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Short communication

Selectivity effects in semi-polar columns. II

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Abstract

Significant selectivity differences between semi-polar capillary gas chromatographic columns involving the elution order of acyclic and polycyclic lipid molecules have been observed. These differences occur despite these columns being reported to be "equivalent" in catalogue specifications. In Part I, the changes in retention indices between columns manufactured by different companies were described. In this paper, it is shown that these variations in selectivity may even be observed among semi-polar columns originating from the same manufacturer.

1. Introduction

In Part I [1], we showed that the use of different capillary columns in the trace organic analysis of environmental samples may give rise to significant changes in the relative retentions of several major compounds. These selectivity changes essentially concerned the elution order of linear vs. polycyclic molecules such as squalene and benzopyrenes and alkan-1-ols and sterols, and were observed among semi-polar stationary phase columns (5% phenyl–95% methyl type) which are reported to be equivalent in commercial catalogues. The study involved a comparison of semi-polar columns from J & W Scientific (DB-5), Carlo Erba (SE-52 and SE-54), Chrompack (CP-Sil 8 CB) and Hewlett-Packard (HP-5).

In the context of the chromatographic work on environmental problems regularly performed in our Department, we have found that these previously described differences [1] may even involve columns manufactured by the same company. In this paper we present one of these cases, which extends the previous description of selectivity differences between semi-polar columns.

2. Experimental

2.1. Materials

Chromatography quality *n*-hexane, methanol, dichloromethane, isooctane and neutral silica gel (Kieselgel 40, 70–230 mesh) were obtained from Merck (Darmstadt, Germany). The silica gel was extracted with dichloromethane–methanol (2:1, v/v) in a Soxhlet apparatus for 24 h. After solvent evaporation, the silica was heated for 12 h at 120°C. A total of 5% (w/w) of water obtained with a Milli-Q system (Millipore) was then added to the chromatographic adsorbents for deactivation. The purity of the solvents was checked by concentrating under vacuum 100 ml of solvent to 10 μl for GC analysis. Blank

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requirements were as follows: splitless injection of 2 μl should result in chromatograms with no unresolved GC envelope and only a few peaks, representing up to 1 ng in terms of their flame ionization detector response.

2.2. Sampling, extraction and fractionation

Samples were obtained by sediment coring in high-altitude lakes. The cores were divided into sections at the sampling site and frozen at −20°C until analysis in the laboratory. Lipids were extracted by sonication after freeze-drying. About 1 g of sediment was extracted with dichloromethane–methanol (2:1, v/v) (3 × 20 ml; 20 min), the extract was vacuum and nitrogen evaporated almost to dryness and diluted to 0.5 ml with *n*-hexane, then fractionated by column chromatography according to previously established methods [2]. A column filled with 2 g of 5% water-deactivated silica was used. The hydrocarbon and alcohol fractions were obtained by successive elution with 8 ml *n*-hexane–dichloromethane (1:1) and 12 ml of dichloromethane–methanol (18:2), respectively. These fractions were vacuum and nitrogen concentrated almost to dryness and silylated with isooctane–bis(trimethylsilyl)trifluoroacetamide (1:1) (60 min, 70°C).

2.3. Instrumental analysis

The samples were analysed by GC and GC–MS. These analyses were performed with a Carlo Erba HRGC Mega 2 Series gas chromatograph

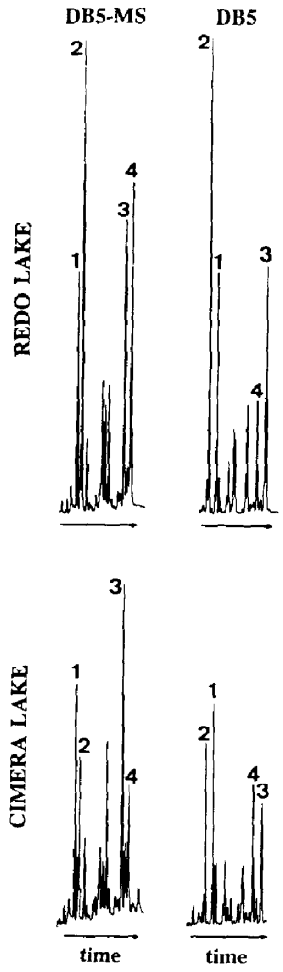

Fig. 1. GC profiles showing the elution order of the TMS ether derivatives of alkan-1-ols and Δ^5-sterols with the DB-5 and DB5-MS semi-polar columns. Peaks: 1 = cholest-5-en-3β-ol; 2 = octacosan-1-ol; 3 = β-sitosterol; 4 = triacontan-1-ol.

Table 1
Semi-polar capillary columns compared in this study

Column	Stationary phase[a]	Dimensions	Film thickness (μm)	Manufacturer
DB-5	5% phenyl–95% methyl	30 m × 0.25 mm I.D.	0.2	J & W Scientific (Folsom, CA, USA)
DB5-MS	5% phenyl–95% methyl	30 m × 0.25 mm I.D.	0.2	J & W Scientific

[a] As defined by the manufacturer.

equipped with a flame ionization detector and with a Carlo Erba GC8000 Series gas chromatograph coupled to a Fisons MD 800 mass spectrometer, respectively. The GC analyses were performed with the capillary columns described in Table 1. The oven temperature was programmed from 90 to 300°C at 6°C/min, and the injector and detector temperatures were set at 280 and 330°C, respectively. Hydrogen was used as the carrier gas at a flow-rate of 50 cm/s. The oven temperature programme for the GC–MS analyses was from 90 to 300°C at 4°C/min, the injector and transfer line temperatures were 280 and 300°C, respectively, and helium was used as the carrier gas at a flow-rate of 50 cm/s. Data were acquired in the electron impact mode (70 eV), scanning from 50 to 550 mass units at 1 s per decade. In both instances the injector was in the splitless mode (1 μl; hot needle technique), the split valve being closed for 35 s.

3. Results and discussion

C_{20}–C_{30} alkan-1-ols constitute the dominant compounds in the polar fractions of the sediments considered in this study. In addition to these, sterols are also major compounds, encompassing a mixture of C_{27}–C_{29} homologues dominated by cholest-5-en-3β-ol and β-sitosterol. The gas chromatograms corresponding to the analysis of the alcohol–sterol mixtures with the two columns indicated in Table 1 are shown in Fig. 1. These chromatograms illustrate that important changes in relative retention are observed when using the different columns. Thus, with the DB-5 column, the trimethylsilyl (TMS) ethers of octacosan-1-ol and triacontan-1-ol elute before than the TMS ethers of cholest-5-en-3β-ol and β-sitosterol, respectively, whereas with the DB-5 column the elution order is the reverse.

Repeated analyses with several columns and comparison of the resulting relative retention indices (I) of the sterols (Table 2) showed that the discrepancy is maintained despite using columns from different manufacturing batches. The I dispersion is low (relative standard deviation 0.17–0.26%) in comparison with the DB-5–DB5-MS I discrepancies (1.4–1.8%), which results in very significant mean I differences between the two column types (confidence level in Student's t-test $\gg 0.9995$).

These selectivity effects parallel the elution order changes observed in our previous work [1] and show that this new J & W Scientific column, DB5-MS, behaves similarly to CP-Sil 8 CB, SE-54 and HP-5, with which the elution of the linear compounds follows that of the polycyclic molecules occurring at close retention times (Table 3). In particular, the retention index of β-sitosterol with this column is coincident with that with CP-Sil 8 CB.

The difference in selectivity is independent of the specific functionality of the analyte mole-

Table 2
Relative retention indices (I) of alkan-1-ols and sterols with the DB-5 and DB5-MS columns

Column	I			
	Octacosan-1-ol	Cholest-5-en-3β-ol	Triacontan-1-ol	β-Sitosterol
DB-5	2800.0	2831.1	3000.0	3037.7
	2800.0	2825.6	3000.0	3035.2
	2800.0	2827.9	3000.0	3035.8
	2800.0	2819.7	3000.0	3020.3
DB5-MS	2800.0	2781.6	3000.0	2973.2
	2800.0	2791.7	3000.0	2983.5
	2800.0	2787.5	3000.0	2979.2
	2800.0	2781.4	3000.0	2974.0

I values relative to alkan-1-ols.

Table 3
Average relative retention indices (I) of alkan-1-ols and sterols with the semi-polar columns listed in Table 1 and those considered in a previous study [1]

Column	I			
	Octacosan-1-ol	Cholest-5-en-3β-ol	Triacontan-1-ol	β-Sitosterol
DB-5	2800.0	2826.1	3000.0	3032.2
SE-52	2800.0	2803.6	3000.0	2998.2
CP-Sil 8 CB	2800.0	2776.9	3000.0	2974.1
SE-54	2800.0	2782.5	3000.0	2985.3
HP-5	2800.0	2774.4	3000.0	2969.1
DB5-MS	2800.0	2785.6	3000.0	2977.5

I values relative to alkan-1-ols.

cules. Thus, the comparison of the elution order of C_{29}–C_{32} n-alkanes and several triterpenes and triterpanes [taraxer-14-ene, olean-12-ene, hop-17(21)-ene, glutinane, heterolupene, diploptene and (22R)-17α(H),21β(H)-homophopane] in the two columns shows the same displacement towards higher I for the polycyclic molecules eluting in the DB-5 column (Fig. 2 and Table 4). With these hydrocarbons, the general displacement effect gives rise to elution order inversions between hop-17(21)-ene and n-hentriacontane and between heterolupene and n-dotriacontane.

The same type of I differences between the DB-5 and the CP-Sil 8 CB, SE-54 and HP-5 columns was observed in our previous study [1] when comparing the elution time of squalene and benzopyrenes. Hence the selectivity effect

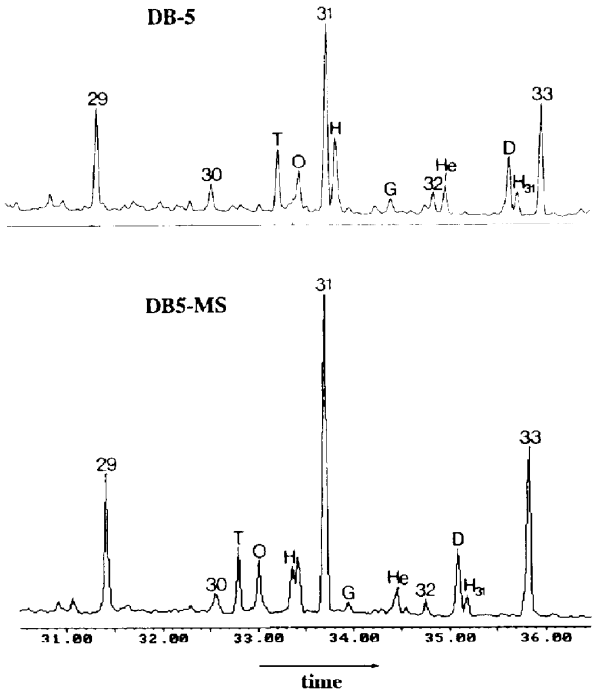

Fig. 2. GC profiles showing the elution order of n-alkanes and triterpanes with the DB-5 and DB5-MS semipolar columns. Numbers on peaks refer to n-alkane chain length. T = taraxer-14-ene; O = olean-12-ene; H = hop-17(21)-ene; G = glutinane; He = heterolupene; D = diploptene; H_{31} = (22R)-17α(H),21β(H)-homohopane. Time scale in min.

Table 4
Relative retention indices (I) of linear and polycyclic hydrocarbons with the DB-5 and DB5-MS columns

Compound	I	
	DB-5	DB5-MS
n-Triacontane	3000.0	3000.0
Taraxer-14-ene	3059.0	3020.7
Olean-12-ene	3077.0	3040.7
n-Hentriacontane	3100.0	3100.0
Hop-17(21)-ene	3109.8	3070.0[a]
Glutinane	3161.0	3124.1
n-Dotriacontane	3200.0	3200.0
Heterolupene	3210.8	3170.4[a]
Diploptene	3271.4	3231.2
(22R)-17α(H),21β(H)-Homohopane	3278.4	3240.4
n-Tritriacontane	3300.0	3300.0

I values relative to n-alkanes.
[a] Compounds showing reversal of elution order with respect to the n-alkane with the nearest retention.

which differentiates the DB-5 column essentially concerns the elution time of cyclic vs. polycyclic molecules.

4. Conclusions

The important changes in relative retention on analysis of organic compounds with different semi-polar columns described in Part I [1] are also observed even with columns produced by the same manufacturer. Catalogue specifications do not allow differentiation between semi-polar columns corresponding to one or another group. Hence, the use of a standard mixture containing linear and polycyclic molecules with similar retention times is recommended as a guideline for environmental applications where both types of molecules are easily encountered. This is particularly relevant when comparing GC traces obtained with different detectors, such as flame ionization and mass spectrometric types. Important peak assignment errors may be produced from the straightforward comparison of "equivalent" semi-polar capillary columns such as those described here.

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