Sulfite, UV method

Alternative Procedures Brochure

Introduction

This kit has been developed to work in cuvettes with a standard pathlength of 1 cm, as described in the respective "Product Brochure". However, it can be adapted for use in 96-well microplates or in auto-analysers (micro-volume formats) with minimal assay optimisation. Basically, the assay volumes for the cuvette format have to be reduced approximately 10-fold for use in microplate format or in auto-analyser format. However, when using these micro-volume formats, you must be aware that the radiation pathlength is usually smaller than the standard cuvette pathlength of 1 cm. Thus, to perform the calculation of the amount of analyte in the samples under analysis follow one of the three strategies described in the section below.

Strategies for analyte calculation

Auto-analysers use reaction volumes of approximately 0.315 ml and pathlengths from 4 to 8 mm, which is similar to a standard 96-well microplate in which the same reaction volume would have a pathlength of 6 or 7 mm (similar assay volumes). Therefore, in both formats (96-well microplate and auto-analysers systems), the calculation of the analyte must be done by one of the three possible methods described below:

1. Using the pathlength conversion factor

This is the easiest method to perform the calculation of the analyte. However, it requires a microplate reader with pathlength conversion capacity, i.e., the apparatus can detect the pathlength of each well and convert the individual readings to a 1 cm pathlength (cuvette format). In the case of auto-analysers, the absorbance readings should be directly converted to a 1 cm pathlength. This will allow the calculation of the analyte content as described in the "Product Brochure", provided with the kit and available at the NZYTech website.

2. Using one standard curve

In this method, it is necessary to perform a standard curve of the analyte on each microplate that contains the test samples, or in the auto-analyser, and calculate the result from the standard curve of analyte concentration *vs.* absorbance. The standard curve can be performed by using the control solution provided in the kit.

3. Using two standard curves

The most complicated method is to perform standard curves of the analyte in both the cuvette format (i.e. with a 1 cm of radiation pathlength) and the 96-well microplate or auto-analyser formats, and use these results to obtain a mean conversion factor between the cuvette procedure values and the alternative procedure values. The standard curves can be performed by using the control solution provided in the kit.

Please enquire info@nzytech.com to obtain any additional information about this kit, including additional specific procedures and applications.

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www.nzytech.com

Sulfite, UV method

Catalogue number: AK00071, 30 tests

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AK0007_IFU_EN_V2302

Sulfite, UV method

Catalogue number	Presentation
AK00071	30 tests

Application

This rapid and simple specific enzymatic method is used for the determination of sulfite ("total SO_2") in foodstuffs such as wine, beer, fruit and vegetables, as well as in pharmaceuticals and water.

Introduction

Sulfur dioxide, sulfurous acid and its salts (sulfites) occur in very low concentrations in nature. However, they have been used for a very long time as a preservative in the industrial production of foodstuffs ("sulfurating") in order to prevent microbial spoilage. In the production of wine sulfuric acid is well known to improve stability and taste. Sulfite is regarded as being poisonous for cells and thus, during metabolism, it is rapidly oxidized to sulfate and excreted. The sulfite content in foodstuffs is legally prescribed in a number of countries and the content has often to be declared on the label.

Principle

$$SO_3^{2-} + O_2 + H_2O \xrightarrow{SOD} SO_4^{2-} + H_2O_2$$

$$H_2O_2 + NADH + H^+ \xrightarrow{NADH\text{-}POD} 2\,H_2O + NAD^+$$

Measurement of sulfite results from its oxidation to sulfate by sulfite oxidase (SOD) in the presence of oxygen as shown above. The amount of NADH consumed through the combined action of SOD and NADH-peroxidase (NADH-POD), measured at 340 nm, is stoichiometric with the amount of sulfite in sample volume.

Specificity

Sulfite oxidase reacts with sulfites, isothiocyanates and their glycosides. Organic sulfonic acid compounds can give rise to a degree of creep reaction. Sulfides, thiosulfates, sulfat and organic sulfinic acid compounds do not react under the assay conditions. Purified reagents, such as sodium sulfite, sodium disulfite and potassium disulfite absorb moisture and are easily oxidized. In addition, aqueous solutions are instable. Thus, under these conditions, values below 100% should be expected.

Sensitivity and detection limit

The sensitivity of the assay is based on 0.005 AU and a sample volume of 2.00 mL. This corresponds to a sulfite concentration of 0.1 mg/L sample solution when measured at 340 nm. The detection limit of 0.30 mg/L is derived from the absorbance difference of 0.010 (340 nm) and a maximum sample volume of 2.00 mL.

Linearity and precision

Linearity of the determination exists from 1 to 30 µg sulfite per assay. In a double assay using one sample solution, a difference of 0.005 to 0.010 AU may occur (0.15-0.30 mg/L of sulfite, v = 2.00 mL). The CV is approx. 1 to 4% in the measuring range.

Kit composition

Solution 1. Triethanolamine buffer (30 mL, 0.8 M, pH 8.0) and sodium azide (0.02% w/v) as a preservative. Store at 2 °C to 8 °C.

Solution 2. NADH tablets (~0.4 mg each). Store at 2 °C to 8 °C (up to 1 month). For long-term storage store desiccated at -15 to -30 °C.

Dissolve 1 tablets per mL of Solution 1; use forceps for taking the tablets out.

Suspension 3. NADH-peroxidase (NADH-POD, EC 1.11.1.1; 0.4 mL). Store at 2 °C to 8 °C. Swirl bottle before use.

Suspension 4. Sulfite oxidase (SOD, EC 1.8.3.1; 1,6 mL) in 3.2 M ammonium sulphate. Store at 2 °C to 8 °C. Swirl bottle before use.

Protocol (endpoint analysis)

Wavelength: 340 nm

Cuvette: 1 cm light path (glass or plastic)

Temperature: ~25 °C

Final volume: 3.060 mL

Sample solution: 1-30 µg sulfite/assay (as SO_2; in 0.10 - 2.00 mL sample volume)

Read against air (without a cuvette in the light path) or against water

PIPETTE INTO CUVETTES (mL)	**BLANK**	**SAMPLE**
Solution 1+2	1.00	1.00
Sample	-	0.10
Distilled water	2.00	1.90
Suspension 3 (NADH-POD)	0.010	0.010
Mix, measure the absorbance of the solutions (A1) after ~5 min and start the reaction by addition of		
Suspension 4 (SOD)	0.050	0.050
Mix, measure the absorbance of the solutions (A2) at the end of the reaction (approx. 30 min)*		

Mixtures can be obtained with a plastic spatula or by gentle inversion after sealing with a cuvette cap or Parafilm®.

* if necessary, continue to read the absorbances at 5 min intervals until absorbances decrease constantly over 5 min..

Calculation

Determine the absorbance difference for both blank and sample (A1-A2). Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{Sulfite}$. The concentration of sulfite (as SO_2), based on the ε of NADH at 340 nm (6300 $L \times mol^{-1} \times cm^{-1}$), is calculated as follows:

$$C\ (Sulfite) = 0.3111 \times \Delta A_{Sulfite} \qquad [g/L]$$

If the sample has been diluted or a different sample volume was used during the reaction, the result must be multiplied by the corresponding dilution/concentration factor.

Alternative procedures (micro-volumes)

Although this kit has been developed to work in cuvettes, it can be easily adapted for use in 96-well microplates or in auto-analysers. Basically, the assay volumes for the cuvette format have to be reduced approximately 10-fold for use in microplate format or in auto-analyser format. However, when using these micro-volume formats, you must be aware that the radiation pathlength is usually smaller than 1 cm, which is the standard cuvettes pathlength. Thus, to perform the calculation of the amount of analyte in the samples follow one of the three possible strategies described in the “Alternative Procedures”, available on the NZYtech website.

Interferences

L-Ascorbic acid inhibits sulfite oxidase. Higher concentrations of L-ascorbic acid in the assay reacts with hydrogen peroxide which is formed as an intermediary product and thus produce results that are too low if L-ascorbate is not removed by means of ascorbate oxidase before the determination of sulfite.

Aqueous sulfite samples are typically unstable, thus recoveries of less than 100% should be expected. This is consistent with results obtained with other enzymatic sulfite test kits.

Sulfite control solution

A sulfite solution may be prepared to quantify method accuracy.

A control sulfite solution (~0,3 g sulfite /L) can be prepared as follows:

- Using Sodium Sulfite (NA_2SO_3; M=126,04g/mol; 50,8% SO_2): Dissolve approx. 60 mg of sodium sulfite (~30 mg sulfite) in 100 mL of citric acid solution (1g/L);

- Using Sodium disulfite ($Na_2S_2O_5$; M=190,10 g/mol; 67,4% SO_2): Dissolve approx. 45 mg of sodium disulfite (~30 mg sulfite) in 100 mL of citric acid solution (1g/L);

- Using Potassium disulfite ($K_2S_2O_5$; M=222,33 g/mol; 57,6% SO2): Dissolve approx. 50 mg of sodium disulfite (~30 mg sulfite) in 100 mL of citric acid solution (1g/L);

Store in a well-sealed bottle and use on the day of preparation. Usually, the recovery of the control solution will be under 100% since sulfite standard materials are easily oxidized.

General information on sample preparation

The amount of sulfite present in the cuvette should range between 1 and 30 µg. Thus, if a sample volume of 0.10 mL is used the sample solution must be diluted to yield a sulfite concentration between 10 and 300 mg/L. However, the sample volume can vary from 0.10 to 2.00 mL, by replacing water.

To implement this assay use clear, colourless and practically neutral liquid samples directly, or after dilution; filter turbid solutions; degas samples containing carbon dioxide (e.g. by filtration); adjust acid samples, which are used undiluted for the assay, to pH 8 by adding sodium or potassium hydroxide solution; adjust acid and weakly coloured samples to pH 8 and incubate for approx. 15 min; measure "coloured" samples (if necessary adjusted to pH 8) against a sample blank; treat "strongly coloured" samples that are used undiluted or with a higher sample volume with PVPP; crush or homogenize solid or semi-solid samples, extract with water or dissolve; extract samples containing fat with hot water.

Since sulfurous acid is volatile, reactive and easily oxidized, please take special care when preparing the samples and performing the analysis. Moreover, due to sulfite solutions instability, samples should be analysed as soon as possible after preparation.

Examples of sample preparation

Determination of sulfite in white wine

Use white wine directly for the assay with a sample volume of v = 0.10 mL.

Determination of sulfite in red wine

Adjust 25 mL of red wine to pH 7.5-8.0 with sodium hydroxide (2 M) and dilute to 50 mL with distilled water in a volumetric flask (dilution factor of 2). Incubate for approx. 10 min at ~25 °C. Use 0.10 mL for the assay.

Determination of sulfite in beer

Open bottle and filter beer sample immediately at ~25 °C. Add 0.7 g bentonite to 10 mL beer in a 50 mL beaker, stir for 1 min and filter. Use 1.00 mL of the filtrate for assaying.

Determination of sulfite in jam

Homogenize approx. 100 g jam in a homogenizer for 30 s. Weigh precisely approx. 5 g of the homogenous sample into a 50 mL volumetric flask and add 40 mL double distilled water. Close the volumetric flask and incubate at 60 °C for 5 min. Cool to ~25 °C, fill up to the mark with distilled water, mix and filter. Use the clear solution diluted, if necessary, for the assay.

Determination of sulfite in potato products

Mince and homogenize dried potato products using a mortar or mixer. Weigh precisely approx. 5 g of minced and ground potato chips (or approx. 2 g ground dried potato material intended for production of potato dumplings) into a 100 mL volumetric flask and add 80 mL hot (65 °C) distilled water. Close the volumetric flask and shake rigorously for 5 min or stir with magnetic stirrer. Let stand for 15 min. After cooling to ~25 °C, fill up to the mark with distilled water, mix and centrifuge (10 min at 4000 rpm). Use the clear solution with a sample volume of up to 0.50 mL or diluted, if necessary, for the assay.

Determination of sulfite in spices and coffee products

Mince and homogenize the spice sample using a mortar or mixer. Weigh precisely approx. 100 mg sample into a 50 mL volumetric flask and add 30 mL distilled water. Close the volumetric flask and incubate at approx. 60 °C for 5 min. Cool to ~25 °C, fill up to the mark with distilled water, mix and filter. Use the clear solution diluted, if necessary, for the assay.

References

Beutler, H. O. (1988). Sulfite. In: Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), 3rd ed., Vol.VI, pp. 585-591, VCH Publishers (UK) Ltd., Cambridge, UK.

Recommendations

This method is recommended/approved by the:

- German food law;

- European and German standards (EN, DIN);

- Nordic Committee on Food Analysis (NMKL);

- Mitteleuropäische Brautechnische Analysen commission (MEBAK).

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