

Separation of Tannin from Non-tannin Phenolics

Ann E. Hagerman © 2002

Tannins adsorb to Sephadex LH 20 in alcohol while small phenolics elute from the material. Tannins can then be eluted with aqueous acetone. We have used this method, coupled with Prussian blue assays, to estimate tannin vs. non-tannin phenolics in crude plant extracts.

Reagents

- 95% ethanol
- 70% acetone (70:30 acetone:water).
- Sephadex LH-20 Pharmacia 17-00-0-01, 25-100 micron
- Column 1.5 cm x about 8 cm.
- Fraction collector.
- Continuous UV monitor--useful for continuous monitoring of the eluate, but not essential.

Method

Plant extracts are applied to the column after removing all acetone from the sample, and reducing the volume as much as possible. Add ethanol to keep the sample in solution. Centrifuge before applying to the column to remove any insoluble materials (you can redissolve this insoluble fraction and assay with Prussian blue; we find this fraction accounts for less than 10% of the total phenolics). About the equivalent of 90 μmol equivalents of gallic acid (Prussian blue assay) can be loaded onto the column described here. Apply the sample to the column and elute with ethanol (1 mL/min). Elute until the absorbance at 280 is no longer changing and is near baseline.

The ethanol eluate can be combined, rotary evaporated to reduce the volume, and assayed with the Prussian blue assay to determine non-tannin phenolics. It can also be dried to constant weight and non-tannin phenolics determined gravimetrically (Zhao, M.S. Thesis, Miami University 1995). Elute the column with 70% acetone; tannins are usually visible as a brown band of pigments. This eluate cannot be monitored in the UV because of the strong UV absorbance of acetone. The Prussian blue spot test can be used to establish when all of the tannin has been eluted. Combine the tannin fractions and use the Prussian blue to determine tannin. The tannin can also be determined gravimetrically after drying to constant weight. We find that total recovery is routinely greater than 75%. Most of the unrecovered material is tannins which irreversibly sorb to the column.