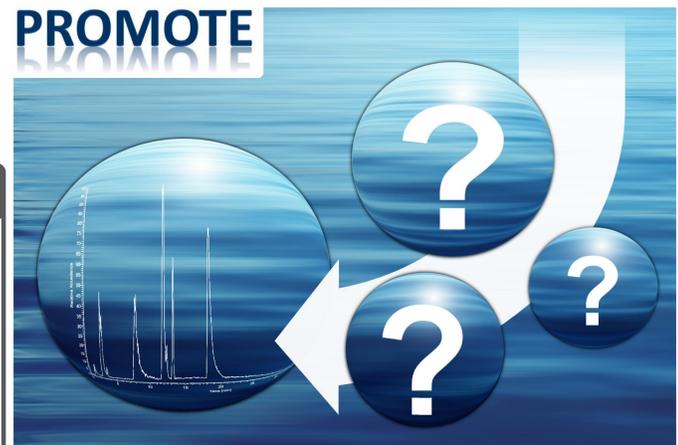


Stationary phase selection in HILIC: a study about stationary phase retention properties and the influence of mobile phase parameters



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Introduction

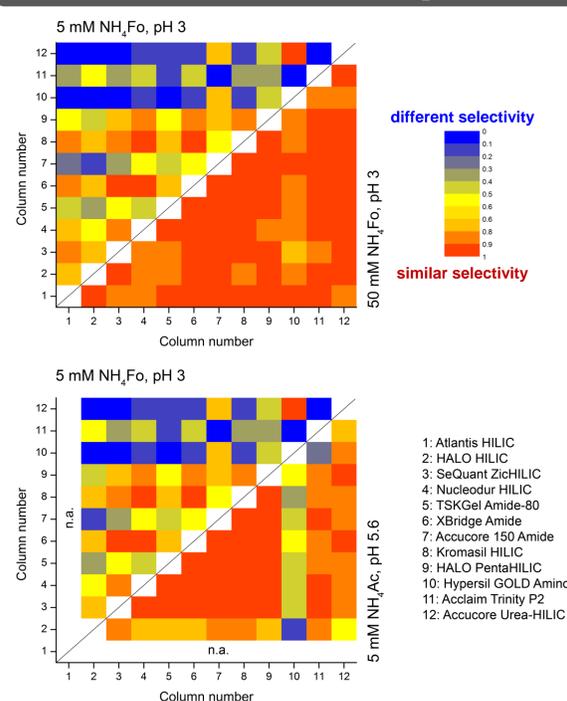
Persistent and mobile organic micropollutants may enter raw and drinking water and thus need to be monitored and regulated. A high polarity, the property that increases the chance of a substance to be mobile in the water cycle, also exacerbates its analysis. While GC-MS and RP-HPLC-MS, the two predominantly deployed methods in organic trace analysis are rarely applicable for very polar substances, **Hydrophilic Interaction Liquid Chromatography (HILIC)** is not only a versatile tool for the separation of such substances but can also be easily interfaced with mass spectrometry.

As a consequence of its evident advantages the number of HILIC applications increased significantly. However, **column selection and method optimization** may still cause problems, especially for

less experienced operators. Reasons for this may be the complex multimodal retention mechanism, the availability of a variety of HILIC stationary phases and the lack of an almost universally applicable stationary phase chemistry like C18 in RP-HPLC.

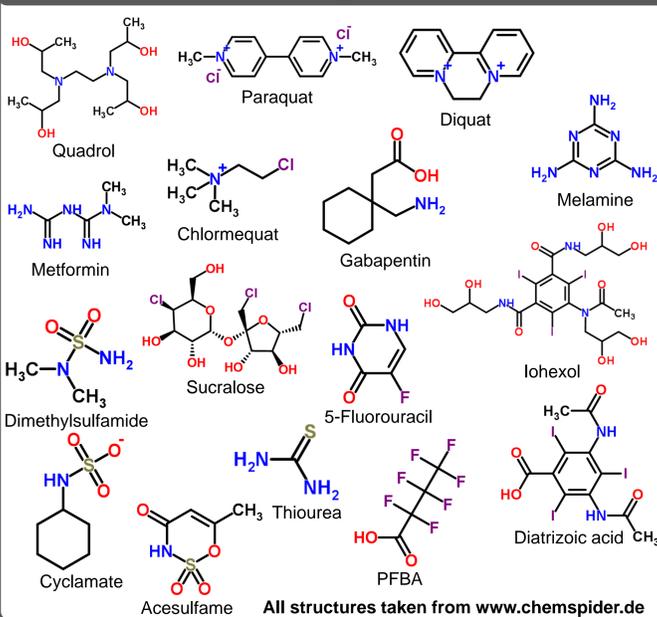
While many studies investigated the retention properties of HILIC stationary phases, this was often done using model substances specifically selected to test for certain interactions. In this study we investigated the retention behavior of **19 environmentally relevant analytes on 12 HILIC stationary phases** with different mobile phase compositions (acetonitrile/water 90:10 (V:V), 5 or 50 mM salt, pH 3 or 5.6, column 1 was only tested at pH 5.6 due to supplier recommendations) and compared our results with previous studies.

Selectivity



- The Pearson coefficients of determination ($\log k$ vs $\log k$) are plotted as a heatmap
- A high correlation indicates a similar selectivity
- Elevated salt concentrations and a change in pH from 3 to 5.6 decrease differences in selectivity between the columns, which can be attributed to a suppression of electrostatic interactions (salt concentration) or the formation of additional active centers (SiO^-) for cation exchange (pH)
- Columns of the same group (see column grouping) often show a similar selectivity

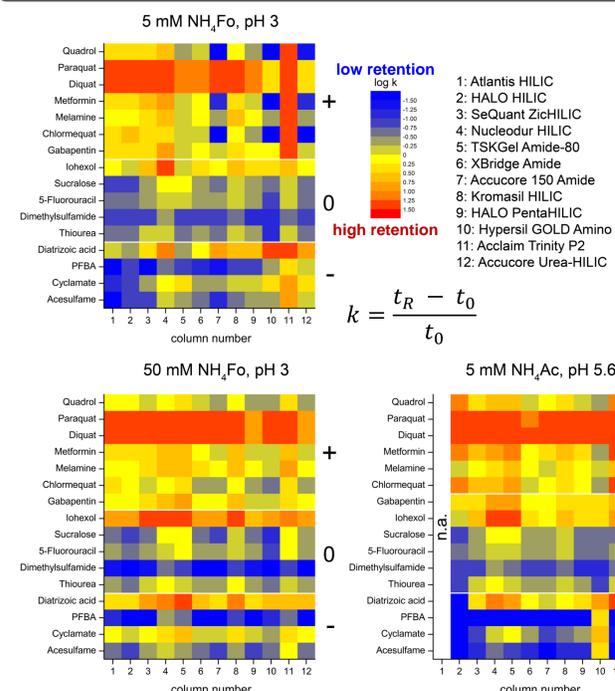
Analytes



Conclusion

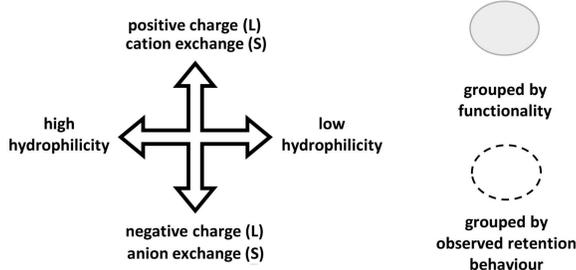
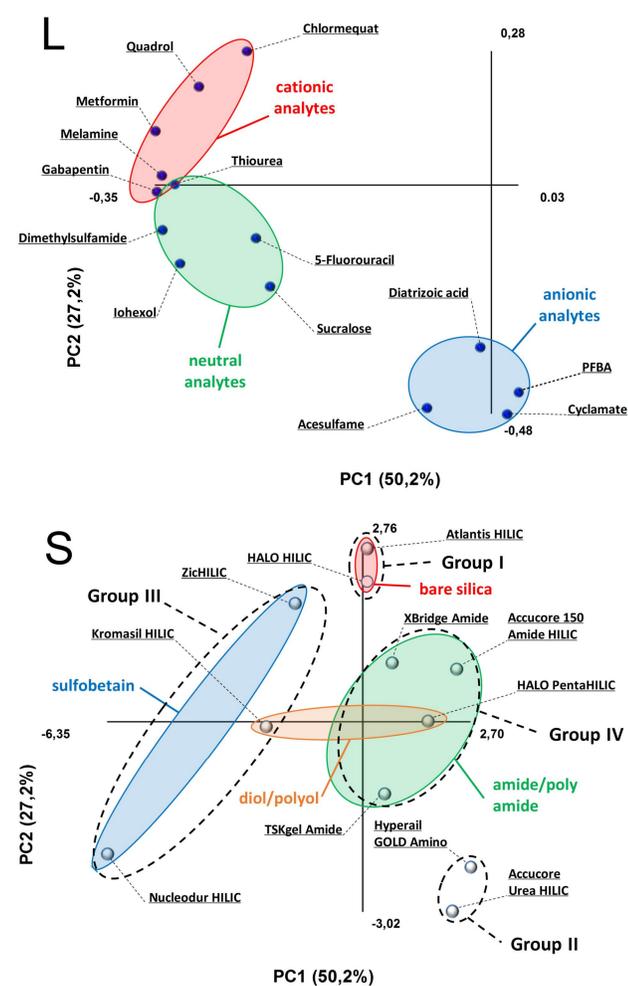
- The observed effects on retention and selectivity of changes in salt concentration^{1, 2} and pH^{1, 3} are in compliance with previously published literature
- The column grouping showed many similarities with other similar attempts⁴ despite using a different set of columns and analytes, which indicates a general applicability of this grouping system rather than a limitation to the tested columns
- Results from studies with model analytes can easily be transferred to environmentally relevant contaminants

Retention behavior



- The logarithm of the capacity factor k of all analytes on every column is plotted as a heatmap. The charge state of analytes (0 = neutral, + = positive, - = negative) at the given pH is indicated.
- Increasing the salt concentration from 5 to 50 mM results in an increase in retention for the majority of substances on all columns. Only in cases where retention seems to be predominantly governed by electrostatic attraction (e.g. PFBA on columns 10, 11 and 12) retention decreases with elevated salt concentrations
- Changing the pH from 3 to 5.6 increases the retention of positively charged analytes while simultaneously decreasing the retention of negatively charged analytes. Neutral analytes remain mostly unaffected. This observation can be attributed to a significantly increased quantity of deprotonated silanol groups at pH 5.5 that act as additional active centers for electrostatic interactions

Column grouping



- A principle component analysis (PCA) of the generated data was performed resulting in the depicted loading (L) and score (S) plot. Diquat, paraquat and column 11 were excluded.
- PC 1 correlates with the polarity ($R^2 = 0.5$) of the analytes while PC 2 correlates well with the charge state ($R^2 = 0.8$), indicating that hydrophilicity and charge state are the two major factors in HILIC retention
- A grouping of columns by observed retention behavior results in slightly different groups than a grouping by functionality

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