

# Simultaneous Continuous Flow Analysis of Free and Total Sulfur Dioxide in Wine

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A rapid, reliable method (60 samples/h) for the simultaneous determination of free and total sulfur dioxide (SO<sub>2</sub>) in wine has been developed employing a third-generation continuous flow analyzer, the TRAACS 800. Continuous flow analysis measured 75-100% free SO<sub>2</sub> compared to the Ripper method. Recoveries from sulfite-spiked wine samples ranged from 62 to 104%, with an average precision of 4.5%.

## INTRODUCTION

It is accepted practice in most wineries throughout the world to add sulfur dioxide (SO<sub>2</sub>) to the grapes, usually as sodium or potassium metabisulfite, during or immediately after crushing. SO<sub>2</sub> is traditionally used as an antiseptic in wine-making to inhibit the growth of spoilage bacteria and undesirable wild yeast as well as to prevent discoloration by oxidative or nonoxidative browning.

Free SO<sub>2</sub> is defined as the total of the molecular and ionic forms that are unreacted (SO<sub>2</sub>, HSO<sub>3</sub><sup>-</sup>, SO<sub>3</sub><sup>2-</sup>). Bound SO<sub>2</sub> is that which can be released by hydrolysis and/or heat and distillation. In wines the bisulfite ion reacts with acetaldehyde to form acetaldehyde hydroxysulfonate. It also reacts with aldose sugars (i.e., glucose), with glyoxylic, pyruvic,  $\alpha$ -ketoglutaric, and galacturonic acids, with some unsaturated compounds, and with phenolic compounds such as caffeic and *p*-coumaric acids (Ough and Amerine, 1988). The anthocyanins in red wine also bind SO<sub>2</sub>. Total SO<sub>2</sub> is the sum of the bound and free SO<sub>2</sub>.

Since the Liquor Control Board of Ontario (LCBO) sells wines from around the world, our interest in the determination of SO<sub>2</sub> arises from the following: (1) The differences in the maximum levels of SO<sub>2</sub> that are allowed, as established by legislation in different countries. Canadian government regulations (Canadian Food and Drug Act, 1991) limit both free (maximum 70 ppm) and total SO<sub>2</sub> (maximum 420 ppm). (2) The U.S. Food and Drug Administration (FDA, 1986) requires that the presence of sulfite at or above 10 ppm be declared on the label of food products because of reported adverse reactions in a select proportion of the population that is hypersensitive to sulfites. (Similar legislation may be enacted in Canada in the near future.)

Enologists, however, are primarily concerned with the amount of free SO<sub>2</sub> because the combination of SO<sub>2</sub> with aldehydes or pigments reduces the antimicrobial and antioxidant powers of SO<sub>2</sub>.

The analysis of free and total SO<sub>2</sub> is conventionally carried out directly by the Ripper method (Vahl and Converse, 1980) or with the prior separation of the analyte using the Monier-Williams method (AOAC, 1984) or the aeration-oxidation method (Buechsenstein and Ough, 1978). Recently, other methods for determining SO<sub>2</sub> based on ion chromatography (Moore et al., 1987), polarography (Stonys, 1987), and redox titration (DeVries et al., 1986) have been reported.

Flow injection analysis (FIA), now widely developed, is based on the reproducible injection of samples into a flowing stream of a carrier or a reagent solution (Ruzicka

Table I. Comparison of TRAACS vs Ripper SO<sub>2</sub> Measurements

TRAACS		Ripper	
free SO <sub>2</sub> , ppm	total SO <sub>2</sub> , ppm	free SO <sub>2</sub> , ppm	total SO <sub>2</sub> , ppm
Red Wine			
3.8	34.1	4.2	38.2
6.4	102.3	6.7	105.6
25.6	107.3	26.1	109.4
3.1	26.8	3.9	30.1
17.2	117.8	18.1	120.1
13.0	111.2	13.9	115.7
9.5	58.9	9.9	61.3
11.6	66.1	11.7	69.4
10.4	75.1	10.8	78.1
10.9	29.5	12.3	33.1
White Wine			
13.1	44.8	13.7	48.9
14.7	47.1	15.1	49.1
19.6	73.8	20.3	77.5
12.5	63.1	13.4	68.1
23.2	53.5	24.2	59.4
9.8	52.7	11.1	56.3
16.8	57.2	17.3	66.1
15.2	57.3	16.7	64.1
16.7	137.5	18.1	155.6
26.0	74.9	28.1	83.1
Ports and Sherries			
<3.0	27.2	<3.0	35.2
<3.0	28.1	<3.0	36.4
<3.0	64.1	<3.0	75.1
<3.0	<10.0	<3.0	11.4
<3.0	<10.0	<3.0	<10.0

and Hansen, 1975). FIA techniques have been described for a variety of analytes in wines (Maguierira et al., 1987) including sulfites (Sullivan et al., 1990).

Procedures for the estimation of free and total SO<sub>2</sub> content in wine have presented researchers and industry with the dilemma of accuracy vs speed. Since the LCBO analyzes over 6000 wines annually, we required a method that was both rapid and accurate. This paper describes a method for the simultaneous determination of free and total SO<sub>2</sub> in wine. The methodology is based on the formation of a colored compound between the analyte, formaldehyde, and *p*-rosaniline (Dasguta et al., 1980). Refinements to the original Technicon procedure (Technicon, 1987), which measured free and total SO<sub>2</sub> individually, included an elaborated optimization of all pertinent parameters. The operating conditions are fully described for determining free and total SO<sub>2</sub> simultaneously using a third-generation continuous flow analyzer, the TRAACS 800. The analyzer is completely integrated with a personal

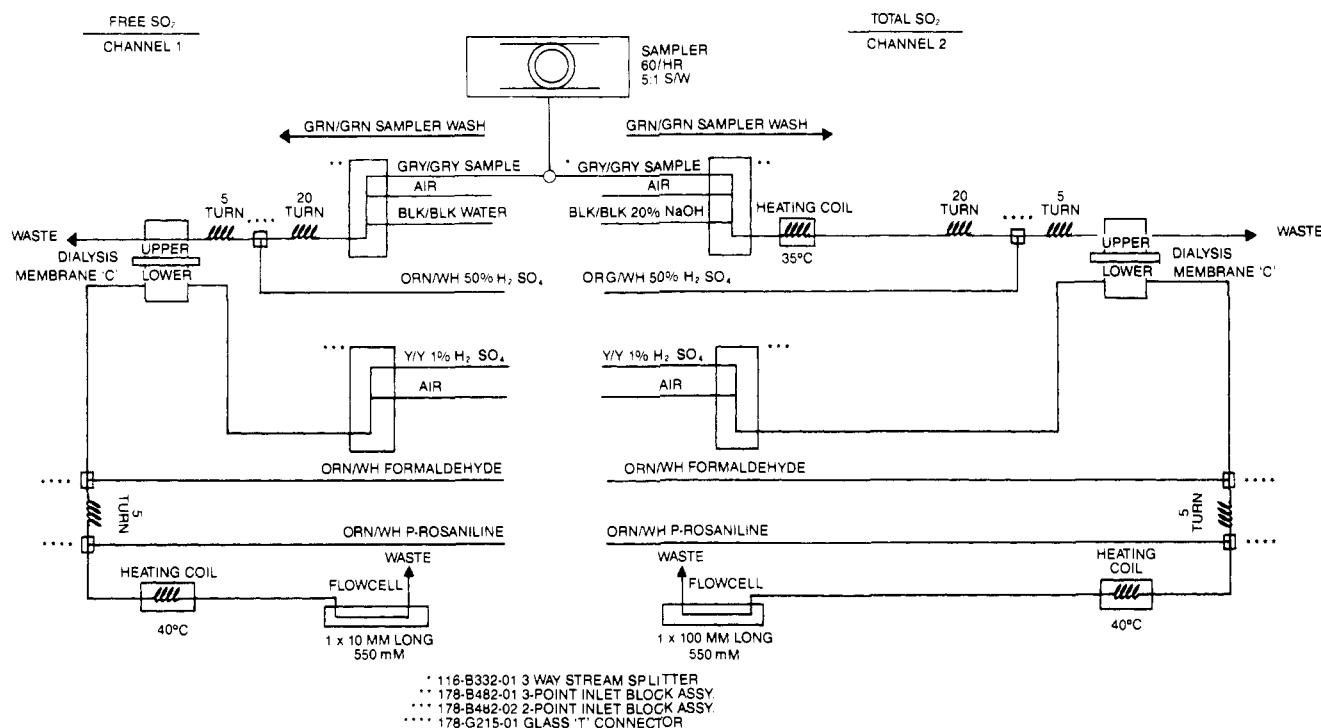


Figure 1. Free and total SO<sub>2</sub> manifold.

Table II. TRAACS Total SO<sub>2</sub> Recovery<sup>a</sup>

wine	SO <sub>2</sub> added, ppm	wine + SO <sub>2</sub> added, ppm	recovery	% recovery
cabernet sauvignon	0	88.45 ± 2.73		
	10	98.32 ± 2.73	9.87 ± 2.07	99
	50	138.80 ± 1.22	50.35 ± 1.77	101
chardonnay	0	79.62 ± 1.04		
	10	87.42 ± 0.32	7.80 ± 0.72	78
	50	123.00 ± 1.07	43.38 ± 2.10	87
port	0	31.16 ± 2.13		
	10	37.37 ± 0.12	6.21 ± 2.21	62
	50	70.93 ± 0.57	39.77 ± 2.43	80
icewine	0	49.08 ± 0.69		
	10	58.07 ± 0.52	9.00 ± 0.84	90
	50	91.79 ± 1.76	85.43 ± 1.69	85

<sup>a</sup> Average of three replicates.

computer, which makes possible an intelligent sampler with a great deal of instrument flexibility.

## MATERIALS AND METHODS

**Apparatus.** The autoanalyzer TRAACS 800 continuous flow autoanalyzer (Bran & Luebbe, Technicon Industrial Systems, Elmsford, NY) with manifold is illustrated in Figure 1. The manufacturer's instructions were followed for start-up and shut-down of the instrument. The sample tray was filled with the standards arranged in descending order followed by the wine samples.

**Caution.** Use necessary safety measures when handling the reagents specified in this paper. Protective gloves and a laboratory coat should be worn to prevent skin contact with these reagents.

**Reagents.** Sodium hydroxide and sulfuric acid were purchased from BDH Chemicals (Toronto, Canada). A 1.85% formaldehyde solution was prepared daily as required from 37% formaldehyde (BDH). A stock solution of *p*-rosaniline was prepared by dissolving 1.0 g of *p*-rosaniline hydrochloride (Fisher Scientific, Toronto) in 500 mL of deionized water (18 MΩ). After 3 days

of standing, this solution was filtered and stored in an airtight brown glass bottle. The working solution of *p*-rosaniline was prepared by transferring 80 mL of the stock solution to a 1-L volumetric flask. While mixing, 125 mL of concentrated phosphoric acid (Fisher) was added. The volume was made up to 1 L with deionized water. A 1000 ppm stock standard of SO<sub>2</sub> was prepared by dissolving 0.1483 g of sodium metabisulfite (BDH) in 100 mL of deionized water. Working standards of 200, 100, 50, and 25 ppm SO<sub>2</sub> were prepared by serial dilutions of the stock SO<sub>2</sub> standard. Triton X-405 (BDH) was used as a wetting agent. All reagents were filtered prior to use to eliminate excessive baseline noise. Wine samples were run as received.

**Principle.** The sample (wine or standard) is drawn up by the autosampler and split, with half going to channel 1 (free SO<sub>2</sub>) and the other half going to channel 2 (total SO<sub>2</sub>) (see Figure 1). For the determination of total SO<sub>2</sub> the sample is first made basic with NaOH to liberate the bound SO<sub>2</sub>. After this point, the chemistry for both the free and total SO<sub>2</sub> determination is identical. The sample is made acidic with H<sub>2</sub>SO<sub>4</sub> to convert all forms of free SO<sub>2</sub> to gaseous SO<sub>2</sub>, a portion of which diffuses across a gas-permeable membrane into a stream of 1% H<sub>2</sub>SO<sub>4</sub>. This stream is mixed with a solution of formaldehyde, and finally this intermediate adduct is reacted with *p*-rosaniline. The resulting colored complex is measured at 550 nm.

## RESULTS AND DISCUSSION

The optimum NaOH and H<sub>2</sub>SO<sub>4</sub> concentrations were 5 and 6 M, respectively. This NaOH concentration along with an extra 20-turn heated (35 °C) coil was found to be sufficient to accomplish the fast dissociation of bound SO<sub>2</sub>. The H<sub>2</sub>SO<sub>4</sub> concentration (6 M) is the best for neutralizing the NaOH/sample stream and for providing an acidic medium for the conversion to gaseous molecular SO<sub>2</sub>. A dialysis type C membrane (Pulse Instrumentation, Saskatoon, Canada) was used to separate color pigments in the wine from the color development section of the manifold.

**Free SO<sub>2</sub>.** To determine how accurately the method was measuring free SO<sub>2</sub>, results were compared to those obtained by the Ripper method (Table I). Although the Ripper method is not recognized as an official method by the AOAC, it is the standard method recommended by the EEC (OIV, 1978). We chose to compare the results to those obtained by the Ripper method because approx-

Table III. Round-Robin Results of Free and Total SO<sub>2</sub>

		range, <sup>a</sup> ppm		average, <sup>a</sup> ppm		TRAACS, ppm	
		free	total	free	total	free	total
white	A	31.0–37.0	98.0–107.0	33.9 ± 2.6	101.7 ± 2.8	29.5	103.0
	E	46.0–53.0	150.6–160.0	50.8 ± 2.8	154.1 ± 3.2	52.4	151.1
	AA	51.5–55.0	137.0–150.0	54.2 ± 2.3	144.6 ± 5.4	54.4	155.0
red	D	49.0–52.8	145.0–154.0	51.3 ± 1.9	149.4 ± 3.4	54.4	147.0
	C	22.0–25.7	134.0–200.0	24.7 ± 1.9	155.3 ± 21.9	28.3	159.0
rose	F	12.0–26.0	110.0–126.0	18.5 ± 4.2	119.7 ± 4.5	18.3	121.3
	B	70.0–80.7	138.0–152.0	75.5 ± 3.6	145.5 ± 8.1	79.7	143.0

<sup>a</sup> Results from six different laboratories using the Ripper method of analysis.

imately 90% of the wines analyzed by the LCBO are from Europe. As shown in Table I, the results by both methods are consistent. The precision (percent relative standard deviation) averaged 1.7%. Although not significant, it should be pointed out that the TRAACS results are consistently slightly lower than the Ripper results. The reason for these discrepancies is likely differences in handling for the two methods. In addition, we tried to measure the accuracy of the method by determining the recovery of free SO<sub>2</sub> from spiked wine samples. Unfortunately, recoveries were low (about 50%), because addition of SO<sub>2</sub> shifts the equilibrium of free vs bound SO<sub>2</sub> in favor of the bound form (Robinson, 1991).

**Total SO<sub>2</sub>.** To assess the accuracy of the method to measure total SO<sub>2</sub>, wine samples were spiked with 10, 50, and 100 ppm of SO<sub>2</sub> (Table II). The data indicate that the recoveries were quite good, 62–104%, with an average precision of 4.5%, when samples are spiked with a known quantity of SO<sub>2</sub> and analyzed immediately. Different types of wines (dry, sweet, sherry) were chosen to demonstrate the versatility of the method with respect to color and matrix of the sample. Fortified wines showed a decreased sensitivity by the TRAACS method (Table II) for the 10 ppm spike. This was not concluded to be a poor reflection of the TRAACS method because on average the method detected the same level of SO<sub>2</sub> as the Ripper method (Table I). Since the maximum legal amounts of total SO<sub>2</sub> range from 125 to 475 ppm (Ough and Amerine, 1988), lower recoveries of the 10 ppm spikes do not pose a serious problem for determining illegal levels of total SO<sub>2</sub> in sweet or fortified wines.

As a further test of the method, we have used it in a round robin in which six other laboratories participated in the determination of free and total SO<sub>2</sub> in wines. The results we obtained (Table III) agree favorably with the average results obtained by the other six laboratories.

**Conclusion.** The direct simultaneous determination of free and total SO<sub>2</sub> on the TRAACS instrument represents a definite improvement in analytical quality. Elimination of the necessity of sample preparation represents a reduction in labor requirements and the elimination of a source of error. Like other published FIA methods for sulfite determination in wine (Sullivan et al., 1990), the method requires no sample preparation and is generally free of interferences.

Among the advantages of the method described in this paper over other available FIA methods are the following: (1) The method is extremely rapid, allowing for the simultaneous determination of free and total SO<sub>2</sub> at a rate of 60 samples/h. (2) The instrument is completely automated. (3) There is no need for a dual-injection system; a single-injection port with a sample splitter is used instead. This method can be applied to any routine laboratory analysis used for quality control of alcoholic beverages.

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#### LITERATURE CITED

- AOAC. *Official Methods of Analysis*, 14th ed.; Association of Official Analytical Chemists: Arlington, VA, 1984; pp 229, 391–392.
- Buechsenstein, J. W.; Ough, C. S. SO<sub>2</sub> Determination by Aeration-Oxidation: A Comparison with Ripper. *Am. J. Enol. Vitic.* 1978, 29, 161–164.
- Canadian Food and Drug Act. Part B Food Division 1, Section B.02.100(vii), 1991.
- Dasguta, P. K.; DeCesare, K.; Ullrey, J. C. Determination of Atmospheric Sulfur Dioxide without Tetrachloromercurate-(II) and the Mechanism of the Schiff Reaction. *Anal. Chem.* 1980, 52, 1912–1922.
- DeVries, J. W.; Ge, H.; Ebert, F. J.; Magnuson, J. M.; Ogawa, M. K. Analysis for Total Sulfite in Foods by Using Rapid Distillation Followed by Redox Titration. *J. Assoc. Off. Chem.* 1986, 69, 827–830.
- FDA. *Fed. Regist.* 1986, 51, 25012–25020.
- Maguierira, A.; Luque de Castro, M. D.; Valcarcel, M. Determination of Reducing Sugars in Wine by Flow Injection Analysis. *Analyst* 1987, 112, 1569–1572.
- Moore, L. F.; Bates, R. P.; Marshall, M. R. A Direct HPLC Analysis of Total Sulfur Dioxide in Wine. *Am. J. Enol. Vitic.* 1987, 38, 28–34.
- OIV. *Recueil des Methode Internationales d'Analyse des Vins*, 5th ed.; Office International de la Vigne et du Vin: Paris, 1978; pp 196–198.
- Ough, C. S.; Amerine, M. A. *Methods for Analysis of Musts and Wines*, 2nd ed.; Wiley: New York, 1988; pp 222–225.
- Robinson, D. The Chemistry of Sulfur Dioxide and Other Antioxidants in Wine. *Am. J. Enol. Vitic.* 1991, 42, 81.
- Ruzicka, J.; Hansen, E. H. Flow Injection Analysis. Part 1. A New Concept of Fast Continuous Flow Analysis. *Anal. Chim. Acta* 1975, 78, 145–157.
- Stonys, D. B. Determination of Sulfur Dioxide in Foods by Modified Monier-Williams Distillation and Polarographic Detection. *J. Assoc. Off. Anal. Chem.* 1987, 70, 114–117.
- Sullivan, J. J.; Hollingworth, T. A.; Wekell, M. M.; Meo, V. A.; Saba, H. H.; Etemad-Moghadam, A.; Eklund, C. Determination of Total Sulfite in Shrimp, Potatoes, Dried Pineapple and White Wine by Flow Injection Analysis: Collaborative Study. *J. Assoc. Off. Anal. Chem.* 1990, 73, 35–42.
- Technicon. Industrial Method Can 4-87WN, Free Sulfur Dioxide in Wines, Industrial Method Can 5-87WN, Total Sulfur Dioxide in Wines; 1987.
- Vahl, J. M.; Converse, J. E. Ripper Procedure for Determining Sulfur Dioxide in Wine: Collaborative Study. *J. Assoc. Off. Anal. Chem.* 1980, 63, 194–199.

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