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Study of the selectivity of reversed-phase columns for the separation of polycarboxylic acids and polyphenol compounds

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Abstract

A systematic investigation was undertaken into the relative separation performance of five reversed-phase chromatography columns including some commercially new hybrid packed columns for a series of polycarboxylic acids and polyphenol compounds. Information theory (IT) and factor analysis (FA), together with a basic evaluation of retention information (band shape, retention factor and elution order) were used to compare four columns to a conventional C18 column. The results revealed very little difference in retention behaviour between the Phenomenex Aqua C18 column, the Waters XTerra RP C18 column, and the conventional Phenomenex Luna C18 column. However, there were notable differences in the retention processes between the Phenomenex Synergi polar-RP column, which is an ether-linked phenyl base with polar endcapping, and the Luna C18 column. The most significant differences were observed between the Luna C18 column and a Phenomenex Luna Cyano column. However, the limited degree of retention of the polycarboxylic acids and polyphenol compounds on the Luna Cyano column permits only limited use for the separation of these types of compounds. Overall, the Phenomenex Synergi polar-RP column exhibited the best performance for the separation of the test solutes compared to that of the conventional C18 column, with IT yielding an Informational Similarity of 0.99 and FA a moderate correlation coefficient of 0.70. The Phenomenex Synergi polar-RP column gave the best peak shape and offered substantial selectivity differences thereby providing a good alternative over the conventional C18 column for separating polycarboxylic acids and polyphenols.
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1. Introduction

Polycarboxylic acids and polyphenols are compounds commonly found in humic substances, wines, foods and natural and industrial waters. They affect both the capacity of soils and waters to hold cations and pollutants [1,2], and the taste and colour of wine [3,4] and food [5,6]. In the alumina industry, they affect the precipitation yield and crystal size of alumina [7].

Reversed-phase high-performance liquid chromatography (RPLC) is commonly used for the analysis of polycarboxylic acids and polyphenols and is chosen due to the wide range of applicability, convenience, and the ease with which the retention factor can be altered by manipulation of the mobile phase. To some degree, the selectivity of a chromatographic system can be varied by changing the solvent or pH, or by changing the experimental conditions such as temperature or the choice of the chromatographic column. The retention of polycarboxylic acids and polyphenols in RPLC is highly dependent on the degree to which these types of compounds are ionized and therefore on the pH of the mobile phase [8]. A number of studies have discussed the fundamental retention mechanisms of these types of compounds [8–10].

Since the inception of RPLC in the early 1950s, this technique has evolved into the most popular mode of liquid chromatography. The popularity of RPLC can be attributed largely to the

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resolving power of modern day column technology and to the ease with which the experiments can be undertaken. The supply of spherical, smaller particulate matter, new hybrid packing materials, optimised surface modifications, advanced column packing, and monolithic technology have all led to improvements in this separation technique [11,12]. In addition, due to advances in column technology the reproducibility of column packing and the mechanical stability of the packed bed are no longer an issue [13–19].

The most widely used column packing materials for modern liquid chromatography are bonded-phases, most commonly on a silica support. There are many types of reversed-phase bonded-phase stationary phases available today, the most common of which is the C18 or C8, with a C4 being particularly useful for protein separations. Cyano and nitrile columns are often used for more polar solutes and can be operated in reversed phase or normal-phase mode. The phenyl column often finds use as an alternative to the C18 column and can take advantage of π–π type interactions. In addition there are a multitude of speciality type reversed-phase stationary phases also on the market.

The continual search for new and improved stationary phases has led to new bonded phase supports that have alleviated some of the limitations of the traditional silica-based packing materials, such as improving the pH stability and minimising interactions with residual surface hydroxyl groups. The new hybrid packing materials now available, such as the Waters XTerra column, have the advantages of both silica and polymer packing materials. They are stable over a wider pH range, with a working range of pH 1–12 and have a high efficiency, mechanical strength and high temperature stability [8]. There have also been a number of advances in the design of stationary phases that can help retain polar analytes under highly aqueous conditions including hydrophilic, polar-endcapped and polar-enhanced stationary phases and polar-embedded alkyl phases [20].

Little work has been done in evaluating these new phases for separating polycarboxylic acids and polyphenols relevant to the industries noted above. In this study we compared the resolving power of five new generation stationary phases that were selected according to their potential differences in retention mechanism and hence possible changes in selectivity, band shape and performance. Using a probe of 24 standards comprised of a mixture of polycarboxylic acids and polyphenols, we compared the retention behaviour of five different columns using information theory (IT) and factor analysis (FA) to determine the degree of orthogonality and correlation between the four different columns and the conventional C18 column.

2. Theory

2.1. Information theory

The development and subsequent practical applications of IT, also known as communication theory, have grown dramatically since the code-breaking abilities during World War II were demonstrated. Scientific achievements and inventions in signal processing, advanced communication technology, and cybernetics are just a few IT applications of thousands.

Informational entropy, *I*, which is mathematically described below, is the measurable information content of a signal or band. In chemistry, IT has become a powerful tool for measuring and comparing the results of multiple methods of analysis in spectrometry, spectroscopy, chromatography, and other types of analysis. We have shown that IT is especially useful in chromatography where different types of modes of separation or chromatographic columns yield different amounts of information [21].

Firstly, to compensate for differences between the columns, such as manufacturer, base silica and particle size, the retention data is normalised according to Eq. (1) [22,23]. This yields scaled retention factors (X_a) that allow independent systems to be directly compared.

$$X_a = \frac{t_r - t_{r(0)}}{t_{r(f)} - t_{r(0)}} \quad (1)$$

In Eq. (1) $t_{r(i)}$ is the retention time of any solute (*i*), $t_{r(f)}$ is the retention time of the last eluting solute and $t_{r(0)}$ is the retention time of an unretained solute.

In IT, "information" is defined as a measure of the uncertainty of the incidence of an event [24]. In this instance, the "information" or informational entropy, *I*, is a measure of the reduction in the uncertainty about the nature of the substance and is a quantity that is measured in units of bits [22]. IT allows a mathematical evaluation of qualitative methods by the calculation of the expected or average amount of information obtained from an analysis [25]. Steuer et al. [23] compared HPLC, supercritical fluid chromatography (SFC) and capillary zone electrophoresis (CZE) for the analysis of drugs using IT to describe the informational orthogonality between the chromatographic systems. Huber et al. [26] applied IT to retention data to determine the optimal selection of gas chromatographic columns for the analysis of chemical warfare agents. IT has also been used to describe the "informational orthogonality" of two-dimensional chromatographic separations of complex mixtures [22,27].

The informational entropy of a measurement, *I*, whose unit of measure is the "bit", is a probabilistic quantity described by Eq. (2)

$$I = \sum_k (-p_k \ \log_2 p_k) \quad (2)$$

where p_k is the probability of the incidence of a single possible result, *k*, out of *n* possible results [22,24].

In this work, a statistical measure of the "Similarity" of the informational entropies was calculated from the retention behaviour of a set of compounds separated on a number of pairs of new generation RPLC columns. In other words, the "informational similarity" was calculated to determine the degree of data overlap between two dimensions. With the chosen compounds, informational similarity provided a numerical description of the informational orthogonality of the pairs of columns studied.

In the case of comparing two different types of chromatographic columns, the informational entropy is first calculated from the normalised retention time data for the first chromatographic column, $I(k)$, and then the second chromatographic column, $I(k, l)$, where *k* and *l* represent the two columns being

compared [22]. This is achieved by summing the informational entropy for each normalised retention time (X_a). The information of a correlated state can then be calculated using Eq. (3);

$$I(1, 2, 3, \ldots, j) = \sum_{j=1}^{n} I(j) - I(1; 2; 3 \ldots; j) \quad (3)$$

where $I(1; 2; 3; \ldots; j)$ is the mutual information that represents correlation [22].

To compare the informational entropy of the two chromatographic columns k and l, the fractional informational content, $h(k, l)$, is calculated by [22];

$$h(k, l) = 1 - \frac{I(k; l)}{I(k, l)} = 1 - \frac{\text{mutual information}}{\text{total 2D informational entropy}} \quad (4)$$

where $I(k; l)$ represents the mutual information between the chromatographic columns k and l, and $I(k, l)$ the total informational entropy. The informational similarity $H(k, l)$ of the two chromatographic columns can then be calculated using Eq. (5).

$$H(k, l) = [1 - h^2(k, l)]^{1/2} \quad (5)$$

The informational similarity of the two chromatographic columns, $H(k, l)$, is a measure of the degree of solute crowding of the sample components being separated on a normalised two-dimensional (2D) retention plot, with a value of unity indicating high solute crowding, while a value of zero no solute crowding and hence utilising all of the separation space [22,27].

A second tool used in comparing the retention behaviour of a set of compounds on different columns is the 2D retention plot where normalised retention factors, calculated using Eq. (1), of the sample components separated on column 1 are plotted against normalised retention factors of the sample components separated on column 2.

The percent synentropy (%synentropy), is another important IT parameter for determining the informational orthogonality (the divergent retention behaviour) between any two liquid chromatographic columns. The %synentropy is a measure of the 2D informational entropy that is clustered along the diagonal represented on the normalized retention plots and is used to determine the retention mechanism equivalency between two different columns [22].

This allows a comparison of the retention mechanisms of the systems under investigation, which in this study was the separation of the polycarboxylic acids and polyphenols. The %synentropy is calculated by dividing the informational entropy from data diagonally aligned on the normalised retention plots by the total 2D informational entropy [22].

2.2. Factor analysis

Factor analysis is a mathematical tool that is used to examine a wide range of data sets, taking large amounts of data and resolving them into distinct patterns of occurrence that can be used to indicate any type of correlation or relationship between variables [28]. A geometric approach to factor analysis as described by Liu and Patterson [29] and Gray et al. [27], can be applied to the chromatographic data. Using this geometric approach to FA correlations between different factors and their variables can be determined and then displayed in a geometric manner.

In the present study, pairs of chromatographic columns were compared resulting in two sets of retention data. Each set of retention data can be considered as an independent vector that represents the interaction between the solutes, mobile phase and stationary phase [29]. For example, if we compare two different columns, columns 1 and 2, two sets of retention data are generated for each column. This can be represented in a matrix form K;

$$K = \begin{vmatrix} k_{11} & k_{12} & \cdots & k_{1n} \\ k_{21} & k_{22} & \cdots & k_{2n} \end{vmatrix} \quad (6)$$

where k_{12} is the normalised retention data of the second component separated on the first column, 1. A geometric approach to factor analysis enables the calculation of correlations between a pair of vectors. In order to find the angles between the vectors the cross product of the normalised matrix represented in Eq. (6) needs to be formed where the entries in the matrix must be scaled so that their mean is zero and their variance one [29]. This results in a correlation matrix that has as its elements the cosines of the angles between vectors. The scaled matrix can be calculated using Eq. (7);

$$k'_{nn} = \frac{k_{ij} - m_i}{s_i} \quad (7)$$

where m_i is the mean of the original entries of the ith vector, s_i is the standard deviation of the original entries in the ith vector and k is the value of each retention time of the components. The new scaled matrix is represented by K' and the transposed matrix by K'^{T}. The sample by sample correlation matrix is then calculated by Eq. (8) [29];

$$C = \left(\frac{1}{N-1}\right) K'^{\mathrm{T}} K' \quad (8)$$

where N is the number of entries found in each data vector. A square diagonal matrix where $C_{12} = C_{21}$ is produced with the matrix acting as a quantitative measure of the vector correlations as seen in Eq. (9) [29].

$$C = \begin{vmatrix} 1 & C_{12} \\ C_{21} & 1 \end{vmatrix} \quad (9)$$

Eq. (9) is important as it enables us to define the degree of retention correlation between any two columns. For example when comparing the first column, 1, and the second column, 2, perfect correlation would exist when $C_{12} = 1$ and a truly orthogonal separation would be obtained when $C_{12} = 0$ as C_{12} is the cosine of any two unit length vectors [29]. The retention correlations, C, for each of the cases investigated here are reported in Table 2 and are a measure of the orthogonality of the two chromatographic columns. The correlation matrix generated using Eqs. (6)–(9) provides a measure of the interaction between the stationary phase and a chosen parameter for a group of solutes

and this information is useful in aiding the selection of a column or in the optimisation of a particular separation [29].

3. Experimental

3.1. Chemicals

HPLC-grade methanol was obtained from Mallinckrodt Australia. Formic acid (BDH) was analytical grade (98%) and was purchased from Sigma–Aldrich. The polycarboxylic acids and polyphenol standards used in this study were oxalic acid (**1**), catechol (**2**), glutaric acid (**3**), 3-hydroxybenzoic acid (**4**), 4-hydroxybenzoic acid (**5**), 2,3-dihydroxybenzoic acid (**6**), 3,4-dihydroxybenzoic acid (**7**), 2,6-dihydroxybenzoic acid (**8**), 2,5-dihydroxybenzoic acid (**9**), 3,5-dihydroxybenzoic acid (**10**), phthalic acid (**11**), 3-hydroxy-4-methoxybenzoic acid (**12**), 2,4,6-trihydroxybenzoic acid (**13**), 3,4,5-trihydroxybenzoic acid (**14**), 2,3,4-trihydroxybenzoic acid (**15**), suberic acid (**16**), 3,4-dimethoxybenzoic acid (**17**), sorbitol (**18**), 4-hydroxyisophthalic acid (**19**), 5-hydroxyisophthalic acid (**20**), 3,5-dimethoxy, 4-hydroxybenzoic acid (**21**), 1,2,4-benzenetricarboxylic acid (**22**), 1,2,3-benzenetricarboxylic acid (**23**) and 1,2,4,5-benzenetetracarboxylic acid dianhydride (**24**). They ranged in molecular weight from 90 to 218 Da and were purchased from Sigma–Aldrich Australia.

3.2. Equipment

A Waters LC system was used for all chromatographic separations and included a 717plus autosampler, 600 pump and controller, 2487 dual wavelength detector and Millennium software run using a Pentium 4 1.60 GHz processor. Five reversed phase columns were chosen for this work, a Phenomenex Luna C18 (150 mm × 4.6 mm, 5 μm), Phenomenex Luna Cyano (150 mm × 4.6 mm, 5 μm), Waters XTerra RP_{18} (150 mm × 4.6 mm, 5 μm), Phenomenex Aqua C18 (150 mm × 4.6 mm, 5 μm) and a Phenomenex Synergi Polar-RP (150 mm × 4.6 mm, 4 μm).

3.3. Sample preparation and chromatographic separations

A set of 24 standards containing a mix of polycarboxylic acids and polyphenols were used in this study. The polycarboxylic acids and polyphenol standards were dissolved in methanol/water (50:50, v/v). The concentration of the standards were 1 g L^{-1} except for oxalic acid, glutaric acid, 4-hydroxybenzoic acid, 2,6-dihydoxybenzoic acid, suberic acid and sorbitol that were made to a concentration of 2.5 g L^{-1} due to there low absorbance.

All separations on the five reversed-phase columns were carried out using different mobile phase mixtures consisting of methanol and 0.1% formic acid. The flow rate was set at 1.5 mL/min for all separations and 5 μL duplicate injections were performed for each standard. All reversed-phase columns were thermostated to 40 °C and UV detection was set at 220 nm.

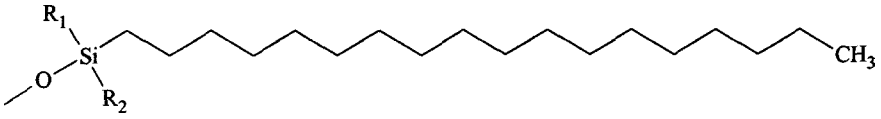
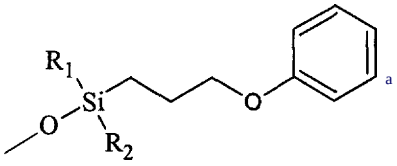
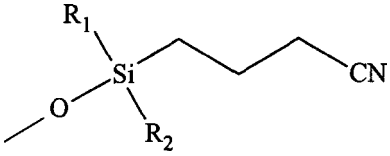
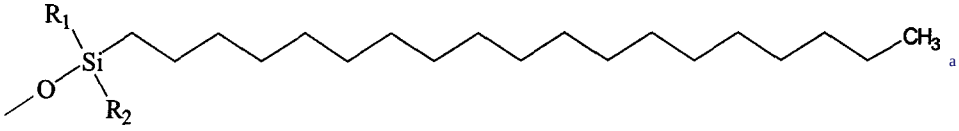
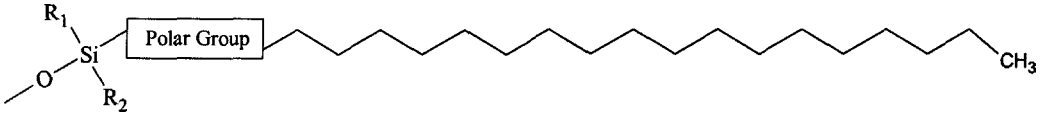
4. Results and discussion

Throughout this study the Phenomenex Luna C18 column was used as reference for comparing the Phenomenex Luna Cyano, Waters XTerra RP_{18}, Phenomenex Aqua C18 and Phenomenex Synergi Polar-RP columns. Table 1 summarises the bonded stationary phase supports used in this study. The reference Phenomenex Luna C18 column offers high quality silica that improves the structural stability of the silica particles and the stability of the column bed. It contains dense bonded phase coverage and enhanced endcapping to improve peak shape as well as an extended pH working range of 1.5–10. The Phenomenex Luna C18 was picked as the reference for this study due to the popularity of the C18 stationary phase. The Phenomenex Luna Cyano was selected because of the probable difference in retention mechanism compared to a C18 column and is made using the same silica base as the Luna C18, but with a pH working range of 1.5–7.0. The Phenomenex Aqua C18 column is packed using a bonded phase akin to the C18, but incorporates polar endcapping. It was chosen for the possible differences that this polar endcapping group could have on the retention mechanism of the polycarboxylic acids and polyphenol compounds. The Synergi polar-RP column was also manufactured by Phenomenex, and is essentially an ether-linked phenyl base with polar endcapping. The Synergi polar-RP phase was chosen for this study as it was specifically developed for separating extremely polar, aromatic analytes or mixtures, and is reported to improve the peak shape of both acids and bases and is stable in 100% aqueous conditions. Unfortunately, both the Aqua C18 and Synergi polar-RP phases are not available on Luna silica.

The Waters XTerra RP_{18} column was the only non-Phenomenex column employed in this study and was chosen due to its unique new generation stationary phase that provides a number of advantages. This new generation stationary phase material is a silicon organic/inorganic hybrid, made by reacting methyltriethoxysilane with tetraethoxysilane to form methylpolyethoxysilane. The methyl groups are incorporated into the silica backbone, improving the pH stability of the structure over that of conventional silica used in reversed phase columns. The silicon–oxygen backbone provides the packing with the mechanical strength normally associated with silica supports [8]. The XTerra RP_{18} stationary phase contains a monofunctional silane with an embedded carbamate group [8].

To compare the resolving power of the conventional C18 column with the other four columns and to determine the retention mechanism equivalency between these columns, a molecular probe consisting of 24 standards of polycarboxylic acids and polyphenols were used. For each column a method was developed to separate the 24 standards using a mobile phase mixture consisting of methanol and formic acid (0.1%). The mobile phase compositions for each of the columns were: methanol:formic acid (0.1%) (20:80; v/v) for the Luna C18, Aqua C18, and Synergi polar-RP columns: methanol:formic acid (0.1%) (10:90; v/v) for the Luna Cyano and the XTerra RP_{18} columns. These mobile phase compositions were chosen such that the minimum retention factor of the least resolved solute was 0.15 and the maximum retention factor of the most

Table 1
List of bonded stationary phase supports used in this study

Column	Structure
Phenomenex Luna C18	
Phenomenex Synergi polar-RP	 [a]
Phenomenex Luna Cyano	
Phenomenex Aqua C18	 [a]
Waters XTerra RP_{18}	

[a] With polar encapping.

strongly retained compound was 20. While we acknowledge a retention factor of 20 is excessive, such conditions were required in order to gain some degree of resolution between the less retained species. Gaining resolution for the early eluting compounds consequently increased the retention factor of the later eluting species.

Using the Phenomenex Luna C18 column as the reference for comparing each of the four chromatographic columns, IT and FA were then used to determine the correlation between the four different columns in comparison to the conventional C18 column. Table 2 lists the system attributes used to determine the measure of orthogonality or correlation for each of the columns studied compared with the Luna C18 column. This information was then used to determine if there were any differences in retention mechanism and hence possible changes in selectivity, band shape and performance between these columns.

Fig. 2 shows a normalised 2D retention plot of the transformed retention data for the comparison of the Luna C18 column with the Luna Cyano column. Each of the plotted points represents the normalised elution time of each of the standards. Using IT to assist in the interpretation of the retention data obtained we can make a comparison of the resolving power of each of the columns in relation to the Luna C18 column. In calculating the informational similarity, the standard compounds were considered to be separated when their X_a values differed by more than 0.01 X_a [22,27]. Table 2 reports the informational similarity between the Luna C18 column and the Luna Cyano column as 0.98 indicating a high degree of solute crowding (1 indicating complete solute crowding, conversely 0 indicating complete utilisation of the separation space). This can also be seen on examination of the normalised plot in Fig. 1.

As previously described, the %synentropy IT parameter is a measure of the Informational Entropy equally contributed from both column dimensions and therefore, a measure of the retention mechanism equivalency. In this study, the %synentropy was used as a numerical tool for the comparison of the retention mechanisms involved in the separation of the polycarboxylic acids and the separation of polyphenols with selected stationary phases. A %synentropy value of 0.0% indicates that there is no retention mechanism equivalency between the two chromatographic systems, whereas a %synentropy value

Table 2
System attributes used to determine the measure of orthogonality for the four column combinations in association with the Luna C18

Attributes	Luna C18/Luna Cyano	Luna C18/XTerra RP_{18}	Luna C18/Aqua C18	Luna C18/Synergi polar-RP
Informational similarity	0.98	1.00	0.99	0.99
Percent synentropy[a]	12.5	34.0	72.1	50.0
Correlation (C)	0.56	0.80	0.86	0.70

[a] A X_a factor variance of ±0.05 of the normalised retention factor (X_a) was assumed when using the informational entropy for the calculation of %synentropy.

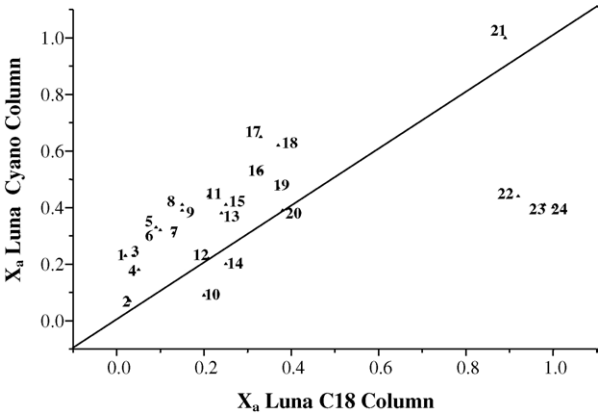


Fig. 1. Normalised plot of the Luna C18 column vs. the Luna Cyano column, number according to order of elution on the Luna C18.

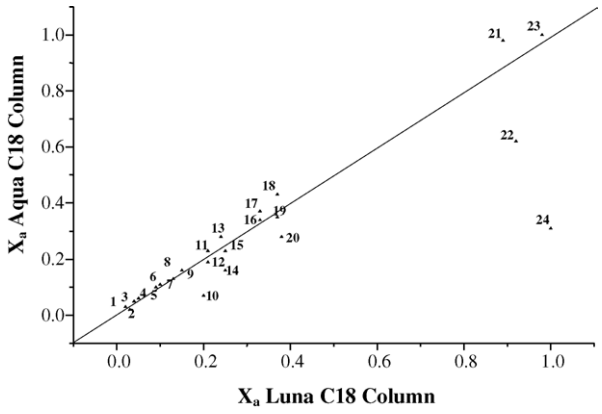


Fig. 3. Normalised plot of the Luna C18 column vs. Aqua C18 column, numbered according to elution order of the Luna C18.

of 100% indicates complete retention mechanism equivalency between the two chromatographic systems. When comparing the Luna C18 and Luna Cyano columns the %synentropy was found to be 12.5% indicating that there was only a limited degree of retention mechanism equivalency between the two columns.

FA can also be used to help in the comparison of the retention behaviour on different stationary phases. In this work, however, we have limited our application of FA to describe the retention equivalency of the two systems under comparison. The retention correlation coefficient between the Luna C18 and the Luna cyano columns was 0.56 and is given in Table 2. A value of 1.00 represents complete retention correlation; conversely a correlation of 0.0 indicates perfect orthogonality.

The Luna C18 column and the XTerra RP_{18} column performance are compared in Fig. 2. The normalised retention plot of this couple was similar to that of the Luna C18/Luna Cyano couple with both displaying a small degree of scatter. Although on closer inspection of the data scatter it could be seen that there were significant changes in selectivity for compounds on an individual basis. The Informational Similarity between the Luna C18 and the XTerra RP_{18} was 1.00, the highest degree of solute crowding of any of the column comparisons. There

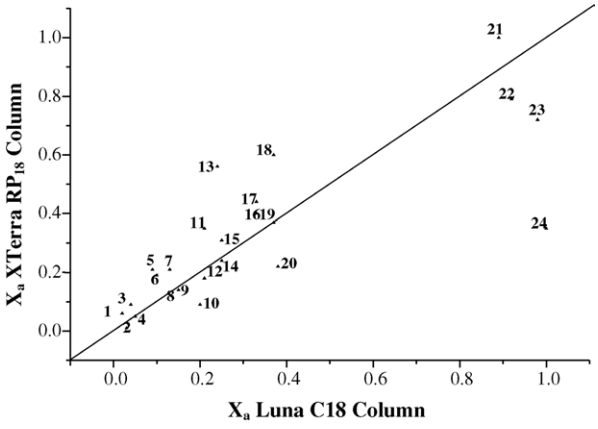


Fig. 2. Normalised plot of the Luna C18 column vs. Waters XTerra RP_{18} column, numbered according to elution order on the Luna C18.

was some retention mechanism equivalency between the Luna C18 and the XTerra RP_{18} as indicated by a %synentropy value of 34%, although it is still quiet low. The retention correlation coefficient was 0.80. The large difference between the %synentropy of 34% and the correlation coefficient of 0.80 is easily explained by remembering that the correlation coefficient is a measure of how well the retention data of each dimension match exactly, while the %synentropy is a measure of how well the retention data of each dimension cluster along the diagonal represented on the normalised retention plots.

Fig. 3 shows the normalised retention plot of the Luna C18 column versus the Aqua C18 column. On inspection of the retention plot it can be seen that the data was more ordered along the diagonal compared to the previous two column couples discussed, with a higher degree of data overlap and clustering with an informational similarity of 0.99. The %synentropy was 72.1% displaying the highest degree of retention mechanism equivalency for this study. The retention correlation coefficient was 0.86 and is approaching the value of unity, indicating a high degree of correlation between these two columns. This was in fact the highest reported in this study. Essentially the Luna C18 and Aqua C18 columns exhibited nearly the same chromatographic information.

The Luna C18 column and the Synergi polar RP column were compared and the normalised retention plot for these two columns is shown in Fig. 4. The retention plot once again showed a moderate degree of alignment along the diagonal with a %synentropy of 50.0%, which was less than the % synentropy for the Luna C18/Aqua C18 couple (72.1%). This is reflected in the retention correlation coefficient of 0.70 determined via FA, which represents a moderately high system correlation. An Informational Similarity value of 0.99 was experienced with these columns. This indicated a high degree of overlap and clustering between these columns.

Evaluating the statistical distribution of the compounds' retention in a hypothetical separation space only goes part way to assessing the performance of these columns with respect to that of the Luna C18 reference. In evaluating the IT and FA very little attention is paid to the true band shape of the eluting solutes. Peak tailing effects could, for example, transform an

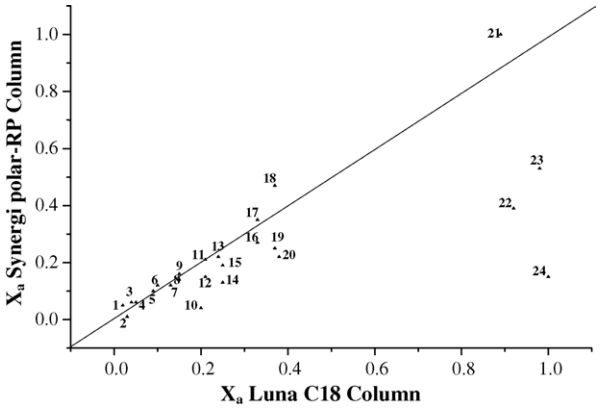


Fig. 4. Normalised plot of the Luna C18 column vs. Synergi polar-RP column, numbered according to the elution order of the Luna C18.

apparently useful separation system (as gauged by IT and FA) into one that is essentially impractical in reality. Hence, the study would be incomplete without paying due regard to the physical nature of the band shape. Table 3 reports the USP tailing factors for the five columns studied. When studying the peak shape of the standards on the five different columns, overall the Synergi polar-RP column gave the best results for the averaged USP tailing factors, followed by the XTerra C18 column. The Luna C18 compared to the other four columns gave the worst results for averaged peak shape with higher USP tailing factors. For example, the averaged USP tailing factor for all test compounds on the Synergi polar-RP column was 1.24 compared to 1.74 on the Luna C18.

There were a number of significant changes in elution order when comparing the Luna C18 column with the other four columns used in this study. Table 4 illustrates the elution order changes for the four chromatographic columns in comparison to the order of elution for the Luna C18 column. For instance, on the Luna C18 1,2,4-benzenetricarboxylic acid eluted 14th, but on the XTerra RP_{18}, Synergi polar-RP, Aqua C18 and Luna Cyano it eluted 13th, 9th, 11th and 4th, respectively. The Synergi polar-RP and Aqua C18 columns had similar elution orders to each other, but still differed when compared to the Luna C18 elution order. The XTerra RP_{18} and Luna Cyano columns differed the most from all the other columns showing significant re-ordering of all the standards.

The analysis of the four column couples using IT allowed us to examine the retention mechanism equivalency between the Luna C18 and the other four columns in this study. The values for the informational similarity were almost identical for all four comparisons approaching a value of 1.00 indicating high solute crowding. These appear to be in conflict with the other reported system attributes, but it is important to remember that Information Similarity is a measure of the crowding of the system, not a measure of the difference in retention behaviour. For that matter, the normalised retention plots showed a significant degree of solute grouping within the 2D separation plane, especially along the main diagonal for the Luna C18/Aqua C18 couple. This high degree of solute crowding masked some of the

Table 3
Summary of the retention factors and USP tailing factors for each of the chromatographic columns studied

Compound name	Retention factors (k') and USP tailing factors (k)									
	Luna C18		Luna Cyano		XTerra RP_{18}		Aqua C18		Synergi polar-RP	
	k	USP	k	USP	k	USP	k	USP	k	USP
Oxalic acid	0.582	2.086	0.175	1.018	0.166	1.154	0.257	2.537	0.297	1.972
Catechol	2.783	1.266	0.855	1.610	2.707	1.089	2.340	1.059	2.891	1.103
Glutaric acid	0.914	1.212	0.429	1.609	0.968	1.089	0.866	1.084	1.285	1.135
3-Hydroxybenzoic acid	5.940	1.391	1.296	1.650	7.695	1.176	5.120	1.144	5.338	1.127
4-Hydroxybenzoic acid	3.783	1.568	1.068	1.639	6.720	1.391	3.478	1.395	4.279	1.247
2,3-Dihydroxybenzoic acid	6.806	1.762	1.135	1.697	7.171	1.216	5.243	1.188	5.034	1.065
3,4-Dihydroxbenzoic acid	1.813	1.264	0.780	1.615	3.627	1.151	1.723	1.105	2.313	1.079
2,5-Dihydroxybenzoic acid (gentisic acid)	4.593	1.990	0.998	1.640	6.009	1.321	3.389	1.288	3.790	1.086
3,5-Dihydroxybenzoic acid	1.610	1.294	0.797	1.628	4.020	1.215	1.569	1.145	2.069	1.120
Phthalic acid	6.981	2.836	0.949	1.607	4.332	1.604	4.219	2.115	4.405	1.199
3-Hydroxy-4-methoxybenzoic acid	5.953	1.351	1.595	1.689	8.519	1.147	5.630	1.127	6.997	1.120
2,4,6-Trihydroxybenzoic acid	0.427	1.173	0.565	2.090	1.134	1.083	0.455	1.078	0.971	1.099
3,4,5-Trihydroxybenzoic acid	0.652	1.149	0.555	1.608	1.783	1.104	0.684	1.166	1.147	1.120
2,3,4-Trihydroxybenzoic acid	2.301	1.358	0.759	1.654	4.046	1.122	2.010	1.118	2.481	1.099
Suberic acid	17.778	1.570	1.005	1.634	13.985	1.548	15.036	2.607	10.496	1.358
3,4-Dimethoxybenzoic acid	16.202	1.347	2.440	1.698	19.357	1.184	14.724	1.279	19.935	1.282
Sorbitol	2.805	1.203	0.964	1.454	2.700	1.075	2.338	1.173	3.138	1.037
4-Hydroxyisophthalic acid	16.777	2.840	1.082	1.694	15.310	2.180	9.297	2.607	7.836	1.678
5-Hydroxyisophthalic acid	4.439	1.484	0.918	1.734	10.790	1.251	4.244	1.279	4.331	1.157
3,5-Dimethoxy-4-hydroxybenzoic acid	6.745	1.387	1.511	1.712	11.680	1.179	6.492	1.173	9.445	1.140
1,2,4-Benzenetricarboxylic acid	4.566	2.884	0.477	1.460	4.565	2.092	2.478	2.784	2.551	1.410
1,2,3-Benzenetricarboxylic acid	3.796	1.664	0.526	1.489	3.471	1.119	2.890	1.339	3.041	1.094
1,2,4,5-Benzenetetracarboxylic acid dianhydride	3.607	3.952	0.213	2.307	1.658	2.264	1.125	5.393	0.843	1.704
Average USP tailing factor	1.74		1.65		1.34		1.66		1.24	

Table 4
Elution order comparison of the Luna C18 column with the four chromatographic columns chosen for this study

Compound elution on Luna C18 Column	Elution order comparison			
	XTerra RP_{18}	Synergi polar-RP	Aqua C18	Luna Cyano
(**1**) 2,4,6-Trihydroxybenzoic acid	2	2	2	2
(**2**) Oxalic acid	4	10	1	10
(**3**) 3,4,5-Trihydroxybenzoic acid	1	1	3	4
(**4**) Glutaric acid	10	3	4	14
(**5**) 3,5-Dihydroxybenzoic acid	3	4	10	12
(**6**) 3,4-Dihydroxybenzoic acid	9	5	5	3
(**7**) 2,3,4-Trihydroxybenzoic acid	8	6	6	1
(**8**) Catechol	12	7	7	7
(**9**) Sorbitol	6	14	9	6
(**10**) 1,2,4,5-Benzenetetracarboxylic acid dianhydride	5	8	8	5
(**11**) 4-Hydroxybenzoic acid	7	12	14	13
(**12**) 1,2,3-Benzenetricarboxylic acid	20	24	12	20
(**13**) 5-Hydroxyisophthalic acid	14	9	15	9
(**14**) 1,2,4-Benzenetricarboxylic acid	15	15	11	24
(**15**) 2,5-Dihydroxybenzoic acid (gentisic acid)	24	11	20	8
(**16**) 3-Hydroxybenzoic acid	11	13	13	15
(**17**) 3-Hydroxy-4-methoxybenzoic acid	19	20	24	23
(**18**) 3,5-Dimethoxy-4-hydroxybenzoic acid	16	19	16	11
(**19**) 2,3-Dihydroxybenzoic acid	17	16	19	22
(**20**) Phthalic acid	13	17	17	19
(**21**)3,4-Dimethoxybenzoic acid	18	22	18	16
(**22**) 4-Hydroxyisophthalic acid	23	18	22	18
(**23**) Suberic acid	22	23	21	17
(**24**) 2,6-Dihydroxybenzoic acid	21	21	23	21

differences between each of the systems even though there were obvious visual differences. The values for the %synentropy on the other hand indicated that there were changes in the mechanisms by which these columns separate in comparison to the Luna C18 column. This was also supported by what we see on examining the normalised plots as well as the changes in the elution order of the standards as compared to the Luna C18.

Although the information provided by IT and FA is different, with IT describing the retention mechanism equivalency of the Luna C18 column compared to the other four columns and FA determining a correlation of the chromatographic retention data provided by each of the columns studied, both are related and can be combined to determine any equivalency or differences between the Luna C18 and the Luna Cyano, XTerra RP_{18}, Aqua C18 and Synergi polar-RP columns. The correlation coefficient, calculated using FA is impacted by the combined effects of solute crowding as reported by the informational similarity and the retention mechanism equivalency which is measured as the %synentropy, the clustering of the data along the diagonal represented on the normalised retention plots.

The informational similarity for the Luna C18/Luna Cyano couple was 0.98 reflecting a system where the solute crowding was high. On examination of the normalised retention plot in Fig. 1 we see that although the solute crowding was high, the majority of the bands were crowded to the left, above the diagonal. This scatter in the data resulted in the %synentropy being relatively low (12.5%). The three combined effects; high solute crowding, low %synentropy and the crowding of data away from the diagonal overall resulted in a low correlation coefficient of 0.56.

For the Luna C18/XTerra RP_{18} couple the informational similarity was higher at 1.00, but in this case the bands were more evenly distributed either side of the diagonal on the normalised retention plot (Fig. 2) with a higher degree of scatter. This resulted in a moderately low %synentropy value of 34.0%. The increase in the %synentropy and the informational similarity for the Luna C18/XTerra RP_{18} couple as compared to the Luna C18/Luna Cyano couple, as well as the re-distribution of the bands around the diagonal had an accumulative effect on the correlation coefficient and this resulted in a higher correlation of 0.80.

The informational similarity for both the Luna C18/Aqua C18 couple and the Luna C18/Synergi polar-RP couple was 0.99; however, the %synentropy for each couple differed significantly with the Luna C18/Aqua C18 reporting the highest selectivity similarity at 72.1% whereas the Luna C18/Synergi polar-RP couple only had a value of 50.0%. Although both systems exhibited high solute crowding, the difference in the %synentropy values was seen in the normalised retention plots for both these cases. The %synentropy value of 72.1% for the Luna C18/Aqua C18 couple was a result of greater alignment and order of the bands along the diagonal as seen in the normalised retention plot in Fig. 3. Whereas the Luna C18/Synergi polar-RP couple exhibited more alignment along the diagonal compared with the Luna C18/Luna Cyano and Luna C18/XTerra RP_{18} couples, the majority of bands fell below the diagonal in the normalised retention plot shown in Fig. 4, resulting in a lower %synentropy of 50.0%. Due to the higher %synentropy value of the Luna C18/Aqua C18 couple as well as the greater order in the distribution of the bands along the diagonal, the correlation

coefficient for this system was higher at 0.86 than that of the Luna C18/Synergi polar-RP couple, whose lower %synentropy and more chaotic distribution of the bands below the diagonal resulted in a lower correlation of 0.70.

When looking at the correlation coefficients, the Luna Cyano seemed to show the greatest difference in retention mechanism and in the chromatographic information provided. However, these results can be quite misleading. On examining the retention data for the Luna Cyano separation most of the standards eluted close to the void within the first three minutes of the separation displaying little resolution and an overall decrease in peak shape compared to the other columns studied. As such employing the Luna Cyano column for the separation of these types of compounds would yield limited separation. However, such a column may be of use for higher molecular weight species.

Out of all the columns studied the Luna C18/Aqua C18 gave the highest correlation coefficient of 0.86 followed closely by the Luna C18/XTerra RP_{18} couple that had a correlation of 0.80. Both these columns displayed a high degree of retention mechanism equivalency and provided similar chromatographic information to the Luna C18 column.

The Luna C18/Synergi polar-RP couple gave the best results with a moderate correlation coefficient of 0.70. Overall the Synergi polar-RP column recorded the best results in terms of peak shape as well as a low degree of retention mechanism equivalency and provided different chromatographic information as compared to the Luna C18 column.

5. Conclusion

Analysis of retention information using FA and IT has demonstrated that the Phenomenex Aqua C18 column and the conventional Phenomenex Luna C18 column statistically exhibit the highest degree of retention mechanism equivalency for a series of polycarboxylic acids and polyphenol compounds. Hence, the chromatographic information produced with each column was almost identical. The Luna C18 column exhibited the lowest overall performance in terms of average peak shape followed by the Phenomenex Aqua C18 column. In general, the Phenomenex Aqua C18 offered little as an alternative to that of a conventional Luna C18 column in terms of the separation of polycarboxylic acids and polyphenol compounds.

Although the differences in selectivity and retention mechanism equivalency were maximised between the Luna C18 and Luna Cyano columns, the overall retention of the test solutes employed in this study on the Luna Cyano column was limited with most of the analytes eluting near the column void volume. Consequently the Luna Cyano column is not recommended for the separation of polycarboxylic acids and polyphenol compounds under the conditions tested.

Overall the Phenomenex Synergi polar-RP column displayed the best performance for the separation of the test solutes. Band shapes generally exhibited less peak tailing and in fact, IT and FA indicated that there was a moderate correlation coefficient of 0.70 compared to that of the Luna C18 column. Consequently, the Phenomenex Synergi polar-RP column would provide a good alternative for separating polycarboxylic acids and polyphenol compounds in contrast to the conventional C18 column.

References

[1] M.A. Wilson, G.S. Kannangara, D.E. Smeulders, J. Proc. R. Soc. NSW 133 (2000) 71.
[2] Asan, I. Isildak, J. Chromatogr. A, 988 (2003) 145.
[3] M. Lopez, F. Martýnez, C. Del Valle, C. Orte, M. Miro, J. Chromatogr. 22 (2001) 359.
[4] C. Mongay, A. Pastor, C. Olmos, J. Chromatogr. A 736 (1996) 351.
[5] I. Molnar-Perl, J. Chromatogr. A 891 (2000) 1.
[6] K. Robards, J. Chromatogr. A 1000 (2003) 657.
[7] T.J. Whelan, A.V. Ellis, G.S.K. Kannangara, C.P. Marshall, D. Smeulders, M.A. Wilson, Rev. Chem. Eng. 19 (2003) 431.
[8] U.D. Neue, C.H. Phoebe, K. Tran, Y. Cheng, Z. Lu, J. Chromatogr. A 925 (2001) 49.
[9] M.G. Kiseleva, P.N. Nesterenko, J. Chromatogr. A 898 (2000) 23.
[10] M. Waksmundzka-Hajnos, J. Chromatogr. B 717 (1998) 93.
[11] R.E. Majors, Am. Lab. (October) (2003) 46.
[12] F. Rabel, K. Cabrera, D. Lubda, Am. Lab. (December) (2000) 20.
[13] U.D. Neue, HPLC Columns: Theory, Technology and Practice, Wiley-VCH, New York, 1997.
[14] M. Kele, G. Guiochon, J. Chromatogr. A 830 (1999) 41.
[15] M. Kele, G. Guiochon, J. Chromatogr. A 830 (1999) 55.
[16] M. Kele, G. Guiochon, J. Chromatogr. A 855 (1999) 423.
[17] M. Kele, G. Guiochon, J. Chromatogr. A 869 (2000) 181.
[18] M. Kele, G. Guiochon, J. Chromatogr. A 913 (2001) 89.
[19] M. Kele, G. Guiochon, J. Chromatogr. A 960 (2002) 19.
[20] R.E. Majors, M. Przybyciel, LC–GC Eur. (December) (2002) 2.
[21] F. Dupuis, A. Dijkstra, Anal. Chem. 47 (1975) 379.
[22] P.J. Slonecker, X. Li, T.H. Ridgway, J.G. Dorsey, Anal. Chem. 68 (1996) 682.
[23] W. Steuer, I. Grant, F. Erni, J. Chromatogr. 507 (1990) 125.
[24] J.F.K. Huber, E. Kenndler, G. Reich, J. Chromatogr. 172 (1979) 15.
[25] D.L. Massart, B.G.M. Vandeginste, S.N. Deming, Y. Michotte, L. KaufmanL., Chemometrics: A Textbook, Elsevier, Amsterdam, London, UK, 1988.
[26] J.F.H. Huber, E. Kenndler, G. Reich, W. Hack, J. Wolf, Anal. Chem. 65 (1993) 2903.
[27] M. Gray, G.R. Dennis, P. Wormell, R.A. Shalliker, P. Slonecker, J. Chromatogr. A 975 (2002) 285.
[28] R.J. Rummel, J. Conflict Resolut. (1967) 444.
[29] Z. Liu, D.G. Patterson, Anal. Chem. 67 (1995) 3840.