

# *Purification of Gallotannins*

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Gallotannins can be purified to serve as chromatographic standards or as standards in various assays. Tannic acid, a commercially available gallotannin is the most convenient starting point for purification of gallotannins. Commercial tannic acid can be fractionated chromatographically to yield specific galloyl esters or can be methanolized to yield homogeneous pentagalloyl glucose.

Commercial preparations of tannic acid vary significantly in their composition, with some having only very small galloyl esters (mono-tetra galloyl glucose) and others having much larger esters. The larger esters precipitate protein more effectively than the small esters. The molecular weight reported by the manufacturer is usually a theoretical mol wt based on a presumed composition. The reported purity indicates how much material other than gallotannin is present. Neither value can be reliably used to determine the composition of the commercial preparation.

Preparative scale HPLC or column chromatography can be used to fractionate commercial tannic acid to yield specific galloyl esters or a mixture enriched in the esters of interest. Methanolysis can be used to produce pentagalloyl glucose from some preparations of tannic acid. Recall that gallotannins such as tannic acid consist of a glucose (or similar polyol) core which is esterified to up to five gallic acid groups. These core gallic acid groups may be linked via "depside" bonds (ester bonds involving a phenolic OH instead of alcoholic OH) to additional gallic acids. The ester bonds are somewhat more difficult to hydrolyze than the depside bonds. Treatment of the gallotannin under mildly acidic conditions in the presence of methanol selectively methanolizes only the depside bonds. Products of methanolysis are the core galloyl glucose and methyl gallate. If each of the available groups on the glucose is esterified to a gallic acid, the core galloyl glucose is pentagalloyl glucose (PGG). Methanolysis does not always yield PGG. For example, a some preparations of commercial tannic acid

are based on a tetragalloyl glucose core (e.g. 1,2,4,6 galloyl glucose). PGG, hexa galloyl glucose, heptagalloyl glucose etc. in these preparations methanolize to yield tetragalloyl glucose and methyl gallate.

It is essential to use an independent technique, such as nmr, to confirm the identity of standards used to calibrate your HPLC.