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Note

Headspace liquid chromatographic technique for the determination of sulfite in food

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Sulfiting agents have long been used in the food and beverage industry. They have recently come under some criticism because of allergic reactions of sulfite in some individuals, particularly asthmatics. As a result, regulatory agencies in many countries have imposed restrictions on the use of sulfiting agents as well as limits on the concentration of sulfite in finished food products.

The concern over sulfiting agents has led to a re-examination of the analytical methodology for sulfite and, as a result, a number of papers have appeared in the recent literature. The most common methods in use to date involve distillation of sulfur dioxide from a highly acidified sample followed by either titration[1], colorimetric[2], polarographic[3] or ion chromatographic[4,5] determination. Direct colorimetric methods[6,7] are useful for certain food products. A flow-injection method using colorimetric detection has also been studied[8]. Several direct methods involving chromatography have been reported. Headspace gas chromatography[9,10] has been evaluated for a variety of foods. However, we found difficulty in obtaining satisfactory chromatograms using a variety of columns. Direct ion chromatography with electrochemical detection appears to be particularly suited to sulfite analysis. Several reports have appeared on the determination of sulfite directly[11] or as formaldehyde-stabilized adducts[4,5]. We have investigated this approach and found that by combining the headspace sample preparation technique of Hamano *et al.*[9] with an ion chromatographic separation technique we were able to determine low parts per million (ppm) levels of sulfite in both liquid and solid foods with no interferences from sample matrices. The approach is particularly novel considering that headspace sampling is not normally associated with liquid chromatography (LC).

EXPERIMENTAL

Reagents

All standards and reagent solutions were prepared with distilled water and degassed before use. A 10000-ppm (as sulfur dioxide) solution was made by dissolving 1.62 g sodium bisulphite in water in a 100-ml volumetric flask and diluting to volume. The acetaldehyde sodium bisulphite adduct was prepared by dissolving 1.62 g bisulphite in 8 ml of water in a 100-ml volumetric flask and adding 0.8 g (15% more than required) acetaldehyde slowly and allowing to stand for 1 h. The solution

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was made to volume, mixed and kept in the refrigerator for 4 h. This solution was used to prepare working solutions of the adduct. The sample extraction solution consisted of 0.04 *M* disodium monohydrogenphosphate containing 0.1 *M* D-mannitol[12] and 0.25% iron(II) sulfate (added fresh, daily) in water. The alkaline mannitol solution used to raise the pH of the sample extraction solution was 2.5 *M* sodium hydroxide in 0.1 *M* mannitol in water. The headspace trapping solution consisted of mobile phase without acetonitrile.

Instrumentation

The LC system consisted of a Beckman Model 112 solvent delivery module, a Model 420 controller and a Model 340 organiser with a 100-μl loop. A Bioanalytical Systems LC-4B amperometric detector with a glassy carbon electrode at 0.6 V and Ag/AgCl reference electrode was used for detection. A 150 × 4.1 mm I.D. Hamilton PRP-X100 anion chromatographic column was used for the separations. The mobile phase employed was 0.03 *M* methanesulphonic acid (made to pH 11 with sodium hydroxide) containing 5% (v/v) acetonitrile added as organic modifier. A flow of 2 ml/min was maintained, with sulfite eluting in about 7 min.

The headspace set-up (Supelco) consisted of 30 ml crimp-seal headspace vials, PTFE-silicone septa, tear away seals and a 5-ml pressure-lock gas syringe with a push-button valve and a side-port needle. The vials were placed in a glass water bath at room temperature on a magnetic stirring plate.

Sample analysis

For liquid food samples, a suitable aliquot was diluted with extraction solution to fill about nine-tenths of a 100-ml volumetric flask. The solution was brought to pH 11 by addition of alkaline mannitol solution, made up to the mark with extraction solution and mixed. In the case of solid food, 2–20 g of chopped sample was weighed into a 250-ml beaker and about 60 ml of extraction solution was added. The sample was homogenised (Polytron) for 30 s. The pH of the solution was adjusted to 11 as above, brought to a known 100 g weight with extraction solution and mixed.

A 3-ml (or 3-g) aliquot of well mixed sample slurry was carefully placed at the bottom of the headspace vial containing a small stirring magnet. The vial was sealed and 3 ml of air was removed with a plastic syringe and a 22-gauge needle. The vial was allowed to stand for 15 min after which time 3 ml of 50% phosphoric acid was added with a glass syringe. The vial was left in the water bath for 15 min with gentle magnetic stirring.

A 0.5-ml volume of trapping solution was pulled into the pressure-lock syringe, the push-button valve closed, and the needle replaced with a clean one. With the syringe in a vertical position and the valve open, 4.5 ml of headspace was withdrawn over a period of 20 s. The valve was closed and the syringe removed from the vial. The syringe was shaken by hand for 10 s, the contents emptied into a 1-ml glass vial and 100 μl injected into the LC system. Quantitation was done by carrying standards through the same procedure. The acetaldehyde–sulfite adduct was used for spiking purposes as well as for standards.

RESULTS AND DISCUSSION

The headspace approach to sampling for LC analysis of sulfite proved to be particularly simple and yet quite sensitive. The principle involves liberation of sulfite as sulfur dioxide from highly acidified samples, then collecting the gas in an alkaline trapping solution as SO_3^{2-}. The trapping solution is then analysed by LC. In almost all cases chromatograms of samples were as clean as those of the standards.

The final experimental conditions were obtained after many studies on optimizing times, temperatures and pH. The use of mannitol was found to be absolutely necessary to prevent sulfite oxidation due to molecular oxygen[11,12]. In addition we also used ferrous sulfate which helped inhibit the destruction of sulfite when added to samples for recovery studies. This addition was necessary at the basic pH required to liberate sulfite from complexes with aldehydes and sugars so that a total sulfite determination could be performed. Since a total sulfite result was desired we used the acetaldehyde–sulfite adduct as the standard to ensure that conditions were optimal for the liberation of reversibly bound sulfite. Free (unbound) sulfite could be determined directly from the acidified samples without first incubating with base.

Fig. 1 shows typical results for a standard of acetaldehyde–sulfite adduct and a wine sample. The wine was diluted five times before being analysed. Recovery of sulfite at 30 ppm spiked in the wine was 88%. Fig. 2 compares results of a spiked (6 ppm) and unspiked apple juice, both diluted twice before analysis. Recovery was calculated to be 94%. Fig. 3 shows the analysis of two raisin samples, one of which (golden raisins) had a label declaration indicating sulfite use. The sulfite can clearly be seen and was estimated to be 157 ppm in the golden raisins. Recoveries of sulfite from spiked red and white wines, potato flakes, raisins and apple juice were generally over 90% at levels between 6 and 200 ppm. Low recoveries (40%) were observed with shrimp samples spiked at 12 ppm. It appears that the added sulfite reacts with

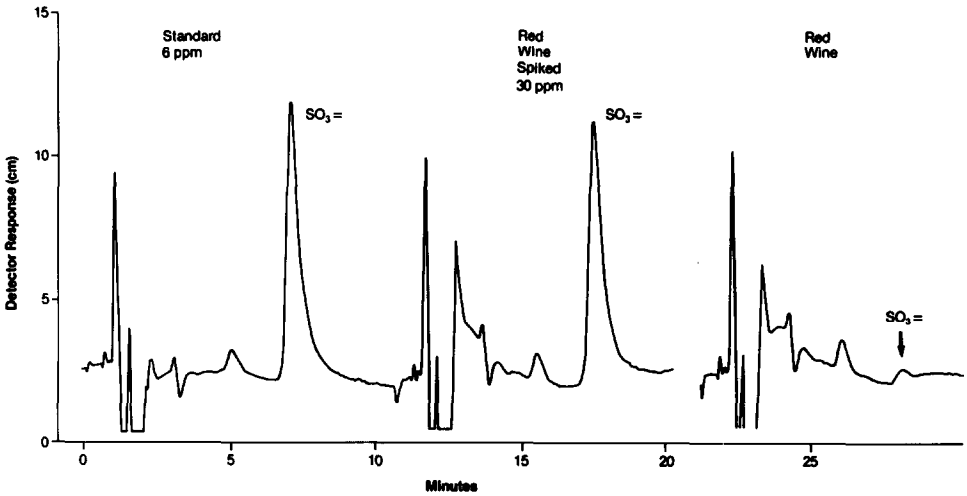

Fig. 1. Chromatograms of a sulfite standard (6 ppm), a spiked red wine (30 ppm) diluted 5 times and the same wine unspiked. 20 nA full scale.

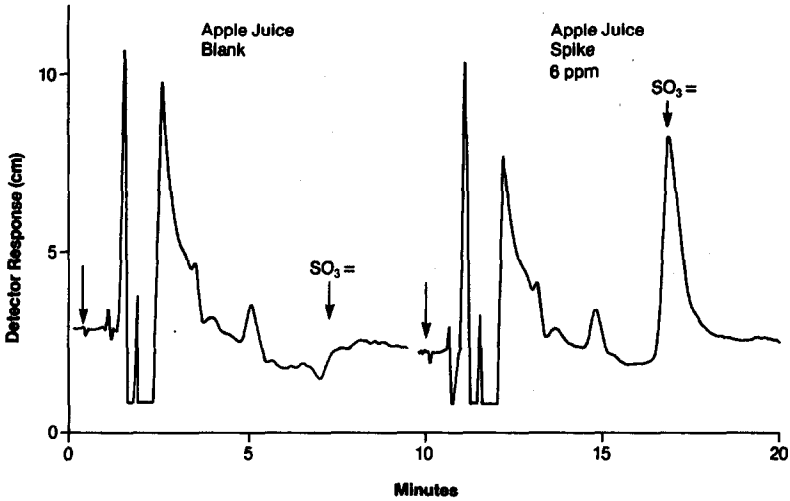

Fig. 2. Chromatograms of apple juice and spiked (6 ppm) apple juice, diluted twice. 20 nA full scale.

the matrix yielding the low values. Efforts to minimize this effect in shrimp are continuing.

The method was found to provide linear results from about 6 ppm up to 1000 ppm. The detection limit was about 0.5 ppm in the samples studied.

The chromatography column provided several months of consistent separations of sulfite which always exhibited rather broad peaks even when the column was

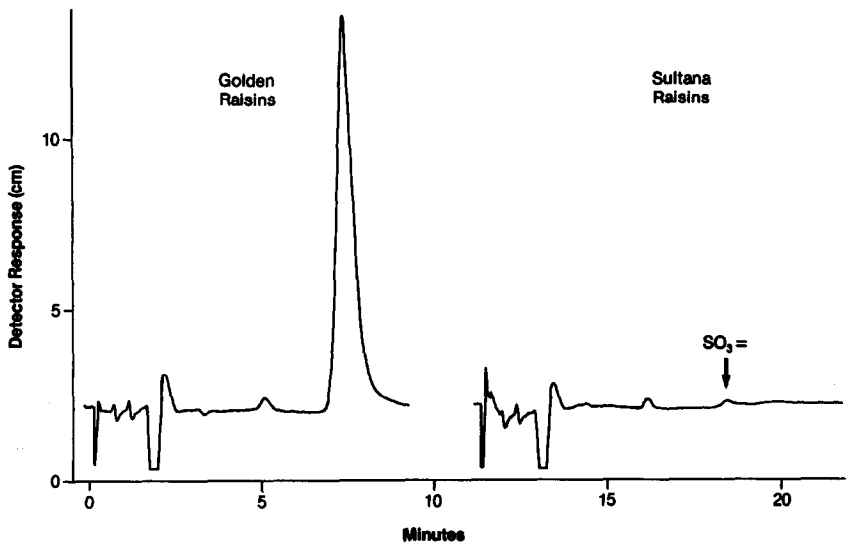

Fig. 3. Chromatograms of sulfited golden raisins, containing 157 ppm sulfite, and unsulfited sultana raisins 50 nA full scale.

new. However, this in no way affected the results. The retention times were very consistent varying by less than 2% with any given batch of mobile phase. Slight variations occurred between batches due to minor differences in methanesulfonate content and pH. The high pH oif the mobile phase was necessary to elute sulfite in a reasonable length of time. Lower pH values caused a significant increase in retention time.

The method described above has the potential to be used for the routine screening and quantitation of free and reversibly bound sulfite in foods. Further work on application of the technique to a variety of food products with direct comparison to the FDA-modified Monier-Williams[1] method is in progress. The headspace LC approach should be applicable to other volatile substances including many organics as well as carbon dioxide and ammonia.

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