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Sulfite Analysis of Fruits and Vegetables by High-Performance Liquid Chromatography (HPLC) with Ultraviolet Spectrophotometric Detection

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Free and total sulfite were analyzed in acidified vegetable products, instant mashed potatoes, and dried apples. Sulfite was separated by HPLC and quantified with a UV−vis detector. Resolution from components of food samples was achieved by varying the acid concentration of the eluant solution and by appropriate choice of the analytical wavelength. The minimum detectable levels for sulfite were 0.5 mg/L for a 10-cm analytical column and 1.5 mg/L for a 30-cm column. For analyses done with a 30-cm column, the coefficient of variation was <2% for analysis of free sulfite and total sulfite in acidified vegetables. For dried apples and instant potatoes, it ranged from 1 to 6.5%. The corresponding analytical errors were <4% and 1.2−5.6%, respectively, for the 10-cm column.

KEYWORDS: Pepper; cucumber; apple; potato; high-performance liquid chromatography; sulfur dioxide

INTRODUCTION

Sulfite is an important additive in many food products because it inhibits development of both enzymatic and nonenzymatic browning in a variety of processing and storage situations (*1*). It is also a very effective microbial inhibitor in acid or acidified foods (*2*, *3*). Sulfite is also an additive that can cause asthmatic reactions in a small proportion of people (*4*, *5*). Excessive sulfite is responsible for off-flavor in food products (*1*). Finally, some of the sulfite added to foods often disappears as a result of reversible and irreversible chemical reactions. Thus, it is often important to measure both free and bound forms of sulfite that are present in foods.

Kim and Kim (*6*) first demonstrated the use of high-performance liquid chromatography (HPLC) with an electrochemical detector to analyze free and total sulfite with high sensitivity and selectivity in food samples. HPLC, sometimes combined with preseparation steps, has now been used for sulfite analysis in a number of food products, including avocado, broccoli, cabbage, catsup, sweet corn, mushrooms (*7*), lemon juice, wine, dehydrated fruits and vegetables (*8*, *9*), grapes (*10*), fresh sausage (*11*), and shrimp (*12*). Pizzoferrato et al. (*13*, *14*) used indirect photometric detection with HPLC by first distilling sulfite from a variety of foods, converting it to sulfate with hydrogen peroxide, and then chromatographing the resulting sulfate ions. With the exception of situations when volatile components in the food co-distill with sulfite or when sulfite concentrations are below the sensitivity of the Monier−Williams method (*15*), HPLC techniques give results similar to that obtained with the Monier−Williams distillation method for sulfite.

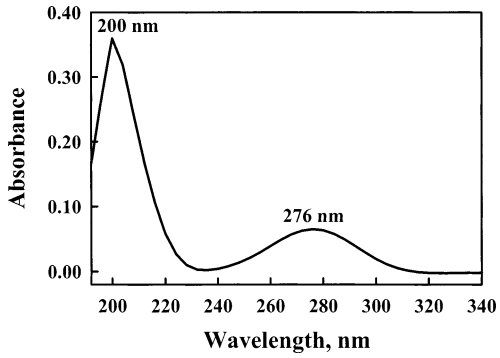

Figure 1. Sulfite spectrum at pH 1.6.

Electrochemical detection of sulfite is very sensitive and selective. However, fouling of the electrode occurs such that frequent restandardization of the detector is required (*16*). In addition, when a problem occurs with the electrodes or electronics of electrochemical detectors, it can be time-consuming and expensive to isolate and correct the problem. Wygant et al. (*17*) were able to very substantially reduce electrode fouling with a complex waveform that repeatedly applies cleaning potentials to the working electrode.

Sulfite has substantial UV absorption with absorption maxima at 200 and 276 nm (**Figure 1**). The absorption at 276 nm is due to free dissolved SO_2 (*18*), which is present as a substantial fraction of the sulfite forms in solution when the pH is in the vicinity of the first $pK_a = 1.86$ of sulfite (*18*, *19*). Despite the fact that ultraviolet detectors for HPLC are very stable, reliable,

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and commonly available, UV absorption has not been used as a detector after chromatographic separation of sulfite in food samples. UV detection has been used for gas phase detection of SO_2 in lemon juice with a flow injection system (*20*). Sample was injected and mixed with sulfuric acid to convert sulfite ions to SO_2, which was purged into a gas phase quartz flow cell with nitrogen gas. The gas phase SO_2 was measured by its absorbance at 200 nm.

The objective of this study was to demonstrate procedures for the quantitative analysis of free and total sulfite in fruit and vegetable products using HPLC with UV detection.

MATERIALS AND METHODS

Sodium sulfite (99.99%), sodium hydroxide, *n*-propanol, mannitol, and sulfuric acid were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). Indigo carmine ($pK_a = 12.8$) was obtained from Fisher Chemical Co. (Fair Lawn, NJ). A pH indicator solution of 1% indigo carmine in 50% aqueous ethanol was prepared. The 30-cm Bio-Rad HPX-87H and 10-cm Bio-Rad Fast Acid columns were purchased from Bio-Rad Laboratories (Hercules, CA).

Two liquid chromatographic systems were used. Both systems had a UV6000 diode array detector with a 50 mm light path and an AS 3000 autosampler (ThermoQuest, Inc., San Jose, CA). The system with the Fast Acid column used a Dionex GPM-2 gradient pump (Dionex Corp., Sunnyvale, CA) at a flow rate of 1.0 mL/min. The system with the HPX-87H column used a ThermoQuest P2000 pump at a flow rate of 0.8 mL/min. The columns for both systems were heated to 60 °C in the autosampler column oven. Samples were held in the autosampler trays at 8 °C prior to injection. Twenty microliter samples were injected onto both columns. Both columns were eluted isocratically with dilute sulfuric acid solution. The specific concentrations used are given under Results and Discussion.

Calibration was done with 6, 15, 38, and 96 mg/L sulfite solutions prepared by dilution from a 960 mg/L stock solution of 99.99% purity Na_2SO_3 in 0.02 N sulfuric acid. Ten millimolar *n*-propanol was added to the stock solution to inhibit oxidation. A set of standards was run before and after each group of samples. Sulfite concentrations were calculated using the standard curve from the eight standard injections.

Free sulfite samples were prepared simply by centrifuging the cover liquid or cheesecloth-filtered slurries at 15000*g* for 5 min in a 1.5 mL microcentrifuge tube to remove suspended particles. After centrifugation, the samples were transferred to autosampler vials and diluted, if necessary, with water.

Samples for total sulfite analysis required treatment with NaOH to release reversibly bound sulfite (*9*). When a new sample matrix was analyzed, a preliminary sample was first titrated with 3 N NaOH to determine the amount of base required to raise the pH to 12 and then titrated with 6 N sulfuric acid to lower the pH to 3. This sample was not analyzed. For analysis, 1.2 mL of a sample solution was added to a 1.5 mL microcentrifuge tube, along with 40 μL of 333 mM mannitol solution, 5 μL of indigo carmine indicator, and the amount of 3 N NaOH, determined by the preliminary titration, needed to raise the pH to 12. The indigo carmine provided a visual confirmation that the NaOH addition was correct for each sample. After 15.0 min of incubation at pH 12, 6 N sulfuric acid, on the basis of the preliminary titration, was added to lower the pH to 3−4. Water was added to bring the total volume to 1.5 mL. Samples were then centrifuged for 5 min at 15000*g*, transferred to autosampler vials, and diluted, if necessary.

Recovery of sulfite was evaluated by the addition of sodium sulfite (64 mg/L calculated as SO_2) to the cover solution of acidified green bell peppers that contained ~60 mg/L sulfite. Quadruplicate samples were prepared from the cover solution and analyzed for both free and total sulfite before and after spiking.

Commercial samples of jalapeño pepper rings, hot pepper sauce, dehydrated mashed potatoes, and dried apples were purchased from local food stores. Five containers of a single lot of each product were analyzed. Duplicate samples for analysis of free and total sulfite were prepared from each container of each product. Acidified red bell peppers and cucumbers stored in 0.6 and 0.9% acetic acid, respectively, with added sodium metabisulfite were prepared in the laboratory. Triplicate brine samples from each of three jars of red bell peppers were prepared and analyzed for free and total sulfite. Cucumbers were analyzed for sulfite by taking triplicate samples from duplicate jars at three sulfite concentrations. Sample preparation was done at room temperature.

Samples of the dehydrated potatoes were prepared by stirring 10 g of potato flakes with 100 mL of water for 10 min. The slurry was filtered through a three-ply layer of cheesecloth. Dried apples (30 g) were blended in a Waring blender with 300 mL of water and also filtered through cheesecloth. For the acidified vegetables, samples of the cover liquid from freshly opened jars were analyzed. To analyze the commercial products from the same containers on both the 10- and 30-cm columns, duplicate sets of autosampler vials were prepared and run simultaneously in the two chromatographic systems.

Statistical calculations were done with PROC GLM of SAS version 8 software (SAS, Inc., Cary, NC).

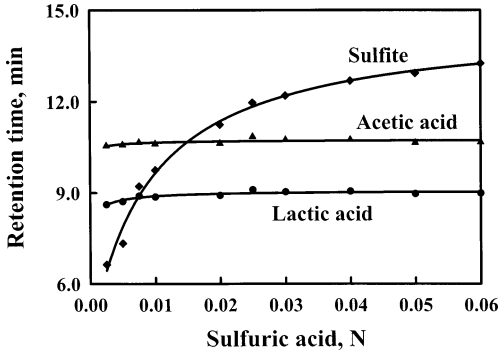

Figure 2. Effect of eluant sulfuric acid concentration on the retention time of sulfite ($pK_a = 1.86$), acetic acid ($pK_a = 4.76$), and lactic acid ($pK_a = 3.86$) on a 30-cm Bio-Rad HPX-87H ion exchange column.

RESULTS AND DISCUSSION

The selectivity of UV detection for sulfite was less than that for electrochemical detection (*6*) because a wide range of naturally occurring compounds absorb in this region of the spectrum. However, **Figure 2** shows that the retention time of the sulfite peak changed by over 6 min as the sulfuric acid concentration in the eluant solution was varied from 0.0025 to 0.06 N (eluant pH range from about 2.5 to 1.2). This large variation in retention time was due to the fact that the SO_2 to HSO_3^{-1} conversion has a pK_a of 1.86, which is in the middle of the eluant pH range of 1.2−2.5. In contrast, organic acids have pK_a values higher than the highest eluant pH (lactic acid $pK_a = 3.73$, acetic acid $pK_a = 4.76$), so their extent of ionization changed very little, and, consequently, their retention times changed by <0.5 min over the same range of sulfuric acid eluant concentrations (**Figure 2**). Nonionizable components in the samples, such as sugars or alcohols, showed almost no change in retention times as the sulfuric acid concentration changed (data not shown). This variable elution behavior by sulfite allowed the location of the sulfite peak relative to other sample components to be adjusted to minimize interferences. In addition, sulfite could be measured in the region of either absorption maximum (**Figure 1**) to minimize spectrophotometric interference. For this investigation, 210 nm was routinely used because this wavelength was also used for organic acids.

Figure 3 shows chromatograms of organic acids and sulfite standards separated on 30- and 10-cm columns with detection wavelengths of 210 and 276 nm. The run time for a chromatogram was only 8 min for the short column compared to 20 min for the 30-cm column. The absorbance at both 210 and 276 nm was linear with concentration, although the intercept was nearly always slightly positive **(Figure 4)**. Linear correlation coef-

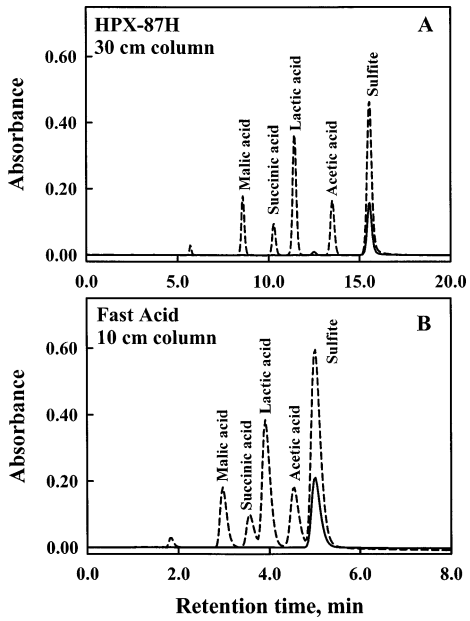

Figure 3. Comparison of organic acid and sulfite resolution on a 30-cm HPX-87H column and a 10-cm Fast Acid column. Absorbance data were collected at 210 nm (- - -) and 276 nm (—).

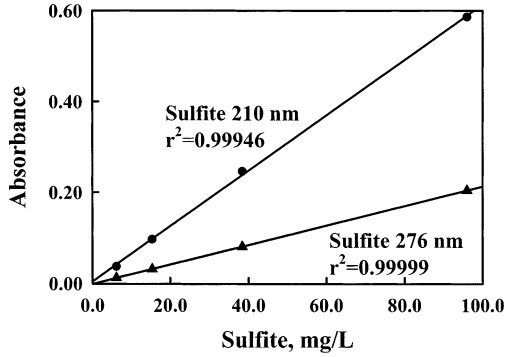

Figure 4. Calibration curves for sulfite at 276 and 210 nm.

ficients (r^2) for standard curves at both wavelengths were always ≥0.9990. **Figure 5** shows the separation on a 10-cm column of 19 mg/L sulfite in a red pepper brine, compared to the same brine without sulfite present. The minimum detectable level for sulfite dissolved in water, based upon a peak height at least 3-fold greater than the noise level, was found to be 1.5 mg/L with the 30-cm HPX-87H column but only 0.5 mg/L with the identical, but shorter, 10-cm Fast Acid column. The lower minimum detectable level for the shorter column was the result of less band spreading with a shorter elution time.

Measurement of total sulfite as the summation of free sulfite and reversibly bound sulfite requires the release of the reversibly bound sulfite either by refluxing in strong acid (Monier−Williams) or by raising the pH with NaOH. Several studies have recommend the pH of food samples be increased to between 9 and 12 to ensure complete release of bound sulfite (*6*, *11*, *12*). For the products analyzed in this work, a pH 12 treatment for 15 min was found to give maximum release of sulfite. The recovery of sodium sulfite added to green pepper brine with some sulfite present and then subjected to pH 12 treatment to measure total sulfite was 110% on the 30-cm column and 105% on the 10-cm column. Recovery of added sulfite from green pepper brine analyzed for free sulfite was 97% on both columns.

To evaluate the reproducibility of sulfite analysis in foods, commercial samples of dried apples, instant mashed potatoes, hot pepper sauce, and jalapeño pepper rings were analyzed.

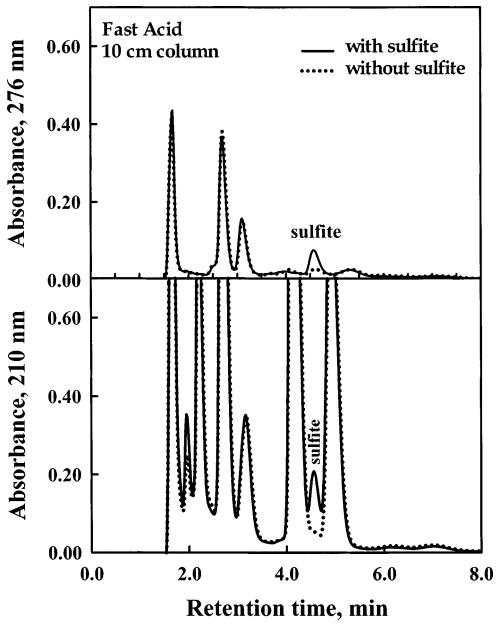

Figure 5. Chromatography of red pepper brine on a 10-cm Fast Acid column with and without added sulfite.

Table 1. Sulfuric Acid Concentration of Eluant Solutions Used on a 30-cm HPX-87H Column and a 10-cm Fast Acid Column To Minimize Interference from Components of Commercial Food Samples

column	dried apples	instant mashed potatoes	hot pepper sauce	jalapeño pepper rings
HPX-87H	0.030 N	0.030 N	0.024 N	0.024 N
Fast Acid	0.030 N	0.029 N	0.036 N	0.036 N

Table 1 shows the sulfuric acid concentration of the eluant used for each product and column. Eluant concentrations were selected to minimize interference from other components at 210 nm. The presence of interfering peaks was evaluated by the addition of 6 mM H_2O_2 to remove sulfite from appropriately diluted samples by oxidizing it to sulfate (*21*).

Table 2 shows the concentrations of free sulfite and total sulfite and the analytical error calculated over all four products and also for each product analyzed on the 30-cm HPX-87H column. Brine samples from the two pepper products had only ∼1% analytical error for both free and total sulfite. The dried products, which had to be reconstituted and extracted with water prior to analysis, had greater analytical errors of ∼2% for free sulfite and 5−6.5% error for total sulfite. For this column, the detection wavelength had no effect on the concentration of sulfite measured. With the exception of the instant potatoes, the total sulfite was greater than the free sulfite, indicating the presence of a significant amount of reversibly bound sulfite.

Analysis of the same samples on a 10-cm Fast Acid column is shown in **Table 3**. There were some resolution problems on the shorter column. This was particularly the case with the instant potatoes in which the measured sulfite was 60−70% greater than that observed with the HPX-87H column. The analytical error on the shorter column varied within a range of 1.1−5.6% for the free and total sulfite over the four products.

The concentration of sulfite in the commercial products was very high relative to the detection sensitivity that was possible with aqueous sulfite solutions. To evaluate the use and reproducibility of UV detection to analyze lower concentrations of sulfite in acidified vegetables, red bell peppers and cucumbers were analyzed on the HPX-87H column. **Table 4** shows that

Table 2. Parameters of the Analysis of Free and Total Sulfite from Commercial Fruit and Vegetable Products Separated on a 30-cm HPX-87H Column

product	free or total sulfite	wavelength (nm)	analytical error (%)	concn (mg/L)
calculated over four products	free	210	1.72	622
	free	276	1.87	619
	total	210	6.43	744
	total	276	6.21	746
dried apples	free	210	1.72	1137
	free	276	1.89	1127
	total	210	6.30	1505
	total	276	6.08	1509
instant mashed potatoes	free	210	0.59	218
	free	276	0.64	235
	total	210	5.37	212
	total	276	4.85	234
hot pepper sauce	free	210	1.10	406
	free	276	1.07	401
	total	210	0.78	466
	total	276	0.84	463
jalapeño pepper rings	free	210	0.84	736
	free	276	0.90	723
	total	210	0.64	793
	total	276	0.65	779

Table 3. Parameters of the Analysis of Free and Total Sulfite from Commercial Fruit and Vegetable Products Separated on a 10-cm Fast Acid Column

product	free or total sulfite	wavelength (nm)	analytical error (%)	concn (mg/L)
calculated over four products	free	210	3.10	642
	free	276	5.28	674
	total	210	1.70	816
	total	276	1.71	821
dried apples	free	210	2.96	1069
	free	276	5.44	1163
	total	210	1.30	1667
	total	276	1.30	1633
instant mashed potatoes	free	210	4.55	354
	free	276	5.65	380
	total	210	3.97	364
	total	276	3.32	378
hot pepper sauce	free	210	3.89	427
	free	276	3.28	409
	total	210	1.15	483
	total	276	1.79	482
jalapeño pepper rings	free	210	1.23	747
	free	276	1.38	733
	total	210	1.13	786
	total	276	1.23	757

<30 mg/L sulfite in red bell peppers could be measured with an analytical error of <2%. Analysis of cucumbers with a range of sulfite from 10 to 350 mg/L resulted in an analytical error of ~1%. Therefore, it was concluded that lower concentrations of sulfite in food matrices could also be reproducibly measured.

The analytical error observed for HPLC analysis with UV detection was equal to or less than that found for other methods used for analysis of sulfite in foods. Kim and Kim (*6*) found analytical errors of 7.7−11.1% for 239−524 mg/L free sulfite in instant mashed potatoes, freeze-dried pears, and freeze-dried green bell pepper. For total sulfite in these three foods, the analytical error ranged from 4.1 to 11.1%. For shrimp with 325 mg/L sulfite, the coefficient of variation was 7.7% for HPLC with electrochemical detection and 4.9% with the optimized Monier−Williams distillation (*12*). Analysis of sulfite in sausage by HPLC with electrochemical detection had analytical errors of 3.3% for 300 mg/L of free sulfite and 5.0% for 360 mg/L total sulfite (*11*). Gas phase UV spectrophotometry of SO_2 after transfer of the sulfite from the liquid phase to the gas phase was used to detect 30 mg/L sulfite in lemon juice (*20*). The coefficient of variation for six analyses of a single sample was 2.8%.

Table 4. Analysis of Brine Samples from Acidified Peppers and Cucumbers for Free and Total Sulfite

	brine	
	free	total
acidified red bell peppers, 30-cm column		
sulfite (mg/L)	22.8	29.8
jar to jar variation (%)	32.6	23.9
analytical variation (% of mean)	1.1	1.5
acidified cucumbers, 30-cm column		
sulfite (mg/L)	9.6–333	11.5–358
jar to jar variation (%)	NA	NA
analytical variation (% of mean)	1.0	0.9

This research has shown that both free and total sulfite can be quantitatively measured in acidified vegetables, dehydrated mashed potatoes, and dried apples using HPLC with a UV detector at either 210 or 276 nm. Variation of eluant acid concentration and selection of the analytical wavelength could be used to minimize the interference from other components in food samples. The analytical errors were either equal to or less than those of previously published procedures for sulfite analysis in food samples. The only previous reported use of UV detection of sulfite in an HPLC application used a gas phase cell (*20*). Use of a 10-cm analytical column had the advantage of reducing the time per analysis compared to a 30-cm column. Its disadvantage was lower resolution of sample components. Comparison of the sulfite concentrations measured on the two columns (**Tables 2** and **3**) shows that higher sulfite concentrations were usually observed with the shorter column. The sulfite concentration differences between the columns were ≤10% of the levels obtained on the 30-cm column. However, 61−72% higher sulfite concentrations were measured with the 10-cm column for instant mashed potatoes. This was a result of incomplete resolution of the sulfite from interfering components.

Compared to the Monier−Williams distillation procedure for sulfite analysis (*15*), much less labor is required. This is particularly the case if an autosampler is used to inject samples. Also, if volatile acids are present in food samples, the Monier−Williams technique will overestimate sulfite unless proper precautions are taken. These advantages are shared by other HPLC techniques that are used for sulfite analysis. Electrochemical detectors for HPLC are more sensitive and selective for sulfite than a UV detector. However, the sensitivity of UV detection was <2 mg/L. UV detectors are more commonly available on HPLC systems than electrochemical detectors, because they are the detector of choice for a wide variety of chemical analyses. Compared to electrochemical detectors, they tend to be very stable and reliable over time. When detector breakdowns occur, UV detectors tend to be easier to troubleshoot and repair.

NOTE ADDED AFTER PRINT PUBLICATION

The word “Performance” in the title was misspelled when this paper was published on the Web (ASAP) on February 15,

2003, and in the March 12, 2003, issue of the print edition. The electronic file was corrected on April 17, 2003, and an Addition and Correction appears in the May 21, 2003, issue.

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