REPORT

Analysis of Free Hydrogen Sulfide in Wines Using Gas Detection Tubes

Cite this article:
Allison RB, Montgomery A and Sacks GL. 2021. Analysis of free hydrogen sulfide in wines using gas detection tubes. Catalyst 6:1-8.

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Supplemental data is freely available with this article.

Manuscript submitted April 2021, revised July 2021, accepted July 2021

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doi: 10.5344/catalyst.2021.21003

Summary

Goals: Hydrogen sulfide (H$_2$S, “rotten egg” aroma) concerns winemakers due to its contribution to sulfur-like off-aromas, and convenient methods are needed for quantitation of H$_2$S in wines at or below its reported odor threshold. The use of selective, colorimetric gas detection tubes (GDTs) for measurement of H$_2$S during fermentation has been previously described, but this approach has not been adapted and validated for finished wines. We developed and validated protocols for rapid, inexpensive analysis of H$_2$S using GDTs and aeration-oxidation (A-O) glassware commonly available in wineries. Video demonstration of the approaches is provided (Supplemental Video 1).

Key Findings:

• Two approaches were validated for GDT-based quantitation of H$_2$S in wine. In the first approach, H$_2$S was sparged from the sample with N$_2$ gas, analogous to Monier-Williams analyses of SO$_2$. In the second approach, H$_2$S was sparged by a vacuum-generated air stream, analogous to A-O analyses of SO$_2$.

• Both approaches require <15 min/sample and achieve excellent linearity. The calibration curve for the N$_2$ Method was identical to the curve predicted from the manufacturer’s markings. The Aspiration Method was less sensitive, likely because of oxidative losses, but this method was simpler to set up, operate, and adapt to samples with higher concentrations.

• The limits of detection were 12 to 13 ng H$_2$S for the methods, or ~0.2 µg/L using a 60-mL sample. The mean coefficients of variance were <5% for both approaches.

• Using these methods, we observed that commercial wines stored in aluminum cans have significantly higher H$_2$S content than commercial wines in glass packaging.

Impact and Significance: Wineries can use these novel methods for routine H$_2$S analysis without significant equipment investment. In addition to cost savings, the ability to test H$_2$S onsite, rather than sending samples to an external lab, decreases risk of H$_2$S losses through oxidation or volatilization. These new analytical tools can be used for benchmarking, diagnosing faulty wines, or evaluating the effects of winemaking parameters, such as yeast selection, remediation treatments, and packaging options, on H$_2$S.

Key words: gas detection tubes, reduced aromas, sulfide analysis, wine fault

Overview

Sulfur-like off-aromas (SLOs) are one of the most common faults observed in commercial wines. Although several sulfhydryls can contribute to SLOs, hydrogen sulfide (H$_2$S) is the S-compound most frequently reported to be in excess of its sensory threshold (~1 µg/L) in wines with SLOs. H$_2$S may contribute directly to SLOs due to its rotten egg aroma, or its presence could serve as a marker for related malodorous sulfhydryls. H$_2$S can be produced during fermentation through several pathways, including as an intermediary step in the biosynthesis...
of S-amino acids,\(^2\) as a degradation product of elemental sulfur (S\(^0\)) fungicide residues,\(^3,4\) and as a product of the catabolism of S-amino acids (especially cysteine).\(^5\) H\(_2\)S formed during fermentation will be partially lost due to CO\(_2\) entrainment\(^6\) and can be further diminished postfermentation by winemaking approaches such as copper addition or aeration.\(^7\) However, these approaches can generate precursor compounds (copper sulfide complexes and organopolysulfanes) capable of releasing H\(_2\)S during the reductive storage conditions typical for bottled wine (i.e., low oxygen in the presence of SO\(_2\)).\(^8,9,10\) Finally, H\(_2\)S is anecdotally reported to form through storage of wine in aluminum cans, possibly from the reaction of SO\(_2\) with the aluminum metal, as well as from the previously described precursors. Thus, enologists may wish to quantify H\(_2\)S not only to determine its potential contribution to faulty wines but also to evaluate winemaking parameters such as yeast selection, fermentation conditions, remediation treatments, and packaging options.

Levels of H\(_2\)S in wines are in the range of 1 to 20 µg/L at the end of fermentation,\(^11\) and its low concentration and high reactivity require the use of specialized analytical approaches. Early reports on H\(_2\)S quantification in wines generally relied on laborious wet-chemical approaches, such as capturing sparged H\(_2\)S with a Cd(OH)\(_2\) solution followed by redox titration with methylene blue.\(^12\) More recent reports have used gas chromatography (GC) coupled with a range of detectors, including pulsed-flame photometric detection\(^13\) (PFPD) and sulfur chemiluminescence detection.\(^1\) These methods offer excellent detection limits (<1 µg/L) and high selectivity but are inappropriate for use in most commercial wineries due to the expense of the equipment and the specialized skill necessary for their operation. Furthermore, H\(_2\)S is highly volatile and readily oxidized, requiring considerable precautions during sample handling.

A modern version of classic colorimetric approaches uses gas detection tubes (GDTs) for selective H\(_2\)S quantification. Originally developed for the mining industry, GDTs for H\(_2\)S consist of glass tubes filled with an inert packing coated with an appropriate indicator compound (e.g., lead acetate). As H\(_2\)S flows through the tube, it reacts irreversibly and causes a discoloration such that the length of the stain is proportional to the mass of H\(_2\)S passing through the tube. An example of discoloration in different GDTs is provided in Supplemental Figure 1.

The first reported use of GDTs to measure H\(_2\)S in enological studies was the measurement of total H\(_2\)S produced by yeast strains during small-scale fermentations.\(^14,15\) In those studies, the CO\(_2\) produced during fermentation was used to force H\(_2\)S through the GDTs, an approach that is not viable for postfermentation wines without CO\(_2\). For postfermentation H\(_2\)S analysis, our

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**Figure 1** Apparatus for measuring hydrogen sulfide (H\(_2\)S) using (A) inert gas (N\(_2\)) sparging and (B) vacuum aspiration. The sample flask is connected to an impinger, and the gas detection tubes are connected to the outlet, in series.
Major Observations and Interpretations

Apparatus and materials. The principle for the proposed H$_2$S detection method is based on GDT protocols previously developed for measurement of S$^0$ residues in grape must or H$_2$S formed following release from wine precursors or during the course of fermentation.\textsuperscript{14,15,16,17} The current approach uses either inert nitrogen gas (N$_2$ Method) or vacuum aspiration (Aspiration Method) to sparge H$_2$S from the sample and through the GDT tube (Figure 1A and 1B), resulting in a discoloration of the tube (Supplemental Figure 1). The flask and tubes are connected in series through PVC tubing. A sufficient length of PVC tubing (15 cm or more) between the sample flask and the first GDT is recommended to prevent splashing of water droplets and fouling of the GDT inlet. A demonstration of these approaches can be found in the accompanying video (Supplemental Video 1). In contrast to earlier studies that relied on antacid tablets to evolve CO$_2$ gas in situ, these new approaches did not cause pH changes or dilution of the sample that could have risked release of H$_2$S from precursors such as copper-sulfhydryl complexes.\textsuperscript{16} The antacid-based approaches also required opening and resealing the apparatus to sequentially add tablets, which risked loss of H$_2$S due to volatilization.

As described in detail below, the N$_2$ and Aspiration Methods both yield satisfactory results. However, the Aspiration Method is easier to use in practice, as the N$_2$ Method can overpressure and dislodge the GDT or other components, resulting in leaks. The Aspiration Method also allows easy replacement of the GDT if the tube becomes saturated during a run. Unlike the Aspiration Method, the N$_2$ Method produced a calibration curve identical to the curve calculated from the manufacturer’s GDT markings and thus may require less frequent calibration (Figure 2).

GDT selection and interference from SO$_2$ and other sources. The Gastec 4LL and 4LT GDTs are not susceptible to interference from most wine components, including sulfate, acetic acid, or water vapor.\textsuperscript{3} Several other compounds listed by the specification sheets for these GDTs (such as ozone, nitrogen dioxide, nitric acid, hydrogen chloride, hydrogen fluoride, and ammonia)\textsuperscript{18}
are unlikely to be important to wine analyses. However, there are two potentially relevant interferences: thiols (mercaptans) and SO₂. Thiols are reported to be a source of interference for the more sensitive 4LT tubes (which contain mercuric chloride [HgCl₂]) but not for the 4LL tubes (which contain Pb(CH₃COO)₂). Although the 4LT tubes respond more strongly to thiols than to H₂S, thiols are also less volatile and are typically found at lower concentrations in wine.1 We previously estimated that interference from methanethiol (the thiol of greatest concern due to its volatility and concentration) in a typical wine would be only 25% of the H₂S signal.17 However, in situations where interference from methanethiol or other thiols is of concern, 4LL tubes can be substituted at the expense of decreasing the sensitivity approximately three-fold.³

SO₂ is described as a source of interference for both 4LL and 4LT tubes.18 Earlier reports by our group using GDTs relied on antacid tablets to generate a gas stream, which buffered the pH to ~6 and strongly favored non-volatile forms of SO₂ (i.e., bisulfite and sulfite). However, in initial studies at native pH, we observed considerable interference from model solutions containing SO₂ (data not shown). To remove SO₂ from the gas stream, we investigated three approaches: adding hydrogen peroxide (H₂O₂) or acetaldehyde directly to the sample, using an inline SO₂ GDT to scrub the gas stream, and using an inline acetaldehyde solution.

Direct addition of acetaldehyde was not effective at eliminating the interference (data not shown). Addition of H₂O₂ (0.18% and 0.35% v/v) to the wine sample was effective at removing the SO₂ interference but was not pursued further due to concern about H₂S oxidation.

SO₂ GDTs were effective in eliminating interference from SO₂. The Gastec 5Lb and 5L SO₂ tubes were both tested, and the 5L was ultimately selected for its higher capacity. We also evaluated the use of an inline solution of acetaldehyde (1.5% v/v; Figure 1B, Item C). Alone, the inline acetaldehyde solution was unable to fully remove SO₂ interference, but inclusion of an acetaldehyde trap before the SO₂ GDT had the advantage of preserving the lifetime of the SO₂ GDT. If this approach is used, the acetaldehyde solution should be replenished when the SO₂ GDT is replaced.

GDTs respond to the initial mass of H₂S, not the initial concentration, and we were able to vary the sample volume of model wine or Milli-Q water over a wide range (6 to 66 mL) without affecting the relative response (Supplemental Figure 2). We also observed that the stain length stopped increasing after 5 min for volumes ≤10 mL. However, volumes ≥60 mL required at least 10 min analysis time before tube staining (and, thus, H₂S sparging) was complete. We selected a 10-min analysis time for further experiments, but shorter analysis times of 5 min may be appropriate for sample volumes of 10 mL or less.

**Calibration and figures of merit for H₂S GDTs.** Figures of merit for the N₂ and Aspiration Methods are summarized in Table 1. We observed a linear relationship (r² > 0.99) between the length of color change on the H₂S GDT and the nominal concentration of the calibration standards for both methods (Figure 2).

(i) **N₂ Method**: Length of color change (mm) = 0.100 × H₂S (in ng)

(ii) **Aspiration Method**: Length of color change (mm) = 0.068 × H₂S (in ng)

Theoretical calibration curves based on the manufacturer-provided markings (Supplemental Figure 2) were also plotted, along with a curve from a previous report which used antacid tablets as an in situ gas source (Figure 2).¹⁷ The slope of the theoretical curve based on manufacturer markings (dotted line) was identical to the slope observed for the N₂ Method. However, the slope of the Aspiration Method was ~30% lower than the theoretical slope, with a similar value reported for the earlier antacid-tablet method (dashed line).

The close agreement between the N₂ Method slope and the theoretical slope based on manufacturer markings is a potential advantage for this method over the Aspiration Method, as the N₂ method could potentially be used with less extensive calibration. The lower sensitivity of the Aspiration Method could be due to partial losses of H₂S from oxidation during the analyses, as the sample was not protected from air in this analysis. The reason for the lower-than-theoretical sensitivity of the antacid method is less clear, as the evolved CO₂ gas should have created an anoxic environment. However,
the antacid method required opening and closing the reaction flask to add additional tablets, which could have introduced air.

Coefficients of variance (%CV) were calculated for each of the five calibration standards for each method (n = 3 replicates for each standard), and the repeatability calculated as the mean %CV. We observed excellent repeatability (%CV <5%; Table 1). We also observed excellent within-lab reproducibility (Figure 3). Two standard solutions (n = 16 total) were run at regular intervals over a four-week period without recalibrating the tubes. Data were normalized to the expected value. We observed a 95% confidence interval of 92 to 97% recovery (ideal = 100%) of the expected signal over the four-week experiment.

Based on noise calculations for the lowest calibration standard on the 4LT GDTs, we calculated limits of detection of 13 ng H$_2$S for the N$_2$ Method and 12 ng H$_2$S for the Aspiration Method. As previously reported for GDT methods, the limit of detection (LOD) is primarily determined by the smallest observable change (0.5 to 1.0 mm) in stain length. This can be more challenging for the 4LT tubes than for other tubes due to the subtle color change (yellow to pink). Using a 60-mL sample volume, we could achieve a detection limit of 0.2 µg/L. This value is below the reported odor threshold for H$_2$S in wine$^1$ and compares favorably to detection limits reported with more expensive technologies. For example, a detection limit of 0.2 µg/L was reported for H$_2$S using GC and a sulfur chemiluminescence detector,$^1$ whereas quantitation limits of 1.0 and 1.7 µg/L were reported using GC-PFPD.$^{13,14,15,16,17,18,19}$

As mentioned above, 4LT tubes achieve the best sensitivity and detection limits but suffer from interference from thiols,$^{14}$ and the use of the less-sensitive Gastec 4LL tube may be prudent when thiol interference is suspected. Furthermore, the cost of a box of 10 GDTs is ~$70 USD, and tubes cannot be regenerated. Although multiple samples can be analyzed on one GDT until the tube is exhausted, high H$_2$S concentration samples can quickly exhaust the capacity of a Gastec 4LT (~500 ng of H$_2$S). In practice, we typically used a GDT only once before disposal, particularly when handling samples of unknown H$_2$S concentration, because we did not want to risk saturating the GDT. However, we did reuse tubes that were not yet saturated when the expected concentration was known (such as with calibration standards). In general, we recommend using the 4LT tube and 60-mL sample size for low-concentration samples (less than ~5 µg/L), and using the 4LL tube when measuring higher-concentration samples or when high levels of thiol interference are expected.

**Analysis of commercial wines.** A convenient sample of 12 commercial wines (six bottled, six canned) were purchased from local stores and evaluated using the GDT methods (Figure 4). We observed an average H$_2$S con-
Concentration in the bottled wines of 1.1 ± 0.9 µg/L, a range consistent with values reported elsewhere. The mean H$_2$S in the canned wines was 13.5 ± 9.9 µg/L—significantly higher than that in the bottled wines ($p < 0.05$). The highest concentration of H$_2$S in the canned wines was nearly 30 µg/L, comparable to the highest values observed in a survey of commercial wines described as “reduced.” These observations concur with recent anecdotal observations that wines stored in aluminum cans will develop H$_2$S during storage due to anoxic conditions and degradation of H$_2$S precursors, as well as reaction of SO$_2$ with the aluminum metal to form H$_2$S. As a caveat, the effects of packaging may have been confounded with other variables, such as production practices used for wines destined for cans; the higher levels of H$_2$S in canned wine are not necessarily due to interactions between the can and the wine.

**Broader Impact**

We have described and validated two convenient, inexpensive approaches to measuring free H$_2$S in commercial wine samples using selective GDTs and glassware from an A-O unit. With 60-mL sample volumes, limits of detection were achieved that are below the sensory threshold in wine. The calibration produced using the N$_2$ Method is identical to the calibration calculated from the manufacturer’s markings on the 4LT GDT, suggesting that the method could be used without the need for regular calibration. In contrast, the Aspiration Method was easier to perform, making it more practical in winery settings, but should be regularly calibrated with standard solutions. By allowing on-site measurement with minimal resources, both methods avoid the risks of H$_2$S loss associated with sending samples to off-site labs. In addition to diagnosing faulty wines, these analytical tools can be used for benchmarking and evaluating the effects of winemaking choices (e.g., packaging and fining trials). Finally, although not the subject of the current investigation, the method could also likely be adapted for analysis of H$_2$S precursor forms by appropriate pretreatment of the wine (e.g., addition of brine to release H$_2$S from copper-sulphhydryl complexes).

**Experimental Design**

**Chemical reagents.** Ethanol (140 and 190 proof) was purchased from Koptec. L(+)-tartaric acid (99%) and H$_2$O$_2$ (35% w/w) were purchased from Acros Organics. Sodium hydroxide solution (NaOH, 50% w/w) was purchased from Fisher Chemical. Sodium sulfide nonahydrate (Na$_2$S·9H$_2$O, 98%) was purchased from Beantown Chemical. Silicone oil was purchased from Sigma-Aldrich. Acetaldehyde (99%) was purchased from Alfa Aesar. Deionized, distilled water with a resistance of 18.2 MΩ × cm at 25°C was provided by a Milli-Q system (Millipore Sigma) and used for all experiments. Nitrogen gas (N$_2$, UHP) cylinders were supplied by Airgas USA LLC. Samples were held in a temperature-controlled incubator at 10°C until use.

**GDTs.** Commercially available GDTs (Gastec International) for the analyses of H$_2$S (Gastec 4LT) and SO$_2$ (Gastec 5L) were purchased from Airgas and W.W. Grainger, respectively. The H$_2$S 4LT tubes rely on the reaction of H$_2$S with HgCl$_2$, resulting in a color change from yellow to pink. The SO$_2$ 5L tubes rely on the reaction of SO$_2$ with BaCl$_2$ to generate HCl, resulting in the appearance of a yellow color. In the method presented here, SO$_2$ GDTs are used only for filtering the gas stream of any volatilized SO$_2$ to prevent interference in the H$_2$S tube; the Gastec 5L GDTs are not appropriate for quantification of SO$_2$ in this method. GDTs should be stored at a cool temperature, refrigerated at or below 10°C, or as indicated on the package. After opening, a GDT may be used reliably for several sequential analyses within one day (data not shown) but should be replaced with a new tube each day.

**Quantitation of free H$_2$S in wines using GDT with N$_2$ sparging (N$_2$ Method).** The N$_2$ Method is depicted in Figure 4.
in Figure 1A. For each analysis, a volume of wine sample (up to 60 mL) was added to a 100-mL round-bottom flask (A). For red wines, four to five drops of silicone oil were added to decrease foaming. One neck of the flask was fitted with a fritted impinger (B) from an A-O unit (Adams & Chittenden Scientific Glass Coop); the impinger was connected to an N₂ cylinder on the inlet side (C). The outlet side was connected by PVC tubing to an SO₂ scrubber (see next subsection), such as an SO₂ GDT (D) followed by an H₂S GDT (E). The sample was sparged with N₂ for 10 min at ambient temperature (~20°C), and the length of color change on the 4LT tube was measured. The flow rate was ~100 mL/min, as faster flow rates would occasionally dislodge the GDT. Under these conditions, negligible change in the length of the GDT stain was observed after 10 min.

Quantitation of free H₂S in wines using GDT with vacuum aspiration (Aspiration Method). In the Aspiration Method (Figure 1B), wine samples were added to a 100-mL pear-shaped flask (A) and attached to a fritted impinger (B) left open to atmosphere. The outlet side was connected by PVC tubing to an SO₂ scrubber (see next subsection), such as a flask containing a 1.5% by volume acetaldehyde solution (C) and an SO₂ GDT (D) followed by an H₂S GDT (E). The outlet of the H₂S GDT was attached by PVC tubing to a vacuum source (F). The sample was vacuum aspirated for 10 min, and analyses were carried out at room temperature (~20°C), with the length of color change on the 4LT tube measured. Unlike the N₂ method, we encountered no issues with dislodging the tubes, as the highest gas flow rate we could achieve was <100 mL/min.

Removal of SO₂ interferences in N₂ and Aspiration Methods. Three strategies were evaluated for preventing SO₂ interference in the Gastec 4LL and 4LT tubes: inserting an SO₂ GDT in series before the H₂S GDT, as shown in Figure 1A Item D or Figure 1B Item D; pretreating the sample with H₂O₂ or acetaldehyde; or inserting a 100-mL pear-shaped flask containing 10 mL of 1.5% (v/v) acetaldehyde solution between the sample flask and the GDT, as shown in Figure 1B Item C. In our evaluations, the first and third strategies were carried out as described in the methods above. For the second strategy, samples were pretreated by adding an aliquot of the oxidizing agent directly to 60 mL of a commercial white wine previously observed to cause interference in the 4LT GDT. H₂O₂ solution was prepared at 3.5% (v/v) and added in quantities of 1, 3, and 6 mL, corresponding to 0.06 to 0.35% (v/v). Acetaldehyde was added in quantities of 5 µL, 25 µL, 50 µL, and 100 µL, corresponding to 0.01 to 0.17% (v/v).

Effects of sample volume and analysis time. To evaluate the effects of sample volume on response, we varied the sample volume from 6 to 66 mL while analyzing the H₂S calibration standards (described below) by the N₂ Method. A response factor (length of stain in mm/ng H₂S) was then calculated for each condition. Analysis time was initially determined by recording the time when the color change ceased on the H₂S GDT for 6 and 10 mL samples of model wine or Milli-Q water spiked with H₂S. Aliquots of H₂S standard solutions were added to the selected sample volume, and analysis of free H₂S was carried out as previously described. For sample volumes ≥60 mL, the analysis time was increased to 10 min to reach the point when no additional color change was observed. Following optimization, analysis times of 10 min were used for all runs.

Method calibration. A working solution of Na₂S · 9H₂O (50 µM) was freshly diluted from a standardized stock solution (5 mM) every 48 hrs, and the stock solution was newly prepared every 2 wks. Stock solutions were stored in the refrigerator when not in use. Calibration standards of 0.017, 0.025, 0.050, 0.100, and 0.150 µM H₂S were prepared in model wine (1% alcohol v/v, 5 g/L tartaric acid, pH adjusted to 3.5 by dropwise addition of NaOH). Calibration curves were prepared by plotting “length of stain (mm)” versus “mass H₂S (ng)”.

The observed slopes from each approach were compared with the slopes indicated by the manufacturer’s markings on the GDT tubes. Because the GDT markings are reported in units of ppm (v/v), the values were converted to units of “ng H₂S” using the Ideal Gas Law and the manufacturer’s suggested 100 mL gas volume for air sampling. Details of the conversion calculation are provided in the Supplemental Calculation.

Reproducibility. In-lab reproducibility was assessed for the N₂ Method. An addition of Na₂S · 9H₂O working solution (50 µM) was measured in Milli-Q water at two concentrations, 1.55 µg/L (n = 9) and 3.10 µg/L (n = 7), over the course of four weeks.

Figures of merit. Calibration curves were used to determine the linear range. The LOD was calculated as 3.3 × standard deviation for the lowest concentration standard (0.6 µg/L, or 34 ng using a 60-mL sample volume) within the linear range (0 to 5.1 µg/L, or 0 to 307 ng using a 60-mL sample volume). Standard deviations were determined for each set of calibration standards, and the coefficient of variance was calculated as the mean of these values.

Evaluation of commercial wines. A convenient sample of commercial bottled (n = 6, two red, four white) and canned (n = 6, two white, two rosé, two rosé sparkling)
wines was purchased from local retailers (Ithaca, NY) and represented a range of regions and cultivars. Vintages ranged from one to three years old, although a few products were labeled as "non-vintage". Bottled samples were analyzed for H₂S in duplicate, and canned samples were analyzed in triplicate.

**Statistical Analysis.** JMP Pro 14 (SAS Institute, Inc.) was used for statistical analysis.

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