

Anomalous behavior of selected methyl-substituted polycyclic aromatic hydrocarbons in reversed-phase liquid chromatography

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ABSTRACT

In the reversed-phase liquid chromatographic separation of polycyclic aromatic hydrocarbons (PAHs) on C_{18} phases, methyl-substituted PAHs are expected to elute after the unsubstituted parent PAH based on the increase in hydrocarbonaceous contact area of the methyl-substituted PAHs. However, we have observed that several methyl-substituted PAHs elute prior to the parent compound, *e.g.*, 1-methylperylene, 1-methylpicene and 13-methylpicene. To investigate this anomalous retention behavior, the retention characteristics of all methyl-substituted isomers of chrysene, picene and perylene were compared on a series of sixteen commercially prepared C_{18} phases. The anomalous retention behavior was observed only on polymeric C_{18} phases (*i.e.*, those prepared using trifunctional silanes) whereas the methyl-substituted PAHs elute after the parent PAH, as would be expected, on monomeric C_{18} phases (*i.e.*, those prepared using monofunctional silanes). The early elution of some of these methyl-substituted isomers is related to the non-planarity of these PAHs due to the presence of the methyl group in the so-called "bay-region" of the PAH structure. The non-planarity of these methyl-PAHs can be characterized by the dihedral angle of distortion between the aromatic rings.

INTRODUCTION

Reversed-phase liquid chromatography (LC) on C_{18} stationary phases provides excellent separations of polycyclic aromatic hydrocarbons (PAHs), particularly isomeric PAHs^{1–4}. Polymeric C_{18} phases (*i.e.*, those prepared using trifunctional silanes with the addition of water) have been shown to provide greater selectivity for the separation of isomeric PAHs than the more commonly used monomeric C_{18}

phases (*i.e.*, those prepared using monofunctional silanes with the exclusion of water)²⁻⁴. The factors responsible for the unique selectivity of polymeric C₁₈ phases have been discussed in several previous publications^{1,3}. The excellent selectivity of polymeric C₁₈ phases for PAH separations has also been demonstrated for methyl-substituted PAH isomers. Comparisons of retention for methyl-substituted phenanthrene, pyrene, fluoranthene, chrysene, benz[*a*]anthracene, benzo[*c*]phenanthrene and benzo[*a*]pyrene isomers on monomeric and polymeric C₁₈ phases have been reported^{1,4}. In general, these methyl-PAH isomers were resolved on polymeric C₁₈ phases and eluted in order of increasing rod-like shape, which was characterized by the length-to-breadth (*L/B*) ratio of the isomer⁴.

In the reversed-phase LC separation of PAHs on C₁₈ phases, methyl-substituted PAHs are generally expected to elute after the unsubstituted parent PAH because of the greater hydrocarbonaceous contact area (*i.e.*, hydrophobic character) of the methyl-PAH. However, we have observed that several methyl-PAHs elute prior to the parent compound, *e.g.*, 1-methylbenzo[*c*]phenanthrene⁴, 13-methylpicene¹ and 1-methylperylene⁵. The anomalous behavior of these methyl-PAHs led to the investigation of the retention characteristics of all methyl-substituted isomers of chrysene, perylene and picene on a series of sixteen commercially prepared monomeric and polymeric C₁₈ columns.

EXPERIMENTAL^a

PAH standards

The methylchrysene isomers were obtained from the Community Bureau of Reference (Brussels, Belgium). The methylperylene isomers were synthesized as reported previously⁶. The methylpicene isomers (except 13-methylpicene) were obtained from oxidative photocyclization of the appropriate 1,2-diarylethylene⁷. 13-Methylpicene was obtained from the Rare Chemical Collection of Aldrich (Milwaukee, WI, U.S.A.). Standard Reference Material (SRM) 869, "Column Selectivity Test Mixture for Liquid Chromatography" was obtained from the Office of Standard Reference Materials at the National Institute of Standards and Technology (Gaithersburg, MD, U.S.A.).

LC columns

The following C₁₈ columns were used in this study: Vydac 201TP reversed-phase C₁₈ and Vydac 218TP protein and peptide C₁₈ (Separations Group, Hesperia, CA, U.S.A.); Bakerbond C₁₈ and Bakerbond C₁₈ wide pore (J. T. Baker, Philipsburg, NJ, U.S.A.) (the Bakerbond C₁₈ is on a narrow pore silica substrate, 120 Å pore diameter, whereas the Bakerbond C₁₈ wide pore is on a silica with a 300 Å pore diameter); Erbasil C₁₈/H (Farmitalia Carlo Erba, Milan, Italy); Sepralyte C₁₈ (Analytichem, Harbor City, CA, U.S.A.); Zorbax ODS (Mac Mod, Chadds Ford, PA, U.S.A.); and Hypersil ODS (Keystone, State College, PA, U.S.A.). Three different Vydac 201TP

^a Certain commercial equipment, instruments or materials 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 the best available for the purpose.

columns were used. Vydac 201TP (normal) has the selectivity of C_{18} columns typically obtained from the manufacturer. Columns designated as Vydac 201TP (high) and (intermediate) are columns with percent carbon loadings higher than those typically obtained from the manufacturer. The Vydac 218TP phase has selectivity that is designated as "low" when compared to the "normal" Vydac 201TP columns. Five different Bakerbond C_{18} (wide pore) columns were used. These columns were experimental columns (A–E) which had been prepared by the manufacturer with varying percent carbon loadings on two different batches of silica (column A: $104 \text{ m}^2/\text{g}$ and 10.4% C; column B: $78 \text{ m}^2/\text{g}$ and 7.1% C; column C: $78 \text{ m}^2/\text{g}$ and 6.8% C; column D: $78 \text{ m}^2/\text{g}$ and 6.2% C; column E: $104 \text{ m}^2/\text{g}$ and 5.6% C)⁸; columns B and C are typical of the carbon loading and selectivity for Bakerbond C_{18} (wide pore) columns.

LC conditions

Each of the LC columns was characterized using the Column Selectivity Test Mixture for Liquid Chromatography (SRM 869), as described previously^{9,10}, and selectivity ratios for tetrabenzonaphthalene (TBN) and benzo[*a*]pyrene (BaP) ($\alpha_{\text{TBN/BaP}}$) were calculated. The $\alpha_{\text{TBN/BaP}}$ values were determined with a mobile phase of acetonitrile–water (85:15) at a flow-rate of 1.5 ml/min and at a column temperature of 28°C. The selectivity ratios for the methyl-PAHs, relative to the unsubstituted parent PAH, were determined using a mobile phase of acetonitrile–water (80:20) for the methylchrysenes and methylperylene and acetonitrile–water (95:5) for the methylpicenes. A multiwavelength ultraviolet absorption detector (Spectroflow 783, Kratos, Ramsey, NJ, U.S.A.) or a fluorescence spectrometer (LS-4, Perkin-Elmer, Norwalk, CT, U.S.A.) was used for detection of the solutes.

Calculation of solute shape and planarity parameters

The length-to-breadth (L/B) values for each of the methyl-PAH isomers were calculated using the approach described previously⁴. A new computer program was written to calculate L/B values using coordinates generated for space filling models of the PAH as shown in Fig. 1. The space filling models of the PAHs were determined using the X1CAMM Molecular Modeling program (XIRIS Corporation, New Monmouth, NJ, U.S.A.). The dihedral angles between aromatic rings were determined from calculations of the geometry of the molecule at the energy minimum using the MM2MP2 (version 1985) empirical force field method¹¹ on a VAX computer at the University of Bordeaux. Dihedral angles represent the angle formed by the bonds which link carbon atoms belonging to three aromatic rings, *i.e.*, it is the angle defined by the two aromatic rings connected to a common aromatic ring. For example in Fig. 1, α represents the dihedral angle of the two planes defined by ring A and ring C, whereas β represents the angle between the two planes defined by ring B and ring D. The sign (+ or –) represents dihedral distortion relative to an average molecular plane (– for down, + for up).

RESULTS AND DISCUSSION

In previous studies^{1,2,4,12}, the retention behavior of isomeric PAHs has been shown to be significantly different on monomeric C_{18} phases compared to polymeric C_{18} phases. Polymeric C_{18} phases are prepared using trifunctional silanes with the

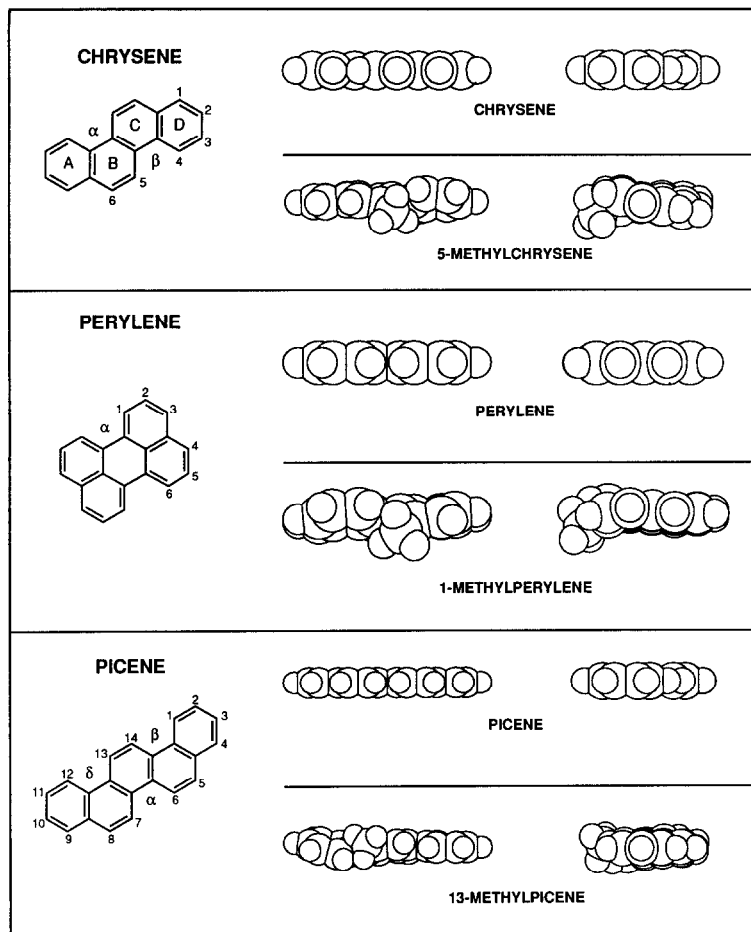


Fig. 1. Structures and space filling models of chrysene, picene and perylene and space filling models of selected non-planar methyl-substituted chrysene, picene and perylene isomers. The symbols α , β and δ designate the specific dihedral angles of distortion (see Table II).

addition of water, whereas monomeric C_{18} phases are prepared using monofunctional silanes, generally with the exclusion of water during the synthesis². Methyl-substituted PAH isomers have different selectivity characteristics on monomeric and polymeric C_{18} phases^{1,4}. In the present study, a series of sixteen commercially prepared C_{18} columns was selected and used to investigate the selectivity of several isomeric sets of methyl-substituted PAHs. These columns were characterized using the selectivity ratio (α) of tetrabenzonaphthalene and benzo[*a*]pyrene, which has been used previously to classify phase selectivity for the separation of PAHs^{9,10}. Based on a previous study¹⁰ in which the $\alpha_{\text{TBN/BaP}}$ values were determined for 25 different commercial C_{18} columns, this series of columns was selected to cover a broad range of selectivity characteristics. The $\alpha_{\text{TBN/BaP}}$ values for the sixteen columns used in this study are summarized in Table I. The majority of the phases used in this study were designated as polymeric C_{18}

TABLE I
LC COLUMNS INVESTIGATED FOR SELECTIVITY OF METHYL-SUBSTITUTED PAHs

Column	Selectivity ratio $\alpha_{\text{TBN/BaP}}^a$	Phase type ^b
Bakerbond C ₁₈ (wide pore) (A) ^c	0.27	Polymeric
Vydac 201TP (high) ^d	0.39	Polymeric
Vydac 201TP (high) ^d	0.46	Polymeric
Vydac 201TP (intermediate) ^d	0.60	Polymeric
Bakerbond C ₁₈ (wide pore) (B) ^c	0.62	Polymeric
Vydac 201TP (intermediate) ^d	0.63	Polymeric
Vydac 201TP (normal) ^e	0.72	Polymeric
Bakerbond C ₁₈ (wide pore) (C) ^c	0.78	Polymeric
Bakerbond C ₁₈ (wide pore) (D) ^c	1.00	Polymeric
Erbasil C18/H	1.00	Polymeric
Vydac 218TP (protein and peptide)	1.12	Polymeric
Bakerbond C ₁₈	1.32	Polymeric
Bakerbond C ₁₈ (wide pore) (E) ^c	1.43	Polymeric
Sepralyte C ₁₈	1.72	Monomeric
Zorbax ODS	1.94	Monomeric
Hypersil ODS	1.98	Monomeric

^a Selectivity factor (α) for tetrabenzonaphthalene and benzo[*a*]pyrene as described by Sander and Wise¹⁰.

^b Phase designation based on manufacturers' information regarding phase preparation.

^c Columns designated as A–E were Bakerbond C₁₈ (wide pore) that had been prepared with varying % carbon loadings; columns B and C are typical of the selectivity for Bakerbond C₁₈ (wide pore) columns.

^d Columns designated as Vydac 201TP (high) and Vydac 201TP (intermediate) are columns with % carbon loadings higher than those typically obtained from this manufacturer.

^e Vydac 201TP (normal) has the selectivity of columns typically obtained from this manufacturer.

phases based on information from the manufacturers regarding the phase preparation. Based on the scheme proposed by Sander and Wise¹⁰, which utilizes the $\alpha_{\text{TBN/BaP}}$ values to classify the characteristics of C₁₈ phases for PAH selectivity, ten of the columns were classified as "polymeric" ($\alpha_{\text{TBN/BaP}} \leq 1$), three columns were classified as "intermediate" ($1 < \alpha_{\text{TBN/BaP}} < 1.7$), and only three were classified as "monomeric" ($\alpha_{\text{TBN/BaP}} \geq 1.7$). The $\alpha_{\text{TBN/BaP}}$ values for the sixteen columns in this study varied from 0.27, which indicates extremely high polymeric-like behavior, to 1.98 which indicates conventional monomeric-like selectivity. To provide a wide range of phases with polymeric-like selectivity, several columns, which had been prepared with varying percent carbon on the same wide-pore silica substrate, were obtained from two of the manufacturers. Even though the majority of commercially available C₁₈ phases are designated as monomeric phases¹⁰, only three monomeric C₁₈ phases were included in this study since all monomeric C₁₈ phases generally have very similar selectivity characteristics for the separation of isomeric PAHs.

The retention characteristics of three sets of methyl-substituted PAH isomers, *i.e.*, methylchrysenes, methylpicenes and methylperylene (see Fig. 1), were investigated in this study. For each of these sets of methyl-PAHs, all possible isomers were available, *i.e.*, chrysene (six isomers), perylene (three isomers) and picene (seven isomers). These methyl-PAHs are of particular interest in the study of geochemical

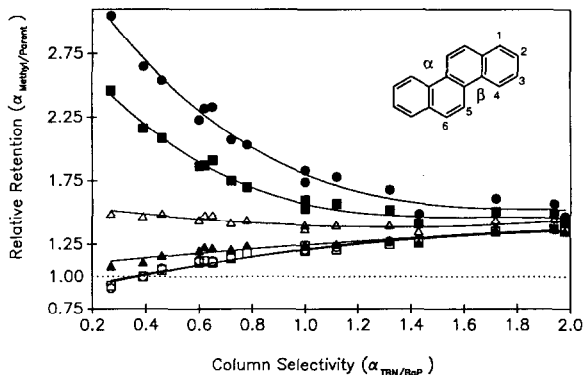


Fig. 2. Plot of retention of the methylchrysene isomers relative to chrysene *versus* column selectivity ($\alpha_{\text{TBN/BaP}}$) for sixteen different C_{18} columns. ● = 2-Methyl; ■ = 1-methyl; △ = 3-methyl; ▲ = 4-methyl; □ = 5-methyl; ○ = 6-methyl; ····· = chrysene.

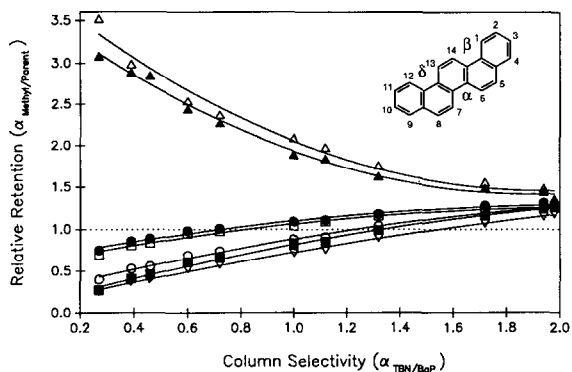


Fig. 3. Plot of retention of the methylpicene isomers relative to picene *versus* column selectivity ($\alpha_{\text{TBN/BaP}}$) for eleven different C_{18} columns. △ = 3-Methyl; ▲ = 4-methyl; ● = 2-methyl; □ = 5-methyl; ○ = 1-methyl; ■ = 6-methyl; ▽ = 13-methyl; ····· = picene.

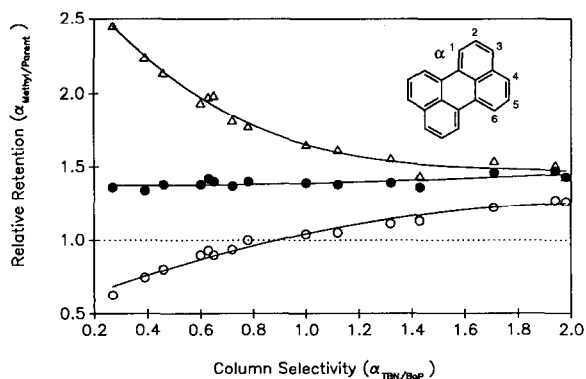


Fig. 4. Plot of retention of the methylperylene isomers relative to perylene *versus* column selectivity ($\alpha_{\text{TBN/BaP}}$) for fifteen different C_{18} columns. △ = 3-Methyl; ● = 2-methyl; ○ = 1-methyl; ····· = perylene.

processes. They have been identified in sediments, petroleum and several rare PAH minerals¹³ as diagenetic maturity indicators¹⁴, and in marine sediments as pollution origin markers¹⁵.

The selectivity ratios (*i.e.*, relative retention) of the methyl-substituted isomers to the unsubstituted parent PAH were plotted *versus* the $\alpha_{\text{TBN/BaP}}$ values for the different LC columns (*i.e.*, the monomeric or polymeric nature of the stationary phase). These selectivity plots are shown in Figs. 2, 3 and 4 for the methylchrysene, methylpicene and methylperylene isomers, respectively. Several trends are apparent for all three groups of methyl-PAHs. For the three monomeric stationary phases, all of the methyl-substituted isomers elute after the parent PAHs, as would be expected based on the increased molecular weight and contact area of the PAH solute, and the methyl-PAHs tend to elute as a group (*i.e.*, very little separation of the individual isomers). As the polymeric nature of the phase increases (*i.e.*, decreasing $\alpha_{\text{TBN/BaP}}$ values), the relative separation of the methyl isomers increases. This increased relative separation of the methyl-PAHs and the parent PAHs cannot be attributed to an increase in absolute retention. In fact, the absolute retention of chrysene, picene and perylene is generally greater on the monomeric phases than on the polymeric phases. These trends will be discussed in more detail below for each group of isomers.

The elution order of methyl-PAHs in reversed-phase LC correlates with the shape of the solute as described by the L/B ratio⁴. In addition to the shape, the planarity of the solute also affects the elution behavior of PAHs³. Recently, Garrigues *et al.*¹⁶ calculated the dihedral angle of distortion for dimethylphenanthrene isomers and found that the isomers with large distortion angles eluted earlier than predicted by the L/B ratio. The distortion angle is defined as the dihedral angle between the aromatic rings opposite each other (*i.e.*, not adjacent rings) in the bay region. For example in Fig. 1, α represents the dihedral angle of the two planes defined by ring A and ring C, whereas β represents the angle between the two planes defined by ring B and ring D. The calculation of an angle of distortion by Garrigues *et al.*¹⁶ represents the first attempt to quantify a parameter associated with non-planarity for correlation with chromatographic behavior. The L/B ratios and the angles of distortion for the methylchrysene, methylpicene and methylperylene isomers are summarized in Table II. For each of the PAHs, substitution of a methyl group in the "bay regions" (designated as α , β and δ in Fig. 1) results in varying degrees of distortion between the carbon atoms in the aromatic rings. For example, 4-methyl- and 5-methylchrysene have angles of 8.7 and 11.4 in the β bay region. For picene, which has three bay regions, distortion of varying amounts occurs in all three regions for 1-methyl-, 6-methyl- and 13-methylpicene.

Methylchrysene isomers

The selectivity ratios of the methylchrysenes relative to chrysene are plotted *versus* the stationary phase characteristics of sixteen C_{18} columns in Fig. 2. The chromatograms for the separation of the six methylchrysene isomers and chrysene on three different C_{18} phases, which have $\alpha_{\text{TBN/BaP}}$ of 0.27, 0.60 and 1.98, are illustrated in Fig. 5. The elution order of the methylchrysene isomers follows increasing L/B ratio. As shown in Fig. 2, the selectivity generally increases as the phase becomes more polymeric in nature (*i.e.*, decreasing $\alpha_{\text{TBN/BaP}}$ values). For the isomers with the largest L/B ratios (1-methyl and 2-methyl), the selectivity relative to chrysene increases as the

TABLE II

LENGTH-TO-BREADTH (L/B) RATIOS AND DIHEDRAL ANGLES OF DISTORTION FOR METHYLCHRYSENES, METHYLPICENES AND METHYLPERYLENES

See Experimental section for detailed definitions of the terms used. Methyl isomers listed in order of elution from Vydac 201TP (normal) column.

Isomers	L/B^a	Angle of distortion		
		α	β	δ
<i>Chrysene</i>				
5-Methyl	1.48	4.3	-11.4	
6-Methyl	1.48	0.0	0.0	
4-Methyl	1.51	-3.6	-8.7	
3-Methyl	1.63	0.0	0.0	
1-Methyl	1.71	0.1	0.0	
2-Methyl	1.85	0.0	0.0	
<i>Picene</i>				
13-Methyl	1.75	-14.1	3.8	-25.4
6-Methyl	1.70	-26.9	-11.2	-12.0
1-Methyl	1.76	-14.0	-25.4	3.7
5-Methyl	1.76	-0.2	-0.7	0.4
2-Methyl	1.83	-1.5	-1.4	-0.4
4-Methyl	2.00	0.4	-1.0	0.0
3-Methyl	2.13	-1.1	-0.2	-0.2
<i>Perylene</i>				
1-Methyl	1.14	9.0		
2-Methyl	1.18	0.0		
3-Methyl	1.37	0.0		

^a L/B is the length-to-breadth ratio of the solute as described previously⁴.

phase becomes more polymeric in character; whereas for the isomers with the lowest L/B ratios and/or some non-planarity (as indicated by the angle of distortion), the selectivity ratio decreases and eventually becomes less than 1 (*i.e.*, elutes prior to chrysene) for the 5-methyl and 6-methyl isomers on the most highest loaded polymeric phase. For 3-methylchrysene, which has no distortion from planar (distortion angle of 0.0), the retention relative to chrysene changes very little on the different phases. The 5-methyl- and 6-methylchrysene have very similar behavior even though the 5-methyl isomer has angles of distortion of 4.3 and 11.4 degrees, whereas the 6-methyl isomer has no distortion. However, Sander and Wise¹⁷ reported a separation of 5-methyl- and 6-methylchrysene at subambient temperatures with the 5-methyl isomer eluting first as would be expected based on non-planarity considerations.

Methylpicenes

The selectivity ratios of the methylpicenes relative to picene are plotted *versus* the stationary phase characteristics of eleven C_{18} columns in Fig. 3. As with the methylchrysenes, the α values for the isomers with the largest L/B ratios (3-methyl and 4-methyl) increase as the phases become more polymeric, and the retention relative to picene for the remaining isomers decreases. The chromatograms for the separation of

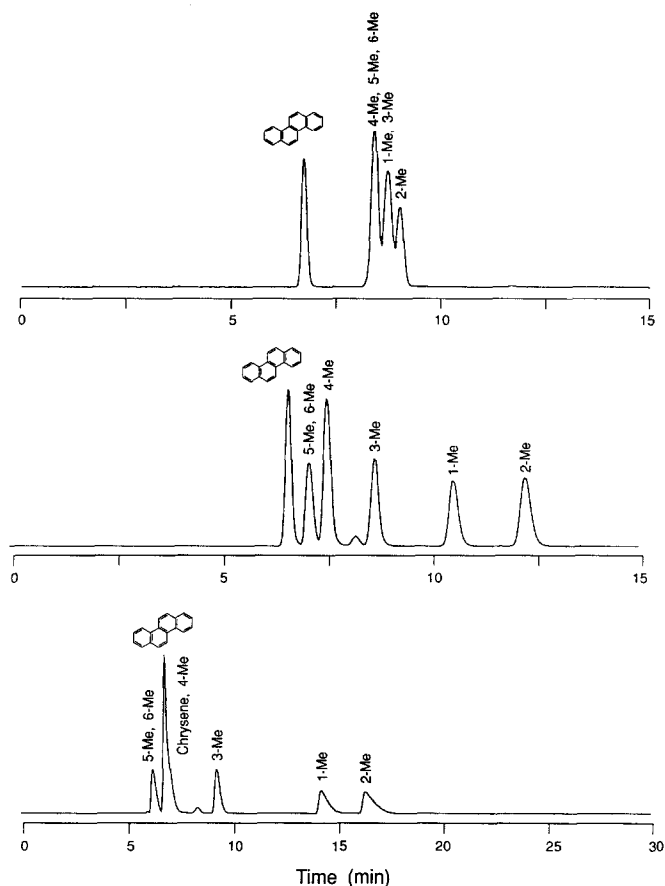


Fig. 5. Reversed-phase separation of chrysene and methylchrysene isomers on Hypersil ODS, $\alpha_{\text{TBN/BaP}} = 1.98$ (upper chromatogram); Vydac 201TP, $\alpha_{\text{TBN/BaP}} = 0.60$ (middle chromatogram); and Bakerbond C_{18} (wide pore) (A), $\alpha_{\text{TBN/BaP}} = 0.27$ (lower chromatogram). Mobile phase: acetonitrile-water (80:20) at 1.5 ml/min. Fluorescence detection at 265 nm excitation and 365 nm emission. Me = Methyl.

the seven methylpicene isomers on three different C_{18} phases are shown in Fig. 6. The three methylpicene isomers that elute the earliest (13-methyl, 6-methyl and 1-methyl) have large angles of distortion (about 12 to 27 degrees) in the α , β and δ bay regions as well as the smallest L/B ratios for the isomer set (see Table II). As the polymeric nature of the phase increases (*i.e.*, decreasing $\alpha_{\text{TBN/BaP}}$ values), the relative separation of the methyl isomers increases and several methyl isomers eventually elute before picene, *i.e.*, 13-methyl-, 6-methyl-, 1-methyl-, 5-methyl- and 2-methylpicene.

Methylperylene

The selectivity ratios of the methylperylene relative to perylene are plotted *versus* the stationary phase characteristics of fifteen C_{18} columns in Fig. 4. The chromatograms for the separation of the three methylperylene isomers and perylene

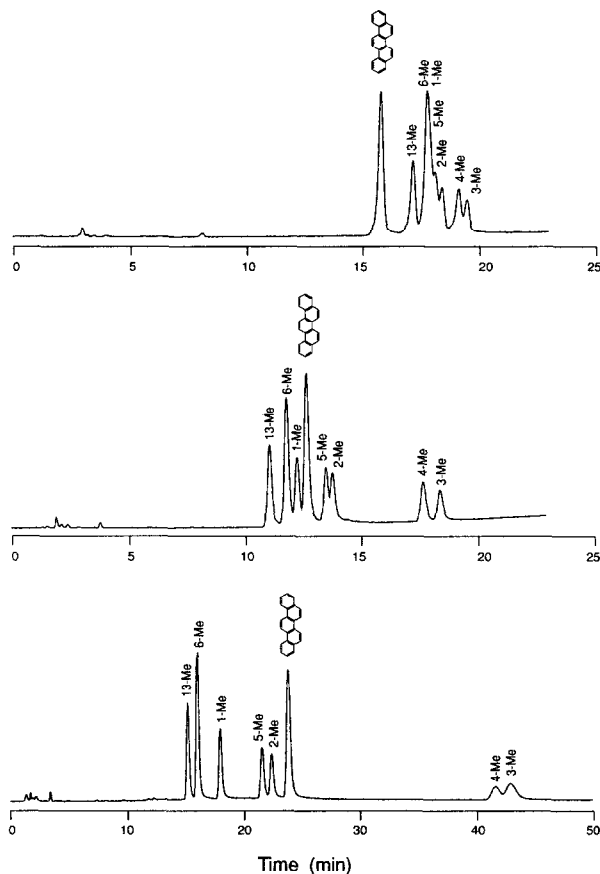


Fig. 6. Reversed-phase separation of picene and methylpicene isomers on Zorbax ODS, $\alpha_{\text{TBN/BaP}} = 1.94$ (upper chromatogram); Vydac 218TP, $\alpha_{\text{TBN/BaP}} = 1.12$ (middle chromatogram); and Vydac 201TP, $\alpha_{\text{TBN/BaP}} = 0.39$ (lower chromatogram). Mobile phase: gradient from acetonitrile-water (80:20) to 100% acetonitrile at 1%/min at 1.5 ml/min. Fluorescence detection at 284 nm excitation and 382 nm emission.

on two different C_{18} phases are shown in Fig. 7. Both of the C_{18} phases used in Fig. 7 were from the same manufacturer, and both columns had selectivity characteristics outside the normal range. Using a column with typical selectivity for this manufacturer ($\alpha_{\text{TBN/BaP}}$ value between approximately 0.70 and 0.90) would have resulted in 1-methylperylene coeluting with perylene (see Fig. 4). Garrigues *et al.*⁵ first observed that 1-methylperylene eluted prior to perylene using a polymeric C_{18} column with a higher than normal carbon loading. The elution order of the three isomers follows increasing L/B ratios. The elution of the 1-methylperylene before perylene on the polymeric C_{18} phases can be attributed to the non-planarity of this isomer since the methyl group in the 1-position results in a distortion angle of 9 degrees.

The following trends can be summarized for all three groups of methyl-substituted isomers investigated: (1) The relative separation of the methyl-substituted PAH isomers increases as the C_{18} phase becomes more polymeric in nature ($\alpha_{\text{TBN/BaP}}$

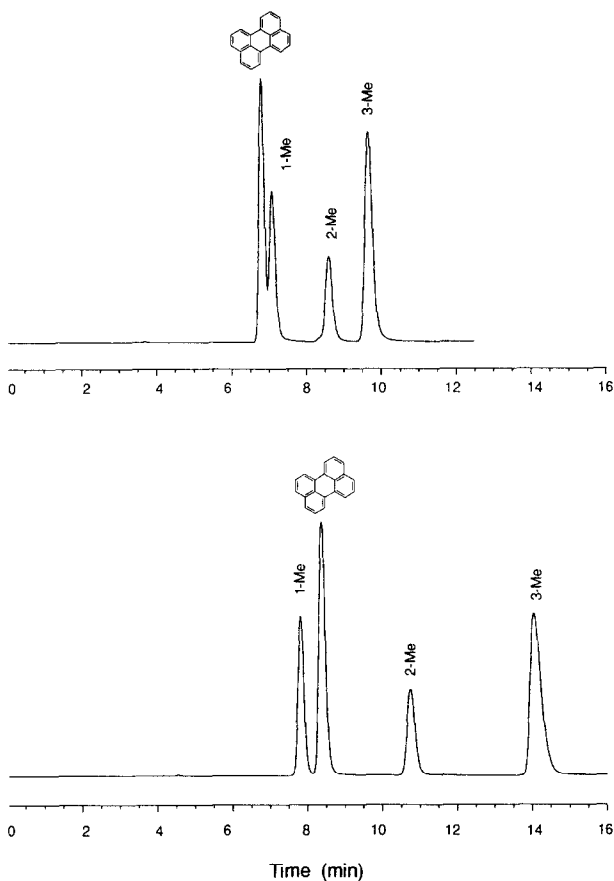


Fig. 7. Reversed-phase separation of perylene and methylperylene isomers on Vydac 201TP, $\alpha_{\text{TBN/BaP}} = 1.12$ (upper chromatogram); and Vydac 210TP, $\alpha_{\text{TBN/BaP}} = 0.60$ (lower chromatogram). Mobile phase: acetonitrile–water (80:20) at 1.5 ml/min. Fluorescence detection at 406 nm excitation and 440 nm emission.

decreases). (2) The order of elution of methyl-PAH isomers generally follows increasing L/B ratio, particularly on polymeric C_{18} phases. (3) On monomeric C_{18} phases, the methyl isomers elute after the parent PAH and tend to elute as a group, with little separation of individual isomers. (4) As the polymeric nature of the phase increases, isomers with some non-planarity and small L/B values eventually elute prior to the parent PAH. (5) As the polymeric nature of the phase increases, the retention of the isomers with large L/B values and planar structures increases relative to the parent PAH.

Retention model

The behavior of these non-planar methyl-substituted PAHs can be explained in terms of the “slot model”, as proposed previously³. In this model the bonded alkyl phase is represented schematically as a surface with slots into which the PAH solute molecules can penetrate. Long, narrow solutes (large L/B values) can penetrate to

a greater extent than more compact, square solutes. Non-planar PAH solutes are partially excluded from the narrow slots resulting in reduced retention relative to planar PAHs.

CONCLUSIONS

For methyl-substituted PAH isomers, the characteristics of the C₁₈ stationary phase and the planarity of the solute significantly influence the selectivity of the reversed-phase LC separation. By understanding the behavior of these isomers on various C₁₈ phases, the appropriate phase can be selected for a particular application. For example, using a monomeric C₁₈ phase provides a separation of the unsubstituted parent PAH from the methyl-substituted isomers with little differentiation among the methyl isomers. Garrigues *et al.*^{18,19} have used this approach to isolate the methylphenanthrene isomers as a group prior to determination of the individual isomers using low temperature fluorescence spectroscopy. However, a C₁₈ phase with extreme polymeric-like behavior may be required to provide an adequate separation of all methyl isomers of a particular PAH to determine the relative distributions of each isomer in various samples.

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