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Hydrophilic interaction liquid chromatography: mixed-mode with advanced π–π selectivity realization

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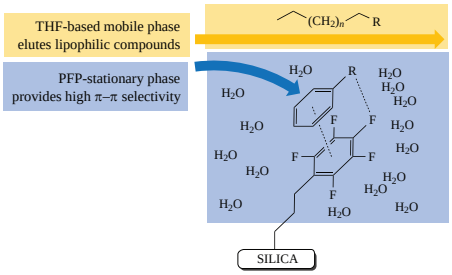
Aprotic polar solvents do not suppress π–π interactions and polar stationary phase containing aromatic groups. The THF additives to MeCN/phosphate buffer mobile phase for hydrophilic interaction liquid chromatography operating in a mixed-mode eliminate lipophilic compounds interference saving unique π–π selectivity.



Keywords: hydrophilic interaction liquid chromatography, acetonitrile, tetrahydrofuran, pentafluorophenyl stationary phase, π–π interactions, lipophilic matrix.

Most medicines and relative biologically active compounds contain simultaneously polar functional groups and aromatic systems. However, HPLC analysis of such compounds in the presence of lipophilic compounds within a sample (*e.g.*, blood plasma or lipophilic auxiliary substance) is a complicated task.[1,2]

The hydrophilic interaction liquid chromatography (HILIC) mode features increased retention and selectivity to polar compounds relative in reversed phase mode that associated with orthogonal complex retention mechanism.[3] HILIC is based on the analytes partition between the immobilized water enriched layer and the bulk of more hydrophobic mobile phase (commonly, 70–95% MeCN).[4] Thus, HILIC mode allows for eliminating the lipophilic compounds interference without gradient elution and sample pretreatment.[4] Quasi-normal phase (QNP) mode is based on π–π interactions between stationary phase aromatic groups [*e.g.*, phenylhexyl, naphthylethyl (COSMOSIL πNAP), nitrophenylethyl (COSMOSIL NPE), 2-(1-pyrenyl)ethyl (COSMOSIL PYE), and pentafluorophenyl (PFP) groups] and aromatic analytes. The π-interactions involve many interacting groups (cation, anion, halogen, hydrogen bonding, lone electron pair, CH, OH, NH, *etc.*),[5,6] which would provide extraordinary selectivity.

Pentafluorophenyl stationary phase contains polar electron withdrawing group that are simultaneously able to participate in π–π interactions (Figure 1) and to form the water-rich layer[7] to combine HILIC and QNP modes selectivity. However, MeCN molecules are capable of forming electron and donor-acceptor complexes[8] leading to the prevention of π–π and dipole–dipole interactions between the aromatic solutes and aromatic groups, which explains the incompatibility of HILIC and QNP modes.[8–10]

Since only aprotic polar solvents (commonly MeCN) can be used in HILIC, protic solvents with a hydrogen-bond donor functional groups (*e.g.*, alcohols) are not suitable.[11–13] Tetrahydrofuran is another aprotic weakly polar water-miscible organic solvent[14,15] that does not prevent π–π interactions, allowing to realize HILIC-QNP mixed-mode. The possibility of using organic modifier other than MeCN both as an independent organic modifier[9,10,15] and as third solvent of ternary system[16] under HILIC was also studied. Needham[17] investigated the use of PFP stationary phase in the HILIC mode for the separation of compounds containing aromatic systems. Aral[18–20] synthetized stationary phases containing phenyl rings and polar amide functional groups to work under HILIC. Ferreira[21] synthesized PFP stationary phase containing polar linker. Methanol was also tested despite its poor suitability for HILIC.[11,13] Some authors used a methanol-based mobile phase when working in reverse phase mode to avoid the MeCN influence on π-interactions.[9] Also, stationary phases containing aromatic group were used in reverse phase mode where methanol was applied as an organic modifier along with acetonitrile when increased aromatic selectivity was needed.[9,16,22–27] Nevertheless, the solution to the compatibility issue of QNP and HILIC modes and enhancing π–π interactions has not yet been found. The implementation of π-interactions in HPLC and the corresponding investigations are limited to the few pioneering studies.[28–31] Recent review articles also do not reveal similar studies investigating π–π interactions in HPLC, especially using the HILIC mode.[32,33]

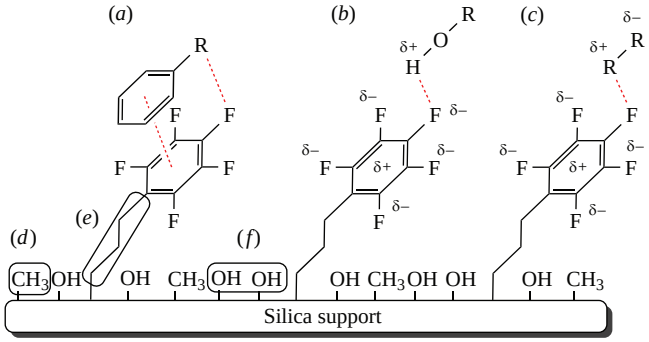


Figure 1 PFP phase structure and interacting groups: (*a*) π–π interactions; (*b*) hydrogen bond interactions; (*c*) dipole–dipole interactions; hydrophobic interactions with (*d*) end-capping groups; and (*e*) propyl linker; (*f*) ion-exchange interactions with residual silanol groups.

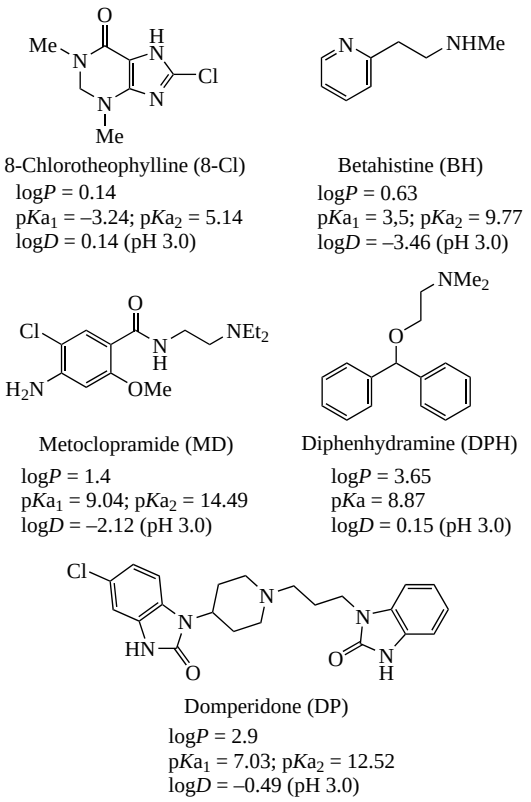


Figure 2 Model compounds. The log*D* values at pH 3.0 were obtained using 'Log*D* Predictor ChemAxon'.

In this work, we attempted to overcome the abovementioned obstacles. Drugs for vomiting and motion sickness containing polar artificial pharmaceutical ingredients with aromatic system (Figure 2) were used as model target compounds (0.1 mg ml^{-1}) and were sequentially dissolved using the mobile phase by ultrasound and then centrifuged. Used equipment and chemicals are indicated in footnote,† and chromatographic conditions are indicated in the Figure captions.

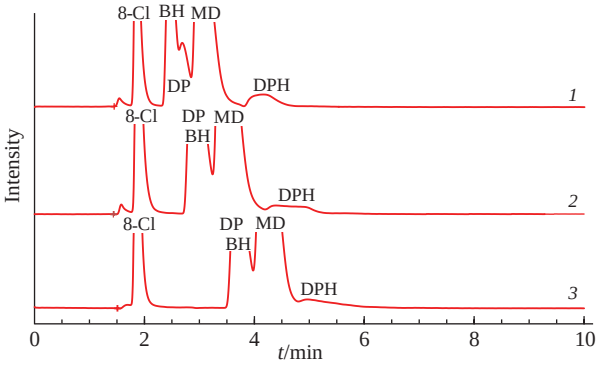


Figure 3 Chromatograms of model compounds using MeCN/ammonium phosphate buffer (pH 3.0; 5 mM) mobile phase (v/v ratios): (*1*) 70:30, (*2*) 75:25, (*3*) 80:20. Column 'dead' volume t_0 is indicated by (|). Flow rate 1.0 ml min^{-1}. Detection: UV at 265 nm.

† Luna PFP(2) column (3.5 μm particle size, 4.6 mm i.d. × 150 mm in length) was purchased from Phenomenex (Phenomenex, St. Torrance, USA). Chromatography was performed with an a CBM-20A system controller equipped with an DGU-20A3 on-line degassing unit, a LC-20AD HPLC pump, a SPD-20A UV-VIS detector, and Shimadzu LC Solution software (Shimadzu corporation, Kyoto, Japan). A manual injector (Rheodyne 7725i) and a 10 μl stainless steel sample loop and fittings for the RH-7725i manual injector were used for sample injection.

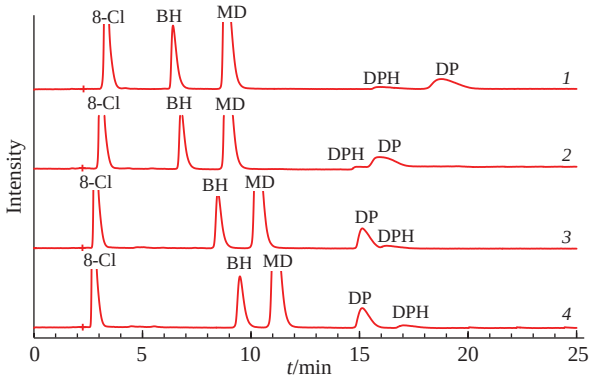


Figure 4 Chromatograms of model compounds using THF/ammonium phosphate buffer (pH 3.0; 5 mM) mobile phase (v/v ratios): (*1*) 50:50, (*2*) 55:45, (*3*) 65:35, (*4*) 70:30. Column 'dead' volume t_0 is indicated by (|). Flow rate 0.7 ml min^{-1}. Detection: UV at 265 nm.

Acetonitrile-based mobile phase did not provide selectivity (Figure 3) because of its ability to prevent the selective π–π interactions. Moreover, as MeCN concentration is increased, the retention time increases according to the HILIC single mode behavior except for 8-Cl.

Tetrahydrofuran-based mobile phase provides notably stronger retention (which required reducing the amount of mobile phase organic part) and selectivity of model compounds in comparison with MeCN except for 8-Cl (Figure 4). As THF concentration is increased, the retention increases for the most hydrophilic compounds (MD and BH, log*D* values at pH 3.0, see Figure 2) according with HILIC mode. However, DP retention decreases indicating multiple forces contribution.

A small amount of MeCN in the mobile phase allows weakening π–π interactions and adjusts retention and selectivity of aromatic substances (Figure 5). Acetonitrile modifier weakly affects the substances retaining by hydrogen bonding, but substantially affects substances retaining by π–π interactions. Compound 8-Cl is eluted around the 'dead' volume regardless of the MeCN modifier amount and solvent type because both 8-Cl and PFP group are electron-deficient aromatic systems and, as a consequence, do not show attractive interactions. Compounds with various electron-rich aromatic system participate in π–π interactions with electron-deficient PFP groups of stationary phase. Moreover, the presence of polar functional group in a molecule, besides the electron-rich aromatic system, provides easy penetration through the water-enriched layer. It results in an additional increase in substance retention due to the interactions with residual silanol groups.

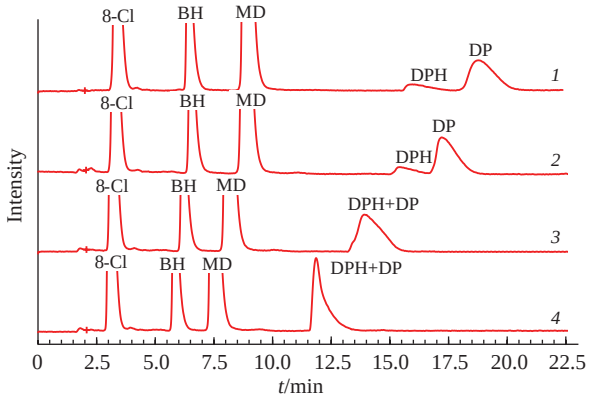


Figure 5 Chromatograms of model compounds using 50:50 (v/v) THF/ammonium phosphate buffer (pH 3.0, 5 mM) mobile phase with MeCN additive (%): (*1*) 0, (*2*) 1, (*3*) 5, (*4*) 10. Column 'dead' volume t_0 is indicated by (|). Flow rate 0.7 ml min^{-1}. Detection: UV at 265 nm.

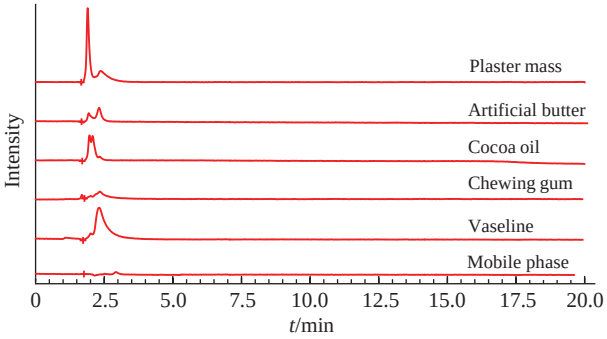


Figure 6 Chromatograms of different model lipophilic substances obtained with 85 : 15 (v/v) THF/ammonium formate buffer (pH 3.0, 10 mM) mobile phase. Flow rate 0.8 ml min^{-1}. Column 'dead' volume t_0 is indicated by (|). Detection: UV at 220 nm.

A crucial problem that might be solved by the mixed mode is the analyses of compounds with moderate polarity within complex lipophilic matrix such as ointments, suppositories, chewing gum, plaster mass, oil-based injection, *etc*. The mode allows for eluting the lipophilic compounds closely at the 'dead volume' because of the hydrophilic interactions (Figure 6). It helps to eliminate the interfering influence of the matrix and as a result to avoid both time-consuming gradient modes and additional sampling. At the same time, applying THF-based mobile phase facilitates the dissolution of lipophilic compounds, which adds to the improvement of target aromatic compounds extraction.

During model mixture analysis, active pharmaceutical ingredient (metoclopramide) demonstrates adequate retention while lipophilic matrix substance (cocoa oil) elutes around the 'dead' volume that allows one to avoid time-consuming gradient elution and to minimize sample pretreatment to extraction by mobile phase and centrifugation (Figure 7). Ammonium phosphate is replaced by ammonium formate for buffer preparation due to better solubility. UV-detection was carried out at 220 nm to increase sensitivity to matrix compounds and ensuring the specificity of target substance determination.

In summary, the use of polar stationary phase containing aromatic groups and mobile phase based on the aprotic polar solvent like THF allows implementing HILIC mixed-mode with advanced π–π interactions compared with MeCN-based mobile phase. Adding a small amount of MeCN in the mobile phase based on aprotic polar solvent makes it possible to adjust π–π interactions strength and, as a result, retention and selectivity of aromatic compounds. The HILIC-QNP mixed mode provides elution of lipophilic substances around the 'dead' volume and adequate retention and fine tuning selectivity for aromatic compounds.

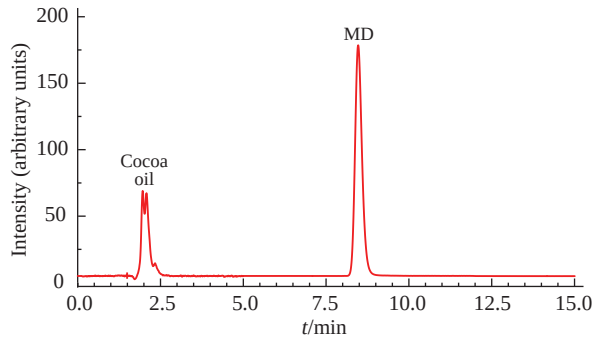


Figure 7 Chromatogram of model mixture obtained with 85 : 15 (v/v) THF/ammonium formate buffer (pH 3.0, 10 mM) mobile phase. Column 'dead' volume t_0 is indicated by (|). Flow rate 1.0 ml min^{-1}. Detection: UV at 220 nm.

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