The Hydrophobic-Subtraction Model: Using the Model to Generate Orthogonal Separations

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Abstract
The Hydrophobic-Subtraction Model is a powerful tool that has proposed six terms to define retention in reversed-phase chromatography. When dealing with straight-chain alkyl phases, four of these retention terms are attributed directly to the underlying silica. These silica interactions are often viewed as undesirable and uncontrolled, however, they are strongly present in even the purest of chromatographic silica. When the silica is held constant, a determination can be made on the effects of both the silica and the stationary phase in the retention of specific solutes. In this study a variety of phases were bonded to the exact same silica and subjected to the Hydrophobic-Subtraction Model protocol. The experimental bonds included both short and long chain alkyl groups, phenyl, and fluorinated phases. By isolating the silica the effects of the individual phases can be studied.

This presentation will briefly review the model and calculations. The empirical and calculated results of the Hydrophobic-Subtraction Model will be shown, and the selectivity highlighted terms contribute will be demonstrated for a particular phase. This presentation will demonstrate how knowledge of column phase, silica, as well as phase-solute interactions aid in choosing the most selective and orthogonal columns for solute confirmation, methods screening, and development.

Scope
Using the testing protocol of the hydrophobic-subtraction model we will empirically define column classes as they pertain to retention profiles. By extending this treatment to define solute retention for substituted alkyl and non-alkyl phases, we will define an orthogonal column set to exploit column selectivity in reversed-phase columns. Finally, this extended treatment will provide a simplified guideline for column selection and create a needed link between analyte chemistry and stationary phase.

Experimental Design
The conditions and calculations described in the hydrophobic-subtraction model were followed to define a basis for stationary phase and silica contributions to selectivity, as well as a qualitative approach to column equivalency. Figure 1 illustrates the test probes and calculations used to define silica and stationary phase contributions to retention.

From the $F_s$ value, we empirically determined the column “dissimilarity” as the reverse of column equivalency, relative to a monomeric C18. For these experiments, the base silica (Ultra column line) was kept constant to not add dissimilarity from changes in the silica, and as a means to focus on the contributions of stationary phase on the A,B and C terms. This, then defined the practical column set for further experimentation. Figure 2 illustrates the equation for column equivalency and the experimental results used to define column dissimilarity. Figure 3 shows the experimental column set used to further define phase-solute interactions.

Table 1  Phase-Solute Interaction Contributing to Reversed-Phase Selectivity

<table>
<thead>
<tr>
<th>Solute Interaction</th>
<th>Selectivity</th>
<th>$F_s$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrophobic</td>
<td>100 x T^T</td>
<td>1.7</td>
</tr>
<tr>
<td>Phase Interactions</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results
Once an orthogonal column set was defined, and the contributions of silica were kept constant, we could then characterize the types and extent of intermolecular interactions as they contribute to retention differences in stationary phases, or selectivity. Table 1 illustrates the characterization of phase-solute interactions largely contributing to reversed-phase selectivity in our defined column set and the calculations employed to quantify the results of the hydrophobic-subtraction model. In Figure 4 we define the 4 major retention mechanisms employed by reversed-phase liquid chromatography. Although not directly measured by the H-S model, polarizability and dipolar retention were inferred by extending the model via the measurements of anisole and benzonitrile.

Discussion
To determine a simplified guideline for column selection, we analyzed empirical selectivity data generated by the H-S model for the experimental column set (Figure 2) against the reversed-phase molecular interactions (Table 1, Figure 4). Through matching stationary phases to specific solute types (Figure 5) based on these measured intramolecular attractions, we can aid method development and shorten screening time in 2 significant ways:

- Employ a small set of columns with a wide range of alternate selectivity
- Define a process for selecting columns based on the chemical properties of the analytes

Extrapolating the retention data for the solute probes in the H-S model allowed us to correlate the retention characteristics of specific solutes to stationary phase types which is represented in Figure 5. Using Figure 4 for column selection or method development is a matter of matching the functionality of the solute or mix of solutes to the retention mechanism(s) that creates the greatest difference. This process is beneficial for LC/MS and LC/MS/MS method development.

We can then further quantify the range of selectivity as described by Neue et al. [2]. By looking at the retention characteristics of the H-S model solute probes, we can define selectivity as the degree of scatter along the regression line when comparing stationary phases of conventional C18 benchmark. Figure 6 illustrates a regression plot of selectivity and the equations used to quantify the selectivity range of our orthogonal column set.

Conclusion
The hydrophobic-subtraction model can be extended to describe the phase-solute interactions of modern reversed-phase columns. This can prove to be a useful tool in defining a column set to provide the widest possible range of selectivity within the smallest column set. This could offer a simplified approach to practicing method developers. We were able to demonstrate how knowledge of reversed-phase (RPLC) column phases and phase-solute interactions can aid in choosing the most selective columns for column screening, solute confirmation, and methods development.

References

Innovative Chromatography Products