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# Short Communication

# Possible value for the gas chromatographic analysis of essential oils of some unusual phase commercial capillaries

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ABSTRACT

Analyses of sweet fennel and mace oils using a toroid or a liquid crystal phase are compared with results from conventional phases, identifying peaks by mass spectra and retention times. The toroid phase may be useful for resolving some terpene hydrocarbons, and the liquid crystal the choice for some aromatics, which include minor toxic oil constituents. They provide useful confirmatory results to those from conventional phases, and possibly better separation of some substances.

## INTRODUCTION

I have previously made gas chromatographic (GC) studies of some volatile oil constituents using commercially available capillaries coated with unusual stationary phases. These were "MPMS", a mesogenic (liquid crystal) polymeric methyl siloxane [1] with triaromatic ester side chains in equal number to methyl groups; and a toroid molecule, dipentylated α-cyclodextrin "Chiraldex-A-DA" [2]. Fully tri-O-pentylated larger cyclodextrins have been used in dilute mixture in a polysiloxane to resolve terpene hydrocarbons of ginger oil, and esters of grapefruit essence [3].

I was interested to see how useful they might be for the analysis of complex volatile oils in comparison with traditional phases, particularly in relation to determining possible toxic constituents. Many volatile oils present a difficult mixture for chromatographic separation which includes various terpene hydrocarbons of low polarity, intermediate-polarity oxygenated terpenoids, and more polar propenyl aromatic ethers. This is their normal elution sequence, although there may be overlap between some terpenoids and aromatics.

Sweet fennel oil was selected for study, having only about 20% monoterpene hydrocarbons and similar rapidly eluting substances. The dominant part of the remainder comprises aromatics, with less than 10% terpenoids. *trans*-Anethole is the major aromatic flavour component, and occurs with traces of its toxic *cis*-isomer which needs to be determined. Anethole-containing oils are readily oxidised, and should be checked for the main product, anisal. Anethole never occurs without some of its (precursor?) allyl isomer estragole, and as a poten-

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tial carcinogen, this requires checking. Some fennels have estragole dominating [4]. The presence of the bicyclic saturated terpenoid ketone fenchone distinguishes all fennels from anise oils.

The other oil chosen was mace, in which the composition is the reverse of fennel, with over 85% monoterpene hydrocarbons, etc. Here the minor component aromatics are hallucinatory myristicin and carcinogenic safrole, requiring control. Terpenoids present include terpineols.

Stationary phases chosen for comparison with the novel two were the classic non-polar fully methyl polysiloxane and polar polyethylene glycol.

## EXPERIMENTAL

### *Apparatus and methods*

*Gas chromatographic comparison of the various capillaries.* A Hewlett-Packard 5790A gas chromatograph was used, fitted with a capillary control unit, and a splitter injection port and flame ionisation detector both set at 235°C. A Hewlett-Packard 3380A recorder/integrator gave the chromatograms.

The MPMS Heliflex fused-silica capillary (Alltech, Deerfield, IL, USA) was 25 m × 0.25 mm I.D. It was heated slowly by programming up at 4.5°C $min^{-1}$ and was cooled slowly after use (as recommended by Alltech) by switching off the oven and not using the 5790A rapid cooling mode. Its mesogenic property should only become apparent after melting to a liquid crystal at about 145°C.

The Chiraldex-A-DA fused-silica capillary (Advanced Separation Technologies (AST), Whippany, NJ, USA) was 10 m × 0.25 mm I.D. It was heated and cooled at less than 25°C $min^{-1}$ as recommended by AST. Although capable of use at up to 200°C, the phase was not heated above 150°C to avoid possible loss of low temperature performance (AST suggestion).

Two conventional phase Hewlett-Packard fused-silica capillaries 25 m × 0.21 mm I.D. were also used, containing (a) Carbowax 20M (polyethylene glycol), or (b) high-performance cross-linked methyl silicone (methyl polysiloxane).

Helium was used as mobile phase at about 1 ml $min^{-1}$ with all capillaries except Chiraldex, where 2 ml $min^{-1}$ was required; and as make-up gas for the detector. Injections were made with a microsyringe; of about 0.2 μl of the volatile oils, or trace residues from an "emptied" syringe of reference substances. The injection split ratio was 60–100:1. Chromatographic conditions are given in Table I. To protect the mass spectrometric (MS) detector, only a dedicated new methyl polysiloxane capillary was used with it as the other phases here had considerable prior use.

*GC–MS identification.* A Hewlett-Packard 5791 mass selective detector and interfaced 5890 gas chromatograph were used, with autoinjection, and computer control and recording of the ion chromatogram. Spectra obtained for peaks were compared to the Wiley "library" [14].

The Hewlett-Packard fused-silica capillary of HP-1 cross-linked methyl silicone gum (polysiloxane) 0.33 μm film thickness was 12 m × 0.20 mm I.D. Helium was the mobile phase. Solutions of 0.1% of the two oils in methanol were autoinjected (1 μl).

Constituents of the oils were identified by MS using the Wiley library computer programme where possible, and in some other cases by reference to published spectra [5,6]. Identifications were checked against retention times/indices with adjacent peaks. Authentic substances were run for other identifications. A few peaks could be only provisionally identified without authentic references by using GC retention time sequences (ref. 5, on DB5, a methyl polysiloxane with only 5% phenyl groups), and retention index lists (ref. 6, on 20M and OV-101, the latter being a fully methyl polysiloxane). Other lists are available (ref. 7 just gives terpenes, for example).

### *Materials*

Sweet fennel oil (Bush, Boake, Allen ref. 46-4488) of unknown geographic origin, and mace oil (Bush, Boake, Allen ref. 48-0802) English distilled; both old samples, originally 1 lb. (= 0.4536 kg), kept in fairly full brown glass bottles.

## RESULTS AND DISCUSSION

Polarity determination of the MPMS capillary indicated it had intermediate polarity (neither very low nor very high) at 120°C, shown by the close sequence of *n*-butanol–2-octyne–pyridine (last). With low-polarity phases like fully methyl polysi-

TABLE I

AVERAGE ANALYSES OF SWEET FENNEL OIL

| | MS identification quality[a] *or ions (m/z)* | | Stationary phase Methyl polysiloxane | | Chiraldex-A-DA | | Carbowax 20M | | MPMS liquid crystal | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | % | $t_R$[b] | % | $t_R$[b] | % | $t_R$[b] | % | $t_R$[b] |
| α-Pinene | Same $t_R$ as mace | | 1.7[c] | 3.93 | 1.8[c] | 2.17 | 1.8[c] | 0.81 | 1.5 | 1.88 |
| ?β-Pinene | $t_R$ used | | 0.9 | 5.12 | 1.6[c,d] | 2.96 | 1.1[c] | 1.05 | 1.8[d] | 1.95 |
| ? Monoterpene | | *93, 136* | 0.7 | 5.68 | | | 0.2[c] | 1.24 | | |
| α-Phellandrene | 83 | *77, 91, 93, 136* | 3.6 | 6.02 | 3.4 | 3.50 | 4.4[c] | 1.36 | 4.0 | 2.04 |
| *p*-Cymene | Same $t_R$ as mace | | 1.1 | 6.41 | 11.5[d] | 4.03 | 1.1 | 2.22[f] | 13.3[d] | 2.12 |
| Limonene | 94 | *67, 68, 136* | 10.9 | 6.73 | | | 11.2[c] | 1.62 | | |
| Fenchone | 90 | *69, 81, 152* | 6.2 | 7.70 | 6.2 | 5.31 | 6.5 | 4.06 | 6.8[d] | 2.52 |
| Linalol | | *55, 69, 71, 93* | 0.7 | 8.08 | 0.7 | 6.45 | 0.7 | 6.59 | | |
| Estragole | 94 | | 4.1 | 9.61 | 4.1 | 7.65 | 4.1 | 7.70 | 4.0 | 3.98 |
| *cis*-Anethole[e] | | *117, 147, 148* | 0.3 | 10.35 | 0.3 | 8.07 | 0.3 | 8.64 | 0.3 | 5.07 |
| *trans*-Anethole | 98 | | 66.0 | 11.08 | 67.2 | 8.85 | 65.6 | 9.79 | 66.8 | 7.17 |
| Anisal | | *77, 92, 107, 135, 136* | 0.4 | 10.28[f] | 0.7[c] | 9.14 | 0.8 | 11.44 | 0.6 | 9.26 |
| ? Feniculin[e] | | *133, 134, 202* | 0.9 | 15.15 | 0.9[c] | 16.95 | 0.7 | 13.80 | 0.8 | 14.56 |
| Temperature programme | | | 60°C for 5.0 min then 12.5°C $min^{-1}$ | | 60°C for 3.0 min then 15.0°C $min^{-1}$ to 150°C (9.0 min) | | 50°C for 2.0 min then 10.0°C $min^{-1}$ | | 100°C for 0 min then 4.5°C $min^{-1}$ | |

[a] The quality of the MS identification has more assurance as it approaches 100. Values over 90 are good, but lower values with significant ions are also distinctive.
[b] Typical retention times ($t_R$) in minutes to show potential resolution of sequential solutes.
[c] Peaks emerging under isothermal conditions.
[d] Merged peaks.
[e] No authentic reference.
[f] Peak not in time sequence on this phase.

loxanes, low-polarity octyne emerges last [8] as it does on Chiraldex-A-DA, considerably after butanol–pyridine. Studies with cuminal and caryophyllene confirm these polarity ratings [9].

Temperature-programmed operating conditions for the four capillaries needed individual design, and were adjusted to yield the final significant peak from fennel in about 15 min, whilst giving best resolution of the early peaks. An initial isothermal period was found appropriate for all phases except MPMS. To preserve the lower-temperature selectivity of Chiraldex-A-DA, a final isothermal period was applied; this phase needed double the normal mobile phase flowrate.

Average analysis results are given in Tables I and II, and Figs. 1 and 2 present typical chromatograms. Linalol provided "standard" retention times in all chromatograms. An internal standard could not be added as neat oil injections were used to avoid solvent overlap with early peaks.

The first peak that could be identified, in both oils, was α-pinene —nearly 24% of mace on the low-polarity phases, but less than 2% of fennel from all four phases. The larger amount caused a shift in retention time —advanced on Chiraldex, but delayed, as more usual, on methyl polysiloxane, from which it was identified by MS in mace. It was preceded on the low polarity phases (best resolved on Chiraldex) by less than 2% of what could be, if it is a monoterpene hydrocarbon, α-thujene. Jennings and Shibamoto [6] record this as the only such substance with a retention index just less than α-pinene on methyl polysiloxane. These two peaks are unresolved on the polar Carbowax 20M; and blurred even more on the MPMS phase which cannot perform well as a liquid crystal at just over 100°C attempting to resolve the large proportion of mace oil constituents which are fast-eluting. The MPMS phase does partly separate the much smaller amount of such constituents of fennel oil, but with some unresolved mixtures.

A second big monoterpene hydrocarbon peak of mace was identified from methyl polysiloxane as sabinene in a double or more complex peak which was better resolved on Chiraldex, where it was evaluated as just under 25% of the oil. By retention times,

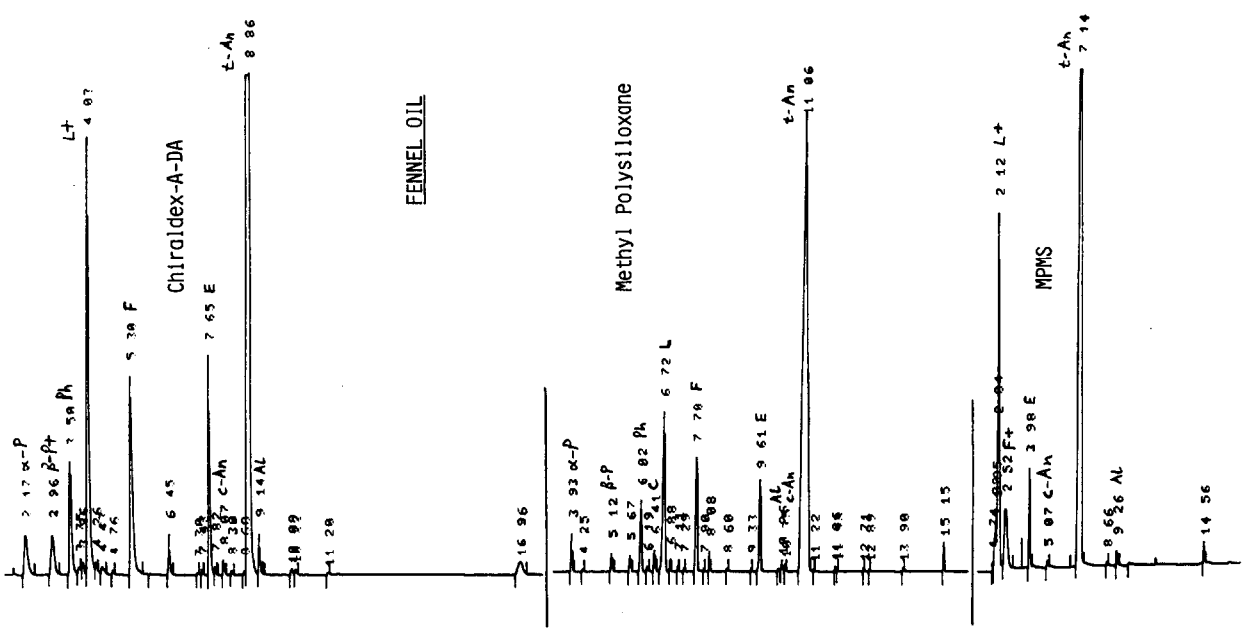


Fig. 1. Chromatograms of sweet fennel oil on three different capillary phases. Left, Chiraldex-A-DA; middle, methyl polysiloxane; right, MPMS. Operational conditions in Table I. c-An = *cis*-anethole; t-An = *trans*-anethole; Al = anisal; C = *p*-cymene; E = estragole; F = fenchone; L = limonene; α-P = α-pinene; β-P = β-pinene; Ph = α-phellandrene; + = merged peak. Numbers at peaks are retention times in min.

TABLE II

AVERAGE ANALYSES OF MACE OIL

See Table I for temperature programme details and footnote explanations.

| | MS identification quality[a] *or ions (m/z)* | | Stationary phase | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Methyl polysiloxane | | Chiraldex-A-DA | | Carbowax 20M | | MPMS liquid crystal | |
| | | | % | $t_R$[b] | % | $t_R$[b] | % | $t_R$[b] | % | $t_R$[b] |
| ? α-Thujene[e] | | | 1.2[c] | 3.80 | 1.5[c] | 1.82 | 25.6[c,d] | 0.86 | | |
| α-Pinene | 94 | | 23.3[c] | 4.13 | 23.9[c] | 2.05 | | | | |
| Sabinene[e] | 95 | | 35.1[d] | 5.39 | 24.7[c,d] | 2.70 | 43.7[c,d] | 1.25 | 73.9[d] | 1.95 |
| ?β-Pinene | $t_R$ used | | 7.7[d] | 5.46 | 19.6[c,d] | 2.84 | | | | |
| ? Monoterpene | | *93, 136* | 2.3 | 5.79 | | | | | | |
| α-Phellandrene | Same $t_R$ as fennel | | 0.6 | 6.04 | | | 1.9[c,d] | 1.33 | | |
| ? Monoterpene | | *93, 136* | 2.0 | 6.27 | | | | | | |
| α-Terpinene | | *121, 136* | 1.1 | 6.39 | 1.6 | 3.53 | 4.1[c,d] | 1.45 | | |
| ? Monoterpene | | *93, 136* | | | 1.8 | 3.74 | | | | |
| *p*-Cymene | | *119, 134* | 2.5 | 6.49 | 8.8 | 4.06 | 2.5 | 2.29[f] | 12.0[d] | 2.13 |
| Limonene | Same $t_R$ as fennel | | 6.4 | 6.75 | | | 6.5[c] | 1.65 | | |
| γ-Terpinene | 90 | | 1.7 | 7.35 | 1.7 | 4.45 | 1.7 | 2.05 | | |
| Terpinolene[e] | 83 | *121, 136* | 1.4 | 7.93 | 1.3 | 4.77 | 1.3 | 2.43 | 1.5 | 2.29 |
| Linalol | Same $t_R$ as fennel | | 0.2 | 8.08 | 0.2 | 6.44 | 0.4 | 6.59 | 0.4 | 2.46 |
| 4-Terpineol | 90 | *71, 111, 154* | 2.9 | 9.40 | 3.1 | 7.42 | 2.8 | 7.07 | 2.8 | 3.30 |
| α-Terpineol | 78 | *59, 93, 121, 136* | 0.5 | 9.54 | 0.5 | 7.80 | 0.5 | 8.23 | 0.6 | 3.67 |
| Safrole | 96 | | 2.1 | 10.86 | 2.1 | 8.81 | 2.1 | 9.91 | 2.1 | 5.37 |
| Methyl eugenol | | *135, 147, 163, 178* | 0.3 | 12.22[f] | 0.2[c] | 9.91 | 0.1 | 11.57 | 0.4[d] | 7.67 |
| Eugenol | 91 | | 0.2 | 11.67 | 0.3[c] | 10.44 | 0.2 | 12.93 | | |
| Myristicin[e] | 91 | | 6.0 | 13.60 | 6.2[c] | 12.96 | 5.6 | 13.85 | 6.1 | 10.96 |

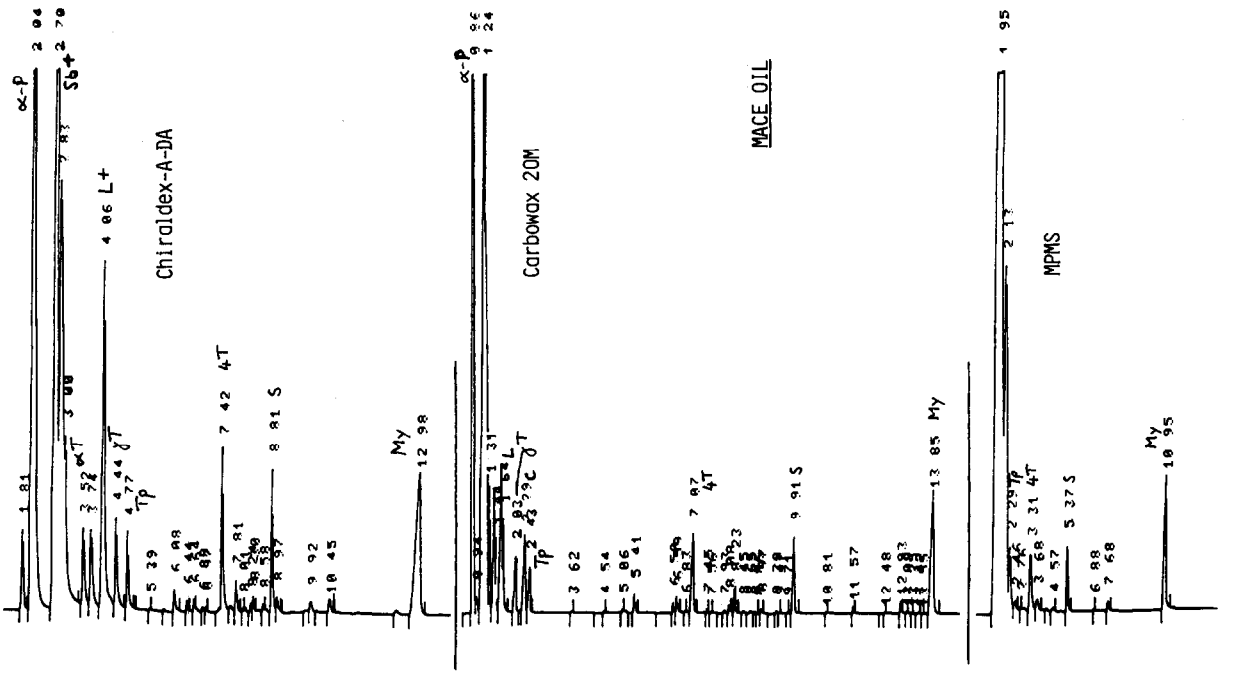


Fig. 2. Chromatograms of mace oil on three different capillary stationary phases. Left, Chiraldex-A-DA; middle, Carbowax 20M; right, MPMS. Other details as in Fig. 1, plus My = myristicin; S = safrole; Sb = sabinene; αT = α-terpinene; γT = γ-terpinene; 4T = 4-terpineol; Tp = terpinolene.

β-pinene seems to be part of this complex of peaks in mace, and has been previously reported in it [10]. It can also be detected in fennel using conventional phases and retention times. These four phases also more or less resolve subsequent peaks from the oils of α-phellandrene; α-terpinene in mace; *p*-cymene; limonene forming about 11% of fennel and over 6% of mace; and γ-terpinene and terpinolene, in mace. Only the lattermost seems resolved on all four phases, being the last monoterpene hydrocarbon to emerge. However, a perfect analysis of all such substances was not the point of this study, nor the identification of every peak. Better results should be achieved by more experienced oil analysts than the author.

In mace, subsequent polar substances total about only one-eighth of the oil, including about 6% myristicin and about 2% safrole. So about one-twelfth of mace consists of hallucinatory or carcinogenic aromatics, which can be evaluated by all four phases for confirmation. Other trace aromatics present were eugenol and methyl eugenol. The principal terpenoid present was 4-terpineol about 3%, preceded and followed respectively by small amounts of linalol and α-terpineol. All the above have been identified by GC–MS in mace oil previously [10].

The principal aromatic in fennel was confirmed as *trans*-anethole, forming about 66% of the oil, together with about 4% of estragole. Its oxidation product anisal (identified by its distinctive pair of MS ions at *m/z* 135 and 136, and others) formed less than 1%, and appeared after anethole, except on methyl-polysiloxane where it was unfortunately close to the toxic *cis*-anethole (identified by ions at *m/z* 117, 147 and 148) which formed merely 0.3% of the fennel oil. This peak, provisionally identified by its amount on the other three phases, seems best resolved from the main *trans*-anethole peak by MPMS. The principal terpenoid is about 6% fenchone with less than 1% linalol, identified by retention times, and MS. These constituents have all been reported from fennel oils [11]. The last fennel constituent from all phases formed less than 1% of the oil. It was not a sesquiterpene hydrocarbon by

the long retention times, which were similar to the sesquiterpenol eudesmol. Its main ions were of $m/z$ 133 and 134, with a small one at $m/z$ 202. 202 is the molecular mass of feniculin, an aromatic isoprenyl ether identified in the last fractions of fennel oil in 1938 [12]. It would yield a MS fragment of $m/z$ 133 like desmethyl anethole, so this late-appearing fennel constituent was provisionally identified as feniculin. It could not be found in an eight-peak MS index [13].

It thus seems that the two novel stationary phases used here may offer some advantages for volatile oil analysis, compared to conventional phases. Chiraldex gives best resolution of an early peak preceding $\beta$-pinene —it may be the phase of choice for monoterpene hydrocarbons. The true resolving character of MPMS does not come into operation at the low temperatures required for such substances, but seems optimum for aromatics, especially problems like resolving a trace of *cis*-anethole from the main *trans*-isomer peak. The advantages of a confirmatory analysis on a phase quite different to conventional ones is also apparent. Results for the minor constituents safrole and estragole are consistent from all four phases, although some other oil components show slightly differing values. However, no phase can be presumed to give "correct" results, and this is another reason for using a diversity of phases in analysis.

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