Published in final edited form as:
J Chromatogr A. 2020 March 15; 1614: 460732. doi:10.1016/j.chroma.2019.460732.

Characterization of Triacontyl (C-30) Liquid Chromatographic Columns

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Abstract

Differences in the characteristics of seventeen commercial C-30 liquid chromatographic columns were studied for the separation of carotenoid isomers. A mixture consisting of nine xanthophyll and hydrocarbon carotenoids were separated under conditions carefully chosen to reveal changes in selectivity. The influence of the mobile phase composition, column temperature, and mobile phase flow rate were evaluated. Shape selectivity was characterized with Standard Reference Material (SRM) 869b Column Selectivity Test Mixture, for correlation with carotenoid retention behavior. Regular changes were observed across a broad spectrum of shape selectivity characteristics as indicated by SRM 869b. Better separations of carotenoid isomers were achieved with C-30 columns than were possible with C-18 columns, even after optimization of separation conditions.

Keywords

C-30 stationary phases; Shape selectivity; Stationary phase synthesis; Column characterization

1. Introduction

It has been estimated that over 90 % of all small molecule chromatographic separations are performed with alkyl modified silica columns operated in the reversed-phase mode, and most of these separations utilize octadecyl (C-18) stationary phases [1]. It is widely recognized that the properties of C-18 columns vary significantly among columns from different manufacturers and even among different lots of a column from the same manufacturer [2, 3, 4]. Dissimilarities among commercial columns can be attributed to variations in the physical and chemical properties of the microparticulate silica sorbents and the synthetic approaches used in the preparation of these materials. Distinctions in column properties are often intentionally created to address specific separation needs and to broaden choices in column selection for unique applications (e.g., endcapped and nonendcapped stationary phases). As such, the availability of columns with dissimilar properties provide opportunities for the development and optimization of new chromatographic methods.

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6. Declarations of Interest: none

The synthesis and characterization of triacontyl (C-30) stationary phases for liquid chromatography was first described by Sander et al. [5] This early study addressed the need for improved separations of polar and nonpolar carotenoid isomers and was an outgrowth of research on shape selectivity for other classes of compounds with constrained molecular shapes [6, 7]. Changes in column selectivity were observed for C-18 stationary phases prepared with polymeric surface modification chemistry ("polymeric phases") compared with stationary phases prepared with monomeric surface modification chemistry ("monomeric phases") [6, 8]. Polymeric syntheses utilize trifunctional silanes in combination with water in solution to form silane oligomers that then react with the silica. Monomeric syntheses are usually carried out under anhydrous conditions with monofunctional silanes to result in surface modification without the formation of oligomeric silane percursors. Changes in selectivity were also noted for different length alkyl stationary phases [9]. The initial C-30 column developed by Sander et al. utilized a polymeric surface modification scheme of silica with trichlorotriacontylsilane, in a manner analogous to the preparation of polymeric C-18 columns [8].

Both polymeric C-18 and polymeric C-30 columns exhibit enhanced separations of constrained-shape solutes compared with corresponding columns prepared with monomeric synthesis schemes [10]. Polymeric C-18 columns have proven to be especially useful for the analysis of polycyclic aromatic hydrocarbon (PAH) isomers [11, 12], whereas polymeric C-30 columns are well suited to the analysis of carotenoid isomers and certain vitamins [13, 14]. The selectivity differences among monomeric and polymeric C-18 and C-30 columns have been attributed to changes in stationary phase order that result from differences in the surface modification chemistry employed, bonding density, alkyl phase length, and column temperature [9, 15–18].

The recent availability of C-30 columns from commercial sources has made possible an examination of variations in performance that are characteristic of this class of columns. In previous studies, SRM 869b was used to provide a metric of shape selectivity for monomeric and polymeric C-18 columns, particularly for application to separations of PAHs [19]. This test is now utilized for C-30 columns with correlation to separations of carotenoids to characterize shape selectivity. Representative columns were further studied to evaluate flow-related kinetic performance for correlation with stationary phase properties.

2. Material and methods[2]

2.1 Reagents.

All solvents were HPLC grade obtained from commercial sources. SRM 869b Column Selectivity Test Mixture for Liquid Chromatography was obtained from the National Institute of Standards and Technology (NIST) Office of Reference Materials. Carotenoid reference standards were obtained from commercial sources: lutein (CAS 127-40-2), β-cryptoxanthin (CAS 472-70-8), *trans*-α-carotene (CAS 7488-99-5), and *trans*-β-carotene

[2]Certain commercial equipment, instruments, or material are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

(CAS 7235-40-7) were obtained from Sigma-Aldrich (St. Louis, MO); zeaxanthin (CAS 144-68-3) was obtained from Indofine Chemical Co, (Belle Mead, NJ); and *apo*-8'-carotenal (CAS 1107-26-2), 15-*cis*-β-carotene (CAS 19361-58-1), 13-*cis*-β-carotene (CAS 6811-73-0), and 9-*cis*-β-carotene (CAS 13312-52-2) were obtained from CaroteNature (Müsingen, Switzerland). Different test solutions containing these carotenoids were prepared over the course of the studies. Levels of the carotenoids in the replicated solutions differed slightly, and variations in the relative responses can be attributed to these differences.

2.2 Instrumentation and software

Separations were performed with two chromatographic systems. A Dionex Ultimate liquid chromatographic system consisting of a 3000 pump, 3000 RS autosampler, 3000 RS column compartment, and 3000 RS diode array detector (Thermo Fisher Scientific, Waltham, MA) and a system consisting of an Agilent G1312B binary pump, G1322A solvent degasser, B1329A autosampler, G1314A variable wavelength detector (Agilent Technologies, Santa Clara CA) and a Dionex Ultimate 3000 column oven were used. Instruments were controlled and data was collected using a Chromeleon 6.80 SR11 data system. The columns utilized in this study are identified in Table 1 and sources are presented in Supplemental Table S1. To facilitate comparisons with the C-30 columns, two commercial C-18 columns (columns R and S) were obtained with custom configurations of 4.6 mm × 250 mm. Separation conditions are provided in Table 1 or in figure captions. For selectivity comparisons among columns, isocratic mobile phase conditions were adjusted to provide similar retention of α-carotene; the details of these conditions are provided in Table 1. Solutions of the reference standards were prepared in ethanol, and the injection volume was 5 μL. For temperature studies, a 50 cm length of 254 μm (0.010") i.d. tubing was added to the inlet of the column within the column oven to assist in equilibration of the mobile phase temperature. Van Deemter coefficients were determined with Excel 2016 Solver and principal component analysis was performed with XLStat version 2019.3.2.61916 (Addinsoft, Inc. New York, NY, USA; www.xlstat.com).

3. Results and Discussion

To assess the scope of characteristics exhibited among commercial C-30 columns, representative examples were acquired and studied. For comparison, monomeric and polymeric C-18 columns were included to illustrate the influence of chain length and bonding chemistry. Differences in column selectivity towards carotenoid isomers were examined under various environmental and mobile phase conditions, and aspects of column kinetic performance related to flow were evaluated for the van Deemter model.

3.1 Differences in column selectivity.

A mixture of nine polar and nonpolar carotenoids were utilized in the assessment of column selectivity (see Figure 1). Lutein and zeaxanthin are isomeric, as are the hydrocarbon carotenoids. As with other classes of isomers, the separation of complex carotenoid mixtures represents a considerable challenge that can be addressed through column selection. Better separations of isomers often can be achieved with columns that exhibit enhanced shape recognition, such as those that are prepared with polymeric surface modification chemistry.

For carotenoids, better separations are usually possible with C-30 stationary phases compared with C-18 stationary phases, but both types of columns exhibit a wide range of selectivity differences that can be used to advantage in the development of separation methods [20, 21]. SRM 869b Column Selectivity Test Mixture was originally developed to provide a metric for use in comparing shape selectivity characteristics among C-18 columns, but applications to other columns have been made, including C-30 columns [10, 15, 19, 22]. The separation factor $\alpha_{TBN/BaP}$ represents the *k* ratio for dibenzo[*g, p*]chrysene (tetrabenzonaphthalene) and benzo[*a*]pyrene, and this value is correlated with shape selectivity, particularly for isomeric compounds. Each of the C-30 columns studied were evaluated by use of SRM 869b under standard conditions (i.e., 85:15 acetonitrile:water, 1.5 mL/min), and the resulting values ranged from $\alpha_{TBN/BaP} = 0.43$ to $\alpha_{TBN/BaP} = 1.59$ (Table 1). It should be noted that in the current study, the terms “monomeric-like” and “polymeric-like” are used to describe C-30 column characteristics that are attributed to these synthetic approaches based on classification with SRM 869b – the actual synthetic procedures used in the preparation of the commercial columns are not known or reported.

Carotenoid separations are illustrated in Figure 2. For this comparison, mixtures of acetone and methanol were utilized for the mobile phase (see discussion below), with absorbance detection at 450 nm, at ambient temperature. The mobile phase composition was adjusted for each column to provide similar retention for α-carotene (compound 7), and the graphical representation for each chromatogram was normalized to the retention of this compound. Retention data is provided in Table S2. The use of different mobile phase conditions (i.e., different proportions of acetone and methanol) is not expected to significantly influence selectivity for carotenoid isomers. This supposition was examined for column N for mobile phase compositions ranging from 20:80 to 70:30 volume fractions of acetone:methanol (Figure S1) and for the most retentive column (I) and the least retentive column (Q) (Figure S2). For each comparison, similar selectivity was observed with different mobile phase conditions.

The separations in Figure 2 are presented in order of increasing values for $\alpha_{TBN/BaP}$ as determined with SRM 869b (i.e., order of decreasing shape selectivity). Regular and gradual changes in the separations of the nine carotenoids were observed for this ordering, which indicates that the separation factor $\alpha_{TBN/BaP}$ can be a useful indicator of C-30 column selectivity for carotenoid separations. The separation of xanthophyll isomers (1) lutein and (2) zeaxanthin can be challenging with certain C-18 columns, and the same is true for C-30 columns. For column A ($\alpha_{TBN/BaP} = 0.43$) lutein and zeaxanthin are easily resolved. With increased values of $\alpha_{TBN/BaP}$, resolution gradually decreases, and the isomers are unresolved for $\alpha_{TBN/BaP} > 1.05$. The relative elution of the hydrocarbon isomers is also observed to vary as a function of $\alpha_{TBN/BaP}$. For most of the columns studied, the geometric isomers of β-carotene elute in the order 15-*cis*-, 13-*cis*-, *trans*-, 9-*cis*-β-carotene. For $\alpha_{TBN/BaP} > 1.05$, the changes in relative elution become sufficient to result in coelution or changes in elution order of these isomers. For column Q, the elution order for the carotenoids is nearly reversed with the sequence *trans*-, 9-*cis*-, 13-*cis*-, and 15-*cis*-β-carotene, and the isomers are fully resolved. Resolution of both the xanthophyll and the hydrocarbon carotenoid isomers was achieved with columns F, G, and H, which corresponds to an interval of 0.5 to 0.66 for $\alpha_{TBN/BaP}$.

3.2 Mobile phase composition.

The choice of the mobile phase composition is one of the most influential decisions made in the development of an LC method that can affect retention and potentially, the selectivity of samples. Because hydrocarbon carotenoids are highly retained in reversed-phase separations, solvents not commonly used in RPLC are sometimes employed to reduce overall retention and to enhance selectivity. Examples of such solvents include acetone, *t*-butyl methyl ether, and ethyl acetate (i.e., stronger solvents) in mixtures with acetonitrile, methanol or water (i.e., weaker solvents). Several such solvent systems were utilized in this study to evaluate the influence of mobile phase composition on selectivity for carotenoid isomers with C-30 columns. An example of changes resulting from the use of dissimilar mobile phases are shown in Figure 3. A column with intermediate selectivity (column N; $\alpha_{TBN/BaP} = 1.29$) was selected for this comparison. Mixtures of acetone, acetonitrile, methanol, ethyl acetate, and water were adjusted to provide comparable retention of α-carotene (compound 7) (Figures 3A–3E). Small differences in the resolution of the carotenoids are apparent for mobile phases that contain mixtures of acetone:methanol, acetone:acetonitrile, acetone:water, or ethyl acetate:methanol. A somewhat larger change is observed with the use of *t*-butyl methyl ether as the strong solvent, which results in loss of resolution of the geometric isomers of β-carotene (Figure 3E). Other examples of the influence of mobile phase composition on carotenoid separations are provided in Supplementary Information (Figures S1 and S2).

3.3 Column temperature.

The influence of column temperature on selectivity was studied for selected monomeric-like and polymeric-like C-18 and C-30 columns. Values for the SRM 869b separation factor $\alpha_{TBN/BaP}$ were determined at five-degree intervals over the range 5 °C to 50 °C, for the four columns (see Figure 4). Changes in selectivity with temperature are nearly identical for comparisons of the C-18 and C-30 monomeric-like columns, as are changes in selectivity with temperature for the polymeric-like columns. In each case, values of $\alpha_{TBN/BaP}$ increase with increased temperature, indicating a reduction in shape recognition at higher temperatures. The shapes of the temperature response curves for each type of column are distinct, which may be characteristic of fundamental differences in the alkyl chain conformational order observed for monomeric and polymeric stationary phases [17].

Examples of the influence of temperature on separation of the carotenoid isomers are provided in Figure 5. For this study, the mobile phase composition was held constant for each column so that the influence of temperature was not conflated with this variable. A two-fold to three-fold increase in retention is observed over the interval 40 °C to 10 °C. Modest changes in selectivity are apparent that may be in part a consequence of this shift in retention. For the polymeric-like C-30 column (Figure 5A), values of the separation factor $\alpha_{TBN/BaP}$ ranged from about 0.3 to 0.8 over the temperature interval studied. The carotenoids are resolved at 10 °C and 25 °C; however, at 40 °C 15-*cis*-β-carotene and 13-*cis*-β-carotene coelute. Several of the carotenoids coelute with the polymeric-like C-18 column at different temperatures (Figure 5B). The relative elution also changes with temperature, most notably for *trans*-β-*apo*-8'-carotenal. Very similar overall separations of the carotenoids are obtained for the C-18 and C-30 monomeric-like columns (Figures 5C, 5D). For both columns, 13-*cis*-β-carotene and 9-*cis*-β-carotene co-elute at reduced temperature;

otherwise the selectivity remains relatively constant. In terms of the shape selectivity separation factor, values for $\alpha_{TBN/BaP}$ ranged from about 1.2 to 1.8 over the temperature interval 10 °C to 40 °C. A comparison can be made to the carotenoid separations for columns M through Q (Figure 2), which are illustrative of the same separation factor interval (assessed at room temperature). For these columns, the changes in selectivity result from differences in column manufacture, rather than changes in temperature. Larger variations in the separation of the carotenoid isomers are apparent among this group of C-30 columns than are observed for the two monomeric-like columns operated at different temperatures.

3.4 Stationary phase chemistry.

The influence of surface modification chemistry on shape selectivity has been studied in detail for applications to polycyclic aromatic hydrocarbon (PAH) isomers [23]. In general, better separations of complex mixtures of PAHs can be achieved with alkyl phases prepared with high density surface modification approaches. Surfaces modified with trifunctional silanes in the presence of water can have bonding densities of 5 μmol/m^2 or greater for octadecyl stationary phases; surface coverages are more limited for longer alkyl length phases due to steric effects [9]. Shape selectivity is enhanced for increases in both bonding density and alkyl chain length, but selectivity distinctions between C-18 and C-30 columns are sometimes apparent even for columns deemed to be highly shape selective.

Representative examples of separations of carotenoid isomers are illustrated in Figure 6 for monomeric-like and polymeric-like C-18 and C-30 columns. These designations are based on column categorization scheme of SRM 869b [19]. Similar separations are observed for monomeric-like C-18 ($\alpha_{TBN/BaP}$ = 1.61) and monomeric-like C-30 ($\alpha_{TBN/BaP}$ = 1.59) columns (Figure 6A and 6B), with only a change in the resolution of 9-*cis*- and 13-*cis*-β-carotene isomers. More significant differences are evident for polymeric-like C-18 ($\alpha_{TBN/BaP}$ = 0.49) and polymeric-like C-30 ($\alpha_{TBN/BaP}$ = 0.50) columns (Figures 6C and 6D). Both columns provide resolution of lutein and zeaxanthin; however, better overall separation of the hydrocarbon carotenoids can be achieved with the polymeric-like C-30 column.

Changes in the elution order of polar and nonpolar carotenoids are observed for comparisons of the polymeric-like C-18 and C-30 columns, and for comparisons of the polymeric-like columns with the monomeric-like columns. Notably, *trans*-β-*apo*-8'-carotenal elutes prior to β-cryptoxanthin for all columns except the polymeric-like C-18 column, in which case coelution with α-carotene is observed. The geometric isomers 15-*cis*-, 13-*cis*-, and 9-*cis*-β-carotene elute after *trans*-β-carotene for each column except the polymeric-like C-30 column, which exhibits the elution order 15-*cis*-, 13-*cis*-, *trans*-, and 9-*cis*-β-carotene.

The relative elution of the β-carotene geometric isomers is indicative of retention mechanism differences for C-18 and C-30 columns related to the solute shape. For polymeric-like C-30 columns, the extended isomers (9-*cis*-β-carotene and *trans*-β-carotene) elute after the bent isomers (15-*cis*- and 13-*cis*-β-carotene), indicating stronger interactions for the extended isomers with the stationary phase. In previous work, the thickness of a monomeric C-30 phase was measured to be 2.5 nm ± 0.4 nm [24]. The thickness of a polymeric C-30 phase was not measured but based on the results for monomeric and

polymeric C-18 phases, this thickness can be estimated to be about 3.1 nm. By comparison, the end-to-end length of *trans*-β-carotene is 2.7 nm. Interactions of 9-*cis*-β-carotene and *trans*-β-carotene can be envisioned to occur completely within the polymeric C-30 stationary phase layer. Because 15-*cis*- and 13-*cis*-β-carotene elute early, interactions of these isomers are weaker than are interactions with the extended isomers. The bent shape of these isomers may preclude full contact with the stationary phase. The thickness of monomeric and polymeric C-18 phases have also been measured (1.7 nm ± 0.3 nm, and 2.1 nm ± 0.3 nm, respectively) [24]. The reduced retention of 9-*cis*-β-carotene and *trans*-β-carotene with C-18 phases may result from the inability of these solutes to fit fully within the thinner stationary phase. Relative to these solutes, 15-*cis*- and 13-*cis*-β-carotene interact more strongly with the C-18 phase, possibly due to combined interactions within and at the surface of the alkyl layer.

3.5 Principal Component Analysis

Differences in column selectivity were further evaluated by principal component analysis (PCA) for the studied columns. Separation factors were calculated for each of the 36 possible pairwise combinations of the nine carotenoid solute probes for the separations shown in Figure 2. PCA was performed on these variables; the first two principal components (F1 and F2) carried 90.8% of the variability expressed by the separation factors (Figure 7). F3 carried an additional 7.1% of the variability for these variables.

An observations plot is shown in Figure 7A for projections onto F1 and F2. The monomeric-like C-30 columns (P and Q) and monomeric-like C-18 column (R) are grouped at one edge of the plot, and the remaining C-30 columns are distributed across the rest of the plot without obvious groupings. The polymeric-like C-18 column (S) is separated from the rest of the columns, as expected from the distinct selectivity demonstrated by this column (see Figure 6C). Column K is also separated from the other C-30 columns; however, no obvious differences in selectivity are apparent in Figure 2.

A variables plot for projections onto F1 and F2 is shown in Figure 7B. The separation factor $\alpha_{TBN/BaP}$ was not used as a variable in the computation of the principal components, but it is informative to plot this data as a supplemental variable. It is notable that this projection falls on the axis of the first principle component (F1), with a correlation of 0.917 with factor F1. Thus, SRM 869b provides a useful indication of differences in column selectivity for the carotenoids studied. Columns with similar values for $\alpha_{TBN/BaP}$ exhibit similar separations of carotenoids, and columns with dissimilar values for $\alpha_{TBN/BaP}$ can be expected to provide dissimilar separations.

3.6 Kinetic performance.

To characterize changes in column performance that occur as a function of flow rate, efficiency was determined for monomeric-like columns (Q and R) and polymeric-like columns (F and S) at different linear velocities. For this study, efficiency was measured for *trans*-β-*apo*-8'-carotenal under mobile phase conditions that resulted in retention of approximately k=1; chromatographic run times ranged from 3 minutes to 5 hours. The

corresponding relationships are plotted in Figure 8 and Supplemental Figures S3 – S6, and van Deemter equation coefficients (i.e., A, B, and C) are provided in Table 2.

The uncertainties in the van Deemter coefficient determinations can be estimated as a relative standard deviation for determinations of each coefficient from independently replicated data (n=4), using column R at 25 °C as a representative example: coefficient A = 15 %; coefficient B = 6 %; coefficient C = 3 %. Using the same approach, the uncertainty in h is estimated to range from 1% to 4% for high and low linear velocities, respectively. Significant differences are observed among the four columns. The two polymeric-like columns exhibit the largest resistance to mass transfer, with values for C ranging from 0.70 to 2.11. For the monomeric-like columns, values for C range from 0.11 to 0.56. The corresponding changes in column efficiency (i.e., reduced plate height) are most apparent at lower temperature (10 °C; Figure 8C).

For example, at 10 °C and a flow rate of 1 mL/min, values of h range from 3.4 (column R) to 16 (column S), whereas at 40 °C, h values range from 3.4 (column R) to 6.4 (column Q) (see Figure S4). The optimum flow rate can be determined graphically, or it can be calculated from the van Deemter coefficients: $\nu_{opt} = (B/C)^{1/2}$ (see Table 2). For three of the columns (F, R, S), the optimum reduced velocity increases with increased temperature (Figure S5). Notably, the opposite trend is observed for column Q, and other aspects of this column are also unusual. Values for C are very small, and the values decrease with decreasing temperature (Figure S6). Among the four columns studied, the optimum reduced velocities range from about 0.6 (column S) to 3.6 (column Q).

It is interesting to examine trends in column efficiency determined at the optimum reduced velocities, as a function of temperature. The reduced plate height at the optimum reduced velocity (designated h_{min}) is plotted as a function of temperature for the four columns (Figure S5). At elevated temperature, h_{min} is observed to decrease significantly for the polymeric-like C-18 column. Thus, column efficiency increases for the polymeric-like C-18 column at elevated temperature, due in part to improved mass transfer kinetics. Changes in h_{min} as a function of temperature are small for the other columns studied. Values for the longitudinal diffusion coefficient B range from about 0.8 (column S) to 2.1 (column R). For each column, relatively small increases in B are observed with increased temperature, as expected for longitudinal diffusion (Figure S6).

4. Conclusions

A broad spectrum of performance characteristics are exhibited by the C-30 columns included in this study. For these columns, the elution order and retention behavior of polar and nonpolar carotenoid isomers is well correlated with the shape separation factor ($\alpha_{TBN/BaP}$) as determined with SRM 869b. C-30 column selectivity spans the range of properties exhibited by monomeric-like and polymeric-like C-18 columns; however, unique separations are possible with C-30 columns that are not achieved with C-18 columns. Solute mass transfer kinetics are reduced for polymeric-like C-18 and polymeric-like C-30 stationary phases compared with corresponding monomeric-like stationary phases. The distinctive properties exhibited by the commercial C-30 columns evaluated in this study

provide unique opportunities for method development and optimization for singular applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors thank David Duewer for assistance with numerical methods used to determine van Deemter coefficients and David Duewer and David Sheen for helpful discussions on principal component analysis. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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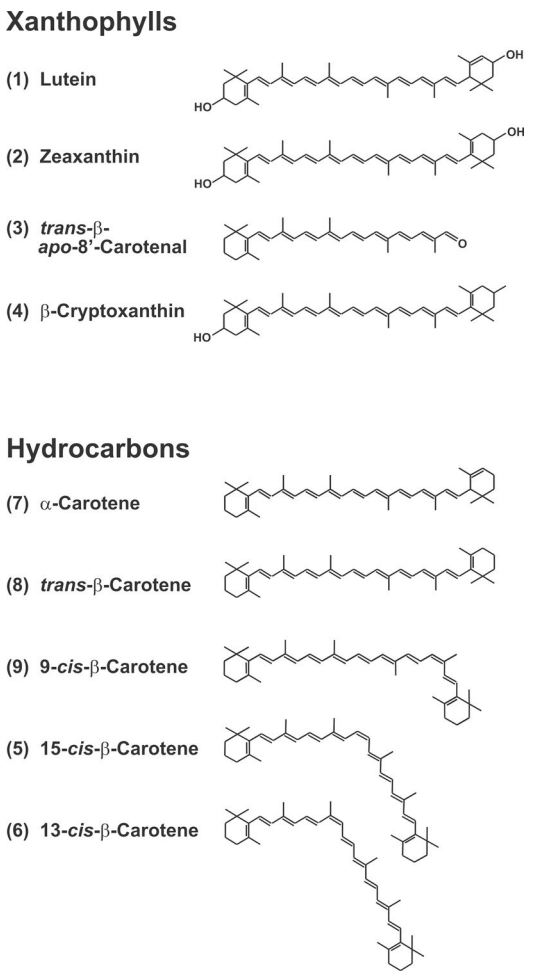

Figure 1.

Structures of xanthophylls and hydrocarbon carotenoids utilized as probes of chromatographic selectivity. Number designations are used for compound identification in all figures.

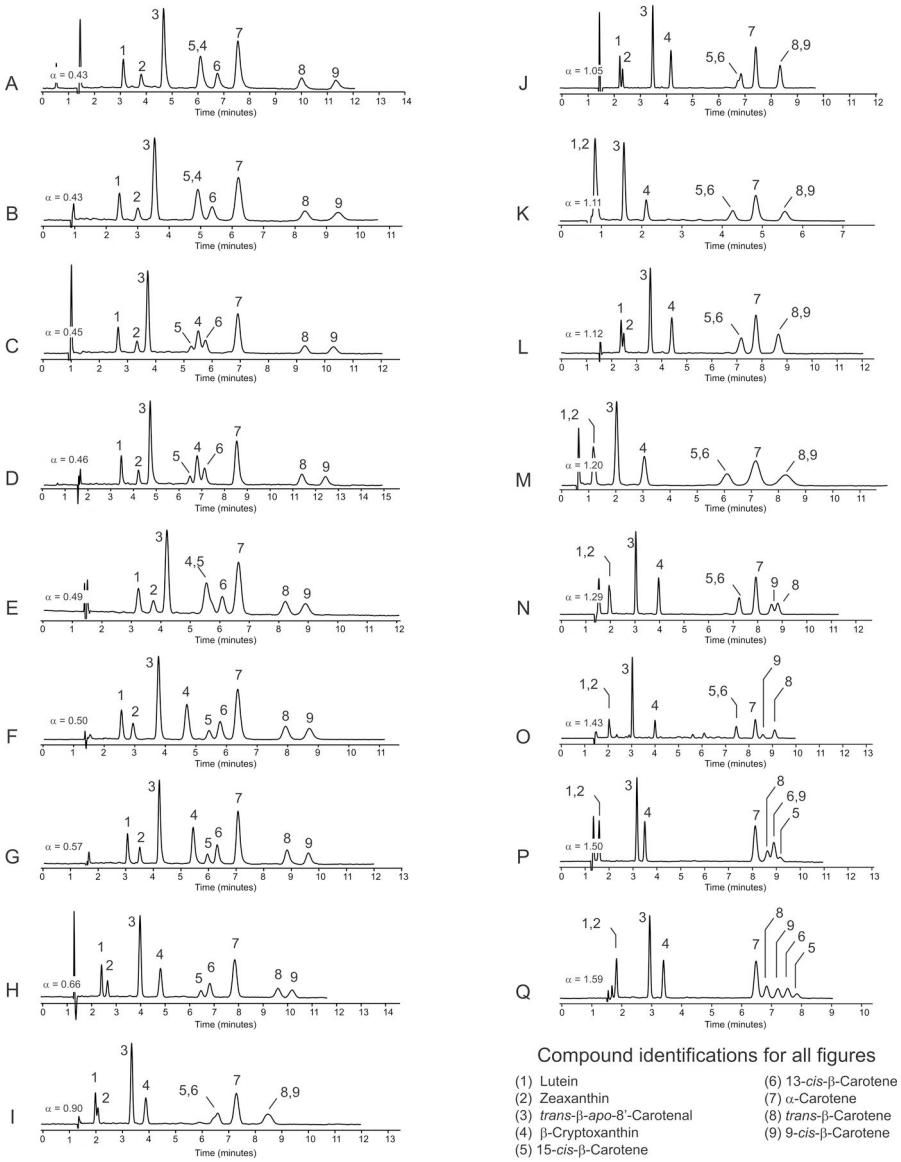

Figure 2.

Separations of carotenoids with different C-30 LC columns, ordered as a function of the shape separation factor ($\alpha_{TBN/BaP}$). Labels A to Q correspond to data in Table 1.

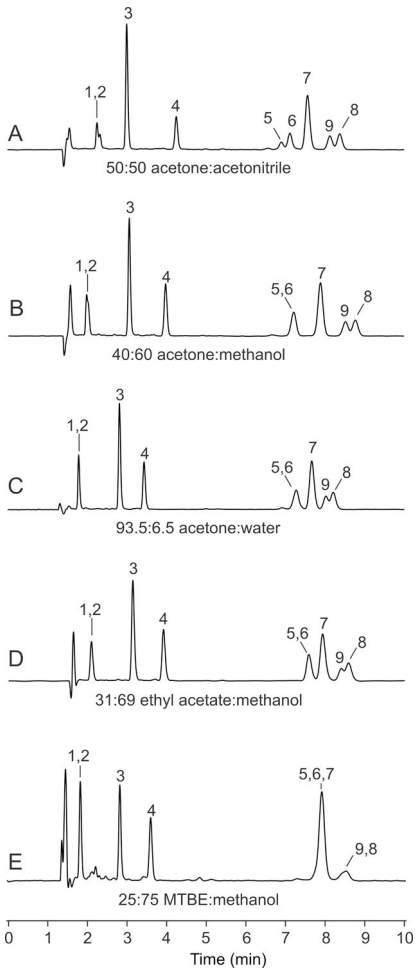

Figure 3.

Separations of carotenoid standards with a C-30 LC column (N; see Table 1) with different mobile phase solvents. Conditions: flow rate 1 mL/min; column temperature ambient.

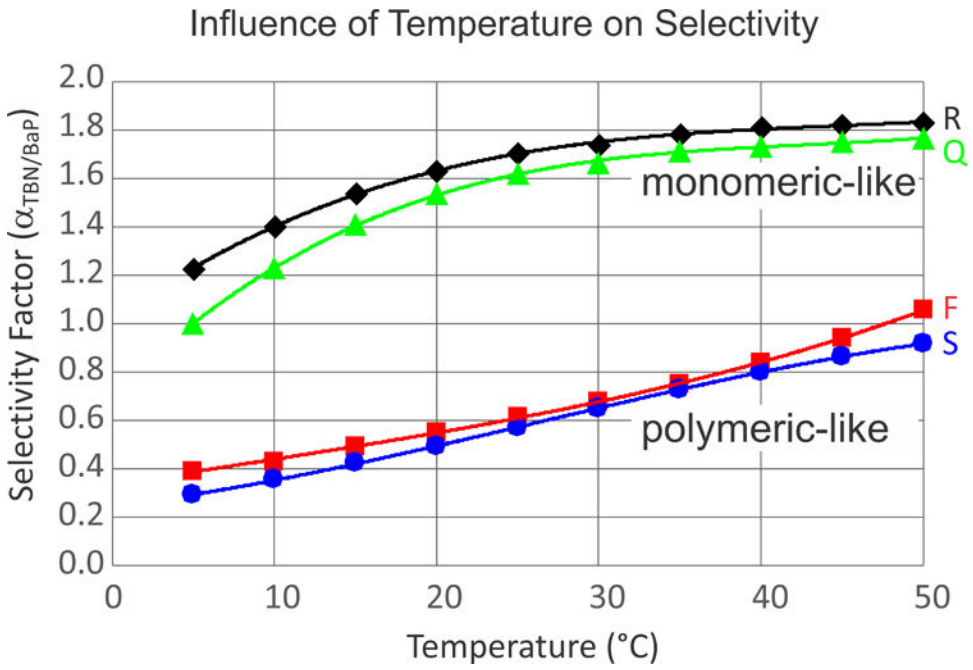

Figure 4.

Shape selectivity ($\alpha_{TBN/BaP}$) plotted as a function of temperature for monomeric-like and polymeric-like C-18 and C-30 columns. Column identification: (▲) monomeric-like C-30 (column Q); (■) polymeric-like C-30 (column F); (◆) monomeric-like C-18 (column R); (●) polymeric-like C-18 (column S). The uncertainty (relative standard deviation) of $\alpha_{TBN/BaP}$ values is estimated to be 0.2 % based on replicate measurements (n=5) of SRM 869b with column R at 20 °C.

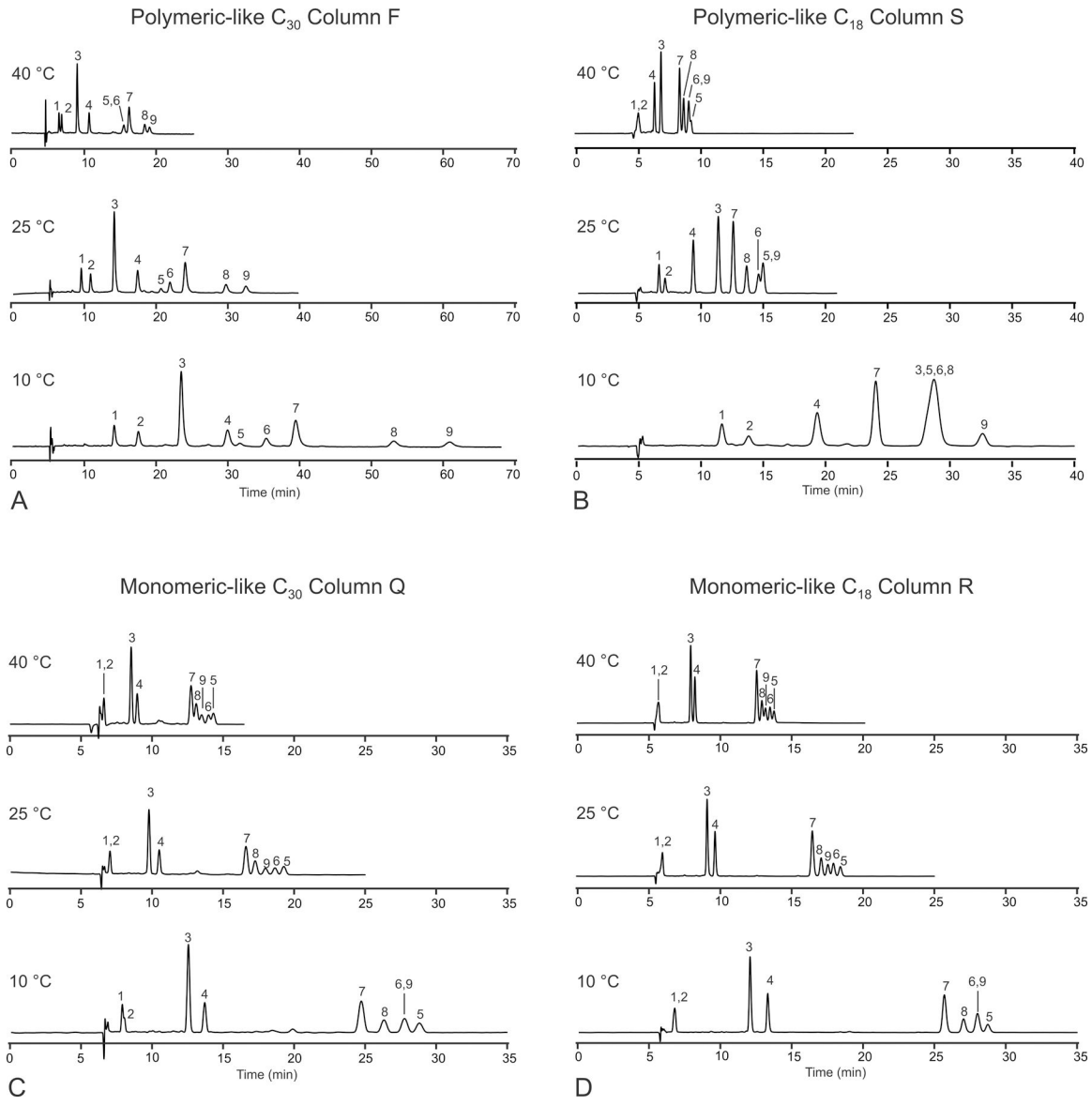

Figure 5.

Influence of temperature on retention and selectivity for the separation of carotenoid isomers. (A) 60:40 acetone:methanol; (B) 50:50 acetone:methanol; (C) 40:60 acetone:methanol; (D) 60:40 acetone:methanol. Flow rate 0.5 mL/min.

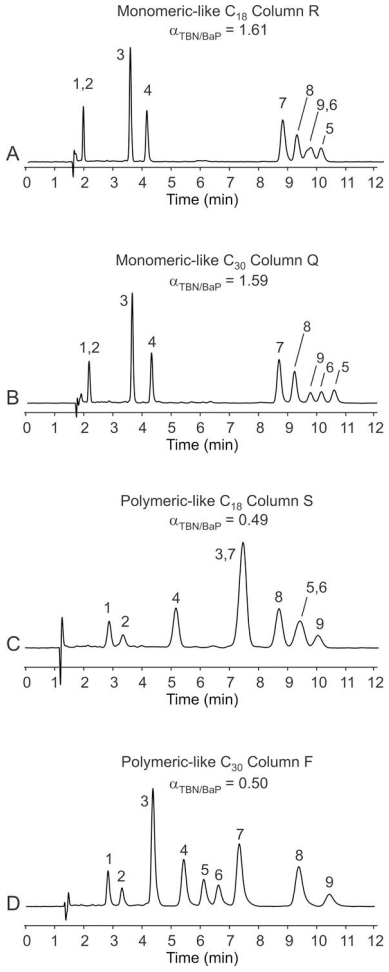

Figure 6.

Separation of carotenoid isomers on monomeric-like and polymeric-like C-18 and C-30 columns. Separation conditions are listed in Table 1.

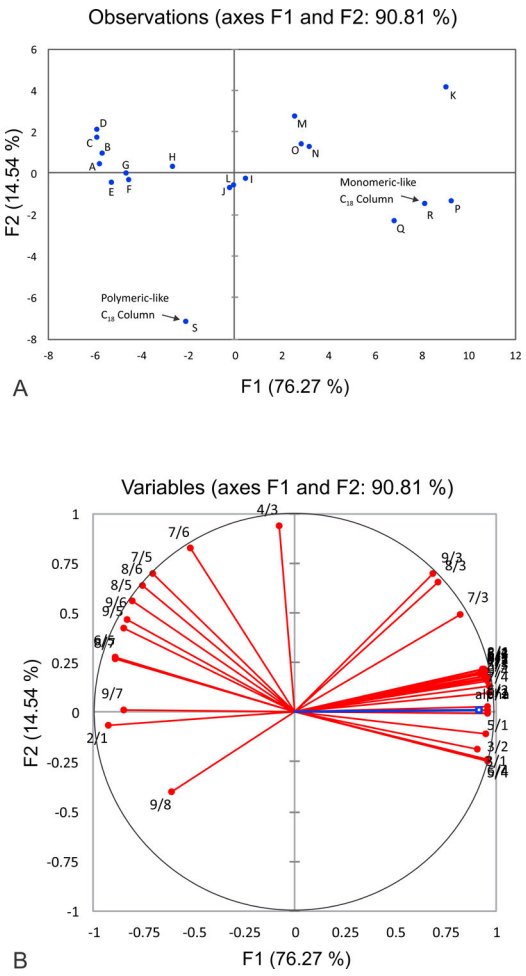

Figure 7.

Principal component analysis plots for columns A – S, based on separation factors for separations shown in Figures 2 and 6. A) Observations plot, showing projections for columns A to S, onto axis F1 and F2. B) Variables plot for separation factors for the nine carotenoid solutes. The blue line (on the F1 axis) is for a supplemental variable represented by the separation factor $\alpha_{TBN/BaP}$.

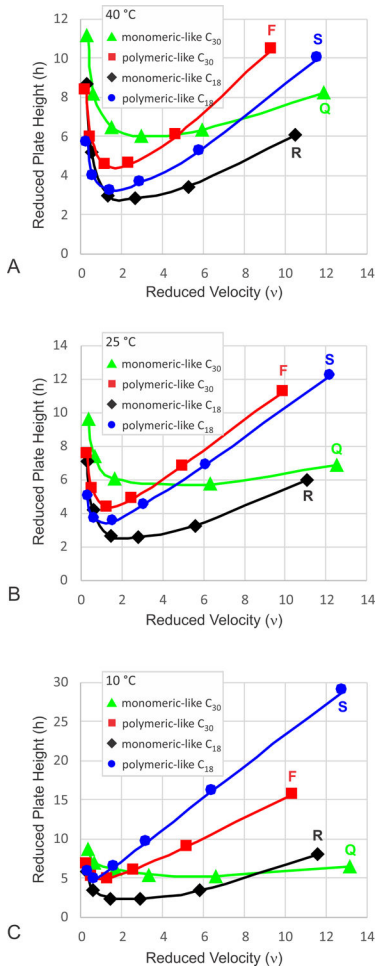

Figure 8.

Van Deemter plots for representative C-18 and C-30 columns at different temperatures. See the text for a discussion of uncertainty. Mobile phase conditions: column S 50:50 acetone:methanol; column F 60:40 acetone:methanol; column R 40:60 acetone:methanol; column Q 15:85 acetone:methanol.

Table 1.

Designations and configurations of C-18 and C-30 columns

	Column Designation	Particle Diameter (μm)	Column Configuration (mm)	Shape Selectivity Factor[a]	mobile phase composition acetone:methanol (volume fraction)[b]
C-30 columns					
A	PrincetonSPHER C30	3	4.6 × 250	0.43	50:50
B	Cogent C30	5	4.6 × 150	0.43	40:60
C	YMC Carotenoid	3	4.6 × 150	0.45	30:70
D	ProntoSIL 300-3-C30	3	4.6 × 250	0.46	30:70
E	Inertsil C30 S-Select	5	4.6 × 250	0.49	50:50
F	ProntoSIL 120-3-C30	3	4.6 × 250	0.50	60:40
G	ProntoSIL 200-3-C30	3	4.6 × 250	0.57	30:70
H	Wako Navi C30-5	5	4.6 × 250	0.66	60:40
I	Develosil RPAQEOUS-AR 5 C30	5	4.6 × 250	0.90	70:30
J	Ultisil XB-C30	3	4.6 × 250	1.05	60:40
K	Accucore C30	2.6	3.0 × 150	1.11	30:70[c]
L	Chromenta C30	5	4.6 × 250	1.12	40:60
M	ReproSIL 100 C30	5	3.0 × 100	1.20	20:80[c]
N	Acclaim C30	3	3.0 × 250	1.29	40:60[c]
O	Halo C30	2.7	4.6 × 250	1.43	40:60
P	Chromegabond C30 120A	3	4.6 × 250	1.50	40:60
Q	Chromegabond C30 300A	3	4.6 × 250	1.59	20:80
C-18 columns					
R	ACE C18	3	4.6 × 250	1.61	40:60
S	Zorbax Eclipse PAH	3.5	4.6 × 250	0.49	30:70

[a] Shape selectivity factor ($\alpha_{TBN/BaP}$) defined as k'_{TBN}/k'_{BaP}; for test conditions see reference [19]

[b] Flow rate 2 mL/min, ambient temperature, except as noted

[c] Flow rate 1 mL/min

Table 2.

Van Deemter Coefficients for C-18 and C-30 Columns

Column Type	ID	Temp	Van Deemter Coefficients[a]			ν_{opt}
		(°C)	A	B	C	
Polymeric-like C-18	S	10	2.43	0.83	2.11	0.63
		25	1.58	0.96	0.86	1.06
		40	1.26	1.27	0.70	1.35
Polymeric-like C-30	F	10	2.45	1.05	1.25	0.92
		25	2.23	1.28	0.89	1.20
		40	2.21	1.46	0.81	1.34
Monomeric-like C-18	R	10	0.28	1.61	0.56	1.70
		25	0.70	1.78	0.44	2.01
		40	0.74	2.12	0.47	2.13
Monomeric-like C-30	Q	10	4.70	1.35	0.11	3.57
		25	4.70	1.56	0.16	3.16
		40	4.57	1.99	0.28	2.67

[a] $h = A + B/\nu + C\nu$; where h is the reduced plate height and ν is the reduced velocity. The coefficients A, B, and C reflect eddy-diffusion, longitudinal diffusion, and resistance to mass transfer, respectively.