.6	1362	3.9
13. β-elemene	1388	2.0	1388	2.8
14. β-caryophyllene	1418	0.5	1418	0.5
15. germacrene D	1480	2.3	1480	2.2
16. δ-cadinene	1516	2.1	1516	2.5
17. elemol	1536	3.1	1537	4.3
18. caryophyllene oxide	1576	1.0	1576	1.0
19. α-cadinol	1643	0.9	1642	0.9
		95.0		94.4

for essential oil distillation in Indonesia, Vietnam, the Guanxi province of China (*C. winterianus*) and Sri Lanka (*C. nardus*). Typically, the fresh grass is cut and allowed to wilt before it is used for the steam distilled oil production. Oils from these origins have a somewhat different composition—Vietnamese being similar to Chinese, but with much higher citronellal contents than those of the Sri Lankan origin.

Oil of citronella is a mobile, pale yellow liquid with a powerful fresh, floral-herbal odor that is quite substantive with a sweet, woody back-note. Traditionally, it has been used as a masking agent and perfume in low-cost household products, and also as an insect repellent in candles and consumer products. Commercially, it is a useful source of terpene alcohols and aldehydes by fractionation.

There are two international standards for this oil—ISO3848:2001 *C. winterianus* and ISO3849:2003 *C. nardus.* In addition, the European Pharmacopoeia includes a monograph for *C. winterianus.* The physical constants mentioned in the two international standards are as follows:

Oil of citronella (Sri Lanka) **F-1**



	ISO 3848:2001	ISO 3849:2003
Relative density at 20˚C	0.880 to 0.893	0.891 to 0.910
Refractive index at 20˚C	1.467 to 1.473	1.479 to 1.490
Optical rotation at 20˚C	0˚ to -5˚	-25˚ to -12˚

The samples analyzed by the subcommittee were of Vietnamese, Chinese (See **T-2** and **F-2**) and Sri Lankan (See **T-1** and **F-1**) origins and conformed to these specifications.

Oil of Lemongrass

Lemongrass, *Cymbopogon citratus* (DC.) Stapf and *C. flexuosus* (Nees ex Steud.) Wats., are members of the Poaceae family. They are medium-sized grasses grown commercially for essential oil distillation in several parts of the world, but mainly in Guatemala (*C. citratus*) and Cochin (*C. flexuosus*). For oil production, the grass is cut and allowed to wilt before being used for the steam distillation. Depending on their origins, the oils have a somewhat different composition—lemongrass oil from Guatemala has higher limonene content than that from Cochin.

Moreover, oil of lemongrass, like that from *Litsea cubeba*, is used as a source of natural citral. However, it is more expensive and contains a higher level of geraniol, which is difficult to remove by distillation due to the closeness of its boiling point to geranial. As a result, except for its slightly different odor and flavor profile, it is now less popular than *Litsea* oil. Moreover, removal of the geraniol by the chemical absorption and regeneration of the citral results in the citral losing its natural status.

The three isocitral peaks in oil of lemongrass and the *Litsea* oil are formed by thermal isomerisation of citral under distillation conditions. The generation and identity of these compounds has been confirmed by GCMS analysis of fractions produced in the rectification of lemongrass and litsea oils.[5]

There are two international standards for these oils, ISO 4718:2004 *C. flexuosus* and ISO 3217:1974 *C. citratus*. The physical constants given in these standards are as listed below:

	ISO 4718:2004	ISO 3217:1974
Relative density at 20˚C	0.885 to 0.905	0.872 to 0.897
Refractive index at 20˚C	1.483 to 1.489	1.483 to 1.489
Optical rotation at 20˚C	-4˚ to +1˚	-3˚ to +1˚

The samples of oil of lemongrass analyzed by the subcommittee were of Guatemalan (See **F-3**) and Cochin origins (See **T-3** and **F-3**), and conformed to these values.

Oil of *Litsea cubeba*

Litsea cubeba (Lour.) Pers., a member of the Lauraceae family, is a medium-sized tree grown commercially for essential oil distillation in the Guangxi province of China; it is locally known as May Chang.

The fresh berries of *Litsea cubeba* are harvested and used for the steam distilled oil production. Compositions of the oils derived from only berries and that from only leaves differ, and so distillation of a mixture leads to a variable composition. Similarly, the collection of wood should be avoided as this can introduce low levels of safrole into the oil.

The primary use of this oil is as a source of natural citral, which has several applications—in lemon flavors, it reinforces the main note, but has limited stability in functional fragrance products. Citral has also been used for the synthesis of vitamin A and several ionone aroma chemicals.

ISO 3214:2000 is the international standard for this oil. The citral content specified in this standard is a minimum of 70% by gas chromatography using the internal standard method, and a minimum of 74% using the chemical determination. The results obtained by chemical determination are slightly higher due to the reaction of other minor carbonyl components. Accordingly, a commercial term often used to describe this oil is "70/75."

The physical constants of *Litsea cubeba* as per ISO 3214:2000 is as follows:

	ISO 3214:2000
Relative density at 20˚C	0.880 to 0.892
Refractive index at 20˚C	1.480 to 1.490
Optical rotation at 20˚C	+3˚ to +12˚

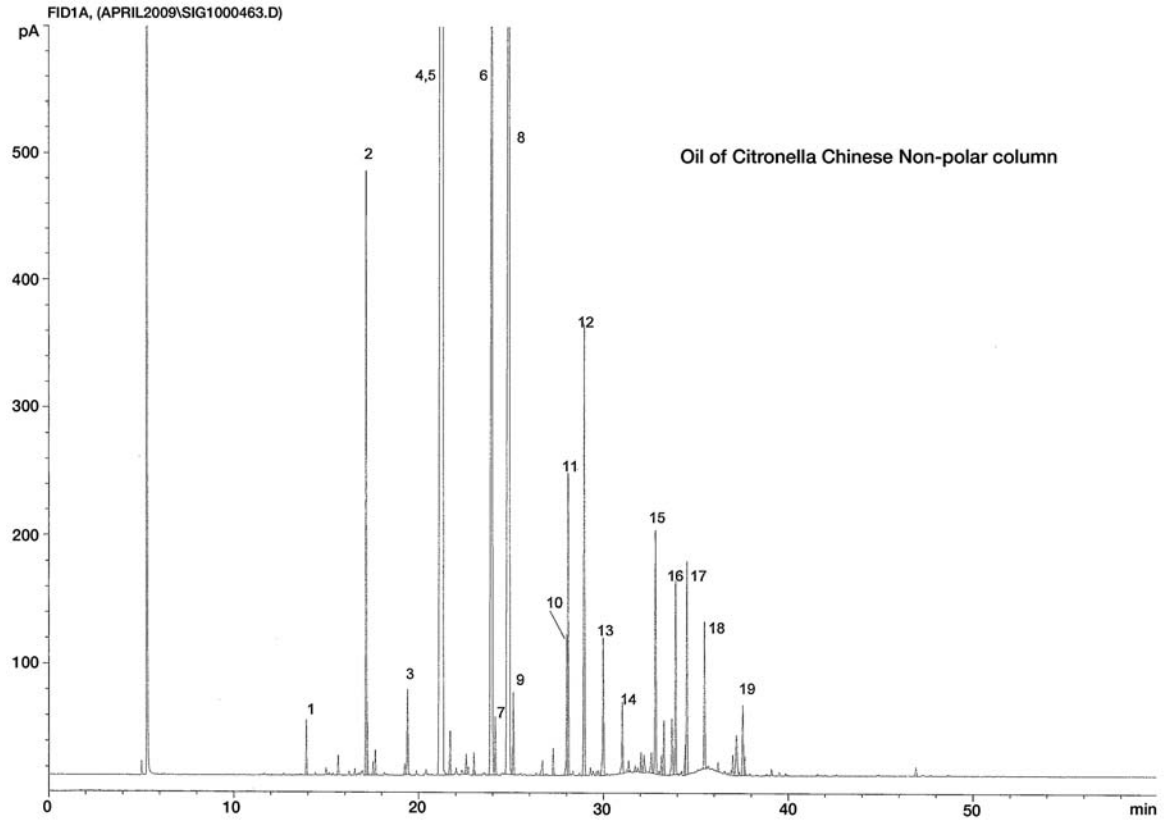
Sample analysis of oil of lemongrass from Cochin and Guatemala **T-3**

Peak identity	Cochin RRI	Cochin Area%	Guatemala RRI	Guatemala Area%
1. camphene	944	0.6	944	< 0.05
2. 6-methyl-5-hepten-2-one	959	2.2	959	0.9
3. limonene	1021	0.2	1022	8.4
4. (Z)-β-ocimene	1027	0.5	1027	< 0.05
5. (E)-β-ocimene	1038	0.3	1038	< 0.05
6. 4-nonanone	1053	0.9	1053	1.2
7. linalool	1083	1.0	1083	1.1
8. isocitral #1	1119	0.3	1119	0.2
9. citronellal	1132	0.3	1132	0.3
10. isocitral #2	1142	1.1	1142	1.2
11. isocitral #3	1160	1.9	1159	1.9
12. decanal	1185	0.3	1185	< 0.05
13. nerol	1220	0.2*	1220	0.2*
14. neral	1220	33.6*	1220	31.1*
15. geraniol	1239	3.2	1239	2.3
16. geranial	1251	43.7	1251	41.8
17. geranyl acetate	1361	2.0	1361	1.4
18. β-caryophyllene	1418	1.8	1418	2.2
19. γ-cadinene	1510	0.6	1510	< 0.05
20. caryophyllene oxide	1576	0.8	1574	1.0
		95.2		95.2

* calculated by proportion by analysis on a polar column

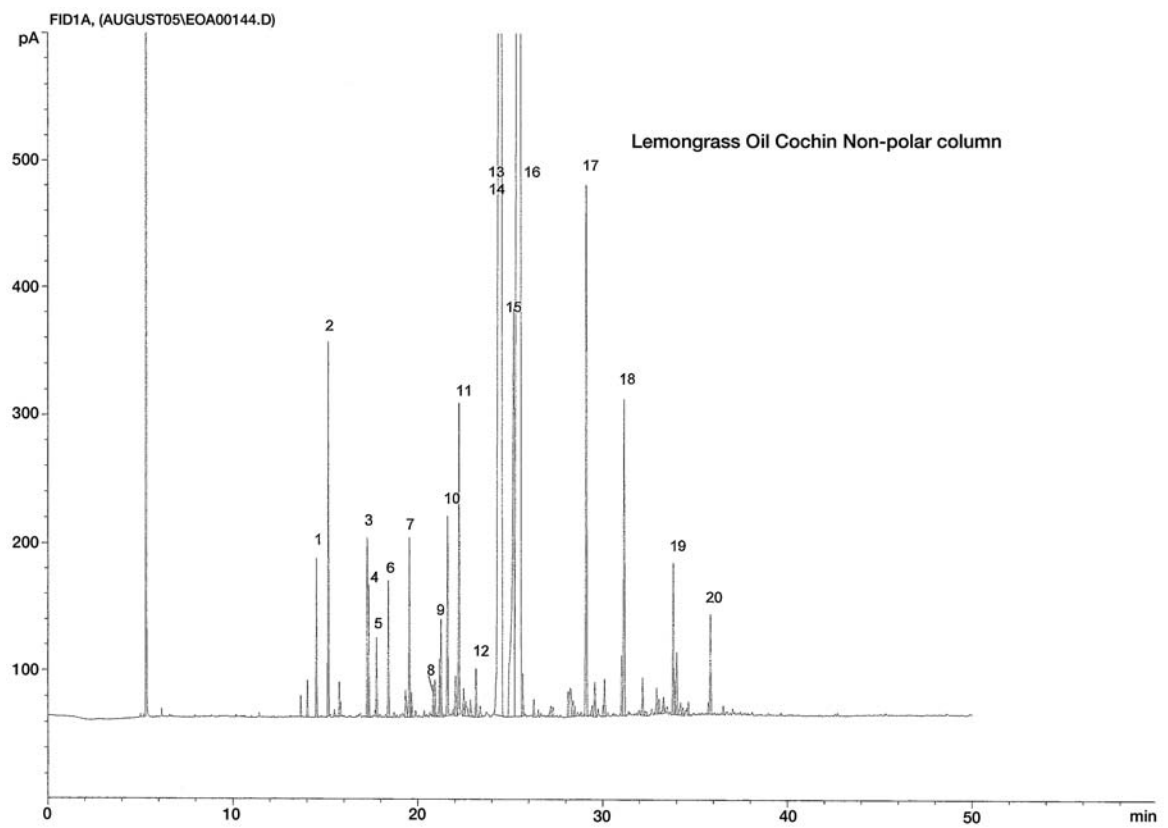
Oil of citronella (Chinese)

F-2

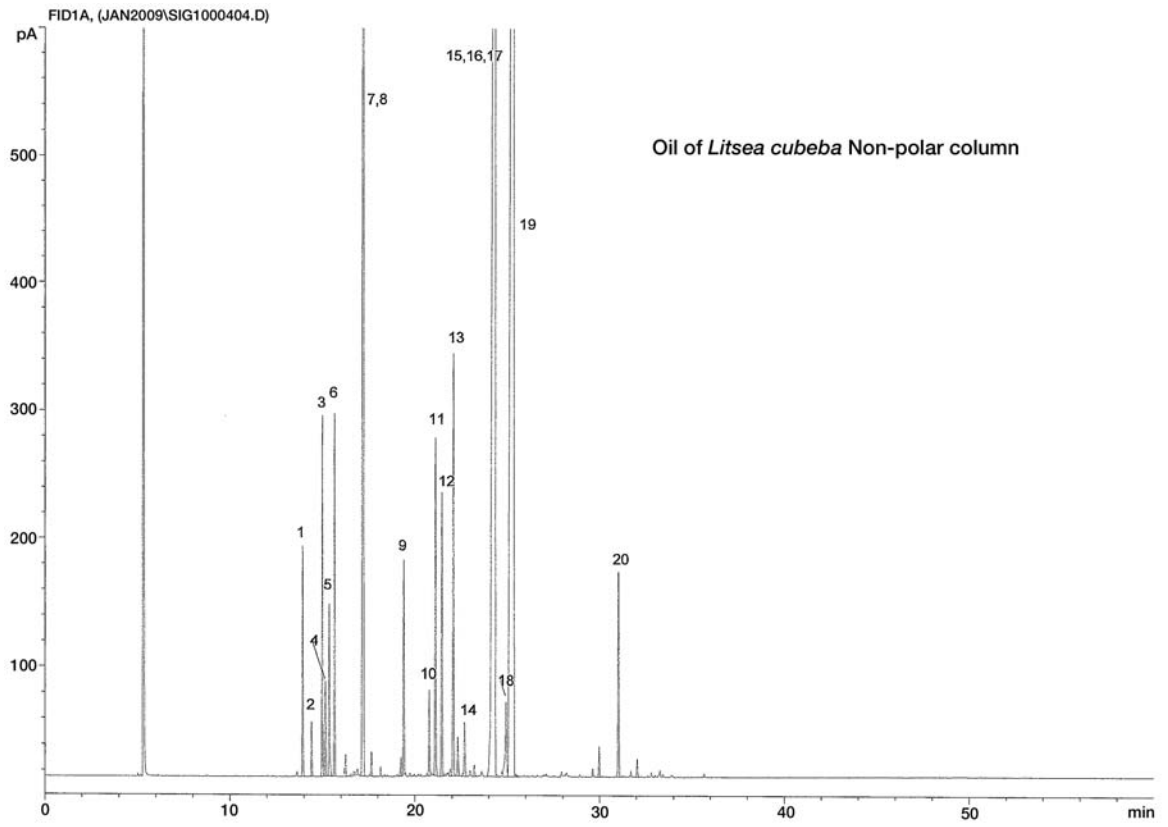


Oil of lemongrass (Cochin)

F-3



Oil of *Litsea cubeba* (Chinese) F-4



Sample analysis of oil of *Litsea cubeba* Chinese T-4

Peak identity	RRI	Area%
1. α-pinene	927	1.1
2. camphene	944	0.3
3. 6-methyl-5-hepten-2-one	959	1.9
4. sabinene	964	0.9
5. β-pinene	969	0.9
6. myrcene	981	1.2
7. limonene	1023	12.0*
8. 1,8-cineole	1023	0.8*
9. linalool	1083	1.5
10. isocitral #1	1124	0.2
11. citronellal	1134	0.8
12. isocitral #2	1142	0.8
13. isocitral #3	1159	1.4
14. α-terpineol	1170	0.5
15. citronellol	1222	0.2*
16. nerol	1222	1.4*
17. neral	1222	29.3*
18. geraniol	1241	0.8
19. geranial	1253	39.6
20. β-caryophyllene	1422	0.7
		96.3

* calculated by proportion by analysis on a polar column

The sample analyzed by the subcommittee was of Yunnan Chinese origin (See **T-4** and **F-4**) and conformed to these values.

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