

# ***Modified Vanillin Assay (for Molecular Weight Estimation)***

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## ***Introduction***

One major problem with the vanillin assay (methanol) is the different kinetics of the reaction of catechin and tannin, which makes it difficult to use catechin as a valid standard for determining tannin (Butler, Price and Brotherton *J. Agric. Food Chem.*, 30, 1087-1089, 1982). It is well known that changing the solvent for a reaction can dramatically change rates of the reaction, and the modified vanillin assay takes advantage of this effect of solvent on kinetics.

When the vanillin reaction is run with glacial acetic acid as the solvent, tannin (proanthocyanidin) and catechin react with similar kinetics. The similar kinetics are probably the result of the specificity of the reaction between vanillin and condensed tannin in glacial acetic acid. Only the terminal units of the tannin react with vanillin in glacial acetic acid.

The site-specificity of the reaction can be exploited to estimate the degree of polymerization of purified condensed tannins. The absorbance obtained in the vanillin (glacial acetic acid) assay for equal weights of tannin and of tannin monomer is directly compared to estimate degree of polymerization of the tannin. As with the vanillin assay itself, this method cannot reliably be used with 5-deoxy proanthocyanidins (e.g. quebracho), since a meta-substituted ring is required for reaction with vanillin.

Methanol diminishes the color yield of the reaction. If the tannin monomer and polymer solutions do not dissolve in glacial acetic acid, they can be dissolved in a minimal volume of methanol and then diluted with glacial acetic acid. For example, 1 part methanol to 4 parts glacial acetic acid can be used as the solvent. The amount of methanol in all samples that are to be compared must be the same.

It is not recommended that the vanillin assay (glacial acetic acid) be used to quantitate tannin in crude extracts. The vanillin assay (methanol) is preferable for quantitation for two reasons: First, the vanillin assay (methanol) is not sensitive to methanol in the extracts. Second, monomers which may be present in crude plant extracts yield less color in the vanillin (methanol) assay than do polymers, and so cause less interference.

## ***Reagents.***

- 1% vanillin in glacial acetic acid (1.0 g vanillin brought to 100 mL with glacial acetic acid). Store in a dark bottle at 4°C.
- 8% concentrated HCl in glacial acetic acid (8.0 mL concentrated HCl brought to 100 mL with glacial acetic acid).
- 0.05 mg/mL tannin monomer (catechin, for the common procyanidins) (0.5 mg catechin dissolved in the minimum volume of methanol, and then brought to 10.0 mL with glacial acetic acid). Store in a dark bottle at 4°C for up to three days.
- 0.1 mg/mL condensed tannin with unknown molecular weight (1 mg purified tannin dissolved in the minimum volume of methanol and then brought to 10.0 mL with glacial acetic acid). Prepare fresh each day.
- Constant temperature water bath set at 30°C. (If this is not available, there will be temperature-dependent variation in the data).

## ***Preparation of Working Reagents***

The working vanillin reagent must be prepared daily from the solutions described above. One part of the 1% vanillin solution is mixed with one part of the 8% HCl solution. The working vanillin reagent is brought to 30°C in the water bath before starting the analysis.

## ***Method***

1. 0 to 1.0 mL aliquots of the samples (catechin and condensed tannin) are dispensed into culture tubes
2. each sample is brought to 1.0 mL by the addition of glacial acetic acid.
3. Tubes are incubated in the water bath for a brief period to bring them to temperature equilibrium.
4. 5.0 mL of the working vanillin reagent is added at 1.0 min intervals to the samples, which are immediately returned to the water bath.
5. The samples are left in the water bath for exactly 20.0 min, and are then
6. the absorbance at 510 nm is read. Because the color continues to develop as time passes, you cannot go back and re-read any sample. You must maintain the strict 1.0 min intervals for reading that you used in the addition of reagents.

## ***Analysis***

The absorbance of the blank (reagent with no tannin) is subtracted from the absorbance of the corresponding vanillin-containing sample.

Two response curves are constructed, and each is checked for linearity and for zero intercept. The absorbance value obtained with a certain weight of catechin is divided by the absorbance value obtained with the same weight of condensed tannin to estimate the absolute degree of polymerization. Butler, Price and Brotherton *J. Agric. Food Chem.*, 30, 1087- 1089, 1982 provides data which can be used to validate the assay.

This method has not been validated for branched chain condensed tannins. It is likely that all the terminal units of a branched chain condensed tannin will react with vanillin, so the apparent molecular

weight of these polymers will be low. However, further investigations into the chemistry of the reaction are required to confirm this assumption.