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APPLICATION NOTE 71451

# Fast and accurate automated method for free sulfite analysis in wine


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**Keywords**

Discrete Analyzer, Photometric Analyzer, Gallery Analyzer, Arena


## Goal

To develop a method for free sulfite analysis in wine using automated discrete photometry

## Introduction

Sulfur dioxide ($SO_2$) is added to control the process of wine making. It serves many useful functions, for example, it acts as an enzyme inhibitor in musts preventing juice from browning. As a microbiological control agent, $SO_2$ is added to the winemaking process to prevent oxidation in the finished product.

Sulfur dioxide can be found in wine in its free forms, $SO_2$ (gas) and bisulfate ion ($HSO_3^-$), or bound to compounds that incorporate a carbonyl group, such as acetaldehyde. Free forms of $SO_2$ are pH and temperature dependent and because of the acidic nature of wines, $SO_2$ is usually present and measured as bisulfate ion ($HSO_3^-$). Results are reported as $SO_2$.

The presence of total $SO_2$, both free and bound, is regulated and, as a result, a warning statement is required on wine labels because sulfite is considered an allergen. The European Union established a maximum permitted level of total $SO_2$ in wine varying from 150 to 500 mg/L which is dependent upon the sugar level of the product.

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In the USA, the maximum level of total $SO_2$ permitted is 350 mg/L.[1] The measurement of both total and free $SO_2$ can be automated using Thermo Scientific™ system reagents and Thermo Scientific™ Gallery™ discrete analyzers[2].

In this study, an automated method to measure free $SO_2$ in wine samples is presented. The method is based on the reaction between sulfur dioxide, *p*-rosaniline hydrochloride, and formaldehyde. This method is designed to use optimal reagent concentrations and volumes to provide accurate results. The concentration of free $SO_2$ in the sample is calculated automatically from a calibration curve. This method enables a laboratory to fully automate $SO_2$ determinations and replace traditional time consuming reference and distillation methods.

As a result of using bar-coded system reagents, this new automated method to measure free $SO_2$ is very quick and easy to use. Analysis of 60 samples takes 35 min with only an additional 10 min for daily calibration and analyzer start-up. From the same samples, various sugars, acids, color, and total $SO_2$ can be run automatically.[3] Compared to Flow Injection Analysis (FIA), the photometric method requires only small volumes of reagents, thus becoming the more economically and environmentally friendly choice.[4]

## Experimental

### Materials and methods

#### Instruments

A Gallery discrete analyzer was used for this study.

Reference values were obtained from a commercially available FIA analyzer and the reference method was based on *p*-rosaniline hydrochloride and formaldehyde reactions.

#### Method principle to analyze free $SO_2$

The method is based on the reaction between $SO_2$, *p*-rosaniline hydrochloride, and formaldehyde and is performed at 37 °C, using a 575 nm filter and a 700/750 nm filter for side wavelength. The purpose of measuring a side wavelength is to remove the effect of bubbles which may appear in cuvettes. The side wavelength is determined from the spectrum area where no reaction occurs. See Figure 1 for the reaction details.

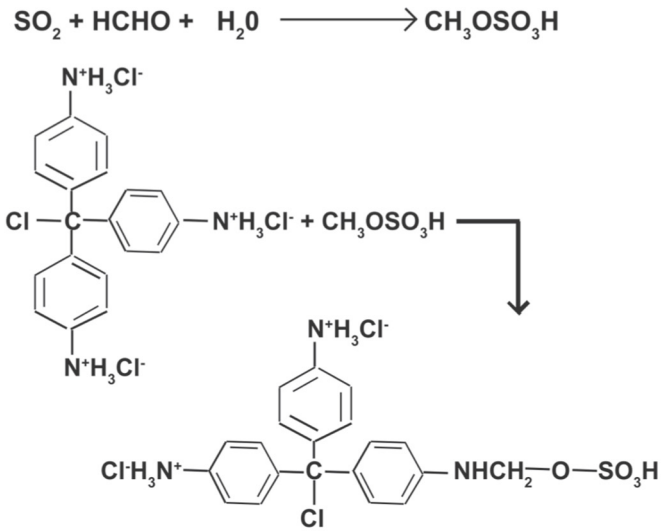


**Figure 1. Method principle to analyze free $SO_2$**

#### Application to wine samples

The automated application to measure free $SO_2$ on Gallery discrete analyzers consists of two reagents, an end-point measurement with a sample blank, and a second order calibration curve used for result calculation. The measuring range of the method is from 2 to 100 mg/L and can be extended by automated dilution of the sample.

First, 100 µL of $SO_2$ Free R1 reagent and 10 µL of sample with 10 µL of water are incubated for 30 seconds and the sample color is blanked. Then, 100 µL of R2 reagent is added. After 75 seconds of incubation the reaction is measured at 575 nm with a side wavelength measurement at 700/750 nm.

#### Reagents and calibrator

Thermo Scientific system reagents for Gallery discrete analyzers were used. The $SO_2$ Free kit is available using the P/N 984634.

Calibration was performed with a self-made standard prepared from sodium metabisulfite ($Na_2S_2O_5$ MW = 190.11 g/mol). The standard concentration was 200 mg/L and calibration points were automatically diluted by the analyzer (Figure 2).

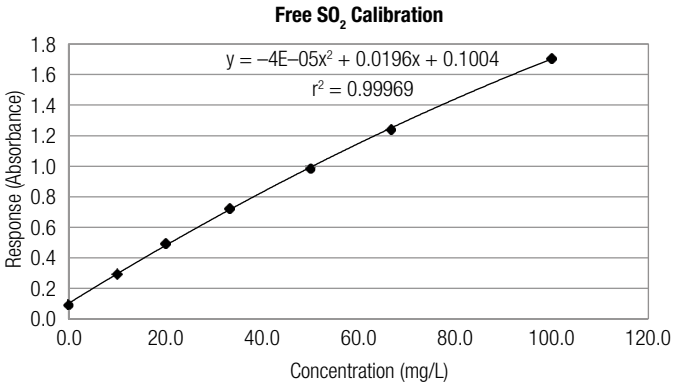


**Figure 2. Method calibration for free $SO_2$ using an automated dilution of the calibration stock solution**

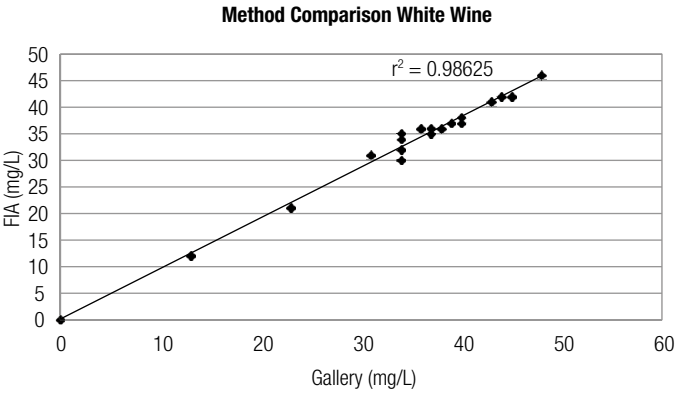


**Figure 4. White wine sample comparison between Gallery and FIA methods**

### Samples

Fifty-seven (57) wine samples were analyzed. Sample types included both red (N = 35) and white wines (N = 22). Reference values were determined using the *p*-rosaniline method on a FIA analyzer. No sample pretreatment was done prior to the analysis.

## Results and discussion

The results of free $SO_2$ in 35 red wines and 22 white wines using the Gallery discrete analyzer and FIA are compared in Table 1 (page 4). The concentration of free $SO_2$ in samples varied from 0 to 46 mg/L with an average of 28 mg/L. The average bias was +3 mg/L when calculated using red wine samples and +1 mg/L when calculated using white wine samples.

The method correlation study is shown graphically in Figures 3 and 4. Concentrations of red wine samples varied from 20 to 40 mg/L and concentrations of white wine samples from 35 to 50 mg/L.

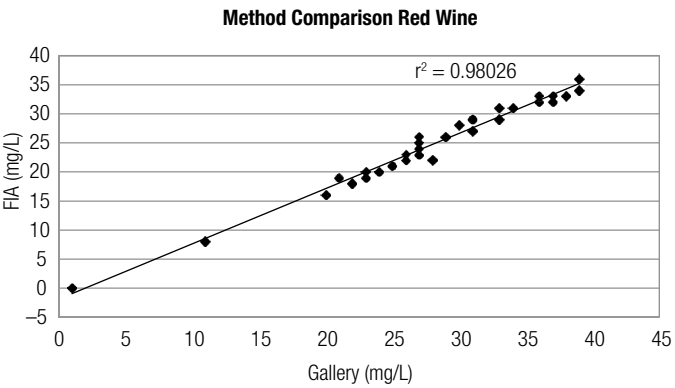


**Figure 3. Red wine sample comparison between Gallery and FIA methods**

Very good correlation between the Gallery discrete analyzer and the FIA analyzer was shown by both sample types.

The coefficient of determination was slightly better for white wines than for red wines, respectively $r^2$ = 0.9863 and 0.9803. When the data from all samples were combined into a single plot, the correlation coefficient ($r^2$) was 0.9755.

In addition, precision for one of the red wine samples (N = 40) was calculated (data not shown) using the Gallery method and was 1.8% within run, and 2.1% between runs, which correlates with the allowed EBC/ASBC standard ranges.

## Conclusions

The automated photometric Thermo Scientific $SO_2$ Free method correlates well with the FIA method with a coefficient of determination ($r^2$) of 0.9755. The concentration of samples varied from 0 to 46 mg/L with an average of 28 mg/L. Samples (N = 57) including different types of wines were tested. Precision for the red wine samples (N = 40) was 1.8% within run, and 2.1% between runs.

This new automated $SO_2$ Free method is very quick and easy to use. Analysis of 60 samples takes only 35 min and allows simultaneous analysis of various sugars and acids, color, and total $SO_2$. Compared to the FIA method, the photometric method requires only small volumes of reagents, thus being the more economically and environmentally friendly choice.

**Table 1. Bias of the two Free $SO_2$ methods**

| Red Wine Sample | Gallery (mg/L) | FIA (mg/L) | Bias (mg/L) | White Wine Sample | Gallery (mg/L) | FIA (mg/L) | Bias (mg/L) |
|---|---|---|---|---|---|---|---|
| Red Wine 1 | 38 | 33 | 5 | White Wine 1 | 40 | 37 | 3 |
| Red Wine 2 | 1 | 0 | 1 | White Wine 2 | 43 | 41 | 2 |
| Red Wine 3 | 11 | 8 | 3 | White Wine 3 | 39 | 37 | 2 |
| Red Wine 4 | 20 | 16 | 4 | White Wine 4 | 34 | 34 | 0 |
| Red Wine 5 | 22 | 18 | 4 | White Wine 5 | 37 | 35 | 2 |
| Red Wine 6 | 26 | 22 | 4 | White Wine 6 | 34 | 30 | 4 |
| Red Wine 7 | 23 | 20 | 3 | White Wine 7 | 38 | 36 | 2 |
| Red Wine 8 | 23 | 19 | 4 | White Wine 8 | 34 | 32 | 2 |
| Red Wine 9 | 24 | 20 | 4 | White Wine 9 | 40 | 38 | 2 |
| Red Wine 10 | 31 | 29 | 2 | White Wine 10 | 37 | 35 | 2 |
| Red Wine 11 | 29 | 26 | 3 | White Wine 11 | 36 | 36 | 0 |
| Red Wine 12 | 27 | 26 | 1 | White Wine 12 | 34 | 35 | -1 |
| Red Wine 13 | 37 | 32 | 5 | White Wine 13 | 34 | 35 | -1 |
| Red Wine 14 | 39 | 36 | 3 | White Wine 14 | 13 | 12 | 1 |
| Red Wine 15 | 39 | 34 | 5 | White Wine 15 | 23 | 21 | 2 |
| Red Wine 16 | 36 | 33 | 3 | White Wine 16 | 0 | 0 | 0 |
| Red Wine 17 | 34 | 31 | 3 | White Wine 17 | 31 | 31 | 0 |
| Red Wine 18 | 36 | 33 | 3 | White Wine 18 | 48 | 46 | 2 |
| Red Wine 19 | 33 | 29 | 4 | White Wine 19 | 37 | 36 | 1 |
| Red Wine 20 | 31 | 29 | 2 | White Wine 20 | 39 | 37 | 2 |
| Red Wine 21 | 25 | 21 | 4 | White Wine 21 | 45 | 42 | 3 |
| Red Wine 22 | 21 | 19 | 2 | White Wine 22 | 44 | 42 | 2 |
| Red Wine 23 | 21 | 19 | 2 | | | | |
| Red Wine 24 | 36 | 32 | 4 | | | | |
| Red Wine 25 | 28 | 22 | 6 | | | | |
| Red Wine 26 | 30 | 28 | 2 | | | | |
| Red Wine 27 | 26 | 23 | 3 | | | | |
| Red Wine 28 | 27 | 23 | 4 | | | | |
| Red Wine 29 | 27 | 24 | 3 | | | | |
| Red Wine 30 | 27 | 25 | 2 | | | | |
| Red Wine 31 | 33 | 31 | 2 | | | | |
| Red Wine 32 | 36 | 33 | 3 | | | | |
| Red Wine 33 | 37 | 33 | 4 | | | | |
| Red Wine 34 | 31 | 27 | 4 | | | | |
| Red Wine 35 | 25 | 21 | 4 | | | | |

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