

Modified Prussian Blue Assay for Total Phenols

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The Price and Butler method as modified by H. D. Graham [*J. Agric Food Chem.* 40, 801-805 (1992)] to give greater color stability. The volumes are also scaled down in this method. We are now using this method.

Reagents

- 0.02 M FeCl₃ in 0.10 M HCl
 1. Dilute concentrated HCl to 0.10 M by bringing 8.3 mL of the concentrated acid to 1 L with distilled water.
 2. Make the ferric chloride by dissolving 3.24 g of anhydrous ferric chloride in 1 L of the 0.10 M HCl. This will make a pale yellow solution.
- 0.016 M K₃Fe(CN)₆ Dissolve 5.26 g of potassium ferricyanide in 1 L of distilled water. This will make a yellow solution.
- Stabilizer (Stable for 1 week in refrigerator). You need 5 mL/sample. 30 mL distilled water, 10 mL 85% H₃PO₄, 10 mL 1% gum arabic.
 1. 85% H₃PO₄ is the typical, commercially available phosphoric acid.
 2. 1% gum arabic is prepared by suspending 1.0 g gum arabic (or gum acacia; Sigma G-9752 or equivalent) in about 80 mL distilled water and boiling the suspension for 25 min.
 3. Vacuum filter the mixture with #1 paper and bring the volume of the filtrate up to 100 mL with distilled water. Refrigerate the solution.

Method

1. Dispense 0.10 mL sample (or a smaller appropriate volume of sample made up to 0.10 mL with sample solvent) into a test tube.
2. Add 3.00 mL distilled (deionized) water and vortex the mixture. Poor quality water, especially iron-containing water, will give high blanks and unacceptable results.
3. To each sample, add 1.00 mL K₃Fe(CN)₆ followed immediately by 1.00 mL FeCl₃ and immediately vortex the mixture. The interval between handling each sample should be approximately 1 min, although exact timing is not as critical as with the Price and Butler method.
4. Fifteen min after adding the reagents to a sample, add 5.00 mL stabilizer to the sample and vortex. Each sample should have a reaction time of 15 min before the stabilizer is added.
5. Read the absorbance at 700 nm; after the addition of stabilizer the colors are stable, so timing is not critical. Include solvent-only blanks, and either "blank" the spec with them or subtract the absorbance of the blank from the absorbance obtained for each sample.
6. Standardize the assay against an appropriate phenolic, for example 0.001 M gallic acid (0.019 g gallic acid monohydrate dissolved in 100.0 mL methanol).
7. The standards must be dissolved in the same solvent that the samples are dissolved in. The values obtained with this method are similar to those obtained with the Price and Butler method, but not identical, probably due to the complexity of the reactions involved in phenolic oxidation and in formation of Prussian blue.