

AWRI Tannin Portal – Sample Preparation Guide



Part (a) – Calculation of tannin, total pigment and total phenolics

Materials

- 10 mm path length quartz cuvettes
- 1 M HCl
- 10ml test tubes
- Pipettes, tips, wipes
- UV/VIS Spectrophotometer
- QC Reference standard cuvette – provided by AWRI

Method

NOTE: This method is suitable for analysing wines that have completed alcoholic fermentation (classified as post-ferment) and ferment samples from Day 3 of fermentation onwards. It is not suitable for analysing juices or ferments earlier than Day 3. If ferments or wines are hazy, clarify first by centrifugation.

1. Add 10 mL of 1M HCl to a 10 mL test tube.
2. Add 200 μ L ferment or wine sample to the tube and mix. Ensure at least 1 tube is set aside containing only 1M HCl to use as a Blank.
3. Incubate Blank & diluted samples at room temperature (18 -21°C) for at least 1 hour. Note that a longer incubation time than 1 hour is fine, but absorbance readings should be taken on the same day as samples are diluted.
4. During incubation period, turn on spectrophotometer to ensure adequate warm up and perform instrument diagnostics if this facility is available.
5. Set instrument for measurement at 250, 270, 280, 290, 315 and 520 nm.
6. Zero with 1M HCl (Blank) in 10 mm pathlength quartz cuvette.
7. Measure diluted samples at 250, 270, 280, 290, 315 and 520 nm using a 10mm pathlength quartz cuvette.

NOTE: if the spectrophotometer is double beam, zero the instrument with no cuvettes in either path, then place a 1M HCl blank in the reference beam and take readings with samples in the sample beam.

8. Re-zero the instrument with an air blank (ie with no cuvette present)
9. Measure your QC Reference standard cuvette at 250, 270, 280, 290, 315 and 520 nm

NOTE: if the spectrophotometer is double beam, zero the instrument with no cuvettes in either path, then leaving the reference beam empty (i.e. air blank) take readings with the reference standard cuvette in the sample beam.

10. Results can then be uploaded to the AWRI Tannin Portal for reporting. (See **Using the AWRI Tannin Web Portal – a quick ‘How-To’ Guide** for instructions)

Part (b) – Optional additional step to calculate pigmented tannin and free anthocyanins

If you wish to calculate free anthocyanins and pigmented tannins for your samples, you will also need to carry out the following additional step. This part of the method cannot be performed in isolation, it should always be done at the same time as part (a) because readings from part (a) are used in the calculations for part (b).

Additional materials

5M NaOH
Tartaric Acid
AR Ethanol
Sodium metabisulfite
pH meter

Buffer preparation (100ml, scale accordingly for larger amounts)

1. Dissolve 0.5 g of tartaric acid in approximately 50 mL of water.
2. Add 12 mL of ethanol and then make up total volume to 100 mL.
3. Using a pH meter adjust the overall solution pH to 3.4 by dropwise addition of 5M NaOH.
4. Add 0.38 g of sodium metabisulfite and dissolve.
5. Place in a tightly stoppered container and store in cool dark place.

Please note that this buffer solution has a shelf life of **one week** after which it must be freshly prepared.

Sample preparation and measurement

1. Place 1 mL of wine or ferment sample in a 10 mL test tube and then add 9 mL of the buffer solution.
2. Mix sample.
3. Set aside one 10mL test tube containing just the buffer solution. This will be your blank.
4. Incubate blank and diluted samples at room temperature for at least 1 hour. As in part (a), a longer incubation time is fine, but readings should be taken on the same day that samples are diluted.
5. After you have completed reading the absorbances of the samples diluted in acid and your QC reference standard as described in part (a), re-zero your spectrophotometer using the blank buffer solution and then measure the absorbance at 520nm of your samples diluted in buffer. This reading should be recorded as A₅₂₀ buffered, and will need to be entered into the sample upload spreadsheet along with the readings generated in part (a).

Safety

- Ensure laboratory staff wear adequate personal protection equipment at all times, including lab coat, safety glasses, closed-in footwear and disposable gloves.
- Diluted samples from part (a) are strongly acidic. Neutralise or dilute with copious amounts of water when disposing. Do not pipette any reagents by mouth.

Help?

If you need help, you can email us at tanninportal@awri.com.au or phone the AWRI on +61 8 8313 6600.