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Review

Chemical characterisation of the different types of volatile oil constituents by various solute retention ratios with the use of conventional and novel commercial gas chromatographic stationary phases

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Abstract

Retention ratios of an unknown solute peak from a volatile oil can help to identify its chemical type. Relative retentions on conventional stationary phases can be used to calculate a polar/non-polar ratio which may suggest it is an aromatic solute or a sesquiterpene hydrocarbon. With dipentyl (DA) cyclodextrin phases, γ -DA/ α -DA ratios can indicate a bicyclic or monocyclic monoterpenoid. Temperature change on α -DA can pick out a non-alcoholic saturated cyclic monoterpenoid, using 150/125°C. Twenty retention ratios are reviewed involving various phases, including other modified cyclodextrins, liquid crystals, “Chirasil-Val” and crown ethers. Applications to six volatile oils are made using a variety of phases. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Oils; Stationary phase, GC; Retention ratios; Reviews

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1. Introduction

A volatile oil is a potentially diverse non-saponifiable lipid in its chemistry. It commonly contains

terpenes, even though they may be minor constituents, and these can be monoterpene (C_{10}) or sesquiterpene (C_{15}) examples of unsaturated hydrocarbons or oxygen-containing terpenoids such as alcohols or carbonyl compounds. A common alternative constituent, major or minor, is aromatics derived from unsaturated allyl- or propenyl-benzenes

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substituted by methyl/methylene-aromatic ethers (frequently C_{10} compounds). Despite this chemical diversity, the Saura-Calixto et al. gas chromatographic linear relationship [1] holds for such constituents:

$$I_R = at + b \quad (1)$$

where I_R is the retention index and t ($^{\circ}C$) is the boiling point of a solute (a particular volatile oil terpene or aromatic constituent) and a and b are constants with the latter having a relationship to the stationary phase. This reviewer has calculated the high correlation coefficients (r) resulting from using Eq. (1) on some conventional stationary phases with literature retention indices [2] of 23 diverse volatile oil constituents against their boiling points, which range from 156 to $236^{\circ}C$. These included the terpene myrcene, the terpenoids camphor and α -terpineol, and the aromatic ether anethole, for example. For the polar polyethylene glycol phase Carbowax 20M, $r = 0.980$, and for low-polarity methyl polysiloxane OV-101 it is 0.990. Because there is this close correspondence to the linear relationship expressed by Eq. (1), conventional phases alone do not provide information about the chemical nature of one unknown solute being resolved from another. For such to be possible, some solutes should diverge away from any good linear relationship, resulting in a lower correlation coefficient, say below 0.90. On conventional phases, if the observed retention time of an unknown peak cannot be matched with that of an authentic sample, tables of retention indices can be used to suggest its identity, but these are not as reliable as their seemingly precise numbers of units within values of many hundreds suggest. They only represent logarithmic relative retentions against a one-carbon standard solute, usually methane ($\times 100$). They give no indication that an unknown is likely to be an acyclic hydrocarbon like myrcene, or a monocyclic alcohol like α -terpineol, or a bicyclic carbonyl compound like camphor, yet this would be useful information for identifying an unknown. Mass spectral evidence, whilst much used for volatile oil studies, will differ in relative ion peak intensities for a given substance from one detector to another, and also according to their settings. Adams' book [3] depicts many mass spectra of volatile oil constituents, and citronellal, citronellol and geraniol can

be seen to have very similar spectra which may not allow them to be reliably "identified" by any computer matching library. Alcohols notoriously do not yield their molecular ions, and the above three solutes, plus citronellyl and menthyl acetates, with molecular masses of 154–198, usually reveal no fragmentation ion heavier than m/z 139. and then at an intensity no more than 5% of the principal ion. Because of these uncertainties, this reviewer has used in the past mass spectrometry (MS) results assisted by retention or I_R sequences of solutes [4] to identify the major and minor constituents of sweet fennel oil (including traces of anisal, toxic *cis*-anethole and feniculin) and of mace oil (including traces of α -terpineol and methyl eugenol). Fresh *Eucalyptus citriodora* leaves processed by solid-phase microextraction [5] were also thus found to have traces of citronellyl acetate and of isopulegol, which are the precursor and decomposition product of the main volatile oil constituent citronellal. However, another method to give confirmation of the chemical nature of newly identified peaks would have been useful. Such information can come from certain ratios which may be applied to retention results from conventional or novel stationary phases which have been commercially available in capillaries. These observations will be expanded below by reference to studies of related phases in packed columns. Applications to named volatile oils will be given.

The term "retention ratios" seems to have been coined by Rohrschneider in 1969 as one of his many methods of characterising stationary phases [6]. To do this he used the logarithms of the retentions of six selected probe solutes (benzene, nitromethane, etc.) against that of his standard, octane. Sixteen years later [7], the same standard was used against octenes by others for the same purpose, without logarithms. This review records various retention ratios which can be calculated using standard solutes that can help to chemically characterise unknown solutes, rather than the phases involved.

2. Retention ratios using conventional stationary phases

Betts in 1988 [8] determined relative retentions

($t_{R,rel}$) against geraniol for some volatile oil constituents with long retention times on two conventional phase capillaries at 135°C. He had found earlier [9] that alcohols were the best monoterpenoids to use as retention standards, and geraniol conveniently had the same retention time of about 1.5 min on both phases. The polar/non-polar ratio (P/N) from the polar Carbowax 20M results vs. the “non”-polar methyl polysiloxane value gave an indication of the chemical nature of an unknown solute peak, see Table 1. A P/N of 0.23 was seen for

three sesquiterpene hydrocarbons; then seven mono/sesquiterpenoids gave ratios of 0.63–0.93. The top end of this range overlapped the lower part of the 0.76–1.34 shown by five aromatic ethers; but two phenols had distinctively higher values. Applied to chenopodium oil, a main peak forming about 65% had a P/N of 0.71 ($t_{R,rel}$ 0.71/1.00), appropriate for the monoterpenoid peroxide ascaridole, which had an identical retention to geraniol on the polysiloxane phase. Ascaridole’s phenolic degradation product thymol (2%) was confirmed as present by the ratio

Table 1
Relative retention times (geraniol=100) at 135°C (from Ref. [8])

Volatile oil constituent (and source)	$t_{R,rel}$ vs. geraniol		P/N	Chemical nature and comment
	Polar (P): Carbowax 20M	Non-polar (N): methyl silicone polysiloxane, cross-linked		
Caryophyllene (Koch-Light)	0.52	2.22	0.23	Sesquiterpene hydrocarbon
Zingiberene (Ginger oil Kelkar)	0.67	2.92	0.23	Sesquiterpene hydrocarbon
Curcumene (Ginger oil Kelkar)	0.76	3.31	0.23	Sesquiterpene hydrocarbon
Eudesmol (Plaimar research)	3.62	5.79	0.63	Sesquiterpenoid mixture, main peak. Very long t_R
Terpinen-4-ol (Dragoco)	0.52	0.80	0.65	Monoterpenoid-detected in nutmeg oil
Carvone (Koch-Light)	0.68	0.99	0.69	Monoterpenoid
Perillal (Koch-Light)	0.82	1.09	0.75	Monoterpenoid
Anethole (Sigma)	0.88	1.15	0.76	Aromatic ether, monomethoxy
Citronellol (BDH)	0.77	0.92	0.84	Monoterpenoid
Farnesol (Aldrich)	6.92	8.14	0.85	Sesquiterpenoid (acyclic). Extremely long t_R
Safrole (Fritzsche)	1.00	1.15	0.87	Aromatic ether, methylene dioxy (detected in nutmeg oil)
Santalol (Sandawood oil Izumi)	6.04	6.46	0.93	Sesquiterpenoid. Extremely long t_R
Methyleugenol (Fritzsche)	1.65	1.75	0.94	Aromatic ether, dimethoxy
Elemicin (synthesised)	3.58	3.46	1.03	Aromatic ether, trimethoxy
Myristicin (Nutmeg oil Bush Boake Allen)	3.83	2.86	1.34	Aromatic ether, methylenedioxy monomethoxy (detected in parsley seed oil)
Cinnamal (Cinnamon oil)	1.73	1.06	1.63	Aromatic aldehyde
Eugenol (Rampre)	2.82	1.50	1.88	Phenol
Thymol (Sigma)	3.15	1.14	2.76	Phenol

Mobile phase: helium at flow-rate about 1 ml min⁻¹ at detector exit, giving t_R for geraniol (Sigma) 1.35–1.55 min on both capillaries.

of 2.77 (3.16/1.14). In addition to the 70% phenolic eugenol in pimento oil with P/N of 1.88 (2.82/1.50), a peak forming about 11% had a ratio of 1.18 (3.07/2.61), indicating an unknown aromatic ether which was not methyl eugenol (ratio 0.94). A 4% peak with P/N 0.24 (0.53/2.18) was clearly a sesquiterpene hydrocarbon, probably caryophyllene.

Earlier work using packed columns [10] had given relative retentions against linalol at 190°C which, if calculated as P/N ratios, showed a similar sequence but with larger values. Three monoterpene hydrocarbons yielded 0.34–0.48; 10 monoterpenoids had ratios 1.04–1.34; three aromatic ethers gave values of 1.26–1.85 (overlap again!); with other aromatics having even larger ratios. In a summary of these studies [9], solutes were divided into two groups; “A” with the highest $t_{R,rel}$ on the low-polarity methyl polysiloxane SE-30 ($P/N < 1.0$), and “B” showing the lowest values on this phase ($P/N > 1.0$). “A” included the low-polarity solutes such as monoterpene hydrocarbons and terpenoid esters. In group “B” terpenoid alcohols could be distinguished from other polar solutes, as they were the only ones which gave a higher $t_{R,rel}$ on Carbowax 20M than on a diethylene-glycol succinate phase. Of the others, if the SE-30 $t_{R,rel}$ was considerably below that on a polar phase, then the solute was aromatic; if not, it was expected to be a terpenoid carbonyl compound.

Another retention ratio which can only be applied to alcoholic solutes of volatile oils is TMS/OH, where the retention time of an alcohol is OH, and that of its trimethylsilyl ether is TMS, from the same phase. A packed column of phenyl–methyl polysiloxane (1:1) at 120°C gave ratios greater than unity (as expected), but only for half of 10 diverse alcohols [11], surprisingly. On such a low-polarity phase, reducing the polarity of the alcohol solute by forming its TMS ether was expected to give a derivative with more affinity for this polysiloxane. However, ratios for four saturated alcohols were only 0.75–0.96, indicating they were less polar to this phase than their ethers! “Appropriate” TMS/OH ratios for two acyclic monoterpene alcohols each with two double bonds were about 1.25, while for the phenolic eugenol it was much higher at 1.40, as anticipated. Thus the various alcoholic constituents of volatile oils may be characterised chemically by using this ratio.

3. Modified cyclodextrin stationary phases and retention ratios

Cyclodextrins (CDs) are fairly large molecular rings of poly- α -glucose consisting most commonly of six (α -CD), seven (β -CD) or eight (γ -CD) monosaccharides, each unit retaining three unbonded hydroxyl groups. “Native” CDs cannot be used as such as stationary phases, but various chemical modifications of them have lower melting points and more suitable properties to permit them to function well, either alone, or mixed in a low-polarity polysiloxane which does not seem to interfere with the responses of the modified CD [12]. The special retention-enhancing character of CDs is due to their molecular ring cavity, allowing them to function as a selective temporary “host” to appropriately sized and shaped “guest” molecules of a solute. The bulkiness and polarity (or lack of it) of various CD substituents provides differing selectivities for diverse solutes.

Less than 10 years after the introduction of gas chromatography (GC), in 1961, Sand and Schlenk first [13] used packed columns of CD esters. They then found that “shifts of retention times of a compound on different (β -CD ester) phases are characteristic for that compound and its class” [14] separating in plots methyl fatty acid-esters from alcohols, aldehydes and olefines. Modified CD phases were thus introduced for the chemical characterisation of unknown solutes, although over 30 years were to pass before such studies, the subject of this review, were continued. The main gas chromatographic use of modified CDs has been in attempts to resolve enantiomeric (racemic) pairs of solutes. In 1993, Betts compared the relative retentions to undecane of some volatile oil constituents using two “ChiralDEX” capillaries which were dipentyl-CDs (having one unsubstituted hydroxyl group remaining) on each α -glucose component [15]. He found the 100 β -DA/ α -DA ratio (the percentage result from dipentyl β -CD against that on the α -CD) for a given monoterpene could be an indication its cyclic nature, see Table 2. At 125°C, four bicyclics showed a 125 to 116% increase in relative retention, whilst four acyclics only gave 25 to –11%. Seven monocyclics exhibited a 101 to 34% increase, but this range was not exclusive as it included members from

Table 2

Relative retention times (*n*-undecane=1.00) on ChiralDEX-DA (Dipentyl) capillaries with percentage increases on changing to the larger molecular ring (from Ref. [15])

Solute	Gas chromatographic phases, used at various temperatures (°C)								
	100			125			150		
	β	α	% inc	β	α	% inc	β	α	% inc
Isoborneol		3.46		7.78	3.45	125	6.35	3.33	91
Borneol		3.95		8.36	3.84	118	6.87	3.57	92
Thujone	3.17	1.08	193	2.69	1.24	117	2.52	1.36	85
Camphor	4.55	1.67	172	4.01	1.86	116	3.84	2.04	88
α -Terpineol		3.65		6.96	3.47	101	5.78	3.19	81
4-Terpineol		2.83		5.26	2.85	85	4.54	2.88	58
Fenchone	2.11	0.98	115	2.02	1.16	74	2.00	1.29	55
Menthone	3.16	1.59	99	2.80	1.73	62	2.65	1.85	43
Menthol		3.87		5.92	3.73	59	4.71	3.33	41
Carvone		3.48		5.30	3.54	50	4.53	3.54	28
Perillal					6.57		7.59	5.95	28
Linalol	2.74	1.57	74	2.26	1.57	44	2.00	1.56	28
Pulegone		2.69		3.83	2.77	38	3.56	2.84	25
Piperitone		3.63		4.96	3.70	34	4.44	3.59	24
Citronellal	2.60	1.82	43	2.26	1.81	25	2.15	1.79	20
Citronellol				6.40	5.11	25	4.94	4.27	16
Geraniol				6.60	6.36	4	4.88	5.04	-3
<i>n</i> -Dodecane	2.06	2.03	1.5	1.83	1.84	-0.5	1.70	1.73	-1.7
Citral				5.27	5.93	-11	4.29	5.02	-14
<i>n</i> -Decanal				2.96	3.78	-22	2.81	3.28	-14
<i>n</i> -Octanol	2.43	3.26	-25	2.01	2.75	-27	1.81	2.37	-24
<i>n</i> -Undecane retention time (min)	0.84	1.00		0.35	0.37		0.17	0.17	

Average results. Percentage increase (inc) shown for solutes on changing from the smaller α - to the larger β -cyclodextrin modification. Helium holdup times on both phases 0.25–0.30 min.

each of the previous two groups, fenchone and linalol.

Results shortly after this [16] allow comparisons between two different α -CDs by the ratio 100 α -DA/ α -PH, using the low-polarity dipentyl-CD against the polar [12] hydroxypropyl, dimethyl modification. At 110°C, four bicyclic monoterpenoids could be picked out by a 176–127% increase in relative retention to linalol, although one acyclic fell in this range, too. Other chemical groups all gave values between 121–102%. Another ratio, 100 α -TA/ α -DA, involving the relative retention on an ester phase, trifluoroacetyl, dipentyl-CD, increased only for seven ketones/aldehydes by 137–104%. The “ChiralDEX” manufacturer claims “unique selectivity (by) the TA series for carbonyl-containing molecules” [17], but this ratio also increased for five hydrocarbons by 111–100%, leaving just increases over 112% to be carbonyl-

associated. Unfortunately, the trifluoroacetate esters of CD are unstable and hydrolyse to DA with use, so the carbonyl selectivity must be sought using a fresh capillary. Nevertheless, the α -TA phase, programmed from 75–150°C, gave a successful chromatogram of the ketone-containing dill oil. 49.4% carvone and 0.5% dihydrocarvone were found [16] along with 43.6% limonene (including about 2% *p*-cymene), 2.8% anethofuran (residual from the flowering stage) and 0.4% dillapiole, an aromatic substance. These last two constituents were not properly revealed by a conventional methyl polysiloxane phase.

Taking relative retentions to undecane, and involving an example of the largest molecular ring, a γ -CD in “ChiralDEX G-DA”, the ratio 100 γ -DA/ α -DA (“A-DA”) at 150°C [18] gave even bigger increases for three bicyclic monoterpenoids of 341–157%

(confirmed by four more at 125°C), with 91–65% for seven monocyclics (with three more at 125°C), and 54–12% for acyclics and aromatics, see Table 3. Ten selected [12] diverse volatile oil constituents (aromatics cuminal and estragole; acyclic monoterpenoids citral, citronellal, citronellol and linalol; monocyclics carvone, menthol and pulegone; and bicyclic fenchone) can have their relative retentions on γ -DA plotted against their boiling points and give an amazingly low correlation coefficient of 0.726 for

Eq. (1). Such a low value indicates that this CD must exhibit considerable extra affinity for some of these solutes, so the large percentage increases in retention ratios given above are not really surprising. This is not so for all CDs as the α -PH modification, for example, gives $r=0.968$, although this value is still less than that of conventional phases.

Relative retentions were now available for all three CD ring sizes of the dipentyl modification, and when plotted gave virtually straight lines of de-creas-

Table 3

Relative retention times (n -undecane = 1.00) on modified dipentyl cyclodextrin capillaries at two temperatures (from Ref. [18])

Solute	Type ^a	Column No. in this table					
		3: Chiraldex-G- DA at 150°C	4: % increase ← α - to γ -CD	5: Chiraldex- A-DA at 150°C	6: Chiraldex- G-DA at 125°C	7: % increase ← α - to γ -CD	8: Chiraldex- A-DA at 125°C
Borneol	B	>12 at 170°C		<i>3.57^b</i>			<i>3.84</i>
Isoborneol	B			<i>3.33</i>			<i>3.45</i>
Camphor	B	9.00 (± 0.07)	341	<i>2.04</i>			<i>1.86</i>
Geraniol	N	7.15 (± 0.18)	42	5.04			6.36
Piperitone	M	6.85 (± 0.18)	91	<i>3.59</i>			<i>3.70</i>
Cuminal	A	6.00 (± 0.11)	20	5.01 ^c			5.09 ^c
Citronellol	N	5.90 (± 0.27)	38	4.27			5.11
Carvone	M	5.85 (± 0.22)	65	3.54			3.54
Menthol	M	5.80 (± 0.23)	74	3.33			$\uparrow 3.73$
α -Terpineol	M	5.75 (± 0.32)	80	3.19			3.47
Citral	N	5.70 (± 0.20)	13	$\uparrow 5.02$			$\uparrow 5.93$
Pulegone	M	5.35 (± 0.12)	88	<i>2.84</i>			<i>2.77</i>
4-Terpineol	M	4.95 (± 0.25)	72	2.88			2.85
Fenchone	B	9.40 (± 0.13)	210	<i>1.29</i>			<i>1.16</i>
Estragole	A	3.55 (± 0.09)	12	$\uparrow 3.18^c$			$\uparrow 3.19^c$
Thujone	B	3.50 (± 0.17)	157	<i>1.36</i>	3.76	203	<i>1.24</i>
Menthone	M	3.35 (± 0.10)	81	1.85			1.73
Cineole	B	2.80 (± 0.10)			2.95 (± 0.16)	288	<i>0.76</i>
Linalol	N	2.40	54	1.56	2.72	73	1.57
Citronellal	N	2.40	34	$\uparrow 1.79$	2.60 (± 0.09)	44	$\uparrow 1.81$
β -Pinene	B				1.84	268	<i>0.50</i>
Camphene	B				1.75	349	<i>0.39</i>
γ -Terpinene	M				1.27	69	0.75
3-Carene	B				1.27	115	<i>0.59</i>
Limonene	M				1.22	85	0.66
α -Pinene	B				1.12	203	<i>0.37</i>
<i>p</i> -Cymene	A				1.10	67	0.66
α -Terpinene	M				0.97	73	0.56
Myrcene	N				0.74	76	0.42

Using γ -(G) or α -(A) cyclodextrins (CDs) with one remaining hydroxyl group per α -glucose unit (-DA). Average values, with range for G-DA phase. Direction of increase shown by arrow.

^a A = Aromatic; B = bicyclic; M = monocyclic; N = acyclic.

^b Values in italics are not in the sequence for Chiraldex-DA (lower, unless with an upward pointing arrow).

^c Estimated.

ing retention from the γ - to α -DA for cyclics menthone, carvone and thujone, and for acyclics linalol, citronellal and citronellol. However, two other acyclics, each with two double bonds in the same molecular position, citral and geraniol, gave distinctive V-shaped plots with lowest relative retentions on the β -DA phase, see Fig. 1. This feature could be detected just by calculating the retention ratios β -DA/ α -DA < 1 instead of the usual result above 1.0.

The accumulation of relative retention values to undecane now allowed Betts [19] to suggest an optimum combination of three modified CD phases from the many available to allow the characterisation of unknown volatile oil constituents using just two retention ratios, see Fig. 2. The initial ratio selected was 100 γ -PN/ β -DA, comparing the stable propionyl ester of dipentyl γ -CD, "ChiralDEX G-PN", against dipentyl β -CD ("B-DA"). Column 9 in Table 4 shows how increases of 0–23%, or values bigger or smaller than this, give an initial partial identification into three non-exclusive solute groups. Then applying the second retention ratio 100 α -PH/ β -DA (involving the hydroxypropyl, dimethyl α -CD "A-PH") differentiated oxygenated aromatics from bicyclic monoterpene/oids, and from non-carbonyl containing monocyclics, see Table 4, column 6. However, carbonyl-monocyclics were not separated from most acyclics. This procedure with two retention ratios only requires three capillaries, but these include the examples of an α -, a β - and a γ -CD, with the β -modification involved in both calculations.

A year later, three β - and two α -CD results were considered, with the emphasis on identifying the type of polar group present [20], for which three β -CDs were chosen. The initial retention ratio selected this time in Fig. 3 was 100 β -PH/ β -DA, when less than 30% increase indicated a hydrocarbon. A 30–80% increase could be from a carbonyl-terpenoid or a monocyclic alcohol. Other substances gave greater increases than 80%, see Fig. 3. The second retention ratio used was 100 β -DA/ β -CDX, with CDX being a "Cyclodex" capillary lined with trimethyl- β -CD, so that a trio, all of β -CDs, was involved here. Cyclic alcohols now gave 41% or more increase, being bicyclic if this was 60% or above. Two composite groupings remained, above and below 8% increase,

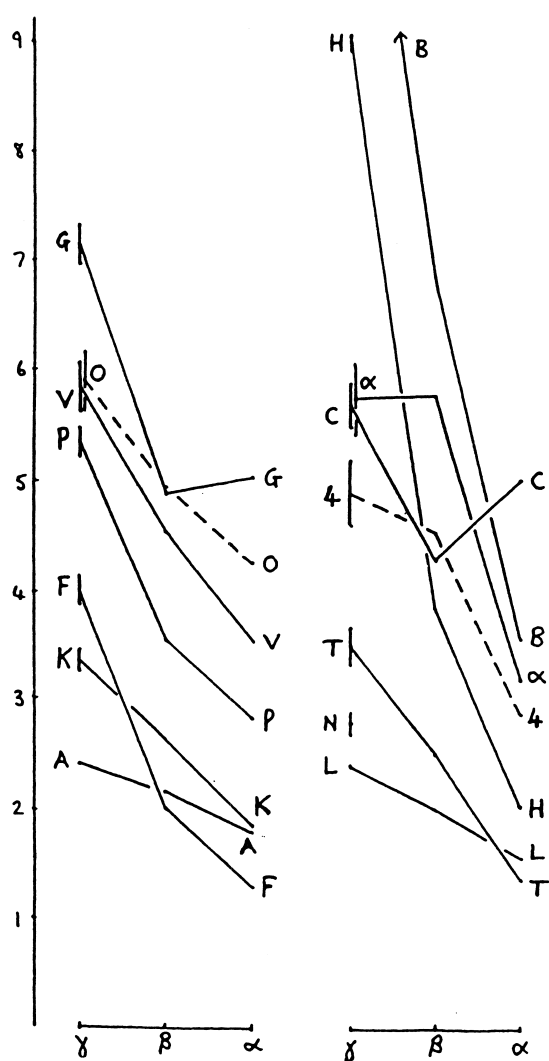


Fig. 1. Linked plots of average relative retention times (*n*-undecane=1.00) (*y*-axis) of some solutes on γ -, β - and α -hydroxydipentylated cyclodextrins (ChiralDEX-DA phases) at 150°C. Ranges of results on the γ -phase shown by vertical lines. A = Citronellal; B = borneol; C = citral; F = fenchone; G = geraniol; H = camphor; K = menthone; L = linalol; N = cineole; O = citronellol; P = pulegone; T = thujone; V = carvone; α = α -terpineol; 4 = 4-terpineol.

which could mostly be resolved by reference back to the initial retention ratio. Unfortunately, monocyclic and acyclic carbonyl compounds could still not be distinguished from each other, although all the other chemical classes of solutes had been differentiated

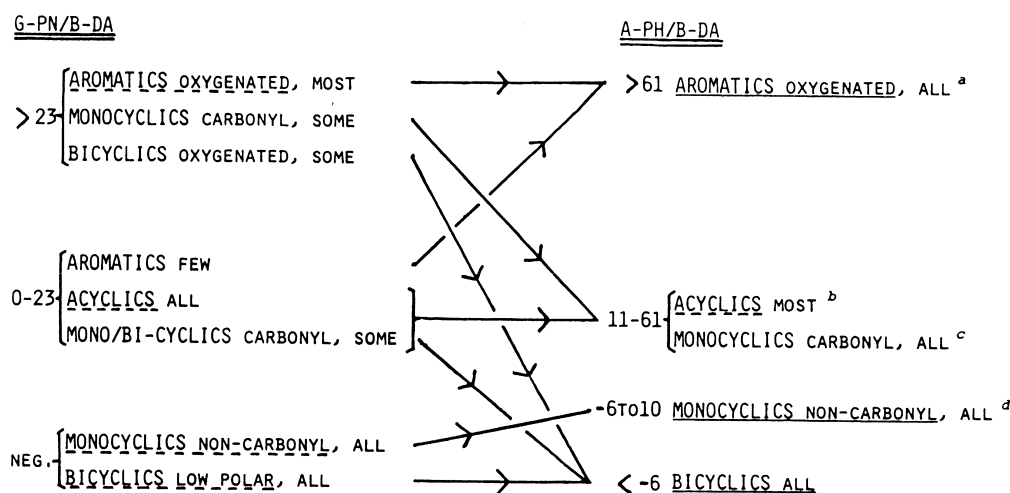


Fig. 2. Solute groupings found for various relative retention time (*n*-undecane=1.00) percentage increases on changing from ChiralDEX B-DA to the other phase indicated (G-PN or A-PH). (a) Together with acyclic geraniol; (b) together with aromatic hydrocarbon *p*-cymene. Acyclic alcohols give over 35% increase A-PH/G-PN. (c) Some carbonyl monocyclics have been in a different group before. (d) Monocyclic alcohols can be detected using ChiralDEX B-PH/A-PH, where they give 40–50% increase.

by these two pairs of retention ratios, with all four comparisons featuring the β -DA phase.

The value of the α -CDs was indicated in the same paper [20] by comparing the retention ratios of each at two temperatures. Table 5 shows that 100·150°C/125°C(α -DA) indicated an increase of 116–107% by five saturated cyclic monoterpenoid carbonyl-compounds and an ether. Also distinctively, a decrease of 85–79% indicated three unsaturated acyclic substances. Fourteen other compounds, including five differing aromatics and other monoterpenoids, clearly fell between these extremes with ratios of 103–88%. The α - were better than the β -CDs for this purpose, and 100·150°C/125°C(α -PH) gave values of 113–98% for the saturated substances, and 80–75% for three unsaturated carbonyl compounds, including now an aromatic. The other solutes gave a non-overlapping range of 91–83%.

4. Liquid crystal stationary phases and retention ratios

Liquid crystals have potential as mesogenic stationary phases which can have extra retention for

some solute molecules by receiving them into an oriented molecular structure. This is their nematic condition, which exists when the solid state melts to give the liquid crystal, up until a higher transition temperature at which it becomes a normal isotropic liquid with the usual randomly oriented molecules. Above this transition temperature, it can still function as a normal liquid phase, but presumably without any extra solute selectivity. The nematic temperature range of some liquid crystal states is quite narrow – from just 112–116°C with cholesteryl acetate, for example [21]. However, such phases can also be used below their melting point, and still function as a liquid crystal [22], possibly due to the passing solute band forming a eutectic mixture with the stationary phase [23]. This property was noted by the first workers to use a liquid crystal as a gas chromatographic stationary phase. In 1964, Dewar and Schroeder explained it by suggesting that supercooling of their liquid crystal had occurred [24]. However, some liquid crystals still function as mesogenic media if heated from cold to less than their melting point [25], when supercooling cannot have happened.

A commercial capillary became available about 10 years ago lined with a film of mesogenic polymeric

Table 4

Relative retention times (*n*-undecane = 1.00) on various modified cyclodextrin phases at 125°C and percentage increases on changing from one to another (italics out of sequence) (from Ref. [19])

Solute	Type ^a	Column No.							
		7, Increase A-PH/B-DA (%)				8 Chiraldex B-DA ^c	9, Increase G-PN ^b /B-DA (%)		
		< -6	-6-10	11-61	>61		<0	0-23	>23
Cinnamal	Aco				209	<i>6.95^c</i>			55 ^b
Borneol	Bol	-17				8.36			25
Geraniol	Nol				101	<i>6.60</i>		17	
Isoborneol	Bol	-23				<i>7.78</i>			26
Safrole	Aet				142	<i>5.1^c</i>			51 ^b
Cuminal	Aco				62	<i>5.9^c</i>		11	
Citronellol	Nol			59		6.40		2	
α-Terpineol	Mol		5			<i>6.96</i>	-17		
Anethole	Aet				128	<i>4.9^c</i>			40 ^b
Carvone	Mco			38		5.30		13	
Citral ^d	Nco			61		5.27		20	
Menthol	Mol		5			<i>5.92</i>	-6		
Piperitone	Mco			53		4.96			45
4-Terpineol	Mol		-3			<i>5.26</i>	-17		
Camphor	Bco	-19				4.01			51
Pulegone	Mco			59		3.83			32
Estragole	Aet				92	<i>3.0^c</i>			31
Linalol	Nol			46		<i>2.26</i>		7	
Menthone	Mco			13		2.80		11	
Thujone ^d	Bco	-17				2.69		6	
Citronellal	Nco			33		2.26		9	
Fenchone	Bco	-7				2.02			28
Cineole	Bet	-31				<i>1.43^c</i>	-3		
γ-Terpinene	Mhc		8			<i>1.19^c</i>	-4		
ρ-Cymene	Ahc			28		<i>0.95^c</i>		9	
Limonene	Mhc		-6			<i>1.10^c</i>	-10		
Camphene	Bhc	-40				<i>0.90^c</i>	-9		
3-Carene ^d	Bhc	-21				<i>1.02^c</i>	-11		
α-Terpinene	Mhc		6			<i>0.88^c</i>	-1		
α-Pinene	Bhc	-33				<i>0.66^c</i>	-3		
Myrcene ^d	Nhc			22		<i>0.58^c</i>		16	

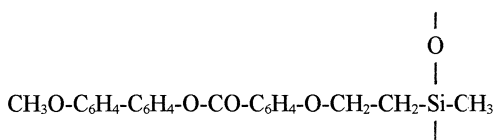
^a Chemical type of solute. A = Aromatic; B = bicyclic monoterpene; M = monocyclic; N = acyclic; co = carbonyl; et = ether; hc = hydrocarbon; ol = alcohol.

^b Previously unpublished relative retention times on Chiraldex G-PN. Other values used are from Ref. [16]; cinnamal 10.75; safrole 7.68; anethole 6.88.

^c Newly determined values. Most aromatics give variable results, and are only averaged to one decimal.

^d Impure solutes. Main peak used.

methylsiloxane ("MPMS") having the following repeating structure:



This liquid crystal side chain is typically multi-aromatic linked in mid-point by an ester bonding, with ethers on either side, including a terminal methoxy group. Thus an affinity for aromatic solutes can be anticipated. At temperatures above or below the "MPMS" melting point (about 145°C, according to the manufacturer, and confirmed by a slight

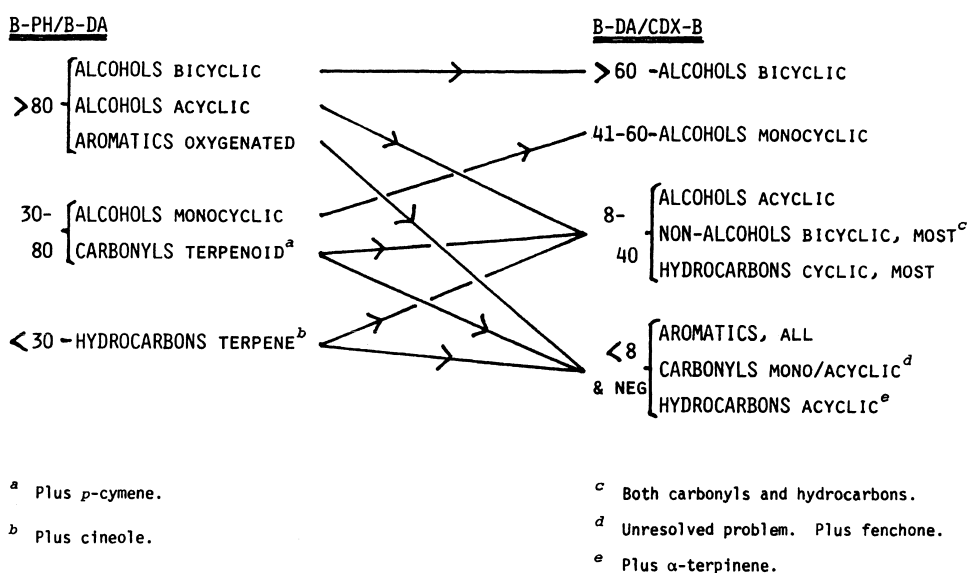


Fig. 3. Solute groupings found for various percentage increases in relative retention times (*n*-undecane=1.00) on changing from Cyclodex-B (CDX-B) or other modified β -CD phases (B-PH or B-DA). These can aid in the identification of unknown peaks from volatile oils which yield ambiguous data by other means, e.g., GC-MS.

angularity at this temperature in the otherwise linear plots of relative retentions to linalol vs. oven temperature [25]), some distinctive solute retention ratios involving aromatics can be calculated, which are both less than unity on conventional phases. These are 1.2 for the allyl, methoxy-benzene estragole/geraniol (acyclic monoterpenoid alcohol) ratio; and a larger 1.5 for the propenyl, methoxy-benzene anethole/safrole (allyl, methylene-dioxy-benzene) relationship. The latter retention ratio ane/saf >1 was realised to be typical of liquid crystals [25], and is not shown by conventional phases, or by modified CDs. The strong affinity of “MPMS” for anethole was confirmed by ane/thymol (a phenolic-cymene noted for strong retention) being 1.2. This suggested that it would be valuable in the analysis of sweet fennel oil for the best separation and quantitative evaluation of the trace amount (0.3%) of the toxic *cis*-anethole in the presence of the main constituent, about 67% *trans*-anethole [4]. The α -value for this separation is 1.41, as opposed to 1.13 on Carbowax 20M, and only 1.07 on methyl polysiloxane. Other aromatics preceding and following the anetholes, estragole and (decomposition product

of anethole) anisal, were also resolved and quantified well, in a shorter time than on the conventional phases. If the relative retentions at 160°C [26] for nine of the 10 selected [12] diverse volatile oil solutes are plotted against their boiling points there is an amazingly low *r* of 0.691 for Eq. (1), suggesting “MPMS” has great extra response to some of these solutes. It also allowed more rapid evaluation of the aromatics 2.1% safrole and 6.1% myristicin in mace oil, although it did not resolve traces of eugenol from methyl-eugenol [4]. The practical results, however, were not not completely satisfying, due to the temperature restrictions of this liquid crystal phase.

As could be anticipated, this “MPMS” was useless for resolving monoterpen hydrocarbons. This task had been achieved on another diaromatic-diether liquid crystal in a packed column, of 3% azoxy-diphenetole (ADP) on silica, used below its melting point of 138°C [27]. Some hydrocarbons could be differentiated using the retention ratio of *S/U*(ADP 125°C), where *U* is the retention of a solute on the “unmelted” non-nematic phase heated just to 125°C, and *S* is the subsequent result on the same column slowly supercooled from above 138°C

Table 5

Changes in relative retention times (*n*-undecane=1.00) as percentages at 150°C of values at 125°C on some modified CD phases (from Ref. [20])

Solute	Chemistry ^a	A-DA	A-PH
Cineole	sBet	116	113
Fenchone	sBco	111	109
Menthone ^b	sMco	107	98 ↓
Thujone ^b	sBco	110	100
Camphor	sBco	110	103
Citronellal	1Nco	99	91
Pulegone	1Mco	103	90
Estragole	1Aet	97	89
4-Terpineol	1Mol	101	88
Carvone	2Mco	100	89
Linalol	2Nol	99	89
Piperitone	1Mco	97	89
Cuminal	sAco	91	88
Safrole	1Aet	91	84
Anethole	1Aet	92	83
α-Terpineol	1Mol	92	85
Citral ^b	2Nco	85 ↓	85
Menthol	sMol	89	89
Cinnamal	1Aco	88	80
Borneol	sBol	93	88 ↑
Citronellol	1Nol	84	76
Geraniol	2Nol	79	75
Mean		97	90

100 means no change in value. Results in italics represent greatest decreases at the foot of the table and least decreases or increases at the top of the table (regions separated by stepped lines). Arrows show solutes “out” of region.

^aA=Aromatic; B=bicyclic; M=monocyclic; N=acyclic; co=carbonyl; et=ether; ol=alcohol; s=saturated; 1=monoene; 2=diene. Monoterpenoids if not aromatics.

^bMain peak of impure solute used.

to the same temperature. Table 6 reports that aromatic *p*-cymene responded to this aromatic mesogenic phase with an *S/U* ratio of 3.0, more than the values of about 2.6 for α- and γ-terpinenes and for limonene. The lattermost is hard to resolve on conventional phases from the low-polarity terpenoid ether cineole, but this had a distinctly different ratio of only 1.5 here. Other monoterpenes had *S/U* ratios about 1.9. Another liquid crystal phase in Table 6, (MBT)₂ explained below, was obviously less satisfactory for applying this retention ratio.

For packed columns, some have claimed [28] that “in the surface film of (liquid crystal) on graphite (carbon), about ten times more molecules are present

Table 6

Relative retention times (linalol=1.00) on columns of 3% melted, slowly supercooled liquid crystals, and ratios to the unmelted values (from Ref. [27])

Solute ^a	ADP at 125°C		(MBT) ₂ at 150°C	
	Time	Ratio	Time	Ratio
<i>p</i> -Cymene	0.36	×3.0	0.53	×1.2
γ-Terpinene	0.35	×2.5	0.53	×1.2
Limonene	0.32	×2.7	0.49	×1.2
α-Terpinene	0.29	×2.6	0.43	×1.1
<i>Terpinolene</i>	0.29	×1.9	0.42	×0.9
<i>Cineole</i>	0.28	×1.5	0.41	×0.9
α-Phellandrene	0.25	×1.9	0.41	×1.0
α-Pinene	0.12	×2.0	0.23	×1.0

^aSolutes named in italics are in a different sequence to the unmelted phase.

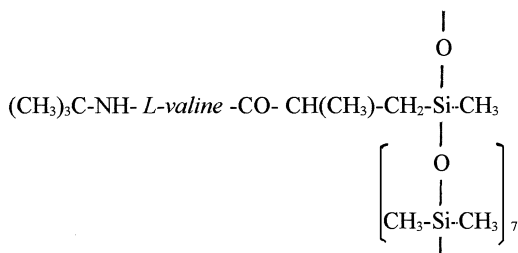
than on silica”. A 3% column packing of bis-(methoxy, benzilidene-bitoluidine) or (MBT)₂, appeared to have lowered polarity on carbon support [29], as the retention ratio of the low-polarity sesquiterpene hydrocarbon caryophyllene/anethole was increased from 0.4 (using silica) to almost 1.0. ane/saf was above 1.3 on both supports, as expected. At about 202°C, the ratios of *t*_{R,rel} (vs. linalol) on silica support compared to carbon SiO/C(MBT)₂ (202°C), were 1.87–1.91 for four well-retained aromatics including ethers, an aldehyde and a phenol, although more-quickly emerging estragole did not fit. Well below this narrow range were a terpenoid alcohol and a hydrocarbon. Programmed up from 110 to 160°C, the carbon-supported (MBT)₂ gave a good chromatogram for terpenoid tea tree oil, with its 34.5% of 4-terpineol nicely resolved from only 3.3% α-terpineol; and also for aromatic sweet fennel oil, which when programmed from 203 to 230°C, gave results similar to those with the “MPMS” capillary [4] but in less than 8 min instead of 15 min.

5. Other novel stationary phases and retention ratios

“Chirasil-Val” (ChV) is a different chirally selective phase invented by Frank et al. in 1977 to resolve enantiomeric amino acid esters [30]. It contains an

optical isomer of valine bound centrally by peptide linkages into periodic side-chains of a methyl polysiloxane.

Its repeating formula is:



ChV was quickly applied to other substances such as sympathomimetics [31], but not to volatile oil constituents for 15 more years [32]. Then, a retention ratio could be calculated involving a CD phase, α -DA/ChV at 140°C for $t_{R,rel}$ linalol. This was 1.36–0.62 for three aromatics and only 0.39–0.22 for three

monoterpenoid alcohols, see Table 7. Three carbonyl-monoterpenoids gave just +0.08 to –0.10, but so did three terpene hydrocarbons. Plotting the boiling points of nine solutes in Table 7 against their retention indices on ChV yields an Eq. (1) with $r=0.948$, distinctly lower than the correlation coefficients for conventional phases. This indicated that ChV gave promise for helping to determine unknowns from volatile oils and possibly other sources. This phase gave good results with Patchouli oil, indicating its value for sesquiterpenes [32]. It resolved contaminants from patchoulol to show that only 28.5% was present at 168°C; and separated well the about 10% α -guainene from 7% seychellene, and also the less than 2% β -patchoulene from other early-emerging sesquiterpenes at 130°C.

Although not commercially available in capillaries, packed columns of crown (cyclic) ethers are another toroid molecular phase (like CDs) which can help to indicate solute molecular structure by selec-

Table 7

Retention indices and relative retention times (linalol=1.0) of various solutes on Chirasil-Val and other phase at given temperatures (from Ref. [32])

Solute		Retention indices (vs. <i>n</i> -alkanes)		Relative retention times			
		Chirasil-Val		Chirasil-Val		inc ^a →	Chiraldex-A-DA
		110°C	140°C	110°C	140°C–		
Caryophyllene	BH ^b		1494		3.56	0.05	3.61
Anethole	AE		1412		2.36	1.36	3.72 ^c
Cuminal	AC		1399		2.19	1.03	3.22
Borneol	BL		1381		1.99	0.39	2.38
α -Terpineol	ML		1375		1.94	0.24	2.18
Isoborneol	BL		1351		1.73		
4-Terpineol	ML	1328	1339	1.65	1.61	0.22	1.83
Estragole	AE	1291	1313	1.35	1.42	0.62	2.04
Camphor	BC	1271	1300	1.17	1.33	–0.07	1.26
Citronellal	NC	1238	1255	0.98	1.06	0.08	1.14
Linalol	NL	1246	1243	1.00	1.00		1.00
Fenchone	BC	1196	1219	0.74	0.89	–0.10	0.79
γ -Terpinene	MH	1110	1120	0.45	0.54	–0.01 ^d	0.44 ^d
Cineole	BE	1096		0.41			0.44
ρ -Cymene	AH	1088		0.39			0.38
Limonene	MH	1078		0.36			0.39

^a Difference in values for a solute between adjacent columns, with increase (inc) direction shown by arrow.

^b Information about the chemical nature of the solutes. A=Aromatic; B=bicyclic; C=ketone/aldehyde; E=ether; H=hydrocarbon; L=alcohol; M=monocyclic; N=acyclic.

^c Numerical values out of descending sequence are italicised.

^d These, and values below them in these columns at 110°C.

tive extra retentions. The “24-crown-8s” have molecular rings of eight “(poly)ethylene glycol” units ($-\text{CH}_2-\text{CH}_2-\text{O}-$), so they consist of 24 toroid carbon-carbon-oxygen atoms, but are devoid of the terminal polar hydroxyl groups of conventional polyethylene glycol phases. Two six-carbon rings (either dibenzo- [bzCr] or dicyclohexano- [cxCr]) are usually locked on opposite sides of the toroid molecule to confer stability, but different properties. The ratio bzCr/cxCr using $t_{R,rel}$ linalol at 155°C calculates as 1.60–1.31 for four non-phenolic aromatic solutes, indicating their extra affinity for the benzene components of the phase [33]. It is 0.97–0.78 for four terpene cyclic hydrocarbons, indicating that they favour the cyclohexane components. Monoterpenoids fall between these ranges.

The same paper [33] allows a comparison between crown ether and mesomeric cholesteryl acetate (ClAc) stationary phases at 155°C. This temperature is far above the transition point (116°C) of the steroid ester from a liquid crystal to a normal isotropic liquid, yet on it $\text{ane/saf}=1.1$, the “test” [25] for the mesogenic condition! Using the retention ratio ClAc/cxCr for $t_{R,rel}$ linalol, the four terpene hydrocarbons now give 1.64–1.50, indicating the even stronger affinity they have for the steroid phase; while the non-phenolic aromatics yield 1.16–0.96.

In one of several attempts to determine the relative polarities of various phases by empirical methods, Betts devised his c ratio, 0.75 of the relative retention of cuminal/caryophyllene [34] or 3cum/4cary. This was a return to the original use of two-solute “retention ratios”, to characterise a stationary phase [7] rather than a solute. Now, ClAc is confirmed to be of lower polarity than cxCr by c ratio. Melted (MBT)₂ on silica at 175°C has a value of 1.44, “more polar” than Carbowax 20M, which is 1.39 at 160°C. The bzCr value at 155°C is less, 1.33; while melted “MPMS” at 160°C is less again with 1.19. The “low-polarity” phases are “ChiralDEX A-DA” with a c ratio of 0.67 at 140°; cxCr with 0.65 at 155°C; ChV with 0.46 at 140°C; and the lowest, 0.40 for ClAc at 155°C; which can be compared to 0.30 for methyl polysiloxane at 160°C. These ratios will alter a bit with changes in temperature, and phases with values of about 1.20–0.80 are considered to be of “intermediate polarity”.

6. Conclusions

Some of the various retention ratios above can be useful aids to identifying unknown constituents of volatile oils. Aromatics can be detected on conventional phases by P/N , and with the ratio pairs of CD trios γ -PN and α -PH/ β -DA and β -PH/ β -DA/ β -CDX, and also α -DA/ChV. The second CD trio is also good for cyclic monoterpenoid alcohols. Bicyclic monoterpenoids are strongly apparent using γ -DA/ α -DA, and so are monocyclics. Acyclics are best deduced from β -DA/ α -DA. Saturated cyclic substances other than alcohols respond to 150°C/125°C(α -DA). P/N is useful for hydrocarbon sesquiterpenes.

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