Technical notes

A new validated method for the determination of free and total sulfur dioxide using a discrete analyser

Introduction

Sulfur dioxide (SO_2) is a preservative widely used in foods and beverages. In wine, SO_2 occurs naturally (generated by yeast) and is a very common additive. Due to the importance of its antioxidant and antimicrobial functions in wine, SO_2 is one of the most commonly analysed wine components and its levels are carefully controlled to ensure effective performance without negative sensory impacts. 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 both in the production of sound and stable wine and to ensure the free and efficient movement of wine between markets.

In Australia, the most common method for the determination of both free and total SO_2 is the aeration/oxidation method (AO) which is also sometimes known as the Monier/Williams or Rankine/Pocock method and is the basis of the OIV approved method OIV-MA-AS323–04A (OIV methods). The free SO_2 AO method involves acidification of a 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 acid 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. Total SO_2 is determined in the same manner with the original sample being heated to release the bound forms of SO_2.

As this method liberates the SO_2 from the sample matrix, it is relatively robust in terms of interference from other wine components. The equipment and reagents required are, however, relatively specialised and need to be carefully used and maintained. Each analysis also takes around 20 minutes and requires the constant attention of a trained technician. Nevertheless, the method is very robust and repeatable, which is why it is the mainstay of SO_2 analysis in most wineries.

There are a number of alternative methods that allow SO_2 analysis to be automated to speed up analysis time and carry out larger batches of samples. Iodic titration (more commonly known as the Ripper method) allows a rapid determination of SO_2 with limited equipment and can be automated for use on autotitators; however, it suffers from significant interferences

from other wine components and often gives artificially high results. There are also several flow injection analysis (FIA) methods on the market and in common use in large wineries. While these give very good throughput and the results compare favourably with those from the reference AO method, the required equipment tends to be expensive, essentially can only be used for SO_2 analysis and requires careful maintenance. This means that such methods are generally restricted to large laboratories with high throughput. There are also a number of kits available for use with discrete analysers (DA), which are commonly used in many winery laboratories to carry out enzymatic analysis of glucose + fructose, malic acid and acetic acid or volatile acidity. The performance of these kits has, however, been very mixed, with difficulties experienced in getting satisfactory results for all wine types.

An automated method which compares well with the reference AO method, is simple to use, fast and carried out on equipment used for other wine analysis would prove a significant boon for medium to large wineries. To meet these requirements the Treasury Wine Estates (TWE) technical team modified available literature methods to overcome problems previously encountered with SO_2 analysis on a DA, using the Thermo Gallery instrument common in the TWE laboratories. In partnership with TWE, AWRI Commercial Services independently validated the methods TWE developed, described below.

The methods

Separate methods were developed for the determination of free and total SO_2. It should be noted that these were developed specifically for the Thermo Gallery DA used by TWE. It may be possible to modify the method for other equipment; however, this will be dependent on the functions and architecture of the individual instrument. The validation data provided in this article are only applicable to the equipment described and should not be considered applicable to other similar equipment without additional validation studies. The full descriptive method for use on a Thermo Gallery DA will be made available on the AWRI website.

Free SO_2 analysis

The free SO_2 method is based on the reaction between free SO_2, in an acidic medium, with a mixture of pararosaniline and formaldehyde to give a pink colour measured at 575 nm. This method is described in OIV-OENO 391–2010 (OIV methods); however, changes were made to eliminate major interferences from polyphenols by measuring duplicate samples concurrently with one sample having had the free sulfur dioxide removed. To achieve this, pyruvic acid was added to a sample to bind free SO_2 without affecting the reaction between the SO_2 and the pararosaniline+formaldehyde reagent. A range of substances were tested for their suitability to remove/bind SO_2, with pyruvic acid proving to be the most effective.

The method requires the two tests to be run concurrently, one without pyruvic acid (FSO_2A) and one with pyruvic acid (FSO_2B). The free SO_2 is then determined using the formula:

$$\text{Free } SO_2 = m * (FSO_2A - FSO_2B) - \text{blank}$$

The value m (the response slope) is determined independently using standards. The blank is determined using 9% sodium chloride solution as in the original OIV method (refer to OIV-OENO 391–2010, OIV methods).

One factor noted during the initial development of the method using a different DA was that the timing of tests A and B was critical to the test accuracy. If the samples were batched together by test, FSO_2A and FSO_2B were not necessarily performed at similar times and the results showed unacceptable variability. Subsequent trials demonstrated that if the two tests for each sample were performed concurrently, as happens in the Thermo Gallery instrument, then acceptable accuracy could be achieved.

Also noted was that the size of the sample tubes placed into the instrument is critical for precise results. The Thermo Gallery instrument can use both 2 mL and 7 mL sample tubes. When the 2 mL tubes were used, a significant low bias on results was seen compared to the AO reference method, particularly for white wines. This bias was eliminated if the 7 mL sample tubes were used – most probably due to more rapid loss of SO_2 from the smaller tube which has greater surface area to volume ratio.

Both these findings highlight how critical it is that the method is optimised for the specific discrete analyser in use and that the equipment chosen considers the needs of the method.

Total SO_2 analysis

The total SO_2 method is based on the method described in OIV-OENO 391–2010 that uses 5, 5′-dithio-bis-2-nitrobenzoic acid (DTNB) as a reagent.

The sample to be analysed is first auto-diluted into a pH 8 phosphate buffer solution. After stabilisation, a zero measurement is taken and DTNB added. The DTNB reacts with a free sulfhydryl group to yield a mixed disulfide and 2-nitro-5-thiobenzoic acid, a yellow-coloured product measurable at 412 nm. DTNB has specificity for -SH groups at this pH, a high molar extinction coefficient and a short reaction time.

The method shows good correlation with the AO method and is suitable for juice, still wine and sparkling wine samples. One item of note found during the method development was

that it was very sensitive to the presence of chlorine in the reagent water. Even relatively low levels of chlorine, (i.e. levels not detectable using standard water testing reagent strips) led to poor results. To be effective, the water used in the test must be free of residual chlorine, usually best achieved by using a dedicated carbon filter before the water purification system.

Method performance

Presented below is an overview of the results collected as part of the validation study performed at the AWRI to accredit the methods to ISO17025 standards. The types of samples analysed for the study included finished still (red, white and rose), sparkling, fortified and botrytis wines, beer, cider, vinegar, spirits and water.

Free SO_2

Figure 1 shows a comparison of results between the new DA method and the reference AO method for free SO_2 for 132 separate samples. There is very good correlation between the new method and the reference, with the average difference just over 2 mg/L, with a standard deviation (SD) of 1.83.

The method was shown to be linear (0.9991) over a range of 0 to 75 mg/L with good repeatability (CV =2.9%) and reproducibility (SD 0.52 mg/L) with a limit of quantification of 3 mg/L. The uncertainty of measurement (UOM) for the method was determined to be ±3 mg/L and the recovery averaged 99.97% across all the standards measured. These results represent an excellent correlation with the standard AO method.

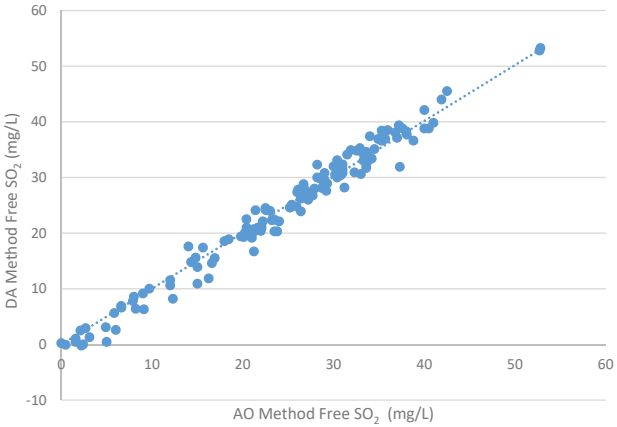

Figure 1. Comparison of the new DA method with the reference AO method for free SO_2 analysis

Total SO_2

Figure 2 shows a comparison between the new DA method and the reference AO method for total SO_2 for 135 separate samples. Very good correlation is again seen between the new method and the reference method, with the average difference being just over 8.6 mg/L (SD 5.74).

The method was shown to be linear (0.9998) over a range of 0 to 300 mg/L with good repeatability (CV =1.72%) and reproducibility (SD 0.76 mg/L) and a limit of quantification of 3 mg/L. The uncertainty of measurement (UOM) for the method was determined to be ±3 mg/L and the recovery averaged 99.60% across all the standards measured. As for the free SO_2 method, these results represent an excellent correlation with the standard AO method. It should be noted that the DA method did show a slight tendency to higher results than that from AO. This appears to be a function of the higher recoveries achieved with this method – in other words, the new method is most likely giving a more representative result for total SO_2, especially at higher total values.

Conclusion

This work has shown that analysis of free and total SO_2 in wines and juices using the Thermo Gallery DA is a viable alternative to other established methods. The new method gives excellent correlation with the industry reference AO method and can process large batches of samples. In fact, it is feasible to run more than 100 free and total samples in a day using this method with only limited staff input. The method also has the advantage that the instrument can also be used to analyse other wine components such as glucose, fructose, malic acid and volatile acidity (as acetic acid).

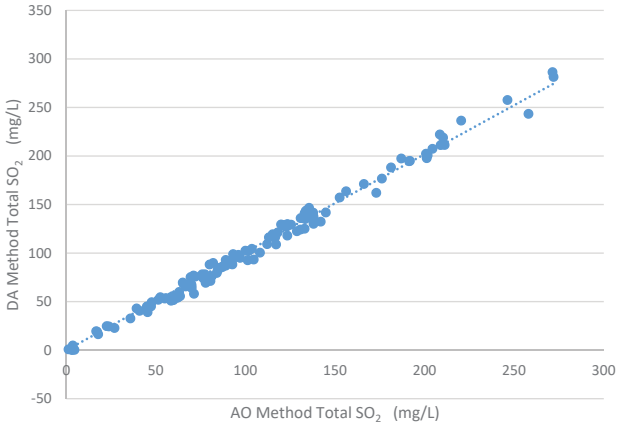

Figure 2. Comparison of the new DA method with the reference AO method for total SO_2 analysis

The new DA method has the potential to be transferred across models and brands of DA instruments; however, care would be needed to develop and validate it for each instrument to address issues such as loss of SO_2 from samples and the sequence and timing of the individual measurements performed. As with all analytical measurements, especially automated ones, well-designed quality assurance measures are essential.

Reference

OIV methods: http://www.oiv.int/en/technical-standards-and-documents/methods-of-analysis

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