Adequately retaining and separating small polar molecules with reversed-phase liquid chromatography (RPLC) is often a challenging task. Alkyl phase LC columns, like C18, are a common starting point for LC method development. However, highly polar analytes are poorly retained on non-polar C18 stationary phases. In order to increase retention of these compounds, there are several techniques that can be tried, such as: adjusting the mobile phase pH when the analytes are ionizable, adding an ion pairing reagent to the mobile phase, or selecting a more appropriate column stationary phase for the analysis. The wide variety of stationary phase chemistries currently available on superficially porous particle columns can facilitate method development; several chemistries are well suited for troublesome polar analytes and can be used under 100% aqueous conditions. Superficially porous particles are known for their ability to generate high efficiency with low back pressure. High efficiency can contribute to resolving closely eluting peaks, while low back pressure allows for flexibility with LC instrumentation. This work will demonstrate a logical, stepwise methodology to enable chemists to retain and separate their polar analytes with superficially porous particle columns.

### Introduction

Agilent InfinityLab Poroshell 120 Columns:

- **Poroshell 120 2.7 µm**: Efficiency 90% of < 2 µm TPP; Pressure 50% of < 2 µm TPP; 2 µm inlet frit
- **Poroshell 120 4 µm**: Efficiency 2x 5 µm TPP; Pressure often below 200 bar; 2 µm inlet frit

A variety of selectivities are available on InfinityLab Poroshell 120 Columns to meet almost any application need.

<table>
<thead>
<tr>
<th>Best all around</th>
<th>Best for low pH mobile phases</th>
<th>Best for high pH mobile phases</th>
<th>Best for alternative selectivity</th>
<th>Best for more polar compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poroshell 120 EC-C18 2.7 µm, 4 µm</td>
<td>Poroshell 120 SB-C18 2.7 µm</td>
<td>Poroshell 120 HPH-C18 2.7 µm, 4 µm</td>
<td>Poroshell 120 Bonus-RP 2.7 µm</td>
<td>Poroshell 120 SB-Aq 2.7 µm</td>
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<tr>
<td>Poroshell 120 EC-CN 2.7 µm, 4 µm</td>
<td>Poroshell 120 SB-CN 2.7 µm</td>
<td>Poroshell 120 HPH-CN 2.7 µm</td>
<td>Poroshell 120 PFP 2.7 µm, 4 µm</td>
<td>Poroshell 120 EC-CN 2.7 µm</td>
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</tbody>
</table>

These phases can be used with 100% aqueous mobile phases to improve retention of highly polar analytes in RPLC mode.

### Compounds of Interest: Water-Soluble Vitamins

- Thiamine, B1
- Riboflavin, B2
- Pantothenic Acid, B5
- Folic Acid, B9
- Ascorbic Acid, C
- Neotenic Acid, B3
- Niacin, B3
- Pyridoxine, B6
- PABA, B10
- Biotin, B7

### Results and Discussion

**C18 columns are usually a good starting point for HPLC method development. However, highly polar analytes can be difficult to retain.**

- Poor overall retention
- For 6 compounds, k’ < 2
- 4 unretained compounds
- Significant coelution

If analytes are ionizable, adjusting the mobile phase pH can alter retention.

- At low pH, acids are neutral (ionized) and more retained.
- At high pH, bases are neutral and more retained.
- Non-ionizable compounds are unaffected by changes in pH.

- pH 2.5 mobile phase allows slightly more retention than pH 7.5
- Most compounds do not change ionization state between pH 2.5 and 7.5
- HPH-C18 is designed for improved lifetime with high pH applications.

Decreasing the mobile phase strength improves retention. C18 columns generally should not be used with too little organic, due to the risk of dewetting. However, there are several stationary phase options that can be successfully used with 100% aqueous mobile phases.

- Dewetting occurs when the chromatographic pores dry out and the non-polar surface expels the pure aqueous polar mobile phase.
- When the pores dry out, the analyte cannot get in and will not be retained by the column stationary phase.
- Dewetting can be observed by a reduction in V_d and a sudden loss of retention.

- Low pH mobile phase was used for the above experiments due to improved retention, compared to high pH
- Using a column compatible with 100% aqueous mobile phase significantly improves overall analyte retention.
- In this case, Phenyl-Hexyl has the best retention, with 7 compounds having k’ > 2; this is improved from the original C18 analysis where only 4 compounds had k’ > 2

### Conclusions

- C18 columns are usually a good column to try first for LC method development, however they may not provide enough retention for polar analytes.
- Poorly retained ionizable compounds, mobile phase pH can be used to adjust retention.
- Several stationary phases are available that can be used with 100% aqueous mobile phase to improve retention of polar analytes without the risk of dewetting.
- Ion pairing agents can be added to mobile phases to improve retention of polar analytes.
- If all else fails with RPLC, try HILIC for polar analytes.
- Similar method development techniques can be applied to HILIC: adjust mobile phase pH & try alternate selectivities.