# Analytical techniques — use of the pipette

1

Never pipette directly from the stock solution container as it may become contaminated.

Pour the solution into a small beaker before pipetting.


2

Using a pipette filler, fill the pipette with a small portion of the solution.

Remove the pipette filler before carrying out Step 3 to avoid any of the solution entering the pipette filler when the pipette is near horizontal.

3

Swirl the solution around inside the pipette. Discard the solution.

Repeat Step 2 and the swirling and discarding steps at least once.

4

Using a pipette filler, fill the pipette with the solution to above the graduation line.

Withdraw the pipette from the solution. Wipe off any liquid adhering to the outside of the pipette with a tissue. Do not let the tissue touch the opening of the tip of the pipette as this may introduce air bubbles.

5

To carry out the operations described in Steps 6, 7, 8 and 9 use the pipette filler mechanism.

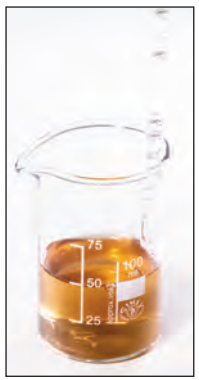
6

Slowly lower the solution in the pipette towards the graduation line.

Match the liquid level to the graduation line by bringing the lowest point of the meniscus (the curved surface of the liquid) to the line. When doing this, your eye must be level with the graduation line.

7

Before delivery, ensure that there are no air bubbles in the pipette. If there are, discard the solution and start again.



8

To deliver the solution:

- hold pipette vertically,
- hold receival vessel on an angle,
- hold the pipette tip so that it touches the wall of the receival vessel,
- use the pipette filler mechanism to allow the liquid to flow out of the pipette, and
- drain the pipette for about 30 seconds. Note, some pipettes have the draining time indicated on them.

9

Remember that after delivery there is a small volume of liquid remaining in the tip of the pipette. Do not blow it out.

Note: Some pipettes are designed to be blown out, e.g. some graduated pipettes.

10

Any solution remaining in the beaker (see Steps 1 and 2) should not be returned to the stock solution.

Note: Automatic pipettors are also available. These provide a convenient and accurate means for delivering volumes of liquid, particularly very small volumes.

# Juice total soluble solids by hydrometry

## Test method

**•Sample preparation**

The test is carried out on a sample of undiluted, settled (or clarified) juice. Ferment samples should be degassed (stirred vigorously) prior to measurement.

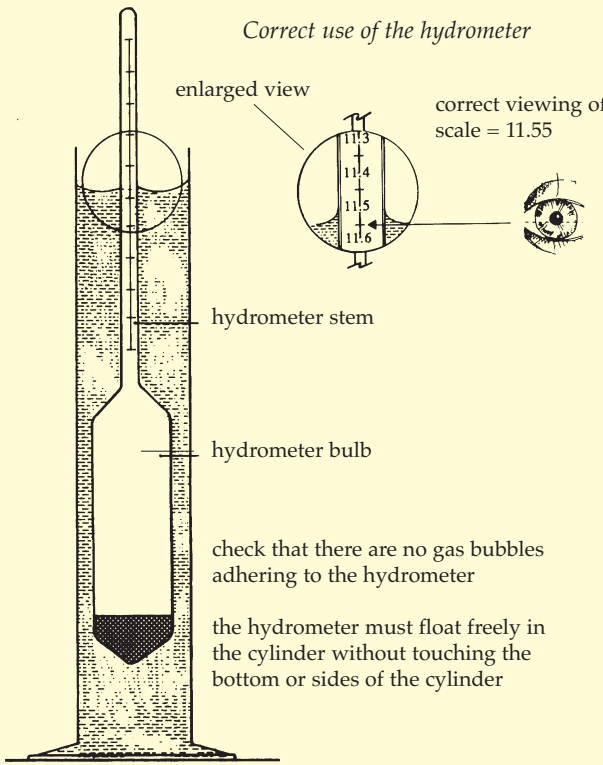
**•Procedure for using a hydrometer**

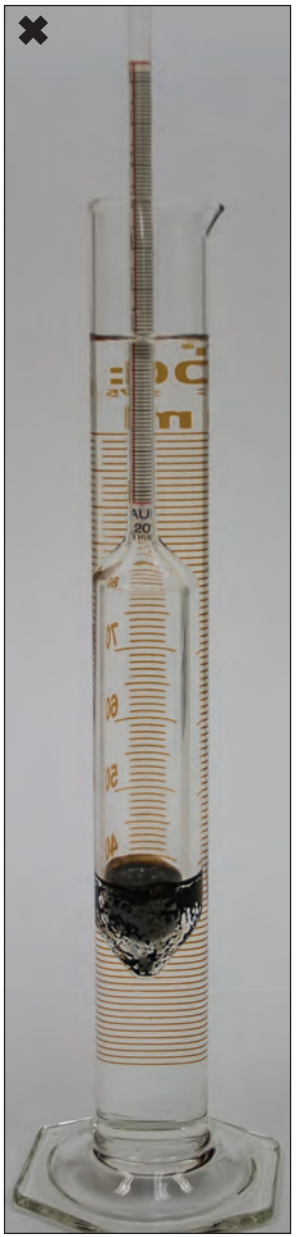
1. Fill a measuring cylinder of an appropriate size with undiluted and settled (or clarified) juice to about 10 cm from the top.
2. Select a hydrometer that is within the expected range. Ensure the hydrometer is clean and dry. Rinse it with a small portion of clarified juice, then gently place the hydrometer in the cylinder and push it down to the bottom — do not let it drop.
3. Move the hydrometer up and down to mix the juice.
4. Spin the hydrometer by hand (to remove adhering air bubbles). The hydrometer (if it is in the appropriate range) will now slowly rise.
5. When the hydrometer settles, read the indicated value with your eye level with the bottom of the meniscus.
6. Insert a thermometer into the juice and measure the temperature. If the temperature is outside the range shown in the table in column 2, cool or warm the sample so that its temperature is within this range and repeat the measurement procedure.
7. Apply the appropriate temperature correction factor to the indicated value obtained in Step 5. This converts the indicated value to the corrected value at 20°C.
8. Record the result in Step 7 to the appropriate values shown below:
   - °Baumé — to one decimal place, e.g. 12.4;
   - °Brix — to one decimal place, e.g. 22.6; and
   - SG — to four decimal places, e.g. 1.0004.
9. Carefully remove the thermometer and the hydrometer and rinse them with purified water.

*Temperature correction table for various hydrometers*

| Temperature of the juice (°C) | Indicated hydrometer value | Corrected value (at 20°C) | | |
|---|---|---|---|---|
| | | °Baumé | °Brix | SG |
| 15 | x | x-0.25 | x-0.30 | x-0.0010 |
| 16 | x | x-0.20 | x-0.24 | x-0.0008 |
| 17 | x | x-0.15 | x-0.18 | x-0.0006 |
| 18 | x | x-0.10 | x-0.12 | x-0.0004 |
| 19 | x | x-0.05 | x-0.06 | x-0.0002 |
| 20 | x | x | x | x |
| 21 | x | x+0.05 | x+0.06 | x+0.0002 |
| 22 | x | x+0.10 | x+0.12 | x+0.0004 |
| 23 | x | x+0.15 | x+0.18 | x+0.0006 |
| 24 | x | x+0.20 | x+0.24 | x+0.0008 |
| 25 | x | x+0.25 | x+0.30 | x+0.0010 |

*Correct use of the hydrometer*



*Incorrect — hydrometer touching the cylinder*

*Correct — hydrometer floating freely*

# Reducing sugars by the Lane & Eynon method

## Helpful hints

**Dilution of the wine prior to analysis**
Diluting the sample can help with accuracy as the titration value will be larger than if an undiluted sample is used — a guide is provided below.

| Reducing sugar concentration (g/L) | Appropriate dilution factor |
|---|---|
| <4 | no dilution required |
| 4 - 20 | 1 in 10 |
| 20 to 50 | 1 in 25 |
| 50 - 100 | 1 in 50 |
| > 100 | 1 in 100 |

**Timing of the titration**
Because of the non-stoichiometric nature of the Lane & Eynon reaction, the titration of the sample with standard glucose needs to be carried out under controlled conditions of heating and timing. It should take as close as possible to 3 minutes from the commencement of boiling until the end point is reached. The solution should be boiling continuously throughout the 3 minutes.

**Mixing during titration**
Mixing during titration can be achieved by different means. The means described in this procedure is by the use of a magnetic stirrer bar in the flask, which is placed on top of a hot plate stirrer to facilitate heating and stirring. The stirrer can be intermittently turned on and off to allow the interfering red precipitate to settle out, making it easier to judge the colour of the solution. Position the flask on the hot plate stirrer so that the tip of the burette is in the centre and aligned with the top of the flask.

Heating can also be achieved by holding the flask (with care) above the flame of a bunsen burner.

**A guide to judging the end point**
Near the end point the blue colour fades and the solution becomes turbid as precipitation of cuprous oxide occurs. At the end point the blue colour disappears; this is best observed at the edge of the solution and in the bubbles (see photographs). The murky solution in (d) has a red appearance but on standing (without stirring) the cuprous oxide settles and the solution appears clear.

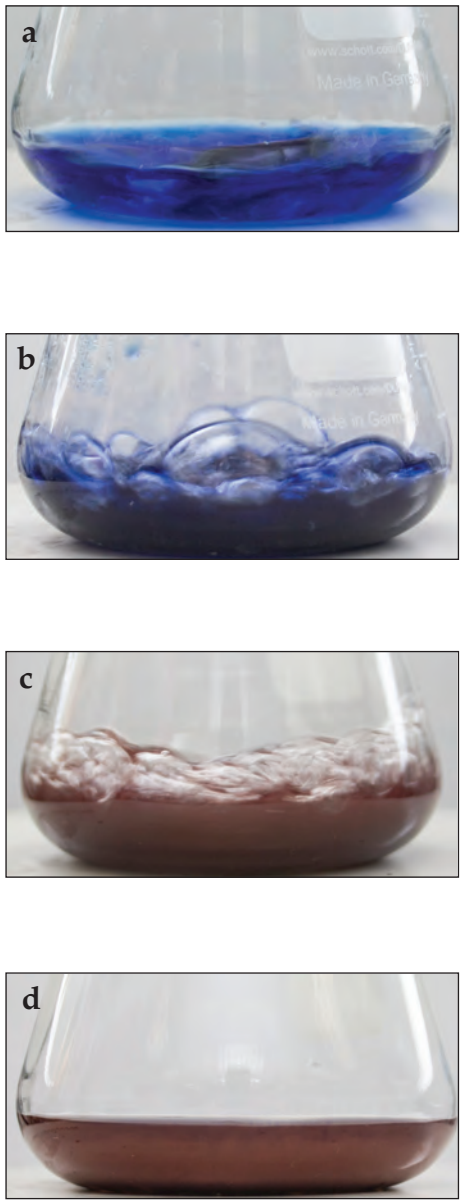
### Alternative approach to adding the methylene blue indicator

In the procedure for standardising the Soxhlet solution (page 63), instead of adding the methylene blue indicator when only a faint blue colour remains in the solution, it can be added after the initial addition of 18 mL of the 0.5% glucose solution. It can be added at this stage because the standard titre is normally between 20 and 23 mL and thus it is being added before the end point.

However for the analysis of the wine sample, the volume of 0.5% glucose solution used in the titration will vary depending on the sugar concentration of the sample being tested. In this case, to avoid overshooting the end point, it is best to add the methylene blue indicator when only a faint blue colour remains in the solution. The exception to this approach would be where an estimate of the sugar concentration is known. In this case a rough calculation can be made of the volume of 0.5% glucose solution to be used in the titration. Knowing this estimate of the titre volume, 75% of the required volume of 0.5% glucose solution could initially be added, followed by the methylene blue indicator. This approach lessens the chance of overshooting the end point. Two examples are given below.

a) If the estimation of the sugar concentration is 4 g/L, according to the calculation formula, the sample titre would be approximately 6 mL and therefore only about 4 mL could be added initially before adding the indicator.

b) If the estimation of the sugar concentration is 10 g/L, according to the calculation formula, the sample titre would be approximately 18 mL and therefore only about 13 mL could be added initially before adding the indicator.



*The sequence of change in the colour of the solution being titrated during a Lane & Eynon titration*

# Following the progress of a malolactic fermentation (MLF)

## Helpful hints

The solvent for paper chromatography should last at least a month. The chromatography solvent can be reused a number of times. With time, water will fall out of the solution and the solvent needs to be rejuvenated. This is achieved by adding a few drops of formic acid to the old solvent, returning it to the separating funnel and carrying out the procedure for separating the water and filtering the solvent as before.

The quality of the solvent can be assessed from the appearance of the chromatogram. A yellow background and/or poor development or separation indicates that there is too much water in the solvent and it should be replaced with a new preparation.

**Applying the sample**
Be careful not to contaminate the capillary tubes or the loop between sample applications. Make the spots as small and as concentrated as possible. It is necessary to work out the best way to achieve this (e.g. how many times to apply each spot) for the particular method of application you are using.

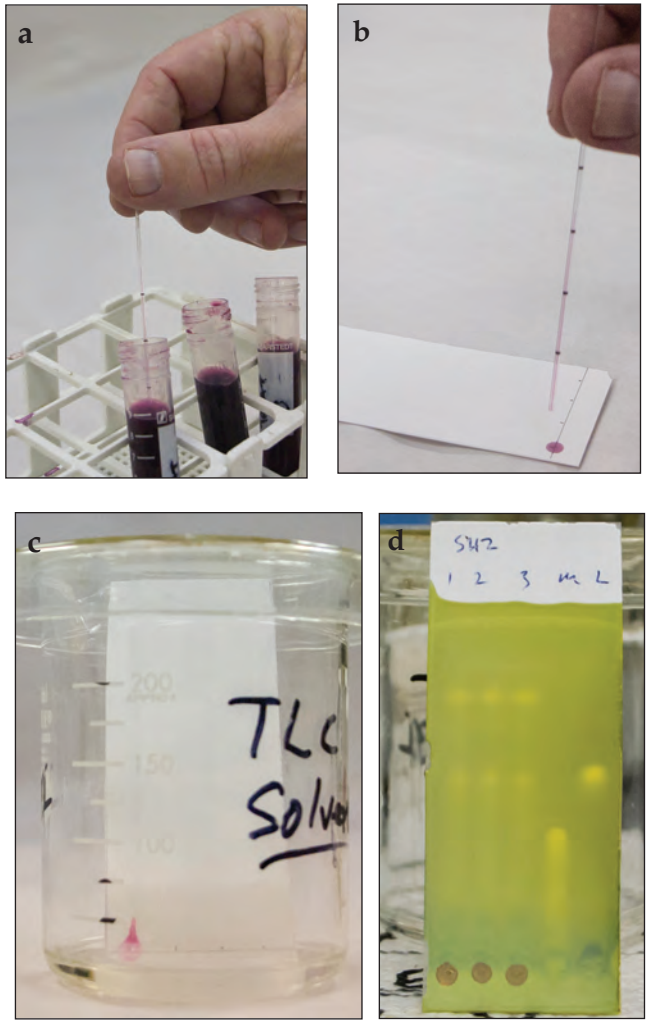
The spots need to be dry before placing the paper or sheet into the chromatography solvent. The streaking in photograph (c) indicates that the spot was not sufficiently dry.

**The chromatographic tank**
The chromatographic tank needs to be clean and dry before use.

**Assessing the chromatogram**
Lactic and succinic acids can be produced during the primary fermentation by yeast, and hence their presence in the chromatogram is not conclusive evidence of a malolactic conversion. The criteria for malolactic conversion are the complete absence of a spot in the position corresponding to malic acid and a more intense spot in the position corresponding to lactic acid.

If DL-malic acid has been used for acidity adjustment during wine production, only the L-malic acid will be converted during malolactic fermentation, and hence the remaining D-malic acid will still appear as a spot in the malic acid position when a chromatogram is run. Therefore, if DL-malic acid has been used, it is not possible to determine if the malolactic fermentation is complete by the technique of paper or thin layer chromatography. In this case assessment of the completion of a malolactic fermentation needs to be by the enzymatic analysis method which is specific for L-malic acid. Note that HPLC methods do not separate L-malic from D-malic acid.

The blue spots near the baseline are produced by pigments in the wine.




*The photographs show some of the steps in the procedure for detecting malic acid and lactic acid by TLC*
*(a) sampling the wine via a capillary tube*
*(b) spotting the thin layer sheet*
*(c) the sheet in a beaker containing the solvent and*
*(d) the developed sheet*

# Protein stability

## Types of tests

A protein stability test is carried out as part of a bentonite fining procedure and prior to bottling.

The presence of unstable proteins in a wine can lead to the development of a haze or deposit. If this occurs after bottling, the wine is unacceptable to the consumer. Since it is difficult to predict the temperature conditions to which a wine will be exposed during transport and storage, wines should be checked for the presence of unstable proteins prior to bottling.

**Tests for estimating protein stability in wine.**
These include the 'heat stability' test and the use of test kits such as Bentotest™, Prostab™ and Proteotest™.

These all involve denaturing the protein either by heating or by the addition of a chemical agent, with or without heating. These agents are not necessarily specific to proteins, as other compounds, e.g. tannins and polysaccharides, may influence the degree of haze formed during the test. Nevertheless the tests provide a guide, based on experience, to the severity of any potential protein instability. The most commonly used method to predict if a wine is protein stable is the 'heat stability' test, where a wine is exposed to an elevated temperature for a period of time and, after cooling to room temperature, assessed for haze formation.

*Recommended temperature and timing*

Previously the recommended 'Heat stability test' utilised a method where the wine was heated for 6 hours at 80ºC and then cooled and assessed for haze formation. McRae et al. (2018) investigated if the test conditions could be modified; they recommend that heating the wine for 2 hours at 80ºC and cooling for 3 hours at 20ºC provides reliable and repeatable heat test results that are similar to those of the longer standard heat test results with 6 hour heating at 80ºC.

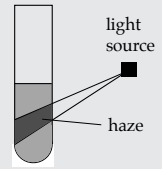
## Heat stability test

**• Procedure for conducting a heat stability test**

1. The wine should be brilliantly clear prior to conducting the test. If necessary, initially centrifuge the wine at approximately 3500 rpm for 10 minutes and then filter the wine through a 0.45 $\mu$m membrane filter using either a syringe or a vacuum filtration system. A prefilter may also need to be used if the wine is very cloudy. Discard the first few mL of filtrate and then collect about 20 to 30 mL of wine.
2. Fill two test tubes of appropriate size or two turbidity meter tubes with filtered wine. Label one tube 'Control' and the other 'Test'. The volume of wine should be such that there is sufficient air space above the wine to allow for expansion under heating. Cap the tubes. The cap should be sufficiently tight that no volatiles are lost from the wine, thus changing its volume, and that no steam or water (if using a water bath) can enter the tube. Caps for tubes used in this test should be PTFE or silicon lined and provide a water-tight seal.
3. Place the 'Test' tube in an 80ºC water bath for 2 hours. (If the tube is an appropriate size, an alternative method of heating is to place it into an electric heating block). Allow time for the temperature of the wine to reach 80ºC then start recording the time — an extra tube containing wine and a thermometer can be used to determine when 80ºC is reached.
4. After 2 hours at 80ºC, immediately remove the tube from the water bath or heating block. Note: the tube will be hot and needs to be handled carefully.
5. Mix the contents of the tube by gently inverting it several times. Cool the samples for 3 hours at 20ºC.

   Repeat the mixing procedure and wipe the outside of the tube.
6. Assess the degree of haze formation *visually (7a) and/or by using a turbidity meter (7b).*

7a. *Assessing the presence of a haze visually*: The presence of a haze can be estimated by shining a strong light through the wine and observing the solution at right angles to the light source (as shown in the diagram. Any internal reflection indicates the presence of a haze. A strong narrow beam of light can be obtained by using a pen light torch or a microscope lamp. Samples pass the test if there is no difference between the test samples and the unheated controls.



*Observation of the haze*

7b. *Using a turbidity meter to assess the presence of a haze*: The outside of the tube must be clean and dry prior to placing it in the turbidity meter and recording the reading. A wine is considered protein unstable if, for example, there is an increase in turbidity of greater than 2 turbidity units (typically NTU units), in the heated sample compared with the control. Note that this criteria may vary for different wine styles. These values are based on experience and the accepted risk of potential haze formation in the wine during storage. Visual estimation of the haze can be used as a preliminary screening prior to using the turbidity meter. If visually there is an obvious haze, there is no point in applying the turbidity meter test.

*If you are using the new recommended 'heat stability test' conditions you should check their performance against the conditions of the previous method that you used.*

# Sensory assessment — some guidelines

## Assessing fining trials

A preliminary assessment is carried out by tasting in the order of the sample treated at the lowest fining level to that treated at the highest fining level. The exception to this rule is for 'off odours'; in this case the tasting order should start at the highest fining level and work backwards in order to avoid adaptation and sensory fatigue.

Generally, the choice of fining level is not based on some complex statistical analysis of the result but rather a judgement based on experience. The response to the fining rates is typically one of 'diminishing effect'. For example, the purpose of the fining trial may be to determine the level of a fining agent which reduces astringency — a value judgement needs to be made between the perception of the reduction of astringency and, for example, the stripping of flavour. When assessing astringency sensations, before carrying out the fining trial it is advisable to adjust the acidity of the wine to the desired level, as acid-tannin interactions can affect the perception of astringency.

## Assembling a blend

Typically, the winemaker has a number of wine parcels which may contribute to a blend, e.g. a Cabernet Sauvignon/Merlot blend. If the exercise is to prepare a wine which is similar from year to year, tasting the wine of the previous year helps to focus on the style characteristics required for the current blend. Obviously the wine is a year older than the wines on the bench, but nonetheless it reminds the taster of the fruit-acid-tannin-alcohol structure/balance.

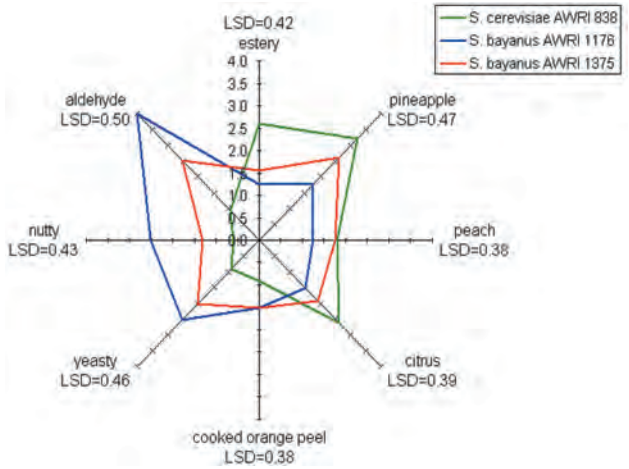
When assembling a blend, it is important to ensure that the final blend complies with the relevant regulations regarding the maximum or minimum allowable percentages of varieties, vintages and regions.

**• A general approach to determining the % components in a blend**

1. Prepare a blend (Blend 1) consisting of the varietal percentage which was used previously, for example 60% Cabernet Sauvignon and 40% Merlot.
2. Initially prepare a blend by adjusting the components up and down by approximately 10%, for example Blend 2 — 66% Cabernet Sauvignon and 34% Merlot.
3. The Paired preference test can be used to determine a preference between two blends.
4. Continue to prepare and compare blends based on the assessment of the above blends until the preferred blend is achieved.

## Presenting data from QDA tests

Data from Quantitative descriptive analysis® (QDA) tests can be visually presented as radar/web diagrams; an example is shown below.




*An example of a radar/web diagram*

*Mean ratings for eight aroma attributes in Chardonnay wine made with S. cerevisiae AWRI 838 (—), S. bayanus AWRI 1176 (---) and S. bayanus AWRI 1375 (—). Each value is the mean score from triplicate wines that were presented to 13 judges on 2 separate occasions (Eglinton et al. 2000, reproduced with permission from the Australian Society of Viticulture and Oenology.*

### Helpful hint

Different segments of the web can be allocated totally to wine attributes or berry attributes or the web can represent a combination of wine and berry attributes and/or vine characteristics to visually show relationships between vines, grapes and wines as shown in the accompanying book, 'Theory and concepts of chemical, physical and sensory analyses and tests of grapes and wine'.