$m0$ = slope of calibration curve for the target analyte
V = volume of test solution, in mL
W = dry weight of sample, in g
D = dilution factor

Individual anthocyanins from liquid samples are quantified in µg/mL using the following equation:

$$\frac{P_0 - b_0}{m_0} \times D$$

The calculations used to determine the Horwitz Ratio (HorRat), a normalized performance parameter used to evaluate overall method precision, are provided below.
RSDr (found, %):

$$RSDr = \frac{SD(r)}{mean} \times 100$$

Where SD(r) = the population standard deviation.

PRSDr (RSDr calc, %):

$$PRSDr = 2C^{-0.5}$$

Where C = the concentration of the analyte expressed as a mass fraction.

4-(dimethylamino)cinnamaldehyde (DMAC) for Quantification of Soluble Proanthocyanidins (PACs) in Cranberry Juice, Concentrated Juice, and Juice Extract Powders

DMAC is one of a few methods used for the quantification of PACs and is only applicable when extracted material has been positively identified as being pure cranberry, because it gives one measure of PAC content (soluble PACs) and cannot differentiate between A and B-type PACs. DMAC is an aromatic aldehyde that reacts with flavan-3-ols and PACs to form a green chromophore with maximum absorbance at approximately 640 nm (Treutter 1989). This wavelength effectively excludes the spectra of anthocyanins, which are a source of interference in other quantifications for PACs. DMAC does not react with hydroxycinnamic acids, hydroxybenzoic acids, flavonones, and flavonols (McMurrough and McDowell 1978; Treutter 1989). DMAC is not an appropriate method for authentication of cranberry or cranberry extract as flavan-3-ols and PACs from any source (cranberry, grape, pine bark, apple, chocolate, peanut skins, etc.) will react with the DMAC reagent.

Some within the cranberry industry use DMAC with a procyanidin A2 dimer reference standard, to standardize the PAC content of extracts and finished products. While DMAC is useful for the quantification of cranberry PACs, there are several limitations to this methodology. DMAC is only capable of quantifying soluble PACs, or PACs that can be readily extracted from cranberry products. Insoluble PACs that remain bound to fruit cell wall constituents such as fiber, pomace, and protein are not quantified by DMAC (Roopchand et al. 2013). Therefore, DMAC quantitation of PACs is most appropriately applied to cranberry juice and cranberry juice powder extracts that contain water-soluble PACs and is not applicable for quantitation of PACs in products such as sweetened-dried cranberries, dried whole cranberry powder, and cranberry pomace (press cake). Because of this, DMAC quantitation results in an underestimation of total PACs (soluble plus insoluble) by approximately half. Additionally, the use of the procyanidin A2 dimer standard is biased toward quantification of PAC oligomers, because these products more closely reflect the structure and reaction kinetics of the A2 dimer (Krueger et al. 2013b). Such methods are designed primarily to ensure consistency of an extract and delivery of a consistent, though not absolute, dose of PACs. For accuracy, it is important to include the A2 reference standard when reporting DMAC results for quantification of PACs in cranberry juice products, as use of different standards will give different PAC levels on the same sample.

The original DMAC method used for quantification of cranberry PACs was published by Prior et al. (2010b) as part of a multi-laboratory validation that included 5 laboratories. The SOPs of the Prior publication are available on the DMAC website www.dmac-asso.org. With the original method, intra-laboratory variation was a mean of 4.1 ± 1.7% RSD (range 2.3–6.1%) and inter-laboratory variability was 16.9 ± 8.5% RSD (range 8–32%). The inter-laboratory variation of almost 17% limited the viability of the original method. For purposes of this monograph, the method of Prior et al. (2010b) was modified by ICT Laboratories (Milford, MA; ICT study number ICT10002; Neotron SpA, Modena, Italy; report 14/10/LRA) for better performance in terms of linearity ($R2 \geq 0.995$), repeatability (relative standard deviation [RSD] ≤ 3%), intermediate precision (RSD ≤ 5%), recovery within 90 to 100%, and robustness (≤3%) for dry cranberry extract. This modified method was validated according to International Conference on Harmonisation (ICH) Guideline Validation of Analytical Procedures: Text and Methodology Q2(R1). Furthermore, a multi-lab validation of this modified method showed an overall RSD of 2.03–3.37 %. Use of this validated method is recommended when DMAC results are desired. In addition to the limitations mentioned, this method requires specialized equipment that can take multiple readings rapidly. Accuracy of DMAC analysis requires strict controls of numerous parameters including development of color reaction.

Experimental Conditions for the Modified DMAC/A2 Method Apparatus: Perkin Elmer Lambda 40 double-beam UV-Visible spectrophotometer, or equivalent.

Reagents

4-dimethylaminocinnamaldehyde-DMAC (reagent grade; Sigma p/n 39421, or equivalent)

Methanol (HPLC grade).

Sulfuric acid (H2S04): reagent grade).

PACs extraction solvent: Methanol.
Reaction Solvent: Carefully measure 12.0 mL of H2S04 into a 50 mL flask. Slowly transfer H_2S0_4 by using a glass Pasteur pipette in a 1 L volumetric flask containing 500 mL of methanol. Add a stir bar and allow to stir at least 30 minutes until the heat generated by addition of H_2S0_4 is completely released. When the temperature of solution reaches room temperature, fill to the volume with methanol and allow stirring for a minimum 10 minutes. Transfer to a glass bottle labeled as 0.4N H_2S0_4 in methanol.

DMAC Solution: Accurately weigh 50 mg of 4-dimethylaminocinnamaldehyde (DMAC) into a 50 mL volumetric flask. Dissolve and dilute to volume with reaction solvent. Prepare fresh and use within 2 hours.

Reference Solutions
Standard stock solution: Prepare a Standard stock solution containing approximately 90 µg/mL of proanthocyanidin A2 (ChromaDex, Boulder, CO) in methanol (for example by weighing approximately 9 mg of proanthocyanidin A2 in 100 mL of methanol).

Prepare 5 reference solutions at different proanthocyanidin A2 concentrations by diluting Standard stock solution with methanol:
Standard 30 µg/mL: pipette 1.0 mL of Standard stock solution into a 15 mL tube and add 2.0 mL of methanol.
Standard 25 µg/mL: pipette 0.75 mL of Standard stock solution into a 15 mL tube and add 1.95 mL of methanol.
Standard 20 µg/mL: pipette 0.5 mL of Standard stock solution into a 15 mL tube and add 1.75 mL of methanol.
Standard 15 µg/mL: pipette 0.5 mL of Standard stock solution into a 15 mL tube and add 2.5 mL of methanol.
Standard 10 µg/mL: pipette 0.25 mL of Standard stock solution into a 15 mL tube and add 2.0 mL of methanol.

Test Solution
In duplicate, accurately weigh about 10 mg of cranberry extract into a 100 mL volumetric flask. Dissolve by sonication and dilute to volume with methanol. If necessary, place on a shaker for 30 minutes at 300 rpm to solubilize and/or extract and centrifuge to clarify the sample.

Procedures and Calculations
Turn on the spectrophotometer and select the reading absorbance at 640 nm.

Pipette 1.0 mL of DMAC solution in a cuvette and place it into the spectrophotometer (cover the top with parafilm). Use this solution as the compensation liquid (reference cuvette in double beam spectrophotometer);

Pipette 1.0 mL of DMAC solution into respective Eppendorf test tubes;

Pipette 0.2 mL of blank (Reaction Solvent), Reference solutions or Test solution(s) into respective Eppendorf test tubes, mix well by vortexing, transfer the solution into the sample cuvette.

Place the cuvette in the spectrophotometer and read the absorbance at 640 nm every 5 seconds for at least 300 seconds.

Record the highest absorbance value of each 300 seconds reading. Subtract the reference reading from the reference cuvette (double beam spectrophotometer should do it automatically). Take the readings within 5 minutes of the addition of the DMAC solution.

Determine regression line in the format y = bx between proanthocyanidin A2 concentration (y) and maximal absorbance at 640 run (x). The correlation factor r2 of the regression curve should be 0.99 (see 11).

From the regression line, calculate the proanthocyanidins % content (PAC%) expressed as proanthocyanidin A2 as follows:

$$PAC\% = \frac{C\ (\mu g/mL)\ x\ V}{P\ (\mu g)}\ x\ 100$$

where:
C (µg/mL) = Proanthocyanidins concentration in µg/mL obtained from the regression line;
V = dilution volume (mL) per l 00 mL.
P (µg) = Sample weight (in micrograms) of the test product.
Express the result as the average of the two determinations.

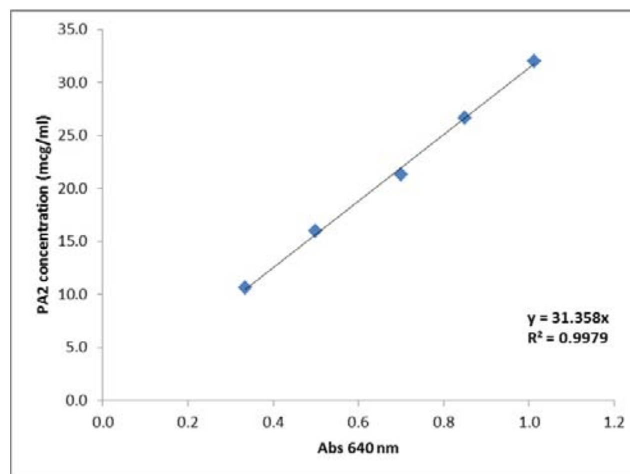

Figure 11 **Typical proanthocyanidin A2 regression line for DMAC analysis**

Limit Tests*

Foreign Organic Matter (fresh):	Free from insects and insect larvae (CFIA 2000). Not to exceed 0.1% infested with worms (USDA 1997).
Foreign Organic Matter (dry):	Not to exceed 3%.†