Measuring Sulfur Dioxide (SO_2) in Wine using a Headspace Gas Detection Tube Method

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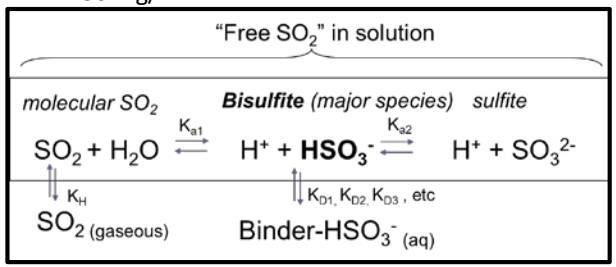
Method adapted from JM Coelho, PA Howe, and GL Sacks. A Headspace Gas Detection Tube Method for Measurement of SO_2 in Wine without Disruption of Sulfur Dioxide Equilibria. *Am. J. Enol. Vitic.* 66:257-265. 2015. doi:10.5344/ajev.2015.14125

Chemical concepts

Sulfur dioxide (SO_2) is widely used as a wine preservative, where it exists in both "free" and "bound" forms (Boulton et al. 1998). The two major forms of Free SO_2 in wine are:

- Molecular SO_2, which contributes anti-microbial activity; Typical targets for molecular SO2 are 0.4-0.6 mg/L for dry wines, and 0.8-1.0 mg/L for sweet wines.
- Bisulfite ions, which are the major participants in reactions with oxidation products, and also the major species (>95%) at wine pH. A typical target for Free SO_2 (bisulfite + molecular) is 20-30 mg/L.



Measuring SO_2 is important during wine production and storage to ensure wine stability, as Free SO_2 forms will decrease due to binding, volatilization and oxidation reactions (Boulton et al. 1998). Common methods used to analyze SO_2 during commercial wine production generally fall into one of two approaches.

- Direct oxidation methods, most commonly iodometric titration ("Ripper method")
- "Acidify-then-separate" methods. For example, aeration-oxidation (A-O), in which SO_2 is stripped from an acidified wine sample by a stream of air, before quantification. A related method is flow injection analysis (FIA).

These standard methods can result in the over-estimation of free and molecular SO_2 (Boulton et al 1998; Coelho et al. 2015). This is due to the dissociation of weakly bound SO_2 adducts, especially anthocyanin-bisulfite adducts in red wine, following acidification, dilution, and/or reacting with other reagents while sampling (Burroughs 1975; Boulton et al. 1998).

The HS-GDT method was developed as a low cost and relatively simple method to accurately measure SO_2 in wine while avoiding disturbances to a wine sample's equilibrium (Coelho et al. 2015). The method relies upon the use of a syringe to draw a sample of wine. A known volume of headspace (HS) is then created above the wine, the syringe is equilibrated, and the headspace expelled through a colorimetric gas detection tube (GDT). Molecular SO_2 concentrations can then be quantified with Henry's law coefficients (K_H). Free SO_2 can then be determined from molecular SO_2 and the pH using the Henderson-Hasselbalch equation (Boulton, et 1998).

Troubleshooting/Sources of Error

Incorrect headspace volume: the volume of wine sampled and headspace drawn must be accurate.

Apparatus leaks: The HS-GDT apparatus should be assessed for leakages by submersion into a water bath. With the stopcock closed, lightly depress the syringe to generate pressure and determine if leakages occur. A spent GDT should then be attached for further assessment for leaks at the GDT-tubing connection.

Rapid expulsion through GDT: Measurement using the Gastec GDT relies upon a reaction of SO_2 with $BaCl_2$ to generate HCl, resulting in a color change of a pH sensitive dye. This reaction occurs at a rate proprietary to their pump device. Expelling the headspace through the tube at a rate great than 50 mL per 10 s will adversely affect measurements.

References

Boulton, R., V. Singleton, L. Bisson, R. Kunkee. 1998. Principles and Practices of Winemaking. Aspen Publishers, Inc., Gaithersburg.

Burroughs, L.F. 1975. Determining Free Sulfur-Dioxide in Red Wine. American Journal of Enology and Viticulture 26:25-29.

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Margalit, Y. 2012. Concepts in Wine Chemistry, 3rd edition. Wine Appreciation Guild, San Francisco.

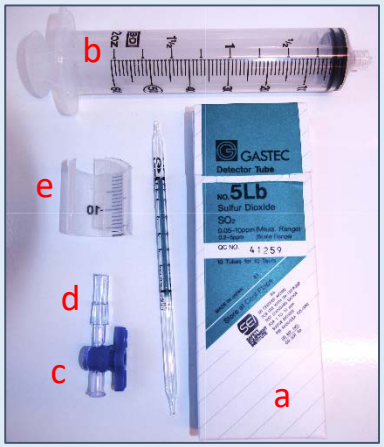
Usseglio-Tomasset, L. and P.D. Bosia. 1984. La prima constant di dissociazione dell'acido solforoso. Vini d'Italia. 26:7-14.

Sulfur dioxide (SO_2) by the Head Space Gas Detection Tube - Protocol

Apparatus components

The HS-GDT apparatus consists of:

a. Gastec #5Lb Sulfur dioxide detector tube ($75 for 10 tubes)
b. 60 ml BD syringe with Luer-Lok tip
c. 1-way polycarbonate stopcock with male Luer-Lok connection
d. a short piece of silicone tubing, approximately 3mm ID x 6mm OD
e. 3 cm plastic dispensing stop (made from cutting a syringe)



Other info required for calculations

- pH of the wine sample
- Temperature during analysis
- Alcohol content of wine
- Measurements of manufacturers tube markings (see next page)

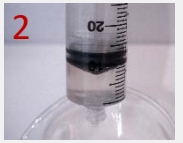
Procedure for measuring SO_2

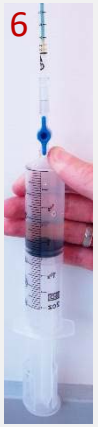
1. Rinse syringe by drawing a 60 mL wine sample into the syringe, then expel.
2. Draw a 10 mL wine sample into the syringe.
3. Invert the syringe, attach the Luer-Lok stopcock in the open position and draw a 50 mL headspace.
4. Close the stopcock immediately and allow sample to equilibrate tip-up for 5 minutes.
5. Affix dispensing stop to syringe. Attach GDT to the syringe apparatus via the piece of silicone tubing connected to the stopcock, ensuring the tube's arrow points in the direction of flow.

   Note: If the GDT has not been used, carefully remove both glass ends. If the GDT is used, ensure the "start" point of measurement is clearly marked with a fine point marker.
6. Once the sample has equilibrated for 5 minutes, open the stopcock and dispense the headspace through the GDT at a constant rate. It should take ~10 seconds to dispense the 50 mL of headspace. DO NOT push liquid into the GDT.
7. Allow the colorimetric reaction to stabilize for 1 minute, and then mark the reaction endpoint with a fine point marker.
8. Measure the length of GDT colorimetric reaction in millimeters with a ruler, from the start point to end point of each sample.

   Note: GDT may be re-used SO_2 analysis may be repeated with the GDT, until the tube is fully used.

   Note: The smallest detectable change for a GDT is ~1 mm. Detection limits for the method can improved by repeating steps 1-6 for the same wine – the Coelho et al. 2015 paper reported using 200 mL (4 repeat sample cycles) for low SO_2 wines





Online calculator tool

An online calculator can then be used to convert ruler measurements to SO_2 concentrations. The calculator also provides a wine matrix corrected conversion between molecular and free SO_2, and shows matrix effects on pK_a.
https://grapesandwine.cals.cornell.edu/extension/grape-and-wine-resources/

Sulfur dioxide (SO_2) by the Headspace Gas Detection Tube – more info

"Why are my numbers lower than for A-O or Ripper , particularly in reds?"

Standard methods cause the dissolution of some weakly bound forms of SO_2, particularly anthocyanin-bisulfite complexes in reds. Using HS-GDT, most red wines will have SO_2 values 30-60% of that measured by standard approaches.

Currently, we recommend lower target values when using HS-GDT on reds: at least 0.2-0.3 mg/L molecular and 10-15 mg/L free SO_2 for dry red wines. Standard recommendations for dry whites (0.4-0.6 mg/L molecular and 20-30 mg/L free) still apply. However, the appropriateness of these recommendations for both standard and HS-GDT methods are still under investigation.

Converting GDT values

The darkening of Gastec gas detection tubes is non-linear with respect the vapor-phase concentration of SO_2. While the ppm concentration can be approximated visually, this can be done more accurately using the online calculator to relate the manufacturer markings of vapor phase concentration (P_{SO2}) of SO_2 (ppm) to the scale length (mm) along the tube.

Thus, as a first step, we recommend measuring the positions of the manufacturers' marks on the GDT tubes and inputting the values into the online calculator. The marks are usually stable within the same lot of tubes.

<u>Example of measuring manufacturer markings of vapor phase SO_2 concentrations (above)</u>

On the online calculator, input 0.2 mm for 0.2 ppm, 4.7 mm for 0.5 ppm, 9.8 mm for 1 ppm, and so on. Then, input start = 0 mm, stop = 22 mm.

Explanation of calculations

SO_2 vapor pressure (P_{SO2}) is calculated from the start ($f(x_{start})$) and end points ($f(x_{end})$) measurements of the sample measured in (mm), and adjusted for the volume of headspace sampled.

$$P_{SO2} = (f(x_{end})-f(x_{start})) * \frac{200\ \text{mL}}{\text{sample headspace (mL)}}$$

Henry's Law coefficient, $K_{H(T)}$, must be temperature corrected for the temperature (T) (^{O}K) during analysis.

$$K_{H(T)} = 0.38\text{ATM M}^{-1} * \exp\left(3100*\frac{1}{294} - \frac{1}{T}\right)$$

Molecular SO_2 (mg/L) is calculated from P_{SO2} using the temperature corrected Henry's Law coefficient and the molecular mass of SO_2.

$$\text{Molecular } SO_2\ \left(\frac{mg}{L}\right) = \frac{P_{SO2}}{K_H} * 0.064$$

Free SO_2 is calculated from molecular SO_2, the sample pH, and a literature value for pK_a of SO_2 as a function of ethanol concentration and ionic strength (Usseglio-Tommasset 1984) .

$$\text{Free } SO_2\ \left(\frac{mg}{L}\right) = [\text{molecular } SO_2](1+10^{pH-pKa})$$

Checking the method
Accuracy and precision

To test precision and accuracy of the method, a model wine and SO_2 solution should be prepared. A model wine may be prepared by adding 5 g/L potassium bitartrate to 10% v/v ethanol.

A 1000 mg/L SO_2 stock solution should be prepared by dissolving potassium metabisulfite (KMBS) in a solution of (10% v/v) methanol or ethanol – SO_2 can autooxidize in pure water.

KMBS is only 57% by weight SO_2. The working standard should be diluted appropriately into the model wine to achieve a 50 mg/L SO_2 model wine solution.

For example:

- Prepare a 200 mL model wine by adding 1 g potassium bitartrate to 10% v/v ethanol.
- Prepare 200 mL of 1000 mg/L SO_2 working standard by dissolving 0.351 g potassium metabisulfite in 10% v/v MeOH or EtOH.
- Accurately dilute 10 mL of SO_2 working standard into model wine.
- The resulting wine will have 50 mg/L free SO_2 . The molecular SO_2 will be approximately 0.8 mg/L at 20 $^{\circ}$ C, but the exact value can be calculated using the calculator (page 2).

To test precision and accuracy, 5 replicates can be run.
For accuracy, the average value should be within ±10% of the expected value, e.g. 0.7-0.9 mg/L in the previous example. For precision, replicates should not differ by more than 0.3 mg/L