ASEV CATALYST REPORT

Analysis of Free Hydrogen Sulfide in Wines Using Gas Detection Tubes

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Acknowledgments: The authors thank the Natural Science and Engineering Research Council of Canada for providing a doctoral research scholarship to R.B.A. Financial support from the Saltonstall Wine Endowment and the New York Wine and Grape Foundation are also gratefully acknowledged. All contributing authors have approved the manuscript submission and that the manuscript is not currently under review with another publication.

Manuscript submitted April 16, 2021, revised July 8, 2021, accepted July 12, 2021

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Summary

Goals: Hydrogen sulfide (H₂S, “rotten egg” aroma) concerns winemakers due to its contribution to sulfur-like off-aromas (SLOs). However, there are a lack of inexpensive, convenient methods for quantitation of H₂S in wines at or below its reported odor threshold. The use of selective, colorimetric gas detection tubes (GDT) for measurement of H₂S 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 H₂S using GDTs and

Aeration-Oxidation (A-O) glassware commonly available in wineries. Video demonstration of the approaches is also provided ([Supplemental Video 1](#)).

Key Findings:

- Two approaches were validated for GDT-based quantitation of H₂S in wine. In the first approach, H₂S was sparged from the sample with N₂ gas, analogous to Monier-Williams analyses of SO₂. In the second approach, H₂S was sparged by a vacuum-generated air stream, analogous to A-O analyses of SO₂.
- Both approaches require <15 min/sample and achieve excellent linearity. The calibration curve for the N₂ Method was identical to the curve predicted from the manufacturer's markings. The Aspiration Method was less sensitive, likely because of oxidative losses. However, the Aspiration Method was simpler to set up, operate, and adapt to higher concentration samples.
- The limits of detection were 12-13 ng H₂S for the methods, or ~0.2 µg/L using a 60 mL sample. The mean coefficients of variance (%CV) were <5% for both approaches.
- Using the new method, we observed that commercially purchased wines stored in aluminum cans have significantly higher H₂S than commercial wines in glass packaging.

Significance: The novel methods can be used for routine H₂S analysis in wineries without the need for significant investment in new equipment. In addition to cost savings, the ability to test H₂S onsite rather than send samples to an external lab decreases the risk of H₂S 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 H₂S.

Keywords: 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 wines¹. Although several sulfhydryls can contribute to SLOs, hydrogen sulfide (H₂S) is reported to be the *S*-compound most frequently in excess of its sensory threshold (~1 µg/L)¹ in wines with this fault. H₂S may contribute directly to SLOs due to its rotten egg aroma and/or its presence could serve as a marker for other related malodorous sulfhydryls. H₂S can be produced during fermentation through several pathways, including as an intermediary step in *S*-amino acid biosynthesis;² as a degradation product of S⁰ fungicide residues;^{3, 4} and through catabolism of *S*-amino acids, especially cysteine⁵. H₂S formed during fermentation will be partially lost due to CO₂ entrainment⁶ and can be further diminished post-fermentation by winemaking approaches like copper addition or aeration⁷. However, these approaches can generate precursor compounds (copper sulfide complexes; organopolysulfanes) capable of releasing H₂S during the reductive storage conditions typical of bottled wine, i.e. low oxygen in the presence of SO₂⁸⁻¹⁰. Finally, H₂S is anecdotally reported to form through storage of wine in aluminum cans, possibly due to reaction of SO₂ with the aluminum metal as well as the previously described precursors. Thus, enologists may wish to quantify H₂S 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.

H₂S is reported to be in the range of 1-20 µg/L in wines at the end of fermentation¹¹, and its low concentration and high reactivity requires the use of specialized analytical approaches. Early reports on H₂S quantification in wines generally relied on laborious wet-chemical approaches, e.g. capturing sparged H₂S with a Cd(OH)₂ solution, followed by redox titration with methylene blue¹². More recent reports utilize gas chromatography (GC) coupled with a range of detectors, including pulsed-flame photometric detection (PFPD)¹³ and sulfur chemiluminescence detection (SCD)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 specialized skill necessary for their operation. Additionally, H₂S is highly volatile and readily oxidized, which necessitates considerable precautions during sample handling.

A modern version of classic colorimetric approaches utilizes gas detection tubes (GDT) for selective H₂S quantification. Originally developed for the mining industries, GDTs for H₂S are composed of glass tubes filled with an inert packing coated with an appropriate indicator compound, e.g., lead acetate. As H₂S flows through the tube, it reacts irreversibly and causes a discoloration such that the length of the stain is proportional to the mass of H₂S passing through the tube. An example of discoloration in different GDTs is provided in the Supplemental Information.

The use of GDTs for measurement of H₂S in enological studies was first reported for measurement of total H₂S produced by yeast strains during small-scale fermentations^{14, 15}. These reports use the CO₂ produced during fermentation to force H₂S through the GDT, an approach that is not viable for post-fermentation wines without CO₂. For post-fermentation H₂S analysis,

our group previously reported that CO₂ could be generated *in situ* through addition of carbonate-containing antacid tablets to a flask containing a wine sample and fitted with a GDT. However, this approach results in a shift of the pH to ~6, which could potentially release H₂S from known precursors¹⁶. Additionally, the rapid generation of CO₂ gas can occasionally result in dislodging the detection tube or another connection.

In this work, we describe a rapid, inexpensive method for detecting and quantifying H₂S in still wine samples using a GDT and the widely available Aeration-Oxidation (A-O) apparatus. We report figures of merit, apply the assay to commercial wines, and provide a detailed video description of the protocol ([Supplemental Video 1](#)).

Major Observations and Interpretations

Apparatus and materials. The principle for the proposed H₂S method is based on the GDT protocols previously developed for measurement of S⁰ residues in grape must³ or H₂S formed following release from wine precursors or during the course of fermentation^{14, 17}. The current approach used either inert gas (N₂ Method) or vacuum aspiration (Aspiration Method) to sparge H₂S from the sample and through the GDT tube (Figs 1a and 1b), resulting in a discoloration of the tube (Supplementary Figure A). The flask and tubes are connected in series through PVC tubing. A sufficient length of PVC tubing (15 cm or more) between 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 (see [Supplementary Video](#)). In contrast to the earlier works which relied on antacid tablets to evolve CO₂ gas *in situ*, these new approaches did not cause pH changes or dilution of the sample which could have

risked release of H₂S from precursors like copper-sulphydryl complexes¹⁶. The antacid-based approaches also required the opening and resealing of the apparatus to sequentially add tablets, which risked losses of H₂S due to volatilization.

As described in more detail below, both the N₂ and Aspiration Methods yield satisfactory results. However, the Aspiration Method is easier to use in practice, as the N₂ Method can overpressure and dislodge the GDT or other components, resulting in leaks. Additionally, the Aspiration Method allows easy replacement of the GDT if the tube becomes saturated during a run. However, the N₂ Method produced a calibration curve identical to the curve calculated from the manufacturer's GDT markings, and thus may require less frequent calibration (Fig 2).

GDT selection and interferences from SO₂ and other sources: The Gastec 4LL and 4LT GDTs are not susceptible to interferences from most wine components, including sulfate, acetic acid, or water vapor³. Several other compounds listed by the specification sheets for these GDTs (e.g. ozone, nitrogen dioxide, nitric acid, hydrogen chloride, hydrogen fluoride, ammonia)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 an interference for the more sensitive 4LT tubes (which contain HgCl₂) and not for the 4LL tubes (which contain Pb(CH₃COO)₂). Although the response of 4LT tubes to thiols is greater than for H₂S¹⁴, thiols are also less volatile and are typically at lower concentrations in wine¹. We had previously estimated that interference from methanethiol (the thiol of greatest concern due to volatility and concentration) in a typical wine would be only 25% of the H₂S signal¹⁷. However, in situations

where interferences from methanethiol or other thiols are of concern, 4LL tubes can substituted at the expense of ~3-fold lower sensitivity³. SO₂ is described as an interference for both 4LL and 4LT tubes¹⁸. 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, sulfite). However, in initial studies at native pH, we observed considerable interferences from model solutions containing SO₂ (data not shown). To remove SO₂ from the gas stream, we investigated three approaches – addition of 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 to the wine sample was effective at removing the SO₂ interference for additions of 0.18% and 0.35% by volume but was not pursued further due to concerns about H₂S oxidation. SO₂ GDTs were effective in eliminating interferences 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; Fig 1(b), Item C). By itself, the inline acetaldehyde solution was unable to fully remove SO₂ interferences but including the acetaldehyde trap before the SO₂ GDT had the advantage of preserving the lifetime of the SO₂ GDT. If this approach is used, then the acetaldehyde solution should be replenished when the SO₂ GDT is replaced.

GDTs respond to the initial mass of H₂S, and not the initial concentration, and we were able to vary sample volume of model wine or Milli-Q water over a wide range (6 to 66 mL) without affecting the relative response (see Supplemental Information, Figure B). We also observed that the stain length stopped increasing after 5 min for volumes ≤ 10 mL, but at least 10 min analysis time were necessary for volumes ≥ 60 mL 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 ($r^2 > 0.99$) between the length of color change on the H₂S GDT and the nominal concentration of the calibration standards for both methods (Fig 2).

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

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

Theoretical calibration curves based on the manufacturer-provided markings (see Supplementary Information) were also plotted, along with a curve from a previous report which used antacid tablets as an *in situ* gas source (Fig 2)¹⁷. The slope of the theoretical curve based on manufacturers markings (dotted line) was identical to the slope observed for the N₂ Method. However, the slope of the Aspiration Method was about 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 from oxidation of H₂S during analyses, as the sample is 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 earlier method required the opening and closing of 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 (Fig 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 95% confidence interval of 92-97% 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 a limit of detection of 13 ng H₂S for the N₂ Method and 12 ng H₂S for the Aspiration Method. As previously reported for GDT methods, the limit of detection is primarily determined by the smallest observable change (0.5-1.0 mm) in stain length³. This can be more challenging for the 4LT tubes than 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 H₂S in wine¹ and compares favorably to detection limits reported with more expensive technologies. For example, an LOD of 0.2 µg/L has been reported for H₂S using GC-SCD¹, while quantitation limits of 1.0 and 1.7 µg/L were reported using GC-PFPD^{13, 19}.

As mentioned above, 4LT tubes achieve the best sensitivity and detection limits but suffer from interferences from thiols¹⁴, and the use of the less sensitive Gastec 4LL tube may be prudent when thiol interferences are 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 H₂S concentration samples can quickly exhaust the capacity of a Gastec 4LT (~500 ng of H₂S). In practice, we typically used a GDT only once before disposal, particularly when handling samples with unknown H₂S concentration when we did not want to risk saturating the GDT. However, reuse of tubes that are not yet saturated was practiced when the expected concentration is 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 to use a 4LL tube when measuring higher concentration samples or when high levels of thiol interferences are expected.

Analysis of commercial wines. A convenient sample of twelve commercial wines (6 bottled, 6 canned) were purchased from local stores, and were evaluated using the GDT methods (Fig 4).

We observed an average H₂S concentration in the bottled wines of 1.1 ± 0.9 µg/L, a range consistent with values reported elsewhere¹. The mean H₂S in the canned wines was 13.5 ± 9.9 µg/L significantly higher than the bottled wines (p<0.05). The highest concentration of H₂S in the canned wines was nearly 30 µg/L, comparable to the highest values observed in a survey of

commercial wines described as “reduced”¹. These observations concur with recent anecdotal observations that wines stored in aluminum cans will develop H₂S during storage due to i) anoxic conditions and degradation of H₂S precursors, and ii) reaction of SO₂ with the aluminum metal to form H₂S²⁰. As a caveat, the effects of packaging may have been confounded with other variables, e.g. production practices used for wines destined for cans, and the higher levels of H₂S in canned wine are not necessarily due to interactions between the can and wine.

Broader Impact

We have described and validated two convenient, inexpensive approaches to measuring free H₂S in commercial wine samples using selective GDTs and glassware from an Aeration-Oxidation unit. With 60 mL sample volumes, limits of detection below the sensory threshold in wine could be achieved. The calibration produced using the N₂ 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. The alternative Aspiration Method was easier to operate, making it more practical in winery settings, but should be regularly calibrated with standard solutions. By allowing for onsite measurement with minimal resources, both methods avoid risks of H₂S loss associated with sending samples to offsite 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, fining trials). Finally, although not the subject of the current investigation, the method could also likely be adapted for analysis of H₂S precursor forms by appropriate pre-treatment of the wine, e.g. addition of brine to release H₂S from copper-sulphydryl complexes¹⁶.

Experimental Design

Chemical reagents. Ethanol (EtOH) at 140- and 190-proof was from Koptec (King of Prussia, PA). L(+)-tartaric acid (99%) and hydrogen peroxide (H₂O₂, 35% w/w) were purchased from Acros Organics (Morris Plains, NJ). Sodium hydroxide solution (NaOH, 50% w/w) was purchased from Fisher Chemical (Fair Lawn, NJ). Sodium sulfide nonahydrate (Na₂S · 9H₂O, 98%) was purchased from Beantown Chemical (Hudson, NH). Silicone oil was purchased from Sigma-Aldrich (St. Louis, MO). Acetaldehyde (CH₃CHO, 99%) was purchased from Alfa Aesar (Ward Hill, MA). Deionized, distilled water with a resistance of 18.2 MΩ × cm at 25 °C was provided by a Milli-Q system (Millipore Sigma; Burlington, MA) was used for all experiments. Nitrogen gas (N₂, UHP) cylinders were supplied from Airgas USA LLC (Elmira, NY). Samples were held in a temperature-controlled incubator at 10 °C until use.

Gas detection tubes (GDT). Commercially available gas detection tubes (Gastec International, San Diego, CA) used for analyses of H₂S (Gastec 4LT) and SO₂ (Gastec 5L) were purchased from Airgas (Radnor, PA) and W. W. Grainger (Lake Forest, IL), respectively. The H₂S 4LT tubes rely on the reaction of H₂S with an HgCl₂, resulting in a color change from yellow to pink. The SO₂ 5L tubes rely on the reaction of SO₂ with BaCl₂ to generate HCl, resulting in the appearance of a yellow color. In this method, SO₂ GDTs are used only for filtering the gas stream of any volatilized SO₂, to prevent interferences in the H₂S tube. Gastec 5L GDT in this method are not appropriate for quantification of SO₂. GDTs should be stored at cool temperature, refrigerated at 10 °C or below, or as indicated on the package. After opening, GDTs 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 H₂S in wines using GDT with N₂ sparging (N₂ Method). The N₂ Method is depicted in Figure 1(a). For an analysis, a volume of wine sample (up to 60 mL) is added to a 100 mL round-bottom flask (A). For red wines, 4-5 drops of silicone oil were added to decrease foaming. One neck of the flask was fitted with a fritted impinger (B) from an Aeration-Oxidation unit (Adams & Chittenden Scientific Glass Coop, Berkeley, CA) and the impinger connected to an N₂ cylinder on the inlet side (C). The outlet side was connected by PVC tubing to an SO₂ scrubber (see next sub-section), e.g. 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 measured. The flow rate was ~100 mL/min, as faster flow rates would occasionally dislodge the GDT. Under these conditions, negligible change to the GDT stain length was observed after 10 min.

Quantitation of free H₂S in wines using GDT with vacuum aspiration (Aspiration Method). In the Aspiration Method [Fig 1(b)], wine samples are 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 sub-section), e.g. a flask containing a 1.5% by volume acetaldehyde solution (C) and SO₂ GDT (D), followed by an H₂S GDT (E). The outlet of the H₂S GDT is attached by PVC tubing to a vacuum source (F). The sample is vacuum aspirated for 10 min and analyses were carried out at room temperature (~ 20 °C), and 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 interferences of SO₂ on the Gastec 4LL and 4LT tubes: i) inserting an SO₂ GDT in series before the H₂S GDT, as shown in Fig 1(a), Item D or Fig 1(b), Item D; ii) pre-treating the sample with hydrogen peroxide or acetaldehyde; iii) inserting a 100-mL pear-shaped flask containing 10 mL of 1.5% by volume acetaldehyde solution between the sample flask and the GDT, as shown in Fig 1(b), Item C. In evaluations, i) and iii) were carried out as described in the methods above. For pre-treatment of the samples in ii), an aliquot of the oxidizing agent was added directly to 60 mL of a commercial white wine which was previously observed to cause an interference in the 4LT GDT. H₂O₂ solution was prepared at 3.5% and added at 1, 3, and 6 mL, corresponding to 0.06 - 0.35% by volume. Acetaldehyde was added at 5 µL, 25 µL, 50 µL and 100 µL, corresponding to 0.01 - 0.17% by volume.

Effects of sample volume and analysis time. To evaluate the effects of sample volume on response, sample volume was varied from 6 mL to 66 mL while analyzing H₂S calibration standards (described below) by the N₂ Method. A response factor (length of stain in mm per ng H₂S) was then calculated for each condition. Analysis time was initially determined by recording the point 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 the analysis of Free H₂S was carried out as previously described. When

sample volumes were ≥ 60 mL, the analysis time was increased to 10 min to reach the point where no additional color change was observed. Following optimization, analysis times of 10 min were used for all runs.

Method calibration. $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ working solution (50 μM) was freshly diluted from a standardized stock solution (5 mM) every 48 h, and the stock solution was newly prepared every 2 weeks. 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 as H_2S were prepared in model wine (12% ABV, 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)” vs. “mass H_2S (ng)”.

The observed slopes from each approach were compared against 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 H_2S ” using the Ideal Gas Law and the manufacturer’s suggested 100 mL gas volume for air sampling¹⁸. Details of the conversion calculation are provided in the Supplementary Information.

Reproducibility. In-lab reproducibility was assessed for the N_2 Method where an addition of $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ working solution (50 μM) was measured in Milli-Q water at two concentrations, 1.55 $\mu\text{g/L}$ (n=9) and 3.10 $\mu\text{g/L}$ (n=7), over the course of 4 weeks.

Figures of merit. Calibration curves were used to determine the linear range. The limit of detection (LOD) was calculated as $3.3 \times$ standard deviation for the lowest concentration standard (0.6 $\mu\text{g/L}$ or 34 ng using 60 mL sample volume) within the linear range (0 – 5.1 $\mu\text{g/L}$, or 0 – 307

ng using 60 mL sample volume). Standard deviations were determined for each 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, 2 red, 4 white) and canned (n=6, 2 white, 2 rosé, 2 rosé sparkling) wines was purchased from local retailers (Ithaca, NY) and represented a range of regions and cultivars. Vintages ranged from 1-3 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., Cary, NC) was used for statistical analysis.

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Table 1 Figures of merit for H₂S GDT N₂ and Aspiration Methods.

	Mean %CV ^a	Linear range (ng H ₂ S)	r ²	LOD ^b (ng H ₂ S)	LOD ^b (µg/L H ₂ S for 60 mL sample)
N ₂ Method	4.5	0 - 307	0.9991	13	0.2
Aspiration Method	4.1	0 - 875	0.9993	12	0.2

(a) Mean and range for % coefficient of variation (CV), where %CV for each wine was calculated as (standard deviation / mean).

(b) Limit of detection (LOD) is calculated as $3.3 \times$ standard deviation of the lowest concentration standard divided by the slope.

Figure 1 Apparatus for measuring H_2S using (A) inert gas (N_2) sparging and (B) vacuum aspiration. The sample flask is connected to an impinger and the gas detection tubes (GDTs) are connected to the outlet, in series.

Figure 2 Calibration curves for H₂S [mass of H₂S (y, ng) vs. stain length (x, mm)] on Gastec 4LT gas detection tubes: (i) Aspiration Method from the current study, regression equation is $y = 14.7x$, (ii) N₂ Method from the current study, $y = 10.0x$, (iii) Alka-Seltzer tablet sparging, with regression equation calculated from reported values (Chen et al, 2017), $y = 14.9x$. The Manufacturer's theoretical regression equation (H₂S vs. stain length) calculated from tube markings, $y = 10.0x$, labeled as (iv). For all regressions, the intercept was not significantly different from zero and was omitted.

Figure 3 In-lab reproducibility of N₂ Method measured over 4 weeks with higher concentration (Δ) = 3.10 μg/L (n=7) or lower concentration (●) = 1.55 μg/L ng (n=9) standards. Sample measurements are normalized to the expected value (% Recovery H₂S = H₂S measured / H₂S added). The 95% CI is shown as $\mu = 94.4 \pm 2.9\%$.

Figure 4 Quantitation of free H₂S in a selection of commercial bottled (n = 6) and canned wines (n = 6).

Supplemental Figure A Color change in Gastec 4LT (a, b), 4LL (c, d) and 5L (e, f) gas detection tubes, showing tubes before (b, d, f) and after (a, c, e) use.

Supplemental Figure B - GDT sensitivity as a function of sample volume

The response of the GDT method (mm stain per ng H_2S) with changing sample volume. Measurements were performed by the N_2 Method, and samples are prepared in both model wine and water. The response did not differ significantly (ANOVA, $p > 0.05$) as a function of sample volume.

Supplemental Calculation - Theoretical mass of H₂S determined from 4LT GDT concentration markings (length of color change in mm to ng):

4LT mm markings	4LT concentration markings ("ppm")	4LT markings (mol equivalent)	4LT ng
7	0.5	2.1E-09	69.9
14	1.0	4.1E-09	139.9
21	1.5	6.2E-09	209.8
28	2.0	8.2E-09	279.8
35	-	1.0E-08	349.7

Conversion from "ppm" to "ng" for the manufacturer calibrated quantity of gas on the 4LT GDT, using Ideal Gas Law, $PV = nRT$.

Volume, V =	0.1	L
Pressure, P =	1	bar
Ideal gas constant, R =	0.083144598	L bar/mol K
Temperature, T =	293	K
Moles in 0.1 L of gas, n =	0.004105	mol

Definition of "ppm" = moles of H₂S/ 1,000,000 moles of gas

(a)	1 ppm =	1 mol H ₂ S/ 1,000,000 moles gas
(b)	1 ppm =	4.10E-09 mol H ₂ S / 0.004105 mol gas
(c)	1 ppm =	139.9 ng H ₂ S

The concentration markings in ppm on the GDT were reported with their corresponding length of color change in mm. Then, the ppm markings are converted to what it would be in moles and mass of H₂S, because the tubes are calibrated to measure concentration in 100 mL gas samples. Moles of gas that correspond to a 100 mL gas sample is calculated assuming Ideal Gas Law under the indicated conditions.

A sample containing 1 mol of H₂S in 1,000,000 mol of gas should correspond to the "1 ppm" mark on the GDT (a). Therefore, a 100 mL sample containing 0.004105 mol of gas and producing "1 ppm" on the GDT would contain $0.004105/1,000,000 = 4.10E-09$ mol of H₂S (b).

Finally, moles of H₂S required to get a "1 ppm" mark on the GDT is converted to mass, using the molecular weight of H₂S (34.076 g/mol), meaning that is the total mass H₂S that has been measured on the tube up to that mark (c). Using the amount of H₂S calculated for "1 ppm", the value is scaled to get the mass of H₂S for all ppm markings and their corresponding mm measurements, giving the theoretical relationship **H₂S (ng) = 10.0 x mm on 4LT**.

Supplemental Video 1 – [Analysis of Free Hydrogen Sulfide in Wines Using Gas Detection Tubes](#)