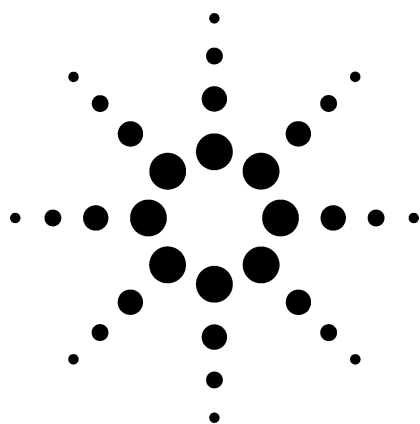




Scale-Up of Anthocyanin Separations and Re-Analysis of Collected Fractions on an Agilent Prep-C18 Column

Application

Biochemical

Cliff Woodward



Anthocyanins, potent anti-oxidants, are now recognized as health important components of many foods. Blueberries, of all natural foods, contain the highest concentrations of these interesting compounds. The purification and identification of various anthocyanins is an important step in understanding which components are most beneficial to human health. Anthocyanins are highly retained on C18 columns; in addition, they require high concentrations of organic solvents for extraction. In attempting to separate large quantities of some of the components for use as standards or for further structural workups, the analyst may encounter solubility issues, which can limit the loadability. The factors affecting loadability of anthocyanins were explored in previous work [1].

The first step in preparative purification is to prove that scale-up works. Once that is known, preparative chromatography is simple. Fractions can be collected and rechromatographed to demonstrate purity. Figure 1 shows the scalability of the Agilent Prep-C18 column. The resolution of the analytical column is, of course, better than the prep column.

Highlights

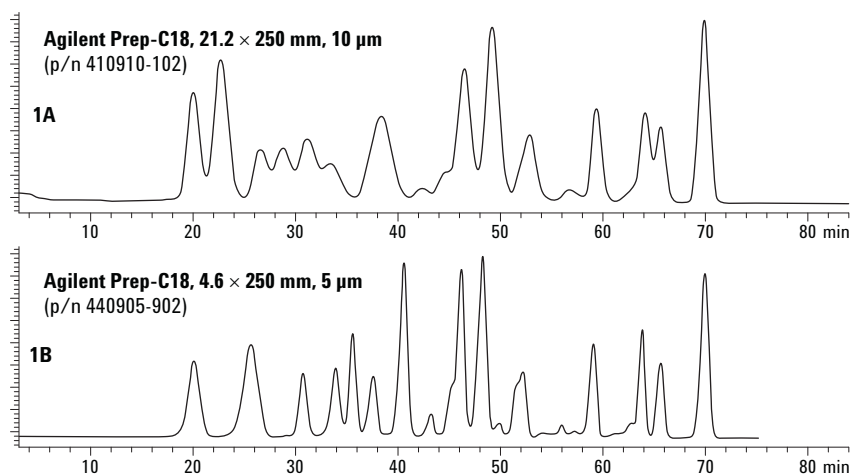
- The Agilent Prep-C18 columns demonstrate excellent scalability, allowing method development on analytical scale columns
- The Agilent Prep-C18 enables high resolution and high purity fractionation
- The Purification software of the Agilent ChemStation combined with the Agilent Prep-C18 makes complex purifications easy



An Agilent Prep-C18 column is shown above with an Agilent 1100 HPLC system.



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1A

Agilent Prep-C18, 21.2 × 250 mm, 10 µ

Temperature: Ambient
 DAD wavelength: 525 nm
 Injection: 2000 µL
 Sample: Blueberry extract, 46.1 mg/mL total dissolved solids
 (~5 mg/mL anthocyanins)
 Flow: 21.2 mL/min

Mobile phase

A = 0.1% TFA in water
 B = 0.1% TFA in methanol

Gradient timetable

Time (min)	% Solvent B
0.00	23.0
35.00	26.0
85.00	53.5

1B

Agilent Prep-C18, 4.6 × 250 mm, 5 µ

Temperature: Ambient
 DAD wavelength: 525 nm
 Injection: 100 µL
 Sample: Blueberry extract, 46.1 mg/mL total dissolved solids
 (~5 mg/mL anthocyanins)
 Flow: 1.0 mL/min

Figure 1. Scalability of Agilent Prep columns.

By using the ChemStation fraction collection software, fractions are obtained that are significantly purer than the separation would seem to allow. See Figure 2. Fraction 1 is >99% pure Delphinidin-3-galactoside and Fraction 2 is >97% pure Delphinidin-3-glucoside. This purity was obtained using the threshold and slope settings of the software to cut the fractions appropriately. Identities of the fractions were verified by liquid chromatography/mass spectrometry (LC/MS) (data not shown).

The conditions for the Agilent Prep-C18 columns below are the same as in Figure 1.

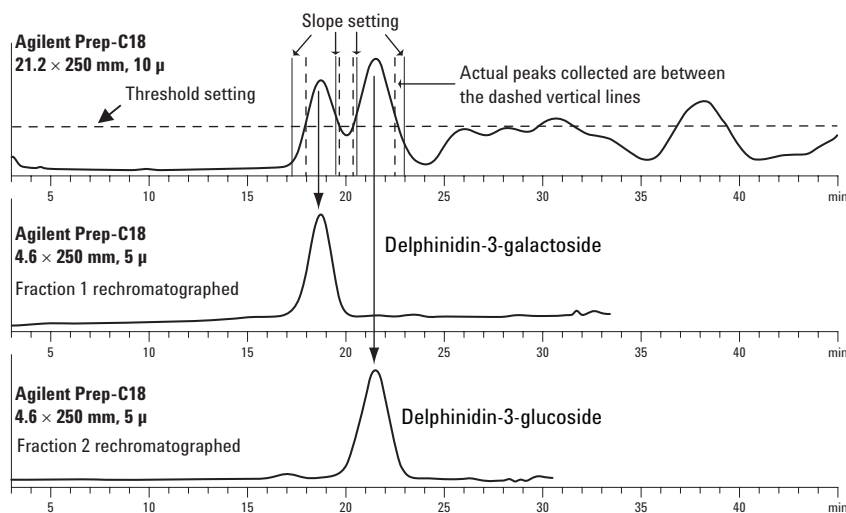


Figure 2. Fraction collection and rechromatography to demonstrate purity.

References

1. "Scalability and Volume Loadability for Highly Retentive Compounds - Anthocyanins", (2004) *Separation Times*, **17**, 1.

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem. Search "Agilent Prep".

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