SO$_2$ determination

Quick and easy distillation & determination of total SO$_2$ in foods and beverages

Abstract

Sulfur-containing compounds, such as sulfur dioxide and sulfites, are widely used as preservatives and anti-oxidants in foods and beverages. Exposure to high levels of sulfites can cause an allergic reaction, difficulty breathing, sneezing, throat swelling, hives and migraine headaches.

Given the health risks associated with sulfite exposure, the amount of sulfites in beverages and foodstuffs is regulated in many countries. Regulations can set the maximum amount of sulfites used and require labels to indicate the presence of sulfites.

Numerous methods are used to determine sulfite concentration. The methods, however, are disadvantageous for various reasons. Disadvantages include the complexity of the methodology, the large amount of time involved, the inaccuracy of results, the use of toxic chemicals, inconvenience, and the required use of special equipment.

A method that overcomes these disadvantages is desirable. The method described herein is simple and fast using the Distillation Unit K-355 or the KjelFlex K-360. The results are accurate, repeatable, and reproducible. The results also compare favorably to results obtained with methods employed by regulatory agencies. In addition, the use of toxic chemicals, such as lead, is avoided. Furthermore, the method is economically and conveniently carried out using reagents commonly found in laboratories.

1. Introduction

Sulfur-containing compounds, such as sulfur dioxide (SO$_2$) and sulfites (SO$_2^2$), are widely used as preservatives and anti-oxidants in foods and beverages (see Table 1). Sulfites also occur naturally in some foods and beverages as a result of fermentation (e.g. beer and wine).

There are individuals, however, who are sensitive to sulfites. Exposure of these individuals to high levels of sulfites can cause symptoms/signs in various systems of the body (see Table 2).

Table 2: Symptoms/signs of individuals which are sensitive to sulfites.

<table>
<thead>
<tr>
<th>System affected</th>
<th>Symptoms/signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circulatory</td>
<td>Migraine headaches, fainting, drop in blood pressure (associated with anaphylactic shock).</td>
</tr>
<tr>
<td>Digestive</td>
<td>Stomach cramps, nausea, diarrhea</td>
</tr>
<tr>
<td>Integumentary</td>
<td>Hives, rash, itching.</td>
</tr>
<tr>
<td>Respiratory</td>
<td>Wheezing, coughing, difficulty breathing, asthma, sneezing, throat swelling, inability to breathe (associated with anaphylactic shock).</td>
</tr>
</tbody>
</table>

Given the adverse reactions to sulfites exhibited by some individuals, the use of sulfites in foods and beverages has become an issue of concern for consumers and regulatory agencies. Investigations into the potential adverse health consequences of sulfite exposure has led agencies to promulgate regulations concerning the usage of sulfiting agents and the labeling of food and beverage products containing sulfites. The use of sulfur dioxide and sulfites as food additives is restricted, for example, in the United States of America (USA), the European Union (EU) and China.

2. Use of sulfites in the USA

The use of sulfiting agents in the USA has been generally recognized as safe (GRAS) since 1959 when used in accordance with good manufacturing practice; excluded is use in meats and other foods recognized as a source of vitamin B1 [1]. GRAS status was revoked in 1986 for fruits and vegetables that are presented fresh or served raw [2]. The U.S. Food and Drug Administration (FDA) requires the label on finished food containing > 10 ppm sulfite to declare the presence of the sulfite (see 21 C.F.R. § 130.9); an
exception is the use of sulfite on minimally processed potatoes sliced or shredded for frying. The U.S. Environmental Protection Agency (EPA) regulates the use of sulfite as a fungicide during the shipment of fresh table grapes; the concentration of $SO_2$ residues on the grapes as consumed must be < 10 ppm total $SO_2$. The Tax and Trade Bureau (TTB) of the U.S. Department of the Treasury requires alcoholic beverages containing sulfites to be labeled accordingly.

**USA:**
label if sulfites > 10 ppm

### 3. Use of sulfites in the EU

The European Parliament and the Council on Food Additives regulate sulfiting agents in the EU under Regulation 1333/2008. The amount permitted varies according to the type of food and the form of the ingredient and ranges from 10 mg/kg to 10,000 mg/kg [1]. The EU requires the label on food containing > 10 mg/kg or 10 mg/L $SO_2$ and/or sulfites to declare the presence of sulfites. Total $SO_2$ is calculated for products ready for consumption or reconstituted.

The European Commission’s Scientific Committee on Food (SCF) set an acceptable daily intake (ADI) for sulfur dioxide and its equivalents at 0.7 mg/kg body weight/day (SCF (1994)). This comports with the ADI established by the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) in 1974 [3].

**EU:**
label if $SO_2 +$ sulfites > 10 mg/kg or 10 mg/L

### 4. Use of sulfites in China

In China the maximum levels set for sulfur dioxide, potassium metabisulfite, sodium metabisulfite, sodium sulfite, sodium hydrogen sulfite, and sodium hyposulfite depend on the category of food [4]. The levels range from 50 to 400 mg/kg or mg/L (residual $SO_2$).

### 5. Codex Alimentarius

The Codex Alimentarius Commission, which is part of the WHO and the FAO program of the United Nations, publishes the Codex Alimentarius. The Codex Alimentarius or “Food Code” sets forth international food standards, guidelines, and codes of practice. Sulfites are one of the food additives included [5].

### 6. Methods currently used to determine total $SO_2$ as a measure of sulfites

In view of the adverse health risks associated with sulfite exposure and labeling requirements, various methods of determining sulfite content are in use, and new methods are continually being proposed. Methods include the Monier-Williams methods and various versions thereof, colorimetry, flow injection analysis, pulse polarography and ion chromatography [6]. Titrimetry, photometry, iodometry, electrochemistry, fluorometry, chemiluminescence spectrometry, gas-liquid chromatography and liquid chromatography also have been used [7]. Still other methods include various electroanalytical techniques, such as voltammetry, amperometry and potentiometry [8, 9].

Since sulfites can convert to $SO_2$, sulfites are measured and expressed as $SO_2$. Most of the procedures, however, are tedious, time-consuming, have a high level of detection and cannot be used on products containing volatile compounds [7]. Other disadvantages can include complex methodology, inaccurate results, inconvenience, the use of toxic chemicals and the required use of special equipment. Chromatographic methods have lower limits of detection and require less time but do not work well on products in which the sulfur dioxide is strongly bound to a matrix; in addition, the equipment is costly and can be difficult to operate [7]. Enzymatic methods can be hard to standardize and are less sensitive [7].

The level of sulfite in finished food can be determined by the modified Monier-Williams (MW) method (see, e.g., Association of Official Analytical Chemists (AOAC) Official Method 962.16). The modified MW method is not applicable to dried onions, leeks, cabbage and certain other vegetables due to the presence of interfering volatile sulfur-containing compounds.

Since the MW method has drawbacks at the 10 mg/L level, the U.S. FDA made minor changes to the procedure to improve accuracy at 10 ppm (21 C.F.R. § 101, Appendix A). Referred to as the Optimized Monier-Williams (OMW) method, the method is the most widely used method for the quantitative determination of sulfites in food and beverages (see, e.g., AOAC Official Method 990.28). The OMW method is the official method for the determination of sulfites in food according to the China National Standard (CNS, 2003).

Using the OMW method, samples are distilled under acidic conditions and then analyzed by iodine or acid/base titration. Complicated sample preparation, which is labor-intensive and time-consuming, precludes using the method for fast or high-throughput analysis. Also, use of the OMW method for analysis of dry fruit and vegetable samples tends to overestimate the level of sulfites because of the abundant presence of volatile acidic compounds [10]. In addition, the OMW method requires the use of custom-made glassware.

A method that overcomes the disadvantages of currently used methods is desirable. The method described herein is simple and fast. The results are accurate, repeatable, and reproducible. The results also compare favorably to results obtained using methods employed by regulatory agencies. In addition, the use of chemicals, such as lead, which are toxic to humans and the environment, is avoided. Furthermore,
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the method is cost-effective and conveniently carried out using reagents commonly found in laboratories.

7. A new method for the determination of total SO₂ —“The BUCHI SO₂ method”

The BUCHI SO₂ method involves the chemical conversion of sulfites into volatile sulfur dioxide. The volatile sulfur dioxide is steam-distilled into a standard solution of iodine. A redox-reaction occurs between the sulfur dioxide and the iodine. After the volatile sulfur dioxide has been completely steam-distilled into the standard solution of iodine, the residual iodine is determined by redox-titration using a standard solution of sodium thiosulfate. The level of quantification is 0.85 mg SO₂ per sample.

For complete details of the BUCHI SO₂ method see European Pat. App. Pub. No. EP 2 515 098 A1, “Method and Device for Determining the SO₂ Content of Food and Drinks” (click on the link for more information).

The method can be carried out by using a distillation unit and collecting the distillate with SO₂ absorption glass as shown in Fig. 1, for example. The SO₂ absorption glass shown in Fig. 1 consists of two connected flasks – the first one contains an iodine standard solution and the second one contains ethanol.

![BUCHI SO₂ Absorption Glass (043070)](image1)

**BUCHI SO₂ method**

Use of the BUCHI SO₂ method in the determination of total SO₂ in wine involves acidification of a wine sample with a mixture of methanol, water, and orthophosphoric acid. SO₂ is efficiently released during steam distillation into the aqueous iodine solution contained in the first of the two connected flasks of the SO₂ absorption glass shown in Fig. 1. SO₂ content is determined by a redox back titration of the iodine solution with a standard thiosulfate solution. A linear correlation equation is determined and applied to the calculation of SO₂ content in a sample of wine.

In distinct contrast to the OIV method, the BUCHI SO₂ method is uncomplicated and can be carried out in about half the time. Using the set-up shown in Fig. 1, a single determination using the BUCHI SO₂ method only requires 5-6 minutes for distillation and 5 minutes for titration. In contrast, a single determination using the OIV SO₂ method requires 15 minutes for distillation and 5 minutes for titration. Comparable results are obtained (see Fig. 2).

**Comparison Total SO₂**

![Graphical comparison of the OIV SO₂ method (green bars) to the BUCHI SO₂ method (blue bars) for 11 wine samples. Experimental repeatabilities r = 2.8. Standard deviations are indicated as error limits.](image2)

**Figure 2:** Graphical comparison of the OIV SO₂ method (green bars) to the BUCHI SO₂ method (blue bars) for 11 wine samples. Experimental repeatabilities r = 2.8. Standard deviations are indicated as error limits.

9. Total SO₂ in beer

**DTNB SO₂ method**

A widely used method for the analysis of total SO₂ in beer is the DTNB (5,5-dithiobis(2-nitrobenzoic acid)) SO₂ method [13]. The DTNB SO₂ method involves release of SO₂ during nitrogen-assisted distillation into a buffered DTNB solution and measurement of the absorbance of the reaction product at 415 nm using spectrophotometry. The method is complicated and time-consuming to carry out.

**BUCHI SO₂ method**

Use of the BUCHI SO₂ method in the determination of total SO₂ in beer involves treatment of a beer sample in the same manner as described above for a wine sample.

To watch a video of the determination of total SO₂ in beer click on the link.
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In distinct contrast to the DTNB SO₂ method, the BUCHI SO₂ method is uncomplicated and can be carried out in about half the time. Using the set-up shown in Fig. 1, a single determination using the BUCHI SO₂ method only requires 5-6 minutes for distillation and 5 minutes for titration. In contrast, a single determination using the DTNB SO₂ method requires 15 minutes for distillation and 5 minutes for titration. Within the limits of standard deviations, similar results are obtained (see Fig. 3).

**Total SO₂ in potato powder by BUCHI SO₂ method**
A sample of potato powder is soaked in ethanol, hydrolyzed with sodium hydroxide, and acidified with ortho-phosphoric acid. SO₂ is efficiently released during steam distillation into the aqueous iodine solution contained in the first of the two connected flasks of the SO₂ absorption glass shown in Fig. 1. SO₂ content is determined by a redox back titration of the iodine solution with a standard thiosulfate solution. A linear correlation equation is determined and applied to the calculation of SO₂ content in a sample of potato powder. Results are shown in Fig. 4.

**Total SO₂ in frozen shrimp by BUCHI SO₂ method**
A sample of frozen shrimp is homogenized, coated with sand, flattened, and folded several times until a multi-layered structure of meat and sand is formed. The multi-layered sample is soaked in ethanol, hydrolyzed with sodium hydroxide, and acidified with ortho-phosphoric acid. SO₂ is efficiently released during steam distillation into the aqueous iodine solution contained in the first of the two connected flasks of the SO₂ absorption glass shown in Fig. 1. SO₂ content is determined by a redox back titration of the iodine solution with a standard thiosulfate solution. A linear correlation equation is determined and applied to the calculation of SO₂ content in a sample of frozen shrimp.

To watch a video of the determination of total SO₂ in dried apricots click on the link.

**BUCHI SO₂ method vs. MW SO₂ method**
In distinct contrast to the MW SO₂ method, which employs 1N hydrochloric acid to release SO₂, the BUCHI SO₂ method releases SO₂ from bisulfite adducts by hydrolysis with 1 mol/L sodium hydroxide solution and acidification with ortho-phosphoric acid. The sample preparation in the BUCHI method results in improved separation of SO₂ from the matrix and avoids corrosion of equipment due to exposure to hydrochloric acid. Also in contrast to the MW SO₂ method, which employs acid-base titration, the BUCHI
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SO₂ method relies on redox titration, which avoids interference with acids. The results obtained with the BUCHI SO₂ method are well in line with the expected values for certified reference material.

The average recovery results of the BUCHI SO₂ method compare favorably with those of the CNS Method. The values obtained with the BUCHI SO₂ method were higher than the values obtained with the CNS method. This could imply an underestimation of the SO₂ when following the CNS method. The CNS method employs hydrochloric acid, which corrodes laboratory equipment, and lead acetate, which is toxic to humans and the environment. In distinct contrast, the BUCHI SO₂ method employs ortho-phosphoric acid, which does not corrode laboratory equipment like hydrochloric acid. In addition, the BUCHI SO₂ method does not employ lead acetate.

11. Reproducibility and repeatability of BUCHI SO₂ method in analysis of total SO₂ in wine

In order to assess the reproducibility and repeatability of the BUCHI SO₂ method, four different laboratories in Switzerland were asked to carry out the procedure strictly as described in the BUCHI Application Note 065/2011 to calibrate individual distillation units using a pre-prepared, stabilized standard solution and to analyze in triplicate a white “Fendant” wine and a red “Gamay” wine. The wine samples were analyzed within a six-day time frame. One laboratory also analyzed the same sample using the OIV SO₂ method.

Two of the labs carried out the calibration for the BUCHI SO₂ method using automated redox titration with a titrator. The other two labs carried out the calibration for the BUCHI SO₂ method using manual redox titration with a starch indicator. A good correlation in the evaluations of the linear regressions was demonstrated by the calibrations performed by the four labs (squared Pearson coefficients (R²) of 0.9990, 0.9998, 0.9980, and 0.9996).

Triplicate analysis of the white “Fendant” wine and the red “Gamay” wine by the labs using the BUCHI SO₂ method yielded higher results for total SO₂ content than analysis of the same wines by the lab using the OIV SO₂ method. The BUCHI SO₂ method was found to be repeatable (within a lab; r) and reproducible (among all labs; R) for wine samples containing total SO₂ at levels within the calibration range (r = 4.1 mg SO₂/L and R = 6.5 mg SO₂/L for the white “Fendant” wine; r = 3.1 mg SO₂/L and R = 7.5 mg SO₂/L for the red “Gamay” wine). Overall, the results obtained using the BUCHI SO₂ method were comparable to the results obtained using the OIV SO₂ method.

12. An ideal method for the determination of total SO₂

Writing with regard to the beer industry, Guido characterized an ideal method for the determination of total SO₂ as one that “should be fast, simple, inexpensive, allow for measurement of free or/and total SO₂ and exclude the use of toxic reagents.” [6] An ideal method, however, should be more than that. The method should generate accurate results and be repeatable and reproducible; in addition, the results should compare favorably with results obtained with methods employed by regulatory agencies. Better still, the method should be convenient and employ common laboratory reagents.

13. BUCHI method for the determination of total SO₂

The BUCHI method for the determination of total SO₂ overcomes the disadvantages of currently available methods. It is simple and fast. Not only are the results accurate, repeatable, and reproducible, but they compare favorably to results obtained with methods employed by regulatory agencies. The use of toxic chemicals, such as lead, is avoided. Economical and convenient, the method is carried out using reagents commonly found in laboratories.

**Table 3: Advantages of BUCHI method for determination of total SO₂**

<table>
<thead>
<tr>
<th>Advantage</th>
<th>BUCHI Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast</td>
<td>✔</td>
</tr>
<tr>
<td>Simple</td>
<td>✔</td>
</tr>
<tr>
<td>Conveniet</td>
<td>✔</td>
</tr>
<tr>
<td>Economical</td>
<td>✔</td>
</tr>
<tr>
<td>Accurate</td>
<td>✔</td>
</tr>
<tr>
<td>Repeatable</td>
<td>✔</td>
</tr>
<tr>
<td>Reproducible</td>
<td>✔</td>
</tr>
<tr>
<td>Results compare</td>
<td>✔</td>
</tr>
<tr>
<td>Using methods</td>
<td>✔</td>
</tr>
<tr>
<td>Employ common</td>
<td>✔</td>
</tr>
<tr>
<td>Avoids use of toxic</td>
<td>✔</td>
</tr>
</tbody>
</table>

Further advantages can be realized by using the BUCHI Distillation Unit K-355 or the BUCHI KjelFlex K-360, for example, in combination with the BUCHI SO₂ Absorption Glass (043070). The app Kjeldahl Reports can be downloaded from the BUCHI Homepage for result calculation and reporting.
14. Conclusion

In conclusion, the BUCHI method for the determination of total SO$_2$ content is a viable alternative to methods currently employed to analyze total SO$_2$ content in a variety of matrices. Not only does the BUCHI method provide results that compare favorably with results obtained using methods employed by regulatory agencies, it offers advantages, such as speed and ease of use.

For more information on the use of the BUCHI method for the determination of total SO$_2$ content and to learn about how BUCHI can provide other solutions for your laboratory analyses, visit www.buchi.com.

15. References


16. Additional resources

- Application Note No. 065/2011, Total SO$_2$ in Wine (http://www.buchi.com/en/content/sulfur-dioxide-determination-wine-0)
- Application Note No.066/2011, Total SO$_2$ in Beer (http://www.buchi.com/en/content/sulfuric-dioxide-determination-beer-0)
- Short Note No.080/2012, Determination of Total SO$_2$ in Certified Potato Powder Reference (http://www.buchi.com/en/content/sulfur-dioxide-determination-potato-powder)
- Application Note No.229/2016, Sulfur Dioxide Determination in Food (http://www.buchi.com/en/content/sulfuric-dioxide-determination-food)
- Case study: Soft drink manufacturer based in the UK needed to measure sodium metabisulfite accurately and consistently during batch production without delay. The BUCHI Distillation Unit K-355 combined with the company’s method provided consistent results that enabled operators to proceed with other tasks while the unit was in operation. Variable settings allowed for optimal time and steam output for the product. (http://www.buchi.com/ch-de/node/1252)
- Case study: "The solution for Kjeldahl as well as sulfur dioxide determination on one single instrument is excellent." Dr. S.S. Marwaha, CEO, Punjab Biotechnology Incubator (http://www.buchi.com/ch-de/node/2316)