Benchmarking of SO_2 Analysis Instruments and Methods in Wine Applications

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Abstract: Free sulfur dioxide (SO_2) is a key parameter monitored throughout the winemaking process and at bottling to ensure wine is adequately protected from enzymatic and chemical oxidative effects and microbial spoilage. The aim of this study was 1) to benchmark accuracy and precision of various instruments and methods, i.e. aeration–oxidation (AO) and Ripper, available on the market for measuring free SO_2 levels in wine, and 2) to determine any impacts from ascorbic acid and tannins as these may interfere with test chemistry. The AO methods measured free SO_2 levels most accurately though some results were outside error margins. Titrets measured free SO_2 levels most accurately and precisely even though they have a high error; however, these cannot be used in red wine due to the high polyphenol content that interferes with the tests. The Vinmetrica SC-300 had good precision; its accuracy was within error margins. The Hanna 84500 unit had variable accuracy and precision. The Quick Tests results were difficult to interpret and therefore their accuracy is uncertain, but tests are precise. Only the AO methods were relatively unaffected by the presence of ascorbic acid.

Key words: sulfur dioxide, sulfite, aeration-oxidation, Ripper method, Hanna, Vinmetrica, Accuvin

Introduction. Sulfur dioxide (SO_2) has long been used in winemaking to protect wine from enzymatic and chemical oxidative effects and microbial spoilage. It can be added in gaseous form or, most common, from a sulfite salt, such as potassium metabisulfite. In aqueous solutions, SO_2, bisulfite (HSO_3^-) and sulfite (SO_3^{2-}) ions exist in equilibrium as per the equation:

$$SO_2 \bullet H_2O \leftrightarrows H^+ + HSO_3^- \leftrightarrows 2\ H^+ + SO_3^{2-}$$

The sum of SO_2, HSO_3^- and SO_3^{2-} concentrations is referred to as free SO_2, or FSO_2, and is the active form that affords protection in wine.

At wine pH, usually in the range 3–4, HSO_3^- is the most abundant form representing about 94–99% of the total, the rest being SO_2; SO_3^{2-} is negligible.

FSO_2 diminishes over time as SO_2 is lost to the atmosphere via tank or barrel headspace or through bottle corks, as HSO_3^- binds with carbonyl (e.g. acetaldehyde and ketone acids) and phenolic compounds (e.g. anthocyanins and tannins), and as HSO_3^- reduces *o*-quinones back to their phenol forms. During alcoholic fermentation, *S. cerevisiae* yeast produces small amounts of FSO_2, in the order of 10 mg/L, but, some strains have also been shown to be able to metabolize HSO_3^- and reduce it into hydrogen sulfide (H_2S) although this trait appears to be rare (Linderholm and Bisson 2005). Winemakers therefore need to add more sulfite to maintain a nominal level based on pH, according to the following relationship, while ensuring that total SO_2 (the sum of free and bound SO_2) never exceeds the maximum set by regulatory agencies, where applicable.

For example, a red wine with a pH of 3.20 and to be protected with 0.5 mg molecular SO_2/L would require approximately 13 mg FSO_2/L. FSO_2 should never be allowed to drop below 8–9 mg/L (Stelzer et al. 2005).

Various apparatus and methods are available for measuring FSO_2 in wine. Although there are several variants, these operate on one of two principles: Ripper chemistry and Monier–Williams method (Zoecklein et al. 1999; Pegram et al. 2013), which is based on aeration–oxidation (AO) chemistry.

The Ripper determination of SO_2 is based on the oxidation–reduction reaction (Ough and Amerine 1988):

$$SO_2 + I_3^- + H_2O \rightarrow SO_3 + 3\ I^- + 2\ H^+$$

The wine sample is first acidified to reduce the oxidation of polyphenols by iodine, then titrated with iodine to a starch endpoint. This method works well with white wines; however, tannins and anthocyanins in reds cause iodine reduction and false results.

A variation of this method generates iodine from an iodate solution, which is more stable, instead of iodine; the reactions are:

$$5\ I^- + IO_3^- + 6\ H^+ \rightarrow 3\ I_2 + 3\ H_2O$$

$$I_2 + SO_2 + H_2O \rightarrow SO_3 + 2\ I^- + 2\ H^+$$

The AO method involves acidifying the wine sample with phosphoric acid to help volatize the SO_2. A stream of air is passed through the acidified sample and the freed SO_2 is

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collected and oxidized in a hydrogen peroxide (H_2O_2) solution to produce sulfuric acid (H_2SO_4) as per the reaction:

$$SO_2 + H_2O_2 \rightarrow SO_3 + H_2O \rightarrow 2\ H^+ + SO_4^{2-}$$

The sulfuric acid solution is then titrated with a base (e.g. 0.01*N* NaOH) to a known endpoint. This method, however, causes ascorbic acid to oxidize to H_2O_2, which then reacts with free SO_2 and therefore yields false results if excessive amounts of the acid are used.

In both methods, FSO_2 in mg/L is determined by the relationship (Ough and Amerine 1988):

$$\frac{V \cdot N \cdot 32 \cdot 1000}{v}$$

V is the volume (mL) of titrant used, *N* is the normality of the titrant and *v* is the volume (mL) of the wine sample.

The purpose of this study was to benchmark six different kinds of apparatus and methods for accuracy (the degree of closeness of measurements of a quantity to that quantity's actual [true] value) and precision (the degree to which repeated measurements under unchanged conditions show the same results) and any impacts from ascorbic acid and tannins: AO method using classic laboratory apparatus and a second method using a scaled-down (home winemaking) version, Ripper-method Titrets® that use an iodide-iodate titrant vacuum-sealed in a bulb, Vinmetrica SC-300™ and Hanna® 84500 titrator units that measure conductivity during Ripper titration with iodate, and Accuvin Quick Tests™ that use a proprietary dye that reacts with SO_3^{2-} in the treated sample.

Materials and Methods

Test Equipment. R&D Apparatus for SO_2 Determination by Aeration-Oxidation purchased from Research & Development Glass Products & Equipment Inc., Berkeley, CA; Vinmetrica SC-300 SO_2 & pH/TA Analyzer Kit purchased from MoreWine! Concord, CA.; Hanna HI 84500 Sulphur Dioxide in Wine Titrator purchased from Hanna Instruments, Laval, Québec, Canada (via Prolab Scientific, Laval, Québec); MT140 Economy Aeration–Oxidation Free SO_2 Test Kit purchased from MoreWine! Concord, CA; CHEMetrics Sulfite in Wine Titrets Kit purchased from Vines to Vintages, Niagara, Ontario; and Quick Tests Free SO_2 purchased from Accuvin, Napa, CA.

Instrumentation. Syringes and other volumetric apparatus supplied with the instruments were substituted for high-accuracy pipettes to minimize sample errors. Test samples were obtained using the same pipette or pipettes of similar accuracy.

Test equipment was calibrated prior to testing. Reagents were purchased fresh or prepared fresh. The 0.01*N* NaOH titrant was standardized against a potassium acid phthalate solution. Potassium metabisulfite (KMS) was purchased fresh. Accuracy and resolution were recorded for all instrumentation.

Model Solutions. Three model solutions with 35 mg FSO_2/L were prepared using a volumetric flask, a 10% sulfite solution and distilled water acidified with tartaric acid to a pH of approximately 3.3. One was a control solution to benchmark FSO_2 measurements and to compare results with a second solution containing 20 mg/L of ascorbic acid, typical of use in white winemaking, and a third solution containing 2 g/L of grape tannins, which represents a highly tannic wine. Solutions were immediately transferred to a sufficient number of 60-mL bottles, fully topped and capped, to run 5 tests for each instrument or method. All samples were held at ambient temperature of approximately 21°C (70°F).

Test Procedure. Each instrument or method was tested by measuring FSO_2 in a sulfited sample and then repeated for a total of 5 times. The tests were repeated using sulfited samples with ascorbic acid. The tests were again repeated using sulfited samples with grape tannins.

Test Errors. Errors on all instrumentation were recorded and factored into test results where possible. Errors that could not be quantified are discussed below.

Results and Discussion

Refer to the data in Tables 1, 2 and 3 and the corresponding graphs in Figures 1, 2 and 3.

Aeration–Oxidation Method. Both the R&D and MT140 test units provided measurements with good precision and accuracy within the margin of error. The tests were relatively unaffected by the presence of ascorbic acid. The results for both units were consistent although they measured lower FSO_2 levels in the presence of grape tannins. As the AO method was not expected to be affected by grape tannins, it is assumed that the lower FSO_2 levels were due to the binding action between SO_2 and tannins.

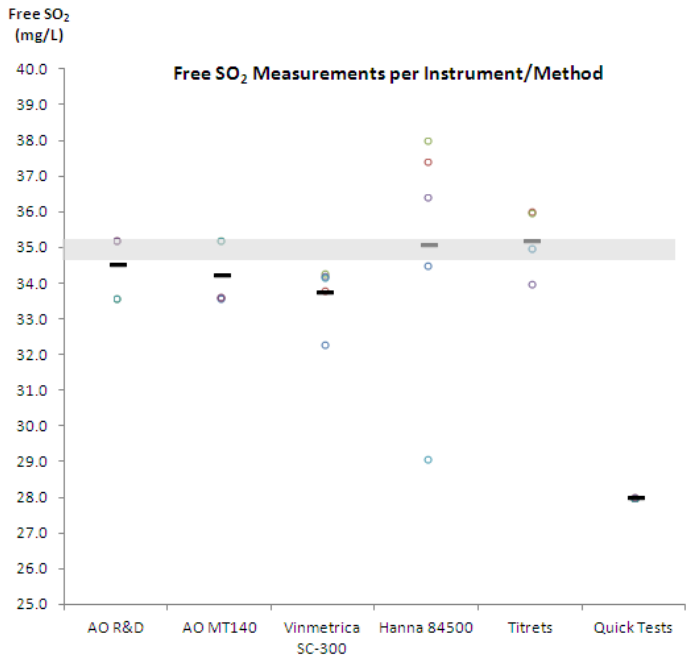

Figure 1 Distribution of free SO_2 measurements (circles) and average (black dashes) per instrument/method using a solution with 35 mg FSO_2/L (gray rectangle, which includes FSO_2 error).

Ripper Method. The Vinmetrica SC-300 unit and Titrets provided measurements with good precision and accuracy within the margin of error, whereas the Hanna unit was less precise. The tests were affected by the presence of ascorbic acid.

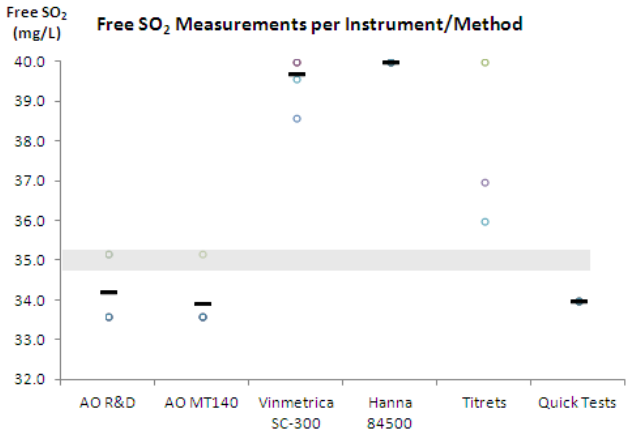

Figure 2 Distribution of free SO_2 measurements (circles) and average (black dashes) per instrument/method using a solution with 35 mg FSO_2/L (gray rectangle, which includes FSO_2 error) and 20 mg ascorbic acid/L.

The Vinmetrica and Hanna units measured lower FSO_2 levels in the presence of grape tannins, but this is assumed to be due to the binding action between SO_2 and tannins. Titrets were significantly affected by the presence of grape tannins, recording measurements in excess of the 100 mg/L test limit. A test using only 200 mg/L of tannins instead of 2 g/L had negligible impact; therefore, white wines with low levels of tannins can still be measured with Titrets.

Test Errors. Test results include instrumentation errors, except for the sample size used with the Quick Tests. For the AO methods, the 0.01*N* NaOH solution was standardized. Instrumentation errors have also been factored into the preparation of the 10% SO_2 solution and model solutions.

The manufacturers' specs on errors for FSO_2 in the 35 mg/L range are: Hanna 84500 (3%), Titrets (±5 mg/L), and Quick Tests (±4 mg/L). No error data was available for the Vinmetrica SC-300 unit; a 2% error was assumed in the calculations.

AO methods, Titrets and Quick Tests rely on color changes to determine the titration endpoint. Test errors can be significant with inexperienced users. Titrets can have an additional error from sampling model solutions as the solution is drawn in by vacuum in the bulbs and cannot be controlled precisely.

The AO methods proved to be the most error prone if solutions are not fresh or if the tests are not performed carefully. Leaks in the aspiration sample may result in a loss of free SO_2 and skewed test results. The aspiration flow rate is also important; an inadequate rate may cause the loss of SO_2 or SO_2 that could not be dissolved in the H_2O_2 solution. The methods should be performed using a flow rate of 1 L/min measured with a flowmeter (Iland et al. 2000). The tests here were not performed using a flowmeter instead relying on user experience. Test results using the model solution containing grape tannins were impacted by the test delays as SO_2 immediately starts binding with grape tannins when the solution is prepared.

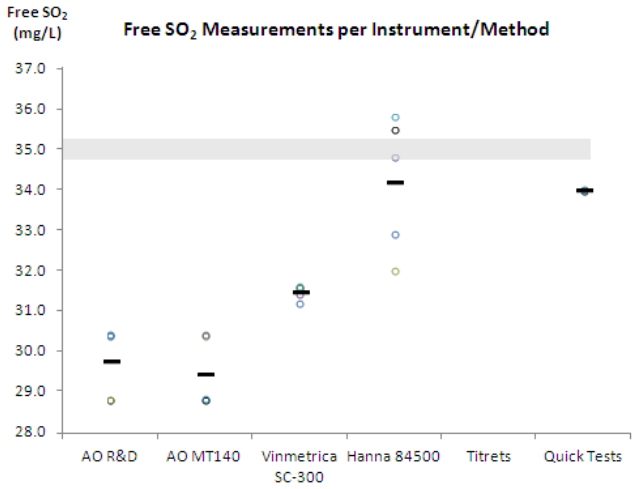

Figure 3 Distribution of free SO_2 measurements (circles) and average (black dashes) per instrument/method using a solution with 35 mg FSO_2/L (gray rectangle, which includes FSO_2 error) and 2 g grape tannins/L.

Conclusions

The AO methods measured free SO_2 levels most accurately though some results were outside error margins. Titrets measured free SO_2 levels most accurately and precisely even though they have a high error; however, these cannot be used in red wine due to the high polyphenol content that interferes with the tests. The Vinmetrica SC-300 had good precision; its accuracy was within error margins. The Hanna 84500 unit had variable accuracy and precision. The Quick Tests results were difficult to interpret and therefore their accuracy is uncertain, but tests are precise. Only the AO methods were relatively unaffected by the presence of ascorbic acid.

Although test results are well outside of the error margins of the model solutions, a free SO_2 error of ±5 mg/L is considered acceptable in the 35 mg/L range at which these tests were executed. As a possible future study, the same tests can be performed in model solutions with, for example, 10–15 mg/L, to assess errors where lower free SO_2 levels may be more of a concern to those wanting to minimize sulfite use in wines.

Another buying consideration is cost effectiveness. Approximate suggested retailer prices (in $US) are: AO R&D Apparatus ($420) can perform free and total SO_2 tests but requires all reagents to be purchased separately; MT-140 kit can perform 2–3 free SO_2 tests, then more reagents must be purchased; Vinmetrica SC-300 ($350) can perform up to 50 free and total SO_2 tests as well as 30 TA/pH tests; Hanna HI 84500 ($850) can perform up to 50 free and total SO_2 tests; disposable CHEMetrics Titrets ($19) can perform 10 free SO_2 tests; and disposable Accuvin Quick Tests ($65) can perform 20 (4 high-range plus 16 low-range) free SO_2 tests.

Sample	AO R&D	AO MT140	Vinmetrica SC-300	Hanna 84500	CHEMetrics Titrets	Accuvin Quick Tests
1	35.2 ± 0.7	33.6 ± 0.7	32.3 ± 1.7	34.5 ± 1.9	35 ± 5	28 ± 4
2	35.2 ± 0.7	33.6 ± 0.7	33.8 ± 1.7	37.4 ± 1.9	36 ± 5	28 ± 4
3	33.6 ± 0.7	35.2 ± 0.7	34.3 ± 1.7	38.0 ± 1.9	36 ± 5	28 ± 4
4	35.2 ± 0.7	33.6 ± 0.7	34.2 ± 1.7	36.4 ± 1.9	34 ± 5	28 ± 4
5	33.6 ± 0.7	35.2 ± 0.7	34.2 ± 1.7	29.1 ± 1.9	35 ± 5	28 ± 4
Avg	*34.6*	*34.2*	*33.8*	*35.1*	*35*	*28*

Table 2 Free SO_2 measurements in mg/L with errors and average per instrument or method using a solution with 35 mg FSO_2/L.

Sample	AO R&D	AO MT140	Vinmetrica SC-300	Hanna 84500	CHEMetrics Titrets	Accuvin Quick Tests
1	35.2 ± 0.7	33.6 ± 0.7	38.6 ± 1.7	40.0 ± 1.9	45 ± 5	34 ± 4
2	33.6 ± 0.7	33.6 ± 0.7	40.0 ± 1.7	40.0 ± 1.9	45 ± 5	34 ± 4
3	35.2 ± 0.7	35.2 ± 0.7	40.4 ± 1.7	40.0 ± 1.9	40 ± 5	34 ± 4
4	33.6 ± 0.7	33.6 ± 0.7	40.0 ± 1.7	40.0 ± 1.9	37 ± 5	34 ± 4
5	33.6 ± 0.7	33.6 ± 0.7	39.6 ± 1.7	40.0 ± 1.9	36 ± 5	34 ± 4
Avg	*34.2*	*33.9*	*39.7*	*40.0*	*41*	*34*

Table 2 Free SO_2 measurements in mg/L with errors and average per instrument or method using a solution with 35 mg FSO_2/L with 20 mg ascorbic acid/L.

Sample	AO R&D	AO MT140	Vinmetrica SC-300	Hanna 84500	CHEMetrics Titrets	Accuvin Quick Tests
1	30.4 ± 0.7	28.8 ± 0.7	31.2 ± 1.7	32.9 ± 1.9	> 100	34 ± 4
2	28.8 ± 0.7	28.8 ± 0.7	31.6 ± 1.7	35.5 ± 1.9	> 100	34 ± 4
3	28.8 ± 0.7	30.4 ± 0.7	31.6 ± 1.7	32.0 ± 1.9	> 100	34 ± 4
4	30.4 ± 0.7	30.4 ± 0.7	31.4 ± 1.7	34.8 ± 1.9	> 100	34 ± 4
5	30.4 ± 0.7	28.8 ± 0.7	31.6 ± 1.7	35.8 ± 1.9	> 100	34 ± 4
Avg	*29.8*	*29.4*	*31.5*	*34.2*	*> 100*	*34*

Table 3 Free SO_2 measurements in mg/L with errors and average per instrument or method using a solution with 35 mg FSO_2/L with 2 g grape tannins/L.

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