Separation of substituted aromatic isomers with porous graphitic carbon in subcritical fluid chromatography

C. West, E. Lesellier*

LETIAM, EA 3343, IUT Orsay, Université Paris XI, Plateau du Moulon, F-91400 Orsay, France

Abstract

The ability of porous graphitic carbon (PGC) to separate structural isomers has been reported in high-performance liquid chromatography (HPLC). This paper presents studies carried out in subcritical fluid chromatography (SubFC). Various polar and nonpolar modifiers were added to the carbon dioxide mobile phase, in proportions ranging from 5 to 40%. The effects of both the nature and the percentage of the modifier on aromatic isomer separations were studied. Two types of selectivity behaviour appear. The first one, related to steric recognition, is due to the number of contact points between the compounds and the flat surface of PGC. In this case, retention orders are often identical to that reported in HPLC. The second is related to the favourable interaction between the polar moieties of the solutes and the stationary phase. In this case, the retention and selectivity strongly depend on the mobile phase composition. Thus, the separations obtained are greatly enhanced, compared to those obtained in HPLC. The retention and selectivity variations observed when the composition of the mobile phase is changed are discussed based on linear solvation energy relationships (LSERs). Practical applications are presented, namely benzene, toluene, ethylbenzene and xylenes (BTEX) and flavour molecules separations.
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1. Introduction

The separation of isomers is a topic of a great interest as isomeric species generally have different biological activities. However, the substituent position on an aromatic ring (*ortho*, *meta*, *para*) or the double bond conformation, or the non aromatic cycle conformation (*cis*, *trans*), induces few differences in interactions between the analytes and the mobile phase or in boiling temperature between isomers. Shape selectivity, when it occurs, arises from interactions in the stationary phase [1], which implies that the isomeric solutes establish different interactions with the stationary phase. Consequently, such separations require especially suited chromatographic system.

In liquid chromatography, this kind of separation first depends on the physical state of the stationary phase, solid or liquid like. In reversed-phase liquid chromatography with solvated bonded octadecyl chains (ODS), the retention is governed by a partition retention mechanism. In this case, solutes are surrounded by the alkyl chains, and generally, conformational changes can not provide the required energetic differences inducing retention variations. However, with large and rigid compounds, such as polynuclear aromatic hydrocarbons (PAHs) [2] or carotenoid pigments [3], the increase in rigidity of the bonded chains, related to a decrease in temperature, favours the separation between planar and non planar or between linear and bent compounds. Thus, some type of isomeric separation is possible on highly ordered polymeric ODS.

Alumina and amorphous silica have geometrically heterogeneous surfaces, which are far from flat at the atomic level but can distinguish molecules on the basis of specific effects, such as dipole and H-bonding interactions [4]. Thus, isomeric separations of polar compounds are possible on these stationary phases.

With solid adsorbents, such as porous graphitic carbon (PGC) and carbon-clad zirconia [5], interactions take place on a flat and rigid solid surface. For compounds which do not possess any functionality group to undergo interactions other than dispersion forces (hydrocarbonaceous compounds), a steric recognition is

* Corresponding author. Tel.: +33 1 69336131; fax: +33 1 69336048.
E-mail address: eric.lesellier@iut-orsay.fr (E. Lesellier).

mainly based on the number of contact points between solute and adsorbent [6]. Enhanced dispersion interactions and overlap of π electrons, achieved for planar aromatic solute molecules, is a critical factor of retention, therefore, a critical factor for separation on PGC [4,7]. For instance, the increase in planarity of cyclohexanes induces a greater retention on PGC [8]. Satisfactory separations were also reported for butyl- and nonylphenol polyethoxylate (NPE) isomers [9]. As expected, the retention of the more branched isomers is lower than that of the linear isomers.

Moreover, any type of functional group, be it polar or not, causes an increase in retention on PGC, making it different from both the polar adsorbents (alumina and silica gel) and the nonpolar bonded phases (ODS). This makes PGC highly suitable for the separation of both polar and nonpolar species.

The nature of the polar substituents (such as OH, NO_2, COOH, NH_2 or halogens), their effects on the electron density distribution in the benzene ring, their ability to create intra- or intermolecular interactions, such as hydrogen bonding, all influence charge transfer, dipole–dipole, acidic or basic interactions between the solutes and the stationary and mobile phases. Therefore, PGC is especially suited for separation of di-*ortho*, mono-*ortho* and non-*ortho* polychlorinated biphenyls (PCBs). Ortho substitutions reduce the planarity of the two rings, as the dihedral angle formed by the two aromatic rings depends on the number of *ortho* substituents [10–12].

Moreover, PGC displays an energetically homogeneous surface [6]. However, despite this surface homogeneity that would simplify the retention phenomenon (as compared to heterogeneous bonded silica), understanding of the retention order of isomers can be unclear, due to the numerous types of interactions occurring on PGC.

Additionally, understanding is further complicated by the fact that the authors, having presented isomeric separations on PGC, used very different liquid mobile phases from one paper to another, thus leading to different retention orders between isomers [6,13–17].

An insight into the retention mechanism occurring in any chromatographic system can be brought by studies based on linear solvation energy relationships (LSER). Using Abraham parameters [18], the classical equation used in high-performance liquid chromatography (HPLC) is:

$$\log k = c + eE + sS + aA + bB + vV \qquad (1)$$

where k is the solute retention factor.

In this equation, capital letters represent the solute descriptors, related to particular interaction properties, while lower case letters represent the system constants, related to the complementary effect of the phases on these interactions. c is a constant, depending on specific column parameters, such as porosity. E is the excess molar refraction (calculated from the refractive index of the molecule) and models polarizability contributions from n and π electrons; S, the solute dipolarity/polarizability; A and B, the solute overall hydrogen-bond acidity and basicity; V, the McGowan characteristic volume in units of $cm^3\ mol^{-1}/100$. The system constants (c, e, s, a, b, v), obtained through a mul-

tilinear regression of the retention data for a certain number of solutes with known descriptors, reflect the magnitude of difference for that particular property between the mobile and stationary phases. Consequently, the coefficients also reflect the system's relative selectivity towards that particular molecular interaction. This model was recently applied in subcritical fluid chromatography (SubFC) with PGC [19,20].

The use of supercritical fluids as a mobile phase is indeed expected to improve the separation efficiency, thanks to increased diffusion coefficients. Wen and Olesik [21] showed that the addition of low amounts of CO_2 to a methanol–water mobile phase created a buffered mobile phase in SFC, thus altering the retention of acidic and basic isomers without modifying their retention order. Obviously, such modifications of mobile phase properties could be dramatically enhanced by the use of super/or subcritical mobile phases, with greater carbon dioxide amounts mixed with different organic solvents used as modifiers. Additionally, the varied nature of modifiers that can be added to carbon dioxide could provide different separations on PGC [20,22], and the LSER could help in understanding isomeric separations.

This paper presents the study of isomer selectivities of di- and tri-substituted aromatic compounds in SFC with PGC. The influence of mobile phase composition on retention order and separation were investigated. The results presented in our two previous papers [19,20] were used to support many discussions in the current paper. Besides, some practical applications of isomeric separation on PGC in SubFC are presented.

2. Experimental

2.1. Chemicals

Solvents used as modifiers were HPLC grade methanol (MeOH), acetonitrile (ACN), tetrahydrofuran (THF), ethanol (EtOH) (VWR Prolabo, Val-de-Fontenay, France), *n*-propanol (nPrOH) (Carlo Erba, Milan, Italy), isopropanol (iPrOH) (SdS, Peypin, France) and hexane (HXN) (J.T. Baker). Carbon dioxide was provided by Alphagaz (Bois d'Arcy, France). The solvents were chosen, as previously discussed [20], so as to provide a wide range of size, polarity, hydrophobicity and hydrogen-bonding ability, therefore, inducing a wide variety of interactions with the solutes.

The series of isomer families were chosen, in the same manner, so as to provide a wide variety of substituents. The isomeric systems chosen were selected for simplicity, to provide some insight as to the retention mechanism, and not for any analytical importance. Differently substituted benzenic species, all commercially available, were studied (see Table 1). Solutions of these compounds were prepared in methanol. For each isomeric group, solutions of both the individual isomers and a mixture of the isomers were prepared. The solute descriptors used in the solvation parameter model were taken from several sources [23–25] and are summarized in Table 1.

Additionally, for application demonstrations, toluene, ethylbenzene, propylbenzene, linalool, nerol, geraniol, camphor and menthone were also used.