

Principle

The total sulphurous acid (dissolved SO₂, sulphite, bisulphite and a fraction of any organically bound sulphite) present in a beverage is driven off as sulphur dioxide by heating the acidified product in a stream of oxygen free nitrogen. The sulphur dioxide is trapped in a receiver flask which contains a dilute, neutralised solution of hydrogen peroxide. The sulphuric acid formed by oxidation of the sulphur dioxide is determined by titration with a sodium hydroxide solution. Since SO₂ may be bound to aldehydes, ketones, anthocyanins or other juice components, adhering to the procedure, in every detail, is crucial. To underline this last point the most critical points have been identified in a separate section.

WORKING INSTRUCTIONS

1. Apparatus and reagents

100 ml capacity heating mantle with, or attached to, a potentiometric control allowing a continuously variable setting. Adjust the setting of the control of the heating mantle as follows.

a) Determination of the correct filling level:

Place the 100ml round bottom flask «A» in the heating mantle and add 65ml of water. Measure the extra volume of water that needs to be added so that the final liquid level is 2 to 3 mm above the rim of the heating mantle. Note the volume, "V" (typically around 10 -15 ml).

b) Determination of the correct setting of the heating mantle:

Preheat the mantle then attach the 100 ml round bottom flask «A» to the condenser, containing (50 + "V") ml of room temperature water. Introduce 15 ml of phosphoric acid from the dropping funnel into the distillation flask and place the flask in the preheated heating mantle. Turn on the nitrogen carrier gas flow for a short moment to allow the acid and water to mix. This point is considered time zero. Note the time it takes for the water to boil vigorously. Adjust the setting up or down and repeat until the time for the water to boil is 7 min +/- 10 seconds.

This setting must be used for all samples. It is crucial that this time interval is achieved reproducibly. If this cannot be achieved the mantle/potentiometer is not suitable!

Distillation apparatus according to the figure given at the end of the method

ortho-Phosphoric acid, p.a., (H₃PO₄ c= 85%)

Hydrogen peroxide solution, (H₂O₂ c = 0.3 %)

Sodium hydroxide solution, (NaOH c = 0.01 mol/l)

Distilled water or similar quality

Sodium metabisulphite (Na₂S₂O₅) p.a. (CAS No 7681-57-4)

Mixed indicator solution:

100 mg methyl red and 50 mg methylene blue are dissolved in 50 ml of 96% ethanol and made up to 100 ml with distilled water.

Purified nitrogen (oxygen free)

Accurate, in-line flow meter

10 ml burette graduated at least in 0.02 ml units.

Note: less accurate gradations will decrease the precision of the method

2. Determination

Pipette 50.0 ml of sample into the 100 ml round bottom distillation flask «A».

Add "V" ml of distilled water as defined above.

Connect the flask to the apparatus.

Place 2 to 3 ml of the 0.3% H₂O₂ solution and 2 drops of the indicator solution into each of the 2 receiver flasks «B»

Carefully neutralise the hydrogen peroxide solutions with the sodium hydroxide solution (0.01 mol/l).

Connect the 2 absorption receivers to the apparatus.

Introduce 15 ml of phosphoric acid (85%) from the dropping funnel into the distillation flask «A». Place the distillation flask into the preheated heating mantle.

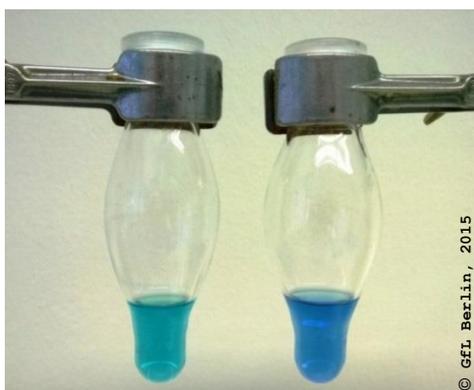
Start the nitrogen carrier gas and adjusted flow to between 30 and 35 l/hour.

This point is considered time zero. End the distillation after 30 minutes.

After the distillation is complete if no colour change is noted in the second flask, only the contents of the first flask needs to be titrated. Remove the first receiver flask and rinse the tip with water, both internally and externally, adding the wash water back into the receiver flask.

If the second receiver flask has changed colour, due to the presence of sulphuric acid, the contents of both flasks are mixed together prior to the titration.

Titrate the sulphuric acid, formed by hydrogen peroxide oxidation of the sulphur dioxide, with 0.01 mol/l sodium hydroxide solution to the green coloured end point.



3. Calculation

The concentration of sulphur dioxide in the sample is calculated by the following expression where 1 ml of 0.01 mol/l NaOH corresponds to 0.32 mg SO₂.

$$\text{SO}_2 \text{ (mg/l) } = a \cdot 6.4$$

where *a* is the titrant volume, in ml, of 0.01 mol/l NaOH

The concentration of the sodium hydroxide solution should be checked using the procedure in IFU No.3 - titratable acidity and if it varies from exactly 0.01 mol/l the concentration of sulphur dioxide in the sample is given by this modified expression:

$$\text{SO}_2 \text{ (mg/l) } = a \cdot 6.4 \cdot c / 0.01$$

where *c* = the actual concentration of the sodium hydroxide in mol/l

4. Statement of results

Express the total sulphurous acid, under reference of this method, in mg/l as sulphur dioxide (SO₂) to one point of decimal.

5. Precision of the method *

Repeatability (r)

$$r = 0.10 x + 0.47$$

Reproducibility (R)

$$R = 0.22 x + 1.62$$

Explanation:

r – repeated analysis of the same sample in one lab, by one person on the same day should not vary by more than 1.5 mg/l, given a theoretical value (x) of 10 mg/l.

R - two results of different labs are expected not to deviate by more than 3.8 mg/l, given a theoretical value (x) of 10 mg/l.

6. Critical control points of the test procedure

The analysis requires special care and experienced users. Adhering to the method in every detail is essential for correct results.

Failure to following the critical control points listed below may lead to false results, both high or low. Quality control measures must be performed with every batch of samples to ensure correct results.

- It cannot be stressed enough how important it is to meet the +/- 10 seconds interval for the “come-up time” to boiling. It has been found that this cannot be achieved reproducibly by all mantels on the market.
- The 7 min +/- 10 sec “come-up time” to boiling must be verified at regular intervals since the performance of heating mantels changes with time.
- The concentration of sodium hydroxide can change over time especially if it is allowed to come into contact with the carbon dioxide in the air. Regular checks of its concentration must be performed (see IFU 3).
- The glassware may contain acidic residues. Verify such residues are absent by performing a distillation using distilled water in place of the sample. All parts of the distillation apparatus must be scrupulously clean.
- Ensure that the apparatus is gastight. Use grease and / or PTFE sleeves and use joining clips.
- The temperature of the water flowing through the reflux condenser should be less than 15°C. Higher temperatures may lead to other substances being recovered in the receiver flask.
- If the heating area of the heating mantle is in contact with the part of the flask above the liquid level there is the possibility of pyrolysis of the sample. This must be avoided at all costs. Pyrolysis of the sample may result in artificially high results.
- The heating mantle must be the correct size for the distillation flask. Use of a heating mantle which is too large may result in inadequate heat transfer.
- At the start of the distillation the liquid level in the flask must be 2 to 3 mm above the rim of the heating mantle.



7. Recovery

The procedure must be subject to periodic verification by the performance of a recovery exercise.

Weigh approximately but accurately 20-30 mg of sodium metabisulphite (Na₂S₂O₅) of analytical grade into a 100 ml volumetric flask and note the accurate weight in milligram, preferably with one decimal place (W). Fill the flask to the mark with distilled water. Use a 1:10 dilution of this standard (5ml in a 50 ml flask) as a normal sample and note the result. This solution is not stable and must be freshly prepared each time a recovery is to be checked.

The recovery is calculated according to the following formula. It should be between 85% and 105%. Values outside these limits must be investigated and the problem rectified. The results of any samples which have been analysed between this recovery check and the previous successful one should be considered as suspicious and these samples should be re-analysed.

$$\text{recovery}[\%] = \frac{\text{Standard sample result [mg/l]}}{0.674 \cdot W}$$

(potassium metabisulphite may also be used; but the given formula has to be corrected for the difference in molecular weight of the two salts)

8. Bibliography

Determination of total sulphur dioxide. Method No. 7a (1987)

In : the collected Analyses of the International Federation of Fruit Juice Producers. Loose-leaf edition as of 1991 - Zug: Swiss Fruit Union

International collection of methods for the analysis of wines, method A 17 Edited by Office International de la Vigne et du Vin, 1, rue Roquepine, F-75008 PARIS

Official journal of the European Communities (3 October 1990), L272, Volume 33, 15 – 34. Measurement of sulphur dioxide in wines and grape musts.

* The original method was tested in a collaborative study, directed by BIPEA Bureau interprofessionnel d'études analytiques, 6-14, Avenue Louis-Roche - F-92230 GENNEVILLIERS – France.

The precision data of this revision was reconfirmed in a small CMA ringtrial

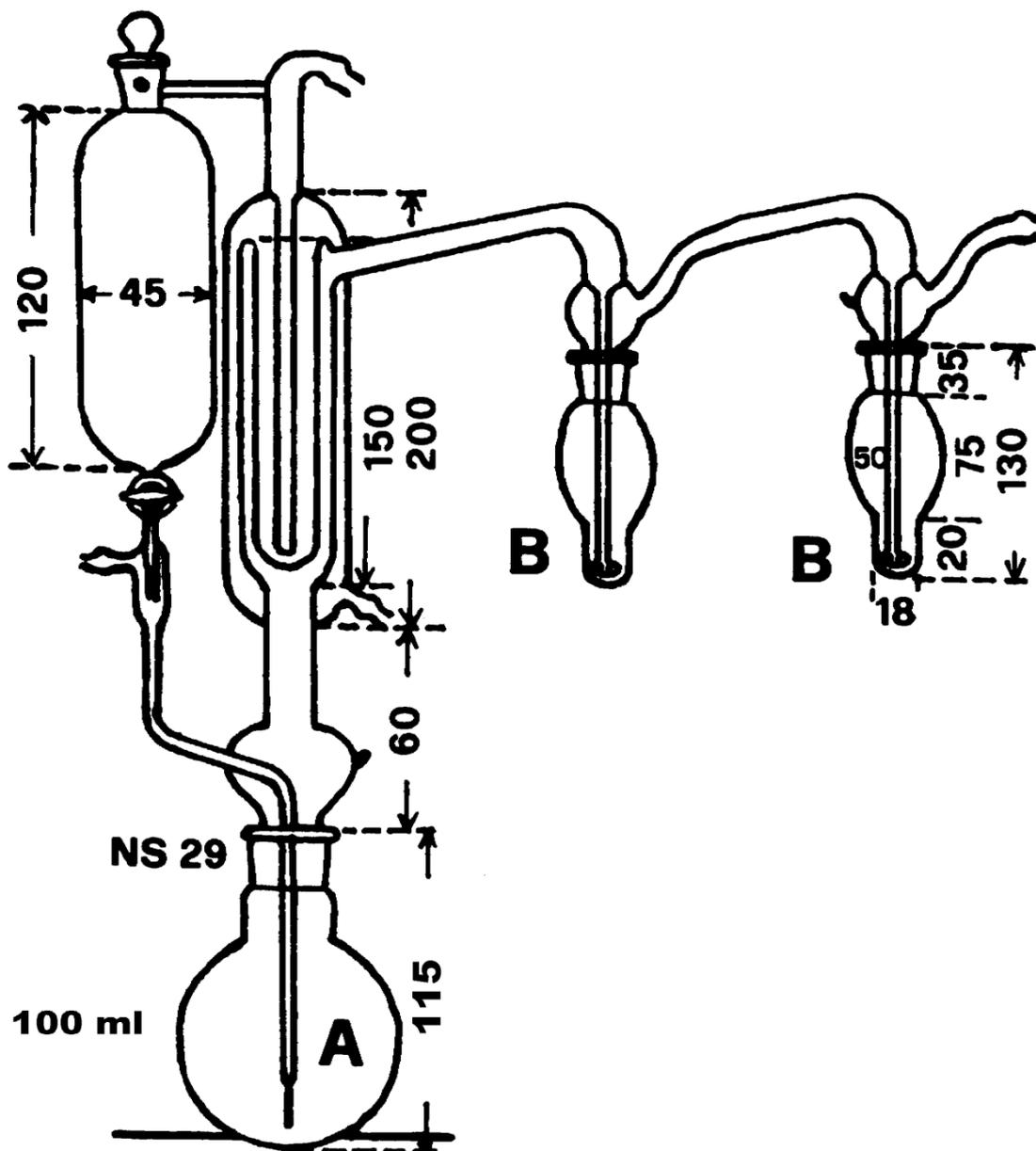


Figure 1: Dimensions are in mm. The internal diameters of the 4 concentrically fixed cooling tubes which form the condenser are 45, 34, 27 and 10 mm.