# Rapid Separation of Anthocyanins in Cranberry and Bilberry Extracts Using a Core-Shell Particle Column

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**Key Words**
Acclaim Column, Accucore Column, Antioxidants, Flavonoids, Polyphenols, Vaccinium Berries

## Introduction

Polyphenols, a large family of compounds, are widely distributed in plants and are the major antioxidants consumed in human diets. The main dietary sources are polyphenol-rich foods and beverages, which include fruits, fruit juices, tea, coffee, and red wine. Studies suggest that diets abundant in fruits and vegetables may provide protection against certain diseases, such as cardiovascular disease and cancer.

Flavonoids are a subclass of polyphenols that includes anthocyanins, anthocyanidins, proanthocyanidins, and catechins. Anthocyanins are powerful antioxidants that are responsible for the red, orange, and blue coloration in fruits and flowers. Major anthocyanin sources include blueberries, pomegranates, and cranberries. The consumption of cranberries is reported to provide various health benefits, such as the prevention of urinary tract infections, stomach ulcers, and dental caries.[1] Therefore, these products are some of the fastest growing herbal dietary supplements in the U.S. Determination of anthocyanins is also useful in authenticating fruits and fruit juices.[2, 3]

High-performance liquid chromatography (HPLC) columns with fully porous 2.2 µm and solid core-porous shell particles are two of the newer developments in separation science. Both particle types deliver separation efficiencies higher than traditional packing materials (i.e., fully porous particles of 3–5 µm nominal diameter). The fully porous 2.2 µm particles are designed to increase peak efficiency, but at the expense of increased system pressure requirements. Core-shell particles improve mass-transfer kinetics by restricting intraparticle diffusion to the thin porous shell while maintaining the hydraulic permeability associated with the total particle diameter. Core-shell particles also have the advantage of having a narrow size distribution compared to fully porous 2.2 µm particles, which come in broader size distributions because traditional sizing methods are ineffective for these particles.[4-7]

This study compares the separation of anthocyanins in cranberry and bilberry extracts using a Thermo Scientific™ Accucore™ C18 HPLC Column and an Acclaim™ Rapid Separation LC (RSLC) 120, C18 Analytical Column. The Acclaim 120, C18 column uses a fully porous 2.2 µm particle designed for fast separations. The Accucore C18 column uses 2.6 µm diameter particles with a solid core and a porous shell to generate high-speed, high-resolution separations without excessive backpressure. The methods developed with these columns are ideal for simple and rapid determination of anthocyanins in natural products.

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## Goal

Develop a method for anthocyanin separations of cranberry and bilberry extracts on an Accucore C18 column to increase speed and reduce solvent consumption per analysis as compared to a previously developed method using an Acclaim 120, C18 column; then compare the methods.

## Equipment

- Thermo Scientific Dionex™ UltiMate™ 3000 RSLC system, including:
  - SRD-3600 Integrated Solvent and Degasser Rack, 6 Channels (P/N 5035.9230)
  - EO Eluent Organizer, including pressure regulator and 2 L glass bottles for each pump (eluents maintained under nitrogen headspace, 5–8 psi)
  - HGP-3400RS Binary Pump with Solvent Selector Valves (P/N 5040.0046)
  - WPS-3000TRS Wellplate Sampler, Thermostatted (P/N 5840.0020)
  - Sample Loop, 25 µL (P/N 6820.2415)
  - TCC-3000RS Thermostatted Column Compartment (P/N 5730.0000)
  - DAD-3000RS Diode Array Detector (P/N 5082.0020)
  - Semi-Micro Flow Cell for DAD-3000 and MWD-3000 Series, SST, 2.5 µL Volume, 7 mm Path Length (P/N 6082.0300)

## Consumables

- Accucore C18, 2.6 µm, 2.1 × 150 mm Column (P/N 17126-152130)
- Acclaim 120, C18, 2.2 µm, Analytical, 2.1 × 150 mm Column (P/N 071399)
- Centrifuge equipped with a ten-place, aluminum fixed-angle rotor
- Thermo Scientific Dionex Viper™ UHPLC Fingertight Fitting incl. Capillary for 10-32 Fitting, ID 0.13 mm/0.005", Length 250 mm, SST (P/N 6040.2325)
- Viper UHPLC Fingertight Fitting incl. Capillary for 10-32 Fitting, ID 0.13 mm/0.005", Length 350 mm, SST (P/N 6040.2335)
- Viper UHPLC Fingertight Fitting incl. Capillary for 10-32 Fitting, ID 0.18 mm/0.007", Length 450 mm, SST (P/N 6040.2365)
- Mixer Kit, mixing volume: 400 µL (P/N 6040.5310)
- Vial Kit, 1.5 mL, Glass with Caps and Septa (P/N 055427)
- Borosilicate glass scintillation vials with closures attached, 20 mL (VWR® P/N 66022-129)

## Reagents and Standards

- Deionized (DI) water, 18 MΩ-cm resistance or better, filtered through a 0.2 µm filter immediately before use
- Acetonitrile, HPLC Grade (Fisher Scientific P/N A9984)
- Hydrochloric acid, ACS Grade (Fisher Scientific P/N A144-500)
- Phosphoric acid, ACS grade (Fisher Scientific P/N A242-1)
- Methanol, HPLC Grade (Fisher Scientific P/N A542-4)
- Formic Acid, ACS grade (Fisher Scientific P/N A118P-500)
- Delphinidin 3-Glucoside (Dp3Glu, Cerilliant® P/N PHY89627)
- Delphinidin 3-Galactoside (Dp3Gal, Cerilliant P/N PHY89506)
- Cyanidin 3-Galactoside (Cy3Gal, Cerilliant P/N C-070)
- Cyanidin 3-Glucoside (Cy3Glu, Cerilliant P/N PHY89616)
- Peonidin 3-Galactoside (Peo3Gal, Cerilliant P/N P-058)
- Malvidin 3-Galactoside (Mal3Gal, Cerilliant P/N PHY80600)
- Peonidin 3-Arabinoside (Peo3Ara, Cerilliant P/N 82247)
- 15 Monoglycosides (Anthocyanins) from Bilberry—includes 5 Anthocyanidins (Polyphenols P/N 1826)
- Cyanidin Chloride (Cerilliant P/N PHY80022)
- Malvidin Chloride (Cerilliant P/N PHY80083)
- Peonidin Chloride (Cerilliant P/N PHY80085)
- Delphinidin Chloride (Cerilliant P/N PHY89625)

*Note: The anthocyanidin standards were only used to confirm retention times.*

## Samples

- Cranberry Extract provided by the National Institute of Standards and Technology (NIST)
- Bilberry Extract provided by NIST

## Conditions

**Acclaim 120, C18 Column Conditions**

| | | | | |
|---|---|---|---|---|
| Column: | Acclaim 120, C18, 2.2 µm Analytical (2.1 × 150 mm) | | | |
| Eluents: | A: 10% Formic Acid<br>B: 10% Formic Acid, 22.5% Methanol, 22.5% Acetonitrile | | | |
| Flow Rate: | 0.475 mL/min | | | |
| Inj. Volume: | 2.0 µL | | | |
| Tray Temp: | 4 °C | | | |
| Column Temp: | 35 °C | | | |
| Detection: | Absorbance, vis, 520 nm | | | |
| System Backpressure: | ~6700–7400 psi during the gradient | | | |
| Gradient Conditions: | Time (min) | A % | B % | Comment |
| | 0 | 91 | 9 | Isocratic |
| | 12 | 91 | 9 | Gradient |
| | 25 | 65 | 35 | — |
| | 25 | 50 | 50 | Step Change |
| | 30 | 50 | 50 | — |
| | 30 | 91 | 9 | Step Change |
| | 35 | 91 | 9 | Equilibration |

**Accucore C18 Column Conditions for Cranberry Extract Analysis**

| | | | |
|---|---|---|---|
| Column: | Accucore C18, 2.6 µm (2.1 × 150 mm) | | |
| Eluents: | A: 10% Formic Acid<br>B: 10% Formic Acid, 22.5 % Methanol, 22.5% Acetonitrile | | |
| Flow Rate: | 0.65 mL/min | | |
| Inj. Volume: | 2.0 µL | | |
| Tray Temp: | 4 °C | | |
| Column Temp: | 42 °C | | |
| Detection: | Absorbance, vis, 520 nm | | |
| System Backpressure: | ~7800 psi during the gradient | | |
| Run Conditions: | Time (min) | A % | B % |
| | 0 | 90 | 10 |
| | 15 | 90 | 10 |

**Accucore C18 Column Conditions for Bilberry Extract Analysis**

| | | | | |
|---|---|---|---|---|
| Column: | Accucore C18, 2.6 µm (2.1 × 150 mm) | | | |
| Eluents: | A: 10% Formic Acid<br>B: 10% Formic Acid, 22.5 % Methanol, 22.5% Acetonitrile | | | |
| Flow Rate: | 0.70 mL/min | | | |
| Inj. Volume: | 2.0 µL | | | |
| Tray Temp: | 4 °C | | | |
| Column Temp: | 35 °C | | | |
| Detection: | Absorbance, vis, 520 nm | | | |
| System Backpressure: | ~7800 psi during the gradient | | | |
| Gradient Conditions: | Time (min) | A % | B % | Comment |
| | 0 | 98.5 | 1.5 | Isocratic |
| | 0.5 | 98.5 | 1.5 | — |
| | 0.5 | 85 | 15 | Step Change |
| | 10.5 | 85 | 15 | — |
| | 10.5 | 50 | 50 | Step Change |
| | 13 | 98.5 | 1.5 | Gradient |
| | 15 | 98.5 | 1.5 | Equilibration |

## Preparation of Solutions and Reagents

### 10% Formic Acid

Transfer 200 mL of formic acid into a 2 L glass volumetric flask containing ~1700 mL of DI water. Bring to volume using DI water and invert flask several times to mix.

### 10% Formic Acid, 22.5% Methanol, 22.5% Acetonitrile

Transfer 200 mL of DI water into a 1 L glass volumetric flask, then add 100 mL of formic acid. Transfer 225 mL of methanol and 225 mL of acetonitrile to the flask. Invert the flask several times to mix the contents and bring to volume using DI water.

### Acidified Methanol (2% Hydrochloric Acid in Methanol)

Transfer 20 mL of hydrochloric acid to 800 mL of methanol in a glass 1 L volumetric flask. Allow the mixture to cool, invert to mix several times, and bring to volume using methanol.

### 10% Phosphoric Acid

Transfer 100 mL of phosphoric acid to 800 mL of DI water in a glass 1 L volumetric flask. Invert to mix several times and bring to volume using DI water.

### 100 µg/mL Standards

Prepare each anthocyanin standard by accurately weighing 1 mg of solid into a 100 mL glass volumetric flask and adding 2 mL of acidified methanol followed by ~90 mL of 10% phosphoric acid. Invert several times to dissolve completely and bring to volume using 10% phosphoric acid. Prepare standards for Dp3Gal, Dp3Glu, Cy3Gal, Cy3Glu, Peo3Gal, Mal3Gal, and Peo3Ara using this procedure. The stock solids can be stored at -40 °C in glass vials until needed. All standard concentrate solutions can be stored for up to six months at -40 °C and protected from light.

### Working Standards and Standards for Method Linearity

To prepare working standards, use a calibrated pipette to deliver the appropriate volume of the 1 mg/mL stock standard into a glass vial containing the appropriate volume of 10% formic acid in water. To prepare mixed anthocyanin working standards, combine appropriate volumes of the stock-mixed anthocyanin standard into a glass vial containing the appropriate volume of mobile phase A. Diluted intermediate standards are stable for three months at -40 °C and working and mixed standards are stable for four weeks at 2–4 °C.

## Sample Preparation

To prepare samples, accurately weigh 125 mg of the solid stored in the glass vial and add 1 mL of acidified methanol. Mix by vortexing to dissolve and add 4 mL of 10% phosphoric acid. Prior to analysis, filter the sample using a 0.2 µm cellulose acetate sterile syringe filter.

## Results and Discussion

The analysis of botanical supplements is challenging due to the complexity of the sample matrices and the lack of consensus in the dietary supplement community regarding the best analytical approach. In 2007, NIST initiated the Dietary Supplements Quality Assurance Program (DSQAP) to improve the accuracy of measurements in the dietary supplements community. The program includes the measurements of nutritional elements, marker compounds, contaminants, and fat- and water-soluble vitamins in foods, botanical supplement ingredients, and finished products. As part of a DSQAP study with NIST, the anthocyanin content in cranberry powder was determined using an Acclaim 120, C18 column.

### Sample Analysis Using Acclaim 120, C18 Column

The cranberry powder provided by NIST was first analyzed for its anthocyanin concentrations using an Acclaim 120, C18 column. Figure 1 shows the separation of the anthocyanins in cranberry extract using a method previously developed to determine anthocyanins in a bilberry extract. The total run time for the Acclaim 120, C18 column separation is 35 min. The Accucore C18 column was then investigated to reduce the run time and solvent consumption. This column decreased the run time from 35 min to 15 min and therefore was used throughout this study (Figure 2).

| | |
|---|---|
| Column: | Acclaim 120, C18, 2.2 µm, Analytical (2.1 × 150 mm) |
| Eluent: | A: 10% Formic Acid |
| | B: 10% Formic Acid, 22.5% Methanol, 22.5% Acetonitrile |
| Gradient: | 0.0–12.0 min, 9% B |
| | 12.0–25.0 min, 35% B |
| | 25.0–50% B Step change |
| | Hold at 50% B for 5 min |
| | 0.0–35.0 min, 9% B |
| Flow Rate: | 0.475 mL/min |
| Inj. Volume: | 2.0 µL |
| Temperature: | 35 °C |
| Detection: | Absorbance, vis, 520 nm |
| Sample: | 25 mg/mL Cranberry Extract |

| Peaks: | Conc. (µg/mL) | Peaks: | Conc. (µg/mL) |
|---|---|---|---|
| 1. Dp3Gal | 0.019 | 6. Peo3Gal | 0.018 |
| 2. Dp3Glu | 0.029 | 7. Peo3Glu | — |
| 3. Cy3Gal | 0.080 | 8. Mal3Gal | 0.070 |
| 4. Cy3Glu | 0.038 | 9. Peo3Ara | 0.160 |
| 5. Cy3Ara | — | | |

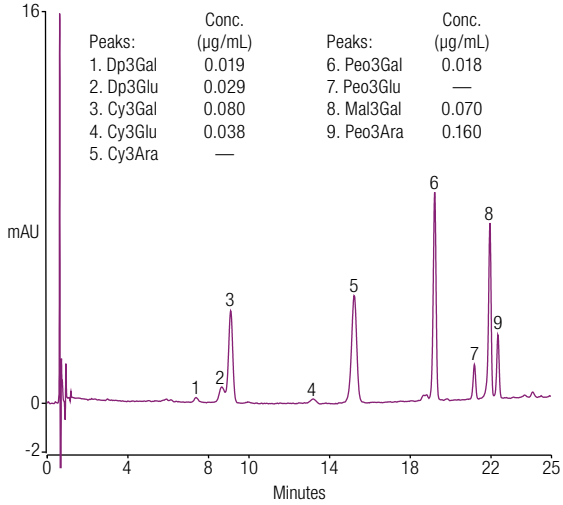


Figure 1. Separation of anthocyanins in cranberry powder using an Acclaim 120, C18 column.

## System Suitability

The linearity, limits of detection (LOD), and limits of quantification (LOQ) were evaluated to determine the suitability of the method for this analysis using an Accucore C18 Column. To determine the appropriate calibration ranges for the target compounds, the sample was analyzed and compared to a mixed anthocyanin standard. The anthocyanins Dp3Gal, Dp3Glu, Cy3Gal, Cy3Glu, Peo3Gal, Peo3Ara, and Mal3Gal showed a linear peak area response in the ranges chosen and produced coefficients of determination between 0.9991–0.9998 (Table 1). The LODs for the anthocyanins were determined based on the concentration of the analyte that provides a peak height of 3× the measured noise (signal-to-noise ratio [S/N] = 3), while the LOQs were determined as the concentration of the analyte that provides a peak height of 10× the measured noise (S/N = 10). The LODs ranged from 0.1 µg/mL for Dp3Gal to 1.56 µg/mL for Peo3Ara and the LOQs ranged from 0.28 µg/mL for Dp3Gal to 3.12 µg/mL for Peo3Ara.

Table 1. Data for linearity, LOD, and LOQ of anthocyanins in cranberry powder.

| Analyte | Range (µg/mL) | Coefficient of Determination ($r^2$) | LOD (µg/mL) | LOQ (µg/mL) |
|---|---|---|---|---|
| Dp3Gal | 0.3–12.5 | 0.9994 | 0.10 | 0.28 |
| Dp3Glu | 0.8–50.0 | 0.9992 | 0.20 | 0.78 |
| Cy3Gal | 1.6–50.0 | 0.9997 | 0.80 | 1.56 |
| Cy3Glu | 0.8–50.0 | 0.9996 | 0.26 | 0.78 |
| Peo3Gal | 1.6–50.0 | 0.9995 | 0.78 | 1.56 |
| Peo3Ara | 3.5–50.0 | 0.9998 | 1.56 | 3.12 |
| Mal3Gal | 0.8–50.0 | 0.9991 | 0.20 | 0.78 |

## Sample Analysis

Dionex (now part of Thermo Scientific) Application Note (AN) 281 describes a sensitive and accurate HPLC method to determine anthocyanins in bilberry-based products and nutritional supplements using a high-resolution silica-based 2.2 µm, Acclaim 120, C18 column and a wavelength of 520 nm. This study evaluates the Accucore C18 column to separate anthocyanins and anthocyanidins in bilberry extract.

The samples here were prepared as described in AN 281.[8] Figure 2 shows the separation of a standard mixture containing 15 anthocyanins and five anthocyanidins derived from bilberries in less than 15 min. Although several anthocyanin pairs are not baseline resolved, the separation is sufficient to perform a qualitative analysis that can be used to authenticate bilberry-based products.

| | |
|---|---|
| Column: | Accucore C18, 2.6 µm, (2.1 × 150 mm) |
| Eluent: | A: 10% Formic Acid |
| | B: 10% Formic Acid, 22.5% Methanol, 22.5% Acetonitrile |
| Gradient: | 0.0–0.5 min, 1.5% B |
| | Step change 0.5 min, 15% B |
| | 0.5–10.5 min, 15% B |
| | 10.5–13.0 min, 50% B |
| | 13.0–15.0 min, 1.5% B |
| Flow Rate: | 0.7 mL/min |
| Inj. Volume: | 2.0 µL |
| Temperature: | 35 °C |
| Detection: | Absorbance, vis, 520 nm |
| Sample: | 125 µg/mL 15 Anthocyanins and 5 Anthocyanidins Standard |

Peaks:

| | | | |
|---|---|---|---|
| 1. Dp3Gal | 6. Pet3Gal | 11. Pet3Ara | 16. Mal3Glu |
| 2. Dp3Glu | 7. Cy3Ara | 12. Peo3Glu | 17. Mal3Ara |
| 3. Cy3Gal | 8. Delphinidin | 13. Mal3Gal | 18. Petunidin |
| 4. Dp3Ara | 9. Pet3Glu | 14. Peo3Ara | 19. Peonidin |
| 5. Cy3Glu | 10. Peo3Gal | 15. Cyanidin | 20. Malvidin |

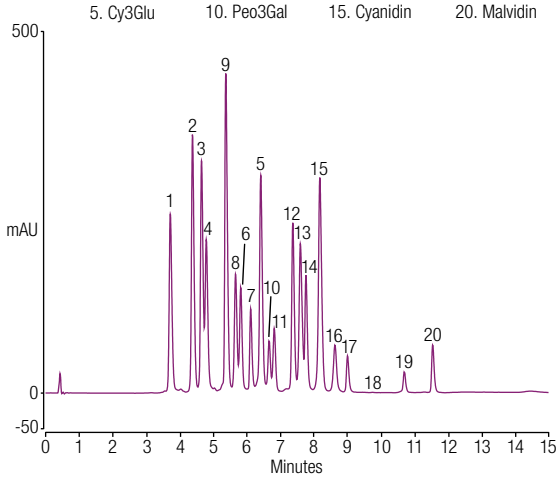


Figure 2. Separation of 15 anthocyanins and 5 anthocyanidins standard using an Accucore C18 column.

If baseline separation of all peaks is desired, then the 35 min separation shown in AN 281 (Figure 3) using an Acclaim 120, C18 column can be used.

| | |
|---|---|
| Column: | Acclaim 120, C18, 2.2 µm, Analytical (2.1 × 150 mm) |
| Eluent: | A: 10% Formic Acid |
| | B: 10% Formic Acid, 22.5% Methanol, 22.5% Acetonitrile |
| Gradient: | 0.0–12.0 min, 9% B |
| | 12.0–25.0 min, 35% B |
| | 25.0–30.0 min, 50% B |
| | 30.0–35.0 min, 9% B |
| Flow Rate: | 0.475 mL/min |
| Inj. Volume: | 2.0 µL |
| Temperature: | 35 °C |
| Detection: | Absorbance, vis, 520 nm |
| Standard: | 15 Anthocyanins and 5 Anthocyanidins |

| Peaks: | Conc. (µg/mL) | Peaks: | Conc. (µg/mL) |
|---|---|---|---|
| 1. Dp3Gal | — | 11. Pet3Ara | — |
| 2. Dp3Glu | 20.1 | 12. Peo3Glu | — |
| 3. Cy3Gal | 11.0 | 13. Mal3Gal | 8.76 |
| 4. Dp3Ara | — | 14. Peo3Ara | 12.7 |
| 5. Cy3Glu | 12.3 | 15. Cyanidin | — |
| 6. Pet3Gal | — | 16. Mal3Glu | — |
| 7. Cy3Ara | — | 17. Mal3Ara | — |
| 8. Delphinidin | — | 18. Petunidin | — |
| 9. Pet3Glu | — | 19. Peonidin | — |
| 10. Peo3Gal | 3.91 | 20. Malvidin | — |

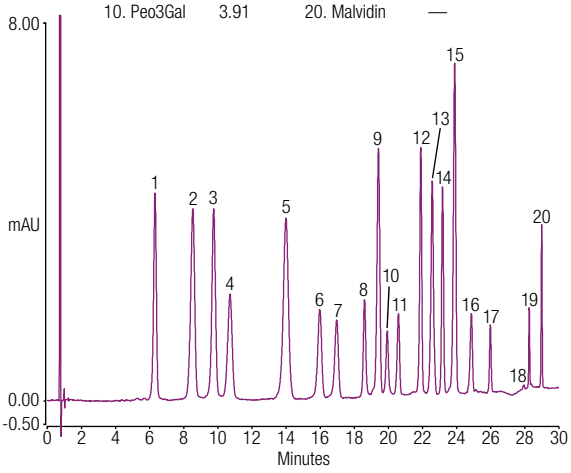


Figure 3. Separation of anthocyanins in bilberry extract standard using an Acclaim 120, C18 column.

A NIST sample of cranberry extract was prepared as described in the Sample Preparation section to determine anthocyanin concentrations. Figure 4 shows the separation of anthocyanins in the cranberry extract provided by NIST. Table 2 compares anthocyanin concentrations determined in the NIST sample to the average concentrations determined by the NIST collaborative study using the Accucore C18 column method. As shown, the concentrations for all the anthocyanins in the cranberry sample were consistent with the average values reported by NIST. The individual anthocyanin concentrations for the NIST sample ranged from 0.021 mg/g for Dp3Gal to 0.176 mg/g for Peo3Gal compared to the average reported values of 0.019 mg/g for Dp3Gal and 0.20 mg/g for Peo3Gal. An unknown peak coeluted with Cy3Gal, which made integration of the Cy3Gal challenging. Therefore, the average concentration for Cy3Gal was

Table 2. Comparison of experimentally determined anthocyanin values in cranberry extract to the average values determined by the NIST collaborative study.

| Analyte | Experimental Values (mg/g) | Average Values Reported by the Collaborative Study (mg/g) |
|---|---|---|
| Dp3Gal | 0.021 ± 0.01 | 0.019 ± 0.029 |
| Dp3Glu | 0.037 ± 0.05 | 0.057 ± 0.062 |
| Cy3Gal | 0.09 ± 0.04 | 0.13 ± 0.039 |
| Cy3Glu | 0.041 ± 0.07 | 0.059 ± 0.078 |
| Peo3Gal | 0.176 ± 0.02 | 0.20 ± 0.043 |
| Peo3Ara | 0.17 ± 0.02 | 0.15 ± 0.048 |
| Mal3Gal | 0.088 ± 0.05 | 0.06 ± 0.087 |

| | |
|---|---|
| Column: | Accucore C18, 2.6 µm, Analytical (2.1 × 150 mm) |
| Eluent: | A: 10% Formic Acid |
| | B: 10% Formic Acid, 22.5% Methanol, 22.5% Acetonitrile |
| Gradient: | 0.0–15.0 min, 10% B |
| Flow Rate: | 0.65 mL/min |
| Inj. Volume: | 2.0 µL |
| Temperature: | 42 °C |
| Detection: | Absorbance, vis, 520 nm |
| Sample: | 25 mg/mL Cranberry Extract |

| Peaks: | Conc. (µg/mL) | Peaks: | Conc. (µg/mL) |
|---|---|---|---|
| 1. Dp3Gal | 0.021 | 6. Cy3Ara | 0.157 |
| 2. Dp3Glu | 0.037 | 7. Peo3Gal | 0.176 |
| 3. Unknown | — | 8. Peo3Glu | — |
| 4. Cy3Gal | 0.090 | 9. Peo3Ara | 0.170 |
| 5. Cy3Glu | 0.041 | 10. Mal3Gal | 0.088 |

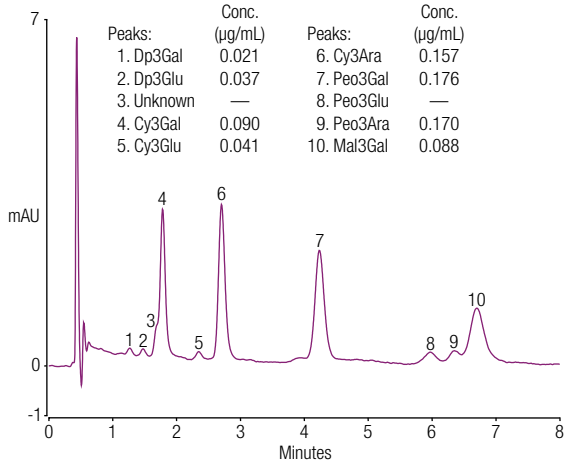


Figure 4. Separation of anthocyanins in cranberry extract using an Accucore C18 column.

0.09 mg/g, which was lower than the average concentration reported by the NIST collaborative study but still within the NIST standard deviation of ±0.039 mg/g. These results agree with the NIST values and therefore suggest that this method is accurate for the determination of anthocyanins in cranberry powder.

### Sample Precision

Three independent preparations of cranberry extract were analyzed in triplicate over three days to evaluate method precision. Table 3 summarizes the results from this analysis. For the samples analyzed in this study, the intraday peak area RSDs ranged from 1.01% for Cy3Glu to 1.31% for Peo3Gal. The between-day peak area RSDs ranged from 1.26% for Cy3Glu to 1.87% for Peo3Ara. These data suggest good method precision.

Table 3. Analysis of three independent preparations of cranberry extract for intraday and between-day precision.

| Analyte | Amount (mg/g) | Intraday Precision RSD | Between-Day Precision |
|---|---|---|---|
| | | Peak Area (n = 3) | Peak Area (n = 3 over three days) |
| Dp3Gal | 0.023 | 1.08 | 1.55 |
| Dp3Glu | 0.035 | 1.17 | 1.43 |
| Cy3Gal | 0.089 | 1.15 | 1.46 |
| Cy3Glu | 0.045 | 1.01 | 1.34 |
| Peo3Gal | 0.176 | 1.31 | 1.36 |
| Peo3Ara | 0.168 | 1.22 | 1.87 |
| Mal3Gal | 0.087 | 1.09 | 1.65 |

## Conclusion

This study describes two simple, sensitive, rapid, and accurate methods to separate and quantify anthocyanins in cranberry extract with a simple solvent extraction using the Acclaim 120, C18 and Accucore C18 columns. This work also describes a method that uses an Accucore column to separate anthocyanins in bilberry extract, which is a complex sample with 15 anthocyanins and five anthocyanidins. Although the Acclaim 120, C18 column can be used to quantify anthocyanins in both cranberry and bilberry extracts, the Accucore C18 column provides a qualitative analysis of bilberry extract and a quantitative analysis of cranberry extract with a 20 min time savings. Anthocyanin concentrations in a cranberry sample provided by NIST were determined using the Accucore method, and the values reported are in agreement with the range of values reported by the collaborative study. Thus, the methods described here are ideal for routine screening and quantification of anthocyanins in many natural products. The Accucore column used for this analysis allows for a faster and lower solvent-consumption analysis (35–40% less) per sample.

## Suppliers

Sarstedt Inc., 1025, St. James Church Road, P.O. Box 468, Newton NC 28658-0468, U.S.A., Tel: 828-465-4000.

Praxair Specialty Gases and Equipment, 39 Old Ridgebury Road, Dansbury, CT 06810-5113, U.S.A., Tel: 877-772-9247.

Cerilliant Corp. (a Sigma-Aldrich Company), 811 Paloma Drive, Suite A, Round Rock, TX 78665, U.S.A., Tel: 800-848-7837.

Polyphenols Laboratories AS, Hanaveien 4-6, 4327, Sandnes, Norway, Tel: +47-4695-3900, www.polyphenols.com.

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