

# ***Normal Phase HPLC of Gallotannins***

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## ***Introduction***

A method particularly useful for separating the constituents of tannic acid in a simple isocratic system. Better resolution can be obtained with reversed phase systems, especially if gradient HPLC is available. Normal phase system described in Hagerman, A. E.; Robbins, C. T.; Weerasuriya, Y.; Wilson, T. C. and McArthur, C. J. *Range Manag.* 45: 57-62 (1992).

## ***Detection***

Gallotannins are conveniently detected with UV detectors.

## ***Column***

Silica (Alltech Econosphere), 150 mm x 4.6 mm, 5 um particles (Alltech, Deerfield IL)

Precolumn containing Perisorb A (Anspec Co., Ann Arbor, MI).

Store the pre-column/column overnight or weekends in isopropanol. Re-equilibrate in 10 volumes of mobile phase before using. Wash in absolute methanol, then equilibrate in isopropanol after using.

## ***Mobile phase***

A mixture of two solvents, hexane and Solvent A, is used to achieve isocratic separation of the various galloyl esters. The proportion of these two solvents can be varied to alter retention times. A good starting point is 58% hexane to 42% Solvent A.

- Hexane. We usually use HPLC grade hexane but analytical grade is often adequate.
- Solvent A. Solvent A contains reagent grade methanol/tetrahydrofuran, 3/1 (v/v) and reagent grade trifluoroacetic acid (0.01 %, by volume). (Original method used citric acid, TFA is a volatile acid and is easier to remove from samples, also eliminates solubility problems).

## ***Sample run***

Run isocratically at 1.0 ml/min. Typical run takes 30 min to ensure elution of all peaks, although longer runs could be necessary if a sample had very high molecular weight tannins.

1. Dissolve the samples in the mobile phase to run. Some samples are not completely soluble in the mobile phase, and insolubles must be removed before chromatography to avoid damaging the equipment. Use a syringe or centrifugal filter for all samples.
2. If the samples contain even a trace of water, they will cause the mobile phase to separate into two layers when you are trying to dissolve the samples. Hexane and water are immiscible. If this happens, dry the sample (under nitrogen is convenient) and try again.
3. Some samples are less soluble than the commercial galloyl esters. In that case it can be helpful to first dissolve the material in methanol, and then add the hexane, THF and TFA. Less polar samples might be dissolved first in hexane, and then the other solvents added. If the sample is dissolved in a solvent other than the mobile phase, its elution time will be altered by the other solvent.
4. Accurate estimates of molecular weight can only be obtained if the system is recalibrated with standards dissolved in that other solvent.

## ***Results***

The galloyl esters are separated according to polarity, with the least polar eluting first. The number of galloyl groups seems to dictate polarity; more galloyl groups makes the molecule more polar, since it has more hydroxyl groups. Methyl gallate elutes first, then gallic acid, monogalloyl glucose, digalloyl glucose, trigalloyl glucose etc. The log of the retention time is a linear function of the number of galloyl groups in the ester (methyl gallate represents no galloyl groups; gallic acid is omitted from the analysis).

Isomers of a given ester may elute at slightly different times, so for example hexagalloyl glucose may be represented by a major peak for the main isomer and several "shoulders" representing other isomers.

Representative chromatograms for several commercial preparations of tannic acid are shown in the paper by Hagerman et al. (1992).