

Determination of Sulfite in Food and Beverages by Ion Exclusion Chromatography with Pulsed Amperometric Detection

INTRODUCTION

Sulfite is a widely used food preservative and whitening agent that received GRAS (generally recognized as safe) status from 1959 until 1986. In 1986, the U.S. Food and Drug Administration (FDA) revoked GRAS status when adverse reactions in sulfite-sensitive individuals were reported. Since then, the FDA has required warning labels on any food containing more than 10 mg/kg of sulfite or beverage containing more than 10 mg/L. Six sulfiting agents are currently approved by the FDA for use as food additives: sulfur dioxide, sodium sulfite, sodium and potassium bisulfite, and sodium and potassium metabisulfite.

The Modified Monier-Williams method¹ is the most widely used method for analyzing the amount of sulfite in various food matrices. However, this method is time-consuming and quite labor-intensive. More recently, the Association of Official Analytical Chemists (AOAC) International adopted a method developed by Kim and Kim that uses ion exclusion chromatography with direct current (dc) amperometric detection.² This method (AOAC Method 990.31) is selective enough that samples need only be homogenized in buffer, filtered, and injected for analysis.

One drawback to the Kim and Kim method is that fouling of the platinum working electrode occurs rather quickly, leading to a significant decrease in detector response over time. As much as a 40% loss of the detector response to sulfite over an 8-h period has been reported.³ Not only does this necessitate frequent polishing of the working electrode, but accurate quantification requires injecting a standard after every sample injection.

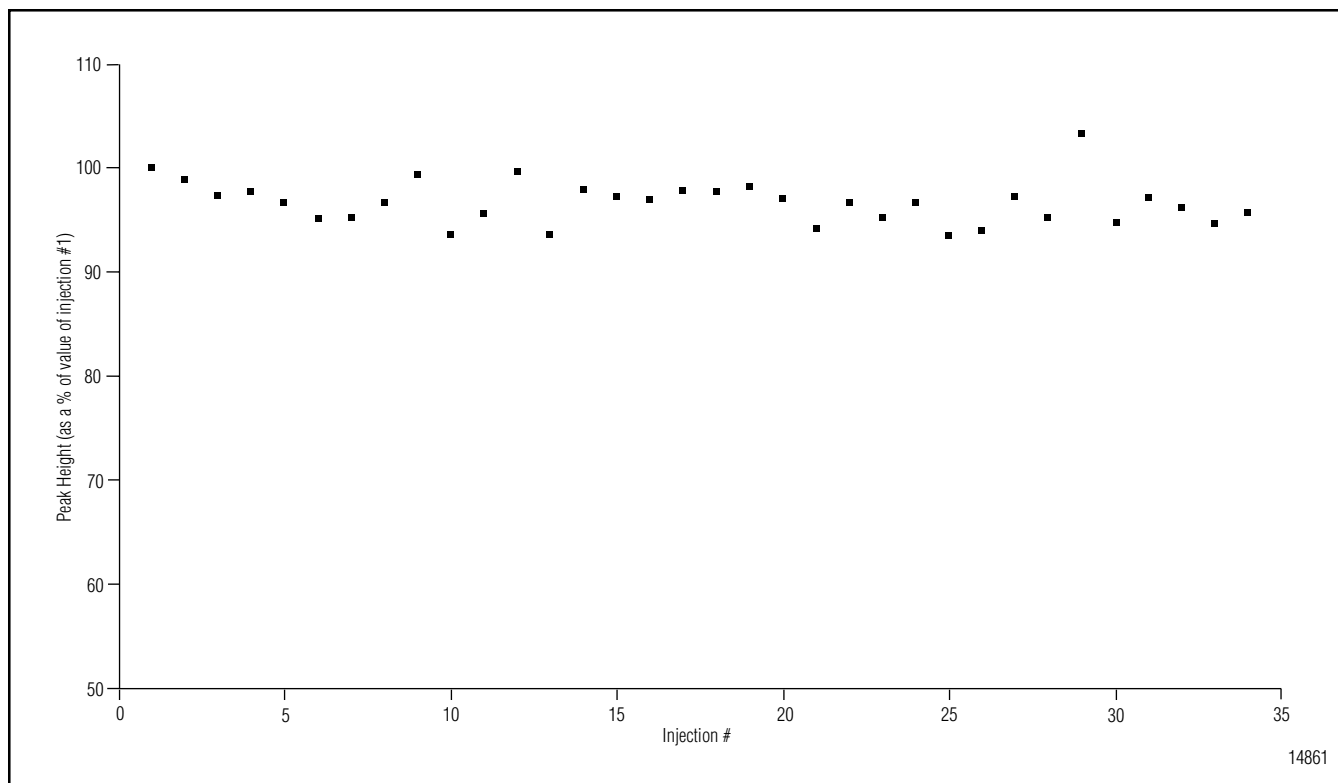
The method described in this Application Note is a modification of the Kim and Kim method. It uses the same sample preparation and chromatographic procedures, but solves the detection problems by using pulsed amperometry instead of dc amperometry. The pulse sequence constantly cleans the working electrode, thus preventing fouling. Detector response remains stable, as shown in Figure 1, resulting in more reliable quantification. In addition, standards can now be injected much less frequently, resulting in higher sample throughput.

Sample Preparation and Preservation

The sample preparation buffer, adopted from AOAC Method 990.31, is alkaline so that both free and bound sulfite can be extracted. Mannitol is included to slow the oxidation of sulfite to sulfate. Food samples are prepared by homogenization in the buffer, followed by filtration. Liquid samples are diluted in the buffer prior to injection.

Summary of Analytical Method

Sulfite is separated from other matrix components by ion exclusion chromatography using a sulfuric acid eluent and detected by pulsed amperometry using a platinum working electrode. Amperometry is a highly sensitive and specific detection method for oxidizable species such as sulfite. The waveform includes oxidizing and reducing potentials, which are constantly cycled to maintain a reproducible working electrode surface.



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Figure 1 Consecutive injections (34) of a 14-mg/L sulfite standard. Peak heights were plotted as a percentage of the peak height from injection #1. Each analysis required approximately 15 min.

EQUIPMENT

Dionex DX-500 HPLC system consisting of:

High Performance Pump (IP25 or GP50)
with vacuum degas

ED40 Electrochemical Detector equipped
with a platinum working electrode

LC20 Chromatography Module

EO1 Eluent Organizer

PeakNet Chromatography Workstation

REAGENTS

Concentrated sulfuric acid, ACS Reagent Grade
(Fisher Scientific, Fair Lawn, NJ)

Deionized water, 18 MΩ-cm

Sodium sulfite, anhydrous, ACS Reagent Grade
(Sigma Chemical Co., St. Louis, MO)

Sodium phosphate dibasic heptahydrate (Na₂HPO₄ • 7H₂O)
(Sigma Chemical Co., St. Louis, MO)

D-Mannitol (J.T. Baker Chemical Co., Phillipsburg, NJ)

REAGENT PREPARATION

20 mN H₂SO₄

Dilute 0.55 mL of concentrated sulfuric acid to 1.0 L with deionized water. Pressurize with helium.

Sample Buffer (20 mM Na₂HPO₄/10 mM Mannitol, pH 9)

Dissolve 5.36 g of sodium phosphate dibasic heptahydrate and 1.82 g of D-mannitol in 1.0 L of water. Filter through a 0.45-μm filter.

STANDARD PREPARATION

Stock Solutions

Prepare a stock solution of sulfite (approximately 1000 mg/L) by accurately weighing approximately 195 mg of Na₂SO₃. Transfer to a 100-mL volumetric flask and dilute to volume with buffer.

Working Standards

Make appropriate dilutions in buffer to bracket expected sample concentrations.

EXPERIMENTAL CONDITIONS

Column: IonPac® ICE-AS1

Eluent: 20 mN H₂SO₄

Flow Rate: 1.0 mL/min

Inj. Vol.: 50 µL

Detection: Pulsed amperometry, Pt electrode;

Waveform: Time (s) Voltage (V) Integration

0.00	0.80	
0.40	0.80	begin
0.60	0.80	end
0.61	1.20	
0.70	1.20	
0.71	0.10	
1.00	0.10	

DISCUSSION AND RESULTS

Food Analysis

Sulfite is found in large quantities as a preservative in dried fruits. To prepare a sample of dried apricots for the chromatogram shown in Figure 2, 100 mL of the mannitol buffer was added to 20 g of sample. The mixture was blended at high speed for about 1 min. After homogenization, the sample was centrifuged for 15 min at 2200 x g. The resulting supernatant was diluted 20-fold in mannitol buffer and 50 µL were injected onto the column. Quantification of the sulfite peak showed that the original dried apricot sample contained 0.8 mg of sulfite per gram of fruit.

Liquid Sample Analysis

For the analysis of lime juice, shown in Figure 3, the lime juice sample was diluted 36-fold in mannitol buffer and injected. Lime juice was found to contain 260 mg/L of sulfite.

Sample Stability

Because sulfite readily oxidizes to sulfate, samples and standards should be analyzed in a timely fashion. Standards should be made fresh daily, and reasonable care should be taken to reduce air exposure of both standards and samples.

Studies indicate that standards and samples prepared in the mannitol buffer should be stable for 24 hours. However, unpreserved samples should be analyzed as soon as possible after opening the sample container. In one study, untreated white wine was injected repeatedly (an example chromatogram is shown in Figure 4) over a period of about 4 h. A plot of sulfite concentration in the

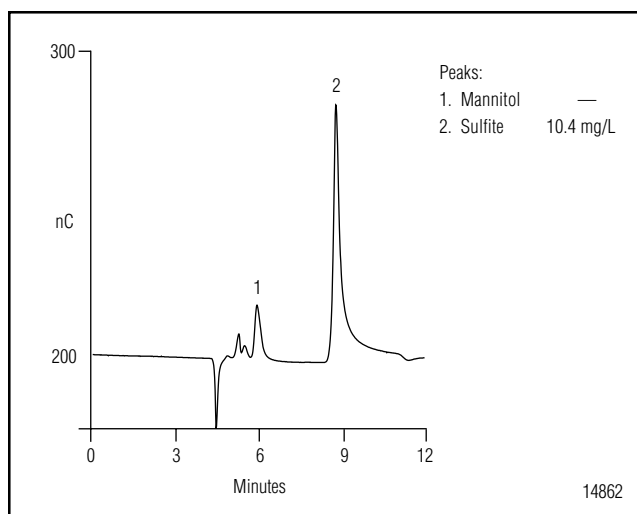


Figure 2 Dried apricot homogenate.

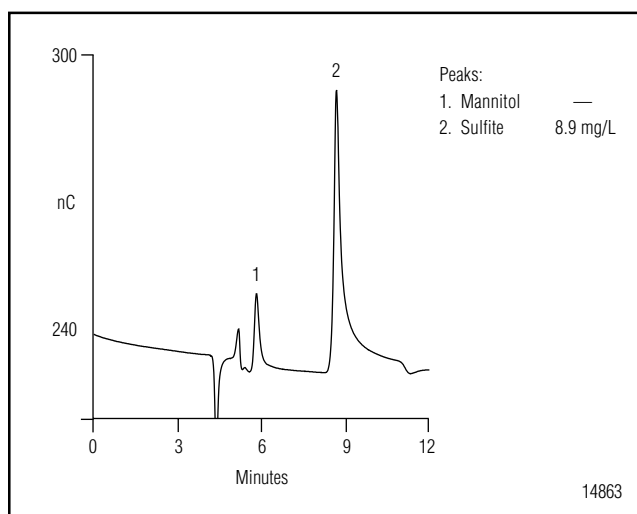


Figure 3 Lime juice, 1/36 dilution.

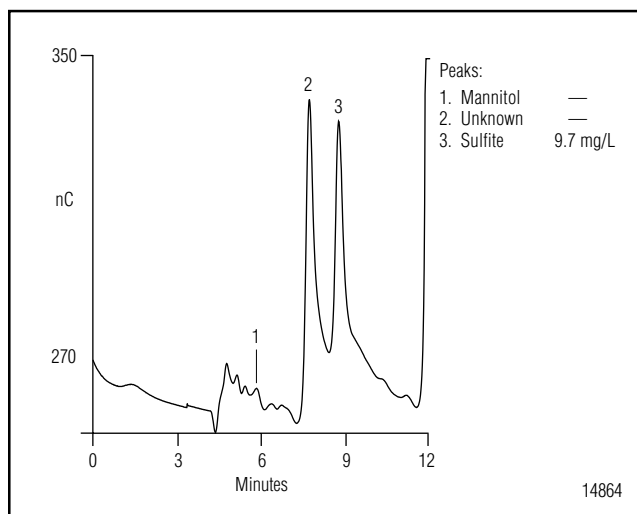
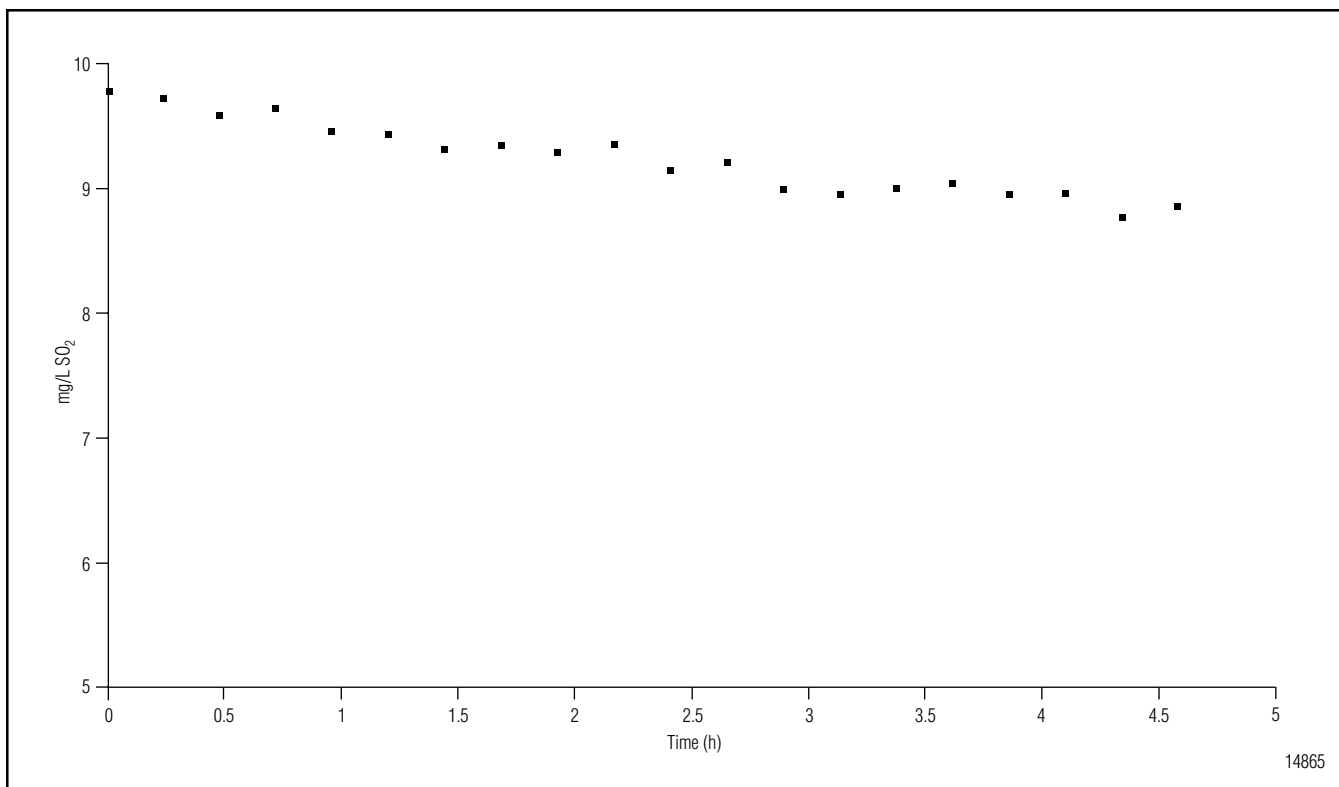


Figure 4 White wine.



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Figure 5 Oxidation of sulfite in white wine over time. Original concentration of SO₂ in fresh sample was 9.7 mg/L.

wine versus time is shown in Figure 5. A linear fit of this plot yields a line with the equation $y = -0.2x + 9.7$, indicating that sulfite is oxidizing at a rate of approximately 2% per hour.

Method Performance

Quantification by peak height, which will give more reliable data for this method, is recommended. All method performance data shown below were calculated using peak height data.

Method Detection Limits (MDL)

A volume of 50 μ L of a 530- μ g/L standard (shown in Figure 6) was injected repeatedly. Using the student's t calculation (99% confidence level, 19 degrees of freedom), the MDL was found to be approximately 40 μ g/L SO₂.

Linearity

Detection of sulfite was found to be linear over the range of 0.9 to 90 mg/L ($r^2 = 0.998$).

Repeatability

Injection-to-injection repeatability was measured by calculating the relative standard deviation (RSD) of the data shown in Figure 1. A 14-mg/L sulfite standard, which had been prepared in mannitol buffer, was injected 34 times. The relative standard deviation for resulting peak heights was 2.2%.

Recovery

Recovery of sulfite from white wine, which as packaged contained 9.7 mg/L of sulfite, was studied. A 20-mL aliquot of wine was spiked with 20 μ L of a 530-mg/L stock solution of sulfite in mannitol buffer. Analysis of the spiked sample showed 104% recovery of the added sulfite ($n=3$).

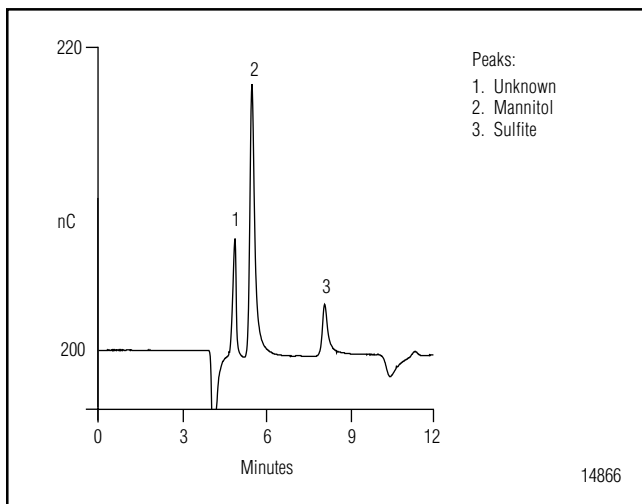


Figure 6 Sulfite standard, 530 µg/L.

PRECAUTIONS

Maintaining Working Electrode

Depending on the cleanliness and complexity of samples analyzed, the working electrode should remain stable for several weeks to months. Typical background is around 100 to 200 nC.

When the background starts to rise and baseline noise increases, it may be necessary to polish the working electrode. Follow the electrode polishing procedure outlined in the ED40 manual. After polishing, it is good practice to condition the electrode for 24 hours (i.e., run eluent at a low flow rate with the cell on) before resuming sample analysis.

On-Line Vacuum Degas

Because dissolved oxygen in the eluent can affect the performance of an amperometric detector, on-line degassing is recommended to maintain an oxygen-free environment. Degassing the eluent and pressurizing it with helium is only partly effective because the Teflon® tubing usually used to carry eluent from the reservoir to the pump is oxygen-permeable.

Instrument Shutdown

For short-term instrument shutdown (less than a week), it is good practice to keep eluent flowing through the system at 0.1 to 0.2 mL/min. The amperometric cell should be left on. Following this recommendation will prevent the need for frequent reconditioning of the working electrode.

For long-term shutdown, the cell should be disassembled and the reference electrode stored in saturated KCl.

CONCLUSION

The method outlined in this Application Note offers a substantial improvement in the detection of sulfite. Using pulsed amperometry, the working electrode surface is continuously cleaned, resulting in a more stable detector response. Good method performance was shown, with injection-to-injection repeatability less than 3%. Using this method, it is possible to achieve more accurate sulfite quantification using fewer standard injections than required for dc amperometric methods.

REFERENCES

1. AOAC Official Method 962.16 in *Official Methods of Analysis of AOAC International*, 16th ed., Vol. II; Cunniff, P., Ed.; 1995.
2. AOAC Official Method 990.31 in *Official Methods of Analysis of AOAC International*, 16th ed., Vol. II; Cunniff, P., Ed.; 1995.
3. Wagner, H.P.; McGarrity, M.J. *J. Chrom.* **1991**, *546*, 119-124.

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