

Sephadex LH 20

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Sephadex LH 20 sorbs tannins in alcohol, and releases them in aqueous acetone. Chromatography on Sephadex is very useful for separating tannin from non-tannin phenolics, or for fractionating hydrolyzable tannins. Condensed tannins (Sorghum or quebracho) can be prepared using Sephadex LH-20. Size-based separation is NOT achieved on any Sephadex for tannins, since phenols sorb to the packing.

Strumeyer and Malin *J. Agric. Food Chem.* 23, 909-914 (1975) first reported on purifications of tannins with Sephadex.

Sephadex LH-20 can be obtained from Pharmacia 17-00-0-01, 25-100 micron or Sigma. Separations can be performed in columns or batchwise in a large sintered glass funnel (medium or coarse sintered glass). If columns are used, they must have plastic connectors and tubing that are resistant to alcohol and acetone.

Equilibrate the Sephadex according to manufacturer's directions in ethanol (absolute or 80%, depending on purification you are following; do not use denatured alcohol). If you plan to use a column, wash with several bed volumes of ethanol after packing the column. Phenolics are applied to the Sephadex in ethanol, and ultimately tannins are eluted with acetone. If a column is used, you must reequilibrate the column in ethanol before using it again. Because the Sephadex beads swell to different volumes in the alcohol and aqueous solvents, it is necessary to use mixtures of acetone/water and ethanol to slowly bring the column back to ethanol. Direct change from aqueous acetone to ethanol will cause cracks to form in the bed of Sephadex and make repacking the column essential.

The Sephadex may be stored indefinitely in the refrigerator (not freezer) in either aqueous acetone or alcohol.