New Formulation for Interference-free Determination of Total Sulfites in Red Wine

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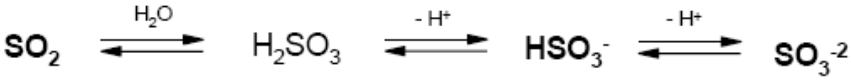
The use of sulfites to preserve foods has been a regular practice in our culture for centuries.[1] In wine, sulfite is a byproduct of some fermentation reactions, but is also added to maintain its properties over time. Sulfites act in three ways[2]:

- To prevent oxidation (antioxidant)
- To halt bacterial growth (antimicrobial)
- To inhibit enzyme reactions that result in color changes (antioxidase)

Because of the pH of wine, sulfites takes several forms at steady state (*Figures 1 and 2*). Free sulfite is found in the form of molecular SO_2 (sulfur dioxide) or HSO_3- (bisulfite) and SO_3^{-2} (sulfite) after reacting with water. The other sulfite fraction occurs in wine in a combined form with unsaturated compounds (sugars, polyphenols, acids, acetaldehyde, etc.), which is in steady state with the free fraction. The sum of free and combined sulfites is total sulfites.[3,4]



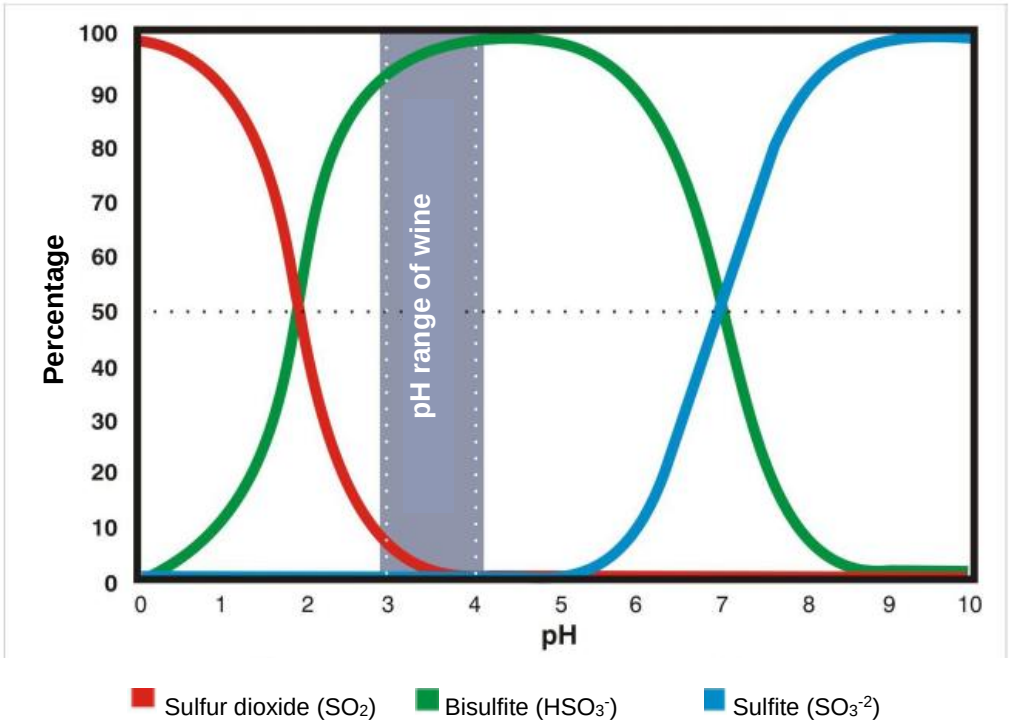


Figure 1. Sulfite species in wine.

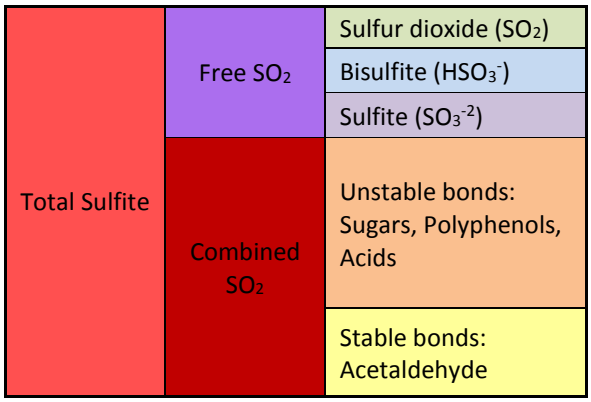


Figure 2. Approximate proportions of free, combined and total sulfites.

Sulfite analysis in wine is of interest for several reasons:

- Current laws in the various countries regulate maximum sulfite levels and sometimes require that the label state this information.
- Sulfite declines in wine should be monitored during ageing (diffusion, oxidation or both), so as to determine the amount of sulfite that must be added.
- Elevated sulfite concentrations that provide unpleasant flavors and odors and inhibit malolactic fermentation must be avoided.[5]
- Several studies indicate that sulfites may be harmful to health. One in every 100 persons in the population is sensitive to these compounds, and some asthma patients are at risk of side effects, some mild (dermatitis, headache, exacerbation of asthma) and some serious (anaphylactic shock, eye injury or brain damage).[6]

Procedures used to measure total sulfites:

Official methods:

- *Modified Monier-Williams (OIV-MA-AS323-04A:2012; Category II and AOAC:990.28)*: This method is recommended by the International Organisation of Vine and Wine (OIV)[7] and the *Association of Official Analytical Chemists* (AOAC).[8] This method first aerates the sample to extract sulfite, then oxidizes it to sulfuric acid by bubbling in a hydrogen peroxide solution and titrating the resulting solution with sodium hydroxide. Free sulfite extraction only requires sample aeration for 15 minutes at 10ºC, whereas the same amount of time is required for total sulfites with a sample at 100ºC.

- *Flow injection analysis (FIA) (AOAC 990.29)*: This technique was recently implemented by the AOAC and readily separates sulfites using various chemical transformations that culminate in SO_2 extraction in gas phase through a Teflon membrane. Once free of interference, SO_2 reacts with the green malachite dye and is detected by spectrophotometry.

- *Ripper method (OIV-MA-AS323-04B:R2009; Category IV)*: This test is less stringent than the Monier-Williams procedure, but is faster and used more often in wine cellars. The method is based on direct titration of the sample with an iodine solution and visual detection of the starch end point.

Nonofficial methods:

The literature also describes a wide array of methods for the determination of sulfites. Most methods fall into one of two groups[2,9]:

- Direct determination by chemical transformation of sulfites: titration, spectrophotometry (chemical or enzymatic), fluorescence, etc.
- Sulfite extraction from the sample and subsequent analysis: high-performance liquid chromatography (HPLC), ion exchange chromatography, capillary electrophoresis, flow injection analysis (FIA), etc.

However, all of the methods described have problems that make them impractical or unreliable[5,10,11] (*Figure 3*).

Modified Monier-Williams method	Slow Manual Analyst-dependent High sample volume
Flow injection analysis (FIA)	Specialized analyst High cost
Ripper method	Manual Analyst-dependent Interference
Chromatographic methods	Specialized analyst High cost

Figure 3. Drawbacks of recommended methods for the determination of sulfites in wine.

Although there are various methods to analyze sulfites, the scientific community does not agree on which method could replace the current official method and would also be rapid, reliable and automated.

Spectrophotometry. 5-5'-dithiobis(2-nitrobenzoic acid) (DTNB)

Spectrophotometry is based on a determination of solution transmittance or absorbance in the ultraviolet and visible spectrum. Molecular absorption methods are perhaps the most widely used among all the quantitative analysis techniques in testing laboratories worldwide.[2] The power of this technique lies in identifying a reaction that is sensitive and selective to the compound to be analyzed. The Beer-Lambert law can then be used to find a relationship between absorbance variation and analyte concentration. Additionally, it is a technique that determines the analyte directly and rapidly, can be automated and requires low-cost instrumentation.

The spectrophotometric method used to determine total sulfites is based on 5-5'-dithiobis(2-nitrobenzoic acid) (DTNB). SO_2 uses its reducing power to cleave the disulfide bond, thus yielding 3-thio-6-nitrobenzoate (TNB), which can be measured at 405 nm (yellow).[13,14]

This reaction is rapid and can be readily automated. However, the method is known to work well with samples of white wine but can show interference with the red wine matrix.

BioSystems: The new Total Sulfites kit

BioSystems has long been aware of the problems associated with the spectrophotometric method and decided to take on the challenge of developing a new reagent to determine total sulfite concentrations in wines regardless of the matrix.

Its R&D and Innovation Department undertook considerable research in collaboration with BioSystems clients worldwide and has now released to the market a unique kit that will change the work of wine cellars and enological laboratories.

The new reagent used to determine total sulfites continues to be based on the DTNB chromogen, but the new formulation prevents interference in both red and white wine samples.

Accuracy study: Monier-Williams (INCAVI) vs. Spectrophotometry (BioSystems)

The new Enology procedure to measure total sulfites was evaluated jointly with the Institut Català de la Vinya i el Vi (INCAVI).

The INCAVI viticulture and enology stations in Reus and Vilafranca del Penedès represent the most specialized Generalitat de Catalunya infrastructure made available to the vine and wine industry.

The testing services provided by its laboratory are approved for official analyses in the wine industry and the services work to ensure the quality specified by the technical and management requirements defined in UNE-EN ISO/IEC 17025. The measurement procedure used to determine total sulfites is the official modified Monier-Williams method accredited by ENAC.

To perform the study, INCAVI collected 40 samples (30 red wines, 9 white wines and 1 rosé wine) from various sources and *Denominaciones de Origen* (D.O.): Penedès, Rioja, Alella, Priorat, Castilla-León, Murcia, DO Cava, Pla de Bages and analyzed all of them simultaneously using both methods.

According to the comparison data *(Figure 4)* from the linear regression study *(Figure 5)*, there were no systematic errors, indicating that there were no significant differences between the results of the two procedures.

	mg/L	
Sample	INCAVI	BioSystems
1594	12	7
1605	38	45
1606	30	27
1607	68	70
1624	22	19
1698	39	42
1801	82	91
1813	30	25
1814	50	53
1949	125	127
1954	5	1
2006	78	80
2011	84	81
2017	117	118
2027	83	84
2044	34	40
2068	30	26
2086	6	12
2094	104	104
2302	28	25
2304	59	60
2346	36	35
2347	22	14
2349	5	0
2352	93	97
2363	68	64
2365	30	29
2381	13	4
2536★	58	61
2539★	66	70
2544★	22	23
2549★	87	97
2552★	107	113
2607★	89	93
2566★	82	81
2589★	90	97
2604★	127	115
2553♦	146	145

★White wine ♦Rosé wine

Figure 4. Comparison of the Monier-Williams procedure and the BioSystems modified Total Sulfites kit.

a	b	r^2
-1.707 (-4.725/1.311)	1.033 (0.990/1.076)	0.985

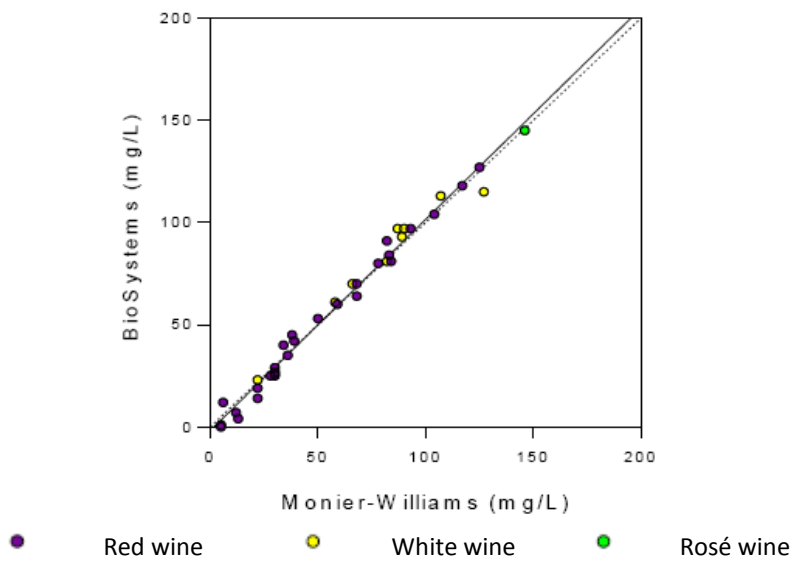


Figure 5. Linear regression comparing the results from the two methods.

CONCLUSIONS

The new BioSystems Total Sulfite reagent provides results equivalent to the official modified Monier-Williams method.

Once again, Biosystems has developed a solution for the enology market that provides laboratories with a reagent for rapid, automatic, low-cost determination of total sulfites regardless of the wine matrix, with results comparable to those obtained with the official method.

The new Total Sulfites kit allows BioSystems to continue offering a unique system to the market (22 reagents and 2 analyzers), thus ensuring enologists of its ongoing efforts to develop and improve its products and maintain its high quality standards.

References

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