WHITE PAPER

Simplified sulfite determination in foods and beverages using ion chromatography

In the food and beverage industry, different additives are used during the manufacturing process to prolong shelf life, increase nutritional content, and more. Such additives may remain unchanged in the final product, while others form new compounds. Currently there are over 300 approved food additives on the market. If added to commercially packaged food items, they must be labelled with an «E» in the list of ingredients. Preservatives are included under the food additive definition. Here, particular attention is placed on the use of sulfites as preservatives.

Sulfites are well-known additives in foods and beverages. The term «sulfites» describes a group of molecules that include sulfur dioxide (SO_2) and chemically related molecules like sodium sulfite (Na_2SO_3), sodium bisulfite ($NaHSO_3$), or sodium metabisulfite ($Na_2S_2O_5$) [**1–3**]. Not only do sulfites inhibit microbial growth (reducing food deterioration), they also enhance the color and have anti-browning and antioxidant properties. Such properties have led to the broad usage of sulfites in a range of foodstuffs like fruits, cereals, vegetables, seafood, juices, alcoholic and non-alcoholic (soft) beverages, and in some meat products.

Despite the benefits of adding sulfite to foods, some negative effects have been reported. Sulfite intake has been correlated with several adverse reactions like hypersensitivity, allergic reactions, or vitamin deficiency. The symptomatic reactions vary, ranging from mild cutaneous symptoms to anaphylactic reactions,

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depending on a variety of factors. Therefore, sulfites are included in the FAO/WHO Codex Alimentarius list which shows all permitted food additives and their maximum concentration level for each foodstuff [**4**].

The labelling of sulfite in foods and beverages is necessary when the total concentration exceeds 10 mg/kg. Different analytical methods have been developed to determine sulfite at this limit in foodstuffs. Metrohm ion chromatography allows the reliable measurement of sulfite in different matrices using either conductivity or amperometric detection.

CHEMICAL PROPERTIES

Depending on the moisture content, pH, or temperature conditions, sulfite can be present in various forms in foods. **Figure 1** shows the many reactions that sulfite can undergo to form other molecules. Usually sulfites are prepared under aqueous conditions by adding an excess of sulfur dioxide and a base such as carbonate or bicarbonate. The formed salts can subsequently release the sulfur dioxide in different amounts depending on the pH or temperature [**5**].

Under acidic conditions (pH 1–2), the most prevalent form is the sulfur dioxide molecule. Because the sulfurous acid (H_2SO_3) is thermodynamically instable, the common form of sulfur dioxide is the hydrate form ($SO_2 \times H_2O$).

In fresh meat products (at pH 4.5–6.8), the most stable form is the hydrogen sulfite ion (HSO_3^-). In products with low water activity, hydrogen sulfite is also in equilibrium with the disulfite ion ($S_2O_5^{2-}$) which is formed after water elimination forms two hydrogen sulfite molecules [**7**]. At higher pH values (above pH 6.5), sulfite (SO_3^{2-}) is the most stable form [**8**].

After meat or other treated foods are exposed to air and microbial activity decreases after they begin to dry out, sulfite oxidizes to form sulfate (SO_4^{2-}). This reaction is the result of the antioxidation process, or can be the result of an enzyme-catalyzed reaction. The estimation is that up to 15% of the total sulfite can be lost due to formation of sulfate. To avoid such problems, antioxidants or chelating agents are added to the food products [**9**].

Depending on the composition and the pH of the food, the sulfites as a chemical reactive additive undergo multiple nucleophilic addition or substitution processes. As a result, their concentration can strongly decrease down to 49% [**10**]. The lowest percentage of the decreasing concentration is related to sulfite oxidation. The main reactions are related to the formation of organic sulfonates [**11**].

When sulfites are applied to foods, only a portion is irreversibly bound to the matrix. The sum of the remaining free and reversibly bound sulfites (total sulfite) therefore becomes the analyte of interest. Reversibly bound sulfites consist primarily of adducts with carbonyl compounds and hydroxy sulfonates. From low pH values until 8, these adducts are quite stable. Above a pH of 8.5, the adducts dissociate into free sulfite.

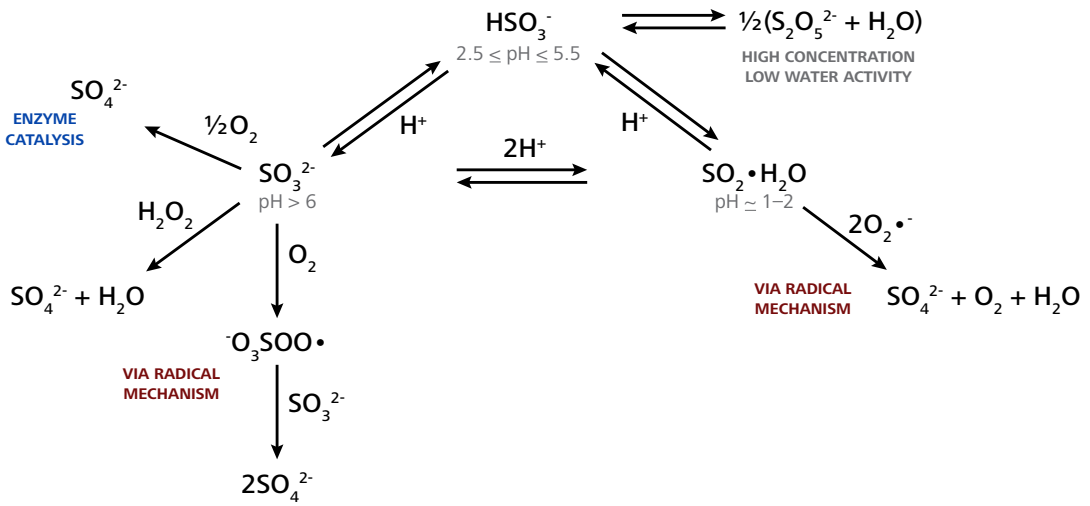

Figure 1. Conversion reaction of sulfite at different pH values [**6**].

OFFICIAL REGULATIONS

Due to the wide range of reported allergenic reactions against sulfites, these additives are strictly regulated and require monitoring in foods and beverages.

The Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) Codex Committee on Food Labelling (CCFL) has listed sulfites among other food ingredients that must be declared as allergens. The ADI value (Acceptable Daily Intake) set by the Joint FAO/WHO Expert Committee on Food Additives for sulfites is **0.7 mg/kg/day body weight** [**12**].

Depending on the country, sulfites can fall under different classifications. European Union Regulation no. 1169/2011 Annex II lists sulfites as «substances or products causing allergies or intolerances» [**13**]. In the United States the Food and Drug Administration (FDA) and USDA-FSIS (US Department of Agriculture Food Safety and Inspection Service) do not classify sulfites in the allergen list, but rather in a list of additives «that may cause intolerance» [**14**]. Furthermore, in the Food Labelling Guidance for Industry the FDA specified that sulfites which may be added with other ingredients that contain sulfites are allowed only when the final concentration in the product is below 10 mg/kg [**15**].

Both the EU Regulation no. 1169/2011 and the FDA (CFR – Title 21 – Part 101) require labelling of any food and beverage containing sulfiting agents at concentration levels **exceeding 10 mg/kg**. This level relates to the total quantity available from all sources, considered as the maximum residual limit [**4, 16–17**]. This applies to all kinds of food except for meat products.

In some countries, sulfites are allowed as additives in meat products. For example, in Europe regulation (EC) no. 1129/2011 [**18**] and (EC) no. 1333/2008 [**17**] specify some meat products which can contain sulfites. These include breakfast sausages, minced meat with cereal or vegetable content above 4%, and traditional Spanish or Portuguese pork meat products (e.g., salsicha fresca or longaniza fresca). The maximum permitted level (MPL level) for the total SO_2 concentration was set to 450 mg/kg as described in EU Regulation no. 853/2004 [**19**]. Other countries like India, South Africa or Australia allow some meat products with sulfite concentrations of 450 to 500 mg/kg. On the other hand, some countries like China, Indonesia, Korea, Brazil, or Saudi Arabia exclude the usage of sulfites in all kinds of meats or meat products [**20**].

HISTORICAL SULFITE DETERMINATION METHODS

Various methods have been used for sulfite determination in foodstuffs. Historically the first AOAC (Association of Official Analytical Chemists) reference method developed by Monier-Williams dates back to 1927 [**21**]. It is based on a traditional distillation procedure of the acidified sample solution. Released SO_2 gas is trapped in a hydrogen peroxide solution where sulfate forms after oxidation. This solution is back titrated using a sodium hydroxide solution. In the following years, this method was slightly modified by changing the distillation or extraction procedure or by replacing the titration step.

Other sulfite analysis methods were developed based on ion exchange or ion exclusion chromatography coupled either with conductivity, direct current, or pulsed amperometric detection [**22–25**]. In addition, a method using a discrete analyzer method was developed [**26**]. All of these methods have been reported only for the analysis of specific food matrices (e.g., wine, beer, or dry fruits) [**27**].

A big step forward in the research of sulfite analysis methods was done by Kim et al. [**28**] to compare Monier-Williams and other IC methods based on either acidic distillation or alkaline extraction. Both of the studied IC methods used electrochemical detection for sulfite determination. It was shown that in comparison to both IC methods, the Monier-Williams method led to falsely positive results. The alkaline extraction determination (pH 9) was easier and faster in comparison to the classical acidic distillation method. Additionally, the alkaline extraction is milder than an acidic distillation. It does not release any sulfites bound to pigments or naturally occurring sulfites in vegetables like leeks or cruciferous plants.

Therefore, this milder analytical method was chosen as the most preferable in a multilaboratory test for sulfite analysis in different food products [**29**]. A major disadvantage was the rapid decrease in detector sensitivity. The applied direct current mode (DC) led to a quick fouling of the electrode when the sample was injected for analysis. This meant that the working electrode required regular manual cleaning.

Another disadvantage of this method was the insufficient extraction of sulfites at pH 9, which precluded the analysis of darkly colored foods or ingredients. Sulfite strongly binds to sugars after the Maillard reaction, which is a chemical reaction between amino acids and reducing sugars that browns food.

Food and beverage testing laboratories require a **single robust analytical method** for sulfite that provides **good sensitivity** and applies to **all kinds of samples**. The **automation** of manual, time-intensive steps would be an additional benefit to such users.

OPTIMIZED SULFITE DETERMINATION: IC WITH AMPEROMETRIC DETECTION

Metrohm has answered the industry need for such an analytical method to determine total sulfite in a variety of foods with sensitive detection even over long sample series.

The general instrumentation setup consists of a basic IC equipped with one high pressure pump, a column oven, and eluent degasser for isocratic analysis. An amperometric detector block is necessary for detection. Automatic sample injection can be managed with an autosampler for low volume analysis with additional cooling function. This allows accurate sulfite analysis over a longer sample series without a significant reduction in sulfite concentration. The developed method uses the direct current (DC) mode for optimal detection in alkaline conditions, however, electrode fouling was prevented by utilizing a specific cleaning procedure. Before getting into such details, we will focus on the experimental configuration setup and reagents needed (**Table 1** and **Table 2**).

Table 1. List of required items for the reported IC analysis of sulfite in food matrices using amperometric detection.

Item	Article number
930 Compact IC Flex Oven/Deg	2.930.2160
889 IC Sample Center – Cool	2.889.0020
IC Amperometric Detector	2.850.9110
IC equipment Wall-Jet cell: without electrodes	6.1257.000
Ag/AgCl reference electrode for amperometric detection	6.1257.720
Au working electrode, 3 mm	6.1257.210
Metrosep Carb 2 -150/4.0	6.1090.420
Metrosep Carb 2 Guard/4.0	6.1090.500
MagIC Net 3.3 Compact	6.6059.331

Table 2. List of solutions used for sulfite analysis via IC with amperometric detection.

Eluent	Sodium Hydroxide 300.0 mmol/L Sodium Acetate 300.0 mmol/L CO_2-free ultrapure water
Rinsing Solution	Ultrapure water (UPW)
Stock stabilization solution	Formaldehyde 1.00 mol/L Sodium Hydroxide 0.20 mol/L
Working stabilization solution (dilution 1:1000 of stock solution)	Formaldehyde 1.00 mmol/L Sodium Hydroxide 0.20 mmol/L

– STABILIZATION OF SULFITE IN SAMPLES

Sulfite is unstable in solutions and gets oxidized to sulfate. To avoid this reaction, the samples and standards are prepared in a stabilization solution. In previous methods, this was made of a mannitol buffer at pH 9. Here, a buffered formaldehyde solution was applied at pH 10.2. It is known that above pH 8.5, the adducts of sulfite dissociate to free SO_3^{2-}. The alkaline extraction at pH 10.2 allowed a reproducible and simple analysis of the total sulfite concentration.

– SAMPLE PREPARATION

Only 1 g of a homogenized sample was used per analysis. After adding 29 g of the freshly prepared stabilization solution the mixture was blended, mixed manually and partly filtrated into a 2 mL sample vial. It was important to fill the vial completely to the top to avoid leaving any headspace which could lead to sulfite oxidation.

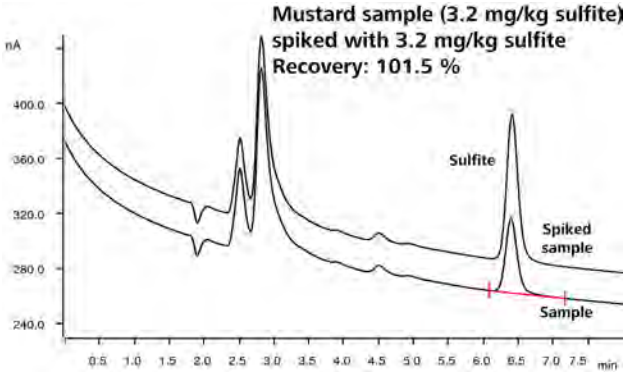

Figure 2. Overlaid chromatograms showing the determination of sulfite in mustard (spiked and unspiked) with a 101.5% recovery.

– AMPEROMETRIC DETECTION

Total sulfite detection was managed using the Metrohm amperometric detector which allows the analyst to oxidize specific molecules of interest. Therefore, this technique is more reliable when analyzing complex sample matrices such as foods.

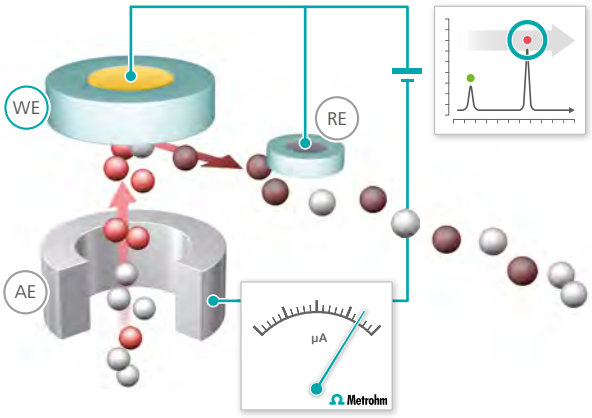

Figure 3. Illustration of the working principle behind amperometric detection (WE: working electrode, RE: reference electrode, AE: auxiliary electrode).

The amperometric detector was operated in DC Mode at a potential of 300 mV. Contrary to all previously described methods, **a potential sweep (patent filed, EP3786628A1) was applied to the electrodes after every chromatographic determination**. This special treatment required only two minutes but **fully reconditioned the electrodes** and helped to overcome otherwise frequent manual cleaning. As a result, the analysis and the reconditioning procedure were completed in 10 minutes (**Figure 2**).

– SAMPLES TESTED

A variety of different food samples were examined for their total sulfite in this study, including unspiked and spiked sample determinations: chickpeas, mustard, cherries, capers, canned garlic, chilli peppers, and red wine.

The spike results in **Table 3** show that the method is applicable for determination of very low and high sulfite concentrations. A summarized comparison of the amperometric determination and the conductivity determination is given later in this paper.

Table 3. Example results of total sulfite determination in different foods with IC and amperometric detection.

Original sample	SO_3^{2-} concentration of original sample (mg/kg)	Spiked SO_3^{2-} concentration (mg/kg)	Determined SO_3^{2-} concentration (mg/kg)	% recovery
Canned garlic	2.6	40	42.7	100.2
Mustard	97.6	100	195.5	98.9
Red wine	86	20	110	103.8

The IC method utilizing amperometric detection exhibits excellent sensitivity, therefore the Method Detection Limit (MDL) for sulfite standard solution was set to **0.2 mg/kg**. In this case, only 3 µL of sample or standard solution were injected for analysis.

Unlike previously published sulfite methods using ion chromatography, a different, **more alkaline stabilization solution** is used here. This modification allows the determination of total sulfite in **almost all sample matrices**. The accurate measurement of total sulfite is necessary on one hand for food manufacturers to control the stability of food and beverages to avoid any microbiological contamination, for example. On the other hand, this determination is necessary for food manufacturers and quality control laboratories to prove the declared sulfite content in foods, a mandatory requirement when sulfite concentration exceeds 10 mg/kg.

Further method improvement was achieved using a high capacity anion exchange column instead of the previously described ion exclusion columns. This leads to short, stable retention times and good peak shapes for the sulfite ion. The used column allows the application of a specific eluent mixture to achieve very good separation between sulfite and the complex sample matrix from different foodstuffs.

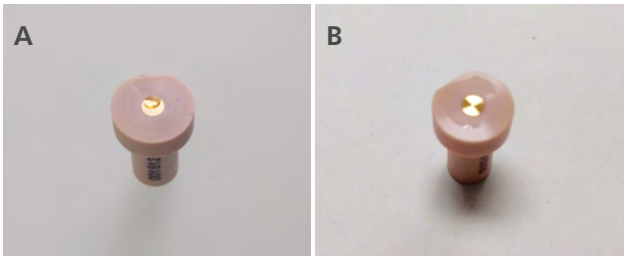

Figure 4. Working electrode before (A) and after (B) running several sulfite analyses with the setup listed in **Table 1** and solutions in **Table 2**. As shown, no fouling can be seen even after three weeks of use, due to the potential sweep/cleaning procedure developed by Metrohm (patent filed, EP3786628A1).

In addition, the newly developed potential sweep/cleaning procedure for the working electrode (patent filed, EP3786628A1) allows the analysis of long sample series without intervention of the operator for manual cleaning (**Figure 4**).

OPTIMIZED SULFITE DETERMINATION: IC WITH CONDUCTIVITY DETECTION

Another straightforward and easy to handle IC method for sulfite determination in foods uses conductivity detection. A drawback to this method is a higher detection limit (**MDL 0.5 mg/kg**) in comparison to the previously described amperometric determination.

Table 4. List of required items for the reported IC analysis of sulfite in food matrices using conductivity detection.

Item	Article number
930 Compact IC Flex Oven/SeS/PP/Deg	2.930.2560
858 IC Sample Processor	2.858.0020
IC Conductivity Detector	2.850.9010
IC equipment: Inline ultrafiltration	6.5330.110
Metrosep A Supp 10 - 100/4.0	6.1020.010
Metrosep A Supp 10 Guard/4.0	6.1020.500
MagIC Net 3.3 Compact	6.6059.331

The combination of a high capacity anion column with an alkaline eluent allows the separation of sulfite not only from the standard anions, but also from different organic acids available in the food product.

The sulfite determination method with conductivity detection is preferable for samples without high organic loads. For samples such as beer or wine, only dilution and filtration steps are necessary before injection. Therefore, conductivity determination for such

samples according to the setup in **Table 4** and solutions listed in **Table 5** can provide good analytical results.

Because the used 858 IC Sample Processor is not cooled, only a few capped sample vials should be placed at room temperature for analysis. The samples can be automatically filtered prior to injection using the inline ultrafiltration procedure. This saves analyst time and laboratory costs because several samples can be automatically filtered using one membrane.

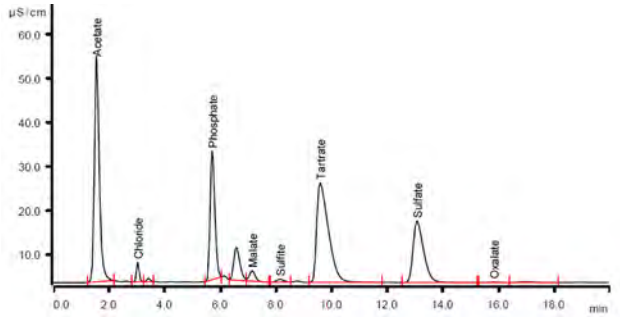

Figure 5. Example chromatogram from a white wine sample showing the complete separation of sulfite from other anions using the conductivity method.

Table 5. List of solutions used for sulfite analysis via IC with conductivity detection.

Eluent	Sodium Carbonate 5.0 mmol/L Sodium Bicarbonate 5.0 mmol/L Perchloric acid 5 µmol/L CO_2-free ultrapure water
Rinsing Solution	Ultrapure water (UPW)
Stock stabilization solution	Isopropanol solution 10%
Working stabilization solution	Isopropanol solution 2%

Table 6. Example results from diluted red wine of sulfite next to standard anions and organic acids with IC and conductivity detection.

Analyte	Chloride	Phosphate	Malate	Sulfite	Tartrate	Sulfate	Oxalate
Mean concentration [mg/kg] n=3	60.0	771.6	92.1	26.7	1755.6	553.0	<10
RSD %	0.03	0.23	0.06	1.9	0.09	0.01	n/a

Table 7. Example results from diluted white wine of sulfite next to standard anions and organic acids with IC and conductivity detection.

Analyte	Chloride	Phosphate	Malate	Sulfite	Tartrate	Sulfate	Oxalate
Mean concentration [mg/kg] n=3	21.5	818.0	105.3	28.8	1534.2	366.7	<10
RSD %	0.04	0.09	0.19	0.44	0.56	0.09	n/a

Table 8. Comparison of both described sulfite determination methods using Metrohm IC.

Sulfite	**Sample type**	
Concentration range	**Liquid samples like beer or wine**	**Complex matrix or liquid samples**
≥10 mg/kg	Sample dilution, direct injection with conductivity detection	Sample preparation (Carrez precipitation) + ultrafiltration, conductivity detection
≤10 mg/kg and ≥10 mg/kg	Sample dilution, filtration, direct injection with amperometric detection	Sample preparation (Carrez precipitation) + dilution, ultrafiltration, and amperometric detection

SUMMARY

Sulfite is a widely used preservative added to foods and beverages to improve the shelf life, prolong the antioxidative properties, and to enhance or preserve color.

Because of the range of allergic reactions reported after exposure to sulfites, these additives are now regulated and need to be monitored. Regulatory laws in the EU and US for example require sulfite to be labeled when its concentration exceeds 10 mg/kg sulfite in foodstuffs. The concentration declaration relates to the total sulfite concentration in the sample matrix.

Previously reported methods for sulfite determination in foods either provide erroneous positive results (Monier-Williams), or do not offer a robust analytical procedure.

Metrohm offers two outstanding choices for sulfite determination in food and beverage matrices with ion chromatography. First, an IC method is offered that utilizes **amperometric detection** allowing accurate quantification at **0.2 mg/kg**, far below the legal limit. The method excels not only due to its low detection limit, but also because of good signal stability and the repeatability of results. The newly developed **automatic cleaning procedure of the working electrode** keeps the results stable and the analysis downtime to a minimum with the elimination of manual electrode polishing steps. This is the preferable method for total sulfite analysis when different types of foods with varying sulfite concentration ranges need to be analyzed.

A second Metrohm IC method has been developed for sulfite analysis using **conductivity detection** for analysis of sulfite at the requested legal limit (10 mg/kg) and for higher sulfite concentrations in foods with a low organic load.

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