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Material Review

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

Fingerprints of 12 essential oils

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GLC fingerprint chromatograms of 12 authentic essential oils were obtained in a collaborative study using the recommended procedure given in Part XVII of this series and are presented here (this paper represents Part XIX).[1] The samples examined were oils of cajuput, eucalyptus, geranium (Bourbon), geranium (Chinese), geranium (Egyptian), lavandin abrialis, lavandin grosso, lavandin super, patchouli (Chinese), patchouli (Indonesian 30 percent), patchouli (Indonesian 35 percent) and thyme. They were selected in consultation with the UK essential oil trade through the British Essential Oil Association (BEOA). The sub-committee gratefully acknowledges the staff time and samples of essential oils provided by the companies involved in this study.

Experimental

Samples of 12 oils together with the NC-hydrocarbon mixture were distributed to all members of the sub-committee with instructions to prepare standard fingerprint chromatograms 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 (this paper represents Part XIX).[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 check that the characteristics of the column did not change during the exercise. A total of 182 chromatograms were returned. Tables of retention times and percent relative peak areas (area percent) values from the flame ionization detector (FID) for each detected peak accompanied the chromatograms. The identities of the components of interest, usually those accounting for more than 1 percent 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 crosscheck the peak identities. The g-pack values were calculated for each column used.[1]

It was noted that 1,8-cineole and limonene are 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 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

[1]The constitution of the sub-committee responsible for the preparation of this report was: M.J. Milchard (chairman), R. Clery, N. DaCosta, R. Esdale, M. Flowerdew, L. Gates, N. Moss, D.A. Moyler, A. Sherlock, B. Starr, J. Webb and J. Wootten, with (the late) J.J. Wilson (secretary).

calculation of relative retention index and component concentration.

Three sub-committee members use a (5 percent) diphenyl (95 percent) polydimethylsiloxane column routinely in their laboratories for essential oil analysis. Although outside the operating conditions for this exercise, results were obtained on these columns in order to determine the feasibility of undertaking a similar exercise on this type of column at a later date. There was good agreement within each laboratory for the RRI of the same component in different oils, for example α-pinene in oils of cajuput, eucalyptus and thyme, but it was considered that the agreement between the laboratories for RRI and g-pack values was not good enough to be able to quote reliable figures for this phase.

The reason for these differences in results may well be due to the differences in the manufacturing processes of this phase as each laboratory uses a column from a different manufacturer. It has been noted previously by the sub-committee that nominally the same phase from different manufacturers can give columns with slightly different characteristics. It may be that for this phase [(5 percent) diphenyl (95 percent) polydimethylsiloxane], individual laboratories need to compile their own database for their own column.[1] However, to be effective, replacement columns would need to be obtained from the same manufacturer and guaranteed to have the same characteristics.

The provision of the same NC-hydrocarbon mixture to each member permitted a comparison between laboratories of the quantitative composition of the mixture. This revealed a significant result for one laboratory which may well have not been detected outside of a collaborative exercise. The content of linalyl acetate was considerably lower than that determined by all of the other laboratories. Close inspection of the chromatogram showed that the shape of the linalyl acetate peak was Gaussian and did not show any signs of degradation. The RRI agreed with the other laboratories' results. However, it was observed that two minor peaks were present, (Z)- and (E)-β-ocimene, known degradation products of linalyl acetate. These were absent from all other NC-hydrocarbon mixture chromatograms. The problem was therefore one of partial decomposition in the injector system and was completely rectified by replacing the injector liner. The decrease in linalyl acetate and increase in (Z)- and (E)-β-ocimene was also observed in the three chromatograms of the lavandin oils from this laboratory.

Observation of the contents of the low and high boiling n-alkane hydrocarbons in the mixture can indicate if any discrimination has 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 is concluded that the observed variation in the RRI for the same component in different oils is due to the loading of that component on the column. A higher loading leads to a later peak apex and hence a greater RRI. If the observed RRI differs significantly from the reported value, it should be checked by diluting the oil and re-running the sample.

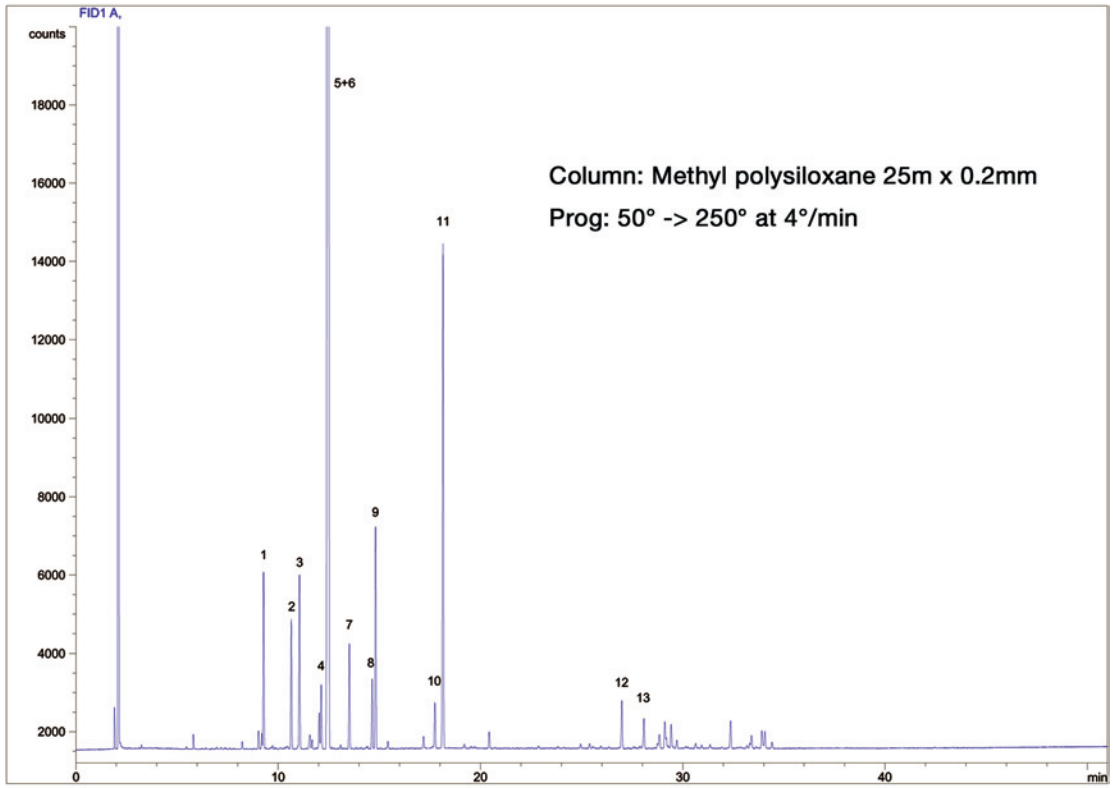
The sub-committee will be investigating the possibility of including other compounds in the standard mixture that can provide indications of the condition of the chromatographic system in addition to those detailed above. Meanwhile, it is recommended that the NC-hydrocarbon mixture be run at regular intervals during essential oils analyses to check the system.

The physical dimensions of the columns used by the laboratories varied between 15 to 60 m in length, 0.2 to 0.32 mm in internal diameter and 0.25 to 1.0 µm in film thickness. It was also noted that the number of peaks obtained on the same oil varied between laboratories, a function of the 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, providing 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.

It was noted that there were some high percentage standard deviations (percent SD) for certain compounds present in the oils at less than 2 percent relative peak area (RPA). This was not related to the functional group nor was it related to known labile materials. These variations are 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 percent RPA the accuracy of relative quantitation below 1 percent RPA should be considered as a guide. If accurate quantitation below

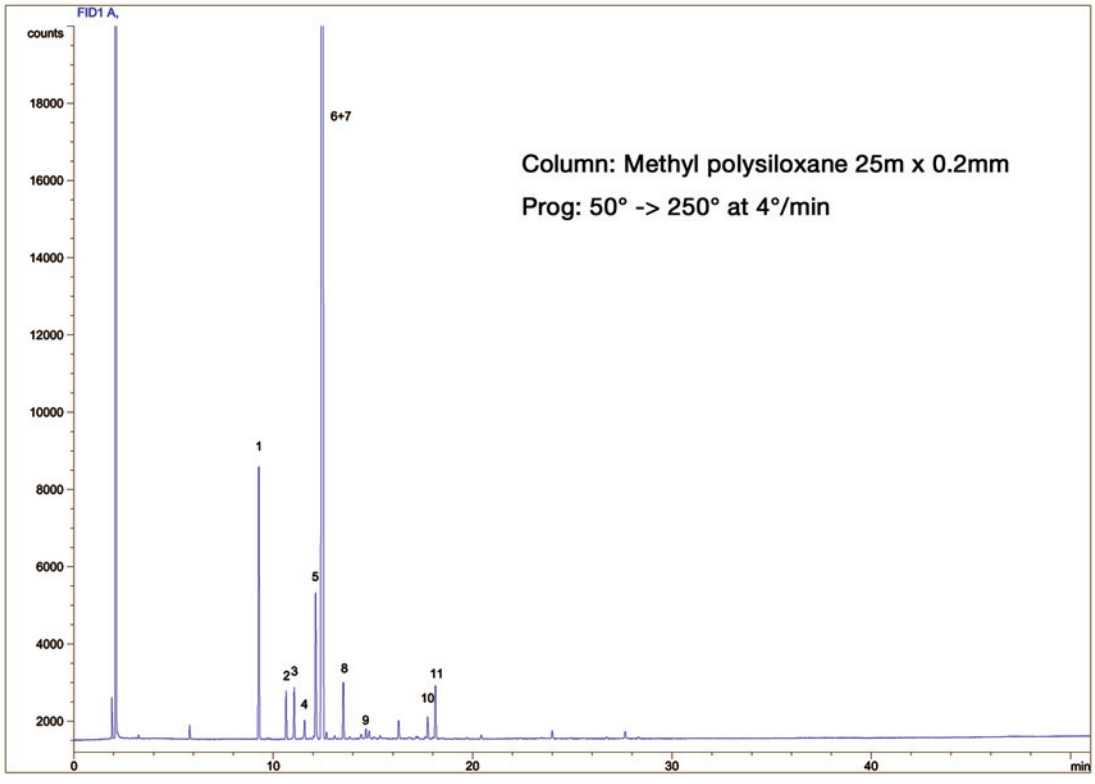
Cajuput Vietnamese

F-1



Eucalyptus Chinese

F-2



this level is required then alternative methods should be considered such as those using internal standards or system calibration.

The results are presented in the following figures and tables for each oil.

Oil of Cajuput

The Cajuput tree Melaleuca cajuputi Powell (syn. M. minor Sm.), a member of the Myrtaceae family, is a medium sized tree which is commercially grown for essential oil distillation in Vietnam, China and Indonesia.

The fresh leaves and the twigs of the tree are used for the steam distilled oil production. Oil from these origins has a somewhat different composition, Vietnamese having a higher 1,8-cineole content than the other origins.

Cajuput oil is mobile, pale yellow-green in color, with a powerful fresh, eucalyptus-camphoraceous odor which is not substantive. Apart from its use as a decongestant and cold remedy in the Far East, it is used as a modifier or milder-sweeter alternative to eucalyptus oil in western pharmaceutical products as well. It was featured in the British Pharmaceutical Codex in 1963. The 1,8-cineole quoted therein, was 50.0-65.0 percent, but genuine Vietnamese oil is sometimes at a higher level than this and some genuine Chinese and Indonesian oil is at a level lower than this.

The physical constants recorded in the BPC 1963 were:

Weight per mL at 20°C	0.910 to 0.923 g
Refractive index at 20°C	1.464 to 1.472
Optical rotation at 20°C	+ 1 to -4

Oil of Eucalyptus

The eucalyptus tree Eucalyptus globulus Labill., a member of the Myrtaceae family, is a medium sized tree which is commercially grown for essential oil distillation in China in the Yunnan province. Although eucalyptus trees are grown in Australia (E. polybractea R. Baker) and the Iberian Peninsula (E. globulus), there is only a limited amount of oil produced in these origins.

There are many species of eucalypts, and some others are used for commercial essential oil production e.g. E. smithii R. Baker, E. dives Schauz, E. polybractea, E. radiata Sieber ex Spreng., all with a high 1,8-cineole content, E. citriodora Hook., with a high citronellal content and E. staigeriana F. Muell. ex Bailey containing citral, geraniol and geranyl acetate.

Some so called "eucalyptus oil" is produced in China from camphor trees, Cinnamonum camphora (L.) J. Presl. After the steam distillation of the oil and in isolation of the camphor crystals by freezing, the remaining "white camphor oil" has less than 1 percent camphor and about 35 percent of 1,8-cineole. This oil is then rectified to remove monoterpene hydrocarbons and increase the 1,8-cineole content to meet the required specification, often a pharmacopoeia. This oil is usually a little cheaper in the market place than genuine E. globulus and can be detected by its different GLC fingerprint.

The fresh leaves and the twigs of the tree are used for the steam distilled oil production. Oils are available with different assays, some having a higher 1,8-cineole content, the usual levels being 70 percent, 80 percent and 85 percent.

Eucalyptus oil is mobile and colorless, with a powerful fresh, camphoraceous odor that is not substantive. Apart from its use as a decongestant and cold remedy it is used

The cajput sample analyzed by the RSC is of Vietnamese origin **T-1**

Peak identity percent	RRI	Area
1. α-pinene	922	2.1
2. β-pinene	967	1.5
3. β-myrcene	979	2.0
4. p-cymene	1012	0.7
5. 1,8-cineole	1025	70.8*
6. limonene	1025	3.8*
7. γ-terpinene	1048	1.2
8. p-2,4 (8)-menthadiene	1079	0.9
9. linalol	1083	2.7
10. terpinen-4-ol	1161	0.6
11. α-terpineol	1174	6.5
12. β-caryophyllene	1419	0.7
13. α-humulene	1452	0.5
		94.0

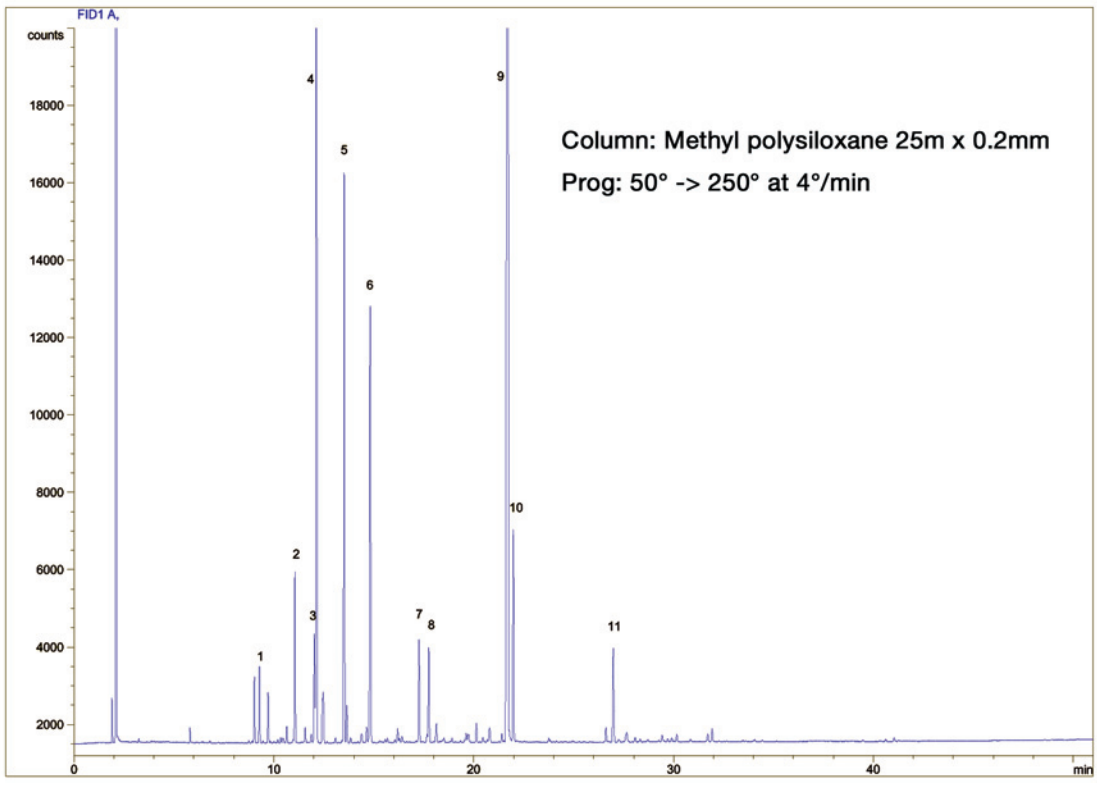
*calculated by proportion by analysis on a polar column

The eucalyptus sample analyzed by the RSC is of Chinese Yunnan Province origin **T-2**

Peak identity percent	RRI	Area
1. α-pinene	922	4.0
2. β-pinene	967	0.7
3. β-myrcene	980	0.7
4. α-phellandrene	997	0.4
5. p-cymene	1014	2.1
6. 1,8-cineole	1026	83.1*
7. limonene	1026	5.4*
8. γ-terpinene	1048	1.1
9. terpinolene	1079	0.2
10. terpinen-4-ol	1161	0.3
11. α-terpineol	1171	0.8
		98.8

*calculated by proportion by analysis on a polar column

Thyme Spanish **F-3**



The white thyme oil sample analyzed by the RSC is of Spanish origin of true botanical source **T-3**

Peak identity	RRI	Area percent
1. α-pinene	922	1.0
2. β-myrcene	980	2.1
3. α-terpinene	1009	1.4
4. p-cymene	1014	17.5
5. γ-terpinene	1049	8.1
6. linalool	1084	6.1
7. borneol	1149	1.4
8. terpinen-4-ol	1162	1.4
9. thymol	1275	48.6
10. carvacrol	1281	2.7
11. β-caryophyllene	419	1.5
		91.8

as a flavor modifier in pharmaceutical and dentifrice products.

Eucalyptus oil is covered by ISO standard 770, with the Australian 80 / 85 percent type as ISO 3065 and the Portugese type as ISO 4732. It is also featured in the 2002 European Pharmacopoeia 4th edition, British Pharmacopoeia and USP 25. The 1,8-cineole quoted therein is 70.0 percent minimum.

The physical constants recorded in the Ph. Eur. 2002 are:

Relative density at 20°C	0.906 to 0.925
Refractive index at 20°C	1.458 to 1.470
Optical rotation at 20°C	0 to + 10°

Oil of Thyme

The thyme herb Thymus vulgaris L. or Thymus zygis L., members of the Labiatae family, are medium sized shrubs which are commercially grown for essential oil distillation in France (T. vulgaris), Spain and Turkey (T. zygis) but the plants grow wild, in many parts of the Mediterranean. There are many different chemotypes of T. vulgaris, T. zygis and other Thymus species, with many having a completely different major component e.g. thymol, carvacrol, eugenol, linalool and methyl cinnamate types.

The fresh flowers, leaves and stems of the herb are used for the steam distilled oil production. Oils from these origins have a somewhat different composition, the most abundant Spanish variety having a higher thymol content than the other origins.

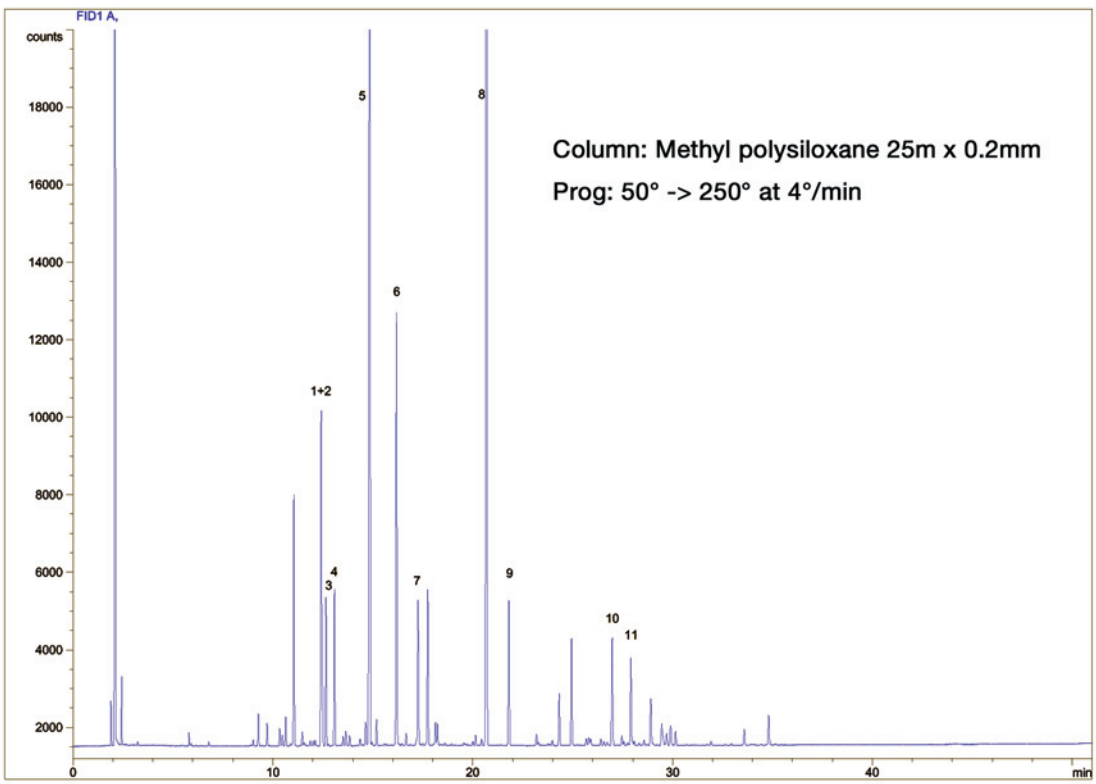
White thyme oil is mobile, pale yellow in color, with a powerful fresh, medicinal odor, which is moderately substantive. The so-called "red thyme oil" is only the white oil containing traces of metals, principally iron;

The samples of lavandin Abrialis, Grosso and Super analyzed by the RSC are all of French origin **T-4**

Peak identity	Abrialis		Grosso		Super	
	RRI	Area percent	RRI	Area percent	RRI	Area percent
1. 1,8-cineole	1021	9.2	1021	5.2*	1020	3.6*
2. limonene	1022	2.5	1021	0.4*	1020	0.5*
3. (Z)-β-ocimene	1026	1.8	1026	0.9	1026	1.5
4. (E)-β-ocimene	1037	3.8	1037	0.2	1037	1.3
5. linalool	1087	31.1	1087	28.0	1087	32.7
6. camphor	1122	8.9	1121	6.6	1121	4.5
7. borneol	1149	2.6	1149	2.4	1149	2.9
8. linalyl acetate	1243	23.0	1245	37.5	1246	38.6
9. lavandulyl acetate	1273	1.5	1273	2.4	1273	1.5
10. β-caryophyllene	1419	3.1	1419	1.9	1419	1.4
11. (Z)-β-farnesene	1448	0.8	1448	1.6	1448	0.9
		88.3		87.1		89.4

*calculated by proportion by analysis on a polar column

Lavandin Grosso **F-4**



which has reacted with the thymol causing it to color orange or red. It is therefore a chemical rather than a botanical distinction.

Apart from its use in medicated shampoos and herbal fragrances, it is used as a modifier for eucalyptus oil in pharmaceutical products as well as toothpaste. There are some reconstituted oils available on the market, with added synthetic thymol.

It has been featured in ISO standard 14715, with wild Thyme oil as ISO 4728. It also appears in the Ph. Eur 4th edition 2002. The total phenols content is typically 60.0 to 70.0 percent, but genuine Spanish oil is

sometimes at a higher level than this and some other genuine oils at a level lower than this.

The physical constants are typically:

Weight per mL at 20°C 0.911 to 0.954 g
Refractive index at 20°C 1.494 to 1.510
Optical rotation at 20°C + 1 to - 5°

Oil of Lavandin

The lavandin shrub (Lavandula x intermedia Emeric ex Loisel.), a member of the Labiatae family, is a hybrid of Lavandula angustifolia Mill. x Lavandula latifolia Medik.. It is medium sized and is commercially grown for essential oil distillation in France. The fresh leaves and flowering tops are used for the steam distilled oil production. There are three oils from three different clones of the hybrid, each having a somewhat different composition and odor.

Lavandin Abrialis has a higher 1,8-cineole content than the other two oils, more like the spike lavender from which is was hybridized. Lavandin Super is sweeter and the most like lavender oil, L. angustifolia. Lavandin Grosso has a composition mid-way between the other two and grown because it has the highest oil yield of all three.

The lavandin oils are mobile, pale yellow in color, with a fresh, floral, slightly eucalyptus-camphoraceous odor, which is not very substantive. They are used as a modifiers or alternatives to lavender oil in the fragrances for toiletry products and soaps.

Lavandins have been featured in ISO standards, but there are ISO standards for only Abrialis 3054 and Grosso 8902. The oils contain esters which are principally linalyl and lavandulyl acetates.

The physical constants are typically:

Weight per mL at 20°C 0.910 to 0.923 g
Refractive index at 20°C 1.464 to 1.472
Optical rotation at 20°C + 1 to -4°

Oil of Geranium

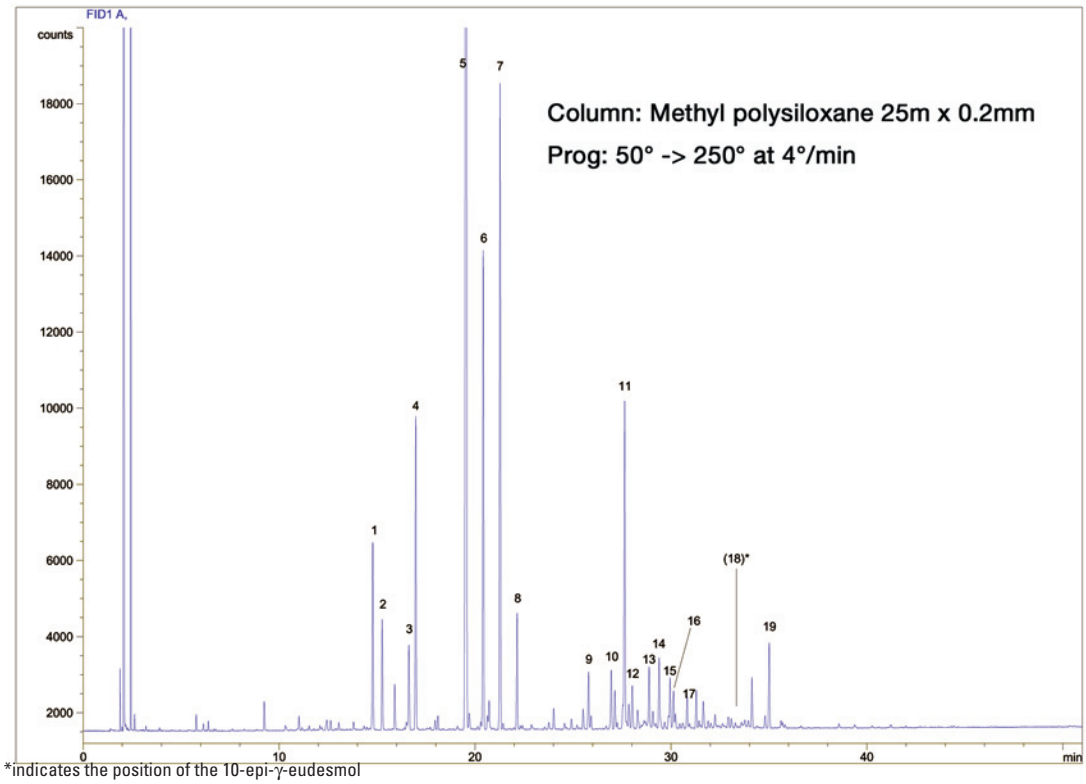
The granium shrub Pelaragonium graveolens L'Herit. ex Aiton is a member of the Geraniaceae family, a medium sized shrub which is commercially grown for essential oil distillation in China, Reunion and North Africa, principally Egypt, Algeria and Morocco.

The fresh leaves are used for the steam distilled oil production. Oils from these various origins have a somewhat different composition; oils from China and Reunion contain about 6 percent of guaia-6,9-diene and the oils from Egypt, Algeria and Morocco contain about 5 percent of 10-epi-γ-eudesmol and little or no guaia-6,9-diene.

Geranium oil is mobile, pale green to yellow in color, with a powerful fresh, green-minty floral odor,

The samples analyzed by the RSC are of Chinese, Reunion and Egyptian origin **T-5**

Peak identity	Chinese		Bourbon		Egyptian	
	RRI	Area %	RRI	Area %	RRI	Area %
1. linalool	1083	2.9	1085	10.2	1084	5.4
2. *cis*-rose-oxide	1096	1.7	1095	0.7	1095	1.3
3. menthone	1132	1.5	1132	0.6	1132	2.0
4. isomenthone	1142	4.9	1143	8.1	1143	5.4
5. citronellol	1215	38.9	1214	21.1	1215	30.7
6. geraniol	1239	7.7	1239	17.7	1240	14.5
7. citronellyl formate	1261	10.7	1261	7.9	1260	6.6
8. geranyl formate	1284	1.9	1284	5.9	1284	2.8
9. β-bourbonene	1384	1.0	1384	0.7	1385	1.2
10. β-caryophyllene	1419	1.3	1419	0.8	1419	1.5
11. guaia-6,9-diene	1441	6.0	1441	5.7	-	-
12. geranyl propionate	1452	0.8	1452	1.5	1452	1.1
13. germacrene D	1477	1.1	1476	0.8	1479	1.4
14. geranyl isobutyrate	1492	1.2	1491	0.6	1494	0.9
15. citronellyl butyrate	1510	1.1	1510	0.5	1510	0.9
16. δ-cadinene	1517	0.7	1517	0.5	1517	1.2
17. geranyl butyrate	1538	0.6	1539	1.3	1540	1.5
18. 10-epi-γ-eudesmol	-	-	-	-	1612	4.6
19. geranyl tiglate	1677	1.5	1676	1.3	1677	1.1
		85.5		85.9		84.1



*indicates the position of the 10-epi-γ-eudesmol

which is quite substantive. It is used as a floral note in perfumes and toiletries and as a modifier or alternative to the more expensive rose otto oils, Rosa damascena Mill. and R. centifolia L.

It has been featured in ISO standard 4731. The terpene alcohols in genuine Bourbon oil from Reunion have a higher ratio of geraniol to citronellol than the Chinese oil.

The physical constants are:

Weight per mL at 20°C	0.884 to 0.893 g
Refractive Index at 20°C	1.462 to 1.472
Optical Rotation at 20°C	-7 to -14°

Oil of Patchouli

The patchouli shrub Pogostemon cablin (Blanco) Benth., a member of the Labiatae family, is a medium sized shrub which is commercially grown for essential oil distillation in China and Indonesia.

The leaves and the twigs of the shrub are used for the steam distilled oil production. Oil from these origins have a somewhat different composition, the Chinese having a lower patchouli alcohol content (15-24 percent) than the Indonesian origin oils (30-33 percent). There is some oil commercially available at a premium price which has a 35 percent patchouli alcohol level, but often this is blended in Indonesia with the cheaper imported Chinese oil to meet the normal grades.

Patchouli oil is mobile, pale yellow to brown in color, with a powerful balsamic woody, slightly camphoraceous odor, which is very substantive, especially on cloth. It is considered by perfumers that this oil improves with age and the odor is disliked if it is too fresh or insufficiently matured. Apart from its use as a soap and detergent fragrance ingredient, it is used as a modifier or blending note in many fine fragrances and aftershaves at levels of 20 percent or more. It has been featured in ISO standard 3757. The typical patchouli alcohol content is 30.0 percent minimum.

The chromatogram of the oil may contain a peak corresponding to α-gurjunene. This suggests the addition of gurjun balsam to the oil. The expected position of this component is indicated on the chromatograms below.

The physical constants are typically:

Weight per mL at 20°C	0.954 to 0.976 g
Refractive index at 20°C	1.507 to 1.512
Optical rotation at 20°C	-47 to -61°

The patchouli samples analyzed by the RSC are two of Indonesian origin and one of Chinese
(a) indicates the position of a-gurjunene if present.

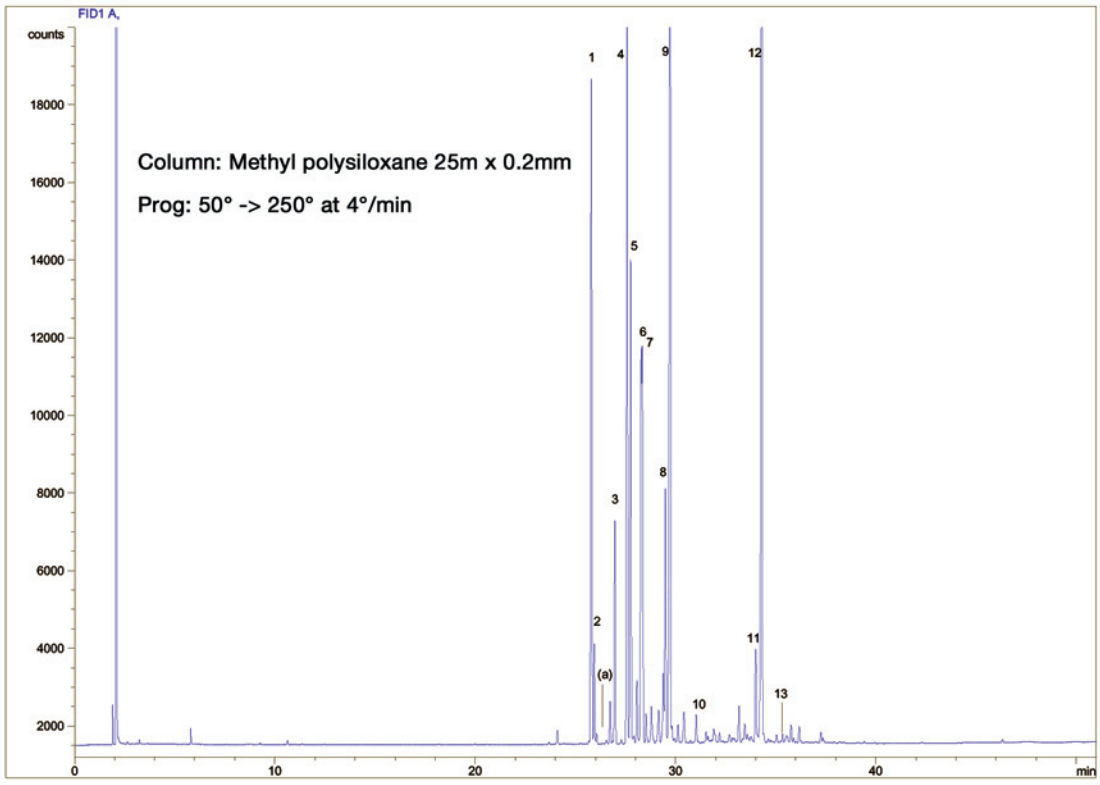
T-6

	Chinese		Indonesian 30%		Indonesian 35%	
Peak identity	**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. γ-patchoulene	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. ledene	1498	3.9	1498	3.7	1498	3.4
9. δ-guaiene	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

Patchouli Chinese

F-6



References

1.Analytical Methods Committee, Analyst, 122, p 1167 (1997).

2.R.P. Adams, Identification of Essential Oil components by Gas Chromatography Mass Spectroscopy. Allured Publ. Corp., Carol Stream, IL (1995).

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