Sentia[™] Analyzer – A New Tool for Measuring Wine Parameters

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DATE: December 8, 2022

ACKNOWLEDGEMENTS: The author wishes to acknowledge Dr. Rich Sportsman (Vinmetrica, Carlsbad, CA) for his guidance and support throughout this project.

SUMMARY

Sentia is a new analyzer for measuring free sulfur dioxide (SO_2) , malic acid and glucose levels in wine. Sentia Analyzer's free SO_2 capability has been shown to have strong correlation with the Ripper method for red wines as well as aeration–oxidation (AO) and enzymatic methods once adjusted for reductones, but less so for white and rosé wines. The analyzer also measured higher values in rosé samples when tested as red wine, and slightly lower values when tested as white wine. Sentia Analyzer's malic acid capability has been shown to have very good correlation with enzymatic analysis for the purpose of assessing MLF progress and completion. Sentia Analyzer's ease of use, portability and fast results make this analyzer an indispensable tool for measuring free SO_2 and malic acid (and glucose) concentrations right in the cellar.

1. PURPOSE OF THIS PROJECT

The purpose of this project was to evaluate the Sentia Analyzer for measuring free sulfur dioxide (SO_2) and malic acid concentrations in wine, and compare it to other apparatus and methods used in the industry.

2. INTRODUCTION

The Sentia Analyzer is a new tool for measuring free sulfur dioxide (SO₂), or FSO2, malic acid and glucose concentrations in wine. This analyzer was developed by <u>Universal Biosensors Inc.</u>



(Melbourne, Victoria, Australia), a company specializing in the design of handheld portable biosensors that use disposable voltammetry-based test strips with applications across several industries including food and drink, human health, animal health, environmental, and agriculture. The company has plans to add fructose and volatile acidity (acetic acid) test strips to its Sentia Analyzer.

Sentia's simple user interface prompts for the type of test (Free SO_2 , Malic Acid, Glucose) to run, type of wine (white or red) to analyze, the sample id for record-keeping purposes, test strip index number to perform calibration when the strip is inserted into the

analyzer, and then guides the user on inserting the strip, diluting the sample if required, i.e., for malic acid analysis, applying a drop of wine sample, and completing the test.



I have a special interest in wine analysis, but I was especially keen in trying out this new analyzer because, not only did the technology promise more accurate results, but it could measure parameters in seconds with no need for additional reagents or elaborate sample pretreatment. And so I decided to purchase a unit from <u>Vinmetrica</u>, a reseller of Sentia products, and try it out to see how the analyzer compares to other test equipment and methods. I was specifically interested in free SO_2 and malic acid parameters; without fructose, measuring glucose only is not useful in assessing alcoholic fermentation completion or the amount of fermentable residual sugars.

3. SULFUR DIOXIDE (SO₂)

Free SO_2 (FSO2) is routinely measured throughout the winemaking process to ensure that juice and wine are adequately protected against enzymatic, chemical and microbiological oxidation and spoilages.

3.1 MEASURING FREE SO₂

Given the complexity and dynamic nature of SO_2 chemistry in wine and the presence of many other substances that can interfere with proper measurements, analyzing SO_2 is anything but straightforward. There are various equipment and methods, each having advantages and disadvantages, but there can be not-insignificant differences in measurements from one method to another, or even among different apparatus using the same method.

Ripper and aeration–oxidation titrametric methods are the most common and most used in winery laboratories as they are relatively easy, quick and cheap to perform.

In one variation of the Ripper method using a meter equipped with an ORP (oxidation– reduction potential) probe, a sample is first treated with dilute sulfuric acid to reduce oxidation of polyphenols by iodine used to titrate a sample. In the reaction, iodine oxidizes the SO₂ in the sample, and the excess iodine at the titration endpoint determines the amount of FSO2, expressed in mg/L. <u>Hanna Instruments</u>' HI902 and HI84500, <u>Vinmetrica</u>'s SC-300 and <u>Chemetrics Titrets</u> are examples of Ripper-method free SO₂-measuring apparatus; the latter three were benchmarked in a <u>separate study</u> (Pambianchi 2014).

The Ripper method has an estimated error of approximately 10% (Margalit 2012) due to the presence of interfering substances (reductones), namely, phenols, sugars and aldehydes, which skew measurements as these substances too are oxidized by iodine during the analytical procedure (Buechsenstein and Ough 1978; Iland et al. 2000). Ascorbic acid, another reductone, sometimes used in white winemaking and particularly at bottling to mitigate oxidation risks, can skew measurements much more significantly. Reductones are discussed in more detail in Section 3.4.

The Ripper method is also affected by the dissociation of weak SO_2 adducts, such as anthocyanin–bisulfite complexes in reds, following changes to wine equilibrium from acidification of samples (Buechsenstein and Ough 1978).

In the aeration–oxidation (AO) method, also known as the Monier-Williams method, a sample is first acidified with dilute phosphoric acid to aid in the release of free SO_2 from the sample, which is kept in an ice bath during the process to minimize evaporation of other volatile substances and prevent dissociation of unbound SO_2 . An air stream is passed through the sample

to aspirate the volatized free SO₂—hence why it is often referred to as the aspiration method—and then collected in a hydrogen peroxide solution to oxidize it into sulfuric acid, which is then titrated with a standard sodium hydroxide solution to determine the amount of FSO2. The R&D (Research & Development Glass Products & Equipment Inc.) AO apparatus and MoreWine's MT140 economy test kit are examples of AO-method free SO₂-measuring apparatus; these were benchmarked in a <u>separate study</u> (Pambianchi 2014).

The AO method gives more accurate measurements since there are no interferences from reductones; the estimated error is about 2.5–5% (Margalit 2012). As with the Ripper method, the AO method too is affected by the dissociation of anthocyanin–bisulfite complexes in reds if the samples are not chilled prior to and during analysis.

To remove the impacts of reductones from FSO2 measurements, samples can be treated with a scavenger, either a 3% hydrogen peroxide or a 40% glyoxal solution, that selectively removes the SO_2 , then re-measuring FSO2 and subtracting that result from the FSO2 measured in an untreated sample (Vinmetrica 2021).

Enzymatic assaying is another method for measuring FSO2 but mainly used in wine analysis laboratories as it requires the ability to dispense small wine and reagent volumes and the use of a spectrophotometer to measure colorimetric (absorbance) changes caused by the enzymatic reactions. In this method, SO₂ is enzymatically oxidized into hydrogen peroxide, which is then enzymatically reduced, and the amount of NADH oxidized determines the amount of free SO₂. <u>Megazyme/Neogen</u>'s (Megazyme) Total and Free Sulfite Assay Protocol and <u>R-Biopharm</u>'s Enzytec Liquid SO2-Free kits are examples of free SO₂-measuring products using enzymatic assaying.

Voltammetry is an electroanalytical method that measures the response of specific substances to a potential difference applied across two electrodes. A test strip is inserted into an analyzer and a single drop is applied to initiate measurement. The technology has already been in use in wine analysis applications in the determination of color and polyphenols, and now, free SO₂, malic acid and glucose using <u>Universal Biosensors</u>' Sentia Analyzer, the focus of this evaluation project.

According to the Sentia Analyzer product data sheet and user guide (Universal Biosensors 2022; Universal Biosensors 2021), the analyzer can measure free SO_2 in the range 3–50 mg/L in white and red wines; it is left to the discretion of the user to choose between white or red when analyzing rosé wines. No pretreatment is required; a drop of wine sample is applied directly to the test strip to initiate measurement. Samples (e.g., greater than 50 mg/L FSO2) should not be diluted with water as it affects the wine matrix and may skew measurements.

3.2 EVALUATION METHODOLOGY

I evaluated the Sentia Analyzer for measuring free SO_2 (FSO2) and compared it to the Ripper method using a Hanna Instruments HI902 Automatic Potentiometric Titration System, to the AO method using R&D's AO apparatus, and to the enzymatic method using Megazyme's Total and Free Sulfite Assay Protocol kit. Enzymatic tests were performed using single-point calibration with a 100-mg/L standard.

Because of the challenges in measuring FSO2 in wines due to the dynamics of SO_2 chemistry, there is no yardstick to determine which equipment or method provides "correct" measurements; rather, we have to look at how close measurements are across equipment and methods.

3.3 EVALUATION RESULTS

I measured FSO2 in red, white and rosé wines and ran each test up to three times to get some sense of precision of each equipment and method. I used samples from my homemade 2022-vintage wines that had just completed alcoholic fermentation.

The red wine was a Petite Sirah (Durif) made from grapes; the rosé was made from the same grapes with no maceration, straight to the press. Wines had not been treated with sulfite yet, and so FSO2 would measure only any free SO_2 generated by yeast during fermentation, which would typically be below 10 mg/L. The white wine was a Chardonnay made from previously sulfited fresh juice; free and total SO_2 had measured approximately 20 mg/L and 80 mg/L in the juice.

At the end of fermentation, small, separate batches were then treated at various SO_2 levels the morning of commencement of this study. I also spiked samples with, for example, 10 and 25 mg/L (as FSO2 using a 10% KMS solution) to have representative test samples across the range supported by the Sentia Analyzer, and then measured each before and after spiking. Because of binding, I would not expect to measure full recovery of the spiking amounts, but I wanted to assess how each equipment and method reacted to newly added SO_2 .

Following are the abbreviations of wine samples tested and in parentheses the amount of FSO2 previously added:

RWA	Red Wine A (none)
RWAs25	Red Wine A spiked with 25 mg/L FSO2
RWB	Red Wine B (35 mg/L)
RWBs20	Red Wine B spiked with 20 mg/L FSO2
WWA	White Wine A (none)
WWAs25	White Wine A spiked with 25 mg/L FSO2
WWB	White Wine B (20 mg/L)
WWBs10	White Wine B spiked with 10 mg/L FSO2
PWA	Rosé Wine A (none)
PWAs25	Rosé Wine A spiked with 25 mg/L FSO2
PWB	Rosé Wine B (30 mg/L)
PWBs10	Rosé Wine B spiked with 10 mg/L FSO2

Figure 1 charts the results showing FSO2 measurements made using a Sentia Analyzer, and using Ripper, AO and enzymatic methods with the test equipment described above.

In the red wine analysis, there was very good correlation between Sentia Analyzer and Ripper measurements throughout the supported Sentia range, and both measured the same recovery in the spiked samples, 17 mg/L and 11 mg/L, respectively for RWAs25 and RWBs20. There was good correlation between the AO and enzymatic methods although these measured lower than the Sentia Analyzer and Ripper method; however, if Sentia and Ripper measurements are adjusted by an amount in the order of 5 mg/L for reductones (see Section 3.4), there is a much stronger correlation now across all apparatus and methods as shown in Figure 2. The AO method had



lower recoveries in the spiked samples, 15 mg/L and 9 mg/L, while the enzymatic method measured recoveries of 12 mg/L and 16 mg/L.



In the white wine analysis, the Ripper method measured much higher levels than the Sentia Analyzer and AO and enzymatic methods, although there was better correlation here among the latter three; AO and enzymatic methods still measured lower than the Sentia Analyzer. The Ripper method did however measure recoveries of approximately 22 mg/L and 10 mg/L for the samples spiked with 25 mg/L and 10 mg/L FSO2, respectively, compared to 14 mg/L and 6 mg/L for the Sentia Analyzer, 15 mg/L and 2 mg/L for the AO method, and 5 mg/L and 4 mg/L for the enzymatic method.

In the rosé wine analysis, Sentia measurements are higher than those using the Ripper method. The wine was tested as a "Red Wine" on the Sentia Analyzer, and it appears to be measuring higher concentrations than expected. The base, unsulfited wine measured 4.9 mg/L and then 32.6 mg/L when spiked with 25 mg/L FSO2 when tested as "Red Wine" and but then measured considerably lower, 19.8 mg/L, when tested as "White Wine," compared to 24.7 mg/L using the Ripper method. These results suggest that the Sentia Analyzer reports higher measurements in rosé wine when tested as red wine, and lower measurements when tested as white wine. There was

poor correlation with both the AO and enzymatic methods, which, here too, measured lower levels, although for this wine, samples were measured later in the day for the AO and enzymatic methods, and therefore there may have been additional binding, which may explain the lower measurements.





Based on three tests (two for the enzymatic method) for each wine samples analyzed using each equipment and method, the Sentia Analyzer had very good precision with most measurements within +/-5 mg/L, consistent with the Ripper and enzymatic methods, while the AO method proved to be the most precise, yielding almost identical results on every test. Figure 3 illustrates an example of the precision of each apparatus and method for a red wine spiked with 25 mg/L FSO2 (RWAs25).



Figure 3: Precision of FSO2-measuring apparatus and methods using a red wine sample spiked with 25 mg/L FSO2 (RWAs25).

The touch-screen user interface is very simple and intuitive albeit very small, making entering data a bit cumbersome, but this is easily overcome by using, for example, the eraser end of a lead pencil.

3.4 ANALYSIS FOR REDUCTONES

To assess reductone impacts, red wine samples were measured before any treatment to scavenge the SO_2 , then treated and re-measured, and FSO2 recalculated as the difference in those measurements.

A red wine sample, similar to RWAs25 above, measuring 19.8 mg/L and 22.9 mg/L with the Sentia Analyzer and Ripper method measured 15.8 mg/L and 16.3 mg/L after a glyoxal treatment and adjustments of 4.0 mg/L and 6.6 mg/L for reductones, while with a peroxide treatment, the samples measured 16.1 and 22.9 mg/L after adjustments of 3.7 mg/L and 0.0 mg/L. These results demonstrate that reductones can introduce an error of up to 30%; however, there is excellent correlation between the Sentia Analyzer and Ripper method when samples are treated with glyoxal. The Sentia Analyzer measured similar levels in adjusted FSO2 using both glyoxal and peroxide treatments whereas the Ripper method did not detect reductones after a peroxide treatment.

A 200-mg/L aqueous solution of ascorbic acid treated with 3% hydrogen peroxide measured approximately 62 mg/L as free SO₂, and so ascorbic acid is left behind as a reductone, consistent with the study by Vinmetrica (2021). However, the Sentia Analyzer measured only 6 mg/L as free SO₂, suggesting that ascorbic acid has no impact as a reductone on FSO2 measurements.

3.5 OTHER TESTS

I performed other informal tests to assess the behavior of the Sentia Analyzer when analyzing unsupported samples.

Any wine sample with more than 50 mg/L FSO2 is reported as "Analysis Error" by the Sentia Analyzer.

Although sample dilution is not supported, a white wine sample measuring on average 14.3 mg/L, measured 8.6 mg/L after a 1:2 dilution, demonstrating that samples can be diluted, for example, to bring expected FSO2 measurements below the 50 mg/L maximum.

A wine model solution (7 g/L TA, 3.58 pH) spiked with 25 mg/L FSO2 measured 18.8, 23.2 and 19.2 mg/L, respectively, using the Sentia Analyzer, and Ripper and AO methods, demonstrating very good correlation when other factors, i.e., interfering substances, are "removed."

3.6 OPERATIONAL CONSIDERATIONS

Table 1 compares various operational considerations for each equipment and method using the apparatus described above; these may vary when implemented using other apparatus.

	SENTIA	RIPPER	AO	ENZYMATIC
Cost per test (US\$)	3.50	0.75	<0.10	3.00
Consumables	Test strips	Sulfuric acid Iodine KI stabilizer powder	Sodium hydroxide Hydrogen peroxide Phosphoric acid Color indicator	Reagent #1 Reagent #2 Sodium sulfite (for standard)
Sample volume	One drop (8 μL)	50 mLª	20 mL	50 μL
Time to Results	30 seconds	~3 minutes	~20 minutes	~15 minutes ^b
Wine Type	Red, White	Any	Any	Any

NOTES:

a. 50 mL using a Hanna HI902; 25 mL using a Vinmetrica SC-300

b. Can test multiple samples at once

Table 1: A comparison of operational considerations for analyzing free SO_2 levels for equipment and methods used in this study.

3.7 CONCLUSIONS

Based on this study's results, Sentia Analyzer's free SO_2 capability has been shown to have strong correlation with the Ripper method for red wines, but less so for white and rosé wines. The analyzer also measured higher values in rosé samples when tested as red wine, and a slightly lower value when tested as white wine. As the analyzer correlated strongly with the Ripper and AO methods in analyzing a model wine solution, further tests should be conducted to determine why the Sentia Analyzer does not correlate to the Ripper method as well for white wines and rosés (tested as white), although there is a better correlation with the AO method when analyzing white wines.

Sample dilution did not seem to be a problem, but more thorough testing is recommended to confirm.

4. MALIC ACID

Red wines, and some whites, such as barrel-fermented Chardonnay, most often go through malolactic fermentation, or MLF, to convert the naturally occurring sharper-tasting L-malic acid (malic acid) into the softer L-lactic acid (lactic acid) by indigenous or cultured lactic acid bacteria. An incomplete MLF and residual malic acid would cause wine to be unstable with possible refermentation or spoilage, whereas lactic acid is stable. Wines with less than 100 mg/L of residual malic acid are considered stable although some winemakers use a threshold of 30 mg/L.

4.1 MEASURING MALIC ACID CONCENTRATIONS

Paper chromatography, a technique for separating dissolved chemical substances by virtue of their different rates of migration across a sheet of paper, is the most common, expedient and inexpensive method for assessing MLF progress and completion. Several drops of wine samples are applied to a sheet, which is then placed in a jar with a small amount of solvent (n-butanol). As the solvent travels up the paper, the major wine acids, i.e. tartaric, malic, lactic and succinic, can be identified by the position of their spots left on the paper after separation. MLF is considered complete when the malic spot for a wine has completely disappeared. This method only provides a qualitative assessment, it takes several hours to results, and requires handling a toxic chemical.

A quantitative assessment can be performed by enzymatic assaying using, for example, <u>Vinmetrica</u>'s SC-50 MLF Kit, which measures pressure resulting from the biochemical MLF reaction, and <u>Megazyme/Neogen</u>'s (Megazyme) L-Malic Acid and <u>R-Biopharm</u>'s Roche L-Malic Acid kits, which measure absorbance differences following biochemical reactions that produce NADH. The pressure and NADH produced are proportional to the amount of malic acid present in a sample. The Vinmetrica SC-50 MLF kit can be used in home winemaking analysis, while the NADH-based kits are mainly used in wine analysis laboratories as these require dispensing small, precise volumes and the use of a spectrophotometer.

According to Universal Biosensors' product data sheet (Universal Biosensors 2022), the Sentia Analyzer can measure malic acid concentrations in the range 0.05–5 g/L (50–5000 mg/L); it is left to the discretion of the user to choose between white and red when analyzing rosé wines. Samples must first be diluted 1:5 accurately with Sentia's malic acid buffer solution, and then a drop of the diluted sample is applied directly to the test strip to initiate measurement. Samples should not be diluted with water as it can affect the wine matrix and may skew measurements.

4.2 EVALUATION METHODOLOGY

I evaluated the Sentia Analyzer for measuring malic acid concentrations and compared it to enzymatic method using Megazyme's L-Malic Acid kit. Enzymatic tests were performed using single-point calibration with a 0.15-g/L standard.

4.3 EVALUATION RESULTS

I measured malic acid concentrations in a red wine that had not undergone MLF, and in both red and white wines that had undergone MLF and which were assessed to have completed malic conversion based on paper chromatography analysis. The wines were also spiked with 0.10 g/L (100 mg/L) and/or 1.0 g/L (1000 mg/L) of malic acid to assess recovery.

Following are the abbreviations of wine samples tested:

RWC	Red Wine C
RWCs100	Red Wine C spiked with 100 mg/L malic acid
RWD	Red Wine D
RWDs1000	Red Wine D spiked with 1000 mg/L malic acid
WWC	White Wine C
WWCs100	White Wine C spiked with 100 mg/L malic acid

Figure 4 charts the results showing malic acid concentrations measured with a Sentia Analyzer and using Megazyme's enzymatic method.



Figure 4: Malic acid measurements comparing a Sentia Analyzer to an enzymatic method

The red wine sample (RWC) with completed MLF measured "<0.05 g/L" with the Sentia Analyzer, as the unit's minimum detection is 0.05 g/L, and an average of 0.003 g/L using the Megazyme kit, demonstrating excellent correlation. When the wine sample (RWCs100) was spiked with 0.10 g/L of malic acid, the Sentia Analyzer measured twice <0.05 g/L and 0.02 g/L on the third test, and therefore not recovering the spike amount at this low level, while Megazyme tests measured an average of 0.09 g/L with a recovery of close to 0.09 g/L.

The red wine sample (RWD) not having undergone MLF measured an average of 1.89 g/L with the Sentia Analyzer and 2.73 g/L with the Megazyme kit demonstrating a good but weaker correlation here. The sample was diluted 1:10 for enzymatic analysis as the expected malic acid concentration was greater than the 0.3-g/L maximum supported by the Megazyme kit; this dilution may possibly have introduced some error. When the wine sample (RWDs1000) was spiked with 1.00 g/L of malic acid, the Sentia Analyzer measured an average of 2.42 g/L with a recovery of 0.54 g/L, while the Megazyme tests measured an average of 3.60 g/L with a recovery of 0.87 g/L.

The white wine sample (WWC) with completed MLF (according to paper chromatography analysis) measured an average of 0.14 g/L with the Sentia Analyzer and 0.09 g/L with the enzymatic method demonstrating a good correlation here although Sentia results would suggest that MLF is not complete given that the result is greater than 0.10 g/L. When the wine sample (WWCs100) was spiked with 0.10 g/L of malic acid, the Sentia Analyzer measured an average of 0.24 g/L with a recovery of 0.10 g/L, while the Megazyme tests measured an average of 0.17 g/L with a recovery of 0.09 g/L, demonstrating good correlation and excellent recoveries.

Based on three tests for each wine samples analyzed using each equipment and method, the Sentia Analyzer had very good precision with measurements within +/-0.09 g/L or better, compared with +/-0.03 g/L for the Megazyme enzymatic method. Figure 4 illustrates an example of the precision of the Sentia Analyzer and Megazyme enzymatic method for a red wine (RWD) not having undergone MLF.



Figure 5: Precision of malic acid-measuring apparatus and methods using a red wine sample (RWD) not having undergone MLF.

4.4 OTHER TESTS

I performed other informal tests to assess the behavior of the Sentia Analyzer when analyzing unsupported samples.

Any wine sample with more than 5 g/L malic acid is reported as ">5.00 g/L" by the Sentia Analyzer.

When the red wine sample (RWDs1000) with no MLF and spiked with 1.00 g/L, which measured on average 2.42 g/L using the Sentia Analyzer and 3.60 g/L using the Megazyme kit, it measured 1.55 g/L and 1.83, respectively, once diluted 1:2, demonstrating that samples can be diluted, for example, to bring expected malic acid measurements below the 5-g/L maximum.

A 0.40 g/L malic standard, included in Vinmetrica's SC-50 product, measured 0.17 g/L (as a white wine) using the Sentia Analyzer and 0.353 g/L using the Megazyme kit.

A laboratory reference standard with 1.10 g/L +/-0.06 measured 0.81 g/L as a red wine using the Sentia Analyzer and 0.82 g/L using the Megazyme kit.

4.5 OPERATIONAL CONSIDERATIONS

	SENTIA	MEGAZYME	PAPER CHROMATOGRAPHY
Cost per test (US\$)	6.20	5.00 up to 7.50ª	0.50 ^c
Consumables	Test strips MLF Buffer	Buffer NAD ⁺ /PVP Enzyme #1 Enzyme #2 Malic standard Pipettor tips	Paper Solvent Capillary tubes Acid standards (optional)
Sample volume	100 µL	100 μL ^ь	3-4 drops
Time to Results	60 seconds	~10 minutes	Hours
Wine Type	Red, White	Any	Any

Table 2 compares various operational considerations for assessing and measuring MLF progress and malic acid levels for each equipment and method using the apparatus described above; these may vary when implemented using other apparatus.

NOTES

a. If sample is to be filtered with syringe filters and using disposable cuvettes

b. Dilution required if malic level >0.3 g/L

c. Based on an average use of 5 wine samples per sheet

Table 2: A comparison of operational considerations for assessing MLF progress and analyzing malic acid concentrations for equipment and methods used in this study.

4.6 CONCLUSIONS

Based on this study's results, Sentia Analyzer's malic acid capability has been shown to have very good correlation with enzymatic analysis for the purpose of assessing MLF progress and completion.

Sample dilution did not seem to be a problem, but more thorough testing is recommended to confirm.

5. REFERENCES

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