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Utilising retention correlation for the separation of oligostyrenes by coupled-column liquid chromatography

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Abstract

The separation of $n = 2$–5 *n*-butyloligostyrenes has been illustrated by reversed-phase reversed-phase (RP–RP) coupled-column liquid chromatography. The coupled-column separation has been achieved by use of a C_{18} column with methanol as the mobile phase followed by a DiamondBond C_{18} column with acetonitrile (ACN) mobile phase. The DiamondBond C_{18} is a hybrid carbon clad zirconia (CCZ)–C_{18} stationary phase. Unlike a C_{18}–carbon clad zirconia two-dimensional liquid chromatographic system, which is orthogonal, the C_{18} and DiamondBond C_{18} columns combination exhibit correlations based upon the molecular weight of *n*-butyloligostyrenes. Using an alternative strategy to two-dimensional liquid chromatography, the molecular weight dependence displayed by both the C_{18} column and DiamondBond C_{18} has been used to increase throughput or decrease analysis time in the analysis of the *n*-butyloligostyrenes. However, this is at the expense of a portion of the two-dimensional peak capacity displayed by the C_{18}–carbon clad zirconia system.

Keywords: *n*-Butyloligostyrenes; Liquid chromatography; C_{18} column

1. Introduction

Two-dimensional liquid chromatography is not a new technique, although interest in its development has only recently increased due to the demands of analytical surety and purification levels. Two-dimensional liquid chromatography is a broad category and includes certain coupled-column techniques, cross-fractionation, heart-cutting and comprehensive methods [1–7].

Multidimensional liquid chromatographic systems are generally designed so that the selectivity difference between the separation dimensions is maximised [8–10]. As retention correlation between each dimension decreases, the peak capacity and hence the amount of chromatographic information gained also increases with a subsequent decrease in the probability of co-eluting components [11]. The term orthogonality is widely used in reference to two-dimensional liquid chromatographic systems. Orthogonality has the implication that an attribute (i.e. chromatographic selectivity) is totally divergent. The chromatographic community has given the term orthogonality the connotation of being a measure of divergence.

Depending upon the final aim of the experiment, chromatographic peaks or samples from the first chromatographic dimension are transferred to the second dimension in a consecutive fashion [12–14]. For example, in the transportation of two first dimension samples (S1 and S2) to the second dimension, S2 is not transported to the second dimension until the completion of the separation of the components contained within S1 on the second dimension. In this manner, wrap around [15] or co-elution of peaks from consecutive first dimension sections are avoided in the second dimension. If sections transported to the second dimension were allowed to overlap, an increase in the chaos of the second dimension chromatogram would occur and software used to plot contour maps would provide erroneous results.

In a number of recent publications, we explored the merits of multidimensional reversed-phase reversed-phase (RP–RP) HPLC [10,14–17]. In these communications, we illustrated the divergent retention behaviour that was apparent between conventional C_{18} columns and new generation carbon clad

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zirconia (CCZ) columns for the separation of polystyrene oligomers and diastereoisomers. Conventional C_{18} columns could separate these oligomeric species according to molecular mass with very little diastereoisomer selectivity if a methanol mobile phase was employed [10]. If an acetonitrile mobile phase was used the diastereoisomer selectivity was expressed to a greater degree [18], but not to the extent achievable on carbon clad zirconia, which displayed a very high degree of shape selectivity and very little molecular weight dependence. Hence, there was a vast difference in the retention of these species on these two types of columns. Furthermore, both columns (especially the C_{18}) were sensitive to the type of end group and *n*-butyl-, *sec*-butyl- and *tert*-butylpolystyrenes could be differentiated [17].

Although the degree of orthogonality observed between retention processes on each of these different stationary phases was high, bonding well for a successful coupling of these systems, the molecular weight retention independence observed on the CCZ column resulted in experimental limitations. Namely, the wrap-around effect [15] was difficult to avoid. That is, once a particular molecular weight component had been transferred from the C_{18} column to the CCZ column, another heart-cut fraction could not be loaded onto the CCZ column until all the components from the previous fraction had eluted. In order to perform comprehensive methods of analysis, the experimental protocol relied on very large differences in flow rates between each of the two dimensions and/or relied on the usage of fortuitous vacant regions within the chromatogram in the second dimension of the components of the first injection for separation of components of a subsequent injection. This second strategy could result in chaotic band displacement.

In this particular study, we addressed the problem of molecular weight independence in the second dimension of CCZ columns by employing instead a hybrid CCZ column; one which contains chains of C_{18} interdispersed throughout the CCZ matrix. The results show that depending upon the required aim, correlation between chromatographic systems can be used as an advantage in order to speed up analysis time. This is accomplished by choosing an alternate approach to the schedule of transporting first dimension samples to the second dimension. We used as our sample base, a complex mixture of *n*-butyloligostyrenes which had two to five styrene configurational repeating units. Examples of the $n = 2$–5 oligostyrenes used in this study are shown in Fig. 1. The sample base can be described in terms of two distinct sample attributes. Firstly, according to variation in molecular mass; and secondly, by variation in the stereochemistry of the oligostyrenes.

2. Experimental

2.1. Chemicals

HPLC-grade methanol and acetonitrile (ACN) were obtained from Mallinckrodt, Australia. *n*-Butylpolystyrene standards with a molecular mass of 580 was purchased from Polymer Labs. The molecular masses of the members of the oligomer series were determined using mass spectroscopy [19]. The $n = 2$–5 oligomer from the polystyrene standard was isolated by fractionation using methods previously described [10]. A Phenomenex (Pennant Hills, Australia) phenosphere (150 mm × 4.6 mm, 5 μm particle diameter) column was used in the first separation dimension. A DiamondBond C_{18} column (100 mm × 4.6 mm) was used as the second separation column and was supplied by ZirChrom Separations (Anoka, MN, USA). Carbon clad zirconia (3 μm particle diameter) was purchased from ZirChrom Separations and packed into columns (30 mm × 4.6 mm) using methods previously described [20]. The stationary phase material was used as supplied from the manufacturer.

2.2. Equipment

All chromatographic experiments were conducted using a Shimadzu LC system (Shimadzu, Rydalmere, Australia) incorporating a LC-10ATVP pumping system, a SIL-10ADVP

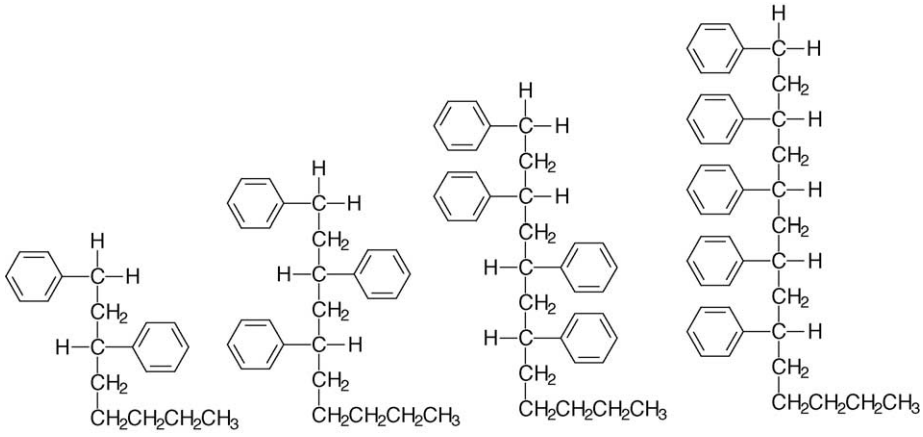

Fig. 1. Diagrammatic representations of $n = 5$ oligostyrenes showing structural and stereochemical variation.

auto injector, SPD-10AVP UV detector, SCL-10AVP system controller and Shimadzu Class-VP version 5.03 software on a Pentium II 266 personal computer. Column switching was achieved using six-port two-position switching valves fitted with micro-electric two position valve actuators (Valco Instruments, Houston, TX, USA). Valve switching was controlled using Shimadzu SCL-10AVP system controller and Shimadzu Class-VP version 5.03 software. Two additional UV–vis detectors (Waters 286; Waters Associates, Milford, MA, USA) were employed to record chromatographic information in the first and second dimensions. A Waters 510 HPLC pump was used to control flow in the second dimension. Data acquisition was achieved using a Lawson Labs model 203 serially interfaced 20-bit data acquisition system with a custom ±5 V gain range operated at 5 Hz (Lawson Labs., Malvern, PA, USA). Columns were packed using a Haskel air-driven fluid pump (Haskel International, Burbank, CA, USA).

2.3. Chromatographic separations

n-Butyloligostyrene standards ($n = 2$–5) were dissolved in methanol. All separations were conducted using mobile phases as described in the text. Mobile phases were sparged with helium. Flow rate for the C_{18} column was 1.0 mL/min as indicated in the text, the flow rate using the DiamondBond C_{18} was 1.5 mL/min and the flow rate for the carbon clad zirconia column was 2.0 mL/min. The carbon clad zirconia column was thermostated at 30 °C. Injection volumes were 10 μL. UV detection was at 262 nm.

3. Results and discussion

In order to minimise replication with other experimental results reported previously, we restrict our study here to a coupled system in which the first dimension is a C_{18} column running a methanol mobile phase, while the second dimension is a DiamondBond C_{18} column running an acetonitrile mobile phase. A two-dimensional liquid chromatographic system was used to perform the coupled-column separations illustrated in this manuscript. This is shown in Fig. 2. The system consists of two chromatographic columns that are ultimately connected by four two-position switching valves. Sample loops located on valves 2 and 3 are used to sample eluent from the first chromatographic column and to transport this sample to the second column according to the switching valve positions. Operation of the switching valves has been described previously [14].

In accordance with the previous studies [10,16], we have used information theory and factor analysis to assess the suitability of these two dimensions when coupled in a multidimensional system. The results reported in Table 1 compare the separation performances of the C_{18}–Diamond system to that of the C_{18}–CCZ system in the separation of *n*-butylpolystyrene. The informational similarity is a measure

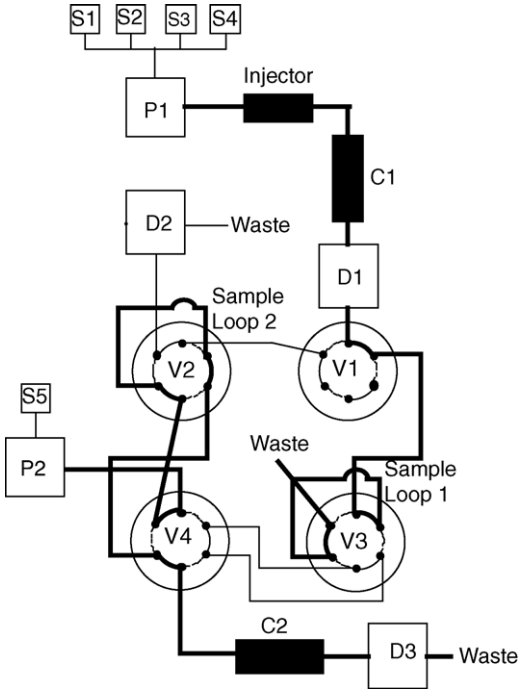

Fig. 2. System configuration of the two-dimensional liquid chromatographic system.

of solute crowding on a normalised two-dimensional retention plot [9]. Values range between zero and one with a value of one representing the highest level of solute crowding. The percentage synentropy, also determined using information theory, is defined as a measure of retention mechanism equivalency [9]. A value of 100% indicates that two chromatographic systems are 100% equivalent. The retention correlation coefficient is a direct measure of orthogonality [11]. Values range between 0 and 1, with a value of 0 indicating that two separation systems are totally orthogonal. The peak spreading angle is calculated from the correlation coefficient and is a relative measure of utilization of the theoretical two-dimensional peak capacity [11].

As the results in Table 1 show, the informational similarities for both the C_{18} (methanol)–CCZ (acetonitrile) and C_{18} (methanol)–DiamondBond C_{18} (acetonitrile) are quite

Table 1
System attributes used to determine the measure of two-dimensional orthogonality for each of the two-dimensional RP–RP systems evaluated

Attribute	C_{18} (MeOH)–Diamond C_{18} (ACN)	C_{18} (MeOH)–CCZ (ACN)
Informational similarity	0.82	0.79
Percentage synentropy	6.9	6.7
Peak spreading angle	47.6	64.8
Practical peak capacity (N_p)	37.5	45.3
Correlation (c)	0.675	0.426
Usage (%)	67	81
Resolved components (/15)	14	15

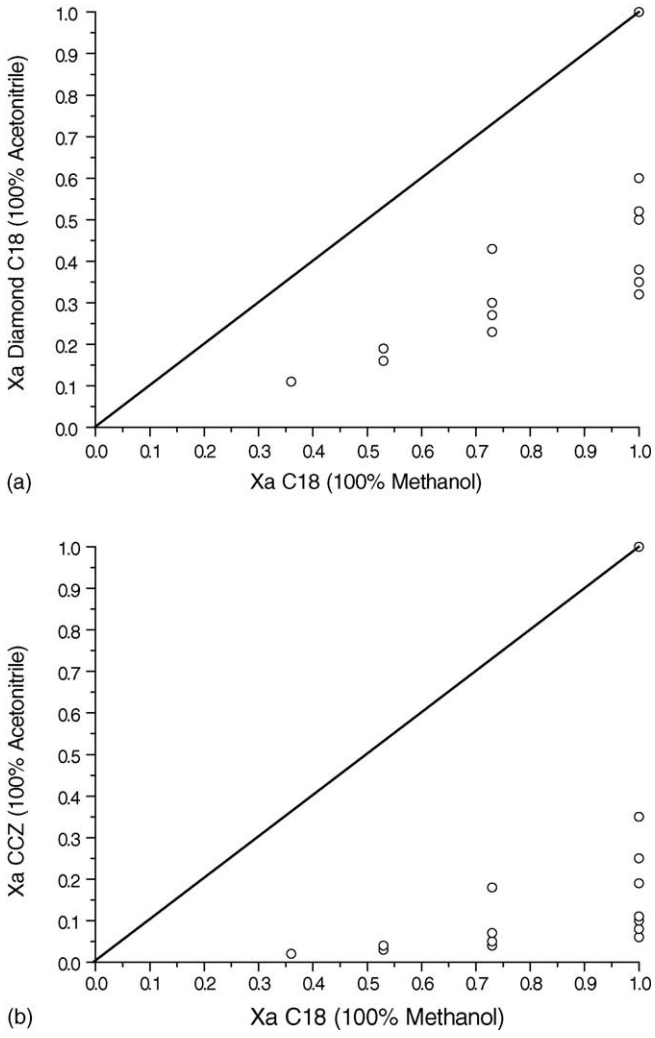

Fig. 3. Normalised two-dimensional retention plot in the separation of the $n = 2$–5 oligostyrene sample for: (a) a C_{18} (methanol)–DiamondBond C_{18} (acetonitrile) theoretical two-dimension chromatographic system; (b) a C_{18} (methanol)–CCZ (acetonitrile) theoretical two-dimension chromatographic system.

similar. However, the peak spreading angle is higher and the retention correlation coefficient is significantly lower for the system that employs a CCZ column and acetonitrile mobile phase in the second dimension. This is clearly illustrated in Fig. 3a where the normalised retention factors (X_a) for the *n*-butyloligostyrenes on the C_{18} (methanol)–DiamondBond C_{18} (acetonitrile) system show a distinct trend in the direction of the main diagonal, whereas for the C_{18} (methanol)–CCZ (acetonitrile) system (Fig. 3b) this trend is absent. As expected from these plots, the percentage usage of the theoretical two-dimensional peak capacity is higher for the CCZ system (81%) compared to the DiamondBond C_{18} system (67%).

The high orthogonality of the C_{18} (methanol)–CCZ (acetonitrile) system has been previously described in detail [10,16] and comes about through the distinctly different sample attributes that are expressed on the C_{18} and CCZ surfaces. The C_{18} column separates the components of the *n*-butylpolystyrene mixture on the basis of molecular mass, whereas the carbon clad zirconia column separates the oligostyrenes on the basis of their stereochemistry with little dependence on molecular mass. The hybrid DiamondBond C_{18} (acetonitrile) column still maintains much of the shape selectivity of the CCZ column, but retention of oligostyrenes is also influenced by molecular mass as shown by the increase in the correlation along the main diagonal in Fig. 3a.

Separation of the $n = 4$ and 5 *n*-butyloligostyrenes is shown in Fig. 4(a–d) for comparison of separation quality. The peak shape and band resolution is significantly superior on the CCZ column even though the data points in the normalised retention plots in Fig. 3 show improved separation between

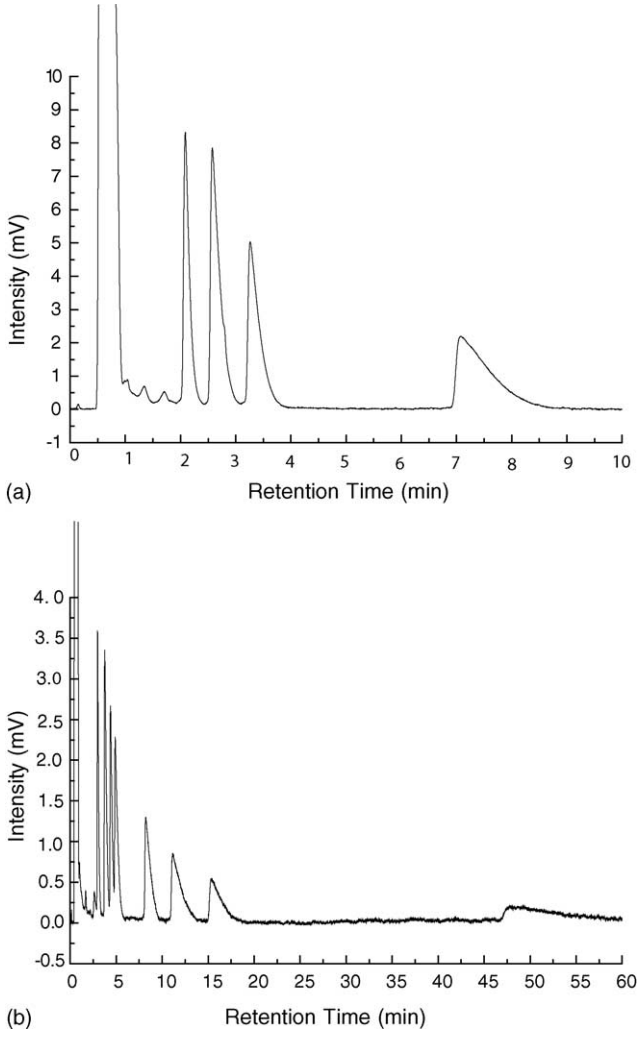

Fig. 4. (a) Chromatogram of the $n = 4$ *n*-butyloligostyrene sample following separation on the C_{18} (methanol)–CCZ (acetonitrile) chromatographic system. *C*1 mobile phase: 100% methanol at 1.0 mL/min, injection volume 10 μL. *C*2 mobile phase: 100% acetonitrile at 2.0 mL/min and thermostated to 30 °C. (b) Chromatogram of the $n = 5$ *n*-butyloligostyrene sample. Conditions as for Fig. 3a. (c) Chromatogram of the $n = 4$ *n*-butyloligostyrene sample following separation on the DiamondBond C_{18} column. Mobile phase: 100% acetonitrile at 1.5 mL/min, injection volume 10 μL. (d) Chromatogram of the $n = 5$ *n*-butyloligostyrene sample following separation on the DiamondBond C_{18} column. Conditions as for Fig. 3c.

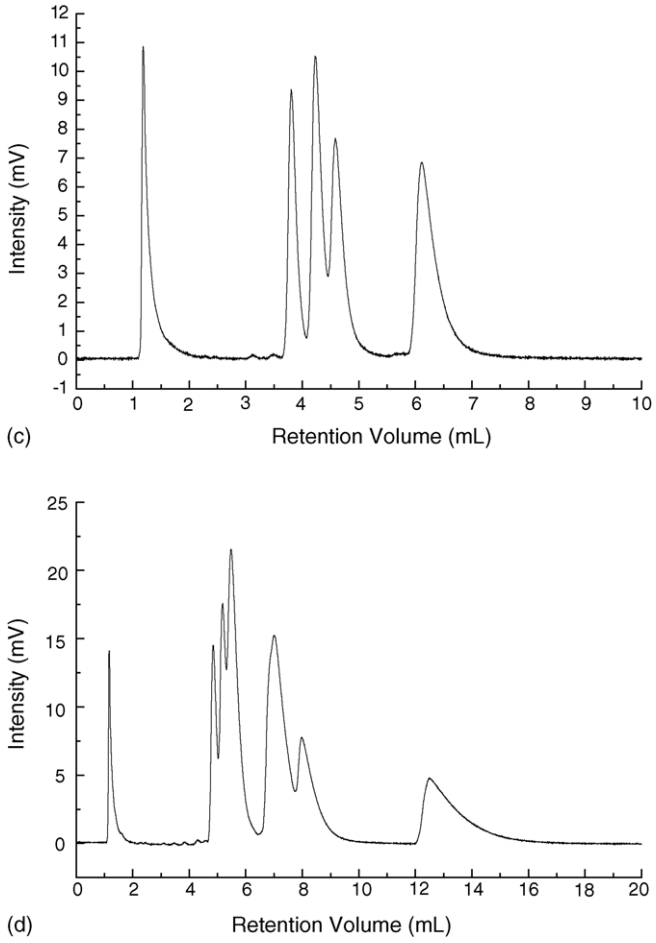

Fig. 4. (*Continued*).

peak maxima on the Diamond column. This occurrence is simply due to compression of the *y*-axis values for the CCZ (acetonitrile) system in Fig. 3b as a result of the long separation time of the last eluting *n*-butyl $n = 5$ diastereoisomer (Fig. 4b), compared to the same component in Fig. 3a using the DiamondBond C_{18} (acetonitrile) system, which exhibits significantly less retention (Fig. 4d) as is seen by comparing the chromatograms for the separation of the *n*-butyl $n = 5$ diastereoisomers shown in Fig. 4(b and d). However, depending upon the type of sample that may be tested, band tailing may not be as significant as is the case for these oligostyrenes and then the DiamondBond C_{18} column may be of benefit in reducing analysis time.

Multidimensional liquid chromatographic systems are designed to maximise dimensional orthogonality [21]. Second dimension chromatographic peaks may then be located at any time within the specified time-window that components from the first dimension are allowed in the second dimension. If selectivity factors in the second dimension distribute peaks across the full peak capacity of the second dimension, this increases the likelihood of co-elution of second-dimension

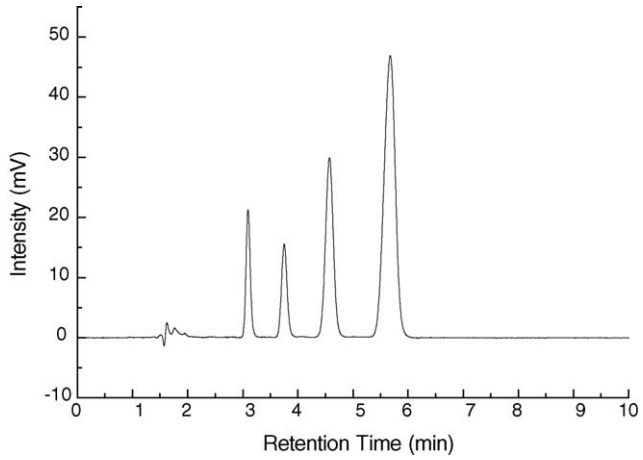

Fig. 5. Chromatogram illustrating the separation of the $n = 2$–5 oligostyrene sample on a C_{18} column. Mobile phase: 100% methanol, flow rate 1.0 mL/min, injection volume 10 μL.

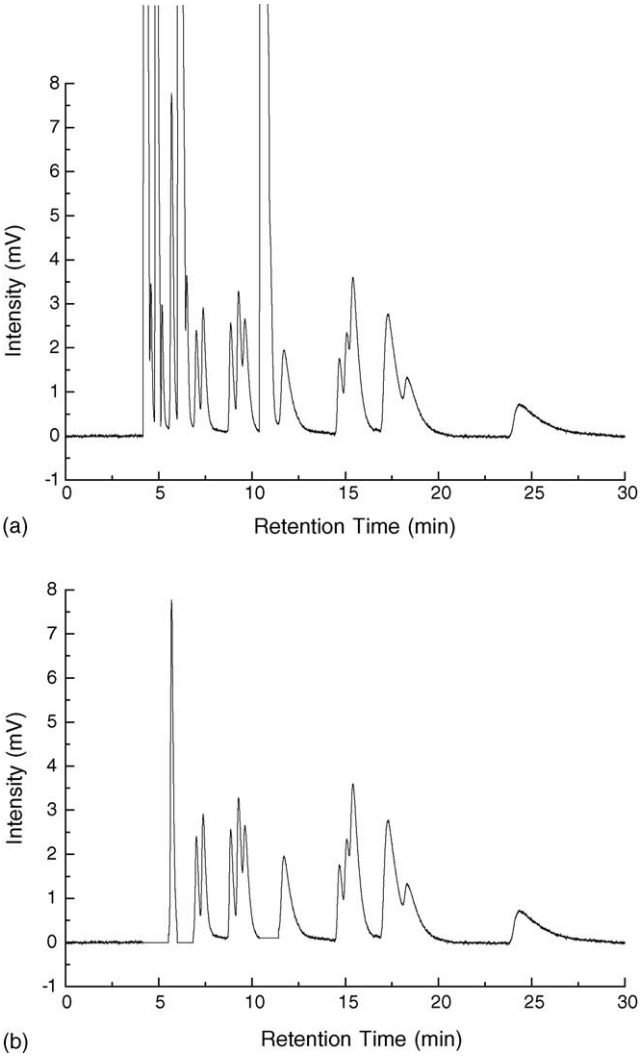

Fig. 6. (a) Chromatogram of the $n = 2$–5 oligostyrene sample following separation on the C_{18} (methanol)–DiamondBond C_{18} (acetonitrile) chromatographic system. *C*1 mobile phase: 100% methanol, flow rate: 1.0 mL/min, injection volume 10 μL. *C*2 mobile phase: 100% acetonitrile, flow rate: 1.5 mL/min. (b) Chromatogram as for Fig. 5a with solvent peaks base-line subtracted.

peaks from different first-dimension samples if each sample is not allowed enough time for complete separation and subsequent elution from the second dimension. Using the molecular mass dependency that both dimensions of the C_{18} (methanol)–DiamondBond C_{18} (acetonitrile) system exhibit, consecutive samples can be transferred to the second dimension in a faster manner. After the first sample is transported to the DiamondBond C_{18} column the second sample can be injected while the first sample is still on the DiamondBond C_{18} column and so on. The positive aspect of this is that components from each consecutive injection do not catch up and overlap with the previous sample that was transferred to the DiamondBond C_{18} column.

Separation of the $n = 2$–5 *n*-butyloligostyrene sample on a C_{18} column with a methanol mobile phase is shown in Fig. 5. The coupled-column separation of the oligomers and diastereoisomers of the sample, following separation on both the C_{18} and the DiamondBond C_{18} columns is illustrated in Fig. 6a. Fig. 6b represents the same chromatogram as that depicted in Fig. 6a, except that solvent peaks associated with solvent transport from the first dimension to the second dimension have been baseline subtracted. For comparison, a separation of the same sample from the C_{18}–CCZ column combination is shown in Fig. 7 (second dimension only). Clearly the advantage of the DiamondBond C_{18} column as a second dimension column is the great reduction in the analysis time for the sample. However, this comes with the sacrifice of some resolution, as predicted by information theory and factor analysis. Consequently, the analyst must have a clear objective in mind when choosing an appropriate combination for separation of complex samples. Factors such as speed, total sample analysis versus partial sample analysis, or perhaps the preparative collection of specific compounds eluting in the second dimension may influence this decision. The importance of each of these different objectives can be argued, but in reality, they will differ for each analyst. As a consequence this study has shown alternative separation strategies that may be implemented by separation scientists who are prepared to explore new types of column selectivities.

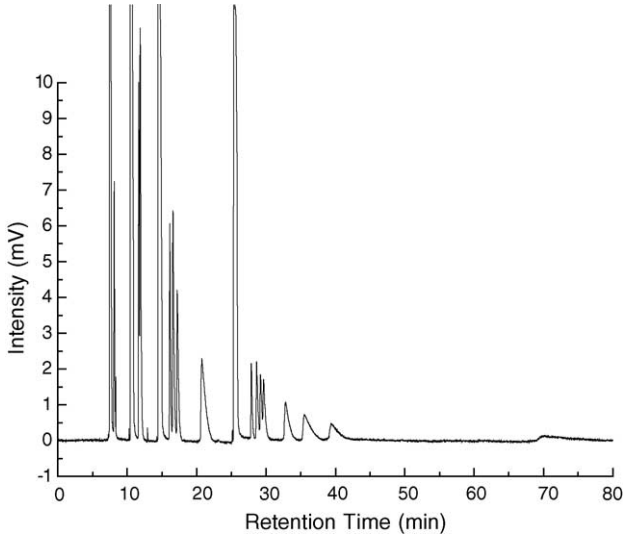

Fig. 7. Chromatogram of the $n = 2$–5 oligostyrene sample following separation on the C_{18} (methanol)–CCZ (acetonitrile) chromatographic system. *C*1 mobile phase: 100% methanol, flow rate: 1.0 mL/min, injection volume 10 μL. *C*2 mobile phase: 100% acetonitrile, flow rate: 2.0 mL/min, thermostated at 30 °C.

4. Conclusion

The results have revealed that by utilising the correlation between the C_{18} (methanol)–Diamond (acetonitrile) system, oligostyrenes eluting from the C_{18} column can be transported to the DiamondBond C_{18} column without the need to wait for all components from one injection to elute from the second dimension. This alternative strategy has allowed the separation of a complex mixture of *n*-butyloligostyrenes with higher throughput and no subsequent wrap-around effects than would be observed if the same strategy was employed when a CCZ column was used in the second dimension [15]. A short-fall of this approach was, however, a reduction in the peak capacity of the system and a decrease in the useable separation space. Hence, a lower number of diastereoisomers were resolved in the system employing the DiamondBond C_{18} column. This may place restrictions on specific applications of this technique, such as fingerprinting methodologies, when correlation between systems increases to the point where uniqueness of retention may be compromised.

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References

[1] F. Erni, R.W. Frei, J. Chromatogr. 149 (1978) 561.
[2] G. Glöckner, J.H.M. van den Berg, N.L.J. Meijerink, T.G. Scholte, R. Koningsveld, J. Chromatogr. 317 (1984) 615.
[3] A. Walhagen, L.-E. Edholm, J. Chromatogr. 473 (1989) 371.
[4] M.M. Bushey, J.W. Jorgenson, Anal. Chem. 62 (1990) 161.
[5] R. Agbaria, H. Ford Jr., J.A. Kelley, F. Xie, P. Politi, J.L. Grem, D.A. Cooney, V.E. Marquez, C.J. Allegra, D.G. Johns, Anal. Biochem. 213 (1993) 90.
[6] O.P. Haefliger, Anal. Chem. 75 (2003) 371.
[7] N. Tanaka, H. Kimura, D. Tokuda, K. Hosoya, T. Ikegami, N. Ishizuka, H. Minakuchi, K. Nakanishi, Y. Shintani, M. Furuno, K. Cabrera, Anal. Chem. 76 (2004) 1273.
[8] J.C. Giddings, J. Chromatogr. A 703 (1995) 3.
[9] P.J. Slonecker, X. Li, T.H. Ridgway, J.G. Dorsey, Anal. Chem. 68 (1996) 682.

[10] M.J. Gray, G.R. Dennis, P.J. Slonecker, R.A. Shalliker, J. Chromatogr. A 1015 (2003) 89.
[11] Z. Liu, D.G. Patterson Jr., M.L. Lee, Anal. Chem. 67 (1995) 3840.
[12] R.E. Murphy, M.R. Schure, J.P. Foley, Anal. Chem. 70 (1998) 1585.
[13] K.K. Unger, K. Racaityte, K. Wagner, T. Miliotis, L.E. Edholm, R. Bischoff, G. Marko-Varga, J. High Resolut. Chromatogr. 23 (3) (2000) 259.
[14] M.J. Gray, G.R. Dennis, P.J. Slonecker, R.A. Shalliker, J. Chromatogr. A 1041 (2004) 101.
[15] M.J. Gray, A.P. Sweeney, G.R. Dennis, P.J. Slonecker, R.A. Shalliker, Analyst 128 (2003) 598.
[16] M. Gray, G.R. Dennis, P. Wormell, R.A. Shalliker, P. Slonecker, J. Chromatogr. A 975 (2002) 285.
[17] M.J. Gray, G.R. Dennis, P.J. Slonecker, R.A. Shalliker, J. Chromatogr. A 1028 (2004) 247.
[18] A.P. Sweeney, V. Wong, R.A. Shalliker, Chromatographia 54 (2001) 24.
[19] A.P. Sweeney, P. Wormell, R.A. Shalliker, Macromol. Chem. Phys. 203 (2002) 375.
[20] A.P. Sweeney, V. Wong, R.A. Shalliker, Chromatographia 54 (2001) 24.
[21] G.J. Opiteck, K.C. Lewis, J.W. Jorgenson, Anal. Chem. 69 (1997) 1518.