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Polarity changes during capillary gas chromatographic and gas chromatographic–mass spectrometric analysis using serially coupled columns of different natures and temperature programming Application to the identification of constituents of essential oils

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Abstract

The polarity and characteristics of systems obtained by connecting two capillary gas chromatography columns of different polarities in series using temperature programming has been determined both in gas chromatography and gas chromatography–mass spectrometry analysis. The influence of the relative length of the two columns and of the column sequence (more polar in front, less polar at the end or the reverse) has been studied. In order to test this method on real world samples, the behavior of different configurations towards a synthetic mixture of nineteen constituents found in essential oils, thirteen of which are not separated on a single column, has been tested. On the best configurations (polar column in front followed by the non-polar one), all of the compounds of the test mixture gave well separated chromatographic peaks. The technique of using capillary gas chromatography columns of decreasing polarity, coupled in series, works efficiently using classical temperature programming from room temperature to the limits of the column used. Therefore, this method is ideally suited for the separation and identification of multicomponent complex mixtures in gas chromatography (or gas chromatography–mass spectrometry).

Keywords: Essential oils; Capillary columns; Coupled columns; Terpenes

1. Introduction

In the last few years, the complete resolution of complex mixtures of organic or inorganic compounds from the environmental field and originating in the cosmetic industry has been a challenge, due to the wide ranges of molecular masses and boiling points of their constituents. In order to obtain the best separation of all of their constituents, the use of coupled column chromatography in series is often required for: (i) the analysis of antiknock additives in gasoline [1]; (ii) the analysis of halogenated pollutants [2–5]; (iii) the analysis of essential oils [6–8]; (iv) the separation of enantiomeric compounds using a chiral column [9–11].

On the other hand, studies have been devoted to establishing the theory of using coupled columns, to optimizing the parameters (temperature, plate height equivalent, pressure, time, etc.) and to predicting them [2,3,12–17]. In addition to the latter, many authors have evaluated systems obtained by coupling two capillary columns in series with, in many if not

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most cases, the same characteristics (length, internal diameter and film thickness) [1,6,8]. The aim of this work was to establish the possibility of using a combination of polar and non-polar columns with different characteristics and then to evaluate the polarity of serial systems obtained in gas chromatography (GC) and GC–mass spectrometry (MS). Constituents of essential oil (terpenic compounds: mono- and sesquiterpenic ones) that were not resolved either on a single polar or non-polar column were used as test mixtures.

2. Experimental

This investigation was carried out using GC and GC–MS. The gas chromatograph was a Hewlett-Packard Model 5890 (Palo Alto, CA, USA) fitted with a flame ionization detector and was used in conjunction with a Hewlett-Packard 3390A integrator. Hydrogen was the carrier gas. The temperature of both the injector and the detector was held at 250°C. Fused-silica capillary columns from Chrompack (Chrompack, Middelburg, Netherlands) and SGE (Victoria, Australia) were used (Table 1). They were coupled with a borosilicate connector (Supelco, Bellefonte, PA, USA).

The GC–MS instrument used was a Trio 1000 (Fisons, Manchester, UK) spectrometer (positive mode; electron impact ionization, 70 eV; scanning from 50 to 400 u in 1 s, with an interscan rate of 0.2 s) coupled with a GC 8000 (Carlo-Erba, Milan, Italy) gas chromatograph. Helium was used as the carrier gas and the temperature source was 150°C.

The chemicals, nineteen compound hydrocarbons and their derivatives, were purchased from Fluka (Buchs, Switzerland). They were injected dissolved in pentane (ratio, 1:1000, v/v, 2 μl and 1:1000, v/v, 0.5 μl for GC–MS and GC, respectively), using the splitless mode. Of the nineteen compounds, there were five pairs and one triplet that were not resolved on a single column. Table 2 regroups them. In addition to these, the melange test contained myrtenol, α-pinene, caryophyllene oxide, fenchone, octanal and linalyl acetate. We added these to the former group of compounds in order to constitute an artificial mixture that was close to the constitution of essential oils.

Table 2
Terpenic compound pairs

Pairs of compounds	Polar phase	Non-polar phase
Limonene	1206	1030
1,8-Cineole	1228	1027
Menthone	1478	1143
Isopulegol	1573	1145
Citronellyl acetate	1645	1335
Terpinyl acetate	1687	1333
Menthyl acetate	–	1333
α-Humulene	1672	1437
α-Cedrene	1600	1436
α-Copaene	1519	1398
Longifolene	1574	1398
Isoborneol	1660	1157
α-Terpineol	1661	1185

The optimum conditions for GC and GC–MS were determined on the basis of the van Deemter graph and the results are given in Table 3. As the analysis must be carried out using GC and GC–MS, we used temperature programming to shorten the total analysis, without sacrificing the resolving power. Therefore, the retention indices were calculated using *n*-alkanes and are given by:

$$I = \frac{t_{RS} - t_{RZ}}{t_{R(Z+1)} - t_{RZ}} \cdot 100 + 100Z$$

where t_{RS}, t_{RZ} and $t_{R(Z+1)}$ are the retention times for

Table 1
Capillary column specifications

Nature of phase	CP SiL 5 CB Dimethylpolysiloxane			CP SiL 8 CB 5% Phenyldimethyl–polysilicone	BP 20 Poly(ethylene glycol)	CP Wax 57 CB Poly(ethylene glycol)
Length (m)	30	50	60	25	50	25
Internal diameter (mm)	0.25	0.25	0.32	0.23	0.32	0.22
Film thickness (μm)	0.25	0.25	1.0	0.12	0.5	0.21
$\Sigma^5 \Delta I$ (McReynolds)		217		323	2188	2424

Table 3
Description of the column coupling configurations

Configuration	P_{min} (p.s.i.)[a]	Column 1	Length (m)	Column 2	Length (m)
1	8	CP Sil 8 CB	25	CP Wax 57 CB	25
2	7	CP Wax 57 CB	25	CP Sil 8 CB	25
3	9	CP Sil 5 CB	50	CP Wax 57 CB	25
4	10	CP Wax 57 CB	25	CP Sil 5 CB	50
5	11	CP Sil 5 CB	60	BP 20	50
6	9	BP 20	50	CP Sil 5 CB	60
7	13	CP Sil 5 CB	30	BP 20	50
8	12	BP 20	50	CP Sil 5 CB	30

[a] 1 p.s.i. = 6894.76 Pa.

the solute and for *n*-alkanes bearing Z and $(Z+1)$ carbon atoms, respectively. The polarity values were obtained using the McReynolds constant, was determined by injecting samples of *n*-alkanes and the first five McReynolds probes at 120°C [5–18].

3. Results and discussion

The specifications of the capillary columns used in different combinations and the McReynolds polarity, $\Sigma^5 \Delta I$ (obtained as the sum of the McReynolds constants, ΔI, of the first five probes, the difference between the retention values of the probes on each phase and those on squalane) are given in Table 1. It will be shown that the difference between the $\Sigma^5 \Delta I$ values of non-polar phases (CP Sil 5 CB and CP Sil 8 CB) is weak, therefore, those two phases would be considered to be similar. Table 4 shows the retention indices obtained by the coupling of various configurations of different columns either in GC or in

Table 4
Retention index values obtained on different configurations

Configuration	GC				GC–MS			
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Limonene	1048	1123	1017	1040	1050	1105	1079	1194
1,8-Cineole	1061	1137	1018	1047	1059	1110	1082	1212
Menthone	1216	1286	1146	1208	1187	1305	1263	1464
Isopulegol	1222	1327	1154	1229	1187	1359	1309	1555
Borneol	1280	1705	1180	1284	1228	1448	1391	1674
α-Terpineol	1332	1552	1203	1279	1237	1438	1404	1664
Terpinyl acetate	1437	1587	1284	1322	1311	1422	1380	1581
Citronellyl acetate	1391	1445	1341	1376	1362	1464	1437	1631
Menthyl acetate	NC	NC	1292	1323	1316	1407	1371	1550
α-Humulene	1512	1547	1476	1504	1525	1618	1542	1688
α-Cedrene	1483	1491	1444	1466	1489	1559	1489	1600
α-Copaene	1421	1443	1396	1413	1429	1458	1428	1513
Longifolene	1467	1487	1432	1458	1487	1553	1485	1603
Linalyl acetate	1295	1343	1247	1278	1264	1356	1342	1525
Octanal	1139	1270	1064	1146	1082	1283	1248	1506
Fenchone	1139	1220	1094	1138	1126	1239	1207	1399
Caryophyllene oxide	1638	1781	1591	1677	1681	1768	1744	1991
α-Pinene	939	989	935	947	963	967	971	1031
Myrtenol	1301	1467	1215	1336	1263	1449	1460	1766
$\Sigma \Delta I$ (McReynolds)	767	894	339	385	497	767	1441	1841

NC, not carried out.

GC–MS. This table also regrouped the McReynolds polarity, $\Sigma^5 \Delta I$, calculated for each arrangement.

The most important observation reported here is that, for all of the compounds, the retention indices increased in going from non-polar–polar (NP–P, configurations 1, 3, 5 and 7) to polar–non-polar (P–NP, configurations 2, 4, 6 and 8). Since *n*-alkanes (non-polar compounds) are not retained in the sequence (P–NP), this order (P–NP) is more polar than the reverse one (NP–P). It is obvious that the solutes move in the first column at a low temperature, which increases as the programme runs, thus, its retention time is longer than in the second column (where it enters near the end of the programmed run, i.e. at high temperature) [4,6,8]. In other words, the first column imposes its polarity on the coupled system. The evolution of McReynolds polarity, $\Sigma^5 \Delta I$ is 767 in order **1** (NP–P) and 894 in order **2** (P–NP), confirms that observation. From Table 4, the highest increment values of ΔI (difference between the retention indices obtained on the two configurations of the same combination) are found in the case of oxygenated compounds. This is probably due to their higher polarities. When both the length and film thickness of one of the two coupled columns increase, this imposes its polarity to the coupled column, either in GC or in GC–MS, without taking account of its position either in front or at the end of the coupled system. Sequences **1** [CP Sil 8 CB–CP Wax 57 CB (25–25)] and **6** [BP 20–CP Sil 5CB (50–60)], having the same $\Sigma^5 \Delta I$ value, should have the same polarity and, therefore, the same behavior. Nevertheless, the retention indices obtained for the two orders are not equal. This result seems obvious as the film thickness of the first column, which imposes its polarity, differs in these two cases. However, configurations **5** and **6** give the weakest separation of the components due to the overall column length. By increasing the film thickness, the retention time becomes greater and the elution temperature higher. This demonstrates that the polarity of the column increases during the run [19,20]. In the same sequence, NP–P (or P–NP), the polarity increases from **3** to **7** (and from **4** to **8**). For mono- and sesquiterpenic hydrocarbons (α-pinene, limonene and α-copaene, longifolene, α-cedrene and α-humulene), the elution order is maintained. However, because of the polarity of oxygenated derivatives, it is not possible to propose an elution order, and a correlation between the molecular mass or structure and the order.

Finally, analysis on the serially coupled columns using temperature programming either by GC or GC–MS enhances the resolution of constituents of essential oils, since a number of unresolved compounds are now separated. Using a single column, the test mixture gives six unresolved peaks. Configuration **5** separates only three of the six couples, and configuration **1** separates only five. On the other hand, configurations **2** and **8** successfully resolve the five doublets and the triplet. Fig. 1 shows the chromatograms of a mixture of terpinyl acetate and citronellyl acetate, obtained using serially coupled columns according to configuration **7** (columns, CP Sil 5 CB–BP 20; length, 30 m, 50 m). These two terpenes, which are not separated in an ordinary column, are eluted at 22.46 and 24.33 min, respectively, corresponding to an increment index, ΔI, of 57. The results obtained for a more difficult case, a mixture of limonene, 1,8-cineole, α-terpineol and borneol, which appeared as two doublets on a classical column, are shown in Fig. 2. Using configuration **8** (columns, BP 20–CP Sil 5 CB; length, 50 m, 30 m), both doublets are cleanly separated without overlap.

In other words, for combinations with different characteristics, the best resolution is achieved when

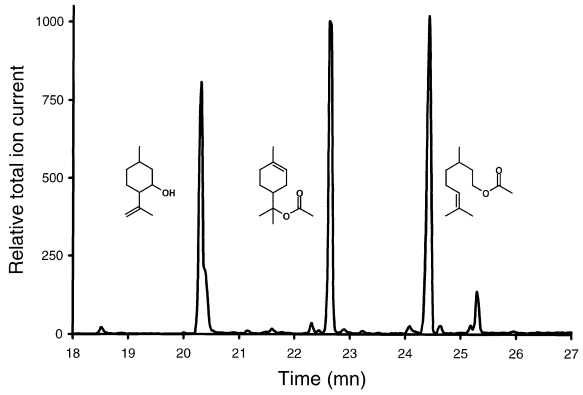

Fig. 1. Separation of a mixture containing terpinyl acetate (t_R = 22.46 min), citronellyl acetate (t_R = 24.33 min) and isopulegol (t_R = 20.7 min) using serially coupled columns in configuration **7** (columns, CP Sil 5 CB–BP 20; length, 30 m, 50 m); temperature programming, 100 to 230°C at 4°C/min.

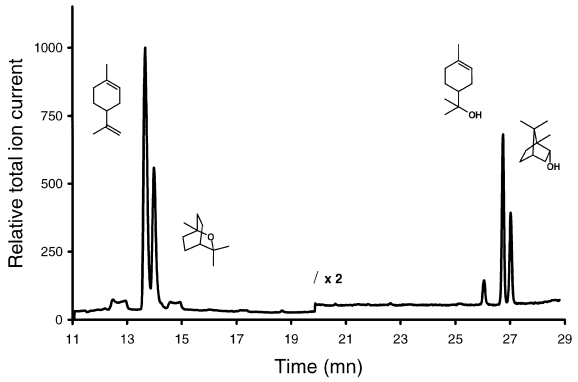

Fig. 2. Separation of a mixture containing limonene ($t_R = 13.63$ min), 1,8-cineole ($t_R = 13.94$ min), α-terpineol ($t_R = 26.73$ min) and borneol ($t_R = 27.00$ min) on serially coupled columns using configuration **8** (columns, BP 20–CP Sil 5 CB; length, 50 m, 30 m); temperature programming, 100 to 230°C at 4°C/min.

the column with the greater film thickness and length is at the front.

4. Conclusion

In order to fully resolve a sample containing mixtures of compounds in essential oils, the use of a system of coupled polar and non-polar capillary columns in series is required, because the properties of the compounds, i.e. boiling points, polarity and molecular masses of the individual constituents of the mixture, are very close. This requires the use of temperature programming. This investigation examined the applicability of combinations of two columns with different characteristics both in GC and GC–MS. The results concerning the resolution of constituents of essential oils on serial systems with dissimilar characteristics lead to the following conclusions: (i) this technique can be used to obtain an improved separation of complex mixtures of compounds that are not separated on simple polar or non-polar column; (ii) the polarity of the serial system is intermediate between those of single columns. On the other hand, the global polarity depends on the contribution made by the physical parameters (length, internal diameter and film thickness) of each column in the series. Bearing in mind that the length of the coupled system should be kept within a reasonable range, the best separation of constituents of essential oils is obtained, for dissimilar columns, when the column with the greater length and film thickness is placed in front. The possibility of modulating polarity provides an additional reason for using serial column coupling in conjunction with retention indices in temperature-programming GC.

References

[1] G. Castello, Chim. Ind. 51 (1969) 700.
[2] T.C. Gerbino, G. Castello, J. High Resolut. Chromatogr. 15 (1992) 428–433.
[3] T.C. Gerbino, G. Castello, J. High Resolut. Chromatogr. 16 (1993) 46–51.
[4] T.C. Gerbino, G. Castello, J. High Resolut. Chromatogr. 17 (1994) 597–602.
[5] G. Catello, G. D'Amato, S. Vezzani, J. Chromatogr. 646 (1993) 361–368.
[6] G.P. Cartoni, G. Goretti, M.V. Russo, Chromatographia 23 (1987) 790–795.
[7] G.P. Cartoni, G. Goretti, B. Monticelli, M.V. Russo, J. Chromatogr. 370 (1986) 93–101.
[8] N. Chanegriha, A. Baaliouamer, J. Chromatogr. A 663 (1993) 163–168.
[9] A. Walhagen, L.E. Edholm, Chromatographia 32 (1991) 215–223.
[10] A.M. Rizzi, J. Chromatogr. 513 (1990) 195–207.
[11] J. Krupcik, M. Grena, I. Spanik, E. Benicka, J. Hrouzek, I. Skacani, P. Sandra, J. Chromatogr. A 779 (1997) 253–262.
[12] E.B. Bakeas, P.A. Siskos, Anal. Chem. 68 (1996) 4468–4473.
[13] G. Guiochon, J.E.N. Gutierrez, J. Chromatogr. 406 (1987) 3–10.
[14] J.R. Jones, H. Purnell, Anal. Chem. 62 (1991) 2300–2306.
[15] R.G. Williams, H.D. Mitchell, J. Chromatogr. 541 (1991) 59–74.
[16] T. Maurer, W. Engewald, A. Steinborn, J. Chromatogr. 517 (1990) 77–86.
[17] W. Engewald, T. Maurer, J. Chromatogr. 520 (1990) 3–13.
[18] W.O. McReynolds, J. Chromatogr. Sci. 8 (1970) 685–691.
[19] K. Grob, G. Grob, Chromatographia 17 (1983) 481–485.
[20] V.G. Berezkin, A.A. Korolev, J. High Resolut. Chromatogr. 12 (1989) 617–619.