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High-performance liquid chromatography and supercritical fluid chromatography of monosaccharides and polyols using light-scattering detection

Chemometric studies of the retentions

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ABSTRACT

Twelve sugars and polyols were analyzed using high-performance liquid chromatographic (HPLC) and supercritical fluid chromatographic (SFC) systems with silica and bonded silica stationary phases with the help of an evaporative light-scattering detector. The separation capacities of the two techniques are discussed. The retention data were studied using different chemometric methods (automatic classification, factor analysis). They clearly show that SFC and HPLC have the same retention process for these compounds and that is the sum of only two mathematically independent physico-chemical phenomena.

INTRODUCTION

Sugars and polyols (sugar alcohols) are widespread compounds found in our biotope. Their use in the food processing, pharmaceutical, cosmetic and chemical industries make them very important from an economic point of view. Thus many studies on their separation by chromatographic methods have been carried out [1–4]. These studies have revealed many difficulties, due principally to two features of these products: their retention mechanism is badly defined, making the development of a new separation difficult, and their detection is not possible or too weak with the UV detectors commonly used in high-performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC).

For several years, our group has been working on these types of compounds [5–7] using a detection technique which is less common but perfectly suitable for them, namely evaporative light-scattering detection. Recently this method of detection was adapted to SFC [8,9] in our laboratory. This is the only detection compatible with polar modifiers in the supercritical fluid phase for solutes which do not absorb in the UV region. Another advantage is that gradient elution can be used in either HPLC or SFC.

This paper concerns the retention results obtained with an isocratic eluent for twelve monosaccharides and polyols on twelve normal-phase chromatographic systems, seven in SFC and five in HPLC. In HPLC an eluent system whose polarity is close to that of the carbon dioxide–methanol mixtures used in SFC was chosen. This was necessary for an objective comparison of the two techniques and an interpretation of the mechanisms because this type of system (eluent–stationary phase) has not yet been studied much. Various statistical analytical methods [10] are used, such as the hierarchical ascending classification (HAC) and factor analysis (FA).

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These methods have been used in our laboratory to analyse the retentions and selectivities of other solutes in other chromatographic systems [11,12]. This allows the establishment of the factors linking the retention in these systems, a better understanding of the "true" complexity of the problem and a clearer visualization of convergences and divergences, and hence the respective advantages, of HPLC and SFC for the separation of these types of products.

EXPERIMENTAL

Apparatus

SFC. Carbon dioxide, kept in a cylinder with an eductor tube connected to a Waters (Milford, MA, USA) Model M45 pump, was passed through a cooling bath (−30°C). The pump head was cooled (0°C) to improve efficiency. The flow-rates and the weight percentages of carbon dioxide and methanol given in the tables and figures have been corrected to take into account the pumping yield and the pump-head temperature. Polar modifier (methanol) was added using a Jasco (Tokyo, Japan) Model 2510 pump. The two solvents were mixed in a Knauer (Berlin, Germany) dynamic mixer. The column temperature (40°C) was controlled with a water-bath. The loop of a Rheodyne (Cotati, CA, USA) Model 7125 valve was immersed in the same water-bath.

A Sedex 45 evaporative light-scattering detector (Sedere, Vitry sur Seine, France) was used with a special interface for the SFC (Sedere). It was connected to a Shimadzu (Kyoto, Japan) CR5A calculator.

HPLC. The chromatographic system consisted of a Gilson (Villiers le Bel, France) Model 303 isocratic pump, a Rheodyne 20-μl sample loop, an Sedex 45 evaporative light-scattering detector and a Shimadzu CR3A calculator.

Columns

The following columns were used: 10-μm LiChrospher Diol (250 × 4.6 mm I.D.), 10-μm LiChrospher Diol (125 × 4 mm I.D.), 5-μm LiChrosorb CN (150 × 4.6 mm I.D.) and 10-μm LiChrospher CN (250 × 4.6 mm I.D.), all from Merck (Darmstadt, Germany), Zorbax CN (150 × 4.6 mm I.D.), Zorbax Phenyl (150 × 4.6 mm I.D.), Zorbax NH_2 (150 × 4.6 mm I.D.), Zorbax TMS (250 × 4.6 mm I.D.), and Zorbax Sil (250 × 4.6 mm I.D.), all from DuPont (Wilmington, DE, USA), 5-μm RSil NO_2 (250 × 4.6 mm I.D.) from RSL (Eke, Belgium) and 10-μm μBondapak CN (150 × 3.9 mm I.D.) from Waters.

Chemicals and reagents

Carbon dioxide (Air Liquide, Paris, France) was of B 50 grade and was flushed through molecular sieves before the pump. Pestipur-grade methanol was purchased from SDS (Vitry, France) for use as a polar modifier. Only a few grades of methanol are suitable as polar modifiers with a light-scattering detection system in SFC and must always be tested before use. Dichloromethane was of HiPerSolv grade (BDH, Poole, UK). Distilled water (Cooperation Pharmaceutique Francaise, Etampes, France) was used throughout.

The solutes (analytical-reagent grade) were dissolved in chloroform–methanol or in pure methanol. The chloroform–methanol mixture was preferable to avoid problems such as band broadening and peak splitting caused by high elution strength solvents.

Data processing

Microcomputers were used for data treatment using commercial programs [13] or specially adapted programs from publications [14,15].

RESULTS AND DISCUSSION

The compositions of the mobile phases differed from one system to another. The compositions for each column had to be adjusted in order to obtain peak shapes as correct as possible, pressures compatible with our devices and to have capacity factors (k') in a suitable range (usually 0.8–10) for every product [9]. A few SFC experiments, using large amounts of methanol in the mobile phase, were performed under subcritical conditions but no dramatic changes concerning selectivity were observed between subcritical or supercritical conditions. Although carbon dioxide is compressible under the conditions applied, the mean density of the carbon dioxide–methanol fluids, calculated from the supplier's data, is quite constant (0.84–0.89). A list of these systems is given in Table I.

TABLE I

LIST OF THE CHROMATOGRAPHIC SYSTEMS

System	Stationary phase	Dimensions (length × I.D.) (mm)	Mobile phase	Composition	Flow-rate (ml/min)	Pressure (p.s.i.)
SYS 1	Zorbax CN	150 × 4.6	CO_2–CH_3OH	93.5:6.5	4.35	3700
SYS 2	μBondapak CN	150 × 3.9	CO_2–CH_3OH	95.9:4.1	3.37	3900
SYS 3	LiChrosorb CN	150 × 4	CO_2–CH_3OH	96.4:3.6	3.35	3900
SYS 4	LiChrospher CN	150 × 4	CO_2–CH_3OH	95.4:4.6	2.97	3000
SYS 5	LiChrospher Diol	250 × 4	CO_2–CH_3OH	83.7:16.3	1.79	3900
SYS 6	RSil NO_2	250 × 4.6	CO_2–CH_3OH	87:13	3.8	3500
SYS 7	Zorbax Phenyl	150 × 4.6	CO_2–CH_3OH	85.7:14.3	3.48	3000
SYS 8	Zorbax NH_2	150 × 4.6	CH_2Cl_2 CH_3OH	65:35	1	
SYS 9	LiChrospher Diol	125 × 4	CH_2Cl_2–CH_3OH	87:13	1	
SYS 10	RSil NO_2	250 × 4.6	CH_2Cl_2–CH_3OH	80:20	1	
SYS 11	Zorbax TMS	250 × 4.6	CH_2Cl_2–CH_3OH	70:30	1	
SYS 12	Zorbax Sil	250 × 4.6	CH_2Cl_2–CH_3OH–H_2O	80:19.8:0.2	1.5	

Table II gives the capacity factors for the twelve compounds with the twelve systems. Direct interpretations and drawing inferences from this type of table are difficult. Fig. 1 presents a graphical representation of these k' values, providing new information. In addition to overall of these k' values among the various systems, abnormal points appear (*e.g.*, xylitol and mannitol in systems 1 and 3) and some retention inversions may be noted. However, this graphical representation does not allow the quantification of these specificities and also hides a large part of the information.

From a practical viewpoint, the chromatographer is essentially interested in the separation capacities

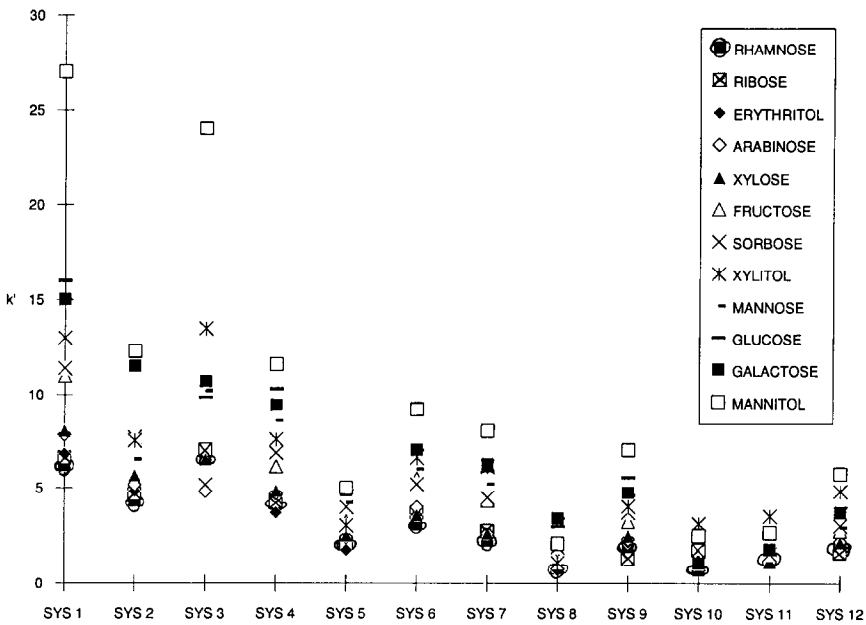

Fig. 1. Capacity factors of the twelve products on the twelve chromatographic systems.

TABLE II
CAPACITY FACTORS OF TWELVE PRODUCTS ON TWELVE CHROMATOGRAPHIC SYSTEMS

System	L-Rhamnose	D-Ribose	*m*-Erythritol	L-Arabinose	D-Xylose	D-Fructose	L-Sorbose	Xylitol
SYS 1	6.2	6.6	6.8	7.8	8	11	11.4	13
SYS 2	4.33	4.67	4.83	5.17	5.67	7.67	7.67	7.5
SYS 3	6.5	7	5	4.83	6.5	5.17	5.17	13.5
SYS 4	4.14	4.28	3.71	4.71	4.86	6.14	6.86	7.57
SYS 5	2.22	2.11	1.78	2.44	2.55	3.22	4	3.05
SYS 6	3.1	3.7	3.6	4	3.6	5.5	5.2	6.6
SYS 7	2.26	2.76	2.62	2.65	2.67	4.35	4.5	6.12
SYS 8	0.882	0.824	0.706	1.82	1.18	2.12	1.94	1.12
SYS 9	2.04	1.32	2.2	2.36	2.52	3.24	3.72	4.04
SYS 10	0.724	1.76	1.24	0.793	0.724	1.07	1.07	3.14
SYS 11	1.13	1.36	1.45	1.45	1.23	1.82	1.59	3.55
SYS 12	1.85	1.65	2.5	2.25	2.2	2.8	3.05	4.85

of a new system. The best criterion would be the calculation of the resolution of every potential pair included in this group of products on each system. In this way the highest resolution system (for this group of products) could be determined and a definitive conclusion could be drawn. However, this would imply the optimization of each chromatographic system. Another problem is linked to this approach, namely that to calculate easily the efficiency of the system, Gaussian or near-Gaussian peaks are required. This is not always the case with these products [9], so we limited ourselves to calculating all the selectivities. For each system we calculated the selectivities (α) of the 66 potential pairs and Table III gives the number of selectivities greater than 1.2, 1.5, 1.7 and 2. These values illustrate, for a system, the more or less regular distribution of the k' values within the range of values. It is obvious that a system giving high values is more likely to separate a given pair than a system giving low values when their efficiencies are equivalent.

Fig. 2 gives a graphical representation of these values, thus allowing an overview of these results. It is worth noting that greater homogeneity of the values is obtained with the supercritical systems compared with the HPLC systems. The average values are slightly greater with HPLC than with SFC, but this is not really significant as indicated by the averages and standard deviations of these values (Table III). Efficiencies are generally slightly higher with SFC, which allows separations with lower selectivities. Therefore, it is not possible to conclude that one of these two systems has an intrinsic advantage over the other, but rather that they are simply complementary. For example, it is worth noting that the products are separable on CN phases in SFC, whereas this is difficult, or even impossible,

TABLE III
NUMBER OF PAIRS OF PRODUCTS WITH A SELECTIVITY GREATER THAN 1.2, 1.5, 1.7 and 2 ON TWELVE SYSTEMS

The last three pairs of lines give the average and standard deviation of these values in SFC, HPLC and SFC + HPLC.

System	$\alpha > 1.2$	$\alpha > 1.5$	$\alpha > 1.7$	$\alpha > 2$
1	51	37	31	20
2	48	35	23	15
3	54	37	30	23
4	52	35	25	18
5	51	34	24	15
6	47	32	22	8
7	49	38	30	21
8	54	47	36	27
9	55	40	33	21
10	48	30	26	21
11	41	20	16	10
12	54	33	22	14
Av. (SFC)	50.29	35.43	26.43	17.14
S.D. (SFC)	2.25	1.92	3.50	4.64
Av. (HPLC)	50.40	34.00	26.60	18.60
S.D. (HPLC)	5.31	9.14	7.26	5.95
Av. (total)	50.33	34.83	26.50	17.75
S.D. (total)	3.84	6.12	5.39	5.28

D-Mannose	D-Glucose	D-Galactose	D-Mannitol
15	16	15	27
6.5	12	11.5	12.3
10.2	9.83	10.7	24
8.57	10.3	9.43	11.6
4.22	4.89	4.88	5
6	7	7	9.2
5.2	5.91	6.25	8
3.41	3	3.41	2.12
4.6	5.56	4.76	7
0.896	0.896	1.07	2.52
1.54	1.64	1.82	2.68
2.95	4	3.72	5.75

in HPLC on the same stationary phases. All the trials we made on these phases with these kinds of mobile phases gave broad peaks with very bad selectivities.

Thermodynamic approach

A comparison of the retention based on the plot of log k'_{ij} *vs.* log k'_{ij} for all the products i on systems j and j' can be made [16,17]. Depending on the relationship between ΔG_j and $\Delta G_{j'}$ (the Gibbs free energy of the transport of the solutes between the mobile and the stationary phases), the slope $a_{jj'}$ and the correlation coefficient $r_{jj'}$ of the regression line $\log k'_j = a_{jj'} \log k'_{j'} +$ constant may vary. If the Gibbs free energies in the two systems j and j' are identical for all the products i, then $a_{jj'}$ and $r_{jj'}$ ought to be close to 1. The retention is called homoenergetic (the same). If these energies are proportional, the correlation coefficient is always close to 1 but the slope is different, and then the retention is called

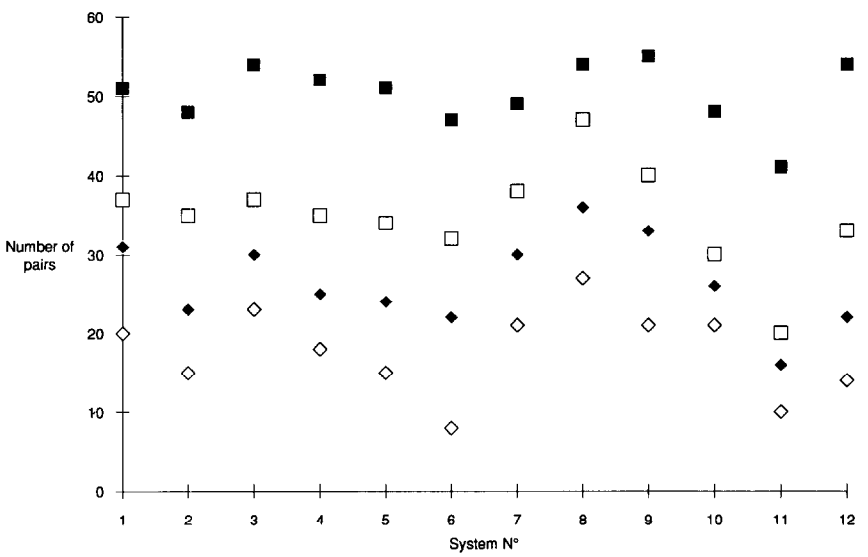

Fig. 2. Representation for the twelve systems of the number of pairs of products with a selectivity greater than (■) 1.2, (□) 1.5, (◆) 1.7 and (◇) 2.

	SYS 1	SYS 2	SYS 3	SYS 4	SYS 5	SYS 6	SYS 7	SYS 8	SYS 9	SYS 10	SYS 11
SYS 2	3	.	.	.	.	.	.	.	.	.	.
SYS 3	3	3	.	.	.	.	.	.	.	.	.
SYS 4	2 (1.12)	3	3	.	.	.	.	.	.	.	.
SYS 5	3	3	3	1 (0.91)	.	.	.	.	.	.	.
SYS 6	2 (1.27)	3	3	2 (0.85)	3	.	.	.	.	.	.
SYS 7	1 (0.99)	3	3	2 (0.86)	3	2 (0.78)	.	.	.	.	.
SYS 8	3	3	3	3	3	3	3	.	.	.	.
SYS 9	2 (0.88)	3	3	3	3	3	3	3	.	.	.
SYS 10	3	3	3	3	3	3	3	3	3	.	.
SYS 11	3	3	3	3	3	3	3	3	3	3	.
SYS 12	3	3	3	3	3	3	3	3	3	3	3

Fig. 3. Comparison of retentions for the 66 pairs of systems. 1 = Homoenergetic; 2 = homeoenergetic; 3 = heteroenergetic. The slope is given in parentheses.

homeoenergetic (similar). If these energies are not proportional then the correlation coefficient is different from 1 and the retention is heteroenergetic (different).

Fig. 3 shows the result of these regressions for all 66 pairs of systems. It is clear that the retentions are mainly heteroenergetic (58 pairs compared with only 6 homeoenergetic and 2 homeoenergetic pairs). This result corroborates the difficulties in the interpretation and prediction process of the retention of the carbohydrates and polyols on such chromatographic systems. However, from another point of view, it is difficult to accept so many different processes for the retention of very similar products on similar chromatographic systems. The limitation of this classification is due to the criterion chosen. The regression analysis is a global approach. For example, it cannot interpret either a specific interaction of a product with a system or the diversity of the chromatographic variables and therefore it gives a bad correlation coefficient. It is for these reasons that the following statistical procedures are more efficient.

Classification approach

Hierarchical ascending classification (HAC) [18] is a statistical method which successively groups together p objects, represented in an N-dimensional observation space, in $p-1$, $p-2$, ..., 2, 1 groups according to their similarity. The programs select the two objects which are the closest to one another, then create a group and replace it with a new point located at its centroid. The procedure is repeated with $p-1$, $p-2$, ..., points until all the objects are agglomerated. The result is presented in the form of a classification tree (dendogram). Several criteria are available to define the inter-object "distance". The average Euclidian distance on raw or reduced data was used because it is straightforward, close to the requirements for developing new separations, and the most natural for quantitative data [19]. This global technique has the advantage of clearly underscoring and even quantifying the similarity between the objects. The lower the agglomeration level is, the closer the objects are; the higher this level is, the more dissimilar the objects are. The basic difference between the classification and the thermodynamic approach is its pragmatic aspect. The aim is not to search for links between the mathematical criteria and the physico-chemical phenomena, but rather a more comprehensible representation of reality.

Our data can be considered as the coordinates of twelve products in a space of twelve chromatographic systems with the products being the objects and the similarity of their retention on this set of chromatographic systems is visualized.

However, these data can also be considered as the coordinates of twelve chromatographic system in a space of twelve products and then the similarity of the chromatographic system in relation to their action on this products is visualized.

Products in the space of systems

In this case, the Euclidian distance on the raw data was selected as a criterion. The classification tree is presented in Fig. 4. The agglomeration level is indicated near each vertical line.

Well known similarities appear, *e.g.*, glucose and

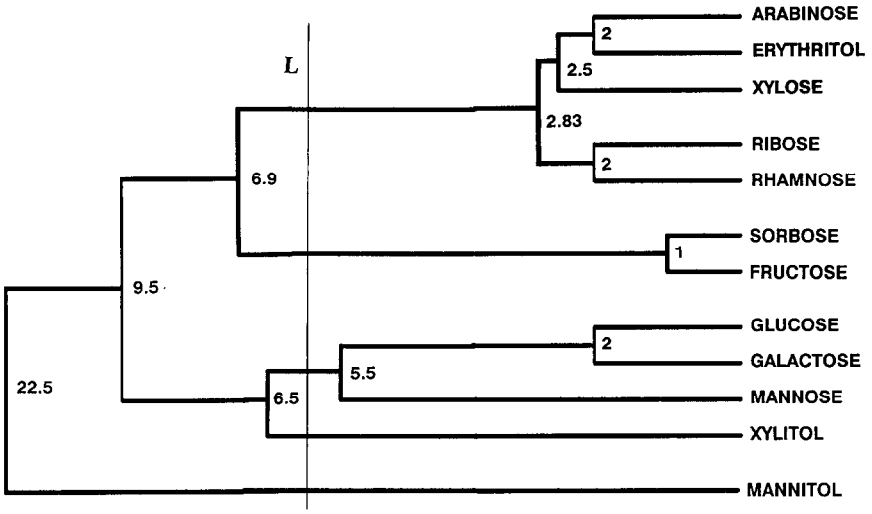

Fig. 4. Classification tree of the twelve products. The vertical line L indicates the partitioning into five groups.

galactose, and xylose and arabinose. It is also worth noting agglomerations which are less obvious: fructose and sorbose (two ketohexoses) are closer to pentoses than to aldohexoses. The division into five classes, dilineated by the line L (Fig. 4), certainly provides the most interesting information as it can be correlated with very simple chemical criteria, *e.g.*, the number of hydroxyl groups on the molecule and its family.

class 1: mannitol	alcohol	6 OH
class 2: glucose, galactose, mannose	aldose	5 OH
class 3: xylitol	alcohol	5 OH
class 4: fructose, sorbose	ketose	5 OH
class 5: other products	sugars and alcohol	4 OH

One can reasonably interpret the proximity of ketohexoses and pentoses by the very poor accessibility of the hydroxyl group located at the C-2 of the furanose form, which leads these molecules to interact with the mobile and stationary phases in a way that is closer to that of the pentoses than that of the aldohexoses. These results indeed show the global influence of the number of hydroxyl groups of the molecule, *i.e.*, its polar area, but it remains a global approach that does not take into account the specific interactions of a system with certain products.

Systems in the space of products

In this case, the agglomeration criterion selected is the Euclidian distance of the reduced data in order to eliminate the scale effect due to products having high retentions (mainly polyols). The classification tree is represented in Fig. 5.

The first noteworthy fact is the non-separation of the SFC and HPLC systems. SFC has been claimed to be superior than HPLC for compounds less polar than sugars. However, regarding selectivity in our experiments, it is clear that there is nothing special about a sub- or supercritical mobile phase in that it is no different from any other mobile phase. The four CN phases in SFC form a group opposed to the other systems but with notable differences within this group (the agglomeration level between the

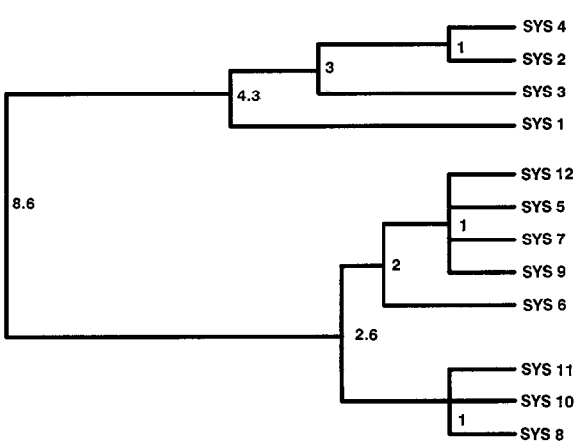

Fig. 5. Classification tree of the twelve systems.

Zorbax CN phase and the other CN phases is equal to 4.3, *i.e.*, the penultimate level). It is also worth noting stranger parallels: LiChrospher Diol HPLC and SFC, Zorbax Sil HPLC and Zorbax Phenyl SFC (SYS 5, 9, 7 and 12) are very similar (level 1). It is not possible to conclude that the mobile or the stationnary phases systematically have a stronger influence because, *e.g.*, the RSil NO_2 in SFC and HPLC systems (SYS 6 and 10) are dissimilar (Level 2.6) and, in contrast, the LiChrospher Diol SFC and HPLC systems (SYS 5 and 9) are very similar (level 1). Contrary to what has been done for the products in the space of systems, it is not possible to classify logically these systems, which are globally very dissimilar. This is in accordance with the results of the thermodynamic approach which indicated a strong complexity of the retentions.

Principal component analysis

Principal component analysis (PCA) is a factor analysis method allowing the exploration of the complete data space and the reduction of this space into a subspace with a much smaller dimension but which still contains the maximum amount of information. The principles, the mathematical bases and the applications to chemical data of these analyses are well known and perfectly explained especially by Malinowski and Howery [20]. Their applications in analytical chemistry are numerous [21] and we have already applied then to chromatographic data [11]. As for the classification, the products in the space of systems or the systems in the space of products can be analysed. The major difficulty in this analysis is choosing the proper number of dimensions in order to keep the maximum amount of information and to eliminate the background noise. We jointly used the imbedded error function (*IE*) and Malinowski's indicator [20], while taking into account the percentage of explained inertia and the comparison between the original matrix and the one reconstituted with the selected eigenvectors.

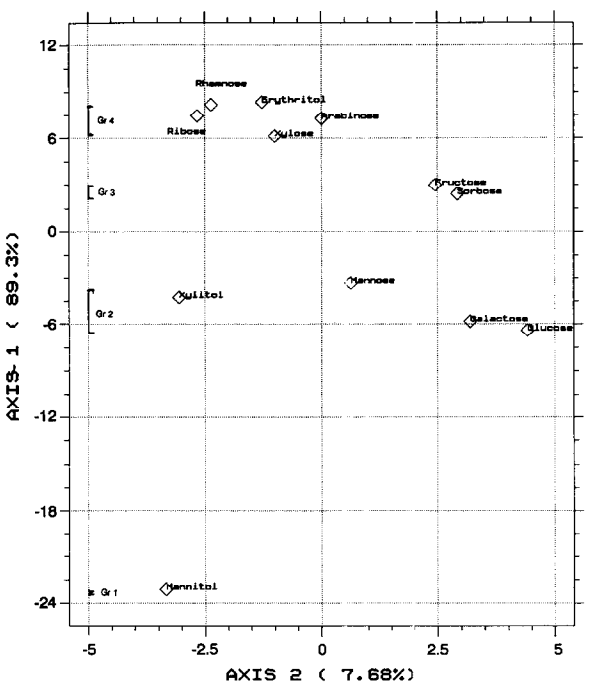

Fig. 6. Projection of the twelve products on the main PCA plane.

PCA of products in the space of systems

The analysis of the k' table of the products in the space of the chromatographic systems was carried out by using the simplest criterion: a canonical metric centred on the mean. The first two components contribute 89.3% and 7.68%, respectively, to the total inertia. The difference between the original and the reconstituted matrices is small and the introduction of additional components does not notably improve it. One can conclude that reduction to a two-dimensional space is possible without a notable loss of information. The plane represents 97% of the inertia of the twelve dimensional initial space and therefore gives a simplified representation, virtually exact, of the relative position of the products in the original space.

Fig. 6 represents a projection of these twelve products on this plane. One notices clusters similar to that with HAC (five groups), but it is much more interesting to note the projections on the two axes, each of which is mathematically independent. On axis 1 (which explains 89.3% of the retention) only four groups can be distinguished: (1) mannitol with six OH, (2) the aldohexoses with five OH and the alcohol with five OH, (3) the products with four OH and, between these two last groups, (4) the ketohexoses with four accessible OH plus one not very accessible OH groups. This proves that this axis, and hence the major part of the retention, are directly linked to the number of accessible OH groups in the molecule and therefore to its polar surface.

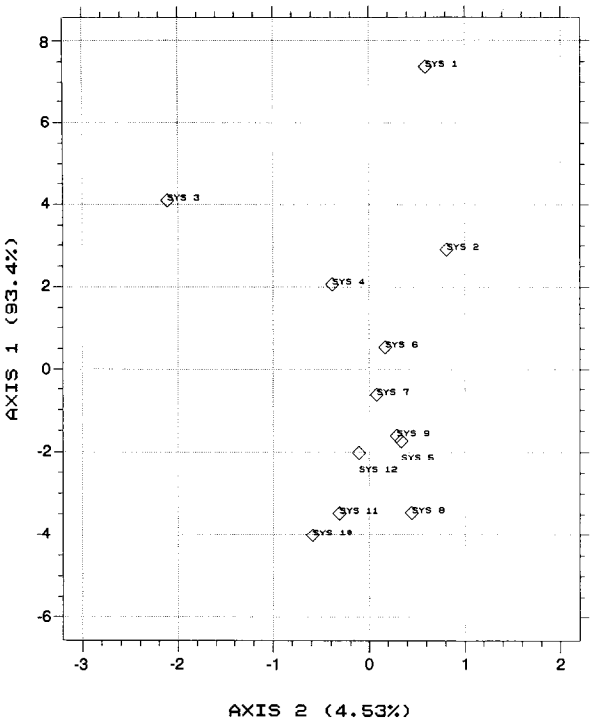

Fig. 7. Projection of the twelve systems on the main PCA plane.

The second axis is smaller (7.68%) and its interpretation is more difficult. One notices mainly an opposition between the hexoses and a group constituted by the alcohols, rhamnose and especially ribose. It is the effect represented by this axis which causes the non-regularity of the retention because although the ratio of the influences is equal to 89:8 for the overall systems, it varies from one system to another. For example, if axis 1 is sufficient to explain the retention of systems 1 or 12 whose correlation with that axis is greater than 0.95, the influence of axis 2 is very important for the systems 3, 8, 10 and 11. Finding a physico-chemical correlation with this axis is difficult. A differentiation between the open forms of the alcohols and the cyclic forms of hexoses can be considered but cannot explain the position of some pentoses on this axis. Such assumptions should be confirmed by structural and conformational studies of the products connected to a rotational study of the two main axes. At present the real conformations of these products in the injection solvent and the kinetics of conversion from one form into another at the time of the transfer from the injection solvent to the eluent phase are unknown, but will be studied.

PCA of systems in the space of products

In a similar way, we can analyse the systems in the space of products. For the same reason as for HAC, we shall use a standardized metric centred on the mean. The two first components contribute 93.4% and 4.5%, *i.e.*, 98% of the total inertia. The other axes, the contributions of which to inertia are very small, do not improve the representation. The plane (Fig. 7) formed by these first two axes is then sufficient to represent the quasi-total amount of information. A clustering made from this plane is in close agreement with the result of HAC.

Axis 1 allows the separation of the CN phases from the others, which is also in agreement with the conclusions of the HAC. It clearly indicates the specificity of these systems. Axis 2 is mainly created by the opposition between one system (LiChrosorb CN) and the others. Its contribution to inertia is only 4.5%, a low value, and a corrective effect of the importance of axis 1 is probable. Giving a physico-chemical explanation to this classification is not really possible with the present state of our knowledge of these systems.

The most significant and most interesting fact, however, is the explanation given by the two factors obtained in the two PCA to the incoherence underlined at the beginning of this paper. Therefore, the variation in the retentions of these similar compounds on similar systems is not due to many different mechanisms but rather to the combination of only two mathematically independent phenomena. The proportions of these phenomena vary from one product to another and from one system to another. This simplicity in the decomposition of the mechanism is even more impressed if these results are compared with those obtained previously [17]. With a homogeneous set of compounds (chalcones), seven significant factors for the interpretation of the retention on eleven chromatographic systems were found, which corresponds to a much greater complexity.

Correspondence factor analysis

Correspondence factor analysis is a factor analysis method allowing the determination of the

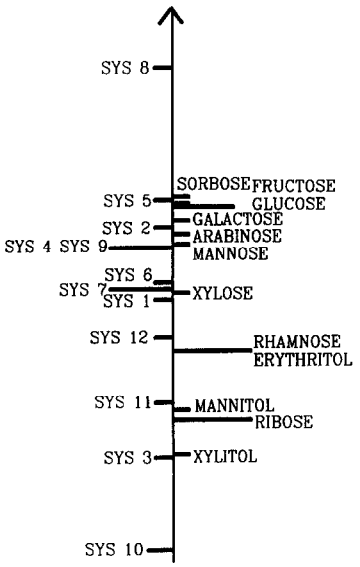

Fig. 8. Projection of products and systems on the main CFA axis.

factors causing the deviation from the state of independence [22] using the χ^2 distance criterion. The number of significant factors in CFA is equal to the number of significant PCA factors minus one because the first one is embedded in the state of independence. One limitation of CFA is the need to have homogeneous data. The major advantages are the possibility of representing the rows and columns of the data matrix on the same space and interpreting the proximity of a row and a column as a specific interaction causing the deviation from proportionality.

Two PCA factors indicate one CFA factor alone. This allows all space to be represented in one-dimensional space, *i.e.*, a line (Fig. 8).

Classification of products is similar to that obtained on axis two of PCA. The deviation from proportionality is caused by specific interactions between certain products (mainly sugar alcohols and some pentoses) and certain systems (SYS 10, 3 and 11). The physical interpretation is not simple; what is the common factor between LiChrosorb CN in SFC and RSil NO_2 and Zorbax TMS in HPLC? It is interesting to note that these interactions were observed not only with alcohols but also with certain pentoses. This proves once again the necessity for a better understanding of the real conformation of these products in the injection solvent and mobile phase, in addition to the solvation state of both the stationary phases and products. This knowledge makes it possible to transform pragmatic information given by these statistical analyses into a rational interpretation.

CONCLUSIONS

The use of a particular system of detection allowed us to obtain a homogeneous set of data on the retention of sugars and polyols in HPLC and SFC. The statistical analysis of the results allows a better definition of the information contained in these data.

First, when we considered the retention mechanism and the separation abilities, neither of the two systems (HPLC, SFC) clearly differed and rather the two techniques are complementary.

However, the most significant fact is that the apparent complexity of the retention mechanisms of these products in these systems is simply caused by the combination of two independent factors, one directly connected to the number of accessible OH groups on the solutes and the other, well quantified, not physico-chemically interpreted.

The knowledge of the components of this retention allows a better choice of which chromatographic system to use for a particular problem. The joint use of HPLC and SFC with a light-scattering detector make it possible to solve frequent separation problems with this set of compounds. This aspect is currently being investigated, principally the interactions between the mobile and stationary phases and the products, to obtain a physico-chemical meaning of these two axes. This information will allow a correct prediction of the retention of a product on a new system using the retention of this product on other systems.

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