Best Practice Principles for Measurement of Total Sulfur Dioxide in Wine

Executive Summary

- Sulfur dioxide (SO_2) is an important component of the wine making process providing both antimicrobial and antioxidant properties.
- In wine, it exists in a range of forms in a dynamic equilibrium with water and other wine components which is independent of the form that the original SO_2 was added to the wine and changes over time.
- It is often only practical to refer to the total SO_2 present when it is deemed necessary to define limits.
- It is essentially impossible to analytically determine the form in which the SO_2 was originally added to the wine.
- To avoid trade issues arising solely from to inconsistent measurement there is a significant need for agreement on best practices for the common methodologies used in the measurement of total SO_2.
- There are a range of methodologies which can be used for the determination of total SO_2, each with different advantages and drawbacks.
- Aeration Oxidation (AO) is most easily implemented in non-specialist laboratories and is free of significant interferences.
- Independent of the method used, the volatile nature of SO_2 and its sensitivity to oxidation mandate that careful sample handling and rigorous quality assurance procedures using matrix matched control samples are required to achieve accurate and precise results.

1. Introduction

Sulfur Dioxide (SO_2) is a substance with both antioxidant and antimicrobial properties commonly used in a vast range of foods and beverages produced around the world. In wine, it occurs both naturally as generated by yeast and as a common additive used both for its antimicrobial and antioxidant properties. Due to the importance of these two functions in wine it is one of the most common analyses during production and its levels in wine are carefully controlled to ensure that effective levels are present without impacting the sensory characteristics of the final product. It is also one of the most commonly regulated wine parameters around the world. As such its accurate and reproducible measurement in wine is of prime importance to ensure the free and efficient movement of wine between markets.

Unfortunately, the quality of measurement of total SO_2 in different markets can vary significantly. This was demonstrated by the results of a ring test programme commissioned by the APEC Wine Regulatory Program in 2015, the results of which are presented graphically in Figure 1.

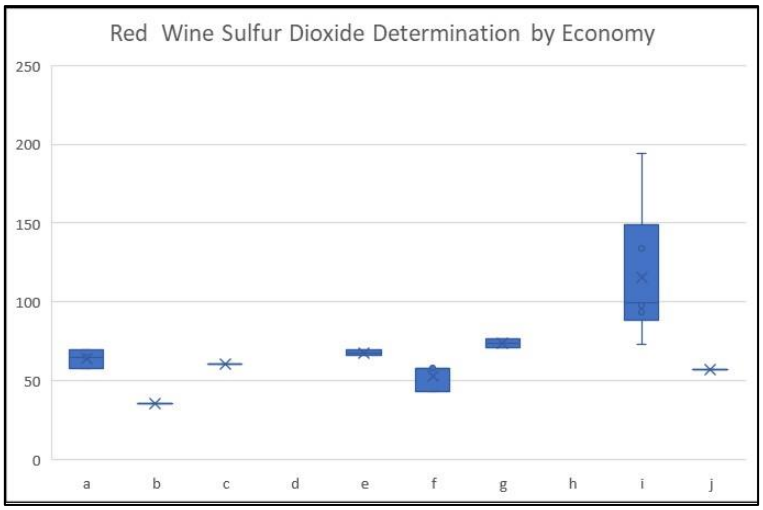

Figure 1, the total SO_2 results determined by 19 different laboratories in 10 economies for the same red wine as part of the APEC Wine Regulatory Forum 2015 ring test programme.

The results for a group of accredited and government nominated laboratories from ten different economies varied between 35 and 194 mg/L, or more than 500%, even though the laboratories were running an "official" method. Obviously, there is a significant need for improved standardisation and quality assurance practices to ensure comparable results between markets.

This paper aims to demonstrate the strengths and weaknesses of the most common methods and the minimum quality assurance steps that should be expected in the performance of the assay. It will provide best practices in order for laboratories to develop guidelines to successfully analyse wine samples based on methods currently in existence.

2. Sulfur Dioxide Pathways to Wine

SO_2 can be added to wine at many points during its production. It is not uncommon for the precursor salt potassium metabisulfite (KMBS, or sometimes PMS), which forms SO_2 on dissolution, to be added to grapes in harvest bins immediately after picking. Once at the crusher SO_2 is once again commonly added either as KMBS or liquid SO_2 to prevent oxidation and inhibit unwanted microbial activity.

Once primary fermentation has begun, SO_2 is produced by wine yeast as an intermediate in its sulfate assimilation metabolic pathway. The amount of SO_2 released into the wine depends on the yeast strain and fermentation conditions, ranging between a few mg/L up to 100 mg/L. SO_2 is not usually added during primary or secondary fermentation in wine due to its potential to inhibit these processes.

Post primary and secondary fermentation, SO_2 can be added to wine either as KMBS or as liquid SO_2 to control any unwanted microbial activity and to limit the effect of oxidation. During wine storage and maturation, particularly in barrel, SO_2 levels are carefully monitored to ensure that sufficient SO_2 is present to achieve the required technical goals. Also changes in levels of SO_2 are an indicator of unwanted oxidative or microbial activity and as such act as a trigger for other interventions in production.

Finally, SO_2 levels are normally adjusted immediately prior to packaging to ensure that sufficient SO_2 is present in wine to ensure that it is adequately protected against oxidation or microbial activity for its foreseeable shelf life. The final levels of SO_2 present in wine from all these inputs are generally well below the typical regulatory limits found in markets around the world and significantly lower than those found in many other packaged foods. High levels of SO_2 in wine are not generally considered favourable as they can begin to have a negative impact on the sensory attributes of the final product.

Once a wine has been packaged, SO_2 performs its functions in a sacrificial manner, and the measured levels decrease over time. The speed of the decrease is a function of range of factors including the wine composition, the amount of oxygen introduced at the time of packaging, the nature of the package and the closure, and the environmental conditions under which the wine is stored.

3. Forms of Sulfur Dioxide Present in Wine

In aqueous solutions such as wine, SO_2 does not exist only as a discrete entity, but rather as a complex mix of related compounds linked by equilibria through reactions with water and other wine components. It should be noted that all the different forms in which SO_2 can be added, liquid, gas or as salt, equilibrate to the same forms in solution. That is, it is not possible in wine to differentiate if the SO_2 present has been naturally generated by yeast, added in the form of liquid SO_2 or as the salt KMBS.

On addition to wine the primary equilibrium that occurs is between SO_2 and water. This leads to three major forms being present in wine as shown in Figure 2.

Figure 2, the equilibria between SO_2 and water.

Of these three forms, at typical wine pH, between 92% and 99% is present in the bisulfite form. It must be noted, however, that these species do exist in equilibrium and the proportions of each will shift with changes in wine pH and other chemistry. The molecular form, usually present in relatively small amounts, is the primary form responsible for the antimicrobial activity of SO_2. The bisulfite form has an important role in the antioxidant properties of SO_2 while the sulfite form is present only in vanishingly small quantities.

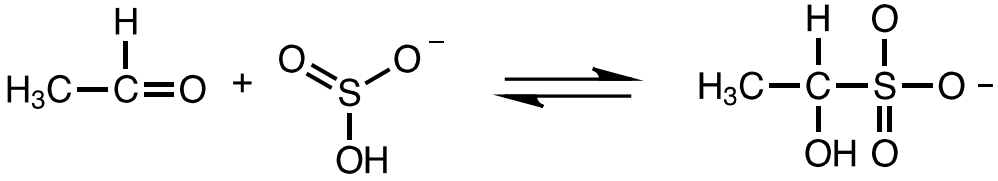

Figure 3, the equilibria between acetaldehyde and bisulfite to form bound SO_2.

Adding to the complexity of speciation of SO_2 in wine is that it is also involved in chemical equilibria with other wine components. Principle amongst these equilibria is that with acetaldehyde (Figure 3), however bisulfite will also bind weakly to other carbonyls, phenolics, colour compounds and sugars found in wine. SO_2 bound strongly to wine components in this way is generally considered to be unavailable for antimicrobial and antioxidant activities and is referred to as the *bound SO_2*. The unbound component is referred to as the *free SO_2* and in wine it consists of molecular SO_2 and bisulfite in equilibrium as described above. The sum of the free and bound quantities of SO_2 is referred to as the *total SO_2* of the wine.

Free SO_2 + Bound SO_2 = Total SO_2

The bound component is usually significantly greater than the free component and in practice, SO_2 tends to be added to a wine to achieve a given free SO_2 level as this is the active constituent. The proportion of free SO_2 will, however, change as the wine ages or reacts with oxygen during storage and given the relatively complex speciation involved, for practical reasons SO_2 regulatory requirements tend to be phrased in terms of total SO_2, disregarding the differing forms in which it may be present.

Levels of total SO_2 are reported in terms of mg/L of wine, although the equivalent parts per million (ppm) is occasionally used. The SI approved mg/L is generally considered preferable.

4. Methods for Measuring Total Sulfur Dioxide.

There is significant debate as to the most appropriate methodology for measuring the proportion of free SO_2 in wine due to the competing equilibria involved. These questions, however, are outside the scope of this paper, which will focus on the best practices for the measurement of total SO_2 as this is the form most commonly regulated and is the most relevant for market access purposes. There are three generally accepted methods for the determination of total SO_2.

Aeration/Oxidation

The most common methodology used for the determination of total SO_2 is the aeration/oxidation method (AO) as described in OIV method OIV-MA-323-04, which is similar to a number of other methods in use (Monier/Williams, Frantz Paul, modified Rankine, etc.). Essentially the analysis involves the heating of an acidified sample of wine to liberate the SO_2 in the molecular form. A stream of air is then used to carry the liberated SO_2 to a reservoir of hydrogen peroxide where it oxidises it to form sulfuric acid. The generated sulfuric is then titrated against dilute sodium hydroxide in the presence of an indicator to determine the amount of acid formed and hence the original quantity of SO_2 in the wine.

As the methodology liberates the SO_2 analyte from the sample matrix, it is relatively robust in terms of the interference from other wine components. The equipment to do the procedure is, however, relatively specialised and needs to be carefully used and maintained, as do reagents and gas flow rates. Each individual analysis also takes in the region of 20 minutes and requires the constant attention of a trained technician. The method is however, very robust and repeatable. A detailed description of the method is given in Iland et.al. referenced below.

Automated Flow Techniques

These techniques use either Flow Injection Analysis or Segmented Flow instruments to automate the determination of total SO_2. Essentially, they all work by passing a heated acidified wine solution past

a gas permeable membrane. This liberates the SO_2 which passes through the membrane into a buffered carrier solution and is then reacted with a SO_2 sensitive dye such as pararosaniline. The interaction of the dye and the SO_2 forms a coloured solution, with the intensity of the generated colour then measured spectrophotometrically. This response is compared to a standard curve to indicate the amount of total SO_2 present in the original wine.

While capable of providing results comparable to AO, these techniques require relatively expensive equipment and lend themselves to facilities running large numbers of samples each day. They also tend to require a high degree of maintenance by trained technicians.

Iodic Titration

Iodic titration, also commonly known as the Ripper method, or OIV method OIV-MA-323-04B, involves the titrating of a wine sample against a standardised iodine solution in the presence of starch (or vitex indicator) after converting the SO_2 present to the bisulfite form by the addition of sodium hydroxide. The bisulfite reduces the molecular iodine to iodate until all the bisulfite present has been exhausted, at which point the solution turns blue due to the interaction of the free iodine with the starch indicator. The total SO_2 is then calculated by the amount of iodine required to reach the blue colour. Some analytical equipment suppliers have automated this procedure using autotitrators and various indicator electrodes.

While much quicker and involving less equipment than the AO methodology, the iodic titration method suffers significant interferences from wine components including phenolics, sugars, aldehydes and other reducing substances. Red wines in particular are known to give erroneously high results due to the reaction of some of the colour compounds. The presence of any ascorbic or erythorbic acid also quantitatively reacts with iodine and this methodology cannot be used in wine which may contain these preservatives, unless they have first been quantified. It is generally acknowledged that iodic titration methods give a less accurate determination of total SO_2 than AO based methods for these reasons. A detailed description of the method is given in Iland et.al. referenced below.

Other Methods

There are a range of other analytical methods available in kit form which tend to rely on the use of coloured dye in buffered wine solutions and measurement in either spectrophotometers or sequential analysers. While holding some promise, all are impacted by wine matrix effects to a greater or lesser extent and have yet to find widespread acceptance. There have also been developed several electrochemical methodologies. These, however, tend to be low throughput and require specialised equipment and training offering little or no advantage over the other methods above for total SO_2.

5. Sample Handling and Management

SO_2 is a relatively volatile compound in the wine matrix, and as such sample handling and management is critical to ensure accurate and precise results. The following are the main considerations.

1. Samples should not be opened until immediately before analysis to avoid loss of SO_2 through volatility or oxidation.
2. If a sample must be decanted, it should be done very carefully to avoid any aeration into a glass container which can be sealed with no air space under the closure.
3. If decanted samples must be stored they should be refrigerated. Freezing samples is not recommended.

4. Refrigerated samples should be allowed to equilibrate to 20°C in sealed vessels before analysis.
5. During transfer and pipetting of samples great care should be taken to avoid aeration of the sample.

It should be noted that these same principles apply to many analyses, but it has been found that these particular considerations are especially important in the analysis of total SO_2.

6. Best Practice Quality Assurance

While for the reasons outlined above it is generally accepted that the AO method is a robust and widely applicable reference method for total SO_2 determination, it is critical that a range of general quality assurance steps are applied to the measurements to ensure the consistency of results and comparable results between laboratories. The steps outlined below are considered the minimum necessary to ensure accurate and precise results. It should be noted that they do not negate the need for a vigorous validation process when the methodology is first introduced to a laboratory or when significant changes are made to equipment, reagents or procedures.

The minimum procedures outlined below are framed in reference to the AO procedure, however they are equally applicable to automated flow methods. Given that iodic titration has associated matrix effects which can vary significantly from wine to wine, reliable quality assurance is much more difficult and it cannot be recommended as a method which would be used for regulatory use.

Control Samples

Every first and tenth sample should be a matrix matched wine of known total SO_2. In the case of less than 10 samples being run on a given day the sample run should be bracketed by control samples. The control wine can be any wine of known total SO_2, either through analysis by another trusted laboratory, or more commonly, by comparative overlap with a previous control wine. Alternatively, the control wine total SO_2 can be determined by the method of standard additions, although great care must be made in the addition of SO_2 (usually in the form of potassium metabisulfite) to ensure none is lost through volatility or oxidation.

Commonly a quantity of commercial wine sealed under screw cap closures can be used to provide a control wine. Wines from a single case under screw cap generally have relatively insignificant variations in total SO_2 as long as the wine has been stored in conditions below 25°C. Wines under cork closure are not generally considered suitable as control wines as the variation in the closures can lead to significant variations in the total SO_2 in bottle.

It is generally acceptable to use a cask (boxed) wine as a control wine allowing a greater interval before the validation of a new control sample. If a cask wine is used, it is important that it is stored in a refrigerator and great care is taken in decanting the wine from the cask to avoid oxidation and the loss of total SO_2. One suggested method is to fit a short section of hose to the cask spout and to fill the transfer container slowly from the bottom with the hose submerged.

The results for the control samples should form the basis of a control chart with the variation from the mean not exceeding 5%. If a cask wine is being used as a control, allowance must be made for the slow degradation of total SO_2 due to oxygen ingress across the cask membrane. This is most easily achieved by using a moving average for the central tendency line of the control chart.

If automated flow techniques are in use, it is recommended that two separate controls, red and white, are used to ensure that there is no difference in matrix dependency. This is not generally considered necessary for AO methods, as colour has not been shown to be a significant issue for this method.

Duplicate Samples

Duplicate samples should also be run regularly, especially in cases where the determination of total SO_2 is not a common occurrence for the laboratory. The use of duplicates should not replace the use of matrix matched control samples.

Known Standard

On a weekly basis, a freshly made standard 100mg/L SO_2 solution should be analysed. The recovery from the analysis of this solution should be within $\pm$5%. Details on the preparation of this standard can be found in Iland et.al. referenced below.

Volatile Acidity Interference Check

The presence of high levels of acetic acid is one of the few possible interferents in the AO method. It is negated by the use of an appropriate condenser between the sample and the hydrogen peroxide solution as well as careful control of the carrier air flow rate, which should be regularly checked to ensure a 1 L/min flow. To check the effectiveness of the equipment, a 1 g/L acetic acid solution should be analysed at least once a week with no more than 1 mg/L of calculated Total SO_2 apparent. This procedure is not applicable for analysis by iodic titration.

7. Conclusion

Sulfur dioxide is an important component of the wine making process providing both antimicrobial and antioxidant properties. In wine, it exists in a range of forms in a dynamic equilibrium with water and other wine components which is independent of the form that the original SO_2 was added to the wine, and changes over time. For this reason, it is often only practical to refer to the total SO_2 present when it is deemed necessary to define limits. This also implies that it is essentially impossible to analytically determine the form in which the SO_2 was originally added to the wine.

Despite the upper limits of SO_2 in wine being regulated in most markets, the performance of laboratories involved in regulatory testing is significantly varied. This would suggest that there is a significant need for agreement on best practice methodologies.

There are a range of methodologies which can be used for the determination of total SO_2, however the one most easily implemented in non-specialist laboratories and free of significant interferences is Aeration Oxidation (AO). Automated flow methods tend to be more suited to high throughput specialist wine laboratories while iodic titration suffers from a range of interferences which can lead to erroneously high results.

Independent of the method used, the volatile nature of SO_2 and its sensitivity to oxidation mandate that careful sample handling and rigorous quality assurance procedures using matrix matched control samples are required to achieve accurate and precise results.

The authors wish to note that large portions of this paper were previously published in the Wine & Viticulture Journal as referenced below.

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