

### 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<sub>2</sub>) and sulfites (SO<sub>3</sub><sup>2-</sup>), 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).

Table 1: Sulfur-containing compounds in foods and beverages [1].

Most common sulfiting agents	Actions	Example food products
Sodium sulfite	Bleaching agent	Beverages: wine, beer, cordials, cider, fruit juice (canned, bottled, and frozen), and soft drinks.
Sodium bisulfite	Anti-microbial agent	Fruits: dried, fruit bars, and maraschino cherries
Sodium metabisulfite	Oxygen scavenger	Vegetables: canned, pickled, dried, and processed (e.g., instant mashed potatoes and potato salad).
Potassium bisulfite	Reducing agent	Seafood: clams (canned), shrimp (fresh, frozen, dried, and canned), lobster (frozen), scallops, and cod (dried).
Potassium metabisulfite	Enzyme inhibitor	Gravies and sauces.
Calcium sulfite	Dough conditioner	Jams, jellies, and fruit toppings.
Calcium bisulfite		Other food: (Maple syrup, Coconut, Gelatin, Vinegar, Beef stew).
Sulfur dioxide (SO <sub>2</sub> )		Dough (pie crust and pizza). Biscuits, cookies, and tortilla shells.

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.

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

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<sub>2</sub> residues on the grapes as consumed must be < 10 ppm total SO<sub>2</sub>. 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<sub>2</sub> and/or sulfites to declare the presence of sulfites. Total SO<sub>2</sub> 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<sub>2</sub> + 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<sub>2</sub>).

### 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<sub>2</sub> 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<sub>2</sub>, sulfites are measured and expressed as SO<sub>2</sub>. 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 also 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,

the method is cost-effective and conveniently carried out using reagents commonly found in laboratories.

### 7. A new method for the determination of total SO<sub>2</sub> – “The BUCHI SO<sub>2</sub> method”

The BUCHI SO<sub>2</sub> 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 SO<sub>2</sub> per sample.

For complete details of the BUCHI SO<sub>2</sub> method see European Pat. App. Pub. No. [EP 2 515 098 A1](#), “Method and Device for Determining the SO<sub>2</sub> 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 SO<sub>2</sub> absorption glass as shown in Fig. 1, for example. The SO<sub>2</sub> 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.



Fig. 1: BUCHI SO<sub>2</sub> Absorption Glass (043070).

### 8. Total SO<sub>2</sub> in wine

#### OIV SO<sub>2</sub> method

A widely accepted method for the analysis of total SO<sub>2</sub> in wine is published by the Organization Internationale de la Vigne et du Vin (OIV) in the Compendium of International Methods of Wine and Must Analysis [11, 12]. The OIV SO<sub>2</sub> method involves acidification of a wine sample with phosphoric acid. SO<sub>2</sub> is released during nitrogen-assisted distillation into hydrogen peroxide. SO<sub>2</sub> content is determined by acid-base titration of sulfuric acid with sodium hydroxide. The method is tedious and time-consuming to carry out.

#### BUCHI SO<sub>2</sub> method

Use of the BUCHI SO<sub>2</sub> method in the determination of total SO<sub>2</sub> in wine involves acidification of a wine sample with a mixture of methanol, water, and ortho-phosphoric acid. SO<sub>2</sub> is efficiently released during steam distillation into the aqueous iodine solution contained in the first of the two connected flasks of the SO<sub>2</sub> absorption glass shown in Fig. 1. SO<sub>2</sub> 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 SO<sub>2</sub> content in a sample of wine.

In distinct contrast to the OIV method, the BUCHI SO<sub>2</sub> 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 SO<sub>2</sub> method only requires 5-6 minutes for distillation and 5 minutes for titration. In contrast, a single determination using the OIV SO<sub>2</sub> method requires 15 minutes for distillation and 5 minutes for titration. Comparable results are obtained (see Fig. 2).

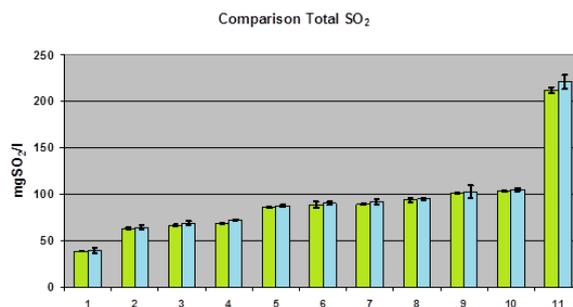


Figure 2: Graphical comparison of the OIV SO<sub>2</sub> method (green bars) to the BUCHI SO<sub>2</sub> method (blue bars) for 11 wine samples. Experimental repeatabilities  $r = 2.8$ . Standard deviations are indicated as error limits.

### 9. Total SO<sub>2</sub> in beer

#### DTNB SO<sub>2</sub> method

A widely used method for the analysis of total SO<sub>2</sub> in beer is the DTNB (5,5-dithiobis(2-nitrobenzoic acid)) SO<sub>2</sub> method [13]. The DTNB SO<sub>2</sub> method involves release of SO<sub>2</sub> 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 SO<sub>2</sub> method

Use of the BUCHI SO<sub>2</sub> method in the determination of total SO<sub>2</sub> 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 SO<sub>2</sub> in beer](#) click on the link.

In distinct contrast to the DTNB SO<sub>2</sub> method, the BUCHI SO<sub>2</sub> 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 SO<sub>2</sub> method only requires 5-6 minutes for distillation and 5 minutes for titration. In contrast, a single determination using the DTNB SO<sub>2</sub> method requires 15 minutes for distillation and 5 minutes for titration. Within the limits of standard deviations, similar results are obtained (see Fig. 3).

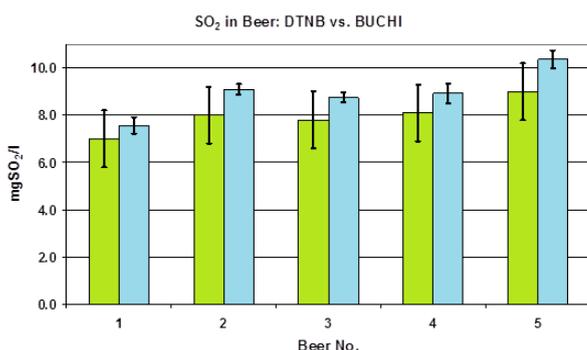


Fig. 3: Graphical comparison of the DTNB SO<sub>2</sub> method (green bars) to the BUCHI SO<sub>2</sub> method (blue bars) for 5 beer samples. Ranges refer to standard deviations.

## 10. Total SO<sub>2</sub> in food

### MW SO<sub>2</sub> method

Methods widely used for the determination of total SO<sub>2</sub> in food involve optimizations of the Monier-Williams (MW) method, which involves the use of custom-made glassware. The MW SO<sub>2</sub> method releases SO<sub>2</sub> from an acidified sample by nitrogen-assisted distillation into a solution of hydrogen peroxide resulting in the formation of sulfuric acid. The sulfuric acid is titrated with a sodium hydroxide standard solution, and the total SO<sub>2</sub> content is calculated.

### China National Standard Method (GB/T5009.34-2003)

The China National Standard Method (GB/T5009.34-2003) involves homogenizing and hydrolyzing the sample with acid. SO<sub>2</sub> is released from the acidified sample by steam distillation into a receiving vessel containing lead acetate. Hydrochloric acid and a starch indicator solution are added, and a redox electrode is used for titration.

### BUCHI SO<sub>2</sub> method

The BUCHI SO<sub>2</sub> method as applied to food differs somewhat from the method as applied to wine and beer. Wine and beer are weak matrices; food, by contrast, can contain bisulfite-aldehyde adducts and, consequently, can present strong matrices. SO<sub>2</sub> is released from bisulfite-aldehyde adducts by hydrolysis with a highly concentrated strong acid/base. For example, the matrix can be degraded by alkaline hydrolysis with 1 mol/L sodium hydroxide as described in the Swiss Book of Food Regulation (SLMB).

### Total SO<sub>2</sub> in potato powder by BUCHI SO<sub>2</sub> method

A sample of potato powder is soaked in ethanol, hydrolyzed with sodium hydroxide, and acidified with ortho-phosphoric acid. SO<sub>2</sub> is efficiently released during steam distillation into the aqueous iodine solution contained in the first of the two connected flasks of the SO<sub>2</sub> absorption glass shown in Fig. 1. SO<sub>2</sub> 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 SO<sub>2</sub> content in a sample of potato powder. Results are shown in Fig. 4.

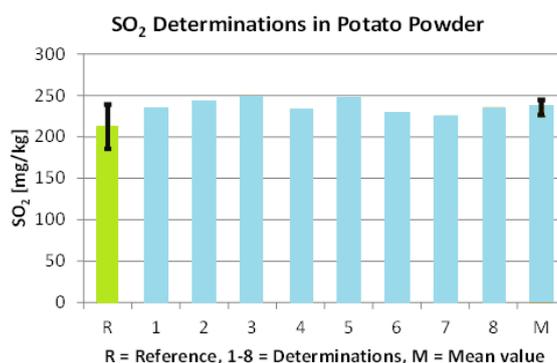


Fig. 4: Is a graphical comparison of the certified reference material (R; green color) to the BUCHI SO<sub>2</sub> method (blue color) for eight samples (1-8) and the mean value (M).

### Total SO<sub>2</sub> in frozen shrimp by BUCHI SO<sub>2</sub> 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. SO<sub>2</sub> is efficiently released during steam distillation into the aqueous iodine solution contained in the first of the two connected flasks of the SO<sub>2</sub> absorption glass shown in Fig. 1. SO<sub>2</sub> 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 SO<sub>2</sub> content in a sample of frozen shrimp.

To watch a video of the [determination of total SO<sub>2</sub> in dried apricots](#) click on the link.

### BUCHI SO<sub>2</sub> method vs. MW SO<sub>2</sub> method

In distinct contrast to the MW SO<sub>2</sub> method, which employs 1N hydrochloric acid to release SO<sub>2</sub>, the BUCHI SO<sub>2</sub> method releases SO<sub>2</sub> 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 SO<sub>2</sub> from the matrix and avoids corrosion of equipment due to exposure to hydrochloric acid. Also in contrast to the MW SO<sub>2</sub> method, which employs acid-base titration, the BUCHI

## SO<sub>2</sub> determination

### Quick and easy distillation & determination of total SO<sub>2</sub> in foods and beverages

SO<sub>2</sub> method relies on redox titration, which avoids interference with acids. The results obtained with the BUCHI SO<sub>2</sub> method are well in line with the expected values for certified reference material.

#### **BUCHI SO<sub>2</sub> method vs. China National Standard (CNS) Method (GB/T5009.34-2003)**

The average recovery results of the BUCHI SO<sub>2</sub> method compare favorably with those of the CNS Method. The values obtained with the BUCHI SO<sub>2</sub> method were higher than the values obtained with the CNS method. This could imply an underestimation of the SO<sub>2</sub> 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 SO<sub>2</sub> method employs ortho-phosphoric acid, which does not corrode laboratory equipment like hydrochloric acid. In addition, the BUCHI SO<sub>2</sub> method does not employ lead acetate.

#### **11. Reproducibility and repeatability of BUCHI SO<sub>2</sub> method in analysis of total SO<sub>2</sub> in wine**

In order to assess the reproducibility and repeatability of the BUCHI SO<sub>2</sub> 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 SO<sub>2</sub> method.

Two of the labs carried out the calibration for the BUCHI SO<sub>2</sub> method using automated redox titration with a titrator. The other two labs carried out the calibration for the BUCHI SO<sub>2</sub> 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 (R<sup>2</sup>) of 0.9990, 0.9998, 0.9960, and 0.9996).

Triplicate analysis of the white "Fendant" wine and the red "Gamay" wine by the labs using the BUCHI SO<sub>2</sub> method yielded higher results for total SO<sub>2</sub> content than analysis of the same wines by the lab using the OIV SO<sub>2</sub> method. The BUCHI SO<sub>2</sub> method was found to be repeatable (within a lab; r) and reproducible (among all labs; R) for wine samples containing total SO<sub>2</sub> at levels within the calibration range (r = 4.1 mg SO<sub>2</sub>/L and R = 6.5 mg SO<sub>2</sub>/L for the white "Fendant" wine; r = 3.1 mg SO<sub>2</sub>/L and R = 7.5 mg SO<sub>2</sub>/L for the red "Gamay" wine). Overall, the results obtained using the BUCHI SO<sub>2</sub> method were comparable to the results obtained using the OIV SO<sub>2</sub> method.

#### **12. An ideal method for the determination of total SO<sub>2</sub>**

Writing with regard to the beer industry, Guido characterized an ideal method for the determination of

total SO<sub>2</sub> as one that "should be fast, simple, inexpensive, allow for measurement of free or/and total SO<sub>2</sub> 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.

It should be fast, simple, inexpensive, allow for measurement of free or/and total SO<sub>2</sub> and exclude the use of toxic reagents.  
(GUIDO, 2016)

#### **13. BUCHI method for the determination of total SO<sub>2</sub>**

The BUCHI method for the determination of total SO<sub>2</sub> 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 SO<sub>2</sub>.*

Fast	✓
Simple	✓
Convenient	✓
Economical	✓
Accurate	✓
Repeatable	✓
Reproducible	✓
Results compare favorably to results obtained using methods employed by regulatory agencies	✓
Employs common laboratory reagents	✓
Avoids use of toxic chemicals	✓

Further advantages can be realized by using the BUCHI Distillation Unit K-355 or the BUCHI KjellFlex K-360, for example, in combination with the BUCHI SO<sub>2</sub> Absorption Glass (043070). The app Kjeldahl Reports can be downloaded from the BUCHI Homepage for result calculation and reporting.

Download the app Kjeldahl Reports from the BUCHI Homepage:

<http://www.buchi.com/en/service-support/scientific-mobile-apps/kjeldahl-reports>

#### 14. Conclusion

In conclusion, the BUCHI method for the determination of total SO<sub>2</sub> content is a viable alternative to methods currently employed to analyze total SO<sub>2</sub> 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<sub>2</sub> content and to learn about how BUCHI can provide other solutions for your laboratory analyses, visit [www.buchi.com](http://www.buchi.com).

#### 15. References

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#### 16. Additional resources

- Application Note No. 065/2011, Total SO<sub>2</sub> in Wine (<http://www.buchi.com/en/content/sulfur-dioxide-determination-wine-0>)
- Application Note No.066/2011, Total SO<sub>2</sub> in Beer (<http://www.buchi.com/en/content/sulfuric-dioxide-determination-beer-0>)
- Short Note No.090/2012, Determination of Total SO<sub>2</sub> in Certified Potato Powder Reference (<http://www.buchi.com/en/content/sulfuric-dioxide-determination-potato-powder>)
- Short Note No.169/2014, Sulfur Dioxide Determination in Shrimp ([http://static1.www.buchi.com/sites/default/files/shortnotes/141104\\_SO<sub>2</sub> in shrimps SN final version .pdf](http://static1.www.buchi.com/sites/default/files/shortnotes/141104_SO2_in_shrimps_SN_final_version.pdf))
- 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 IncubatorIndia (<http://www.buchi.com/ch-de/node/2316>)