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The Influence of the Aromatic Character in the Gas Chromatography Elution Order: The Case of Polycyclic Aromatic Hydrocarbons

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Abstract

A link between the aromatic character of polycyclic aromatic hydrocarbons and gas chromatography elution order in columns with a polysiloxane backbone in the stationary phase is reported for the first time. The aromatic character was calculated using a method that combines the π -Sextet Rule and the Pauling Ring Bond Orders to allow the establishment of the location and migration of aromatic sextets in PAH structures. One GC column with a polysiloxane - like backbone (Rxi-PAH) and three GC columns with a polysiloxane backbone (DB-5, SE-52, and LC-50), were used for the analysis. According to the results of this study, within an isomer group, PAHs that contain a lower number of rings affected by the aromatic sextets tend to elute earlier than PAHs that contain a higher number of rings affected by the aromatic sextets. The PAHs that follow the calculated elution order are 88 % in the Rxi-PAH column, 88 % in the DB-5 column, 93 % in the SE-52 column, and 85% in the LC-50 column. It is expected that future analyses with other aromatic compounds in GC columns with a polysiloxane backbone in the stationary phase will follow a GC elution order that agrees with the aromatic character of the molecules.

Keywords

Polycyclic Aromatic Hydrocarbons; Aromatic Character; π -Sextet Rule; Pauling Ring Bond Order; Gas Chromatography with Mass Spectrometry

1. Introduction

Gas Chromatography (GC) is the most widely used analytical technique for the characterization of polycyclic aromatic hydrocarbons (PAHs). The major factors that influence the separation of solutes in GC are the solute boiling point and the interaction of

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the solute with the column stationary phase. For PAHs with molecular mass below 300 Da, the boiling point, which is usually available [1], is a reliable indication of the GC elution order; i.e., isomers with low boiling point elute before isomers with high boiling point. However, for PAHs with molecular mass of 300 Da and above, the boiling point (if any) is rarely available. In addition, since the number of PAH isomers in groups above 300 Da increase consistently with the increase of the molecular mass, it is likely that some PAH isomers may exhibit similar boiling points. In these cases, the GC separation and elution order depend preferentially on the level of interaction between the solute and the column stationary phase rather than primarily boiling point alone. When the level of interaction is favorable, i.e., due to the existence of solute-stationary phase compatibilities, the presence of dispersion interaction forces or a combination of polar and dispersion interaction forces generate longer elution times [2]. Alternatively, when the level of interaction is unfavorable, i.e., due to the existence of solute-stationary phase incompatibilities, the presence of reduced dispersion forces or negligible combinations of polar and dispersion forces generate lower elution times [2]. Therefore, the properties of the stationary phase and the distribution of aromatic sextets, i.e., rings with inherent delocalized π electrons, in the solute contribute to effective or ineffective solute-stationary phase interactions.

Qualitative and quantitative methods have been developed to elucidate the presence of aromatic sextets in PAHs [3–8]. Among the qualitative methods, the empirical π -Sextet Rule by Clar, which has been verified experimentally [9], has proven to be an exact and a straightforward procedure [6,10]. Another procedure for establishing the presence of aromatic sextets in rings of PAHs is the calculation of the Pauling Ring Bond Orders (RBOs) [11]. Each RBO is representative of a ring and it is calculated by adding the Pauling bond orders of the ring CC bonds [11,12]. The Pauling bond order is a quantitative measure of the content of π electrons in a CC bond [13]. The RBO, in a different way, is a measure of the content of π electrons in a ring [14]. A consistent relationship between the results from the π -Sextet Rule and the RBOs was successfully reported by Randic [12]. In this perspective, the Kekulé structures are essential part of the calculation of the RBOs since these structures describe exactly the arrangement of the carbons inside of the rings using single and double bonds giving a complete panorama of the movement of the electrons.

A method for predicting GC elution order of PAHs uses the correlation of the retention index with the polarizability (α) [15]. As a difference with the aromatic character, i.e., the basis of the methodology reported here, which is an inherent and non-induced behavior of each PAH molecule that depends on the location and migration of the aromatic sextets [6,7,10], the polarizability is defined as the capability to generate instantaneous dipoles (induced and non-inherent behavior) as a response of a bound system to the presence of an external electric field [16]. Lamparczyk et al. [15] studied the relationship between the average molecular polarizabilities of PAHs and their GC retention indices in stationary phases composed of 100 % dimethylpolysiloxanes (SE-30 and OV-101), 5 % diphenyl – 95 % dimethylpolysiloxane (SE-52), 20 % diphenyl – 80 % dimethylpolysiloxane (OV-7), and 50 % diphenyl – 50 % dimethylpolysiloxane (OV-17), and found linear correlations. According to these observations, in GC columns, PAHs with higher polarizabilities are retained longer than PAHs with lower polarizabilities. The advantages of the methodology

based on the aromatic character (also known as aromaticity) explained here in comparison with the method based on the polarizability will be discussed later in this report.

The objective of the reported research was the establishment of a relation between the general elution order in GC and the number of rings affected by the aromatic sextets in PAHs C₁₄H₁₀, C₁₈H₁₂, C₂₀H₁₂, C₂₂H₁₂, C₂₂H₁₄, C₂₄H₁₄, C₂₆H₁₄, and C₂₆H₁₆. Since this investigation is based on experimental measures using GC, only the PAHs with reference standards available and miscible in GC compatible solvents denoted in Figures 1 and 2 are investigated. It is expected that a similar behavior will apply to the GC elution order of PAHs with reference standards not available and of PAHs in isomer groups that exceed the C₂₆H₁₆ formula (MM=328 Da). To the best of our knowledge, this is the first time that the aromatic character has been associated with the elution order of PAHs in GC.

2. Materials and Methods

Chemicals

The compounds under study are identified in Figures 1 and 2.

The PAH standards used in this investigation were obtained from the following sources: Fluka Chemie AG., Buchs, Switzerland (C₁₄H₁₀-a, C₁₈H₁₂-d, and C₂₂H₁₂-a); Eastman Organic Chemicals, Rochester NY (C₁₄H₁₀-b); Chemsyn Science Laboratories, Lenexa, Kansas, USA (C₁₈H₁₂-a); Bureau of Community Reference (BCR), Brussels, Belgium (C₁₈H₁₂-b, C₂₀H₁₂-a, C₂₀H₁₂-b, C₂₂H₁₄-d, C₂₂H₁₄-g, and C₂₂H₁₄-i); Aldrich Chemical Company, Milwaukee, Wisconsin, USA (C₁₈H₁₂-c, C₁₈H₁₂-e, C₂₀H₁₂-c, and C₂₂H₁₄-f); K&K Laboratories, Plainview, New York, USA (C₂₂H₁₂-b); W Schmidt, Institut für PAH-Forschung, Greifenberg, Germany (C₂₂H₁₄-a, C₂₂H₁₄-b, C₂₂H₁₄-c, C₂₂H₁₄-h, C₂₂H₁₄-j, C₂₂H₁₄-k, C₂₆H₁₄-a, C₂₆H₁₄-b, C₂₆H₁₄-c, C₂₆H₁₄-d, C₂₆H₁₄-e, C₂₆H₁₆-b, C₂₆H₁₆-d, C₂₆H₁₆-e, C₂₆H₁₆-f, C₂₆H₁₆-g, C₂₆H₁₆-h, C₂₆H₁₆-i, C₂₆H₁₆-k, C₂₆H₁₆-m, C₂₆H₁₆-n, C₂₆H₁₆-o, C₂₆H₁₆-p, and C₂₆H₁₆-q); Chemical Repository National Cancer Institute, Bethesda, Maryland, USA (C₂₂H₁₄-e); Chiron AS, Trondheim, Norway (C₂₆H₁₆-a); AK Sharma, Penn State University, College of Medicine, Department of Pharmacology, Hershey, Pennsylvania (C₂₆H₁₆-c, C₂₆H₁₆-j, and C₂₆H₁₆-l); Q Miao Department of Chemistry, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, China (C₂₄H₁₄-m). The sources of C₂₄H₁₄-a, C₂₄H₁₄-b, C₂₄H₁₄-c, C₂₄H₁₄-d, C₂₄H₁₄-e, C₂₄H₁₄-f, C₂₄H₁₄-g, C₂₄H₁₄-h, C₂₄H₁₄-i, C₂₄H₁₄-j, C₂₄H₁₄-k, and C₂₄H₁₄-l are stated in reference 17.

The stated purity of the PAH standards is the following: C₁₄H₁₀-a ≈97 %, C₁₈H₁₂-a ≈99 %, C₁₈H₁₂-c ≈98 %, C₁₈H₁₂-e ≈98 %, C₂₀H₁₂-c ≈99 %, C₂₂H₁₄-f ≈99.5 %, C₂₆H₁₄-b ≈99 %, and C₂₆H₁₆-a ≈99.5 %. The purity of the PAH standards C₂₄H₁₄-a, C₂₄H₁₄-b, C₂₄H₁₄-c, C₂₄H₁₄-d, C₂₄H₁₄-e, C₂₄H₁₄-f, C₂₄H₁₄-g, C₂₄H₁₄-h, C₂₄H₁₄-i, C₂₄H₁₄-j, C₂₄H₁₄-k, and C₂₄H₁₄-l is stated in reference 17. The purity of the PAH standard C₂₄H₁₄-m is reported in reference 18. The purity of the PAH standards C₁₄H₁₀-b, C₁₈H₁₂-b, C₁₈H₁₂-d, C₂₀H₁₂-a, C₂₀H₁₂-b, C₂₂H₁₂-a, C₂₂H₁₂-b, C₂₂H₁₄-a, C₂₂H₁₄-b, C₂₂H₁₄-c, C₂₂H₁₄-d, C₂₂H₁₄-e, C₂₂H₁₄-g, C₂₂H₁₄-h, C₂₂H₁₄-i, C₂₂H₁₄-j, C₂₂H₁₄-k, C₂₆H₁₄-a, C₂₆H₁₄-c, C₂₆H₁₄-d, C₂₆H₁₄-e, C₂₆H₁₆-b, C₂₆H₁₆-c, C₂₆H₁₆-d, C₂₆H₁₆-e, C₂₆H₁₆-f,

$C_{26}H_{16}$ -g, $C_{26}H_{16}$ -h, $C_{26}H_{16}$ -i, $C_{26}H_{16}$ -j, $C_{26}H_{16}$ -k, $C_{26}H_{16}$ -l, $C_{26}H_{16}$ -m, $C_{26}H_{16}$ -n, $C_{26}H_{16}$ -o, $C_{26}H_{16}$ -p, and $C_{26}H_{16}$ -q was not assessed.

Instrumentation

GC/MS analyses were performed on an Agilent 6890N Network Gas Chromatograph coupled to an Agilent 5973 Inert Mass Selective Detector (Agilent, Santa Clara, CA, USA) using the following GC columns: (i) a Restek Rxi-PAH, later described as 50 % diphenyl – 50 % dimethylpolysiloxane - like phase (Restek, Bellefonte, PA), 60 m length, 0.25 mm id, 0.10 μ m film thickness, and maximum programmable temperature 360 °C; (ii) an Agilent DB-5 with 5 % diphenyl – 95 % dimethylpolysiloxane (Agilent, Santa Clara, CA, USA), 60 m length, 0.25 mm id, 0.25 μ m film thickness, and maximum programmable temperature 350 °C; (iii) a SE-52 with 5 % diphenyl – 95 % dimethylpolysiloxane of 12 m length, 0.29 mm id, and 0.34 μ m film thickness whose operating parameters are provided in reference 19; (iv) a J&K Scientific LC-50 column (50 % liquid crystal) with dimethylpolysiloxane stationary phase (J&K Scientific, Edwardsville, Nova Scotia, Canada), 20 m length, 0.25 mm id, 0.10 μ m film thickness, and 270 °C maximum temperature; and (v), a J&K Scientific LC-50 of 15 m length whose column dimensions and operating parameters are provided in reference 17.

Experimental Method

The $C_{14}H_{10}$, $C_{18}H_{12}$, $C_{20}H_{12}$, $C_{22}H_{12}$, $C_{22}H_{14}$, $C_{24}H_{14}$, $C_{26}H_{14}$, and $C_{26}H_{16}$ PAH isomers were analyzed using GC/MS with on-column injection and the mass spectrometer programmed in selected-ion monitoring (SIM) mode for the m/z 178, 228, 252, 276, 278, 302, 326, and 328 ions, respectively. The GC was temperature programmed as follows for the study of the $C_{14}H_{10}$, $C_{20}H_{12}$, $C_{22}H_{12}$, $C_{24}H_{14}$, $C_{26}H_{14}$, and $C_{26}H_{16}$ PAH isomers using the Rxi-PAH column and for the study of the $C_{26}H_{16}$ PAH isomers using the DB-5 column: isothermal at 140 °C for 2 min then 20°C/min to 325 °C, and isothermal at 325 °C for 90 min. For the study of the $C_{18}H_{12}$ PAH isomers using the Rxi-PAH column, the GC was temperature programmed as isothermal at 60 °C for 2 min then 5 °C/min to 300 °C, and isothermal at 300 °C for 50 min. Subsequently, for the study of the $C_{22}H_{14}$ PAH isomers using the Rxi-PAH column, the GC was temperature programmed as isothermal at 60 °C for 2 min, 5 °C/min to 300 °C, and isothermal at 300 °C for 30 min. Finally, for the study of the $C_{22}H_{14}$ PAH isomers using the LC-50 column of 20 m, the GC was temperature programmed as isothermal at 100 °C for 2 min, 5 °C/min to 250 °C, and isothermal at 250 °C for 150 min. The details of the GC temperature program used for the study of the $C_{14}H_{10}$, $C_{18}H_{12}$, $C_{20}H_{12}$, $C_{22}H_{12}$, and $C_{22}H_{14}$ using the SE-52 column are reported in reference 19 and for the study of the $C_{24}H_{14}$ PAHs using the LC-50 column of 15 m are reported in reference 17.

Mathematical Methods

1. The method of the π -Sextet Rule

The method used for establishing the locations and migration of the aromatic sextets in PAHs using the π -Sextet Rule is based on the following three points [20].

- i. The aromatic sextets must not be located in adjacent hexagons
- ii. Rings with no capability to accommodate aromatic sextets should accommodate a Kekulé structure
- iii. Conditioned to restrictions (i) and (ii), the maximum number of sextets have to be present in a structure

Two examples of the application of the π -Sextet Rule for **C₂₄H₁₄-f** (a peri-condensed PAH) and for **C₂₆H₁₆-l** (a cata-condensed PAH) are described in Figure 3.

2. The method of the RBOs

The method of the RBOS is based on the following three steps:

- i. Enumeration of the Kekulé structures of the PAHs in Figures 1 and 2 using the information present in references 21, 22, and 23, as it is exemplified in Figure 4A for **C₂₆H₁₆-p**.
- ii. Establishment of the distribution of single and double bonds using the Kekulé structures obtained in step (i) and the binary symbols 0 (for single bonds) and 1 (for double bonds), as it is exemplified in Figure 4B for **C₂₆H₁₆-p**.
- iii. Calculation of the RBOs by application of the programming code given in Figure S1 of the supporting information. In the programming code, first, the number of Kekulé structures is entered using the information defined in part (i). Then, the distribution of single and double bonds is entered using the information established in part (ii). Finally, the RBO is calculated by adding the Pauling bond orders of each bond of a ring. The RBOs obtained by application of the programming code to **C₂₆H₁₆-p** are stated in Figure 4C.

3. Results and Discussion

Establishment of the number of rings affected by the aromatic sextets in PAHs using the π -Sextet Rule and the RBOs approach

It is feasible to establish the number of rings affected by the aromatic sextets in PAHs (NR) by adding the number of stationary sextets to the number of rings in the pathway of a sextet migration. Both the π -Sextet Rule [6,10,20] (qualitative method) and the RBOs approach (quantitative method) provide evidence of the locations and migration of the aromatic sextets in the structures of PAHs. Therefore, a combination of the π -Sextet Rule [6,10] and the RBOs approach is used to establish NR in the PAHs of Figures 1 and 2.

Following the method of the RBOs described in the Mathematical Methods Section and illustrated in Figures S2 and S3, 58 RBOs were calculated. Out of the 58 RBOs, 30 are consistent with the RBOs published in the literature [12]. To the best of our knowledge there is no published RBOs for the remaining 28 compounds included in this study.

The locations and migration of the aromatic sextets in PAHs calculated using the π -Sextet Rule and the method of the RBOs, are then combined to establish NR. The π -Sextet Rule is used as a first method to locate the aromatic sextets and their migration patterns for the PAHs denoted in Figures 1 and 2, while the RBOs approach is used as a second method to establish the location and migration pattern of any other ring affected by the aromatic sextets. Finally, NR is given by the addition of the number of sextets (S), migrating sextets (S-), and induced sextets (IS) in a particular PAH.

The formation of induced sextets is related to the presence of phenylphenanthrene moieties embedded in the structure of PAHs. The presence of a phenylphenanthrene moiety in **C₂₂H₁₄-k**, **C₂₄H₁₄-c**, **C₂₄H₁₄-g**, **C₂₄H₁₄-k**, **C₂₆H₁₄-d**, and **C₂₆H₁₄-e** explain the formation of rings with induced sextets (IS) in these PAHs. An analogous behavior was analyzed and demonstrated by Clar [6] during the study of the asymmetric effects in pyrene derivatives to explain the formation of an aromatic complex in **C₂₄H₁₄-h** due to the existence of a phenylphenanthrene moiety. Therefore, the angular rings in positions 7 of **C₂₂H₁₄-k**, 3 of **C₂₄H₁₄-c**, 2 of **C₂₄H₁₄-g**, 7 and 10 of **C₂₄H₁₄-k**, 7 of **C₂₆H₁₄-d**, and 7 and 11 of **C₂₆H₁₄-e**, which were originally assumed as separate entities from the aromatic complex of the molecules by the π -Sextet Rule, may feasibly be rings that belong to the aromatic complex and thus, they would be capable of holding induced sextets. This observation is supported by the high RBOs (> 2.0) shown in Figures S2 and S3 for the rings in position 7 of **C₂₂H₁₄-k**, 3 of **C₂₄H₁₄-c**, 2 of **C₂₄H₁₄-g**, 7 and 10 of **C₂₄H₁₄-k**, 7 of **C₂₆H₁₄-d**, and 7 and 11 of **C₂₆H₁₄-e**.

Presented in Figures S2 and S3 are the locations and migration of the aromatic sextets using the π -Sextet Rule (CLAR), RBOs, the combined sextet locations and migration (COMB) based on CLAR and RBOs, and NR for the PAHs mentioned in Figures 1 and 2. To give an insight of the calculation procedure, three cases are explained below:

- i. **C₂₄H₁₄-d**. As mentioned in Figure S2, **C₂₄H₁₄-d** has two migrating sextets located in rings 1 and 2 (the first sextet) and in rings 9, 10, and 11 (the second sextet). Thus, COMB, without further analysis, matches with CLAR and RBOs. In this case, NR is equal to 5 because five rings are affected by the presence of the sextets.
- ii. **C₂₄H₁₄-g**. As evidenced in Figure S2, **C₂₄H₁₄-g** has three sextets based on CLAR and RBOs (the three most prominent values); however, ring 2 presents a significant RBO (2.27) in which the presence of a sextet can be explained by the formation of an induced sextet (as it was described previously). Therefore, COMB is in complete agreement with RBOs and in partial agreement with CLAR. In this case, NR is equal to 4 (three sextets and one induced sextet) because four rings are affected by the presence of sextets.
- iii. **C₂₆H₁₄-d**. As observed in Figure S3, **C₂₆H₁₄-d** has four sextets based on CLAR and RBOs (the four most prominent values); however, ring 7 could hold another sextet due to the formation of an induced sextet (as it was described previously). Consequently, COMB is in partial agreement with RBOs and CLAR. In this

case, NR is equal to 5 (four sextets and one induced sextet) because five rings are affected by the presence of sextets.

Interaction between the PAHs and the GC column stationary phase

As stated in the Materials and Methods Section, the Rxi-PAH column is the primary GC column used in this investigation. Although the Rxi-PAH column has a proprietary stationary phase, previous GC separations of PAHs using the Rxi-PAH column [24] followed the same elution order observed in GC columns with 50 % phenylmethylpolysiloxane stationary phases, e.g., DB-17 (50 % diphenyl – 50 % dimethylpolysiloxane stationary phase) [17]. Consequently, a 50 % diphenyl – 50 % dimethylpolysiloxane - like stationary phase behavior for the Rxi-PAH column will be assumed for the rest of this report. Although the common approach points out that the interaction in GC columns occurs between the solute and the liquid part of the stationary phase, a feasible interaction between the solute and the backbone of the stationary phase is also possible, as explained in the following lines.

The polysiloxane backbone of GC columns is composed of two unbalanced poles with a noticeable electronegativity difference ($\chi=1.7$ [13]), including the negative pole composed of oxygen ($\chi=3.5$ [13]) and the positive pole composed of silicon ($\chi=1.8$ [13]). The proposed mechanism of interaction between PAHs with different location and migration of aromatic sextets, e.g., tetracene ($C_{18}H_{12}$ -e) and triphenylene ($C_{18}H_{12}$ -c), and the poles of the polysiloxane backbone monomer at the experimental temperature conditions is illustrated in Figure 5. As described in the mechanisms of interaction of Figures 5A and 5B and in the GC chromatogram of Figure 5C, the interaction between the positive pole (silicon) of the GC stationary phase and PAHs with a migrating sextet that move through the molecule, e.g., tetracene ($C_{18}H_{12}$ -e), is more favorable and longer than the interaction between the positive pole (silicon) of the GC stationary phase and PAHs with only stationary sextets, e.g., triphenylene ($C_{18}H_{12}$ -c). Therefore, the analysis strongly suggests that the proposed interaction would be between the positive pole (located in the silicon atom) of the stationary phase and the negative charge created by the migrating sextet (or sextets) in the PAH. For a successful interaction, the PAH and the positive pole of the silicon do not have to be in contact but close enough to interact. As illustrated in Figure 6 using a molecular model, a noticeable zone generated by the repulsion of the hydrogen atoms in the stationary phase makes feasible the interaction between the positive pole of the stationary phase and the PAH molecule.

Gas chromatography with mass spectrometry analyses using the 50 % diphenyl – 50 % dimethylpolysiloxane - like GC column

The interaction between PAHs composed of rings with different location and migration of aromatic sextets and the polysiloxane backbone of a GC stationary phase has been investigated using GC/MS following the details stated in the experimental section. The chromatograms of the GC/MS analyses of PAHs $C_{14}H_{10}$, $C_{18}H_{12}$, $C_{20}H_{12}$, $C_{22}H_{12}$, $C_{22}H_{14}$, $C_{24}H_{14}$, $C_{26}H_{14}$, and $C_{26}H_{16}$ using the 50 % diphenyl – 50 % dimethylpolysiloxane - like GC column are illustrated in Figure 7. As observed in Figure 7A–7H, the elution order is affected by geometrical constraints that characterize the PAHs under analysis. In the same group of isomers, nonplanar structures with molecular thickness (T) greater than 3.9 Å

(usually 4.5 Å and above) elute before planar structures with T of – 3.9 Å. The T values for the PAHs in Figure 7 are listed in Figures S2 and S3 [25]. In addition to the geometrical effect that chromatographically separates nonplanar PAHs from planar PAHs, the comparison of the elution order in the chromatograms of the GC/MS analyses illustrated in Figure 7 and NR calculated in Figures S2 and S3 shows a direct relation that demonstrates that isomers with low NR elute earlier than isomers with high NR. Therefore, the observations in Figure 7 confirm the favorable interaction between PAHs with high NR and the positive pole of the GC stationary phase, and the unfavorable interaction between PAHs with low NR and the positive pole of the GC stationary phase. The relation is valid for 88% of the PAHs in Figures 1 and 2. The cases that depart from the calculated elution order include **C₂₂H₁₄-f**, **C₂₂H₁₄-h**, **C₂₄H₁₄-f**, **C₂₄H₁₄-i**, **C₂₄H₁₄-m**, **C₂₆H₁₆-i**, and **C₂₆H₁₆-n** whose special behavior will be discussed in the following section. The other case, **C₂₆H₁₆-a**, involves a helical structure which prevents the use of the analysis proposed in this report.

Groups of compounds with special behavior

Out of the 58 compounds described in Figures S2 and S3, seven compounds (**C₂₂H₁₄-f**, **C₂₂H₁₄-h**, **C₂₄H₁₄-f**, **C₂₄H₁₄-i**, **C₂₄H₁₄-m**, **C₂₆H₁₆-i**, and **C₂₆H₁₆-n**) present inconsistencies between the theoretical GC elution orders calculated using the approach discussed in this report and the experimental GC elution orders observed in Figure 7. The inconsistent elution behavior of **C₂₄H₁₄-f** is discussed in the following paragraph. The details of the inconsistent elution behavior of the other six compounds are explained in Figure S4.

The inconsistent GC elution order for **C₂₄H₁₄-f** (NR=5) which appears before (and not after as would be expected) **C₂₄H₁₄-h**, as shown in Figure 7F, demonstrates the favored interaction of the GC stationary phase with the areas of a molecule that contain migrating sextets. As shown in Figure S2, the migrating sextets of **C₂₄H₁₄-f** are moving between two pairs of rings (2–3 and 5–6) located in the same area of the molecule generating a favorable interaction of 4 benzenoid rings with the positive pole (silicon) of the GC stationary phase. In contrast, the remaining ring (12) shows a stationary sextet in an isolated area of **C₂₄H₁₄-f** that presents an unfavorable interaction with the GC stationary phase.

Verification of the calculated elution order using other polysiloxane GC columns

An initial presumption about the direct relation between the GC elution order and the number of rings affected by the aromatic sextets in PAHs for GC columns with polysiloxane stationary phases was demonstrated in the first part of this report for **C₁₄H₁₀**, **C₁₈H₁₂**, **C₂₀H₁₂**, **C₂₂H₁₂**, **C₂₂H₁₄**, **C₂₄H₁₄**, **C₂₆H₁₄**, and **C₂₆H₁₆** PAHs using a 50 % diphenyl – 50 % dimethylpolysiloxane-like column. In an effort to generalize this argument, the analysis is extended to other three columns with the polysiloxane backbone according to the details described in Figure 8A. The results indicate a direct relation between the GC elution order of nonplanar and planar PAHs in the GC columns LC-50 (Figures 8B and 8C), DB-5 (Figure 8D), and SE-52 (Figure 8E), and their respective NR, i.e., isomers with low NR elute earlier than isomers with high NR. The GC elution orders of the **C₂₂H₁₄-f** and **C₂₂H₁₄-h** observed in Figure 8B for the LC-50 column, **C₂₄H₁₄-f** observed in Figure 8C for the LC-50 column, **C₂₆H₁₆-i** and **C₂₆H₁₆-n** observed in Figure 8D for the DB-5 column, and **C₂₂H₁₄-f**

observed in the Figure 8E for the SE-52 column, which are inconsistent with respect to the anticipated elution orders based on NR calculated in Figures S2 and S3, are clarified with the same arguments mentioned in the special cases section for **C₂₂H₁₄-f**, **C₂₂H₁₄-h**, **C₂₄H₁₄-f**, **C₂₆H₁₆-i**, **C₂₆H₁₆-n**. Consequently, from the results presented in Figure 8, the relations between the GC elution order and NR for the LC-50, DB-5, and SE-52 GC columns follow the same direct relation between the GC elution order and NR already established for the 50 % diphenyl – 50 % dimethylpolysiloxane - like column in 85 %, 88 %, and 93 % of the cases, respectively.

Important Observation

The calculation of the GC elution order using the aromatic character of PAHs is an overarching and general procedure to explain the interaction between a PAH molecule and the polysiloxane stationary phase. This effect does not supersede (but complements) any additional parameters that correlate with the separation of PAHs in GC including boiling point, polarizability (discussed later), and shape parameters such as length-to-breadth ratio, planarity, and other geometrical considerations.

Comparison between the method based on the aromatic character and the method based on the polarizability for predicting GC elution order

In comparison with the method based on the aromatic character presented in this report, the following disadvantages are evident in the method based on the polarizability:

- i. The polarizabilities of PAHs with molecular masses higher than 300 Da are usually unknown and due to the limitations in experimental analytical procedures, their calculation depend on complex and expensive computational routines using differential functional theory [27]. On the other side, the method based on the aromatic character described in this report, does not require any complex computational routine but simple logical analysis to establish the location and migration of the sextets in PAHs.
- ii. In the analysis of the GC elution order of PAH isomers, the method based on the polarizability is not completely reliable for the establishment of the elution order of compounds in the same isomer group. For instance, the polarizabilities of the PAH pair **C₂₂H₁₄-f** and **C₂₂H₁₄-h** ($39.18 \times 10^{-30} \text{ m}^3$ and $41.39 \times 10^{-30} \text{ m}^3$) [28] agree with the elution order observed in Figure 7 (Case E), but the polarizabilities of the PAH pair **C₂₂H₁₄-e** and **C₂₂H₁₄-f** ($39.97 \times 10^{-30} \text{ m}^3$ and $39.18 \times 10^{-30} \text{ m}^3$) [28] do not agree with the observed elution order observed in Figure 7 (Case E). On the other side, the method based on the aromatic character presented in this report describe with high precision (85 % or more) the GC elution order of 58 compounds in the eight isomer groups under study.
- iii. The polarizability of PAHs is influenced by topographical effects, such as the presence of phenanthrene-like bays, which could make the cloud of electrons more compact and less susceptible to external electric fields [27]. On the other side, the aromatic character of PAHs does not change due to the existence of topographical effects.

- iv. In terms of reflecting the thermodynamic stability of PAHs, the concept of the aromatic character is more robust than the concept of polarizability. The theoretical comparison between thermodynamic parameters, e.g., heats of formation and relative total energies, and the polarizability for the PAHs **C₁₈H₁₂-c**, **C₁₈H₁₂-d**, and **C₁₈H₁₂-e** shows no conclusive relations [27]. On the other side, the aromatic character, which is related to the highest occupied molecular orbital (HOMO) – lowest unoccupied molecular orbital (LUMO) gap [7], presents a direct correlation with the Hess-Schaad resonance energy per π electron for benzenoid PAHs [29]. The Hess-Schaad resonance energy is a measure of thermodynamic stability due to cyclic conjugation [30].

4. Conclusions

Using experimental and theoretical analysis, a direct relation between the GC elution order in an Rxi-PAH column with 50 % diphenyl – 50 % dimethylpolysiloxane - like stationary phase and the aromatic character of C₁₄H₁₀, C₁₈H₁₂, C₂₀H₁₂, C₂₂H₁₂, C₂₂H₁₄, C₂₄H₁₄, C₂₆H₁₄, and C₂₆H₁₆ PAHs has been established for the first time and verified for 88 % of the cases investigated. The basis of this relation is the suitability of the PAH-stationary phase interaction at the experimental temperature conditions that is influenced by the location and migration of the aromatic sextets in PAHs. The method applied for the calculation of the number of rings affected by the aromatic sextets inside of PAHs is a combination of the π -Sextet Rule and the Pauling Ring Bond Orders. According to this relation, PAHs that contain a lower number of rings affected by the presence of aromatic sextets elute earlier than PAHs that contain a higher number of rings affected by the aromatic sextets. In an effort to generalize the behavior observed with the Rxi-PAH column, the study has been extended to a DB-5 with 5 % diphenyl – 95 % dimethylpolysiloxane for the analysis of the C₂₆H₁₆ PAHs, a SE-52 with 5 % diphenyl – 95 % dimethylpolysiloxane for the analysis of the C₁₄H₁₀, C₁₈H₁₂, C₂₀H₁₂, C₂₂H₁₂, and C₂₂H₁₄ PAHs, and an LC-50 dimethyl (50 % liquid crystal) polysiloxane for the analysis of the C₂₂H₁₄ and C₂₄H₁₄ PAHs. The calculated elution order of the PAHs analyzed is consistent for 88 % in the DB-5 column, 93 % in the SE-52 column, and 85 % in the LC-50 column. It is expected that future analysis with other PAHs in GC columns with a polysiloxane backbone in the stationary phase will follow the GC elution order that closely agrees with the aromatic character of the molecules. In addition, the method described in this report based on the aromatic character presents more advantages than the method based on the polarizability due to its easy approach that demands the usage of simple computational procedures, due to its reliability in describing the elution order of a full range of compounds in a group of isomers, due to the unchanging behavior of the aromatic character in the presence of topographical effects, and due to the suitability of the aromatic character in reflecting the thermodynamic stability. From a practical point of view, the proposed procedure, which is based on the calculation of the aromatic character of PAHs, will be useful so as to predict the elution order of large PAHs (MM > 300 Da) in GC whose boiling points are rarely available.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig 1.

The PAHs $C_{14}H_{10}$, $C_{18}H_{12}$, $C_{20}H_{12}$, $C_{22}H_{12}$, $C_{22}H_{14}$, and $C_{24}H_{14}$ with available reference standards and miscible in GC compatible solvents.

Fig 2.

The PAHs $C_{26}H_{14}$ and $C_{26}H_{16}$ with available reference standards and miscible in GC compatible solvents.

A. $C_{24}H_{14}$ -f (3 aromatic sextets) **B. $C_{26}H_{16}$ -l (3 aromatic sextets)**

Fig 3.
Examples of the application of the π -Sextet Rule for $C_{24}H_{14}$ -f, a peri-condensed PAH; and for $C_{26}H_{16}$ -l, a cata-condensed PAH.

A.

B.

1. 0,0,1,1,0,0,0,1,1,0,1,0,0,0,0,1,0,1,0,0
 2. 1,1,0,0,1,1,1,0,0,1,0,1,1,1,0,0,1,0,1,1
 3. 0,0,1,1,0,0,0,1,1,0,1,0,0,0,0,1,1,0,1,0,0
 4. 0,1,0,0,1,0,1,0,0,1,0,0,1,0,1,0,1,0,0,1,0,0,1
 5. 0,0,1,1,0,0,0,1,1,0,1,0,0,0,0,1,1,0,1,0,0
 6. 1,1,0,0,1,1,1,0,0,1,0,1,1,1,1,0,0,1,0,1,1
 7. 1,0,0,0,0,1,0,0,0,0,0,1,0,1,0,0,0,0,0,1,0
 8. 0,1,1,1,0,1,1,1,1,1,0,1,0,1,1,1,1,1,0,1
 9. 1,0,0,0,0,1,0,0,0,0,0,1,0,1,0,0,0,0,0,1,0
 10. 0,0,0,1,1,0,0,0,1,1,1,0,1,0,0,0,1,1,1,0,1
 11. 1,0,0,0,0,1,0,0,0,0,0,1,0,1,0,0,0,0,0,1,0
 12. 0,1,1,0,0,0,1,1,0,0,0,0,0,0,1,1,0,0,0,0,0
 13. 0,1,1,0,0,0,1,1,0,0,0,0,0,0,1,1,0,0,0,0,0
 14. 1,0,0,1,1,1,0,0,1,1,0,0,0,1,0,0,1,1,0,0,0
 15. 0,1,1,0,0,0,1,1,0,0,0,0,0,0,1,1,0,0,0,0,0

16. 1,0,0,1,1,1,0,0,1,1,1,1,1,1,0,0,1,1,1,1,1
 17. 0,0,0,0,0,0,0,0,0,0,0,1,1,1,0,0,0,0,0,1,1,1
 18. 1,1,1,1,1,1,1,1,1,1,0,0,0,1,1,1,1,1,0,0,0
 19. 0,0,0,0,0,0,0,0,0,0,1,1,1,0,0,0,0,0,1,1,1
 20. 0,0,0,0,0,1,1,1,1,1,0,0,0,1,1,1,1,1,0,0,0
 21. 0,0,0,0,0,0,0,0,0,0,1,1,1,0,0,0,0,0,1,1,1
 22. 1,1,1,1,1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0
 23. 1,1,1,1,1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0
 24. 0,0,0,0,0,1,1,1,1,1,1,1,1,0,0,0,0,0,0,0,0
 25. 1,1,1,1,1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0
 26. 0,0,0,0,0,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1
 27. 0,0,0,0,0,0,0,0,0,0,0,0,0,0,1,1,1,1,1,1,1
 28. 1,1,1,1,1,1,1,1,1,1,1,1,1,1,0,0,0,0,0,0,0
 29. 0,0,0,0,0,0,0,0,0,0,0,0,0,0,1,1,1,1,1,1,1
 30. 1,1,1,1,1,1,1,1,1,1,1,1,1,1,0,0,0,0,0,0,0
 31. 0,0,0,0,0,0,0,0,0,0,0,0,0,0,1,1,1,1,1,1,1

C.

Fig 4.

First and second steps of the method of the RBOs. **A.** Enumeration of the Kekulé structures for **C₂₆H₁₆-p**. **B.** Establishment of the distribution of single and double bonds using the binary symbols 0 (for single bonds) and 1 (for double bonds) for **C₂₆H₁₆-p**. **C.** RBOs of **C₂₆H₁₆-p** obtained by application of the programming code stated in Figure S1.

Fig 5.

A. Proposed mechanism of interaction at the experimental conditions between tetracene ($C_{18}H_{12}$ -e) and the poles of a monomer of polysiloxane backbone. **B.** Proposed mechanism of interaction at the experimental conditions between triphenylene ($C_{18}H_{12}$ -c) and the poles of a monomer of polysiloxane backbone. **C.** Chromatogram of the GC/MS analysis of the five $C_{18}H_{12}$ PAH isomers. Note: The drawings (not in scale) were made to emphasize the interaction (or lack of interaction) between the PAHs and the silicon groups. The CH_3 groups of the polysiloxane (not observed in this figure) are crossing the plane as it is described in Figure 6.

Fig 6.

Three dimensional views of a polysiloxane stationary phase section emphasizing the positive sign of the silicon atoms and the negative sign of the oxygen atoms due to the presence of a covalent bond. **A.** Upper view makes possible to observe the covalent bonds. **B.** Side view makes possible to observe the zone for interaction with PAH. For reference, in the stationary phase, the length of the Si-O bond is appr. 1.63 Å, and the lengths of the Si-C bond is approx. 1.85 Å. In a PAH, the length of the C-C bond is approx. 1.4 Å and the length of the C-H bonds is approx. 1.1 Å. White circle denotes the hydrogen atom. Grey circle denotes the carbon atom. Purple circle denotes the silicon atom. Black circle denotes the oxygen atom. Red circle denotes the connection with the following monomer.

Fig 7.

Chromatograms of the GC/MS analyses of PAHs with available reference standards and miscible in GC compatible solvents using the 50 % diphenyl – 50 % dimethyl polysiloxane column. **A.** C₁₄H₁₀. **B.** C₁₈H₁₂. **C.** C₂₀H₁₂. **D.** C₂₂H₁₂. **E.** C₂₂H₁₄. **F.** C₂₄H₁₄. **G.** C₂₆H₁₄. **H.** C₂₆H₁₆. MM stands for molecular mass in Da. The numbers in superscript denote NR calculated in Figures S2 and S3. The letters in red denote the PAHs that do not follow the expected elution order.

Fig 8.

A. Isomer groups and corresponding columns used to verify the GC elution order as a function of the number of rings affected by the aromatic sextets (NR). **B.** Diagram of NR as a function of the elution order for $C_{22}H_{14}$ PAHs on LC-50 column. **C.** Diagram of NR as a function of the elution order for $C_{24}H_{14}$ PAHs on LC-50 column. **D.** Diagram of NR as a function of the elution order for $C_{26}H_{16}$ PAHs on DB-5 column. **E.** Diagram of NR as a function of the elution order for $C_{14}H_{10}$, $C_{18}H_{12}$, $C_{20}H_{12}$, $C_{22}H_{12}$, and $C_{22}H_{14}$ PAHs on SE-52 column. MM stands for molecular mass in Da. The letters and data points in red denote the PAHs that do not follow the expected elution order.