Hydrophilic interaction liquid chromatography (HILIC) in conjunction with mass spectrometry (MS) has become a powerful tool for the analysis of a wide variety of challenging analytes. Applications of the technique have increased dramatically over the past decade, especially for the analysis of polar compounds where reversed-phase chromatography suffers. HILIC conditions employ a high aqueous layer and thus retain more). Since the ephedrines have the same predicted solubility – Conclusion – close to pure HILIC partitioning

The 2 mM separation shows only a small increase in retention for the probes indicating only a slight increased retention of polar analytes afforded by HILIC provides improved selectivity and decreases molecular interactions. HILIC is known to involve both partitioning of polar molecules in and out of a semi-immobilized aqueous adsorbed layer on the stationary phase as well as direct interactions with the surface of the phase. When a mobile phase rich in organic comes in contact with a polar stationary phase, a semi-immobilized aqueous layer forms from the system. Polar probes partition in and out of the water layer resulting in retention. Due to the close proximity of the water layer to the surface, other interactions such as dipole-dipole and ion-exchange are also likely to occur.

A sound understanding of the interactions in HILIC is paramount to:

- Development of robust and reliable methods
- Intelligent choice of HILIC stationary phase
- Intelligent choice of mobile phase conditions and parameters

This study focuses on the interaction differences that might be expected for three different HILIC stationary phases. PFPF (FS), bare silica (OH5) and a new perfluorinated phase (OSIL). Using ephedrine as a probe molecule, retention as a function of buffer concentration was collected and interpreted. Several related compounds were run simultaneously and retention and selectivity noted. Interpretation of the observed results in terms of the dominant interactions prevalent under each experimental condition

Table 1 shows the structure of the selected probe for the study along with important physiochemical parameters. Each of the compounds are intended to be small, polar, easily derivatizable, of high purity and high solubility in water and methanol. Ephedrine and pseudoephedrine are once again discriminated. For OH5, studies to date have not indicated any true aqueous layer development, presumably due to its relatively nonpolar chemistry. When no aqueous layer exists only surface interactions like IEX are available.

A deep water layer may be expected to occur by the combined presence of long range hydrogen bonding and desorption of the analytes from the silica surface. As the mobile phase percentage increases this surface layer will decrease and the aqueous layer will become more shallow. Euthedrine and pseudoephedrine are once again discriminated. The presence of ion-exchange interactions. Euthedrine and pseudoephedrine are once again discriminated.

Ephedrine and pseudoephedrine are once again discriminated.

In this report, studies investigating the underlying retention mechanisms dominant in HILIC chromatography are presented and discussed. Along with reversed partitioning, HILIC is well known to exhibit non-ideal behavior. The degree of these mechanisms are undefined and used to develop a model of overall retention and selectivity. Interfaces that operate using different stationary phase chemistry and conditions are presented. The impact of analyte polarity and charge as well as the variations caused by high percentages of organic in these physiochemical parameters are highlighted.

The ephedrine pair is not discriminated (partitioning – weakly shape selective) and synephrine elutes after the ephedrine pair (partitioning). Although HILIC has proven useful, it has also been thwarted with complications including difficulties in method development and method robustness.

In order to develop robust and reliable methods using HILIC chromatography, it is essential to understand the retention mechanisms at play. This knowledge leads to:

- Intelligent choice of column and mobile phase
- Robotic choice of mobile phase modifiers and instrument controls

References


Table 1. Selected Probes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>pKa</th>
<th>Log P</th>
<th>Log D</th>
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<td>Ephedrine</td>
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<td>2.91e-8</td>
<td>2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00</td>
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<td>Pseudoephedrine</td>
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<td>3.47</td>
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<td>Synephrine</td>
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<td>2.12</td>
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</tbody>
</table>

**Results**

Figure 1 shows a plot of ephedrine retention on buffer concentration for each of the phases studied. A key feature observed is that the retention is best explained by polar/ionic interactions. In each system, a linear relationship is observed indicating a lack of exchange mechanisms. The linear relationship is best explained by direct interactions with the silica surface. The slope of the linear fit was calculated for the fluorinated ephedrine to be 0.0003 (Slope = 0.0003, R^2 = 0.9984).

Figure 2 shows the retention of the probes on the OH5 column as a function of buffer concentration. The retention is best explained by polar/ionic interactions. The retention on OH5 is best explained by polar/ionic interactions and direct interactions with the silica surface. The slope of the linear fit was calculated for the fluorinated ephedrine to be 0.0003 (Slope = 0.0003, R^2 = 0.9984).

Figure 3 shows the chromatographic results using the OH5 column at 4 and 2 mM ammonium acetate buffer (pH unadjusted) as a function of mobile phase ion concentration shows a considerable contribution from IEX mechanisms. In order to develop robust and reliable methods using HILIC chromatography, it is essential to understand the retention mechanisms at play. This knowledge leads to: