HPLC Column Comparison Screening Study for Reversed Phase Columns

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Introduction

The selection of the proper HPLC column for any given analysis can be an intimidating task for today's analyst. The majority of chemists will begin with some type of C18 chemistry. They will try to develop the separation on that phase by using their knowledge of the analytes and the chromatographic parameters available, such as percent organic or pH. Often times this approach gives adequate retention and resolution in a reasonable amount of time.

At times, however, the above approach does not give the desired separation. The analyst may consider changing to another vendor's C18 chemistry. If this does not give the desired result, a change in the stationary phase might be needed. This will usually yield a change in selectivity and retention of the analytes and give an adequate separation. Choosing this alternative phase may also be a difficult task. In the work to be presented, we will explain the systematic approach we undertook to observe selectivity and retention differences in a variety of phases.

We describe the compound sets used in the column screening data experiments, hardware and results. An example is shown how an analyst can use this screening data in a practical separation.
Purpose of Study

• Develop systematic method to determine selectivity and retention differences in stationary phases as well as overall performance.

• Use screening data as a means to help analysts quickly select a suitable phase as a starting point for methods development.
Compounds chosen to represent basic structure or functional groups of small molecules encountered in various industries utilizing HPLC

**Neutrals**
- Parabens
- Alkyl benzenes
- Functionalized benzenes - 3 groups

**Bases**
- Mix 1 - Simple bases
- Mix 2 - Pharmaceutical bases

**Acids**
- Mix 1 - Simple bases
- Mix 2 - Pharmaceutical bases
### Table of Compounds Used (Partial List Only)*

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>% Organic</th>
<th>pH</th>
<th>k’ C18</th>
<th>k’ RP-AmideC16</th>
<th>k’ C8</th>
<th>k’ Cyano</th>
<th>k’ HS F5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>5% CH3CN</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aniline</td>
<td>5</td>
<td>pH 2</td>
<td>0.7</td>
<td>0.5</td>
<td>0.7</td>
<td>0.4</td>
<td>1.5</td>
</tr>
<tr>
<td>benzyl amine</td>
<td>5</td>
<td>pH 2</td>
<td>1.4</td>
<td>0.8</td>
<td>1.3</td>
<td>0.5</td>
<td>3.1</td>
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<tr>
<td>nizatidine</td>
<td>5</td>
<td>pH 2</td>
<td>1.6</td>
<td>1</td>
<td>1.3</td>
<td>0.7</td>
<td>2.4</td>
</tr>
<tr>
<td>o-aminobenzoic acid</td>
<td>5</td>
<td>pH 2</td>
<td>6.2</td>
<td>4.6</td>
<td>5.8</td>
<td>1</td>
<td>8.3</td>
</tr>
<tr>
<td>procainamide</td>
<td>5</td>
<td>pH 2</td>
<td>0.7</td>
<td>0.5</td>
<td>0.6</td>
<td>0.4</td>
<td>3</td>
</tr>
<tr>
<td>pyridine</td>
<td>5</td>
<td>pH 2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>10% CH3CN</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>codeine</td>
<td>10</td>
<td>pH 2</td>
<td>2</td>
<td>1.2</td>
<td>1.7</td>
<td>0.7</td>
<td>2.8</td>
</tr>
<tr>
<td>hydrochlorothiazide</td>
<td>10</td>
<td>pH 2</td>
<td>3</td>
<td>4.3</td>
<td>2.7</td>
<td>3.1</td>
<td>2.3</td>
</tr>
<tr>
<td>lidocaine</td>
<td>10</td>
<td>pH 2</td>
<td>5.9</td>
<td>3</td>
<td>5.1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>phentermine</td>
<td>10</td>
<td>pH 2</td>
<td>4.8</td>
<td>2.6</td>
<td>4.3</td>
<td>0.8</td>
<td>3.5</td>
</tr>
<tr>
<td>quinididine</td>
<td>10</td>
<td>pH 2</td>
<td>2.1</td>
<td>1.4</td>
<td>1.9</td>
<td>1</td>
<td>8.7</td>
</tr>
</tbody>
</table>

*Refer to Re-Discover Method Development Guide for entire list, T402075A.
HPLC Conditions

Conditions chosen based on simplicity of mobile phase for non-ionic compounds. For ionizable compounds, mobile phases were chosen to cover the pH range of silica based phases.

- Non-ionic compounds: acetonitrile/water
- Ionizable compounds: 25mM phosphate buffers at pH 2 and pH 7
- The concentration of acetonitrile was varied to give a \( k' \) between 1 and 5 for most compounds.
- Columns run using automated switching valve.
Phases Used in This Study

**HS C18, C18**

- \(\equiv\text{Si} - \equiv\text{O} - \equiv\text{Si} - (\text{CH}_2)_7\text{CH}_3\)
- \(\equiv\text{Si} - \equiv\text{O} - \equiv\text{Si} - (\text{CH}_2)_{17}\text{CH}_3\)

**RP-AmideC16**

- \(\equiv\text{Si} - \equiv\text{O} - \equiv\text{Si} - (\text{CH}_2)_3\text{NHCO}(\text{CH}_2)_{14}\text{CH}_3\)
- \(\equiv\text{Si} - \equiv\text{O} - \equiv\text{Si} - (\text{CH}_2)_3\text{CN}\)

**HS F5**

- \(\equiv\text{Si} - \equiv\text{O} - \equiv\text{Si} - \text{F}_5\)

**Cyano**

- \(\equiv\text{Si} - \equiv\text{O} - \equiv\text{Si} - (\text{CH}_2)_3\text{CN}\)
- \(\equiv\text{Si} - \equiv\text{O} - \equiv\text{Si} - (\text{CH}_2)_{17}\text{CH}_3\)
Examples of Data Obtained in Study

- $k'$ values
- USP tailing factors
- Selectivity of compounds on the different phases
- Can compare interactions of compounds between phases, for example:
  - C8 vs. C18 interactions
  - C18 vs. F5 interactions
Example: Column Screening Study Results

log $k'$ C18 vs Log $k'$ C8

$R^2 = 0.9656$

Much less selectivity difference between C8 and C18
Example: Column Screening Study Results

log k' correlation between C18 and HS F5

Much greater selectivity difference with Discovery F5

log k', C18
How Can I Use This in My Work?

• Look up your compound in table. (see Supelco Re-Discover Method Development Guide) If exact compound does not appear in the table, look for one with similar structure or functionality.

• Considering the acetonitrile concentration: If different percentages of acetonitrile used in screening, use the very general rule-of-thumb that an increase of 5% (v/v) of the organic modifier results in a 2-fold decrease in k’.

• Choose pH 2 or pH 7

• Choose column or columns that give the right amount of retention for your compound or representative compound.

• Run the experiments.
What Am I Trying to Accomplish?

Consider the desired end result. Are you looking for:

• certain elution order
• speed
• good retention and resolution
• desire to have flexible method if formulation changes in future
• other requirements
A Practical Example

**Sample:** phenacetin and codeine

**Assume:** preferred elution order is codeine, then phenacetin

**pH:** on the pH 2 chart, the compounds elute at very widely different % acetonitrile (10% and 25%) making an isocratic separation potentially difficult. At pH 7, however, codeine was run at 15% acetonitrile, and phenacetin at 20% acetonitrile. Choose the pH 7 condition.

**Column:** The pH 7 screening data shows the compounds have the preferred elution order (codeine then phenacetin) on all but the Discovery HS F5 column, however, if the preferred elution order was reversed, the HS F5 would be the best choice.
## Applying Column Screening Study Results

<table>
<thead>
<tr>
<th>Column</th>
<th>k’ Codeine@ 15% Acetonitrile</th>
<th>Est. k’ Phenacetin@ 15% Acetonitrile</th>
<th>Alpha</th>
</tr>
</thead>
<tbody>
<tr>
<td>C18</td>
<td>4.4</td>
<td>4.7 x 2 = 9.4</td>
<td>2.1</td>
</tr>
<tr>
<td>RP-AmideC16</td>
<td>3.3</td>
<td>4.8 x 2 = 9.6</td>
<td>2.9</td>
</tr>
<tr>
<td>C18</td>
<td>3.6</td>
<td>4.1 x 2 = 8.2</td>
<td>2.3</td>
</tr>
<tr>
<td>Cyano</td>
<td>1.1</td>
<td>1.3 x 2 = 2.6</td>
<td>2.4</td>
</tr>
</tbody>
</table>
Applying Column Screening Study Results

**Discovery C8**
- Codeine: 9.2 min
- Phenacetin: 15.3 min

**Discovery Cyano**
- Codeine: 3.6 min
- Phenacetin: 4.6 min

**Discovery AmideC16**
- Codeine: 6.5 min
- Phenacetin: 16.0 min

**Discovery C18**
- Codeine: 8.5 min
- Phenacetin: 17.3 min

Columns: 15cm x 4.6mm, 5µm each
Mobile Phase: 25mM H₃PO₄, pH to 7.0 w/NH₄OH:Acetonitrile (85:15, v/v)
Flow Rate: 1mL/min

Detection: UV @ 220nm
Injection Volume: 10µL
Samples: 100µg/mL each, codeine and phenacetin in mobile phase buffer
Analysis of Results: Predicted vs Actual

From the experiments:

• If speed is desired, choose Cyano.
• If formulation contains other compounds, Amide C16 or C18 is adequate due to large amount of peak space between compounds of interest.
• C8 gives adequate separation for a general method.

<table>
<thead>
<tr>
<th>Column</th>
<th>Analyte</th>
<th>Predicted k'</th>
<th>Actual k'</th>
<th>Alpha Predicted</th>
<th>Alpha Actual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discovery C8</td>
<td>Codeine</td>
<td>3.6</td>
<td>3.6</td>
<td>2.27</td>
<td>1.86</td>
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<tr>
<td></td>
<td>Phenacetin</td>
<td>8.2</td>
<td>6.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discovery Cyano</td>
<td>Codeine</td>
<td>1.1</td>
<td>1</td>
<td>2.36</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>Phenacetin</td>
<td>2.6</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discovery RP-AmideC16</td>
<td>Codeine</td>
<td>3.3</td>
<td>2.8</td>
<td>2.9</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Phenacetin</td>
<td>9.6</td>
<td>8.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discovery C18</td>
<td>Codeine</td>
<td>4.4</td>
<td>3.3</td>
<td>2.13</td>
<td>2.33</td>
</tr>
<tr>
<td></td>
<td>Phenacetin</td>
<td>9.4</td>
<td>7.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Conclusion

1. A systematic method has been developed to determine selectivity and retention differences in stationary phases.

2. Column screening can help the analyst quickly select a suitable phase as a starting point for methods development.

3. Comparisons can be made between phases to show which phases are most alike and which are most different.

4. Overall, gain knowledge of separation so that you are better prepared to make adjustments in method if formulation changes in future, knowing that you can change column if necessary to accommodate these changes.