Application Note 126

Analyses of Underivatized Sugars and Oligosaccharides by HPLC

We tested the typical method of analyzing sugars and oligosaccharides, using HPLC, refractive index detection, and buffered mobile phase. However, for fast, selective analyses we found that HPLC with ultraviolet detection and mobile phases that were not buffered provided shorter analysis times and more accurate separations. The SUPELCOSIL LC-NH_2 column is particularly useful for separating these compounds.

Key Words:
- SUPELCOSIL columns • sugars • oligosaccharides
- refractive index detection • ultraviolet detection

Although reversed phase HPLC columns based on n-alkyl bonded groups are extremely versatile, they are not suited to every type of analysis. The introduction of functional groups such as nitrile, amino, or diol at the end of the alkyl groups, however, produces new phases which also have useful properties. A SUPELCOSIL™ LC-NH_2 column, packed with a γ-amino-propylsilyl polar bonded phase, is particularly useful for separating sugars and oligosaccharides.

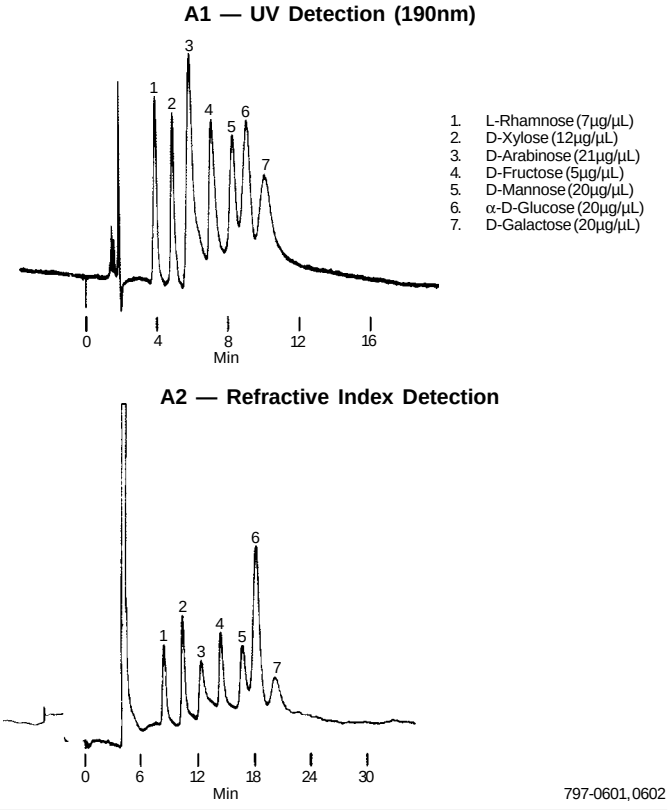
Underivatized monosaccharides are quickly resolved on SUPELCOSIL LC-NH_2 columns through normal phase partitioning, based on the interaction of sugar hydroxyl groups with the amino phase (1). Pentoses (i.e., arabinose, xylose) elute before hexoses (i.e., fructose, galactose, glucose, mannose) because pentoses have four hydroxyl groups while hexoses have five (Figure A). The exception is the hexose rhamnose, which elutes with the pentoses because it has only four hydroxyl groups.

For ease in identification, a refractive index (RI) detector is normally used in sugar analyses. However, RI detection generally has limited sensitivity, while temperature variations and pump pulsations must be carefully controlled. In contrast, ultraviolet (UV) detectors are affected much less by temperature and pressure variations. UV detection also enables analysts to use gradient elution to shorten analysis time and separate complex mixtures, including those containing di- and oligosaccharides. Carbohydrate detection is most sensitive at UV wavelengths of 190nm and lower, although solvent impurities can create noise and limit sensitivity at these wavelengths. Figure A compares the use of UV and RI detectors. UV appears to provide the highest sensitivity for D-arabinose, D-fructose, and D-galactose, while D-xylose and α-D-glucose detection is enhanced using RI.

An 85:15 acetonitrile:water mobile phase resolves the most common monosaccharides well (Figure A). Since water and the sugars compete to form hydrogen bonds with the amino phase, sugar retention decreases as water in the mobile phase is increased.

Figure A. Monosaccharides

Column: **SUPELCOSIL LC-NH_2 (58338), 25cm x 4.6mm ID, 5µm particles, with 2cm guard column**
Mobile Phase: acetonitrile:water (85:15)
Flow Rate: (A1) 2mL/min
(A2) 1mL/min
Det.: (A1) 190nm UV
(A2) RI
Sample: 10µL, component concentrations on figure



1. L-Rhamnose (7µg/µL)
2. D-Xylose (12µg/µL)
3. D-Arabinose (21µg/µL)
4. D-Fructose (5µg/µL)
5. D-Mannose (20µg/µL)
6. α-D-Glucose (20µg/µL)
7. D-Galactose (20µg/µL)

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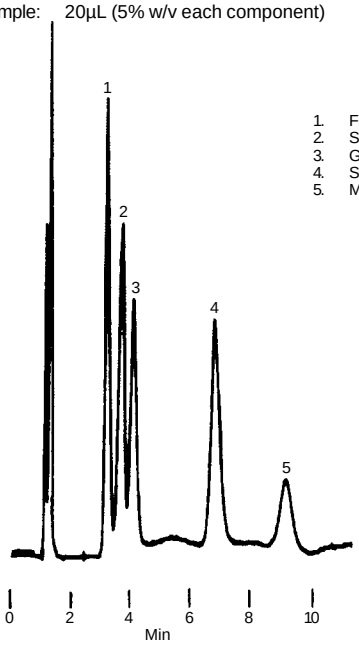
Therefore, water content can be increased to shorten analysis time for less complex samples and, similarly, analysts can separate di- and oligosaccharides on SUPELCOSIL LC-NH_2 columns by varying the amount of water in the mobile phase. Sorbitol and the common mono- and disaccharides of certain fruit and dietetic products are resolved well on SUPELCOSIL LC-NH_2 columns (Figure B). The column also resolves oligosaccharides consisting of 1 to 12 glucose units (DP-1 to DP-12•). Oligosaccharides consisting of 3 and 4 glucose units usually are resolved poorly on other types of HPLC columns, but they were well resolved in beer samples analyzed on SUPELCOSIL LC-NH_2 (Figure C).

Figure B. Food Sugars and Sorbitol

Column: **SUPELCOSIL LC-NH_2, 25cm x 4.6mm ID, 5µm particles**
Cat. No.: **58338**
Mobile Phase: acetonitrile:ethanol:water (75:8:17) (v/v/v)
Flow Rate: 2mL/min
Temp: 30°C
Det.: refractive index
Sample: 20µL (5% w/v each component)



1. Fructose
2. Sorbitol
3. Glucose
4. Sucrose
5. Maltose

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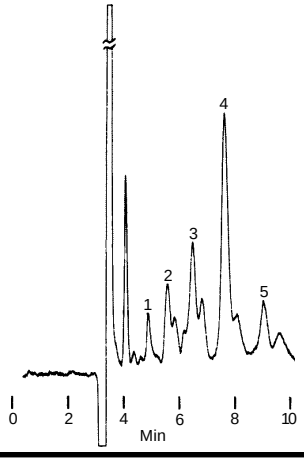
Figure C. Low Molecular Weight Oligosaccharides in Beer

Column: **SUPELCOSIL LC-NH_2, 25cm x 4.6mm ID, 5µm particles**
Cat. No.: **58338**
Mobile Phase: acetonitrile:water (60:40) (v/v)
Flow Rate: 1mL/min
Det.: refractive index
Sample: 10µL of beer



1. Glucose
2. Maltose
3. Maltotriose (DP-3)
4. DP-4
5. DP-5

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In our work with SUPELCOSIL LC-NH_2 columns, we found that buffered mobile phases, which had been suggested for improving monosaccharide peak symmetry (2), in fact did not appreciably improve peak symmetry. Mobile phases buffered to pH 5-6 generally lengthened retention times without improving resolution. In subsequent analyses without buffers, monosaccharide retention times were shorter than values obtained before buffers were used. When boric acid was used to buffer the mobile phase to pH 5, sugars adsorbed irreversibly to the packing. Consequently, we do not recommend buffered mobile phases in monosaccharide analyses.

SUPELCOSIL LC-NH_2 columns contain a five micron spherical packing. In addition to analytical and guard columns, semi-preparative columns filled with this packing are available. For fast, selective LC analyses of mono-, di-, and oligosaccharides, we highly recommend these aminopropyl-bonded phase columns.

Ordering Information:

SUPELCOSIL LC-NH_2 HPLC Columns

Supplied with fittings to connect to 1/16 inch OD stainless steel tubing.

7.5cm x 4.6mm ID, 3µm particles	**58988**
15cm x 4.6mm ID, 3µm particles	**58989**
25cm x 4.6mm ID, 5µm particles	**58338**

Supelguard LC-NH_2 Guard Column,
2cm x 4.6mm ID, 5µm SUPELCOSIL LC-NH_2 packing

Kit, includes one cartridge, holder, connecting hardware	**59558**
Replacement Cartridges, pk. of 2	**59568**

Pelliguard LC-NH_2 Guard Column,
2cm x 4.6mm ID, 40µm pellicular packing

Kit, includes one cartridge, holder, connecting hardware	**59646**
Replacement Cartridges, pk. of 4	**59656**

• Degree of polymerization

References

1. Binder, H., *J. Chromatography*, **189:**414-420 (1980).
2. Rabel, F., et al., *J. Chromatography*, **126:**731-740 (1976).

Acknowledgment

We wish to thank Dr. Eric Coles of the Harvard University Medical School, Boston, for Figure C.