

# ***Isocratic Reversed Phase HPLC of Gallotannins***

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This simple isocratic system is described in Barbehenn and Martin, *J. Insect Physiol.* 38, 973-980 (1992). Resolution is far better if gradient HPLC is used instead.

## ***Detection***

Gallotannins are conveniently detected with UV detectors.

## ***Column***

Ultrasphere 5 RP-18 column (4.6 mm x 25 cm) (Beckman) with a 4mm x 4 mm precolumn with same packing

## ***Mobile phase***

1% acetic acid:acetonitrile, 80:20 (v/v). (Better results might be obtained by substituting 0.1% trifluoroacetic acid for the acetic acid).

## ***Samples and elution***

Isocratic run at 1 mL/min, for at least 15 min. Samples dissolved in the mobile phase.

## ***Results***

Elution is dependent upon polarity, with the most polar molecules eluting first. The most polar compounds are gallic acid, followed by monogalloyl glucose, digalloyl glucose, trigalloyl glucose etc. In this system, additional galloyl groups appear to make the molecule less polar, presumably because of the additional aromatic rings.

It is typical for the order of elution from a RP column to be opposite to the order from a normal phase column, but for the gallotannins the order is the same in both systems. The complex chemical nature of the tannins is reflected in this unusual chromatographic behavior.