Juice total soluble solids by hydrometry

Test method

• Sample preparation

The test is carried out on a representative sample of undiluted, settled (or clarified) juice.

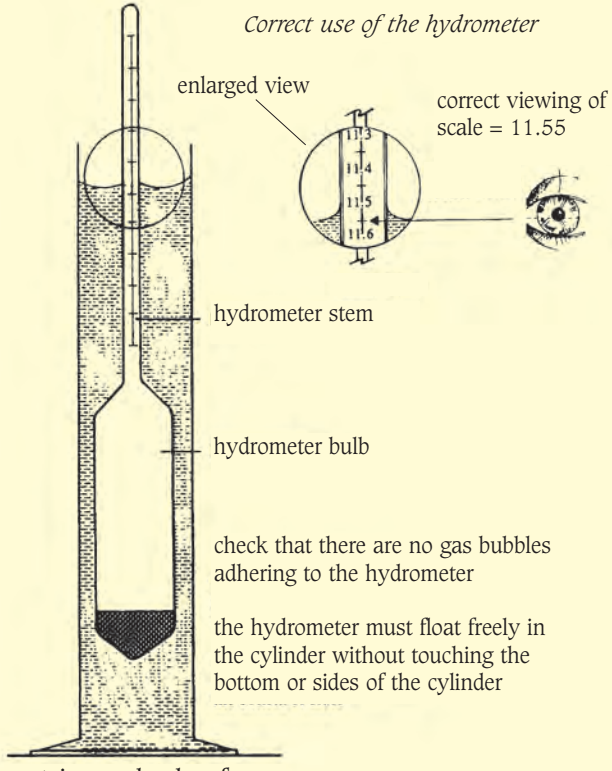
• Procedure for using a hydrometer

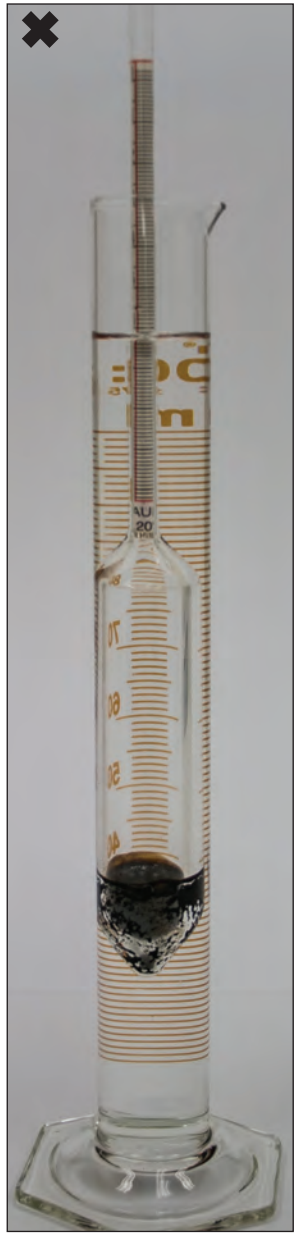
1. Fill a measuring cylinder of an appropriate size with undiluted and settled (or clarified) juice to about 10 cm from the top.
2. Select a hydrometer that is within the expected range. Ensure the hydrometer is clean and dry. Rinse it with a small portion of clarified juice, then gently place the hydrometer 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, eg 12.4;
   - °Brix — to one decimal place, eg 22.6; and
   - SG — to four decimal places, eg 1.0004.

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

Juice and wine pH

Analysis method

● Sample preparation

The determination is normally carried out on a portion of clarified grape juice or clear wine.

The temperature of the juice/wine must be the same as that of the standard buffers used in the standardisation/calibration of the pH meter.

Notes

Commercial buffers may not be exactly pH 4.00 and 7.00. In this case during the standardisation or calibration steps the pH values should be set to exactly the pH values of the standard buffers, eg pH 4.01 or pH 6.86.

After removing the electrode from the buffer solutions, juice or wine samples, the electrode can be rinsed with purified water and gently dried by dabbing it with a tissue. Alternatively the electrode can be dabbed dry with a tissue without the rinsing step. Do not wipe the electrode with the tissue, just dab it dry, as wiping can add charge to the electrode and thus affect the accuracy of the measurement.

For very accurate measurement the electrode can be rinsed with a small portion of the sample to be analysed before immersing the electrode in the sample.

Ideally, when taking pH measures of juice or wine, the stirring conditions and the time until reading the pH value should be the same as those used for the standard buffers.

Checking the pH meter

The accuracy of pH meters may be checked with standard buffers or by preparing and measuring the pH of a saturated aqueous solution of potassium hydrogen tartrate (KHT) which should give a pH value of 3.56 ± 0.02. The precision can be tested by measuring the pH of the same sample a number of times.

● Procedure for calibrating pH meters, which have manual controls, using two standard buffers

1. Pour a sufficient volume of each standard buffer, (eg pH 7.00 and pH 4.00) into separate beakers or vials (of suitable size) so that the electrode bulb and liquid junction will be adequately covered when the electrode is immersed in the solution.
2. Temperature adjustment — measure the temperature of the standard buffers. Set the temperature adjustment dial to the temperature of the standard buffer solutions. Refer to the temperature correction table and obtain the correct pH for that temperature.
3. Set the sensitivity or slope control to the point corresponding to the 100% position (ignore this step for meters that do not have the 100% point indicated on the scale).
4. Initial adjustment — immerse the electrode in the pH 7.00 buffer contained in a small beaker (see Step 1), stir slowly and adjust the buffer or calibration control so the digital display shows pH 7.00. Remove the electrode from the buffer solution (see Notes).
5. Sensitivity or slope adjustment — immerse the electrode in the pH 4.00 buffer contained in a small beaker, stir slowly and adjust the sensitivity or slope control dial so that the digital reading shows pH 4.00 on the display. Remove the electrode from the buffer solution (see Notes).
6. Final adjustment — immerse the electrode again in the pH 7.00 buffer and adjust the buffer control dial so that the digital display shows pH 7.00. Remove the electrode from the buffer solution (see Notes).

● Procedure for measuring the pH of juice/wine

1. Calibrate the pH meter.
2. Place the sample of juice/wine in a small beaker. The volume of juice/wine should be sufficient to cover the junction and bulb when the electrode is immersed in the sample.
3. Measure the temperature of the sample and adjust to the temperature of the standard buffers.
4. Check that the electrode is clean and dry. Place the electrode in the sample of juice/wine contained in a small beaker.
5. Slowly stir the sample.
6. The pH value of the sample will be shown on the digital display. It may take about 20 to 30 seconds to stabilise.
7. After recording the pH value, rinse the electrode with purified water, pat it dry with a tissue and and return it to the storage solution. Do not leave the electrode to stand dry or immersed in juice/wine longer than is necessary.
8. Record the pH value of the juice/wine to two decimal places, eg 3.45.

● Procedure for calibrating pH meters with in-built standardisation

For these pH meters, a similar standardisation or calibration procedure to that described for those with manual controls is followed, but the standardisation or calibration is performed automatically, according to the manufacturer's instructions.

Sulfur dioxide (SO_2) by the Ripper method

Helpful hints

A more accurate titration value is obtained if a micro-burette (eg a narrow bore 10 mL burette) is used for the titration.

Correcting for the presence of ascorbic acid
Any ascorbic acid present in the juice/wine sample will react with iodine during the titration. This increases the volume of iodine used in the titration thus giving an incorrect higher value for the determination of free and/or total SO_2 by the Ripper method. To correct the result obtained by the Ripper method for wines that contain ascorbic acid, the ascorbic acid concentration is determined (as described on page 64 and 65) and the equivalent volume of iodine which would be used in a Ripper titration calculated. This volume of iodine is allowed for when using the formula to calculate the free and total SO_2 by the Ripper method.

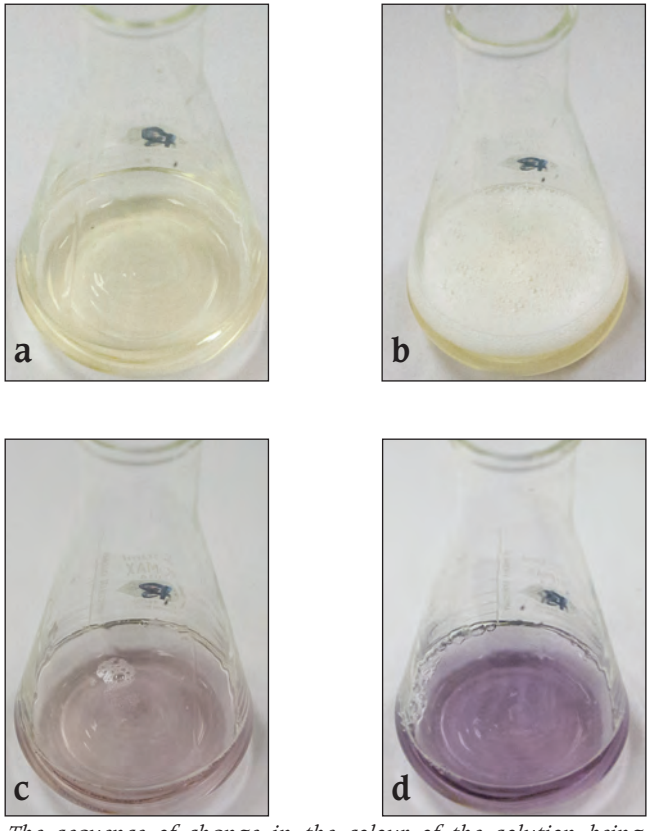
According to the formula for calculating the concentration of ascorbic acid, one mg/L of ascorbic acid is equivalent to 0.0114 mL of iodine. For example, if the concentration of ascorbic acid is 100 mg/L then 1.14 mL of the 0.01 M iodine titre, when determining the SO_2 concentration, is due to the presence of ascorbic acid. This value can be subtracted from the titre value prior to calculating the value for free and total SO_2 by the Ripper method. If the concentration of ascorbic acid has not been determined, the value used in the correction step can be taken as the level of addition of ascorbic acid.

References

Buechsenstein, J.W. and Ough, C.S. (1978) SO_2 determination by aeration – oxidation: A comparison with Ripper. American Journal of Enology and Viticulture 29, 161-164.

A guide to judging the end point
The colour change is more easily observed if a light (eg from a desk lamp) is passed through the solution, while titrating.

The photographs provide a guide to the colour change which occurs at the end point, although in practice the colours may be slightly different.



The sequence of change in the colour of the solution being titrated during a Ripper titration. The foaming in (b) occurs when the sodium bicarbonate is added. The end point of the titration occurs when the colour of the solution changes to a dark blue shade (d) which persists for 30 seconds.

Checking the method

Accuracy and precision
To test the accuracy of the method for your operation recommended standards could be

- free sulfur dioxide — a 20 mg/L solution, while a recommended standard recovery could be ± 10%. This is equivalent to a result between 18 mg/L and 22 mg/L; and
- total sulfur dioxide — a 100 mg/L solution, while a recommended standard recovery could be ± 5%. This is equivalent to a result between 95 mg/L and 105 mg/L.

These are the same solutions which are used for checking the accuracy and precision of the determination of SO_2 by the aspiration method.

Procedures for testing accuracy and precision are given on pages 9 to 12.

Troubleshooting/Sources of errors

Incorrect concentration of 0.01 M I_2 — the iodine solutions can be standardised against a standard sodium thiosulfate solution.

Incorrect volume of wine — use correct pipetting technique.

Interference from air — the titration needs to be completely quickly before losing the CO_2 blanketing effect of the $NaHCO_3$ addition.

Insufficient time to release bound SO_2 — allow 10 minutes after the addition of 1 M NaOH so that all the bound SO_2 is released.

Not allowing for the presence of interfering compounds — any ascorbic acid present in the juice or wine will react with iodine during the titration.

Reducing sugars by the Lane & Eynon method

Helpful hints

Dilution of the wine prior to analysis
The dilution factor should be such that the titration value of the sample titre will be greater than 10 mL. This will increase the accuracy of the titration.

A guide to the dilution required for wines containing greater than 4 g/L reducing sugars

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.

Values of wines fermented to dryness
Wines that have completely fermented to dryness will still contain a small quantity of reducing sugars and thus will not give a zero g/L value when reducing sugars are determined by the Lane & Eynon method. Reducing sugar concentration can vary from 0 to about 2 g/L (see page 66).

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 the solution can also be achieved by holding the flask above the flame of a bunsen burner.

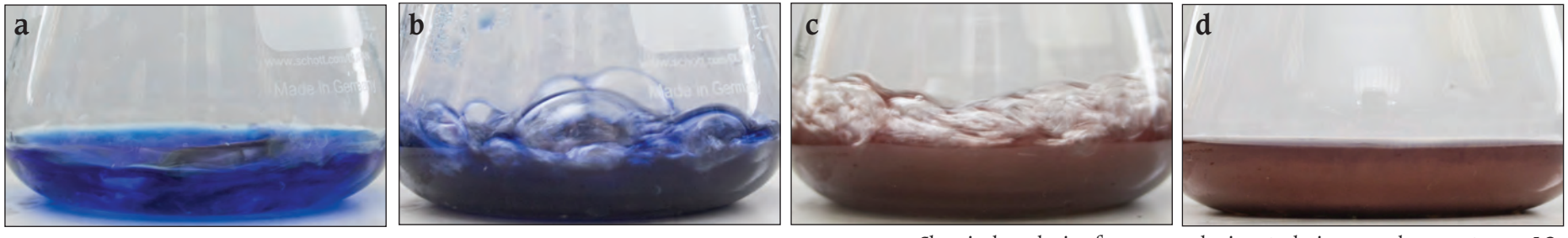
A guide to judging the end point
Near the end point the blue colour fades and the solution becomes turbid as precipitation of cuprous oxide occurs. At the end point the blue colour disappears; this is best observed at the edge of the solution and in the bubbles (see (c) below). The murky solution in (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 69, Step 5), 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 of the sample had been previously obtained, eg by hydrometry or by testing with reducing sugar tablets, eg Clinitest®. 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.



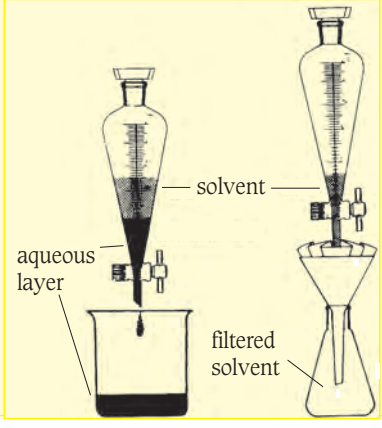
The sequence of change in the colour of the solution being titrated during a Lane & Eynon titration

Detecting malic and lactic acids by paper chromatography

Analysis method

● Procedure for preparing the solvent

1. Place 33 mL of n-butanol, 33 mL of purified water, 3.6 mL of formic acid and 5 mL of 1% w/v bromocresol green indicator solution in a 100 mL separating funnel. Volumes may be measured by measuring cylinders, or graduated pipettes for the smaller volumes.
2. Stopper the funnel and mix the mixture thoroughly by inverting the separating funnel a few times. Invert the funnel so that the tap points upwards and directed away from yourself. Open the tap, release pressure, close the tap and mix again. Repeat this mixing procedure a few times. Allow the mixture to settle and separate into two layers.
3. Remove the stopper at the top of the separating funnel and run the lower aqueous layer out through the tap. This solution is not required and can be discarded.
4. The remaining (top) solvent layer is slowly filtered through several fluted filter papers to remove traces of water. Use either Whatman No. 1 or phase separating filter paper.
5. Collect the filtered solvent and store in a tightly stoppered glass container. Store the solvent in a refrigerator.

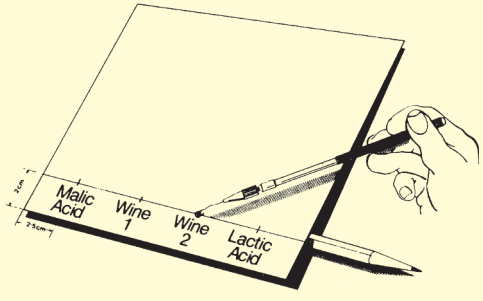


Preparing the solvent

● Procedure for spotting the paper

1. Select a piece of chromatography paper — holding only the top corner with your fingers.
2. Place the chromatography paper on a clean and dry bench area.
3. Make a pencil line from side to side 2 cm from the bottom of the paper. Mark cross lines at 2.5 cm intervals along the line.
4. Write the name of the respective standards and wines below each of the cross lines, using a pencil, NOT a ball point pen.
5. Raise the bottom edge of the paper above the bench by supporting it on, eg a glass rod or pencil.
6. Using either a capillary tube or a wire loop, and keeping the spots as small as possible, spot the samples exactly to the respective crosses 4 times, allowing the spots to dry between applications. Spots may be dried quickly by applying a gentle stream of warm air from a hairdryer.

   If using a wire loop, clean it by washing in purified water, flaming and drying before each application. If using a capillary tube, use a new tube for each sample.
7. When the spots are dry, curl the paper into a cylinder and clip the sides together at the top only (either staple or paper clip).



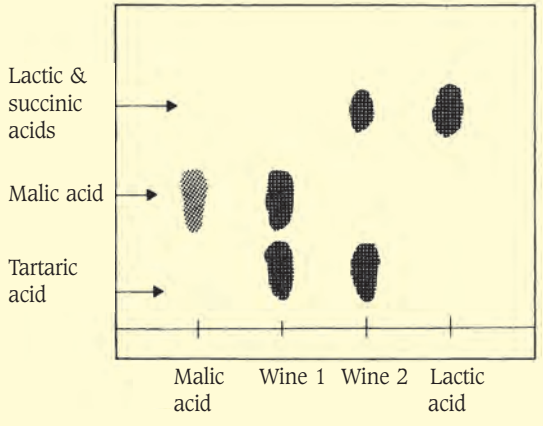
Applying the sample to the paper

● Procedure for setting up the chromatogram

1. Place a sufficient volume of eluting solvent in the chromatography tank (or glass jar with lid) to provide a layer about 0.5 cm deep.
2. Lower the prepared paper into the tank/jar, being careful not to touch the sides of the tank/jar.
3. Place the lid on the tank/jar and leave until the solvent front has risen about 20 cm up the paper (about 3 hours).

● Procedure for assessing the chromatogram

1. After the required time period, remove the paper and allow it to dry in a well ventilated area, away from any contaminating acid or alkaline vapours (about 6 to 8 hours). The paper will develop yellow spots on a green background.
2. Observe the position of each spot and, by comparison with the position of the standard spots, identify the acids present in the tested wine sample (see below). In the example below wine 2 has undergone a MLF.



Assessing the developed chromatogram