Evaluation of series-coupled gas chromatographic capillaries of different polarities

Application to the resolution of problem pairs of constituents in Algerian cypress essential oil

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ABSTRACT

The inability of capillary columns used in GC, GC–MS and GC–Fourier transform IR spectrometry to provide complete resolution of all components of complex mixtures such as essential oils has often been reported. By connecting fused-silica capillary columns of different polarity in series and by changing the temperature programme gradient or the column sequence, a more efficient separation system is obtained. Retention indices of some previously unresolved constituent pairs were calculated and an application to the analysis of cypress essential oil is reported.

INTRODUCTION

Algerian cypress (*Cupressus sempervirens* Linnaeus = *Cupressus fastigiata* De Condolle) essential oil [1] offers some difficulties for the capillary gas chromatographic (GC) separation of pairs of constituents such as limonene–1,8-cineole and 1,8-cineole–β-phellandrene (OV-101 capillary column, GC–MS on FFAP capillary column). To solve this problem, we coupled two capillary columns in series. The analytical use of serially coupled GC columns (packed or capillary) has been the subject of considerable study [2–12]). The advantages of this technique [10] over other techniques such as parallel and mixed-phase capillary columns are the use of just one injector and one detector and the possibility of changing the polarity of the linked columns. How-

ever, it must be noted that this method is efficient only if the difference in polarity of the two columns is high [13].

EXPERIMENTAL

Instruments

The gas chromatograph used was a Hewlett-Packard Model 5730A with a flame ionization detector and an HP 3390 integrator. Two WCOT fused-silica columns with grafted phases were used, nominally 25 m × 0.22 mm I.D., manufactured by Chrompack (Middelburg, Netherlands): a low-polarity (NON) CP-Sil 5 CB (dimethylsiloxane, equivalent to OV-101, film thickness 0.11 μm) and a high-polarity (POL) CP-Wax 57 CB (polyethylene glycol, equivalent to Carbowax 20M, film thickness 0.21 μm). They were linked by a Swagelok 1/16, Ref. 2053 (Supelco, Gland, Switzerland).

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Chromatographic conditions

The columns had different optimum flow-rates as determined by the Van Deemter curve when connected in different orders: CP-wax 57 CB–CP-sil 5 CB (POL → NON) 0.36 ml/min and CP-Sil 5 CB–CP-Wax 57 CB (NON → POL) 0.30 ml/min. The carrier gas was helium. The column temperature programme was 80°C for 8 min increased at 1°C/min to 230°C and held at 230°C for 16 min. The injector and detector temperatures were 250°C. The volume of oil injected was 0.5 μl and the volume of pure sample injected was 0.2 μl. The splitting ratio was 1:20.

Standard compounds were obtained from Fluka (Buchs, Switzerland). Essential oil of cypress was extracted from fresh leaves and twigs by hydrodistillation in the laboratory in March 1989 (d_{20}^{20} = 0.8795, n_D^{20} = 1.4719, α_D^{20} = 5.6°). The oil was analysed by fused-silica capillary GC either alone (25-m OV-101 and 25-m FFAP columns) or coupled with mass spectrometry (GC–MS) (50-m FFAP column) and with Fourier transform IR spectrometry (GC–FT-IR) (25-m column BP 21). Ninety-five compounds have been identified by these techniques [1].

RESULTS AND DISCUSSION

The characteristics of the individual columns and of the columns connected in the two different orders are reported in Table I. The number of theoretical plates of the serially linked columns is about 65% of the sum of the values for the single columns.

The values of $k'_{C_{10}}$, $\alpha_{C_{11}/C_{10}}$ and TZ on series-coupled columns are between those given by the individual columns. The plot of the capacity factor $k'_{C_{10}}$ *versus* column fraction is shown in Fig. 1. The
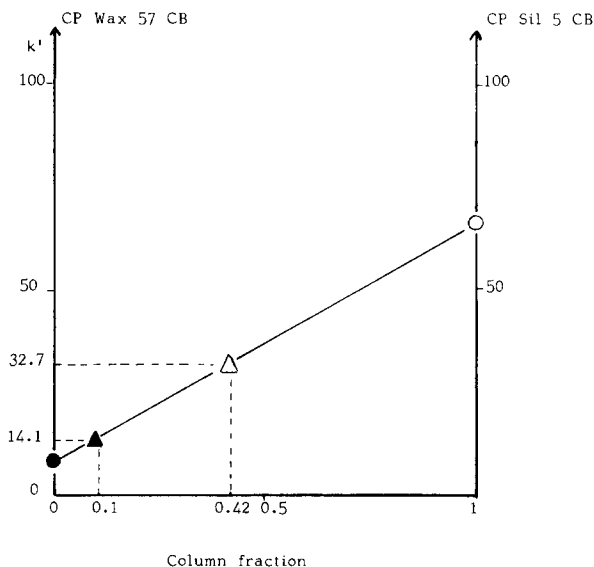

Fig. 1. k' Values *versus* column fraction. $\bigcirc$ = CP-Sil 5 CB; $\bullet$ = CP-Wax 57 CB; $\triangle$ = CP-Sil 5 CB–CP-Wax 57 CB; $\blacktriangle$ = CP-Wax 57 CB–CP-Sil 5 CB.

contribution of each column to the coupled system is not equal to 0.5 as one would have expected. The contribution of the CP-Sil 5 CB column, for example, is 0.42 for order 1 (NON → POL) and 0.10 for order 2 (POL → NON).

Moreover, the Van den Dool retention indices I were calculated for temperature programming [14–16] of standards that are not well separated either on OV-101 (25 m) [1] or Carbowax 20M (25 m) columns [17] or by GC–MS (FFAP, 50 m) [1]. We also calculated the increments ΔI relative to OV-101 and to Carbowax 20M. The results are reported in Table II.

All the retention indices are situated between

TABLE I

CHARACTERISTICS OF COLUMNS (C_{10} at 100°C)

NON→POL = CP-Sil 5 CB–CP-Wax 57 CB; POL→NON = CP-Wax 57 CB–CP-Sil 5 CB.

Column	N	$k'_{C_{10}}$	$\alpha_{C_{11}/C_{10}}$	U (cm/s)	TZ
CP-Wax 57 CB	24 118	8.3	1.1	55	1.60
CP-Sil 5 CB	30 945	65.7	1.4	362	11.37
NON→POL	37 398	32.7	1.3	100	7.08
POL→NON	36 260	14.1	1.3	44	8.17

TABLE II

RETENTION INDICES (I) AND THEIR INCREMENTS (ΔI)

$\Delta I_a = I_{NON \to POL} - I_{OV-101}$; $\Delta I_b = I_{NON \to POL} - I_{CBWax\ 20M}$; $\Delta I_c = I_{POL \to NON} - I_{OV-101}$; $\Delta I_d = I_{POL \to NON} - I_{CBWax\ 20M}$.

Compound	I_{OV-101}	$I_{CBWax\ 20M}$ [17]	$I_{NON \to POL}$	$I_{POL \to NON}$	ΔI_a	ΔI_b [17]	ΔI_c	ΔI_d [17]
Myrcene[a]	982	1156	1006	1128	24	−150	146	−28
Octanal	991	1278	1051	−	60	−227	−	−
p-Cymene[a]	1010	1272	1064	1153	54	−208	143	−119
Limonene[a]	1019	1206	1048	1123	29	−158	104	−83
1,8-Cineole[a]	1020	1228	1061	1137	41	−167	117	−91
β-Phellandrene[a]	1013	1216	1068	1152	55	−148	139	−64
α-Phellandrene[a]	994	1177	1015	1166	21	−162	172	−11
Terpin-4-en-1-ol[a]	1160	1628	1260	1651	100	−368	491	+23
Borneol[a]	1147	1698	1311	1758	164	−387	611	+60
Isoborneol	1137	1660	1280	1705	143	−380	568	+45
α-Terpineol	1185	1661	1332	1552	147	−329	367	−109
Bornyl acetate[a]	1283	1580	1395	1558	112	−185	275	−22
Thymol[a]	1277	2100	1661	−	384	−439	−	−
α-Cedrene[a]	1409	1600	1440	1550	31	−160	141	−50

[a] Constituents of Algerian cypress essential oil.

those given by the individual columns except for borneol, isoborneol and the terpin-4-en-1-ol. For limonene as an example, I_{OV-101} = 1030 < $I_{NON \to POL}$ = 1048, $I_{POL \to NON}$ = 1123 < $I_{CBWax\ 20M}$ = 1206.

These results depend also on the column sequence and advantage can be taken of coupling columns with large polarity differences in series. Indeed, the most retained compounds in one column are the least retained in the other column. Consequently, there are no great changes in the total analysis times when using serially linked columns [10].

The observed values of the retention indices for both coupling sequences 1 and 2 are close to the values given by the first column of the coupled system, e.g. for p-cymene, $I_{NON \to POL}$ = 1064, I_{OV-101} = 1020, $I_{CBWax\ 20M}$ = 1272, $I_{POL \to NON}$ = 1153, ΔI_a = ($I_{NON \to POL}$ − I_{OV-101}) = 54. ΔI_b = ($I_{NON \to POL}$ − $I_{CBWax\ 20M}$) = −208, ΔI_c = ($I_{POL \to NON}$ − I_{OV-101}) = 143, ΔI_d = ($I_{POL \to NON}$ − $I_{CBWax\ 20M}$) = −119. In other words, the first column of the couple imposes its polarity on the coupled system, as has been confirmed by Cartoni et al. [13]. Thus, the polarity of the system increases in the direct OV-101, NON →POL, POL→NON and Carbowax 20M.

In Table III are reported the ΔI values for some previously poorly separated pairs and their resolu-

tions, $R_{A/B}$, for both orders of column coupling. The sequence POL→NON (Table III) includes some of the problem pairs, e.g., p-cymene–β-phellandrene (ΔI = 1, $R_{A/B}$ = 0.3) and α-cedrene–α-terpineol (ΔI = 2, $R_{A/B}$ = 0.4). For this reason, we chose the order NON→POL for the analysis of cypress essential oil.

Application of serial coupling of capillary columns to the analysis of Algerian cypress essential oil

Essential oils are a typical example of very complex mixtures containing many constituents of different nature, polarities and relative amounts. To optimize the resolution for the cypress oil, we used serial coupling of two columns. First, different temperature programmes have been used, as reported in Table IV. Programme A gave the largest number of peaks with both coupling sequences and was therefore adopted.

Fig. 2 shows the chromatogram obtained with the column order NON→POL. A better separation was achieved for the previously poorly resolved pairs: limonene (No. 16)–1,8-cineole (No. 17) (OV-101, 25 m), 1,8-cineole (No. 17)–β-phellandrene (No. 18) (OV-101, 25 m; GC–MS, FFAP, 50 m). However, the use of the serially coupled capillary system to provide optimized separations can

TABLE III

RESOLUTION ($R_{A/B}$) OF PAIRS OF COMPOUNDS AND THEIR RETENTION INCREMENTS (ΔI)

NON→POL = CP-Sil 5 CB–CP-Wax 57 CB; POL→NON = CP-Wax 57 CB–CP-Sil 5 CB; ΔI: $I_A - I_B$.

Compound pairs A/B	ΔI_{OV-101}	$\Delta I_{CBWax\ 20M}$ [17]	$\Delta I_{NON \to POL}$	$\Delta I_{POL \to NON}$	$R\ (A/B)_{NON \to POL}$	$R\ (A/B)_{POL \to NON}$
Limonene[a]/1,8-cineole[a]	1	22	13	14	2.8	5.2
1,8-Cineole[a]/β-phellandrene[a]	7	12	7	15	2.9	7.7
Myrcene[a]/octanal	9	122	45	–	72.7	–
Octanal/p-cymene[a]	19	6	13	–	11.1	–
β-Caryophyllene[a]/β-cedrene[a,b]	11	9	8	16	4.9	6.5
Terpenyl acetate[a]/verbenone[a,b]	252	43	88	–	80.3	–
Isoborneol/α-terpineol	48	1	52	153	6	40
Bornel[a]/α-terpineol [18][b,c]	38	37	21	206	3.9	33.9
p-Cymene[a]/β-phellandrene[a]	3	56	4	1	3.2	0.3
α-Cederene[a]/α-terpineol	224	61	108	2	42.9	0.4

[a] Constituents of Algerian cypress essential oil.
[b] Bad resolution by GC–MS (50-m FFAP column).
[c] Ref. 18: GC–MS (50-m FFAP column).

TABLE IV

DIFFERENT TEMPERATURE PROGRAMMES

NON→POL = CP-Sil 5 CB–CP-Wax 57 CB; POL→NON = CP-Wax 57 CB–CP-Sil 5 CB. Explanation: *e.g.*, 80°(8)1°/min 230°(16) means initial temperature 80°C held for 8 min, then increased at 1°C/min to 230°C, the final temperature being maintained for 16 min.

Programme	NON→POL	No. of peaks	POL→NON	No. of peaks
A	80°(8)1°/min230°(16)	107	80°(4)1°/min230°(8)	99
B	80°(0)1°/min230°(16)	94	80°(0)1°/min230°(8)	96
C	80°(4)2°/min230°(16)	74	40°(0)4°/min100°(0)1°/min230°(8)	94
D	60°(4)2°/min230°(16)	79	70°(0)2°/min230°(32)	92
E	60°(8)2°/min230°(16)	76	60°(0)2°/min230°(16)	96

include some new problem pairs, *e.g.*, peaks Nos. 68/69, 89/90 (Fig. 2).

CONCLUSIONS

The proposed method allows some interesting possibilities for the separation of complex mixtures where the various temperature programmes, the order of column coupling and the polarities of the two columns are chosen according to the constitution of each sample mixture.

This technique can be used as a complementary method, in either qualitative or quantitative analysis of essential oils where the titration of an impor-

tant constituent can be easily made, *e.g.*, 1,8-cineole in eucalyptus oil.

Finally, it is generally preferable to use serial coupling of capillary columns with different polarities than to use only one column with the same length as the total length of the coupled columns but with only one stationary phase, *e.g.*, a 50-m FFAP column in GC–MS [1]. However, in certain circumstances, a single capillary GC column provides a more convenient solution than serially linked capillary columns. For example, limonene, *β*-phellandrene and 1,8-cineole are completely separable on a column of intermediate polarity, such as RSL 300.
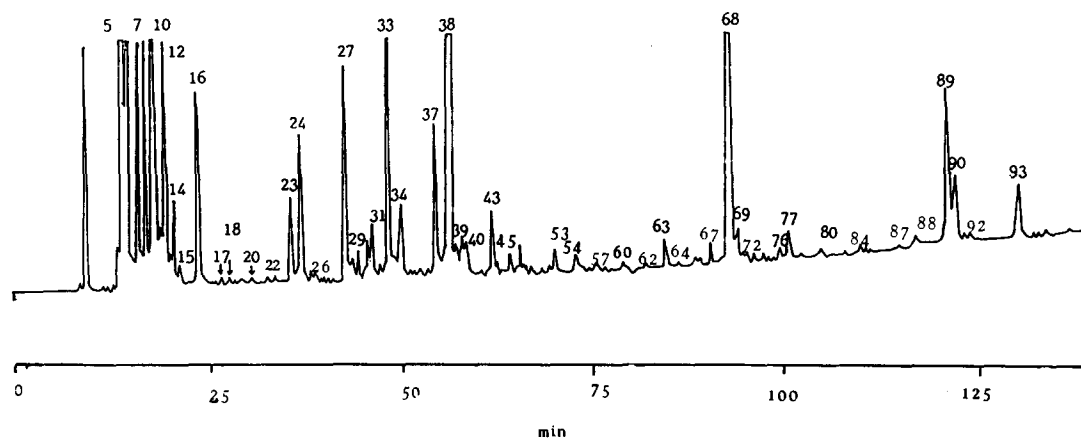

Fig. 2. Chromatogram of Algerian cypress essential oil obtained with the CP-Sil 5 CB–CP-Wax 57 CB (NON→POL) coupled column system. 5 = α-Pinene; 10 = Δ³-carene; 12 = myrcene; 16 = limonene; 17 = 1,8-cineole; 18 = β-phellandrene; 38 = terpenyl acetate; 39 = β-caryophyllene; 40 = β-cedrene; 68 = cedrol; 89 = sandaracopimaradiene; 93 = dehydroabietane.

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