Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1068 (2005) 307–314
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

# Chemometric studies of retention in capillary gas chromatographic separation of hydrocarbons in coupled columns

P. Májek [a], T. Hevesi [a], J. Krupcik [a,*], J.R. Chrétien [b], D.W. Armstrong [c,*]

[a] *Department of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, SK-81237 Bratislava, Slovak Republic*
[b] *Laboratory of Chemometrics and BioInformatics, University of Orléans, 45067 Orléans Cedex 2, France*
[c] *Department of Chemistry, Gilman Hall, lowa State University, Ames, IA 50011-3111, USA*

Received 7 October 2004; received in revised form 19 January 2005; accepted 25 January 2005

**Abstract**

This paper describes how different multivariate analysis and classification methods can be used, to characterize the gas chromatographic separation of complex hydrocarbon mixtures in three columns coupled in series. Principal component analysis (PCA), correspondence factor analysis (CFA), and hierarchical ascending classification (HAC) were used as potential tools for evaluating the experiments on single columns and on column series. It has been demonstrated that: (1) multivariate analysis with PCA and CFA offers a powerful strategy to search for the main factors influencing the separation of hydrocarbons without a priori knowledge of the key factors of the separation. (2) With CFA the contribution of retention due to vapour pressure can be minimized. The use of retention indices, which use the *n*-alkanes as reference compounds, also helps to decrease the dominant focus on vapour pressure in favor of the more selectivity-based interaction forces. (3) CFA helps to analyze the degree of relevance of the chosen experimental design to the most important factors, controlling chromatographic selectivity.


*Keywords:* Chemometrics; Data evaluation; Multivariate techniques; Capillary gas chromatography; Three columns coupled in series; Multicomponent hydrocarbon separation

## 1. Introduction

The main task for the separation of complex mixtures by capillary gas chromatography (CGC) is to find the optimum separation system selectivity. The selectivity of a gas chromatographic separation can be modified by temperature, the stationary phase polarity or by a combination of both [1]. Mixed stationary phases have been proposed in order to develop optimized stationary phase selectivity, initially. However, the final selectivity of mixed stationary phases often do not result from a linear combination of the pure stationary phases due to their mutual physicochemical interactions [2]. This explains why the use of serially coupled columns has been envisaged more successfully [3]. The selectivity of a column series at isothermal conditions can in general be tuned by variation of the lengths of the coupled columns or by control of the carrier gas flow rates in individual columns. Several papers were published dealing with the theory and practice of gas-chromatographic analysis on two-column systems [4–14]. The use of more than two columns may, however, enlarge the experimental dimensions in which the selectivity may be tuned. A theory and its experimental verification have been published for the separation of a complex mixture of hydrocarbons on a gas chromatographic system consisting of three different capillary columns [15].

In gas chromatography, retention is a phenomenon that depends dominantly on solute-stationary phase interactions. For the successful treatment of retention data for a complex mixture, various chemometric techniques can be used

* Corresponding author. Tel.: +421 7 532 5314; fax: +421 7 393 198.
*E-mail address:* jan.krupcik@stuba.sk (D.W. Armstrong).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.01.091

[16,17]. These methods allow the simultaneous evaluation a relatively large amount of data, greatly facilitating the clarification of both practical and theoretical problems. These chemometric procedures have already been extensively employed in chromatography for: (1) identification of the basic factors influencing retention and separation, (2) comparison of various stationary and mobile phases, (3) assessment of the relationship between molecular structure and retention behavior (quantitative structure–retention relationship, QSRR) and (4) elucidation of correlations between retention behavior and biological activity [18–21].

As each chemometric procedure generally highlights only one, or only a few features of the chromatographic problem under analysis, the concurrent application of more than one technique is more the rule than the exception [18,21].

The aim of the present paper is to show how different multivariate analysis and classification methods can be used, to characterize the gas chromatographic separation of complex mixtures of hydrocarbons in three columns coupled in series. Principal component analysis (PCA), correspondence factor analysis (CFA), and hierarchical ascending classification (HAC) were used as potential tools for evaluating the experiments on single columns and on column series.

## 2. Experimental

Three columns with different polarities were used:

A. SE 30, 30 m × 0.32 mm × 0.25 μm (from Machery-Nagel, Germany);
B. SE 54, 25 m × 0.25 mm × 0.25 μm (from RIC, Belgium);
C. Nucol (bonded polyethyleneglycol, SUPELCO, Bellefonte, USA), 15 m × 0.25 mm × 0.25 μm (from Supelco, USA).

The columns were coupled in series by press-fit connectors. The HP 5890 A (Hewlett-Packard, Avondale, USA) gas chromatograph with split injector and FID was used for all measurements.

The inlet carrier gas pressure was measured by an additional U-manometer with an accuracy of 100 Pa. An aneroid manometer was used to measure the outlet pressure with an accuracy of 10 Pa.

The chromatograms were evaluated by HP 3365 ChemStation software (Hewlett-Packard, Avondale, USA).

Hydrogen was used as a carrier gas. The oven temperature was 60 °C.

The characterization of the sample constituents and their corresponding retention times on the three single columns A, B and C and four different column series ABC, CBA, BCA and ACB is listed in Table 1.

The retention factors and retention indices have chiefly been processed for calculations. Retention factors ($k_i$) of each hydrocarbon ($i$) were calculated from corresponding retention times ($t_R$) listed in Table 1 using the equation:

$$k_i = \frac{t_{R,i} - t_M}{t_M}$$

where $t_M$ is a corresponding retention time of methane.

Retention indices ($I_i$) of each hydrocarbon ($i$) were calculated from corresponding retention times ($t_R$) listed in Table 1 using the equation:

$$I_i = 100z + 100\frac{\ln(t'_{R,i}/t'_{R,z})}{\ln(t'_{R,z}/t'_{R,z+1})}$$

where $t'_R$ is adjusted retention time $\{t'_R = t_R - t_M\}$ and $z$ denotes number of carbon atoms in a $n$-alkane elutes before considered hydrocarbon ($i$). The retention times of the $n$-alkanes and other hydrocarbons should increase in the order: $t_{R,z+1} > t_{R,i} > t_{R,z}$.

### 2.1. Chemometrics methods

For the calculations of PCA, CFA and HAC the program Statistica 4.3 for Windows was used [22].

## 3. Results and discussion

### 3.1. Principal component analysis (PCA) and correspondence factor analysis (CFA)

The hydrocarbons in the used model mixture exhibit only slight differences in chromatographic behaviour both on the individual chromatographic columns as well as the column series. This is why a multivariate analysis was used to detect these small differences.

The retention factors ($k_i$) and retention indices ($I_i$) on the column series are expected to be a linear combination of the retention data on single columns [2,3]. As the retention indices for the column series have no direct physical interpretation, we will focus our attention only on the retention indices for the three uncoupled columns. Therefore the calculations were performed using two data matrices. The first matrix containing retention factors was of size 51 × 7, which includes all 51 sample constituents on seven different columns (A, B and C) or column series (ABC, ACB, BCA and CBA). The second matrix containing retention indices was of size 51 × 3 (all 51 sample constituents on three different columns A, B and C. Both for the retention factors and the retention indices PCA and CFA were performed.

The PCA map of retention factors is shown in Fig. 1, which corresponds to the first factorial plan defined by the two main factorial axes. Only these two main factorial axes are significant. This implies that only two interaction mechanisms influence the separation. The variation along the first principal component is related the variation of vapour pressure of the compounds, it takes into account 97.3% of the variance, i.e. of the information content. The second main

Table 1
Retention times of model sample components obtained at 60 °C on individual columns A, B and C and the column series ABC, CBA, BAC and ACB

| No. | Compound name | $t_{R,A}$ | $t_{R,B}$ | $t_{R,C}$ | $t_{R,ABC}$ | $t_{R,CBA}$ | $t_{R,BCA}$ | $t_{R,ACB}$ |
|---|---|---|---|---|---|---|---|---|
| 0 | Methane | 0.797 | 1.867 | 0.856 | 2.635 | 2.061 | 2.067 | 2.640 |
| 1 | *n*-Hexane | 0.992 | 2.401 | 0.920 | 3.255 | 2.500 | 2.548 | 3.180 |
| 2 | Benzene | 1.103 | 2.798 | 1.776 | 3.953 | 3.324 | 3.258 | 4.027 |
| 3 | Cyclohexane | 1.126 | 2.815 | 1.048 | 3.738 | 2.882 | 2.942 | 3.643 |
| 4 | 2,2,4-Trimethylpentane | 1.214 | 3.007 | 0.950 | 3.953 | 2.987 | 3.079 | 3.814 |
| 5 | *n*-Heptane | 1.245 | 3.104 | 0.940 | 4.059 | 3.074 | 3.170 | 3.920 |
| 6 | 2,2-Dimethylhexane | 1.337 | 3.350 | 0.940 | 4.338 | 3.263 | 3.381 | 4.172 |
| 7 | 2,5-Dimethylhexane | 1.384 | 3.472 | 0.940 | 4.478 | 3.324 | 3.488 | 4.300 |
| 8 | 2,4-Dimethylhexane | 1.395 | 3.507 | 0.940 | 4.518 | 3.360 | 3.520 | 4.338 |
| 9 | 2,2,3-Trimetylpentane | 1.395 | 3.540 | 0.940 | 4.553 | 3.391 | 3.554 | 4.374 |
| 10 | 2,3,4-Trimetylpentane | 1.482 | 3.776 | 1.001 | 4.821 | 3.619 | 3.763 | 4.622 |
| 11 | 2,3,3-Trimetylpentane | 1.511 | 3.869 | 1.001 | 4.924 | 3.701 | 3.848 | 4.720 |
| 12 | Toluene | 1.511 | 4.051 | 2.673 | 5.595 | 4.802 | 4.671 | 5.729 |
| 13 | 2,3-Dimethylhexane | 1.539 | 3.927 | 1.001 | 4.992 | 3.735 | 3.893 | 4.776 |
| 14 | 2-Methyl-3-ethylpentane | 1.550 | 3.959 | 1.030 | 5.031 | 3.767 | 3.926 | 4.814 |
| 15 | 2-Methylheptane | 1.565 | 4.008 | 1.072 | 5.082 | 3.790 | 3.959 | 4.856 |
| 16 | 4-Methylheptane | 1.580 | 4.051 | 1.073 | 5.119 | 3.821 | 3.992 | 4.892 |
| 17 | 3,4-Dimethylhexane | 1.595 | 4.098 | 1.075 | 5.186 | 3.879 | 4.048 | 4.958 |
| 18 | 3-Methyl-3-ethylpentane | 1.602 | 4.135 | 1.077 | 5.242 | 3.914 | 4.088 | 5.006 |
| 19 | 3-Methylheptane | 1.620 | 4.140 | 1.080 | 5.242 | 3.920 | 4.088 | 5.006 |
| 20 | 3-Ethylhexane | 1.630 | 4.150 | 1.142 | 5.277 | 3.940 | 4.114 | 5.038 |
| 21 | *n*-Octane | 1.814 | 4.704 | 1.142 | 5.867 | 4.353 | 4.572 | 5.578 |
| 22 | 2,2-Dimethylheptane | 1.994 | 5.178 | 1.142 | 6.407 | 4.713 | 4.976 | 6.065 |
| 23 | 2,2,3-Trimetylhexane | 2.040 | 5.342 | 1.197 | 6.584 | 4.857 | 5.125 | 6.235 |
| 24 | Ethylcyclohexane | 2.110 | 5.678 | 1.466 | 6.965 | 5.220 | 5.471 | 6.637 |
| 25 | 1,1,3-Trimethylcyclohexane | 2.166 | 5.781 | 1.378 | 7.074 | 5.240 | 5.552 | 6.673 |
| 26 | 2,4-Dimethyl-3-ethylpentane | 2.180 | 5.781 | 1.259 | 7.074 | 5.273 | 5.510 | 6.720 |
| 27 | 2,2,3,3-Tetramethylpentane | 2.305 | 6.159 | 1.338 | 7.485 | 5.552 | 5.859 | 7.086 |
| 28 | Ethylbenzene | 2.305 | 6.544 | 4.114 | 8.700 | 7.504 | 7.335 | 8.883 |
| 29 | 3,3,4-Trimethylhexane | 2.332 | 6.218 | 1.378 | 7.567 | 5.577 | 5.905 | 7.150 |
| 30 | 2,3,3,4-Tetramethylpentane | 2.380 | 6.389 | 1.378 | 7.751 | 5.734 | 6.062 | 7.329 |
| 31 | *m*-Xylene | 2.413 | 6.859 | 4.299 | 9.157 | 7.870 | 7.729 | 9.308 |
| 32 | *p*-Xylene | 2.413 | 6.859 | 4.455 | 9.157 | 7.951 | 7.737 | 9.383 |
| 33 | 3,3-Diethylpentane | 2.671 | 7.211 | 1.439 | 8.700 | 6.392 | 6.790 | 8.179 |
| 34 | *o*-Xylene | 2.723 | 7.880 | 5.285 | 10.649 | 9.463 | 9.117 | 11.032 |
| 35 | 1,1,2-Trimethylcyclohexane | 2.723 | 7.491 | 1.689 | 8.987 | 6.699 | 7.075 | 8.503 |
| 36 | *n*-Nonane | 3.093 | 8.332 | 1.439 | 9.934 | 7.221 | 7.737 | 9.308 |
| 37 | Cumene | 3.250 | 9.609 | 5.690 | 12.299 | 10.437 | 10.337 | 12.400 |
| 38 | 3,3,5-Trimethylheptane | 3.300 | 8.831 | 1.439 | 10.491 | 7.609 | 8.150 | 9.811 |
| 39 | Butylcyclopentane | 3.709 | 10.337 | 2.031 | 12.156 | 8.950 | 9.540 | 11.411 |
| 40 | 2,6-Dimethyloctane | 3.841 | 10.399 | 1.466 | 12.299 | 8.822 | 9.510 | 11.411 |
| 41 | *n*-Propylbenzene | 3.927 | 11.614 | 6.464 | 14.820 | 12.620 | 12.478 | 14.947 |
| 42 | 1,3,5-Trimethylbenzene | 4.334 | 12.872 | 7.977 | 16.674 | 14.493 | 14.182 | 17.014 |
| 43 | 1,2,4-Trimethylbenzene | 5.051 | 15.202 | 9.975 | 19.787 | 17.411 | 16.943 | 20.319 |
| 44 | *tert*-Butylcyclohexane | 5.051 | 14.431 | 2.527 | 16.674 | 12.256 | 13.115 | 15.579 |
| 45 | *iso*-Butylbenzene | 5.599 | 16.782 | 7.718 | 20.668 | 17.108 | 17.193 | 20.472 |
| 46 | *n*-Decane | 5.953 | 16.516 | 1.952 | 19.039 | 13.618 | 14.764 | 17.606 |
| 47 | *sec*-Butylcyclohexane | 6.611 | 19.090 | 3.232 | 21.829 | 16.010 | 17.193 | 20.472 |
| 48 | Butylcyclohexane | 7.018 | 20.216 | 3.079 | 23.055 | 17.108 | 18.103 | 21.428 |
| 49 | 1,4-Diethylbenzene | 7.681 | 23.648 | 11.793 | 29.079 | 24.503 | 24.472 | 29.021 |
| 50 | 1,2-Diethylbenzene | 7.911 | 24.549 | 13.415 | 30.514 | 26.137 | 25.887 | 30.718 |
| 51 | *n*-Undecane | 12.315 | 34.925 | 3.075 | 39.345 | 27.841 | 30.452 | 36.054 |

factorial axis takes into account 2.7% of the information content; it corresponds to the ability of the sample constituents to interact with the stationary phase by induction forces. This is not surprising, as the volatility and polarity are well known as the main factors influencing gas chromatographic separations.

As the volatility does not depend on the stationary phase type the selectivity in our particular case can be tuned only by varying the polarity of the column series. For this, two chromatographic columns are enough, the least polar (A) and most polar one (C). The three-column series should be meaningful if the third column exhibited some another interaction mechanism with the sample constituents (for example steric effects, $\pi$–$\pi$ interactions, hydrogen bonding, etc.). Such cases should be indicated by the significance of the third or higher principal components.

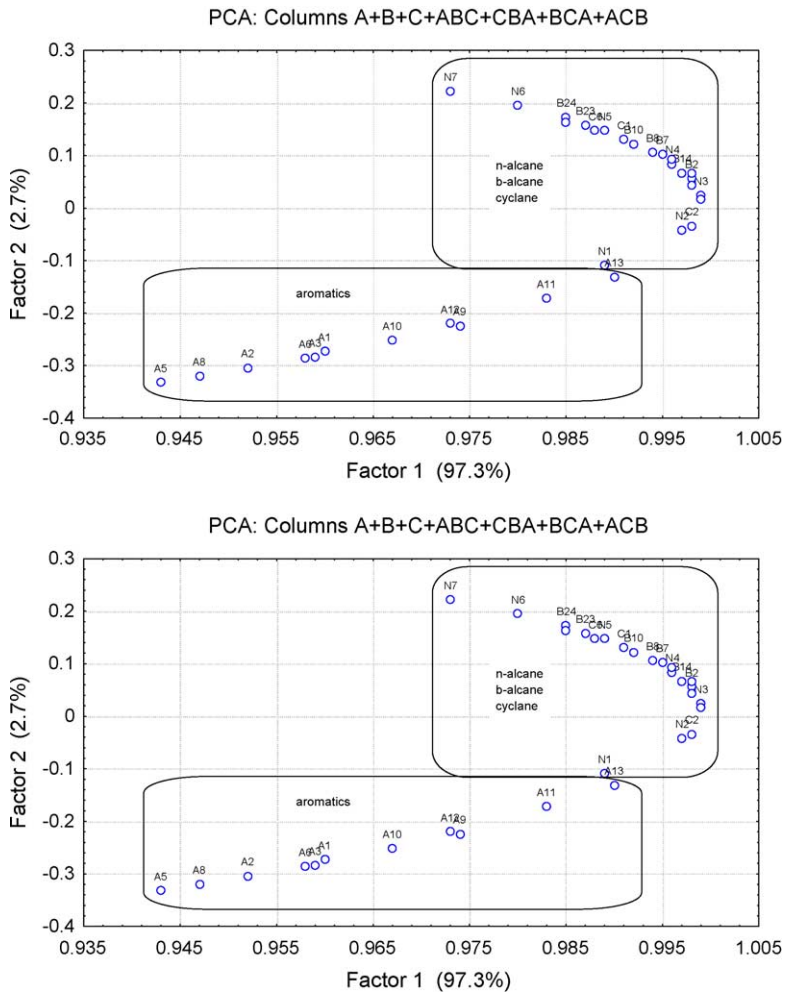


Fig. 1. PCA map of the retention factors of 51 test compounds on three single columns and four column series. The projection of the compounds on the first factorial plane is defined by the main factorial axes 1 and 2. The information content corresponding to the axes are 97.3% and 2.7%, respectively.

It was observed that when using only the three single columns, the corresponding data matrix gave the same results as with PCA. The fact that no additional principal component appears if the extended matrix is used, demonstrates the agreement between observed data and the theoretical assumptions. It illustrates the additivity of the columns or the fact that the role of the column can be expressed by their linear combination [2,3].

Evidently, for a chromatographer, the selectivity of two columns can be easily studied by simpler methods than PCA or CFA. For example, a plot of retention data on the former column versus a plot of the retention data on the latter one, or calculation of the correlation coefficient is frequently used. However, the use of multivariate statistical methods is superior if more than two columns are to be taken into consideration. Moreover, in GC, a high correlation between retention data on two different columns is likely since retention is always highly influenced by volatility, which does not depend on the stationary phase type. By using factor analysis more subtle selectivity mechanisms can be found. These subtle mechanisms may be sufficient for enhancing separations because of the high performance of GC.

The results obtained by PCA of the retention indices on three single columns are shown in Fig. 2. They lead to fairly similar conclusions to those obtained previously when using PCA of retention factors for the seven chromatographic column system. The chemically similar compounds form linear clusters in both cases. However, a visual inspection of Fig. 2 is more convenient because the clusters are parallel and more easily separated. The observed pattern can be easily explained by the mathematical definition of retention indices. They are calculated relatively to the *n*-alkanes. From this point of view the use of these retention indices utilizes the physicochemical differences relatively to the *n*-alkanes and therefore, is more suitable for examining the role of polarity on selectivity. It must be emphasized that a direct data processing of the retention factors of the corresponding data matrix on three single columns leads to similar results. The normalisation done by retention indices calculation or the $\chi^2$ metrics used in CFA have approximately the same effect. They decrease or sup-

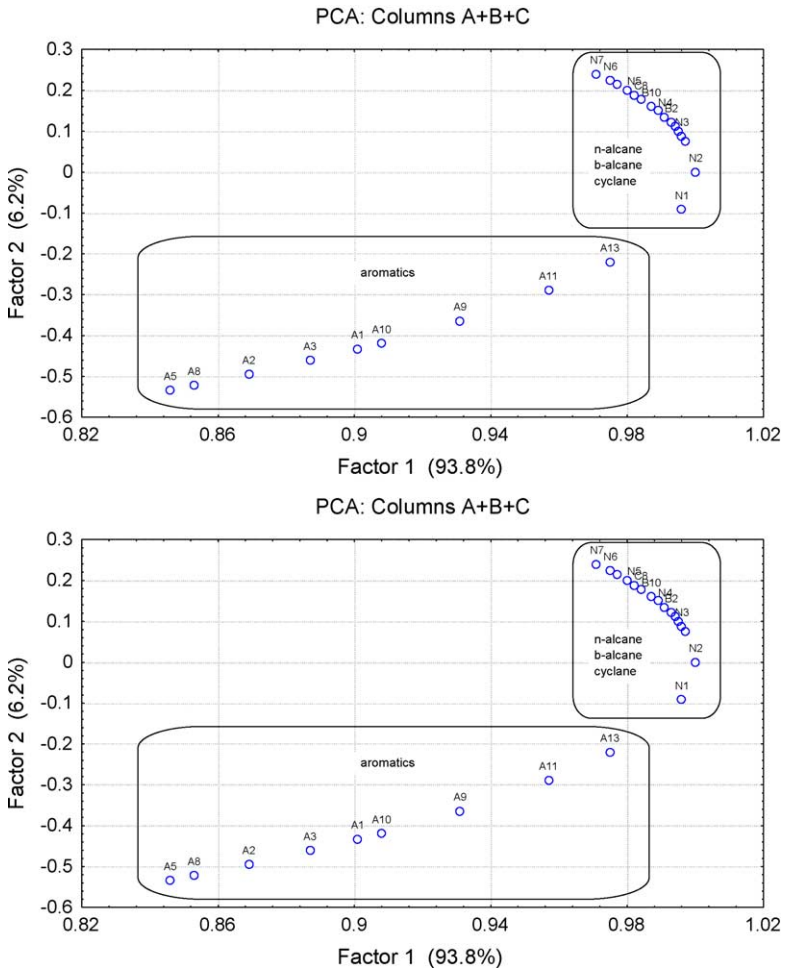


Fig. 2. PCA map of the retention indices of 51 test compounds on three different columns. The projection of the compounds on the first factorial plane is defined by the main factorial axes 1 and 2. The information content corresponding to the axes are 91% and 9%, respectively. (□) *n*-alkanes; (△) branched alkanes; (◯) cyclanes; (◊) aromatics.

press the contribution of retention to overwhelm the weight of selectivity.

The results of CFA of the retention factors matrix are given in Fig. 3. It can be observed that the volatility of the sample constituents plays only a minor role in the variation of the data—it corresponds to the second principal component which accounts for 0.1% of the total variation. The first principal component, this time, is related to polarity, it accounts for 99.9% of the information content. This is in contrast with the results of PCA where the first principal component, related to the volatility, accounts for 97.3% of the total variation and the second principal component related to the polarity accounts for only 2.7%. The mathematical character of the CFA can explain these facts—it focuses on the relative aspects of the data [17,18,23–25]. For the investigation of the selectivity in gas chromatography, CFA is superior as it filters out the basic retention and non-selective factors such as volatility, which has a major influence on the results obtained by PCA. Thus, PCA can obscure secondary effects by emphasizing the major one, i.e. chromatographic retention. For chromatographers involved in the separation

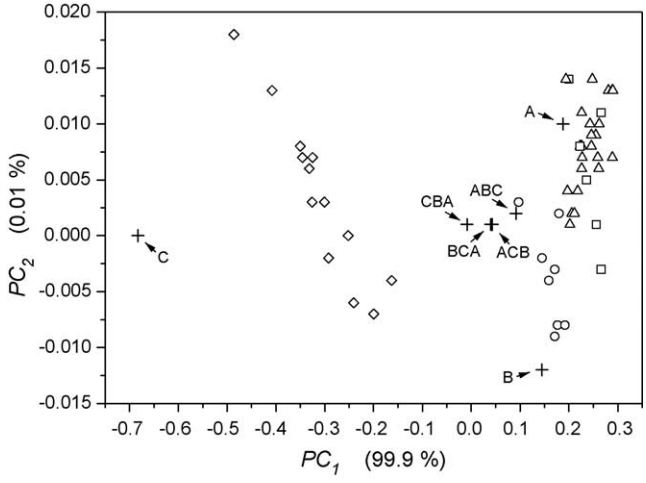


Fig. 3. CFA map of the retention factors of 51 test compounds studied with three single columns and four column series. Simultaneous projection of the compounds and columns on the first factorial plane are defined by the main factorial axes 1 and 2. The information content corresponding to the axes are 99.9% and 0.1%, respectively. (□) *n*-alkanes; (△) branched alkanes; (◯) cyclanes; (◊) aromatics.

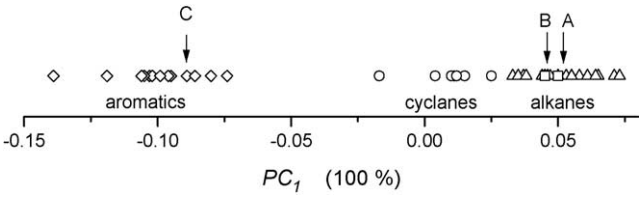


Fig. 4. map of the retention indices of 51 test compounds studied with seven chromatographic columns/column series. Simultaneous projection of the compounds and columns are on the first factorial axis. The information content corresponding to the axis approaches 100 %. (□) *n*-alkanes; (△) branched alkanes; (◯) cyclanes; (◊) aromatics.

of complex mixtures, selectivity remains the preponderant consideration.

This effect is even more evident and interesting for CFA of retention indices. In our case only one significant principal component was obtained as shown in Fig. 4. The clustering of the components according to their ability to interact via polar interactions is now observable. But there is no resolution within the homologous groups.

Also of interest concerning CFA, is the possibility of exploiting the simultaneous projection of the representative points of the compounds and those of the columns. Proximity between representative points of column C with aromatic compounds, of column B with cyclanes and of column A with linear and branched alkanes and the saturated hydrocarbons;

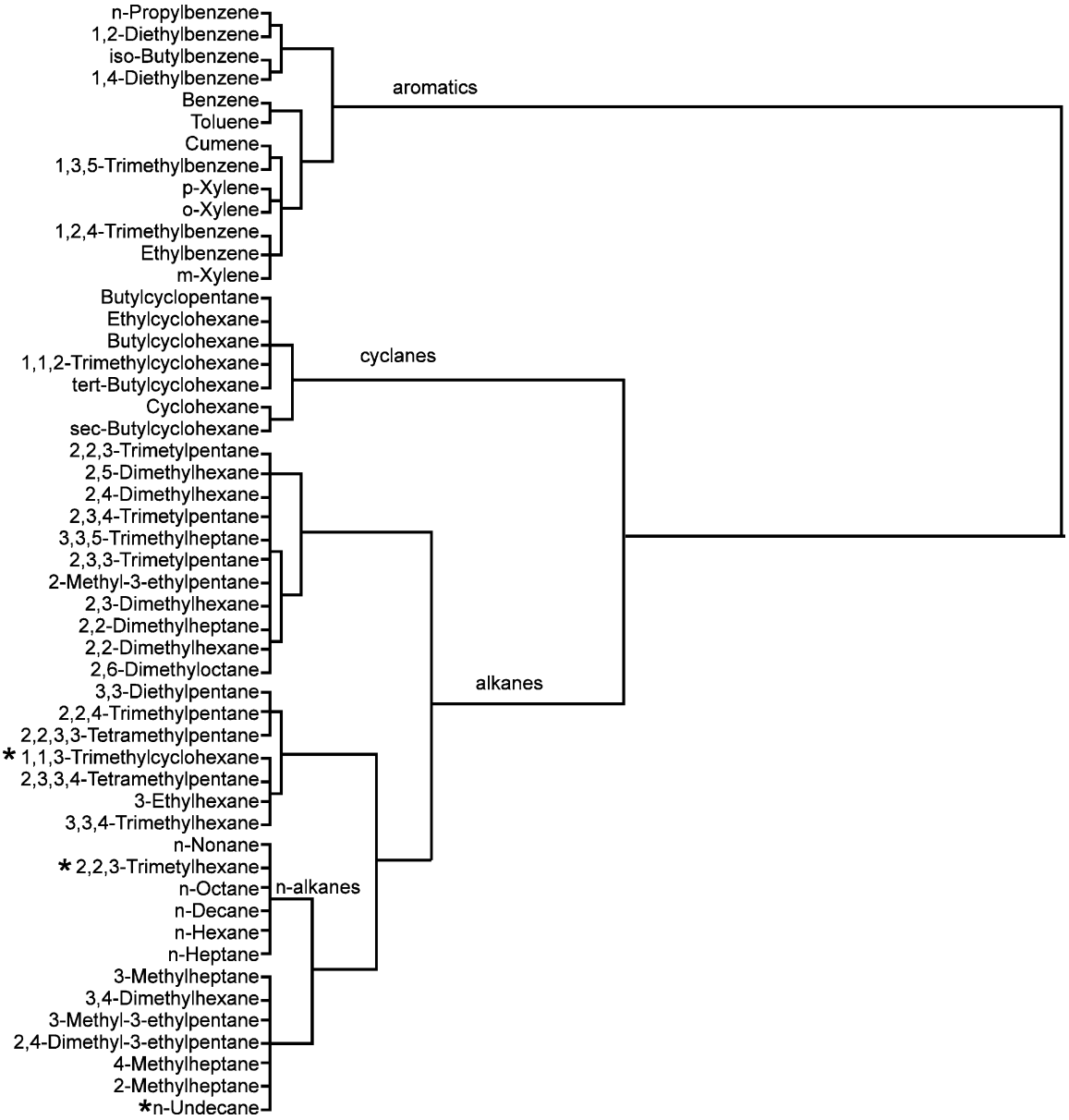


Fig. 5. HAC tree of 51 test compounds according to their retention indices on three different chromatographic columns. For the measurement similarity, chi-squared metrics was used. The clusters are linked by the minimal cluster variance criterion.

indicates that axis 1 corresponds to the ability to develop particular induction forces.

CFA allows simultaneous projection of the sample constituents and chromatographic columns in the same graph and hence to indicate similar structures. The co-ordinate of the point representing the chromatographic column along the first principal component is related to the polarity of the column. It can be observed that column A (very low polarity) lies near the column B (also low polarity) according to the first principal component. However, the C column (which is more polar) is shifted relative to the others. It is evident that aromatic compounds, which are more conducive to polar interactions than the other hydrocarbons in this study, are shifted in the same direction. In contrast, the apolar-saturated hydrocarbons are found in the same region as the chromatographic columns of low polarity.

The displacement of the points corresponding to the column series is interesting as well. Due to the gas compressibility it might be expected that the chromatographic properties of the column series be most influenced by the first (closest to the inlet) column of the series and least by the last column in the series. This is evident in Fig. 3 as the CBA point lies closest to the C point, the ABC point lies closest to the A point and the BCA point lies closest to the B point. Comparison of the ABC and ACB points shows that the ACB point is closer to the C than ABC.

### 3.2. Hierarchical ascending classification (HAC)

HAC is a well-known and widely used method. In investigations of selectivity it can be utilized for a better understanding of the interaction mechanisms between the sample and the chromatographic systems.

For meaningful classification by HAC the selection of the metric for the measurement of distances and the choice of the linking criteria are of critical importance. However, there are no simple rules for this selection. The experience and the chemical intuition of the analyst play an often-important role. In this study some typical behavior of HCA of chromatographic data is demonstrated.

The most common distance measurement used in HAC is the simple Euclidean distance. However, for our particular problem it does not yield meaningful results as the compounds are clustered by their volatility instead of their chemical structure. Usually the volatility of the components is of less interest than their chemical structure. This is the case in the present study as we are trying to investigate the interactions that are most important for chromatographic selectivity.

More definitive results are obtained if the chi-squared metric (reference?) is used for the distance measurement (Fig. 5). The sample constituents are clustered according to their chemical similarity. Applying of chi-squared metric has the same effect in HAC as in CFA—the volatility is filtered out and the relative (selective) properties are emphasized. In Fig. 5 it can be observed that even the *n*-alkanes are separated from branched alkanes and form a distinct cluster. There are only three compounds, which are misclassified. They are highlighted in Fig. 5 by an asterisk.

The linking criteria play only a minor role in our particular case. The best results are obtained if the criterion, which minimizes the variance of the linked clusters, is applied.

## 4. Conclusions

This paper demonstrates the strong hybridization of the data processing (DP) and experimental design (ED) aspects of Chemometrics. The four following points supports this hybridization:

(1) In gas chromatography the chromatographic retention contains the main part of the variability of the chemical information. Selectivity optimization for the separation of complex samples remains a challenge in capillary gas liquid chromatography. But chromatographic selectivity corresponds, in fact, to second order effects in terms of information content. This explains why the prevalent factor controlling retention often hides important second order effects. It also explains why a direct exploitation of a retention data matrix is most often impossible and supports the systematic use of Chemometric tools.
(2) Multivariate analysis with principal component analysis plus correspondence factor analysis offers a powerful tool to search for the main selectivity controlling factors without a priori knowledge of the key factors of the separation mechanism. These factors are basically abstract ones, but often they can identify physicochemical factors.
(3) With CFA the part of retention due to a solute's vapour pressure can be minimized. It is possible to highlight the secondary order effects, which usually play a key role in selectivity, and are necessary in order to optimize the separation of a complex mixture. The use of retention indices, which use the *n*-alkanes as reference compounds, also helps to decrease the dominant focus on vapour pressure in favor of the more selectivity-based interaction forces.
(4) CFA helps to analyze the degree of relevance of the chosen ED to the most important factors, controlling chromatographic selectivity.

## Acknowledgements

The authors would like to thank the Slovak Ministry of Education for a Grant VEGA 1/2461/05 GAV and Agency for International Science and Technology Cooperation in Slovakia for a Grant No. 035/2001.

## References

[1] P.J. Schoenmakers, Optimisation of Chromatographic Selectivity, Elsevier, Amsterdam, 1986, p. 11.

[2] R.J. Laub, J.H. Purnell, J. Chromatogr. 112 (1975) 71.
[3] R.E. Kaiser, R.I. Rieder, L. Leming, L. Blomberg, P. Kusz, J. High Resolut. Chromatogr. Chromatogr. Commun. 8 (1985) 92.
[4] P. Sandra, F. David, M. Proot, G. Diricks, M. Verstappe, M. Verzele, J. High Resolut. Chromatogr. Chromatogr. Commun. 8 (1985) 782.
[5] J.V. Hinshaw, L.S. Ettre, Chromatographia 21 (1986) 561.
[6] D.R. Deans, I. Scott, Anal. Chem. 45 (1973) 1137.
[7] R.E. Kaiser, R.I. Rieder, L. Leming, L. Blomberg, P. Kusz, J. High Resolut. Chromatogr. Chromatogr. Commun. 8 (1985) 92.
[8] T. Tóth, H. van Cruchten, J. Rijks, in: P. Sandra (Ed.), Proceedings of the 6th International Symposium of Capillary Chromatography, Huethig, Heidelberg, 1985, p. 769.
[9] T. Tóth, F. Gray, in: P. Sandra (Ed.), Proceedings of the 8th International Symposium of Capillary Chromatography, Huethig, Heidelberg, 1987, p. 585.
[10] R.J. Laub, J.H. Purnell, J. Chromatogr. 112 (1975) 71.
[11] D. Repka, J. Krupcík, E. Benická, P.A. Leclerq, J.A. Rijks, J. Chromatogr. 463 (1989) 243.
[12] E. Benická, J. Krupcík, D. Repka, P. Kuljovský, R.E. Kaiser, Anal. Chem. 62 (1990) 985.
[13] T. Hevesi, J. Krupcík, J. Chromatogr. 517 (1990) 161.
[14] T.C. Gerbino, G. Castello, J. High Resolut. Chromatogr. 17 (1994) 597.
[15] T. Hevesi, J. Krupcik, J.R. Chrétien, Fresenius J Anal. Chem 352 (1995) 643.
[16] R. Kaliszan, Quantitative Structure–Chromatographic Retention Relationships, John Wiley, New York, 1987.
[17] T. Cserháti, E. Forgács, in: P.R. Brown, E. Grushka (Eds.), Use of Multivariate Mathematical Statistical Methods for the Evaluation of Retention Data Matrices, Advances in Chromatography, vol. 36, Marcel Dekker Inc., New York, 1996, pp. 1–63.
[18] A. Felinger, Data Analysis and Signal Processing in Chromatography, Elsevier, Amsterdam, 1998.
[19] K. Heberger, M. Gorgenyi, J. Chromatogr. A 845 (1999) 21.
[20] B.K. Kochanowski, S.L. Morgan, J. Chromatogr. Sci. 38 (2000) 100.
[21] Tibor Cserhati, Esther Forgacs, Chemometrics in Chromatography, Marcel Dekker Inc., 2003.
[22] STATISTICA for Windows ver. 6.0, StatSoft Inc., 2300 E. 14th St., Tulsa, OK 74104, USA, 2001.
[23] D.L. Massart, B.G.M. Vandeginste, S.N. Deming, Y. Michotte, L. Kaufman, Chemometrics: A Textbook, Elsevier, Amsterdam, 1988, p. 366.
[24] S. Sharma, Applied Multivariete Techniques, J. Wiley, New York, 1996.
[25] M. Otto, Chemometrics, J. Wiley, Weinheim, 1999.