Determination of Organic Acids and Anthocyanins in Cranberry Extract

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Introduction

The dietary supplement industry is a rapidly growing market segment, with U.S. sales estimated at approximately $20 billion annually.[1] Approximately 50% of the U.S. population claim that they regularly use dietary supplements, as more health-conscious consumers seek complementary and alternative medical treatments. The primary mission of the Office of Dietary Supplements (ODS) at the National Institutes of Health (NIH) is to promote the quality, safety, and efficacy of dietary supplements.[2] To accomplish this mission, authentic reference materials that closely match the matrix components of the dietary supplements are needed.

Standard Reference Materials (SRMs) for vaccinium (e.g., cranberries, blueberries, and bilberries) are being developed at the National Institute of Standards and Technologies (NIST) in collaboration with the NIH-ODS to evaluate these types of dietary supplements.[3] Several SRMs with certified values for organic acids are currently available from NIST to aid dietary supplement and juice manufacturers in their analytical method development and QA/QC operations.[4] Further work to certify anthocyanins and anthocyanidins is under way.

The popularity of cranberries is primarily associated with their reported ability to combat urinary tract infection and their high organic acid, anthocyanin, and polyphenol content. The primary health benefits of cranberries are derived from their antioxidant properties, and their anthocyanin profiles are important in determining good quality and authenticity of the products. Cranberry extracts are prone to adulteration with lower value products in order to offer consumers competitive prices, therefore making it important to ensure the quality, safety and efficacy of these products.

Organic acids present in cranberries impart flavor, and their specific ratios can be used to detect adulteration.

Here we present two methods to determine organic acids and anthocyanins in cranberry extract. Method A demonstrates the determination of organic acids in cranberry extract using a hydroxide-selective anion-exchange column with suppressed conductivity detection. Method B demonstrates the determination of anthocyanins present in cranberry extract using a core-shell C18 column and detection at 540 nm. The linearity, limits of detection (LODs), limits of quantification (LOQs), and accuracy of each method for determining the target compounds in these products will be reported.

Methods

Method A: Determination of Organic Acids

A Thermo Scientific Dionex ICS-5000 Ion Chromatography (IC) system was used for determination of organic acids. For preparation of samples for the determination of organic acids refer to Dionex (now part of Thermo Scientific) Application Brief (AB)112.[5]

Method B: Determination of Anthocyanins

A Thermo Scientific Dionex UltiMate 3000 Rapid Separation Liquid Chromatography (RSLC) system was used for this study. For preparation of solutions, standards, and samples and the individual components of the system used, refer to Dionex (now part of Thermo Scientific) Application Note (AN) 281.[6]

Table 1: Method conditions.

	Method A Determination of Organic Acids	Method B Determination of Anthocyanins
Columns	Thermo Scientific Dionex IonPac AG11-HC and Dionex IonPac™ AS11-HC, 2 mm	Thermo Scientific Accucore, C18, 2.6 µm Analytical, 2.1 150 mm
Mobile Phases	KOH delivered using a Thermo Scientific Dionex EGC II KOH with Thermo Scientific Dionex CR-ATC Continuously-Regenerated Anion Trap Column	A: 10% formic acid B: 10% formic acid, 22.5% methanol, 22.5% acetonitrile
Gradient Conditions	-8.0 to 8.0 min-1 mM KOH 8.0 to 20.0 min 1–30 mM KOH 20.0 to 30.0 min 30–60 mM KOH 30.0 to 45.0 min 60 mM KOH	0–15 min 10% B (Isocratic)
Total Run Time	45 min	15 min
Flow Rate	0.38 mL/min	0.65 mL/min
Column Temperature	30 ºC	42 ºC
Detection	Suppressed conductivity via a Thermo Scientific Dionex ASRS 300 Anion Self-Regenerating Suppressor, 2 mm, recycle mode, 57 mA	Vis via a Thermo Scientific Dionex DAD-3000RS Diode Array Detector at 520 nm
Injection Volume	5 µL	2 µL

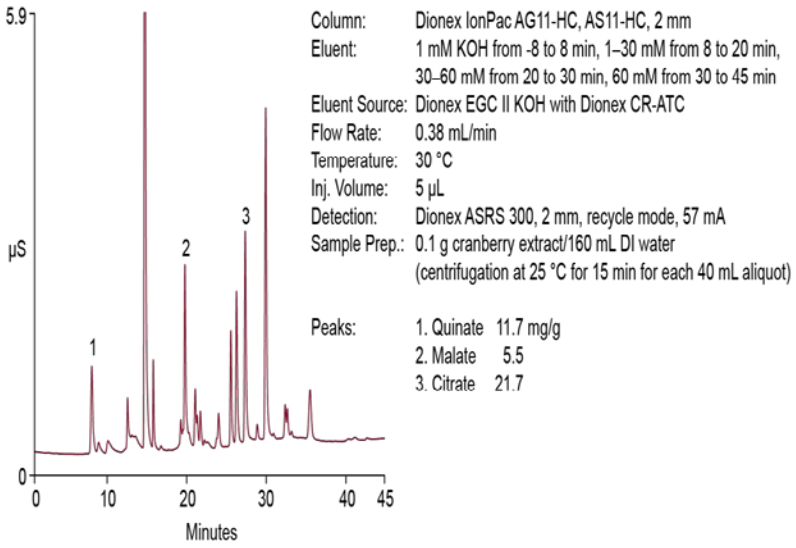
Results

This study demonstrates two methods: an IC method to determine organic acids and an HPLC method to determine anthocyanins in cranberry extract.

Method A: Determination of Organic Acids

The chromatogram in Figure 1 demonstrates the determination of quinic, malic, and citric acids in cranberry extracts using a Dionex IonPac AS11-HC column with suppressed conductivity detection. Although the primary benefits of the Vaccinium species are derived from their antioxidant properties, the relative amounts of organic acids can significantly influence the characteristics of the berries. For example, organic acids are known to impart unique flavors and the specific ratios in fruit juices can be used to detect adulteration. In addition, organic acids are often used to control pH and can be an indicator of product quality.

Figure 1: Separation of organic acids in cranberry powder using Method A.



Method B: Determination of Anthocyanins

Method Performance

The linearity, LOD, and LOQ were evaluated for this analysis. All the anthocyanins showed a linear peak response in the ranges chosen and produced correlation coefficients between 0.9991– 0.9998 (Table 2). 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.

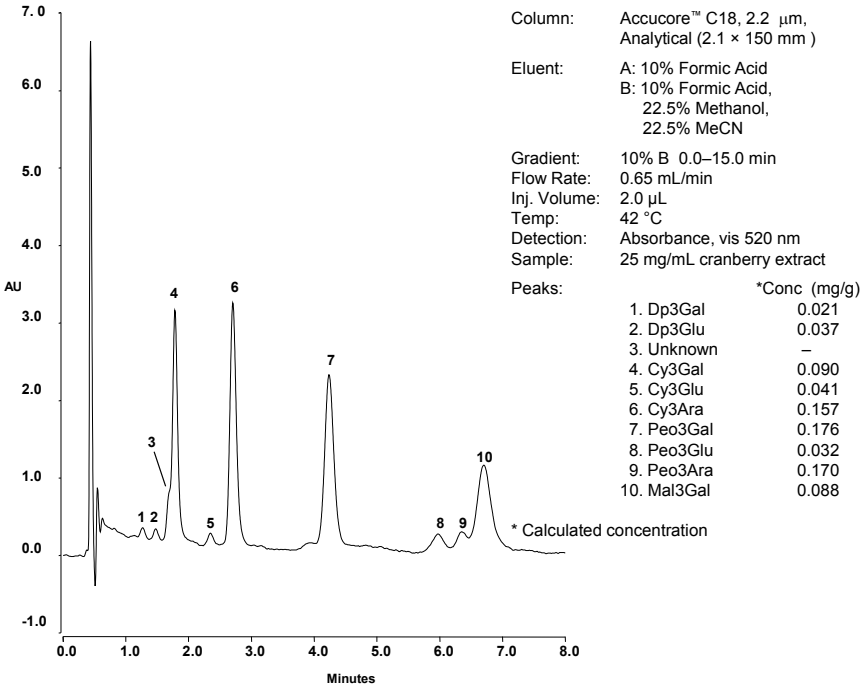
Table 2: Data for LODs and LOQs of anthocyanins in cranberry.

Analyte	Range µg/mL	Correlation Coefficient r^2	LOD (µg/mL)	LOQ (µg/mL)
Dp3Gal	0.3–12.5	0.9994	0.1	0.28
Dp3Glu	0.8–50.0	0.9992	0.2	0.78
Cy3Gal	1.6–50.0	0.9997	0.8	1.56
Cy3Glu	0.8–50.0	0.9996	0.26	0.78
Peo3Gal	1.6–50.0	0.9995	0.78	1.56
Peo3Glu	0.6–50.0	0.9993	0.2	0.56
Peo3Ara	3.5–50.0	0.9998	1.56	3.12
Mal3Gal	0.8–50.0	0.9991	0.2	0.78

Sample Analysis

Figure 2 shows the separation of anthocyanins in cranberry powder investigated in this study. Ten different anthocyanins were identified in this sample, but only nine were quantified due to the availability of standards for the rest of the anthocyanins. The nine anthocyanins quantified ranged from 0.021 mg/g for Dp3Gal to 0.176 mg/g for Peo3Gal.

Figure 2: Separation of anthocyanins in cranberry powder using Method B.



This study included a total of 14 participating laboratories that submitted results to the NIST. An overall consensus of the average reported concentration was observed, indicating validity of the data and the method.

Table 3. Comparison of the experimentally determined anthocyanin values to the average values determined by the NIST collaborative study.

Analyte	Experimental Values (mg/g) n = 3	Average Values Reported by Collaborative Study (mg/g)
Dp3Gal	0.021 ± 0.01	0.019 ± 0.03
Dp3Glu	0.037 ± 0.05	0.057 ± 0.06
Cy3Gal	0.090 ± 0.04	0.13 ± 0.04
Cy3Glu	0.041 ± 0.07	0.059 ± 0.08
Cy3Ara	0.157 ± 0.03	0.20 ± 0.06
Peo3Gal	0.176 ± 0.02	0.20 ± 0.04
Peo3Glu	0.032 ± 0.07	0.047 ± 0.05
Peo3Ara	0.170 ± 0.02	0.15 ± 0.05
Mal3Gal	0.088 ± 0.05	0.06 ± 0.09

Conclusion

- A method to determine organic acids in cranberry extract was demonstrated using suppressed conductivity detection. This information can be used to determine organic acid ratios in fruit juices to detect adulteration.
- A sensitive, accurate, and rapid method to separate and quantify anthocyanins in cranberry extract following a simple extraction was demonstrated.
- Reported anthocyanin values using the method showed an overall consensus with the average concentrations reported by the interlaboratory NIST study.

References

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