Remarkable separations

guaranteed time after time
When developing a new method, one of the most important goals for the chromatographer is to achieve a consistent, reproducible separation. The selection of a highly reproducible HPLC column is essential if this goal is to be attained.

Our Thermo Scientific™ Syncronis™ HPLC columns are manufactured, packed and tested in ISO9000:2008 accredited facilities. Each lot of silica is tested for the physical properties of the silica support and only released for production if it meets the stringent test specifications.

Each bonded lot of chromatographic packing material is rigorously tested for primary and secondary interactions with the bonded phase.

New, enhanced, automated packing methods drive consistency even further and every column is individually tested to ensure that it meets the required quality.

These extensive testing and quality control procedures ensure the delivery of a consistent product, column after column.
Predictability you can count on, it’s a beautiful thing.

Syncronis HPLC columns are available in a range of chemistries to give reproducible separations in reversed phase, HILIC and normal phase chromatography.

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Testing and Quality Control

Silica Characterization

Consistent separations require a rugged, reproducible silica backbone and to achieve this consistency, strict control of the physical properties of the silica particles is essential. Each manufactured lot of Syncronis silica is tightly controlled and extensively tested for its particle size and distribution, pore size and surface area and elemental purity. Only those batches which meet the rigorous quality control specifications are used.

**Particle size and distribution**

Tight control of the particle classification process ensures that a narrow particle size distribution is achieved around the target particle size, an important consideration for consistent chromatographic efficiency. Each manufactured lot of Syncronis silica is tested for its particle size and distribution using a laser particle size analyser. Three particle sizes are available: 1.7 μm for rapid UHPLC separations plus 3 μm and 5 μm for the more traditional HPLC analysis.

**Pore size and surface area**

Well controlled pore size and surface area are key to ensuring consistent carbon load and retentive properties of the chromatographic media. Each batch of Syncronis silica is tested for its pore size and surface area using liquid nitrogen adsorption.

Syncronis columns are based on highly pure 100 Å silica, with a surface area of 320 m²/g, compared to 200 m²/g for typical silica based material. This greater surface area ensures good retention of analytes having a range of hydrophobicity, away from the solvent front. The high surface area also allows for higher sample loading.

**Silica purity (metals content)**

The purity of the silica support is of particular importance when considering the separation of polar and basic compounds. Older, less pure silica supports contain a greater number of metallic impurities. The presence of certain metallic impurities with electron withdrawing properties (particularly aluminium) in silica can activate the silanols so that they become highly acidic, which can lead to peak tailing for basic solutes. Metallic impurities can also complex with chelating solutes, resulting in asymmetrical or tailing peaks. In extreme cases, these interactions may be strong enough to result in complete retention of the solute.

Each batch of Syncronis silica is tested for metals content using atomic emission spectroscopy.
Bonded Phase Characterization

Syncronis HPLC columns are bonded and endcapped with a range of stationary phases to effect different selectivity in separation. Whatever the bonded phase, rigorous testing and precise control of the bonding process are essential to achieve consistent chromatography. Each batch of Syncronis chromatographic media is tested for carbon load and characterized by stringent chromatographic testing before it is used to pack columns.

Carbon loading and surface coverage

The hydrophobic retention of a stationary phase is directly dependent on the carbon loading on the silica. Precise control of the batch to batch carbon loading is therefore a critical factor in ensuring consistent retention times. Syncronis reversed phase columns are densely bonded and double endcapped to minimise the number of residual silanols available to interact with basic analytes. Each batch of bonded phase is tested for carbon load using a total carbon analyzer.

Chromatographic tests

The retention properties of a reversed-phase packing material can be categorized into hydrophobic interactions, which include the measure of the hydrophobicity of the ligand and its density, steric or shape selectivity and secondary interactions such as silanol and surface metal activity. The impact that interactions between analytes and silanols have on the chromatographic performance depends on the pH of the mobile phase. Silanols on the silica surface can hydrogen bond (both as a donor and acceptor) and dissociated silanols can ion exchange with protonated bases.

To ensure consistent, predictable separations, the chromatographic media packed into Syncronis HPLC columns is extensively characterized using a series of diagnostic chromatographic tests, based on those developed by Tanaka. These tests rigorously probe interactions between analytes and stationary phase, measuring hydrophobicity, shape selectivity and secondary interactions with bases, acids and chelators.

Column Packing

Every Syncronis HPLC column is individually tested and will not be released unless it meets the required retention, efficiency and peak symmetry. This testing is used to confirm the quality of the column packing process and the stability of the packed bed inside the column.

To ensure the most consistent results column after column, all Syncronis HPLC columns are packed using automated workstations.

To ensure that the measurements are a true indication of the quality of the packing, the testing of individual columns is performed on a highly optimized system.

Summary of Tests Performed on Syncronis HPLC Columns

<table>
<thead>
<tr>
<th>Test</th>
<th>C18</th>
<th>C8</th>
<th>aQ</th>
<th>Phenyl</th>
<th>Amino</th>
<th>Silica</th>
<th>HILIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silica</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Particle size and distribution</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
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<tr>
<td>Pore size and surface area</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
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<td>Bonded phase</td>
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<tr>
<td>Carbon Load</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
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<td>Y</td>
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<tr>
<td>Chromatographic</td>
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<tr>
<td>Hydrophobic retention</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
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<tr>
<td>Hydrophobic selectivity</td>
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<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
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<td>Steric selectivity</td>
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<td>Hydrogen bonding capacity</td>
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<td>Y</td>
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<td>Y</td>
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<td>Activity towards basic compounds</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
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<tr>
<td>Ion exchange capacity (pH 7.6)</td>
<td>Y</td>
<td>Y</td>
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<tr>
<td>Activity towards acidic compounds</td>
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<td>Y</td>
<td>Y</td>
<td>Y</td>
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<td>Ion exchange capacity (pH 2.7)</td>
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<td>Anion exchange test</td>
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<td>Y</td>
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<td>Normal phase test</td>
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<td></td>
<td>Y</td>
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<tr>
<td>HILIC retention and selectivity</td>
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<tr>
<td>Column packing</td>
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<tr>
<td>Reversed phase packing test</td>
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<td>Y</td>
<td>Y</td>
<td>Y</td>
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<td></td>
</tr>
<tr>
<td>Normal phase packing test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>HILIC packing test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Y</td>
</tr>
</tbody>
</table>
1.7 μm Particles for UHPLC Applications

1.7 μm particles give higher efficiency than 3 μm or 5 μm particles and this efficiency is delivered over a greater range of optimum linear velocity. This makes it possible to operate at higher flow rates without losing performance. Because shorter columns packed with 1.7 μm particles give equivalent efficiency to longer columns packed with 5 μm particles faster analysis and solvent savings for the chromatographer become a reality.

Three Tips for Method Transfer

1. To maintain an equivalent separation when transferring a method it is important to keep the linear velocity constant between the original and new method.

2. Sub-2 μm-based methods are most often transferred to smaller volume columns, so the same injection volume will take up a larger proportion of the new column, possibly leading to band broadening. It is therefore important to scale down the injection volume to match the change in column volume.

3. Geometrical transfer of the gradient requires calculation of the number of column volumes of mobile phase in each segment (time interval) of the gradient in the original method to ensure that the new calculated gradient takes place over the same number of column volumes, for the new column.

System Considerations

With 1.7 μm particles, analyses can be performed with a high linear velocity through the column without loss in performance, provided the LC system is optimized to operate under these conditions. In order to produce fast, efficient chromatography, all system components for the assay should also be considered. Modern ultra high pressure liquid chromatography (UHPLC) instruments, including the Thermo Scientific™ Vanquish™ UHPLC System will take account of these factors.

There are three major system considerations to remember when using short columns packed with 1.7 μm particles.

1. The system volume (connecting tubing ID and length, injection volume, UV detector flow cell volume) must be minimized.

2. The detector time constant and sampling rate need to be carefully selected.

3. When running fast gradients pump delay volume needs to be minimal.

We also offer a convenient HPLC method transfer calculator at the Chromatography Resource Center www.thermofisher.com/SBE
When developing a new method, one of the most important goals for the chromatographer is to achieve a consistent, reproducible separation. The selection of a highly reproducible HPLC column is essential if this goal is to be attained.

- Outstanding reproducibility
- Highly pure, high surface area silica (320 m²/g)
- High carbon load for increased retention
- Double endcapped for extra surface coverage
- Highly inert towards basic compounds
- Rigorously tested to ensure quality

**Syncronis C18**

Outstanding column to column reproducibility

When developing a new method, one of the most important goals for the chromatographer is to achieve a consistent, reproducible separation. The selection of a highly reproducible HPLC column is essential if this goal is to be attained.

- Outstanding reproducibility
- Highly pure, high surface area silica (320 m²/g)
- High carbon load for increased retention
- Double endcapped for extra surface coverage
- Highly inert towards basic compounds
- Rigorously tested to ensure quality

Syncronis C18 HPLC columns show excellent column to column reproducibility, as illustrated here by the analysis of zidovudine using five separate columns. The reproducibility in terms of retention time and peak area is less than or equal to 0.5%, column to column. This indicates that the columns are well packed.

The variation in peak area is 0.27%, which is important for quantitation of analytes.
Application: Chloramphenicol (USP)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>USP specification</th>
<th>Measured (6 replicate injections)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficiency (N)</td>
<td>&gt; 1800</td>
<td>6164</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>&lt; 2</td>
<td>1.06</td>
</tr>
<tr>
<td>%RSD retention time</td>
<td>&lt; 1%</td>
<td>0.03%</td>
</tr>
<tr>
<td>%RSD peak area</td>
<td>&lt; 1%</td>
<td>0.32%</td>
</tr>
</tbody>
</table>

Column: Syncronis C18 5 μm, 100 mm × 4.6 mm
Mobile Phase: water:methanol:glacial acetic acid (54.9:45:0.1)
Flow Rate: 1.0 mL/min
Inj. Volume: 10 μL
Temp.: 25 °C
Detection: 280 nm
Sample: Chloramphenicol

Application: Ibuprofen and Valerophenone (USP)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>USP specification</th>
<th>Measured Valerophenone (5 replicate injections)</th>
<th>Measured Ibuprofen (5 replicate injections)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution</td>
<td>&gt; 2.0</td>
<td>–</td>
<td>3.56</td>
</tr>
<tr>
<td>Relative retention time</td>
<td>~ 0.8</td>
<td>0.84</td>
<td>–</td>
</tr>
<tr>
<td>Efficiency (N)</td>
<td>–</td>
<td>8317</td>
<td>5872</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>–</td>
<td>1.11</td>
<td>1.38</td>
</tr>
<tr>
<td>%RSD retention time</td>
<td>–</td>
<td>0.50%</td>
<td>0.77%</td>
</tr>
<tr>
<td>%RSD peak area</td>
<td>–</td>
<td>0.50%</td>
<td>0.27%</td>
</tr>
</tbody>
</table>

Column: Syncronis C18 5 μm, 150 mm × 4.0 mm
Mobile Phase: water/phosphoric acid (pH 2.5):acetonitrile (66.3:33.7)
Flow Rate: 2.0 mL/min
Inj. Volume: 5 μL
Temp.: 30 °C
Detection: 214 nm
Sample: 1. Valerophenone
2. Ibuprofen
Syncronis C8
Low hydrophobicity columns for less retention than Syncronis C18

Syncronis C8 HPLC columns are less hydrophobic than the C18 and are therefore particularly useful where the lesser degree of hydrophobicity is needed in order to successfully retain compounds of interest. Syncronis C8 HPLC columns can also be used where it is desirable to elute compounds more quickly.

- Outstanding reproducibility
- Highly pure, high surface area silica (320 m²/g)
- Less hydrophobic than C18
- Double endcapped for extra surface coverage
- Highly inert towards basic compounds
- Rigorously tested to ensure quality

Application: Fenoprofen (USP)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>USP Specification</th>
<th>Measured Fenoprofen (5 replicate injections)</th>
<th>Measured Gemfibrozil (5 replicate injections)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution</td>
<td>&gt; 8</td>
<td>–</td>
<td>17.6</td>
</tr>
<tr>
<td>Relative retention time</td>
<td>~ 0.5</td>
<td>0.48</td>
<td>–</td>
</tr>
<tr>
<td>Efficiency (N)</td>
<td>&gt; 3000</td>
<td>9812</td>
<td>10254</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>&lt; 2</td>
<td>1.21</td>
<td>1.22</td>
</tr>
<tr>
<td>%RSD retention time</td>
<td>&lt; 2%</td>
<td>0.13%</td>
<td>0.14%</td>
</tr>
<tr>
<td>%RSD peak area</td>
<td>&lt; 2%</td>
<td>1.6%</td>
<td>1.8%</td>
</tr>
</tbody>
</table>

Column: Syncronis C8 5 μm, 150 mm x 4.6 mm
Mobile Phase: acetonitrile:water:phosphoric acid (50:49.6:0.4)
Flow Rate: 2.0 mL/min
Inj. Volume: 20 μL
Temp.: 30 °C
Detection: 272 nm
Sample: 1. Fenoprofen
2. Gemfibrozil
Application: Uron Herbicides

Column: Syncronis C8 5 μm, 150 mm × 4.6 mm
Mobile Phase:
A: water
B: acetonitrile
Gradient: 35 to 60 % B in 10 minutes
Flow Rate: 1.0 mL/min
Inj. Volume: 20 μL
Temp.: 30 °C
Detection: 240 nm

Sample:
1. Tebuthiuron
2. Metoxuron
3. Monuron
4. Chlorotoluron
5. Diuron
6. Linuron

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>RT (%RSD) (6 replicate injections)</th>
<th>Peak Area (%RSD) (6 replicate injections)</th>
<th>Peak Asymmetry</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - Tebuthiuron</td>
<td>0.31</td>
<td>0.95</td>
<td>1.17</td>
</tr>
<tr>
<td>2 - Metoxuron</td>
<td>0.25</td>
<td>0.64</td>
<td>1.18</td>
</tr>
<tr>
<td>3 - Monuron</td>
<td>0.18</td>
<td>0.20</td>
<td>1.16</td>
</tr>
<tr>
<td>4 - Chlorotoluron</td>
<td>0.12</td>
<td>0.55</td>
<td>1.15</td>
</tr>
<tr>
<td>5 - Diuron</td>
<td>0.10</td>
<td>0.37</td>
<td>1.19</td>
</tr>
<tr>
<td>6 - Linuron</td>
<td>0.05</td>
<td>0.65</td>
<td>1.13</td>
</tr>
</tbody>
</table>
In comparison to a conventionally endcapped C18, the Syncronis aQ polar end-capped C18 stationary phase exhibits superior stability towards aqueous mobile phase. Syncronis aQ shows no degradation in performance after 100 injections in a buffered 100 % aqueous eluent.

- Stability in 100 % aqueous mobile phase
- Outstanding reproducibility
- Highly pure, high surface area silica (320 m²/g)
- Highly inert towards basic compounds
- Rigorously tested to ensure quality

In contrast, the performance of the C18 packing begins to deteriorate appreciably after roughly 50-60 replicate injections of the mixture of analytes. The decline in chromatographic performance is more pronounced for the later-eluting compounds. As shown on the right, there is a 20 % decrease in retention time for adenosine monophosphate on the C18 column.

Comparison of relative retention time for 5-AMP on Syncronis aQ and C18 over 100 injections
### Application: Lamivudine (USP)

Column: Syncronis aQ 5 μm, 250 mm × 4.6 mm  
Mobile Phase: 25 mM ammonium acetate (pH = 3.81):methanol (95:5)  
Flow Rate: 1.0 mL/min  
Inj. Volume: 10 μL  
Temp.: 35 °C  
Detection: 277 nm  
Sample: Lamivudine

### Application: Amoxicillin and Potassium Clavulanate (USP)

Column: Syncronis aQ 5 μm, 300 mm × 4.0 mm  
Mobile Phase: phosphate buffer (pH 4.4):methanol (95:5)  
Flow Rate: 2.0 mL/min  
Inj. Volume: 20 μL  
Temp.: 25 °C  
Detection: 210 nm  
Sample: 1. Amoxicillin  
2. Potassium Clavulanate

<table>
<thead>
<tr>
<th>Parameter</th>
<th>USP Specification</th>
<th>Measured Amoxicillin (6 replicate injections)</th>
<th>Measured K Clavulanate (6 replicate injections)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution</td>
<td>&gt; 3.5</td>
<td>–</td>
<td>12.8</td>
</tr>
<tr>
<td>Efficiency (N)</td>
<td>&gt; 550</td>
<td>7598</td>
<td>6475</td>
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<tr>
<td>Tailing factor</td>
<td>&lt; 1.5</td>
<td>1.15</td>
<td>0.92</td>
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<tr>
<td>%RSD retention time</td>
<td>&lt; 2%</td>
<td>0.29%</td>
<td>0.36%</td>
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<tr>
<td>%RSD peak area</td>
<td>&lt; 2%</td>
<td>0.30%</td>
<td>0.29%</td>
</tr>
</tbody>
</table>
Syncronis Phenyl
Enhanced retention of aromatic compounds

Syncronis Phenyl HPLC columns provide alternative selectivity to C18 and are particularly useful for the retention and separation of aromatic compounds.

- Outstanding reproducibility
- Highly pure, high surface area silica (320 m²/g)
- Alternative selectivity to C18
- Double endcapped for extra surface coverage
- Highly inert towards basic compounds
- Rigoruously tested to ensure quality

Application: Oxacillin Sodium (USP)

![Graph showing mAU vs. minutes for Oxacillin Sodium analysis](image)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>USP Specification</th>
<th>USP Specification</th>
<th>USP Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficiency (N)</td>
<td>&gt; 2000</td>
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<tr>
<td>Tailing factor</td>
<td>&lt; 1.6</td>
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<td></td>
</tr>
<tr>
<td>%RSD retention time</td>
<td>&lt; 2%</td>
<td>0.03%</td>
<td></td>
</tr>
<tr>
<td>%RSD peak area</td>
<td>&lt; 2%</td>
<td>0.29%</td>
<td></td>
</tr>
</tbody>
</table>

Column: Syncronis Phenyl 5 µm, 300 mm × 4.0 mm
Mobile Phase: phosphate buffer:acetonitrile:methanol (70:30:10)
Flow Rate: 1.0 mL/min (2 mL/min in USP method)
Inj. Volume: 10 µL
Temp.: 25 °C
Detection: 225 nm
Sample: Oxacillin Sodium (0.11mg/mL)
Syncronis Amino HPLC columns exhibit excellent chromatographic properties in weak anion exchange, reversed phase, normal phase and HILIC.

- Outstanding reproducibility for reversed phase, normal phase, ion exchange and HILIC
- Highly pure, high surface area silica (320 m²/g)
- Alternative selectivity to C18
- Double endcapped for extra surface coverage
- Rigorously tested to ensure quality

**Application: Lactulose**

![Graph showing chromatography results for Lactulose analysis using Syncronis Amino HPLC columns.]

- Column: Syncronis Amino 5 μm, 150 mm × 4.6 mm
- Mobile Phase: water:acetonitrile (30:70)
- Flow Rate: 1.0 mL/min
- Inj. Volume: 5 μL
- Temp.: 35 °C
- Detection: RI

Sample: Lactulose
Syncronis Silica
For highly efficient, normal phase chromatography

Syncronis Silica HPLC columns serve as a powerful and efficient tool for the chromatography of moderately polar organic compounds by normal phase chromatography.

- Highly pure, high surface area silica (320 m²/g)
- Excellent reproducibility for normal phase chromatography
- Rigorously tested to ensure quality

Application: Cetirizine (USP)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>USP Specification</th>
<th>Measured (6 replicate injections)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tailing factor</td>
<td>&lt; 2.0</td>
<td>1.05</td>
</tr>
<tr>
<td>%RSD peak area</td>
<td>&lt; 2%</td>
<td>0.17%</td>
</tr>
</tbody>
</table>

Column: Syncronis Silica 5 μm, 250 mm × 4.6 mm
Mobile Phase: acetonitrile:water:sulfuric acid (93:6:6:0.4)
Flow Rate: 1.0 mL/min
Inj. Volume: 10 μL
Temp.: 30 °C
Detection: 230 nm
Sample: Cetirizine
Syncronis HILIC
Enhanced retention of polar and hydrophilic analytes

Syncronis HILIC is based on highly pure, high surface area silica particles. The zwitterionic modified stationary phase results in total charge equalisation and therefore a neutral (uncharged) but highly polar surface. Syncronis HILIC HPLC columns offer enhanced retention of polar and hydrophilic analytes.

- Highly pure, high surface area silica (320 m²/g)
- Zwitterionic bonded phase
- Enhanced retention of polar and hydrophilic analytes
- Excellent reproducibility
- Rapid equilibration
- Rigorously tested to ensure quality

Application: Allantoin

![Graph showing retention of Allantoin with Syncronis HILIC](image)

Column: Syncronis HILIC 5 μm, 100 mm × 4.6 mm
Mobile Phase: ammonium formate buffer (pH 3):acetonitrile (10:90)
Flow Rate: 1.0 mL/min
Inj. Volume: 10 μL
Temp.: 30 °C
Detection: 210 nm
Sample: Allantoin
### Ordering Information

**Syncronis HPLC Columns**

<table>
<thead>
<tr>
<th>Particle Size (µm)</th>
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*Other columns dimensions are available. Please contact Customer Services for more details.*

### Syncronis HPLC Guard Columns (5 µm Particle Size)

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Resources for Chromatographers

Thermo Scientific Chromatography Columns and Consumables Catalog
This extensive catalog offers 450 pages of proven chromatography tools and product selection guides. Available online, with a robust search tool and optimized for your iPad®.
Visit www.thermofisher.com/catalog

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