

# **Viticulture and Enology Research Center**

California State University Fresno

## **Sotto Method Comparison Validation Study**

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By

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## Objectives and Principles of the Study

### Sulfur dioxide in wine

Sulfur dioxide ( $\text{SO}_2$ ) in its natural form is a gas that can form a weak acid if dissolved in water. Similar to carbon dioxide, the chemical characteristics in solution are mostly dependent on temperature and pH. Figure 1 illustrates the changes that occur to the molecular configuration between pH 0 and pH 14. This behavior is not only important for the functionality of  $\text{SO}_2$  in wine but also for its analysis.

At very low pH, the active or molecular form is predominantly present in solution, which is essentially the gaseous and most volatile configuration. With increasing pH, the portion of bisulfite and later sulfite increase relative to the active form, making the sulfur dioxide less reactive, less volatile, and much more soluble. In the pH range of wine, only up to 10% of the free  $\text{SO}_2$  is present in the active form while more than 90% are inactive as bisulfite. The higher the wine pH, the lower the percentage of active  $\text{SO}_2$ , and past pH 4, there is practically no active sulfur dioxide left.

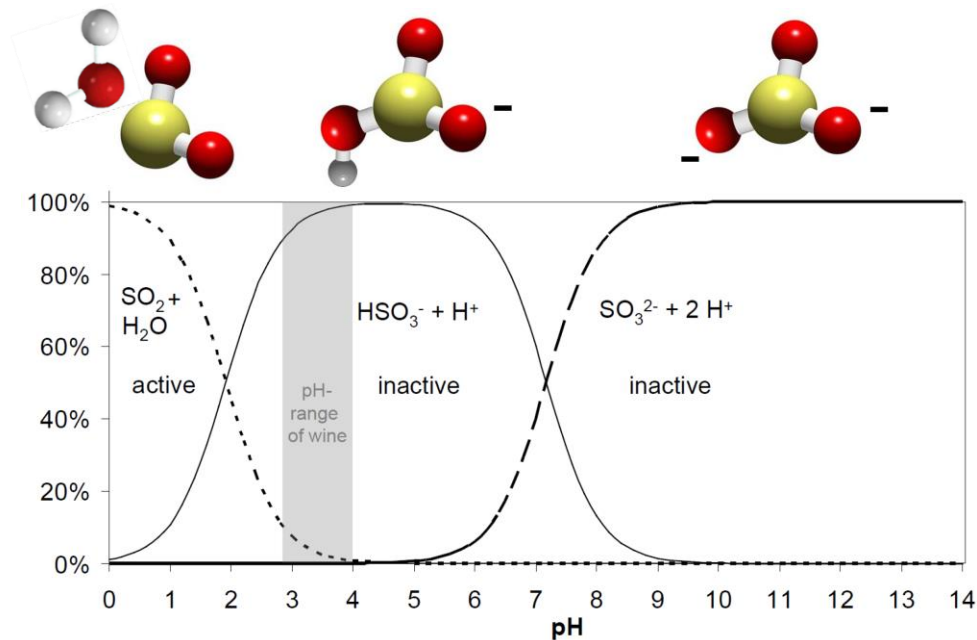


Figure 1 Changes in sulfur dioxide configuration depending on solvent pH.

This lack of active  $\text{SO}_2$  could become a problem since the form is needed to ensure microbial stability. The bisulfite form serves as an oxygen scavenger, inactivates enzymes, and binds undesirable compounds like acetaldehyde but has no protecting effect against microbial spoilage. In order to reach a protective level of sulfur dioxide, winemakers can either lower the pH of the wine or add more  $\text{SO}_2$ . It is important to note that

only unbound sulfur dioxide has any protective function, which is referred to as free SO<sub>2</sub>. Aldehydes, polyphenols, sugars, and many other molecules can bind SO<sub>2</sub> with various bond strengths and therefore lower its efficiency. This study only focusses on free sulfur dioxide, which forms an equilibrium not only with molecules it binds to but also any reduction-oxidation process in wine.

### Analytical principles and validation methods

Methods for analyzing sulfur dioxide in various media besides wine range from traditional wet chemistry like titration <sup>1</sup>, colorimetric determination <sup>2</sup>, and distillation <sup>3</sup>, to more modern methods like gas chromatography <sup>4-5</sup>, liquid chromatography <sup>6</sup>, ion chromatography <sup>7</sup>, gas-diffusion cells <sup>8-9</sup>, optical sensor technology <sup>10</sup>, voltammetry <sup>11-12</sup>, and a combination of several extraction and detection techniques <sup>13-16</sup>. The analytical field is developing rapidly because every methods has its own limitations, sources of error, and disadvantages in a production environment.

Analyzing sulfur dioxide is generally challenging due to the highly reactive nature of the molecule. The reducing power is unmatched by any other preservative used in wine, which is also the basis for most analytical methods that are commonly used in the wine industry. Sulfur dioxide is easily oxidized into sulfate while reducing other compounds in solution. This reaction happens very fast and quantitatively at low pH when the most active and most volatile form is dominant. Most inexpensive methods therefore include an acidification step using a strong inorganic acid. While this allows for a rapid and reproducible analysis of SO<sub>2</sub>, it also introduces the biggest source of error to those methods.

In this volatile state, sulfur dioxide can be easily removed from solution by shaking or any other form of agitation and it will also indirectly react with oxygen in the air. This means that samples need to be analyzed relatively quickly after exposure to air and especially after acidification. While some of the advanced methods can avoid extensive sample preparation, they are often cost prohibitive for wineries. Quality control in a production environment needs to be robust, inexpensive, and manageable by personnel with variable levels of analytical experience. For these reasons, only very few methods have actually been established on a broader basis. The most common methods in the United States of America are probably the Aeration-Oxidation (or Aspiration) method, Ripper Titration, and the WineScan™ unit (FOSS, Hilleroed, Denmark) with SO<sub>2</sub> module. The latter two were chosen as reference methods for this study due to their different analytical principle. Aeration-Oxidation was not selected due to the known reproducibility problems among users and the potential duplication with a separate validation study. The following sections discuss analytical basics and sources of error for each reference method in this study.

## Ripper Titration

The Ripper titration is based on the reducing power of sulfur dioxide (SO<sub>2</sub>) in an acidic environment. Starch is used as an indicator while iodine is added to an acidified sample. As long as there is still SO<sub>2</sub> left in the sample, iodine is reduced to iodide, which does not react with starch. When all of the sulfur dioxide is oxidized, it can no longer reduce the iodine and any excess iodine forms a purple color complex with the starch, indicating the endpoint of the titration.

While the assumption is accurate that SO<sub>2</sub> is the most common reducing substance in wine, there are compounds that can influence the results. Other reducing compounds like ascorbic acid, tannins, sugar, aldehydes, polyphenols, and additives like glutathione can significantly influence the titration results. These compounds have reducing capacity and essentially perform the same reduction reaction as SO<sub>2</sub>. Wines that have high concentrations of reducing substances will show artificially high sulfur dioxide concentrations.

## FOSS FT2 WineScan™ SO<sub>2</sub>

The WineScan™ unit performs a micro-distillation to remove sulfur dioxide from the acidified wine matrix and analyzes free SO<sub>2</sub> in the gas phase. While the method excludes any other reducing substances that could interfere with the results, it is extremely sensitive to dissolved gases like CO<sub>2</sub>. The sample needs to be degassed carefully before it can be entered into the system. There are several ways to remove gas from a liquid, most commonly by agitation or ultrasound. Other methods like vacuum degassing or warming the wine on a stir plate lead to unpredictable losses of SO<sub>2</sub> through evaporation and oxidation.

There are two major sources of error for this method: the degassing procedure and the micro-distillation itself. Degassing always leads to a loss of free sulfur dioxide because any agitation, stirring, or ultrasound treatment oxidizes or evaporates SO<sub>2</sub>. The following micro-distillation heats up the sample, leading to an increased oxidation rate. These two factors are responsible for WineScan™ readings being generally lower to some extent compared to any other method.

The principle of the Wine Scan™ analysis is based upon an increase in sensitivity whenever SO<sub>2</sub> can be removed from the wine matrix and analyzed in a gaseous form without background noise and matrix interferences. Another advantage is the level of automation that eliminates any variability that would be

introduced by the user. The setup can be seen in Figure 2 and includes an optional autosampler, which makes this system attractive for large sample throughput and routine analysis.

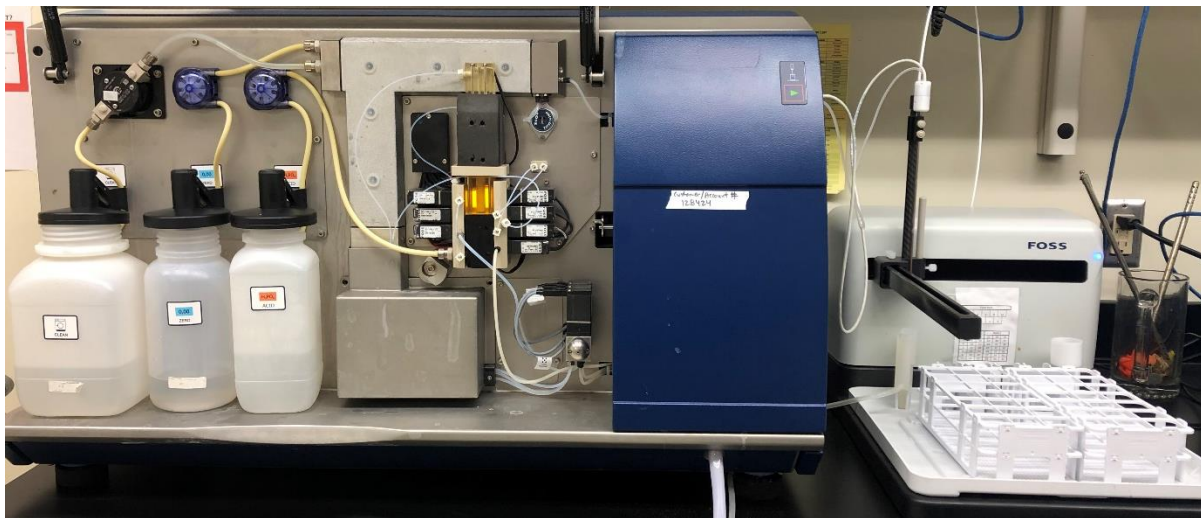


Figure 2 The FOSS FT2 WineScan™ with SO<sub>2</sub> module.

### Sentia™ Free SO<sub>2</sub> Analyzer

The Sentia™ System, which includes the Analyzer and the consumable test strip have been designed and developed using Blood Glucose Monitor expertise. The analyzer uses the same platform as a Blood Glucometer and the test strip is manufactured in the same way.

Unlike the glucose strip though, which utilizes a dried down enzyme, the free SO<sub>2</sub> strip uses voltammetry instead of amperometry, which in this case means observing electrical current as a function of voltage rather than time - specifically, square wave voltammetry. When wine is added to the strip, it dissolves dried down reagents on the strip. The dried down acid automatically adjusts the pH of the sample so that the hydrogen sulfite gets converted to sulfur dioxide. This becomes directly reduced at the electrode when the square waveform is applied. The data obtained is analyzed to generate “differential current vs voltage” data which contains a peak around 600 mV. The height of this peak correlates to the concentration of sulfur dioxide in the wine sample. Figure 3 shows the analyzer in battery mode and the test strips. The charging cable that is included with the instrument is not shown here.



Figure 3 The Sentia™ Free SO<sub>2</sub> Analyzer and test strips

### Scope of the study

The objective of this study is to compare the results for free sulfur dioxide reported by the Sentia™ analyzer to the most common established methods in the US by analyzing a total of 200 commercial wines with all three methods. The goal was to test all wines with the methods in duplicate and predict method agreement as well as calculate key statistical determinates. The wines were selected based on the following specifications: still white and red wines only (no rosé wines), residual sugar less than 30 g/L (no dessert wines), expected free SO<sub>2</sub> levels between 3 and 50 mg/L.

## Results

The following graphs compare two methods each and illustrate the characteristics of the individual method principles. While the accuracy is generally good for all comparisons, the Ripper method generates results that are consistently higher than the Sentia Analyzer due to the impact of other reducing substances. The WineScan™ on the other hand is consistently lower than the Sentia™ Analyzer due to the loss of SO<sub>2</sub> during the sample preparation and analysis itself. All graphs include a black line of best fit and a correlation coefficient as an indicator of the scatter or precision around that black line of best fit. The accuracy of the method (compared to the comparator device) can be estimated by comparing the position of that line to the orange line, which represents perfect accuracy and precision between the two methods.

### White wines

White wines might show a lower correlation between the Sentia™ analyzer and Ripper titration than the red wines due to the tendency of some winemakers to add ascorbic acid to their wines in addition to SO<sub>2</sub>. The majority of Californian Chardonnays and blends are also barrel aged, which increases the concentration of hydrolysable tannins that have some reducing capacity as well.

Even though the Sentia™ analyzer and the WineScan™ are not affected by other reducing substances, Figure 4a indicates that the two methods vary based on the free SO<sub>2</sub> concentration. While the agreement at lower concentrations is high, the slope of the black line is much steeper than the ideal fit, meaning that the WineScan™ is farther off with increasing SO<sub>2</sub> levels. The comparison between Ripper titration and the WineScan™ (Figure 4b) shows a slightly better correlation but also a lower accuracy overall. The line of best fit is entirely removed from the ideal fit, indicating that the titration yields consistently higher values than the micro-distillation.

The best correlation can be seen between the Ripper titration and the Sentia™ analyzer (Figure 4c and d). The only wines that significantly deviate from the line of best fit are those that contain ascorbic acid. The wines show a “moving” endpoint for the Ripper titration since it takes substantially longer to reach a stable purple color. Ascorbic acid can be confirmed by the addition of acetaldehyde to the sample prior to titration. The acetaldehyde binds all free SO<sub>2</sub> and the remaining reducing power then comes from ascorbic acid. However, this confirmation was only



done for wines that were suspected to contain ascorbic acid after the initial Ripper titration. When the wines are removed from the graph that had ascorbic acid additions confirmed, the correlation coefficient increases from 83% to 92%. The line of best fit shows a slight offset at lower sulfur dioxide concentrations, indicating a lack of accuracy for the titration. This is not surprising as the titration is based on a color reaction and a factor calculation, so even small added volumes of iodine result in relatively high calculated numbers. Overall however, the wines that use sulfur dioxide as the main source of preservation, correlate very well.

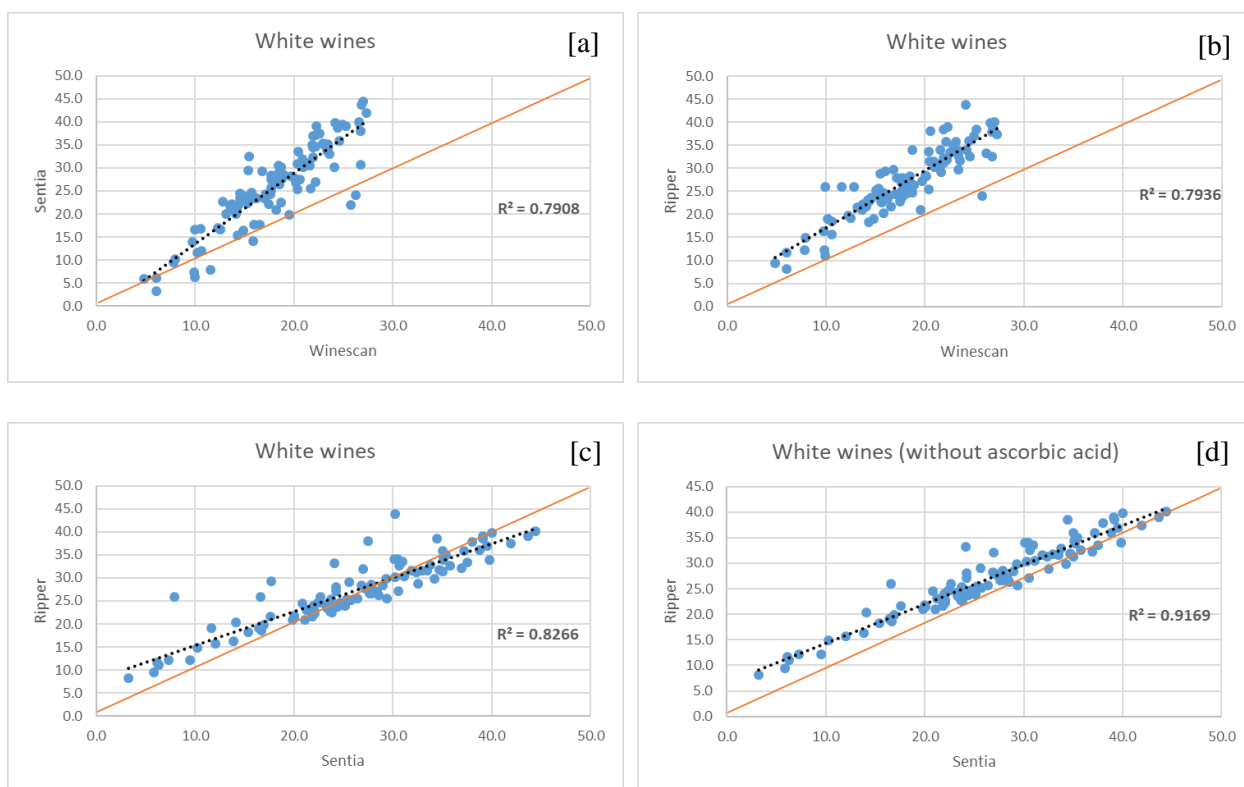


Figure 4 Comparison between analytical results for free sulfur dioxide in white wines (n = 103 in a, b, and c; n = 98 in d).

### Red wines

Red wines generally contain high levels of reducing substances like polyphenols and tannins from the skins, seeds, and various types of barrel aging procedures. Although the antioxidant potential can help to reduce the amount of SO<sub>2</sub> that needs to be added to the wine to protect it from oxidation, some analytical methods

struggle to differentiate any reducing substances from sulfur dioxide. The same trend that was discussed above for white wines, is also visible for red wines. The WineScan™ delivers slightly lower results compared to the Sentia™ analyzer (Figure 5a), while the Ripper titration again overestimates sulfur dioxide especially in lower concentrations (Figure 5c). The overall correlation between Ripper titration and the WineScan™ is better than any other comparison in red wines, however, the accuracy is questionable.

The accuracy offset between the line of best fit and the ideal correlation shown in Figure 5b is larger compared to white wines, which indicates a larger difference between the SO<sub>2</sub> loss during the WineScan™ analysis and the increased titration response due to phenolic material in red wines. The correlation coefficients vary between 76% and 89% for the exact same reasons.

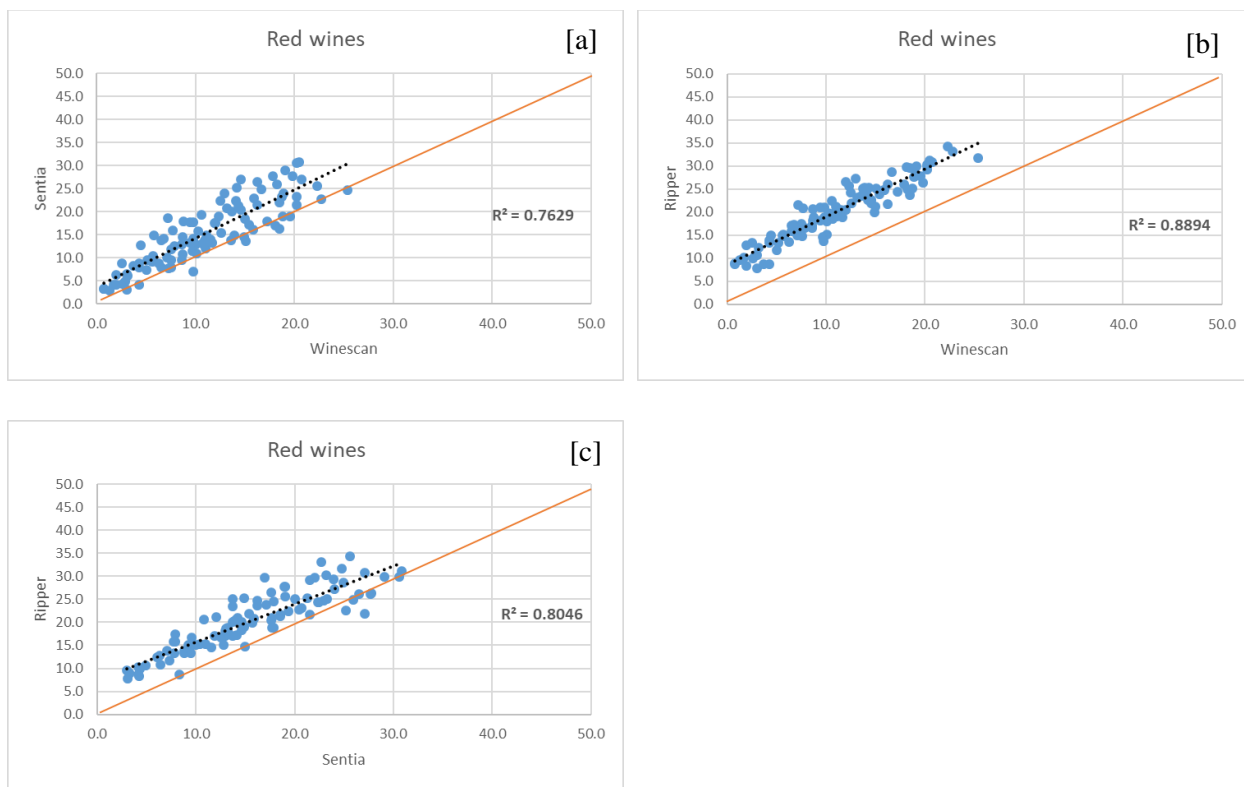


Figure 5 Comparison between analytical results for free sulfur dioxide in red wines (n = 100).

## All wines

When combining all 200 wines into the same correlation matrix, the overall performance of the Sentia™ analyzer in comparison to the other methods shows the same trends. Since no red wines were removed from the original dataset, only the white wines that contained ascorbic acid are missing between Figure 6c and d. Correlation coefficients over the whole dataset vary between 81% and 88%, showing a high degree of agreement considering the broad variability among commercial wines.

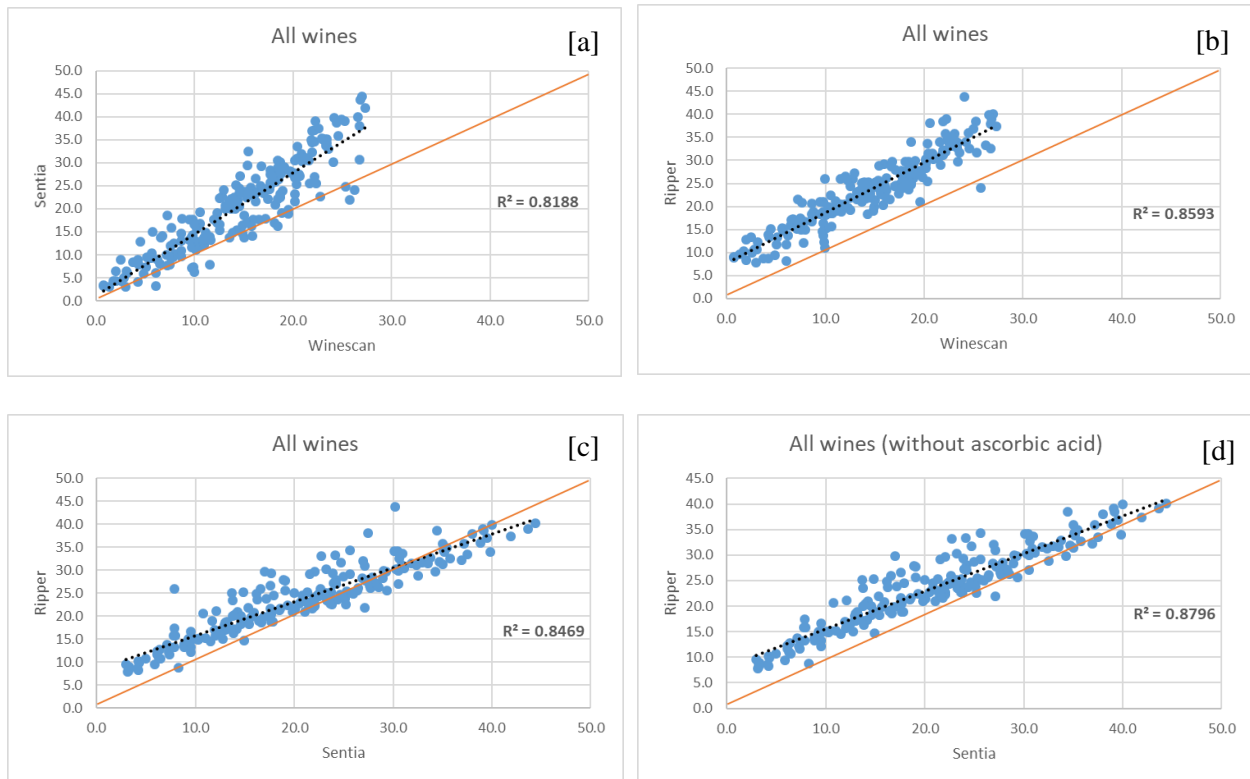


Figure 6 Comparison between analytical results for free sulfur dioxide in all wines (n = 203 in a, b, and c; n = 198 in d).

## Discussion

### Handling of the Sentia™ Analyzer

The Sentia™ analyzer for free SO<sub>2</sub> is designed like a blood sugar monitor which has multiple advantages for a winery production environment with the possibility of untrained personnel doing the analyses. Most importantly, it is a closed system. The sample is not introduced directly into the system but is dosed onto a test strip. That eliminates the cleaning step and ensures that just the right amount of wine is moved through capillary forces, minimizing user error. The liquid wine is never in touch with the instrument itself and can therefore not contaminate the analyzer. Carryover and false positive readings are also highly unlikely this way. The analyzer itself is robust and the only opening is the slot for the test strip, keeping the electronics fairly safe from splashing water in a winery.

Everything from selecting the type of sample, introducing the test strip, and producing the result is intuitive and supported by instructions and images on the display of the instrument. That makes the process very robust and easily understandable for winery interns. With very little practice and basic pipetting skills, workers of all knowledge levels should be able to produce consistent and reproducible results.

The data can either be copied from the display right after the analysis or exported electronically. This flexibility allows the user to move around the winery freely with this handheld instrument, either filling tank cards with the results directly or moving the data into a laboratory information management system (LIMS) or any winemaker software. The analyzer connects to WiFi which helps to incorporate it into a workflow and working environment.

The analyzer can operate in two different main setups, either on battery or in place while plugged in. Since testing the battery life was not part of this study, it was mainly operated on a constant power supply.

### Sources of error

In contrast to the majority of other analytical methods for sulfur dioxide, the Sentia™ analyzer does not require any sample preparation. Besides basic clarification, the sample can be introduced right out of the bottle or tank. This reduces the sources of error to an absolute minimum.

The analysis of sulfur dioxide is generally sensitive to oxygen. Whenever a bottle is opened or a tank sample is pulled, the analysis should be done as soon as possible. From the limited experience from this study, open samples for more than 30 minutes should be avoided. Any sampling technique that introduces oxygen into the wine, such as pouring needs to be avoided. The best sampling technique with the best reproducibility is drawing directly from tank or barrel, or pipetting out of a freshly opened bottle.

If samples are taken from barrels or tanks, mixing is essential. Wine from the surface might have experienced more exposure to oxygen, so homogenizing the vessel to some degree will improve the consistency and quality of results. This is especially true for sensitive techniques like the Sentia™ analyzer.

The only actual user error that could be introduced when operating the analyzer is pipetting of the sample. The best reproducibility could be achieved when using a 20 µL transfer pipette. That way, some excess wine is added to the test strip without flooding it completely. It is not recommended to keep the wine in the pipette tip for an extended period of time, since it seems to promote oxidation of the sample, which is a noted issue with all pipette transference. Rapid pipetting of small volumes is the best way to load the test strip.

### Method comparison data

Table 1 compares the standard deviations of all analytical methods tested in this study. In order to see an overall concentration effect, wines with less than 20 mg/L free SO<sub>2</sub> were separated from wines that were above that level. The third number for each category represents the standard deviation across all wines. It has to be noted that the standard deviation for the Sentia™ analyzer reflects data from two different instruments. The other methods were performed with the same instrument or setup, so the Sentia™ analyzer data introduces an additional source of variability.

Table 1 Comparison of standard deviations [mg/L] between analytical repetitions

	Sentia™ Analyzer		FOSS WineScan™ SO <sub>2</sub>		Ripper Titration	
	White wines	Red wines	White wines	Red wines	White wines	Red wines
Below 20 mg/L	0.37	0.48	0.34	0.30	0.22	0.40
Above 20 mg/L	0.70	0.73	0.50	0.36	0.28	0.36
Whole range	0.63	0.55	0.41	0.31	0.27	0.38

Even though the standard deviation in the higher sulfur dioxide concentration range is higher compared to the lower levels, all average standard deviations are well below 1 mg/L. Given all the challenges and sources of error for this analyte, these results are impressive. With very similar reproducibility, accuracy, and precision, compared to the reference methods, the Sentia™ analyzer provides a reliable and easy-to-use alternative.

In order to understand the influencing factors for this study, it is worth looking at the commercial wines that were included. Figure 6 shows the whole range of over 200 wines that were analyzed. While the majority of white and red wines show very similar total sulfur dioxide levels (Figure 7a), white wines have slightly higher concentrations on average. Most wines were less than three years old, with red wines spanning over seven vintages. This broad range was necessary to ensure that the levels of free sulfur dioxide cover the analytical capacity of the Sentia™ analyzer between 3 and 50 mg/L.

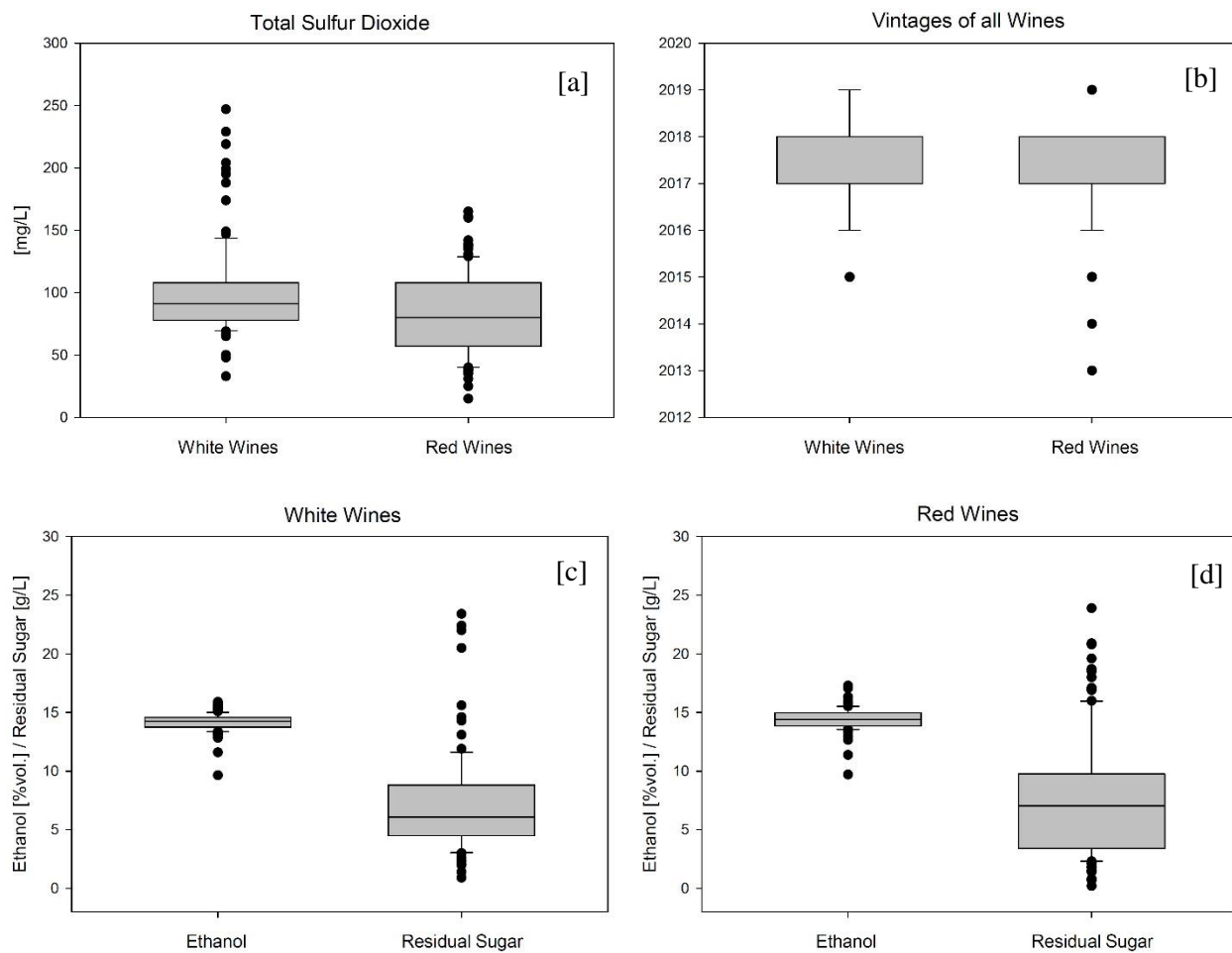


Figure 7 Characterization of all commercial wines included in this study.

Ethanol and residual sugar concentrations were surprisingly similar between red and white wines, also spanning the whole range of specifications. None of these parameters influenced the accuracy of the Sentia™ analyzer.

## Conclusion

The comparison between the Sentia™ analyzer for free SO<sub>2</sub> and two reference methods confirms that the electrochemical analysis in a handheld instrument can provide the same level of accuracy and reliability that could be expected from a standard laboratory method. In addition to that, the compact design and simple procedure eliminate sources of error that can make the analysis of sulfur dioxide extremely challenging. The data range between 3 and 50 mg/L free SO<sub>2</sub> is fully sufficient for the majority of commercially available wines in California. None of the specific properties of Californian wines appears to influence the accuracy of the Sentia™ analyzer. Based on the results of this study, it can be highly recommended for the use in finished and clarified still wines. It should be noted that the wine does not have to be filtered. Wines that are pre-clarified and are still stored in tanks or barrels can also be analyzed.

## Literature

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## Additional Information

### Information about the Viticulture and Enology Research Center (VERC)

Faculty and staff conduct grape and wine research through the Viticulture and Enology Research Center in the Department of Viticulture and Enology at Fresno State. VERC is one of three research centers under the auspices of the California Agricultural Technology Institute within the Jordan College of Agricultural Sciences and Technology.

VERC is widely recognized as an applied research and education institution for the California raisin, table, and wine grape, and wine industry. Through partnerships including industry, state, and government entities, faculty and research scientists conduct solution-driven research and disseminate knowledge to the grape and wine industry and community.

Students may enhance their academic careers by working under the direction of faculty and research scientists on projects designed to benefit the grape and wine industry. In addition to Fresno State's Master's degree program in viticulture and enology, there are also several industry-funded research assistantships available each year to interested students who work and learn under the direction of our research faculty and staff.

With a long history of industry support, the Viticulture and Enology Research Center and Department of Viticulture and Enology building provides research laboratories, administrative and faculty offices, meeting space, and a specialized viticulture and enology library that is available to the campus community and the public. Other unique facilities used for research and teaching include the raisin processing plant, greenhouses, a research winery, a commercial winery, and the campus vineyard as part of the University Agricultural Laboratory.

### Information about the Principle Investigator of this study

Dr. Stephan Sommer joined the Department of Viticulture and Enology as the Director of the Viticulture and Enology Research Center in the Spring 2019 semester. As Director of VERC, Sommer provides research and administrative leadership by planning, promoting, and administering all viticulture and enology research programs. Responsibilities involve collaboration with other research centers within the Jordan College and university, the Department of Viticulture and Enology, other institutions, and the grape and wine industry.

Dr. Sommer earned an Engineering Diploma in Beverage Technology from the Hochschule Geisenheim University (Geisenheim, Germany) and a PhD in Microbiology and Wine Science from the Johannes

Gutenberg University (Mainz, Germany). He managed a federal analytical research laboratory in Germany for eight years before moving to the United States in 2013.

Prior to joining Fresno State's viticulture and enology team, Dr. Sommer was the academic program director and an Assistant Professor of Fermentation Sciences at Appalachian State University in Boone, NC. He also brings more than eight years of industry experience in various fields of beverage production including wineries, sparkling wine companies, breweries, table water production, and related fields including industrial flavor production. His main research focus is on physical and chemical product stability, beverage microbiology, and wine aging.