Retention Parameters of Aromatic Hydrocarbons with Mono-Substituted Polar Groups in Binary RP-HPLC Systems

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Key Words
Column liquid chromatography
Reversed-phase systems
Binary eluents
Mono-substituted aromatics
Retention and selectivity

Summary
Capacity factor (k') values of aromatic hydrocarbons with mono-substituted polar-groups are correlated for reversed-phase systems involving stationary phases with C₁₈ or C₄ ligands chemically bonded to silica and a binary aqueous eluent containing modifiers: methanol, acetonitrile, tetrahydrofuran, isopropanol, dioxane or dimethoxyethane. The relative retention variations of the solutes are interpreted with special consideration of their interactions with non-polar stationary phases and the molecular structure of the modifiers and solutes. Rules for retention and selectivity optimisation in RP-HPLC systems are given.

Introduction
Optimization of retention and selectivity is still a problem in liquid chromatography, especially in reversed-phase systems (RP-HPLC) [1, 2]. An understanding of these problems enables choice of a suitable adsorbent-eluent system, saving time, solvents and materials. There are two main approaches to retention mechanisms in RP-HPLC. One is the solvophobic theory of Melander and Horvath [3] where retention depends on molecular interactions in the mobile phase. As the aqueous mobile phase is very ordered and possesses high cohesive energy the solute molecule is expelled onto the non-polar adsorbent surface coated with hydrocarbon chains. Interactions between solute molecules and hydrocarbon chains are neglected. The second theory [4–6] emphasizes that in the retention process interaction between the stationary phase (hydrocarbon chains) and solute also takes place as well as in the mobile phase. The structure of the hydrocarbon chains modifies the adsorbent surface which can be described as intermediate between a liquid and a hydrocarbon brush comprising Cₙ chains alone; solvent molecules can penetrate the brush depending on their properties. In this way a film of surface phase is formed with characteristic quasi-liquid properties in which solute molecule can be embedded. Retention is governed by partition of solute between the bulk mobile phase and solvated ligand region of the stationary phase.

A pure adsorption mechanism in RP-HPLC has been reported [7, 8]. This approach is good for large molecules but for small ones they seem questionable. Investigations concerned with correlation of retention parameters with physico-chemical properties of solute, mobile phase and stationary phase are important in elucidating the retention mechanism. Examples are: solubility of the sample in the mobile phase [9], van der Waals' volume of sample [10, 11], total surface area of the molecule [12], parameters related to lipophilic and polar additive increments of structural substituents [13], carbon number of the alkyl chain in the solute molecule [14], difference of solvation energies between the mobile and solvated region of the stationary phase [15], activity coefficients in the mobile phase [16], partition coefficients [17], electron density in the molecule [18], surface tension of the mobile phase [19], solvatochromic parameters [20], solubility parameters [21, 22] interaction index [23], proportion of the adsorbent surface covered by alkyl chains [24, 25] length of alkyl chain [24, 26], kind of ligand bonded to adsorbent surface [27].

A very popular correlation for retention is log k' against volume fraction, φ, of modifier in an aqueous mobile phase which shows linear dependence in more or less limited regions of modifier concentration:

\[ \log k' = \log k_w - S \phi \]

where S is a factor dependent on solute and solvent, k_w is solute capacity factor in pure water as mobile phase. Since the plots are often curved, an extended range of validity of the relationship is obtained with the equation:

\[ \log k' = \log k_w - a \phi + b \phi^2 \]
where a and b are constants dependent on solute and solvent. For liquid-liquid partition systems the simpler equation was reported earlier by Sozczewiński and Wachtmeister [28]. The equations are very popular in practice for optimizing chromatographic systems in reversed-phase mode.

Another kind of correlation consists in direct comparison of retention parameters for two or more chromatographic systems which can be represented as a plot of \( \log k' \) against \( \log k'' \) where \( k' \) and \( k'' \) are capacity factors of a solute in the two systems. One of the first Collander-type correlations dealing with selectivity of reversed-phase systems was reported by Bakalyar et al. [29] and Karger et al. [30]. In the latter correlation of RP-HPLC systems with binary eluents consisting of methanol (MeOH), acetonitrile (ACN) or tetrahydrofuran (THF) with water were reported. The data show differences in retention of different compounds for the solvent systems compared. For example, phenols show enhanced retention in THF compared to MeOH. Further work [31] was concerned with ternary solvent systems: MeOH + water and ACN + water plus 1–2 % of another organic modifier chosen to show stronger hydrophobic and proton-donor or electron-donor properties. In this type of system greater extraction of organic components of the mobile phase into the nonpolar stationary phase was expected [32–34]. The authors found stronger retention of phenols relative to proton-acceptor solutes in the system: 2 % THF + MeOH + water and similar results in other systems which was interpreted in terms of specific interaction of phenols with THF extracted into the stationary phase; the interpretation explained data obtained in previous work. Similar effects were reported by Huber et al. [35] who investigated the influence of small concentrations of cyanoalkanes in the eluent phase on the retention of organic compounds with proton-donor groups on a nonpolar-stationary phase. Tanaka et al. [36–38] applied the correlation method of \( \log k' - \log k'' \) (see below) to compare the retention and selectivity of various nonpolar solutes (aliphatic, alicyclic, aromatic hydrocarbons) on various polymer and silica-based, reversed-phase systems containing MeOH or ACN. Differentiated correlation lines were obtained for various classes of compounds investigated. It was interpreted as the result of nonspecific and/or weak specific interactions of ligands alone and extracted modifier with solute in the stationary phase (interphase). Participation of solvated components of the mobile phase with ligands on the stationary phase and its influence on retention seems to be universal in liquid chromatography, especially in RP-HPLC [39, 40]. Besides the above systems others could be mentioned such as ion-pair, reversed-phase systems and chiral separations due to chiral mobile-phase additives; the interaction of mobile-phase components sorbed onto or into the stationary phase with the enantiomers to be separated plays an important role in retention. The effects are also known in polar adsorbent systems with eluents containing small or large quantities of modifiers [41–43]. Participation of modifier adsorbed by a polar stationary phase was observed in systems with silica and dioxan (DX) as a component of the binary eluent, where the retention of phenols relative to quinolines was shown to be stronger in comparison to systems with THF, ethyl acetate or isopropanol due to the molecular structure of dioxan which can be adsorbed with one ether-type oxygen bound to silica and the second ether oxygen directed into the mobile phase can interact specifically with proton-donor solutes [41, 42].

Taking into account the importance of the problem and that some questions of relative changes of selectivity and retention in RP-HPLC are current we decided to compare retention in various reversed-phase systems with various binary eluents for aromatic solutes with one polar functional group (proton-donor and proton acceptor). It is known that \( \log k' - \log k'' \) plots (see below) for compounds with more polar groups are more difficult to interpret. From data of Karger et al. [30] using a C8 stationary phase we have chosen \( \log k' \) values for mono-functional solutes and then plotted: \( \log k'_{\text{ACN}} \) vs. \( \log k'_{\text{THF}} \). We have obtained good correlation for solutes with one polar electron-donor group although two points obtained for phenols show much stronger retention in THF relative to electron donor compounds (Figure 1). Similar correlations were obtained using data from Smith and Garside [44] with similar results. These data inspired us to carry out further investigations with a larger group of monofunctional compounds and a chromatographic system: nonpolar adsorbent – organic modifier + water. A limited number of solutes restricted to monofunctional aromatics, certainly make molecular interpretation of the

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**Figure 1**

\( \log k' \) in 30 % ACN against \( \log k' \) in 25 % THF, C8 stationary phase, legends in Table 1 and 43: benzaldehyde, 44: 3-nitrotoluene, 45: ethyl benzoate, 46: isopropyl benzoate. Data from Ref. [30].