



Additional studies on shape selectivity by using the carotenoid test to classify C18 bonded silica

E. Lesellier*

Institut de Chimie Organique et Analytique (ICOA), Université d'Orléans, CNRS UMR 6005, B.P. 6759, rue de Chartres, 45067 Orléans Cedex 2, France

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ABSTRACT

Numerous chromatographic tests are applied to study the C18 bonded phases, either to classify these phases, or to better determine their properties or their chromatographic behaviours. Because the carotenoid test is developed in supercritical fluid chromatography (SFC), many correlations with high performance liquid chromatography (HPLC) results have been necessary to ensure the trueness of the classification reached in these analytical conditions. Consequently, the analytical conditions of the carotenoid test were chosen to fit with the T_bN/BaP values from the NIST 869a test, which describe the shape selectivity of the alkyl bonded phases. Additional studies performed in this paper by using well standardized silica (J'Sphere, YMC Pack ODS A, Wakosil II), which varied from their surface coverage (J'Sphere), their pore shape (YMC), or their bonding type and carbon content (Wakosil II). The use of these C18 stationary phases allows to reach more accurate conclusion on the comparison of the shape selectivity values provided either by the *cis/trans* β-carotene selectivity or by the TRI/oTER or T_bN/BaP ones. The extension of the studies to many other C18 stationary phases allows clarifying the relationships between the carotenoid test and the tests based on the use of PAH, as well as the usage limit of TRI/oTER and T_bN/BaP selectivity in regards of the bonding density of the stationary phases for both monomeric and polymeric phases. By checking other aromatic compounds, another selectivity (anthracene/oTER) display greater correlation with the carotenoid test, that suggest an improvement of the relevance of this new compound couple for the steric selectivity study.

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1. Introduction

The carotenoid test was introduced to classify varied C18-bonded silica stationary phases based on three main properties: hydrophobicity, steric selectivity and surface polar activity [1–3]. It was later improved by complementary studies using the solvation parameter model (LSER) [4,5].

The first difference between this test and numerous other ones [6] is that analyses are performed in supercritical fluid chromatography (SFC). This specificity ensures a better access to the stationary phase properties: first because the great efficiency (N) encountered with sub/supercritical carbon dioxide, due to the high compound diffusivity, allows to enhance separations of isomeric forms in regards to liquid-phase separations; and secondly, because the absence of water, which rules separation in reverse phase liquid chromatography (RPLC), in the mobile phase favours the compound/stationary phase interactions, especially with polar sites of the stationary phase [7]. Moreover, the fast equilibrium required to stabilize the column in the testing conditions, and the high flow

rate usually selected in SFC, allow completing the testing procedure in less than one hour, whatever the fluid that previously filled the column. This fast equilibrium and this high flow rate are due to the low fluid viscosity, explaining the great mobile phase diffusivity into the porous particles (equilibrium of phases) and a low pressure drop despite a high flow rate. Previous studies underlined that with a 250 mm × 4.6 mm column, and 5 μm particles, at usual temperature (25 °C), 10% of modifier and outlet pressure conditions (15 MPa), the pressure drop was only around 15 bar per mL min⁻¹ [7]. Finally, due to high diffusion coefficient (D_m), the optimum efficiency is reached for flow rates ranged from 3 to 5 mL/min in SFC, warranting satisfactory peak width at these flow rates [8].

The second significant difference between the carotenoid test and other tests is the use of natural and safe compounds, carotenoid pigments, using a single analysis in a CO₂/MeOH 85/15 (v/v) mixture. Three main compounds are involved, 13 *cis*- and all *trans*-β-carotene, and zeaxanthin. They absorb visible light at 440 nm, and are thus easily detected.

This test is mainly used to check and compare the own phase properties, i.e. bonding density or type (monomeric, polymeric), specific surface area, and polar group accessibility (called polar surface activity).

* Corresponding author. Tel.: +33 2 38 49 45 88; fax: +33 2 38 41 72 81.

E-mail address: eric.lesellier@univ-orleans.fr

Nevertheless, the relative stationary phase properties determined in SFC by the carotenoid test, i.e. the greater or lower carbon content and specific area (hydrophobicity), amount of residual silanols (polar surface activity), bonding density and the type of bonding (monomeric vs. polymeric), do not depend on the fluid nature, despite some shifts in the absolute values due to the nature of the mobile phase. However, this test cannot describe the retention behaviour of compounds in extreme pH conditions, or the polar interactions between stationary phases and basic compounds in aqueous mobile phases. In reversed-phase liquid chromatography (RPLC) with hydro-organic mobile phases, the water molecules adsorbed onto residual silanol groups can reduce the interactions between basic compounds and the stationary phase.

The steric selectivity, also called shape selectivity, is determined by the selectivity between the *all trans* β -carotene and the 13-*cis* β -carotene. Those two isomers do not have the same surface contact with the stationary phase, inducing varied selectivity between different stationary phases [2–5]. We observed previously that the selectivity value was equal to 1 for low bonded density monomeric phases. For most of the phases, the *cis* form (bent) was more retained than the *all trans* one (linear), and α 13 *cis*/*all trans* β -carotene ranged from 1 to 1.18 for numerous monomeric phases, and was larger than 1.18 for polymeric ones. Moreover, some phases display a value below 1, showing a retention inversion of the two carotenoid isomers.

Strong correlation with the Sander and Wise test [9–11] using TbN/BaP selectivity was reported [2]. The analytical conditions of the carotenoid test were chosen to fit with the TbN/BaP values from the Sander and Wise test. The two testing conditions were selected so as to achieve an inversion in the retention order of two compounds, indicating a clear transition between two types of stationary phases: monomeric bonded C18 and polymeric bonded C18. Moreover, they allow the determination of bonding density for monomeric phases, between high and low bonded phases.

Among the other steric recognition tests used, one can mention the triphenylene/ortho-terphenyl (TRI/oTER) selectivity [12] and the S^* term from the hydrophobic subtraction model [13]. The last one is called steric resistance and is not correlated to shape selectivity, but rather to the resistance to solute insertion in the stationary phase, depending on solute size and on the stationary phase bonding density.

Despite numerous experiments done on varied stationary phases, systematic studies, using stationary phases provided by the same manufacturer and varying by one parameter had not been performed. Recently, we have obtained such phases, allowing to improve our knowledge on the use and the limits of the carotenoid test. Several series were principally studied: the J'Sphere 80, composed by three phases based on the same silica (80 Å) but having varied carbon content (7%, 14% and 22%), the YMC ODS-A, composed by three phases having varied pore size (120, 200, and 300 Å) but identical type of stationary phase, and the Wakosil II C18 series, having both monomeric and polymeric stationary phases. The first of these series (J'Sphere) was previously used by Neue [14] to achieve a test describing hydrophobicity and polar surface activity.

In this paper, we mainly studied the relationships between our variables and some others used in well-known tests: Tanaka, or Sander and Wise, by using these standardized phases and numerous others to evaluate the limits of our conclusions.

2. Materials and methods

The carotenoid test was described elsewhere [1–5], as well as the apparatus used. All compounds were analysed with CO₂/MeOH 85/15 (v/v), 25 °C, Pout 15 MPa, 3 mL/min. The column list is avail-

able in Table 1. Surface coverage was calculated as described in Ref. [15].

Most of the phases studied were monomeric alkyl-bonded phases: ACE C18, ACE C18 HL, TSKgel ODS-100V, TSKgel ODS-100Z, Wakosil II 5C18 HG, Wakosil II 5C18 RS, J'Sphere 80L, J'Sphere 80M, J'Sphere 80H, YMC ODS-A 120 Å, YMC ODS-A 200 Å, YMC ODS-A 300 Å, Cogent Bidentate C18, Gemini C18, Luna C18(2), YMC Pack Pro C18, YMC Pack Pro C18 RS, Sunfire C18, Atlantis dC18, Atlantis T3, XBridge C18, Hypersil Gold, Synergi Max-RP (C12), Nucleodur Gravity, Ascentis Express (Fused core) and Denali C18.

Some others were polar-embedded or polar-endcapped: XBridge Shield C18, Hypersil Gold AQ, Aquasil, HyPurity Aquastar, Acclaim Polar Advantage, Genesis AQ, Cosmosil PAQ, YMC Hydro-sphere and YMC Pack ODS AQ.

Others were polymeric C18-bonded phases: Wakosil II 5C18 AR, Vydac 201 TP 54, Jupiter 300 C18 and Nucleodur ISIS.

One was a dual phase C18/propyl-phenyl: Nucleodur Sphinx, and three were phenylhexyl bonded phases: Luna, Zorbax Eclipse Plus and Gemini.

Triphenylene and ortho-terphenyl were provided by Sigma-Aldrich (Saint-Quentin, France). The text-mix 869a (NIST, Gaithersburg, MD, USA) was used for tetrabenzonaphthalene and benzo[a]pyrene. PAHs were analysed in SFC in the same conditions as for the carotenoid pigments.

3. Results and discussions

Fig. 1 displays the chromatograms of the carotenoid mixture for the three J'Sphere 80 YMC phases. For the most retentive phase, the analysis is performed in 16 min. One can remark that for the less bonded phases (80L and 80M) the bent compound (13 *cis* β -carotene) is eluted first, and for the higher bonding density (80H), the isomer retention is reversed. This retention inversion for the *cis* and *trans* isomers of β -carotene clearly shows that changes in the bonding density of monomeric bonded phases induces modification of shape selectivity, due to a modification of the mechanisms of solute penetration into the stationary phase.

As described in Section 1, the retention order for the less bonded phases corresponds to the one described by Sander and Wise with TbN/BaP [9–11]. The linear compound can penetrate into slots of the stationary phase, where the bent compound cannot fully enter.

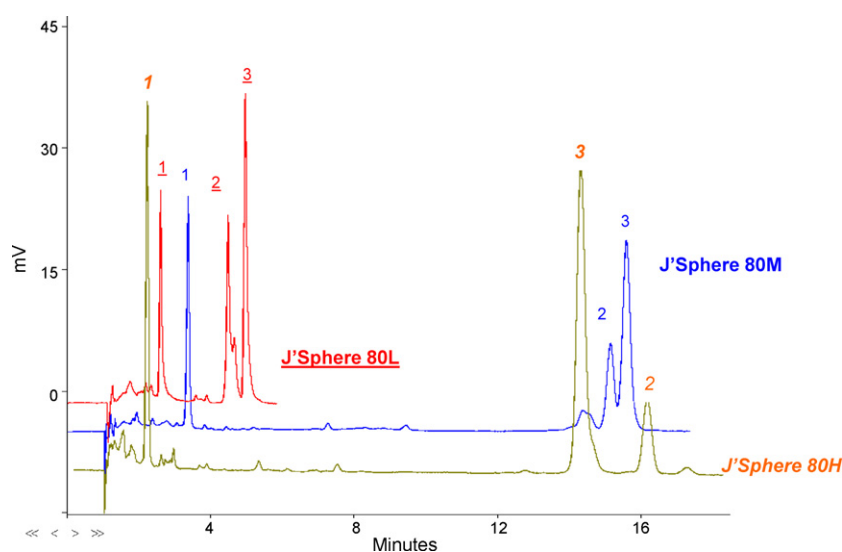
For the J'Sphere 80L, the linear compound being most retained than the bent (13 *cis* β -carotene), this model can be applied. However, this result is surprising because of the clear relationship shown by Sander and Wise, between the increase in retention of the linear PAH and the increase of the bonding density (the opposite in regards of the results reported with carotenoid pigments). Changes in retention order between 13 *cis* and *all trans* β -carotene for the three J'Sphere are easily seen.

The effect of the carbon content (%), i.e. the surface coverage, on the 13 *cis*/*all trans* β -carotene selectivity is presented in Fig. 2. As expected, the increase in the carbon content improves the selectivity between the linear and the bent carotene. A good linear relationship is observed from the correlation coefficient ($R^2 = 0.9993$). For monomeric C18-bonded phases based on identical silica, i.e. identical surface area, the carbon content increase induces a regular increase in the surface coverage, from 0.93 to 1.55 and 2.74 for J'Sphere phases.

Fig. 3 displays the triphenylene/ortho-terphenyl (TRI/oTER) selectivity change vs. the carbon percentage for the three J'Sphere phases. This selectivity was included in the Tanaka test to study the shape selectivity [12], and has often been used until today. It was often correlated to the Sander and Wise test, and explained by the same slot model, despite the smaller size of the used PAHs. As expected, the increase in carbon content favours the TRI/oTER

Table 1
Column list, physico-chemical values and selectivity data.

Manufacturer	Column	Pore diameter (Å)	Carbon content (%)	Surface area (m ² /g)	α TBN/BaP	α TRI/oTER	α Anth/oTER
ACT	ACE 5C18	100	15.5	300	1.25	4.21	1.75
ACT	ACE 5C18 HL	90	20.0	400	1.19	4.39	1.74
ACT	ACE 5C18AQ	100	14.0	300	2.02	2.60	1.34
Agilent	Eclipse Plus Phehex	na	na	na	nd ^a	2.29	1.20
Dionex	ACCLAIM PA	120	17.0	300	nd	6.16	2.00
Discovery Science	VYDAC 201 TP54	300	8.0	90	nd	8.05	2.65
Discovery Science	DENALIC18	120	na	na	1.24	4.35	nd
Discovery Science	GENESIS AQ	120	na	na	1.99	2.85	nd
Macherey-Nagel	NUCLEODUR Gravity	110	18.0	340	1.72	nd	nd
Macherey-Nagel	NUCLEODUR Sphinx	110	15.0	340	2.10	nd	nd
Macherey-Nagel	NUCLEODUR ISIS	110	20.0	340	0.80	5.80	2.15
MacMod	COGENTC18	100	16.0	350	1.42	5.13	1.86
Nacalai	COSMOSIL PAQ	120	11.0	300	1.70	nd	nd
Phenomenex	GEMINI C18	110	14.0	375	nd	2.63	1.19
Phenomenex	LUNA C18(2)	100	13.5	400	nd	2.66	1.28
Phenomenex	JUPITER C18 300	300	13.3	170	nd	4.62	1.91
Phenomenex	LUNAC18(2) Phehex	100	17.5	400	nd ^a	2.15	1.19
Phenomenex	GEMINI Phehex	110	12.0	375	nd ^a	2.12	1.09
Supelco	ASCENTIS express	90	7.5	150*	1.56	3.78	1.64
Thermoelectron	HYPERSIL GOLD	175	10.0	220	1.33	2.98	1.45
Thermoelectron	HYPERSIL GOLD AQ	175	12.0	220	1.00	5.92	2.07
Thermoelectron	AQUASIL	100	12.0	300	1.69	3.82	1.49
Thermoelectron	HyPurity AQUASTAR	190	10.0	200	0.91	6.00	2.04
Tosoh Bioscience	TSKgelODS-100V	100	15.0	450	1.61	3.10	1.34
Tosoh Bioscience	TSKgel ODS100Z	100	20.0	450	1.99	2.57	1.20
Wakosil	WAKOSIL II 5C18AR	120	20.0	300	1.00	4.11	1.56
Wakosil	WAKOSIL IIS18 RS	120	17.0	350	1.86	2.81	1.25
Wakosil	WAKOSIL II 5C18HG	120	15.0	300	1.62	3.57	1.53
Waters	SUNFIRE	100	16.0	340	1.68	3.17	1.47
Waters	ATLANTIS dC18	100	12.0	330	1.92	2.33	1.16
Waters	AtLANTIS T3	100	14.0	330	1.91	2.27	1.14
Waters	XBRIDGE C18	135	9.5	185	1.33	3.45	1.55
Waters	XBRIDGE Shield C18	135	10.0	185	1.27	6.20	2.15
YMC	J'SPHERE 80L	80	9.0	510	1.66	1.69	1.02
YMC	J'SPHERE 80M	80	14.0	510	1.97	2.25	1.13
YMC	J'SPHERE 80H	80	22.0	510	1.35	4.56	1.81
YMC	YMC Pack ODS-A 120	120	17.0	330	1.71	3.42	1.47
YMC	YMC Pack ODS-A 200	200	12.0	175	1.92	3.12	1.35
YMC	YMC Pack ODS-A 300	300	7.0	100	1.74	3.33	1.40
YMC	YMC Pack Pro C18	120	17.0	340	1.97	2.94	1.29
YMC	YMC Pack ProC18RS	80	22.0	510	1.34	3.85	1.57
YMC	YMC HYDROSPHERE	120	12.0	340	1.97	3.29	1.14
YMC	YMC Pack ODS AQ	120	14.0	300	2.00	3.93	1.22

nd^a: not determined.**Fig. 1.** Chromatograms of carotenoid mixture in SFC. Analytical conditions: see text for details. Columns (250 mm × 4.6 mm; 5 μm): J'Sphere 80L (red); J'Sphere 80M (blue); J'Sphere 80H (green). (1) Zeaxanthin, (2) 13 cis β-carotene, (3) all trans β-carotene.

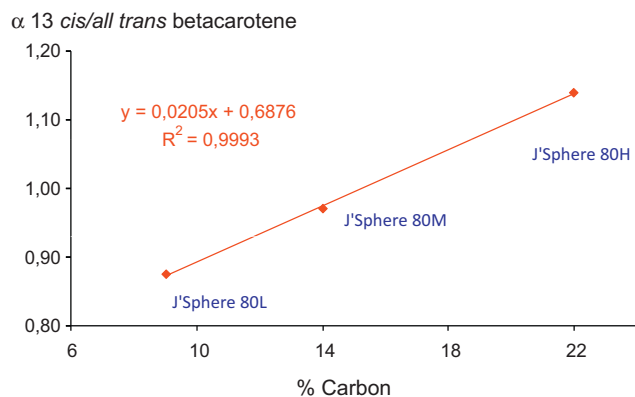


Fig. 2. Effect of the carbon content (in percentage) on the 13-*cis/all trans* β -carotene selectivity for the three J'Sphere phases.

selectivity. The linearity of this relationship is not as good as the one observed for carotenoid pigments in Fig. 2 but rather satisfactory.

The TBN/BaP selectivity vs. the carbon content is also plotted in Fig. 3. The result is unexpected because no correlation appears between the two parameters for these three phases. A closer look at the results indicates that the value of TBN/BaP (1.66) for J'Sphere 80L is underestimated in regards to its low carbon content, while the data obtained for J'Sphere 80M is close to the one obtained in HPLC, i.e. around 2, meaning a monomeric stationary phase with a low surface coverage. One can remark that the results of the TBN/BaP test are highly dependent on the pore size [9–11]. Thus this uncorrelated value for J'Sphere 80L could be due to the very low pore diameter of J'Sphere silica (80 Å). Previous works reported the change in carotenoid selectivity vs. pore size, whereas the TBN/BaP values were close for monomeric stationary phase [16]. However, another point could explain this result: the very low surface coverage value, equal to 0.9 for J'Sphere 80L. This value is somewhat lower than the ones generally studied by this TBN/BaP test (from 2 to 7 mmol/m²) [9–11,17]. For such low values, it can be suggested that the way of penetration of compounds into the stationary phase cannot be appropriately described by the slot model.

Fig. 4 shows the carotenoid separation of another stationary phase family. The higher selectivity for 13 *cis/all trans* β -carotene, is reached for Wakosil II C18 AR despite the lower retention. This behaviour is specific to polymeric phases. On the other hand, despite the greater retention, no separation for the two studied isomers appears for Wakosil II C18 RS.

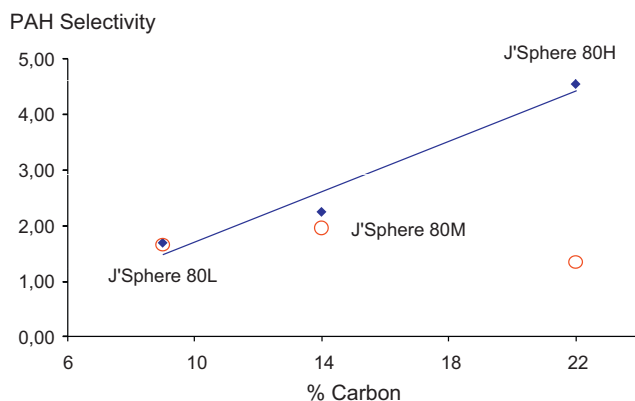


Fig. 3. PAH selectivity vs. carbon content (in percentage) for the three J'Sphere phases. Blue diamonds: α TRI/oTER; open red circles: α TBN/BaP. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

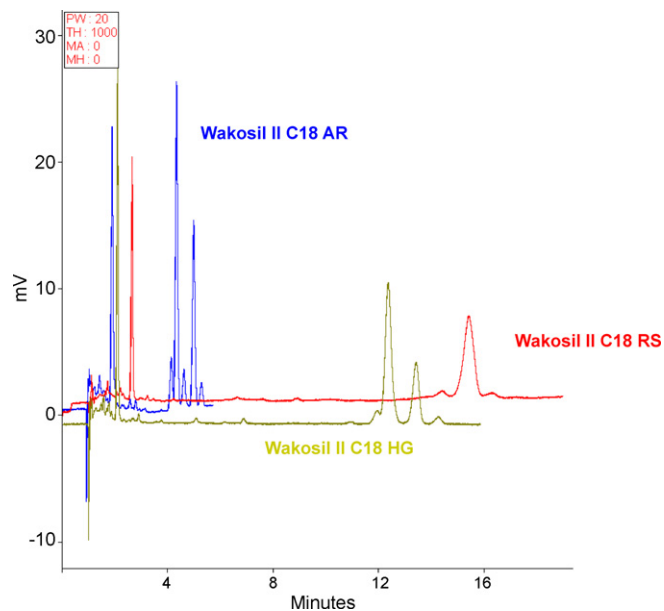


Fig. 4. Chromatograms of carotenoid mixture in SFC. Analytical conditions: see text for details. Columns (250 mm \times 4.6 mm; 5 μ m): Wakosil C18 II RS (red); Wakosil C18 II AR (blue); Wakosil C18 II HG (green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

For these three Wakosil phases, a good correlation between the carotenoid and the TRI/oTER selectivities is observed (Fig. 5), although they differ both in bonding density and in the type of bonded phase, monomeric (Wakosil II C18 RS and HG) and polymeric (for Wakosil II C18 AR). A satisfactory correlation between TBN/BaP and *cis/trans* β -carotene selectivities is also seen. As classically reported, the two PAH selectivities have opposite variations: α (TBN/BaP) decreases from lower to higher bonding density, whereas α (TRI/oTER) increases for an identical density change.

However, Figs. 3 and 4 underline that the TRI/oTER selectivity value is almost the same for the Wakosil II C18 AR and the J'Sphere 80H, even though the two phases are quite different. Indeed, the Wakosil phase is polymeric, while the J'Sphere is monomeric. This point is one of the most important difference between the TRI/oTER test and both the TBN/BaP and the 13 *cis/all trans*- β -carotene selectivity because the values are 1.35 (TBN/BaP) and 1.14 (*cis/trans*) for J'Sphere 80H, meaning a monomeric phase with a high bonding

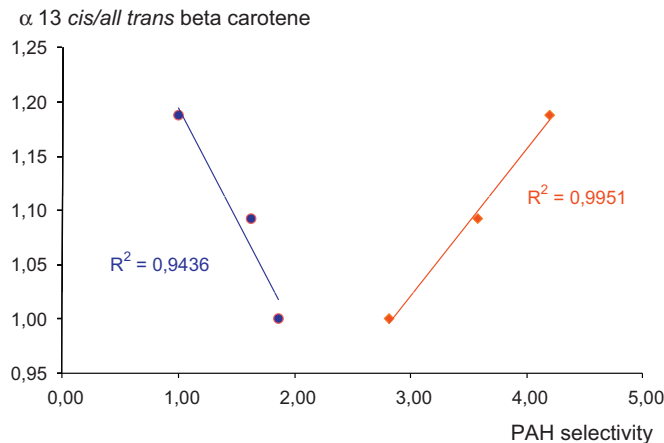


Fig. 5. 13-*cis/all trans*- β -Carotene selectivity vs. PAH selectivity for the three Wakosil (red line) stationary phases. Red diamonds: α TRI/oTER; blue circles: α TBN/BaP. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

density, and 1.0 (TBN/BaP) and 1.19 (*cis/trans*) for Wakosil II C18 AR, in accordance with a polymeric phase.

Fig. 6a and b presents the selectivity values obtained for phases of the same type (YMC Pack ODS-A) having varied pore size, from 120 to 300 Å. A satisfactory correlation between the two PAH tests is achieved (Fig. 6a). The lowest values are obtained with the medium pore size (200 Å), indicating the greater monomeric character of this phase. Changes in TBN/BaP values, in relation to the silica pore size, have been reported by Sander and Wise, but rather for polymeric phases [11].

However, the 13 *cis/all trans*- β -carotene selectivity seems constant for the two lowest pore sizes (120 and 200 Å) despite a bonding density lower for the 120 Å pore phase, and increases between 200 and 300 Å, despite a identical bonding density. This behaviour difference between the carotenes and the PAH is probably due to the shape difference of the varied compounds used (the total length of β -carotene is higher than the ones of the PAH). Whatever the probes, the changes observed from 200 to 300 Å, with identical bonding densities, shows the modification or the phase organization due to the pore radius increase, which induces a greater polymeric phase character. On the other hand, from 120 to 200 Å, the selectivity changes noticed by the two PAH couples is rather due to the decrease in the bonding density, which induces a greater monomeric character, whereas no real variation of *cis/trans* selectivity is observed. Nonetheless, these results indicate that the conclusion of each test could slightly vary depending on the probes used.

A general comparison of selectivity values was performed with all the stationary phases (Fig. 7). The two PAH selectivities were plotted vs. the 13 *cis/all trans*- β -carotene one. The correlation coefficient is better for TBN/BaP than for TRI/oTER. Two main reasons can be evoked:

1. for lower values, the points that are far from the red line are two "Aqua type" phases intended for use with water-rich mobile phases.
2. For higher values, a great dispersion of values appears, showing the lack of accuracy of TRI/oTER selectivity to distinguish the monomeric phases with high bonding density from the polymeric phases (as reported for Wakosil phases). One can suggest that the length of these two PAHs is not sufficient to provide relevant selectivity values for polymeric C18 phases having larger depth, whereas the length of carotenoid pigments is in the range of a C18 bonded chain. In the same way, on C8-bonded phases,

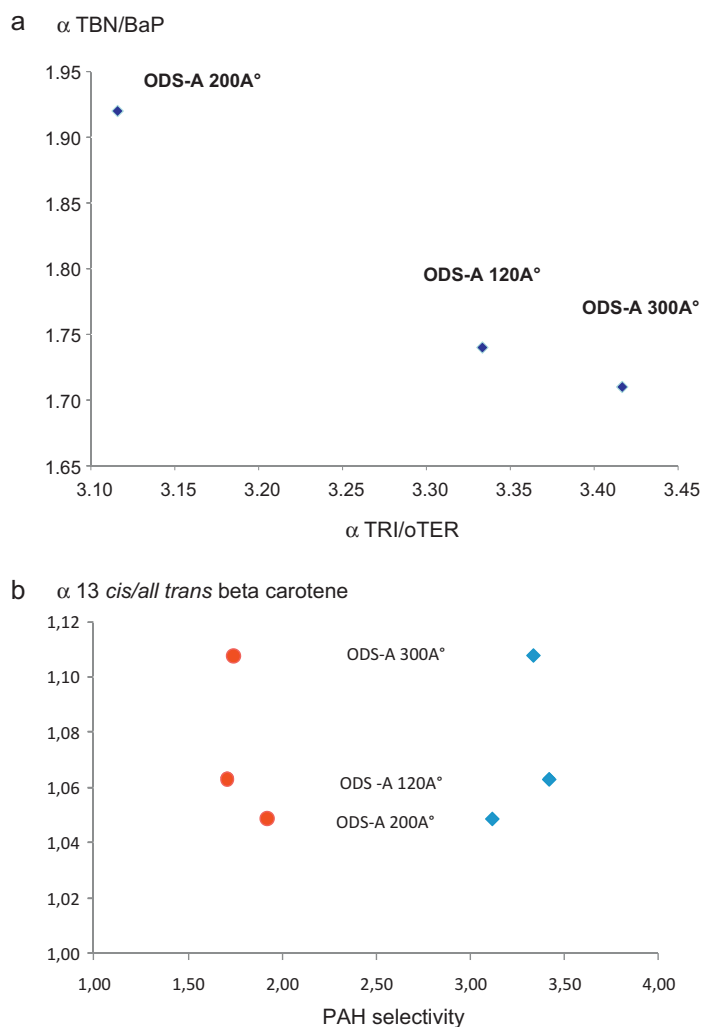


Fig. 6. Effect of the pore diameter on varied selectivities for three J'Sphere ODSA stationary phases. (a) α TBN/BaP vs. α TRI/oTER; (b) blue diamonds: α 13 *cis/all trans* vs. α TRI/oTER; red circles: α 13 *cis/all trans* vs. α TBN/BaP. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

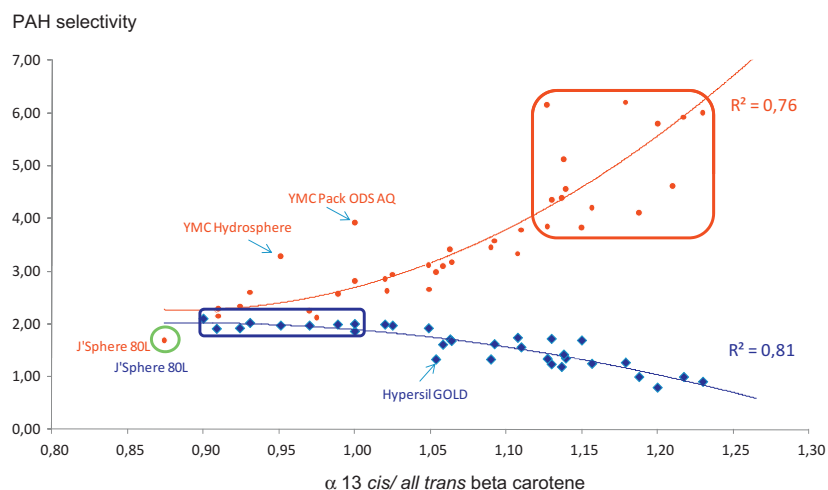


Fig. 7. Plot of the PAH selectivity vs. 13 *cis/all trans* β -carotene selectivity for varied stationary phases (Table 1). Red circles: α TRI/oTER; blue diamonds: α TBN/BaP. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 2

Log of the retention index (I) for two PAH (anthracene and o-terphenyl) on monomeric (Zorbax ODS) and polymeric (Vydac 201 TP) phases in HPLC (ACN/water) (from Ref. [18]).

Compounds	Monomeric phase	Polymeric phase
Anthracene	3.14	3.2
o-Terphenyl	3.31	2.97

no separation of *cis/trans* isomers occurs, do to the small length of the bonded phase, which is unable to distinguish linear and bent forms of longer compounds.

On the other hand, the TBN/BaP variations for the purely monomeric phases (selectivity values around 2), are minor in regards to the one of the 13 *cis/all trans* β -carotene ones (from 0.9 to 1.0). A possible explanation is that low bonding density does not allow the presence of slots having a sufficient size onto the silica surface. Among the points that are not correctly fitted, the Hypersil Gold can be mentioned, as the alkyl chain length is not known to us at this moment (the chain length is of prime importance for most of the probe solutes used in this paper). Of course, as reported previously, the J'Sphere 80L values are out of the regression lines, as well for TBN/BaP as for TRI/oTER, showing a close chromatographic behaviour of the varied PAHs on this phase.

In conclusion of these comparisons, the TRI/oTER selectivity fails to classify the phases having the higher selectivity values, whereas, as expected, the TBN/BaP selectivity is not very sensitive to stationary phase changes for purely monomeric bonded phases.

Next, we studied the retention behaviour of four PAHs classically used for column testing: acenaphthene, used by Neue to check hydrophobicity [10], triphenylene, ortho-terphenyl used in the Tanaka test having three aromatic rings leading to bent compounds, either planar (triphenylene) or non-planar (ortho-terphenyl) and anthracene, which have three aromatic rings placed in a linear, rigid and planar fashion. Anthracene is selected because the retention order between anthracene and o-terphenyl was reversed in HPLC from a monomeric phase (Zorbax ODS) to a polymeric one (Vydac 201 TP) (Table 2) [18]. This retention inversion is identical to the one observed between TBN and BaP, the linear compound (anthracene) was more retained on the polymeric phase, and the bent one (o-

terphenyl) on the monomeric one. This retention inversion was not observed in SFC with the selected mobile phase conditions, but, as expected, the increase or decrease in this selectivity in relation to the stationary phase nature is observed.

On the three J'Sphere phases, several retention inversions appear (Fig. 8), and selectivity varies. Three selectivities were selected, TRI/oTER, TRI/ANTHR, ANTHR/oTER, and correlated to the 13 *cis/all trans* β -carotene one (Fig. 9). No clear correlations were obtained with compound couples including acenaphthene.

The worst correlation coefficient ($R^2=0.54$) was obtained from the triphenylene/anthracene (TRI/ANTHRA) selectivity, and the best one ($R^2=0.80$) for anthracene/ortho-terphenyl (ANTHRA/oTER). On the other hand, the dispersion of the points in regards to the regression line is higher for TRI/oTER selectivity than for the two others. As indicated in Fig. 9, the points that do not really fit are mostly polar-embedded (Acclaim PA, XBridge Shield), polar-endcapped phases (Hydrosphere, YMC Pack ODS AQ, Aquasil, Aquastar) and polymeric ones (Nucleodur Isis, Vydac 201 TP54, Wakosil II 5C18 AR, Jupiter 300 C18).

Besides, YMC Hydrosphere and YMC Pack ODS AQ are not well located on the TRI/ANTHRA regression line, whereas the two polar-embedded phases (Acclaim PA and XBridge Shield) better fit on this TRI/ANTHRA line, as well as the two polymeric C18-bonded Nucleodur Isis and Jupiter 300.

It underlines that some of the polar-endcapped phases induce a shift in the retention of triphenylene. Previous studied on polar-embedded or polar-endcapped stationary phases have shown the specific ability of these phases for steric selectivity [3,4]. For most of the polar-embedded and endcapped phases, the TRI/oTER selectivity seems to provide uncorrelated values in regards to TBN/BaP and 13 *cis/trans* selectivities. Finally, except the polymeric Wakosil II 5C18 AR and Vydac 201 TP 54, the best fitting with 13 *cis/all trans* β -carotene selectivity is obtained for the anthracene/ortho-terphenyl selectivity. Thus, the TRI/oTER selectivity could be replaced by ANTHRA/oTER selectivity, in order to improve the accuracy and relevance in the shape selectivity studies. Moreover, the ability of this new PAH couple to be related to shape selectivity in HPLC due to temperature changes was also reported [19], and could be explained by greater stationary phase reordering for anthracene in regard to the one of triphenylene (entropic behaviour) [20].

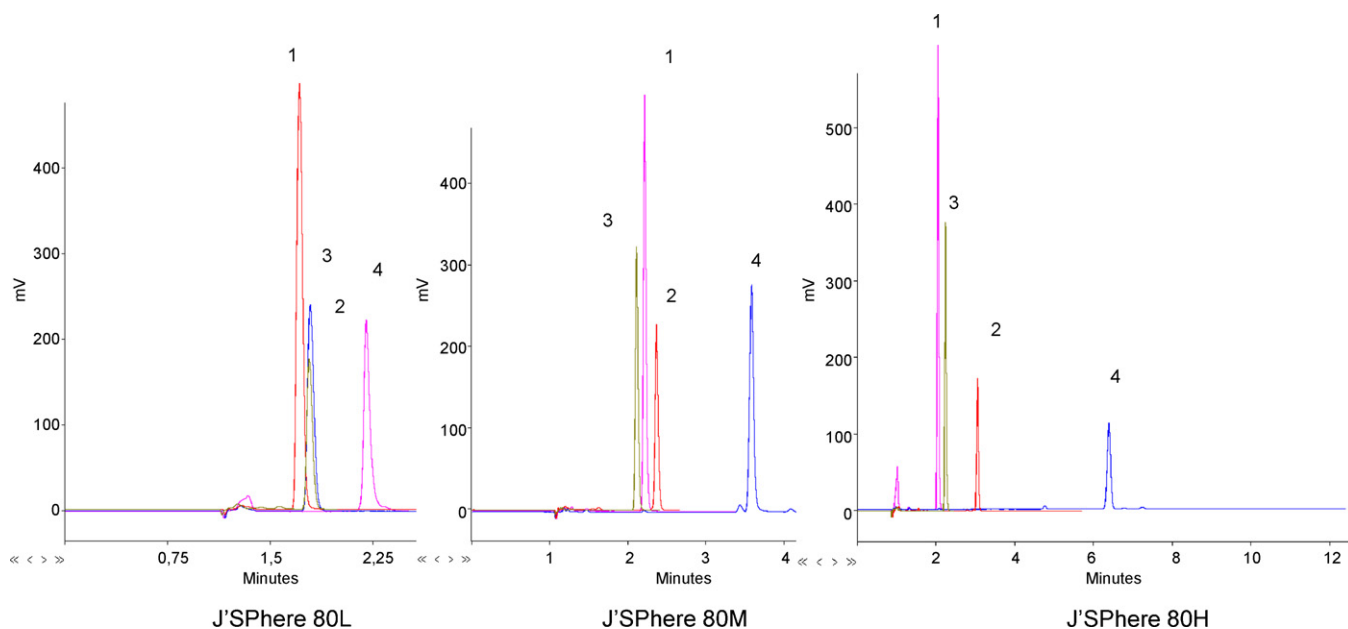


Fig. 8. Chromatograms of PAHs for the three J'Sphere phases. (1) Acenaphthene; (2) anthracene; (3) ortho-terphenyl; (4) triphenylene. Analytical conditions as in Fig. 1.

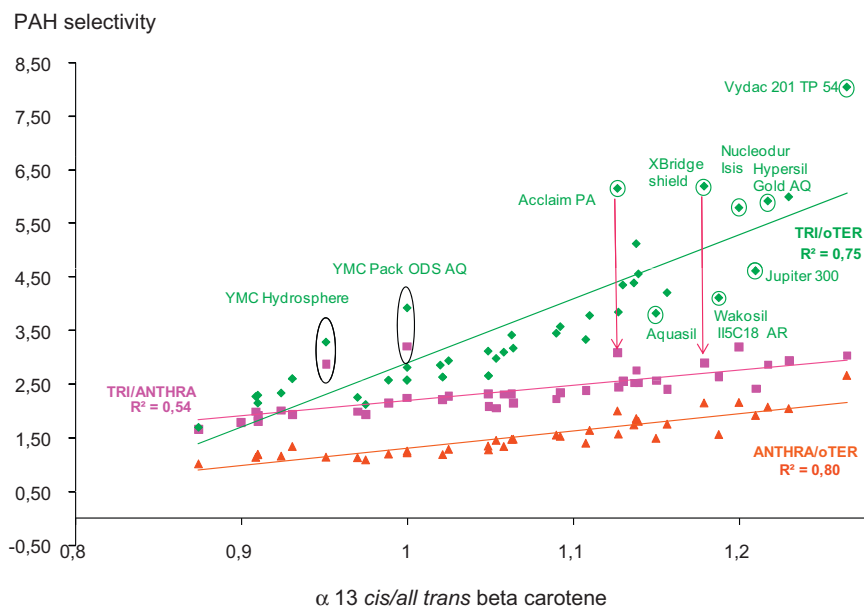


Fig. 9. Selectivities of varied PAHs vs. the 13 *cis/all trans* β -carotene selectivity. Green diamonds: α TRI/oTER; pink squares: α TRI/ANTHRA; orange triangles: α ANTHRA/oTER. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

4. Conclusion

From previous papers, the correlation between the 13-*cis/all trans* β -carotene selectivity and the TBN/BaP one was known, but the correlation between the 13-*cis/all trans* β -carotene, TBN/BaP and the triphenylene/ortho-terphenyl ones was not really studied.

It appears that the TRI/oTER selectivity does not provide accurate fitting both with the 13-*cis/all trans* β -carotene selectivity and with the surface coverage in SFC. Studies of comparable correlations from data obtained in HPLC provide identical conclusions [21].

The use of anthracene/ortho-terphenyl selectivity could be better suited to estimate shape selectivity mainly for polar-embedded and polar-encapped stationary phases. Yet it remains less suited than the 13-*cis/all trans*- β -carotene one to correlate surface coverage. However, surface coverage is determined by calculations whose accuracy depends on numerous parameters, that are probably sometimes over- or under-estimated.

On the other hand, the use of this new couple of PAHs for steric selectivity determination should be studied in HPLC.

The differences observed by using the varied probes could be in part due to their size or length differences. Additional studies on the steric hindrance of the polyaromatic compounds to penetrate into the bulk of the stationary phases should be done in regards of the bonding density for monomeric phases, especially for TRI/oTER, which are supposed to be included into the chains. Additional studies

in regards of the bonded chain length from C8 to C18 could also be interesting to better understand the penetration mechanism of these compounds.

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