

Prussian Blue Assay for Total Phenols

Ann E. Hagerman © 2002

This is the original method described by Price and Butler [*J. Agric. Food Chem.* 25, 1268-1273 (1977)]. The major difference between the method described here and the method as published is the use of ferric ammonium sulfate instead of ferric chloride as the first reagent. Solubility problems are common with ferric chloride, but are eliminated by using ferric ammonium sulfate. We have recently started to use a modified Prussian blue method.

Reagents

- 0.10 M $\text{FeNH}_4(\text{SO}_4)_2$ 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 ammonium sulfate by dissolving 48.2 g of the dodecahydrate salt ($\text{FeNH}_4(\text{SO}_4)_2 \cdot 12 \text{H}_2\text{O}$) in 1 L of the 0.10 M HCl. This will make a pale yellow solution.
- 0.008 M $\text{K}_3\text{Fe}(\text{CN})_6$ Dissolve 2.63 g of potassium ferricyanide in 1 L of distilled water. This will make a yellow 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 125 mL Erlenmeyer flask. Add 50.0 mL distilled (deionized) water. Poor quality water, especially iron-containing water, will give high blanks and unacceptable results.
2. Add 3.0 mL $\text{FeNH}_4(\text{SO}_4)_2$ and swirl. Additions should be timed; 1 min intervals are convenient.
3. Exactly 20 min after the addition of the ferric ammonium sulfate, start timed (1 min intervals) additions of 3.0 mL $\text{K}_3\text{Fe}(\text{CN})_6$. Swirl.
4. Exactly 20 min after the addition of the ferricyanide, read Absorbance at 720 nm, making readings at 1 minute intervals.
5. Include solvent-only blanks. Subtract the absorbance of the blank from the absorbance obtained for each sample. Standardize against 0.01 M gallic acid (0.094 g gallic acid monohydrate per 50 mL methanol).