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Development of a Liquid Chromatography–Tandem Mass Spectrometry Method for the Determination of Sulfite in Food

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Abstract

Sulfites are widely used food preservatives that can cause severe reactions in sensitive individuals. As a result, the U.S. FDA requires that sulfites be listed on the label of any food product containing >10 mg/kg (ppm) sulfite (measured as sulfur dioxide). Currently, the optimized Monier–Williams (MW) method (AOAC Official Method 990.28) is the most common approach for determining sulfite concentrations in food samples. However, this method is time-consuming and lacks specificity in certain matrices. An improved rapid, sensitive, and selective method has been developed using electrospray ionization (ESI) high-performance liquid chromatography–tandem mass spectrometry (LC-MS/MS) for the determination of sulfite in various food matrices. A total of 12 different types of foods were evaluated. These included dried fruits and vegetables, frozen seafood, sweeteners, and juices. The matrix is extracted with a buffered formaldehyde solution, converting free and reversibly bound sulfite to the stable formaldehyde adduct, hydroxymethylsulfonate (HMS). Extracts are prepared for injection using a C_{18} SPE cartridge to remove any lipophilic compounds. HMS is then separated from other matrix components using hydrophilic interaction chromatography (HILIC) and detected using multiple reaction monitoring (MRM). The method was validated at 5 concentrations in 12 food matrices. Accuracy data showed spiked recoveries ranging from 84 to 115% in representative foods. Six commercially available sulfited products were analyzed using the LC-MS/MS method, as well as the MW method, to determine if differences exist.

Keywords

LC-MS/MS; sulfites; food additives

INTRODUCTION

Sulfites are a family of food preservatives that commonly include sulfur dioxide, sodium sulfite, sodium metabisulfite, potassium metabisulfite, sodium bisulfite, and potassium bisulfite. They are used worldwide in a broad range of food and beverage products including dried fruits, dried vegetables, jams, flours, juices, and seafood due to their antioxidant, antimicrobial, and antibrowning properties.[1] Sulfite is present in three forms in food

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systems: free, reversibly bound, and irreversibly bound. Free sulfite, as its name suggests, is not bound to any other food component and is easily quantified by most existing methods. Reversibly and irreversibly bound sulfites exist when adducts form between sulfite and various food components such as acetaldehyde, sugar monomers, or sugar acids.[2] Through sample preparation steps, such as pH adjustment and heating, sulfite can be released from reversibly bound sulfite and quantified. Irreversibly bound sulfite is very stable, making it difficult to quantify.[1]

Although sulfites contribute beneficial properties to a food system, a small subset of the population has an adverse allergy-like reaction upon consumption. In sensitive individuals, sulfites can produce health effects including skin rashes, nausea, and respiratory distress.[1] In 1986, the U.S. Food and Drug Administration (FDA) began requiring that all sulfite be declared on the label of any product in which the concentration exceeded 10 mg/kg (ppm) (measured as sulfur dioxide (SO_2)) and that no sulfites be added to any food product intended to be served raw or presented fresh to the public such as those items found on a salad bar.[3,4] Similar guidance was adopted by other regulatory agencies including those in Europe, Canada, and Korea.[5–7] The FDA identified AOAC Official Method 990.28,[8] the optimized Monier–Williams (MW) method, as the required method for all regulatory analyses. This method allows for accurate determination of sulfites in most food products and requires readily available glassware and reagents.[9] However, this method includes an extended distillation, displays reduced sensitivity at levels below 10 ppm of SO_2, and lacks specificity in some matrices such as *Allium* and *Brassica* vegetables.[10] Various alternatives to this method have been reported in the literature. These include electrochemical methods,[9,11,12] ion exchange chromatography,[13,14] liquid chromatography,[15] flow injection analysis,[16–18] spectrophotometry,[19] and iodometry.[20] Although these methods have shown promise for sulfite determination, there are still several limitations including lack of sensitivity, specificity, and applicability across multiple food types, making them unsuitable for routine regulatory use.

Sulfite is challenging to analyze in its highly unstable free form, leading to the use of chemical derivatization prior to analysis.[21] Warner et al.[21] found that the addition product of sulfite and formaldehyde, hydroxymethylsulfonate (HMS), is easily formed and more stable than the free form. HMS has been previously determined using ion-pairing high-performance liquid chromatography (HPLC).[22,23] Although this HPLC method is sensitive and selective for sulfite, the postcolumn reaction step requires specialized instrumentation and expertise. Liquid chromatography–tandem mass spectrometry (LC-MS/MS) is a technique that affords high specificity and sensitivity and has become widely used for food analysis. The present study describes the development and validation of an improved LC-MS/MS method for sulfite determination in a variety of food matrices. Sulfite is converted to HMS and then separated using hydrophilic interaction liquid chromatography (HILIC) and quantified by LC-MS/MS. Following method development and optimization for a wide range of food categories, validation was performed at five different concentrations ranging from 0.5 to 100 ppm of SO_2. The LC-MS/MS method was then compared to the MW method using commercially available sulfited products. LC-MS/MS proved to be a more sensitive, selective, and rapid method that will enable improved enforcement of the sulfite labeling requirements protecting sensitive individuals from inadvertently ingesting sulfite.

MATERIALS AND METHODS

Reagents and Materials

Both the internal standard, sodium sulfite-^{34}S ($Na_2\ ^{34}SO_3$, 95%), and the surrogate, chloroacetic acid ($ClCH_2CO_2H$, ≥99%), in addition to formaldehyde (37%), ammonium acetate (Sigma Ultra, minimum 98%), and sodium sulfite (Na_2SO_3, ≥ 98%) were acquired from Sigma-Aldrich (St. Louis, MO, USA). LC-MS grade acetonitrile, water, and methanol, in addition to glacial acetic acid, methylene chloride, hydrogen peroxide (30%), sodium hydroxide (certified 0.1 and 0.01 N), and concentrated hydrochloric acid, were purchased from Thermo Fisher Scientific (Pittsburgh, PA, USA). Methyl red from Mallinckrodt Baker (Phillipsburg, NJ, USA) was used in the MW titration. Samples were diluted and extracted using 18 MΩ water obtained from an Aqua Solutions water purification system (Jasper, GA, USA). Unsulfited food samples were purchased online and from grocery stores located in Greenbelt, MD, USA. For comparison of the LC-MS/MS and MW methods, products with sulfite declared on the label were obtained from local grocery stores. All food samples were stored according to the manufacturer's suggested storage conditions.

Standard Preparation

A 2% formaldehyde solution in 0.05 M ammonium acetate was prepared by dissolving 1.925 g of ammonium acetate in 50 mL of 18 MΩ water, adding 27 mL of 37% formaldehyde solution and diluting to 500 mL with 18 MΩ water. The pH of this solution was adjusted to 4.5 by dropwise addition of acetic acid. Working 0.2% formaldehyde extraction solutions were prepared by diluting 2.0% solution 1:10 with 18 MΩ water. The 0.2 and 2% formaldehyde solutions were stable at room temperature for at least 1 week and 3 months, respectively.

Both the chloroacetic acid and $Na_2\ ^{34}SO_3$ (IS) solutions were prepared using the formaldehyde extraction solvent. Briefly, a 25 ppm IS stock solution was prepared by dissolving 2.5 mg of $Na_2\ ^{34}SO_3$ in 100 mL of 2% formaldehyde solution. This stock was further diluted to form a 5 ppm working solution. The chloroacetic acid stock solution (10 mg/mL) was prepared by weighing 100 mg into a tared 10 mL volumetric flask and diluting to volume with 0.2% formaldehyde solution. Working standards of 2.5 mg/mL, 1 mg/mL, and 5 μg/mL chloroacetic acid were prepared by diluting the stock solution with the appropriate volume of 0.2% formaldehyde. Stock solutions were stored for up to 1 year at 4 °C, and working standards were stored for up to 1 month.

Stock solutions of HMS were prepared by weighing 100 mg of Na_2SO_3 into a tared 10 mL volumetric flask and diluting with 2% formaldehyde solution. On the day of analysis, a 1 mg/mL solution was prepared by diluting the stock solution with 18 MΩ water. Further dilutions with 0.2% formaldehyde solutions were made to give final concentrations of 1, 10, and 100 ppm of Na_2SO_3 working standards. Ten standards (0.01, 0.02, 0.05, 0.1, 0.2, 0.4, 0.8, 1.5, 3.0, and 4.5 μg Na_2SO_3/mL) were prepared in 2 mL glass autosampler vials with a PTFE/red silicone septum cap (Agilent Technologies, Palo Alto, CA, USA) by diluting the 1, 10, or 100 ppm working standards with 0.2% formaldehyde to 100 μL and then adding

100 μL of IS working solution, 100 μL of 5 μg/mL chloroacetic acid, and 700 μL of acetonitrile.

Sample Preparation

Due to the wide variety of food commodities analyzed, both liquid and solid preparation methods were developed. Refrigerated samples were allowed to come to room temperature prior to analysis. For solid samples, the sample (50 ± 1 g) was cut into small ¼ in. pieces and homogenized with 100 g of 0.2% formaldehyde solution in a variable-speed laboratory blender (Waring Laboratory Science, Torrington, CT, USA) with a 500 mL glass jar. A portion of the homogenate (15.0 ± 0.5 g) was transferred into 50 mL centrifuge tubes, and 20 mL of 0.2% formaldehyde was added along with 50 μL of 2.5 mg/mL chloroacetic acid. The centrifuge tubes were mixed end-over-end on a tube rotator (Glass-Col, Terra Haute, IN, USA) at 70 rpm for 10 min prior to 8 min of sonication (Branson 3510 ultrasonic cleaner, Sigma-Aldrich). The tubes were then centrifuged at 4000 relative centrifugal force (rcf) for 5 min (Marathon 2100R centrifuge, model 120, Thermo Fisher Scientific), and the supernatant was decanted into a 50 mL stoppered graduated cylinder. The extraction steps were repeated on the same sample portion, the supernatant was pooled, and the final volume was brought to 50 mL with the extracting solvent. A 6 mL Bakerbond C_{18} SPE cartridge with a sorbent bed of 500 mg (MG Scientific, Pleasant Prairie, WI, USA) was used to clean up the extract. The cartridges were conditioned by rinsing sequentially with 3 mL portions of methylene chloride, methanol, and 0.2% formaldehyde solution. The extract (2 mL) was passed through the cartridge and the eluent discarded. An additional 2 mL of extract was passed through and collected into a 4 mL clear glass screw cap vial with a solid cap and PTFE liner (Sigma-Aldrich). The vials were heated at 80 °C for 30 min using a DB3 Dri-Block Sample Concentrator from Techne (Staffordshire, UK) and then allowed to cool to room temperature. Samples were prepared for injection by diluting 200 μL of cooled extract with 100 μL of IS working solution (5 ppm) and 700 μL of acetonitrile in a 2 mL glass autosampler vial with a PTFE/red silicone septum cap (Agilent Technologies). After mixing, the vial contents were filtered with a 0.20 μm PTFE filter (17 mm, Thermo Fisher Scientific) into a new vial. Liquid samples were prepared for analysis by weighing 1.00 ± 0.05 g of sample into a 10 mL volumetric flask and diluting to volume with 0.2% formaldehyde solution. The samples were then analyzed in the same manner as the solid samples beginning with the SPE cleanup step and continuing through the heating and filtering steps. For the validation spiking, samples were fortified after grinding but prior to extraction.

LC-MS/MS

An Acquity Ultraperformance LC System (Waters, Milford, MA, USA) equipped with a SeQuant ZIC HILIC analytical column (150 × 2.1 mm × 5 μm; The Nest Group, Inc., Southborough, MA, USA) was used for separation. The column was thermostated at 30 °C, and a flow rate of 0.30 mL/min was employed. The following 24 min gradient program was used: 6 min hold at 90% mobile phase A (10 mM ammonium acetate in 90:10 ACN/H_2O) and 10% mobile phase B (10 mM ammonium acetate in 50:50 ACN/H_2O), gradient to 50% A in 4 min, 5.75 min hold at 50% A, gradient back to 90% A in 0.25 min, and equilibration at 90% A for 8 min before the next injection. All samples and standards utilized a 5 μL

injection volume. An external valve (Valco Instruments Co., Houston, TX, USA) was directed to waste at 0.0, 4.5, and 9.0 min and to the mass spectrometer at 2.0 and 6.5 min.

An AB Sciex 4000 QTRAP mass spectrometer equipped with an electrospray ionization (ESI) source in the negative ion mode was used for analysis. Analyst 1.5.2 (AB Sciex, Foster City, CA, USA) controlled both systems and data analysis. The source parameters were optimized for the HMS transitions. The curtain gas was set to 35 arbitrary units (au), the collisionally activated dissociation (CAD) gas was run at medium, an ion spray voltage of –1200 V was used, the source temperature was 550 °C, gas 1 pressure was 70 au, and gas 2 pressure was 40 au. The MS/MS data were acquired using the MRM mode (unscheduled) with unit resolution of both Q1 and Q3. Table 1 highlights the MS/MS conditions for each monitored transition including the compound ID, dwell time, declustering potential (DP), entrance potential (EP), collision energy (CE), and collision cell exit potential (CXP). There was a 5 ms pause between each transition, and the total scan time was 0.99 s.

Quantitation

A 10-point calibration curve ranging from 0.01 to 4.5 ppm of Na_2SO_3 was generated for the quantitation of sulfite. The curve was created from the MRM ratios of the peak areas of the analyte to the internal standard ($Na_2\ ^{34}SO_3$). A quadratic fit with $1/x^2$ weighting was used for the calibration curve due to the 3 orders of magnitude range of standard concentrations. The R^2 values of all curves were >0.990. Concentrations obtained using the calibration curve were adjusted by the appropriate dilution factor (250 for solid samples and 50 for liquid samples) and converted from Na_2SO_3 to SO_2. All values were reported as micrograms of SO_2 per gram of food sample.

Optimized Monier–Williams Method

Six different commercially available sulfited products were analyzed using the optimized MW method (AOAC Method 990.28). Briefly, a volume of 400 mL of 18 MΩ water was added to a 1 L round-bottom flask. A 30% hydrogen peroxide stock solution was diluted 1:10 to give 30 mL of 3% hydrogen peroxide. Three drops of a methyl red indicator were added to the solution, and then 0.01 N NaOH was added dropwise until a yellow end point was reached. This solution was added to a 50 mL graduated cylinder and placed under the bubbler. Nitrogen was bubbled through the entire closed apparatus for 15 min. Fifty grams of sample (both solid and liquid) was added to the round-bottom flask, and the transferring container was rinsed with 100 mL of 5% ethanol. Using the dropping funnel, 90 mL of 4 N HCl was added to the flask. The nitrogen flow was restarted, and the heating mantle (Glas-col, Terre Haute, IN, USA) was turned on to a heating level that produced 80–90 drops/min of condensate from the condenser. The contents of the flask were boiled under these conditions for 105 min. Upon completion of the distillation, the cylinder containing the hydrogen peroxide was removed, and its contents were quantitatively transferred to a 125 mL Erlenmeyer flask. The contents were titrated with either 0.1 or 0.01 N NaOH until the yellow end point was again reached. The volume of titrant needed to reach the end point was recorded. The sulfite content (as μg SO_2/ g food sample) was determined by

$$\text{ppm of } SO_2 = \frac{32.03 \times V_B \times N \times 1000}{w}$$

where 32.03 = milliequivalent weight of SO_2, V_B = volume (mL) of NaOH of normality N required to reach end point, 1000 = factor to convert milliequivalents to microequivalents, and w = weight (g) of sample added to the round-bottom flask.

RESULTS AND DISCUSSION

Method Development

During the early stages of method development, there was no commercially available stable isotopically labeled sulfite, which led to the use of chloroacetic acid as an internal standard. However, chloroacetic acid was not ideal because of its much earlier elution time compared to HMS. During method development, a stable isotope sodium sulfite ($Na_2\,{}^{34}SO_3$) became commercially available and was added into the method as the internal standard. Although this particular isotopic labeling was not ideal given that approximately 4% of naturally occurring sulfur occurs as the ^{34}S isotope, the calibration curve accounts for this effect, and the use of a true labeled internal standard greatly improved sample quantitation. However, due to the isotope's cost-prohibitive nature, chloroacetic acid remained in the method as a surrogate to identify any significant losses from the extraction process. The chloroacetic acid transitions are not used for quantitation and serve only as a visual check. The chloroacetic acid elutes first at 3.5 min, and HMS elutes at 7.5 min. A sample chromatogram of a 0.4 ppm of Na_2SO_3 (10 ppm of SO_2 in sample) standard is shown in Figure 1.

Filtration of the extract prior to LC-MS/MS analysis was necessary because a precipitate formed in the autosampler vial for most solid samples after the combination of the cooled extract, IS, and ACN. Initially, an investigation of several types of SPE cartridges was conducted to determine if the precipitation could be prevented. Polystyrene-divinylbenzene, weak anion exchange, strong anion exchange, and primary/secondary amine cartridges from several different manufacturers were examined. Although most resulted in a cleaner extract, none of these cartridges produced consistent sulfite recoveries of >75%. Both the weak anion exchange and strong anion exchange cartridges bound the sulfite too strongly, and no combination of elution solvents could recover the analyte. A mixed-mode cartridge, Isolute HAX (Biotage), yielded high sulfite recovery (>80%) for some matrices, but these results were neither repeatable nor achievable for all required samples. A systematic review of the conditioning and elution solvents did not improve the results. The Bakerbond C_{18} cartridge produced the most consistent and repeatable results and, once coupled with the additional filtering step, produced a clean extract for injection. To determine the best filtering material, both PTFE and nylon filters (0.20 and 0.45 μm) were evaluated with blank matrix samples, as well as 5 and 10 ppm of Na_2SO_3 spikes. At all three concentrations tested, the PTFE filters resulted in lower sample losses. For this reason, only PTFE filters are used in this method.

Complete conversion of reversibly bound sulfite to free sulfite is necessary for accurate quantitation. Previous studies in wine and apricot have shown that sulfites bind easily with

reducing sugars and carbonyls, such as acetaldehyde, galacturonic acid, and glucose.[2,24,25] All of these could potentially be present in the skin and flesh of dried fruits and vegetables. An 8 min sonication step was added in the extraction process to reduce or reverse this binding. The inclusion of this step increased the overall recovery yield in apricot samples. Because there was no indication that its addition would be detrimental to other food matrices, it was permanently added into the method. A heating step was included in the method to ensure that all sulfite–carbonyl adducts remaining after the SPE cleanup were converted to their free form.

On the basis of the large range of samples investigated, slight modifications to the sample preparation method described previously were necessary for a small number of matrices. Solid vegetable samples with low moisture content (i.e., dried potatoes or vegetable mix) formed a thick paste upon the initial addition of extracting solvent making analysis difficult. To avoid paste formation, samples were ground without extracting solvent into a homogeneous powder using the laboratory blender. After grinding, 5 g of the sample was accurately transferred into the 50 mL centrifuge tube, and 30 mL of 0.2% formaldehyde solution was added. The extraction, SPE cleanup, and heating steps were performed in the same manner as described for solid samples. For these matrices, a large amount of precipitate formed with the acetonitrile addition. It was determined that a better cleanup was achieved using a 0.45 μm PTFE filter.

All jam and preserve samples were best analyzed as solid samples. These samples have higher moisture content than the dried fruits and vegetables, so the extracting solvent volume was reduced to 15 mL for both extraction steps. Due to the high lipid content of coconut, after centrifugation three visible layers were present: solid coconut shavings in the bottom of the centrifuge tube, formaldehyde extracting solvent in the middle, and a lipid layer on top. The lipid top layer made pouring off the supernatant difficult. To improve this step, an empty 20 mL reservoir and frit (Agilent) were used to filter the extracting solvent. Additionally, the higher lipid content of the coconut samples necessitated a reduction in SPE extract volume to 1.5 mL. The heating and filtering steps remained the same as detailed previously for the other solid samples.

Two MS/MS transitions were identified for the HMS molecule, but there was some concern as to their validity because the transitions differed by only 1 mass unit. However, previous studies have reported that these two structurally significant product ions are commonly observed during mass spectrometric analysis of sulfonic acids.[26] The MS/MS product ion ratios ranged from 1 to 8% for all of the standards and samples analyzed. For regulatory analyses, all ratios must be within 10% absolute of the value, with a signal-to-noise ratio of >3:1. After several months of analysis with the same column, the peak shape started to degrade and the peaks became extremely broad (>1 min). The column was cleaned using the manufacturer's recommendations: 30 column volumes each of water, 0.5 M NaCl, and water followed by column equilibration. After washing, the peak shape and size returned to their original values.

Initially, a linear regression was used for the calibration curve, but linearity was maintained only over the range from 0.01 to 0.8 μg/mL Na_2SO_3, which equates to a range of 0.25–20

ppm of SO_2 in the food sample. Whereas the 10 ppm of SO_2 regulatory action concentration fits into this range well, there are several foods that commonly contain much more than the 20 ppm of SO_2, which could not be measured with the linear curve. It was found that a quadratic regression maintained a good fit ($r > 0.990$) over the range of 0.25–114 ppm of SO_2, so three additional curve points were added on the upper end. A $1/x^2$ weighting was included to ensure that the lower points on the curve received proper weighting because the curve spanned 3 orders of magnitude.

During analyses of liquid samples, a white powder was observed in the source and on the electrode. The sensitivity also decreased markedly, and the peak shape quickly deteriorated. An external valve was installed to divert the flow to waste when data collection was not necessary. The switching valve was used for all samples, and the white powder was not observed again. Throughout the analysis, the source and the curtain plate were cleaned approximately twice per month during times of heavy analysis.

METHOD VALIDATION

Twelve different matrices representing four commodity categories were selected to determine method accuracy. The four categories investigated included dried fruit (apricot, coconut, crystallized ginger, and pineapple), dried vegetables (dried potato, dried vegetable mix, and canned bamboo shoots), liquids/sweeteners (white grape juice, red wine vinegar, and molasses), and seafood (shrimp). Each unsulfited matrix was spiked prior to extraction in triplicate at five different SO_2 concentrations (0.5, 5, 10, 20, and 100 ppm of SO_2). Recoveries were determined and are reported in Table 2 along with the relative standard deviation (RSD) for the triplicate measurements. The target values for the percent recovery and the RSD were 80–115 and <16%, respectively. All recoveries in all matrices at all concentrations fell within the desired specifications. The only matrix that did not fall within the target RSD value was the 10 ppm of SO_2 spike with the dried potato. The dried potato and dried vegetable mix samples had among the lowest overall recoveries with 87–94 and 87– 92%, respectively. It is believed that these lower values are a result of sample binding, which could produce some irreversibly bound sulfite. The lowest and highest concentration spikes (0.5 and 100 ppm of SO_2) had the largest RSD values on average. At the lowest concentration, the spikes were close to the limit of quantitation for the method, affecting the repeatability. For the 100 ppm of SO_2 spikes, the points lay near the flat portion of the quadratic calibration curve, where small differences in instrument response cause a relatively large variation in the SO_2 value.

One matrix from each category was selected for a stability study. Both the SPE extract and the LC vials were reanalyzed at days 1, 3, and 7. The SPE extract and LC vials were stored at 4 °C and room temperature, respectively. No observable trends or significant differences ($p > 0.05$) were observed for the potatoes or the shrimp. The apricot samples stored in the refrigerator had no significant differences ($p > 0.05$) appear until day 7 of storage, but the samples stored in the autosampler (25 °C) had significant differences for days 1 and 7 ($p < 0.05$). For this reason if any reanalysis needs to be completed, it should be conducted from the cooled SPE extract stored in the refrigerator. The red wine vinegar had no significant differences ($p > 0.05$) except for day 1 with refrigerator storage. Due to these differences in

stability, further investigation into other matrices is necessary prior to any attempt to store samples for reanalysis.

Limit of detection (LOD) and limit of quantitation (LOQ) determinations were difficult due to the presence of a blank response in both the matrix and method blanks. The background concentration in the method blanks is due to the presence of sulfite in both the formaldehyde and labeled stable isotope (purity = 95%). Because this background concentration should be consistent for all samples, the standard curve corrects for the increased area. However, all of the samples investigated had an additional concentration of background sulfite. White grape juice had the smallest background with a mean area response of 80 ppb of SO_2. Figure 2 compares the response of a method blank and an apricot blank. An LOD could not be determined for any of the matrices analyzed because sulfite could be detected even in the blank analyses. Because the signal-to-noise ratio is >10 for any injected sample, the LOQ was calculated by analyzing seven blank samples for each matrix studied. The mean (μ_b) and standard deviation (σ_b) of these analyses were calculated, and the LOQ was determined using the following formula: $LOQ = \mu_b + 3.3\sigma_b$. The LOQ was determined for six different matrices to determine the expected range for common sulfite matrices. The matrices selected were white grape juice, molasses, dried potatoes, crystallized ginger, dried apricots, and frozen raw shrimp; results are shown in Table 3. Due to the variation in the mean response (0.08–0.59 ppm), the LOQ varied from 0.12 ppm in white grape juice to 0.75 ppm in dried apricots (Table 3), all of which are >2 orders of magnitude below the Monier–Williams method.

METHOD COMPARISON

Six of the validated matrices (dried pineapple, dried apricot, crystallized ginger, dried potatoes, white grape juice, and raw shrimp) were analyzed in triplicate by both the LC-MS/MS method and the MW distillation to determine differences between the two methods (see Table 4). Dried potato was the only matrix for which there were no significant differences ($p > 0.05$) between the methods. The dried fruit samples (pineapple, apricot, and ginger) tested produced higher values for the MW titration than the LC-MS/MS, whereas the other three matrices investigated produced lower values for the MW titration than the LC-MS/MS. The product labels listed sulfur dioxide as the sulfiting agent for the dried fruit samples, whereas bisulfites and metabisulfites were used in the other products. It is possible that the type of sulfiting agent, along with the physical properties of the food system, may have an effect on the quantitation via the two methods. Further investigation is necessary to determine the cause of this discrepancy.

The relative standard deviations of the analyses for both methods ranged from 1 to 8% for all matrices with the exception of shrimp. The shrimp RSD values were 11 and 28% for the MW and LC-MS/MS methods, respectively. The large variation in sulfite concentration in lots of shrimp may account for the differences observed between the methods. Hardisson et al.[27] investigated the sulfite concentration of frozen prawns from Spain and Venezuela and reported large ranges, values ranging from 12.8 to 546 ppm sulfite. Similar concentration variation was reported by Iammarino et al.[14] for shrimp samples collected over a four year timespan of 2009–2012, and the authors reported that due to the sulfite application process

used for shrimp, the residual amount of sulfite present can vary greatly. Therefore, the range found in shrimp in this comparison could be expected.

The LC-MS/MS method presented here can be used in determining sulfite concentrations in a wide range of food and beverage matrices, and the sensitivity limitations of other published sulfite methods have been eliminated. Sulfite concentrations between 0.5 and 1.0 ppm of SO_2 can be detected in most matrices. The sample preparation involves basic extraction techniques and allows for as many as 30 samples to be completed by a single analyst in a single day. This method will be a useful tool for food safety laboratories to monitor sulfite concentrations in foods to ensure compliance with U.S. regulations. Further investigation is needed with this method to determine the rate of false positives in those matrices that contain a high quantity of endogenous sulfur compounds such as *Allium* and *Brassica* vegetables.

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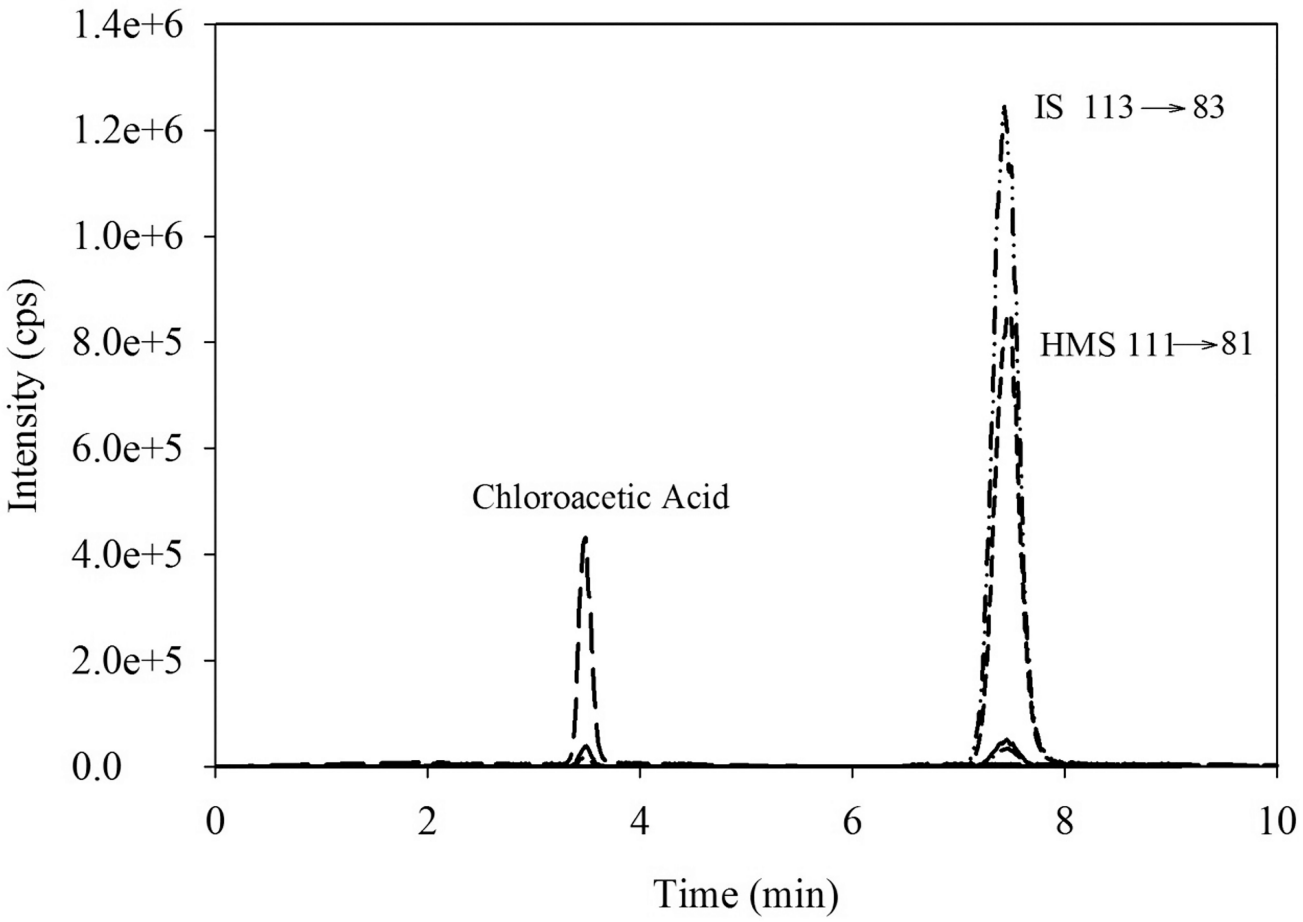

Figure 1.
Sample LC-MS/MS chromatogram for a 0.4 ppm of Na_2SO_3 standard with both the chloroacetic acid surrogate and stable isotope internal standard.

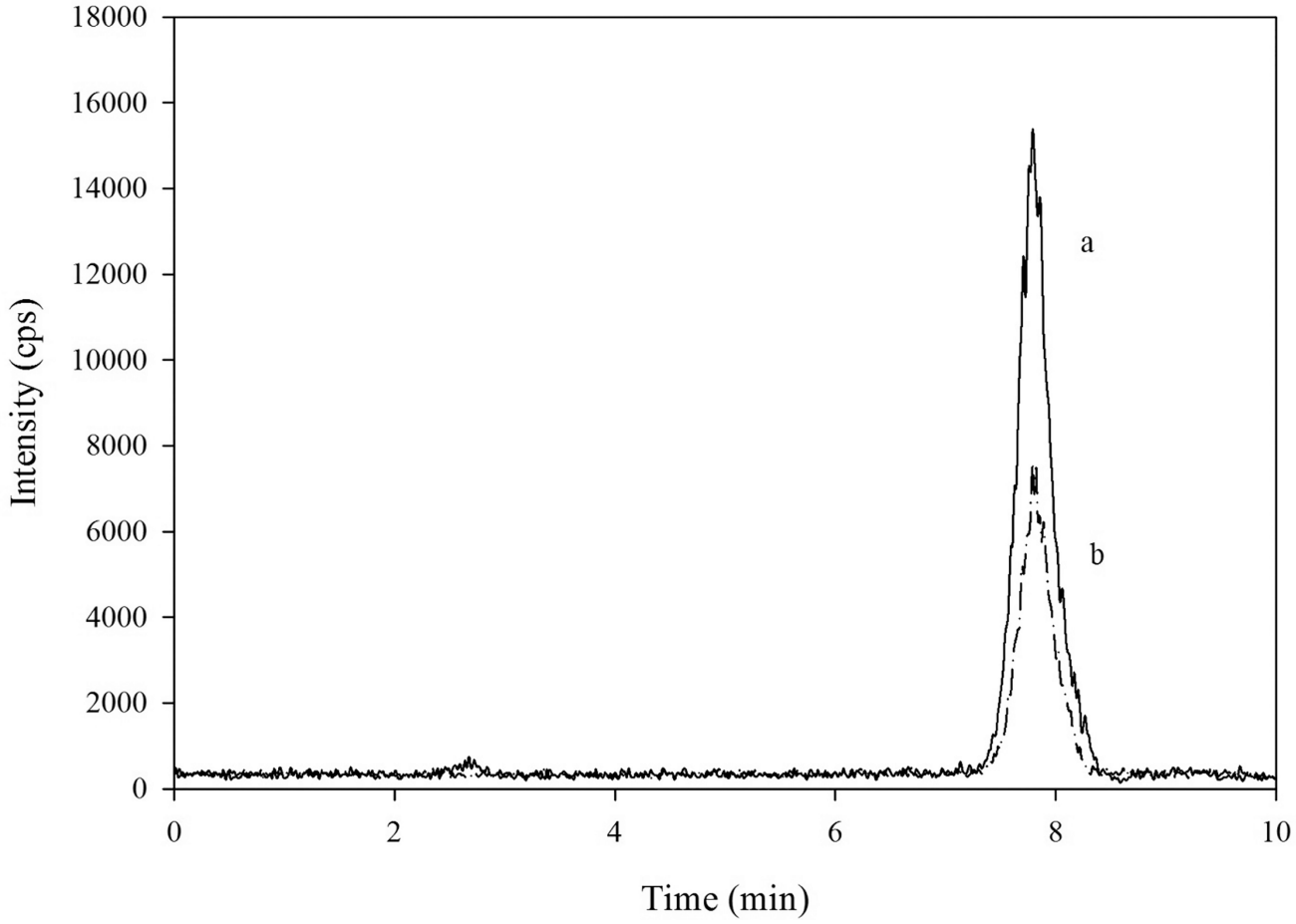

Figure 2.
LC-MS/MS HMS transition for (a) a blank apricot sample and (b) a solvent blank sample.

Table 1

MS/MS Conditions for Monitored Transitions

Q1 mass (*m/z*)	Q3 mass (*m/z*)	ID	dwell time (ms)	DP (V)	EP (V)	CE (V)	CXP (V)
111	81	HMS	170.0	−25.0	−4.00	−15.0	−6.00
111	80	HMS	550.0	−25.0	−4.00	−40.0	−6.00
113	83	HMS (^{34}S)	80.0	−25.0	−4.00	−15.0	−6.00
113	82	HMS (^{34}S)	80.0	−25.0	−4.00	−40.0	−6.00
93	35	chloroacetic acid	25.0	−40.0	−10.0	−12.0	−4.00
95	37	chloroacetic acid	25.0	−40.0	−10.0	−12.0	−4.00
93	93	chloroacetic acid	25.0	−40.0	−10.0	−1.00	−4.00

Table 2

Average Percent Recovery for Five Concentration Spikes of Different Food Matrices[a]

matrix	0.5 ppm of SO_2	5 ppm of SO_2	10 ppm of SO_2	20 ppm of SO_2	100 ppm of SO_2
dried apricot	93 (4)	97 (3)	96 (4)	98 (5)	102 (15)
dried coconut	92 (6)	93 (3)	86 (7)	91 (4)	87 (8)
crystallized ginger	96 (9)	95 (6)	103 (5)	99 (7)	109 (13)
dried pineapple	90 (11)	96 (4)	102 (8)	95 (10)	105 (10)
dried potato	88 (5)	87 (12)	89 (17)	94 (5)	93 (16)
dried vegetable mix	92 (11)	89 (5)	89 (7)	91 (7)	87 (13)
bamboo shoots	89 (10)	106 (9)	106 (7)	108 (5)	109 (16)
white grape juice	104 (6)	100 (8)	98 (7)	109 (16)	97 (7)
red wine vinegar	93 (9)	105 (11)	111 (6)	102 (4)	94 (3)
molasses	93 (15)	105 (1)	104 (15)	105 (4)	115 (8)
apricot jam	84 (15)	102 (4)	100 (4)	101 (3)	91 (6)
shrimp	99 (10)	103 (9)	105 (6)	104 (5)	99 (2)

[a] $n = 3$; % RSD is shown in parentheses.

Table 3

Limit of Quantitation (LOQ) in Six Food Matrices

food matrix	blank concentration[a] (ppm of SO_2)	LOQ[b] (ppm of SO_2)
white grape juice	0.08	0.12
molasses	0.36	0.67
dried potatoes	0.17	0.27
crystallized ginger	0.12	0.30
dried apricots	0.40	0.75
frozen raw shrimp	0.59	0.72

[a] Calculated concentration of seven method blanks in food matrix.

[b] LOQ was calculated using the following formula: $LOQ = \mu_b + 3.3\sigma_b$ where μ_b is the mean blank concentration for seven method blank replicates and σ_b is the standard deviation for these blanks.

Table 4

Comparison of the Monier–Williams Distillation Method and the LC-MS/MS Method for Sulfite Determination in Six Matrices

food matrix	Monier–Williams titration[a] (ppm of SO_2)	LC-MS/MS (ppm of SO_2)
dried pineapple	266 (4)	207 (8)
dried apricot	2596 (3)	2051 (4)
crystallized ginger	227 (8)	168 (4)
dried potatoes	58 (3)[b]	54 (6)
white grape juice	74 (1)	84 (4)
raw shrimp	50 (28)	94 (11)

[a] $n = 3$; % RSD is shown in parentheses.

[b] Dried potato is the only matrix with no significant differences between methods by a two-sample t test ($p > 0.05$).