

PESTICIDE AND INDUSTRIAL CHEMICAL RESIDUES

Determination of Sulfur Dioxide in Grapes: Comparison of the Monier-Williams Method and Two Ion Exclusion Chromatographic Methods

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Results for determination of sulfur dioxide in grapes were compared by 3 methods: the modified Monier-Williams method, acid distillation/ion exclusion chromatography with electrochemical detection (AD/IEC-EC), and alkali extraction/ion exclusion chromatography with electrochemical detection (AE/IEC-EC). An unusual positive response was observed during the later stage of the Monier-Williams distillation of both control grapes and sulfited grapes. Development of volatile acidic compounds in parallel with this Monier-Williams response and darkening of sample was also observed by collection in an alkali trap and analysis using anion exclusion chromatography and photodiode array detection. No parallel increase in sulfite was observed by the more selective AD/IEC-EC method, which clearly demonstrated that the response observed during the later stage of the Monier-Williams method is a false positive, probably due to caramelization reaction products. Monier-Williams results for grapes containing ca 10 ppm sulfite were in reasonably good agreement with those by either the AD/IEC-EC or AE/IEC-EC methods, presumably because the false positive response in the Monier-Williams analysis compensated for the somewhat incomplete recovery of sulfite. The AE/IEC-EC method is recommended because it is rapid, sensitive, straightforward, and free from interference. Accurate results by Monier-Williams analysis could be obtained by limiting distillation to 60 min and correcting for recovery.

Sulfur dioxide is used in grapes as a fungicide. Recently, the U.S. Environmental Protection Agency (EPA) established a pesticide tolerance limit of 10 ppm sulfur dioxide in grapes (1). EPA determined that sulfur dioxide in excess of 10 ppm would pose an unacceptable risk to sulfite-sensitive individuals. When grapes are fumigated with sulfur dioxide gas, a portion of the sulfur dioxide is bound irreversibly to the grape components, and the sum of the remaining free and reversibly bound sulfite (total sulfite) becomes the analyte of interest. Once sulfur dioxide reacts and binds with grape components, it is indistinguishable from sulfite derived from other sulfiting agents.

Even though a number of analytical methods have been reported for sulfite in wine over the years (2-6), determination of sulfite in grapes has not received much attention. Recently, Perfetti et al. (7) reported a liquid chromatographic (LC) method for sulfite in grapes and grape products based on a reverse-phase ion-pairing liquid chromatography and colorimetric detection technique of Warner et al. (8). They observed a generally good agreement between the LC and Monier-Williams methods.

A number of other analytical methods for sulfite in foods have also been published in recent years. The following 4

methods received official first action approval from AOAC: the differential pulse polarographic method (9), the FDA-optimized Monier-Williams method (10, 11), the flow injection method (12, 13), and the ion exclusion chromatography-electrochemical detection method (14-16). The polarographic method and the Monier-Williams method require acid distillation for release of reversibly bound sulfite. The flow injection and ion exclusion chromatographic methods use alkali treatment for release of the bound sulfite. Recently, Holak and Specchio (17) improved the polarographic method by replacing acid distillation with alkali treatment.

Other LC methods include ion chromatography with conductivity detection (18-20) and headspace techniques (21). Recently, Lawrence et al. (22) compared 3 LC methods that use neutral/alkaline extraction and electrochemical (amperometric) detection with the optimized Monier-Williams method and reported good agreement among the 4 methods with a few exceptions. The advantage of the rapid alkali extraction method over the lengthy distillation method is obvious. Kim (23) compared the efficacy of alkali extraction with that of acid distillation in several typical food systems. Alkali extraction is milder than acid distillation. Therefore, sulfite strongly bound to dark pigments or naturally occurring in allium and brassica vegetables is not released by alkali. Except for these 2 cases, a good agreement is usually found unless interference associated with acid distillation leads to overestimation by the Monier-Williams method (7, 15, 23).

While performing Monier-Williams analysis on grapes purchased from a local supermarket, we observed no color change in the trap during the first 60 min and some change after 60 min, which continued to increase during the 105 min distillation. This observation was inconsistent with the known behavior of sulfite in foods. The release of most reversibly bound sulfite in foods takes place during the first 15 min of distillation, which is the basis of many flash distillation methods (9, 18, 19, 24-30). Moreover, more than half the sulfite is released from the strong formaldehyde-sulfite adduct during the first 60 min (31). The same is true with the sulfite occurring naturally in allium and brassica vegetables (23). These considerations suggested that the Monier-Williams response from grapes beyond 60 min might represent some interference that could affect the accuracy of results. The present paper (a) investigates the nature and extent of this Monier-Williams response so that a modification can be suggested to avoid interference and (b) demonstrates the advantages of the rapid AE/IEC-EC method for accurate determination of sulfite in grapes.

Experimental

Reagents

(a) *Hydrochloric acid*.—4N. Dilute AR grade HCl with deionized water.

(b) *Hydrogen peroxide solution*.—3%. Dilute 30% hydrogen peroxide 10-fold with deionized water.

(c) *NaOH solution*.—0.002N. Make fresh daily and standardize with 10 mM potassium biphthalate solution.

(d) *Alkali trapping solution*.—Prepare 0.1M Na_2HPO_4 , 0.05M D-mannitol solution (pH 9.4) in deionized water and degas under vacuum.

(e) *pH 9 buffer*.—Prepare 20 mM Na_2HPO_4 , 10 mM D-mannitol solution in deionized water and degas.

(f) *Sulfite standard solution*.—*Stock solution*.—1000 ppm SO_2 . Dissolve 196.7 mg sodium sulfite (Na_2SO_3) in 100 mL pH 9 buffer. *Working solution*.—Dilute stock solution with pH 9 buffer as needed.

Sample Preparation

California red seedless grapes and green seedless grapes (both Thompson variety) were purchased from a local supermarket. The grapes with skin were cut into ca 0.8 cm dimension. For control experiments, cut pieces were subjected to analysis of sulfite without further treatment. For experiments with sulfited grapes, a 120 g portion of cut grapes was soaked in 200 mL of 200 ppm SO_2 solution in water with occasional stirring. After 10 min, the solution was decanted and the grapes were rinsed 3 times with deionized water (to remove excess sulfite on the surface) and drained. Both red and green grapes were treated similarly. Sulfite in the grapes was analyzed immediately by the 3 different methods.

Monier-Williams Method

A 50 g aliquot of cut grapes (control or sulfited) was distilled with 200 mL deionized water and 45 mL 4N HCl in a 500 mL flask according to the optimized Monier-Williams method (10). Timing was started when a steady reflux was obtained. Condenser temperature was maintained at 5°C using a circulating bath (Forma Scientific, Model 2095, Marietta, OH). Nitrogen flow was maintained at 200 mL/min. For the kinetic study, 30 mL trapping solution (3% hydrogen peroxide, titrated to yellow end point with methyl red indicator) was replaced after 15 min distillation by fresh trapping solution if color change was observed. If there was no color change, the distillation was continued for another 15 min. Each 30 mL trapping solution was titrated with 0.002N, instead of 0.01N, NaOH solution for increased sensitivity. The cumulative result from these 15 min intervals was reported for different distillation times.

Ion Chromatography

Sulfite in the alkali extract or alkali trap was separated by anion exclusion chromatography and detected amperometrically (14, 15). For most experiments, Wescan ion chromatography system (Alltech/Wescan Instruments, Deerfield, IL) was used with Ion-Guard anion exclusion cartridge, anion exclusion/HS column (4.6 × 100 mm) or sulfite analysis column (7.8 × 100 mm), 20 μL Rheodyne injection loop, Model 271 electrochemical detector with Pt working electrode, and computing integrator (Spectra-Physics 4270, Spectra-Physics, San Jose, CA). The packing material for the column is sulfonated polystyrene/divinylbenzene copolymer, which is ideal for separation of weak acids. A 20 mM sulfuric acid solution was pumped at 0.8 mL/min flow rate. Detector voltage was set at +0.6 V vs Ag/AgCl reference electrode. A Waters Model 460 electrochemical detector with glassy carbon electrode was also used. A higher detector

voltage of +0.8 V vs Ag/AgCl reference electrode was used for the glassy carbon electrode.

AD/IEC-EC Method

In the AD/IEC-EC method, the efficiency of acid distillation for release of sulfite is combined with the sensitivity and specificity of the IEC-EC system for determination of sulfite. To determine recovery of authentic sulfite by the AD/IEC-EC method, a 1 mL pH 9 buffer containing 0 to 300 μg SO_2 was added to 500 mL distillation flask and distillation was performed in the same manner as the Monier-Williams method except that nitrogen flow rate was increased to 400 mL/min and 30 mL alkali trapping solution (0.1M Na_2HPO_4 , 0.05M D-mannitol solution) was used instead of hydrogen peroxide solution. After 105 min, the trapping solution was diluted as necessary and injected into the ion chromatography system. The sulfite peak from the trap was compared with 0.5 ppm standard solution. Each measurement was performed in duplicate. The slope obtained by linear regression analysis of recovery data was used as a correction factor for results from grapes.

Sulfite in a 30 g aliquot of cut grapes (control or sulfited) was determined in the same manner. The trapping solution was injected into the ion chromatography system when a steady reflux was obtained and at 15 min intervals thereafter either directly (control grape) or after 20-fold dilution (0.1 mL added to 1.9 mL fresh trapping solution) (sulfited grape). The sulfite peak was compared with the 0.5 ppm standard. When 30 g grape sample and 30 mL trapping solution are used, the sulfite concentration observed from the trap for control grapes corresponds directly to the sulfite concentration in the sample after correction for recovery. Result was multiplied by dilution factor of 20 for sulfited grapes.

AE/IEC-EC Method

For determination of total sulfite by the alkali extraction method, a 5 g aliquot of control grapes was homogenized with 20 mL pH 9 buffer for 1 min using Polytron® (Brinkmann Instruments, Westbury, NY). For sulfited grapes, a 2 g aliquot was extracted with 48 mL pH 9 buffer. The delay between sulfite treatment and analysis was reduced to the minimum. The extract was filtered through a 0.45 μM membrane filter and injected immediately into the chromatograph. The sulfite peak was compared with a 0.4 ppm standard. Details of the method were published (14, 15). For free sulfite, 5 g grape sample was homogenized with 20 mL of 20 mM sulfuric acid solution for 5 s immediately after sulfite treatment (14). Longer extraction leads to a loss of free sulfite.

Photodiode Array Detection of Interfering Compounds

The alkali trapping solution in the AD/IEC-EC method was analyzed for volatile acidic compounds using a Wescan anion exclusion column (7.8 × 100 mm) and Waters Model 990 photodiode array detector. The eluant was a 20 mM sulfuric acid solution and the flow rate was 1 mL/min. The trapping solution was injected directly at 15 min intervals after steady reflux started. Spectral data were obtained by the photodiode array detector in the UV range, from 190 to 340 nm, in 2 s intervals as the compounds eluted from the chromatographic column. Contour diagram was used to identify peaks in the spectrochromatogram. Post-run chro-

matographic analysis or spectral analysis was used to quantitate the eluting compounds.

Results and Discussion

Grapes are usually fumigated with 0.5% sulfur dioxide gas for 20–30 min. According to Peiser and Yang (32), over 90% of the initial sulfite in grapes is oxidized to sulfate after 72 h. Therefore, if initial sulfite level is below the 10 ppm tolerance limit, sulfite concentration in grapes will, under most circumstances, be well below 10 ppm by the time the grapes reach consumers. Nevertheless, it is necessary to be able to accurately determine sulfite in grapes at the 10 ppm level for regulatory purposes. Some of the added sulfite will bind irreversibly with grape components and not be determined, which is why sulfite added to grapes cannot be fully recovered (7). We were only concerned with accurate determination, at the 10 ppm tolerance limit, of residual sulfite, which is the sum of free and reversibly bound sulfite. To simulate this situation, grapes were soaked in a solution containing enough sodium sulfite to yield an initial concentration of ca 10 ppm in the grapes. Ideally, fumigation with sulfur dioxide gas should be used. However, because the chemistry of binding and release of sulfite should be the same irrespective of the source, it was hoped that treating grapes with sulfite solution was acceptable for the purpose of this study. Perfetti et al. (7) reported difficulty preparing grapes with 10 ppm sulfite using fumigation.

Control Grapes

During a typical Monier-Williams analysis of control grapes, no color change was observed in the trap up to 60 min. However, a slight color change started after 60 min and the intensity of the color increased during the 105 min distillation. This behavior was not consistent with rapid release of bound sulfite upon acid distillation and appeared to be due to interference. The apparent sulfite response after the Monier-Williams distillation time of 105 min varied from ca 1 ppm to several ppm. This response is much less than the 10 ppm tolerance limit. However, we felt that, if it represents a false positive response, uncertainty associated with it could seriously affect the accuracy of the results for grapes containing ca 10 ppm sulfite.

To confirm the observation by extrapolation and investigate the nature of this response, distillation time was extended to 3 h. Monier-Williams responses during 15 min periods, obtained by replacing the hydrogen peroxide solution in the trap every 15 min and titrating the trap with 0.002N NaOH solution, were accumulated up to the different distillation times. As shown in Figure 1 for control red grape, no Monier-Williams response was observed during the first 60 min, but the response started after 60 min and continued to grow. For this particular grape, the cumulative response was 0.8 ppm after 105 min and 5.4 ppm after 3 h.

To verify whether this response was due to sulfite, the Monier-Williams distillation was repeated with the same control grape sample using alkali buffer in the trap, instead of the hydrogen peroxide solution, in order to collect sulfur dioxide and other volatile acidic compounds that might be produced upon distillation. Determination of sulfite in the trap by selective IEC-EC method would then yield the true amount of sulfite that could be released by acid distillation.

Overall recovery of this AD/IEC-EC method was established beforehand as shown in Figure 2. The 300 $\mu\text{g SO}_2$

added corresponds to the amount of sulfite present in a 30 g grape sample at the 10 ppm concentration. A linear regression analysis gave a straight line with a slope of 0.947 and an intercept of -5.2 . The average recovery of 94.7% was considered satisfactory and subsequent results obtained by the AD/IEC-EC method were divided by 0.947 for correction. Recovery was slightly higher when a nitrogen flow rate of 400 mL/min was used instead of the 200 mL/min recommended in the Monier-Williams method. Warner et al. (31) reported 85–90% recovery of sodium sulfite by the Monier-Williams method.

When the AD/IEC-EC method was used for the same control grape sample as the Monier-Williams method, a small peak of sulfite <0.1 ppm was observed in the trapping solution when the solution was directly injected every 15 min up to 105 min, and the amount of sulfite in the trap did not increase even after 3 h distillation (Figure 1, top). Therefore, true residual sulfite in the control grape was estimated to be <0.1 ppm. Observation of increasing amounts of sulfite in the trap, in parallel with the Monier-Williams response, would be expected if the Monier-Williams response is due to sulfite strongly bound to certain components of the grape and released only after prolonged distillation. Therefore, it appears that the positive response beyond 60 min in the Monier-Williams analysis is due to interfering compounds.

By the time the Monier-Williams response was observed, the sample in the flask turned dark and became darker as distillation continued. It was reminiscent of the caramelization reaction of erythorbic acid that generated volatile acidic compounds (23). Because such acidic compounds would be trapped in alkali, the alkali trapping solution was analyzed using an anion exclusion column, which is widely used for separating organic acids such as ascorbic acid and acetic acid. Photodiode array detection was used to rapidly detect all UV-absorbing compounds. Figure 3 shows a typical contour diagram corresponding to a 3-dimensional spectrochromatogram (retention time–wavelength–absorbance) obtained from the alkali trap after 105 min distillation of 50 g control grape. Several compounds were observed with retention times of 6.5, 7.8, 9.1, and 12.3 min and with characteristic UV absorption spectra. These compounds were not present in the trap initially. However, they accumulated significantly in the trap after 105 min and continued to increase up to 3 h in parallel with the Monier-Williams response. UV absorbances at 280 nm for the major compound with 12.3 min retention time were 0.13 and 0.33 after 105 min and 3 h distillation, respectively. Identification of these compounds was not attempted. However, the fact that they are separated by the organic acid column and increase with the Monier-Williams response (titrated with alkali) strongly suggests that the Monier-Williams response beyond 60 min is a false positive response due to volatile organic acids produced by caramelization reaction. The interference was not prevented by the low condenser temperature of 5°C. The observation is also consistent with reported production of volatile acids from carbohydrates, such as erythorbic acid, upon distillation (23).

When the sulfite in the same control grape sample was determined by the AE/IEC-EC method using 4-fold excess of the pH 9 buffer for extraction, the sulfite peak observed by the IEC-EC method was <0.02 ppm, which is equivalent to 0.1 ppm in grapes (indicated by an arrow in Figure 1, top). This result is consistent with the AD/IEC-EC result and

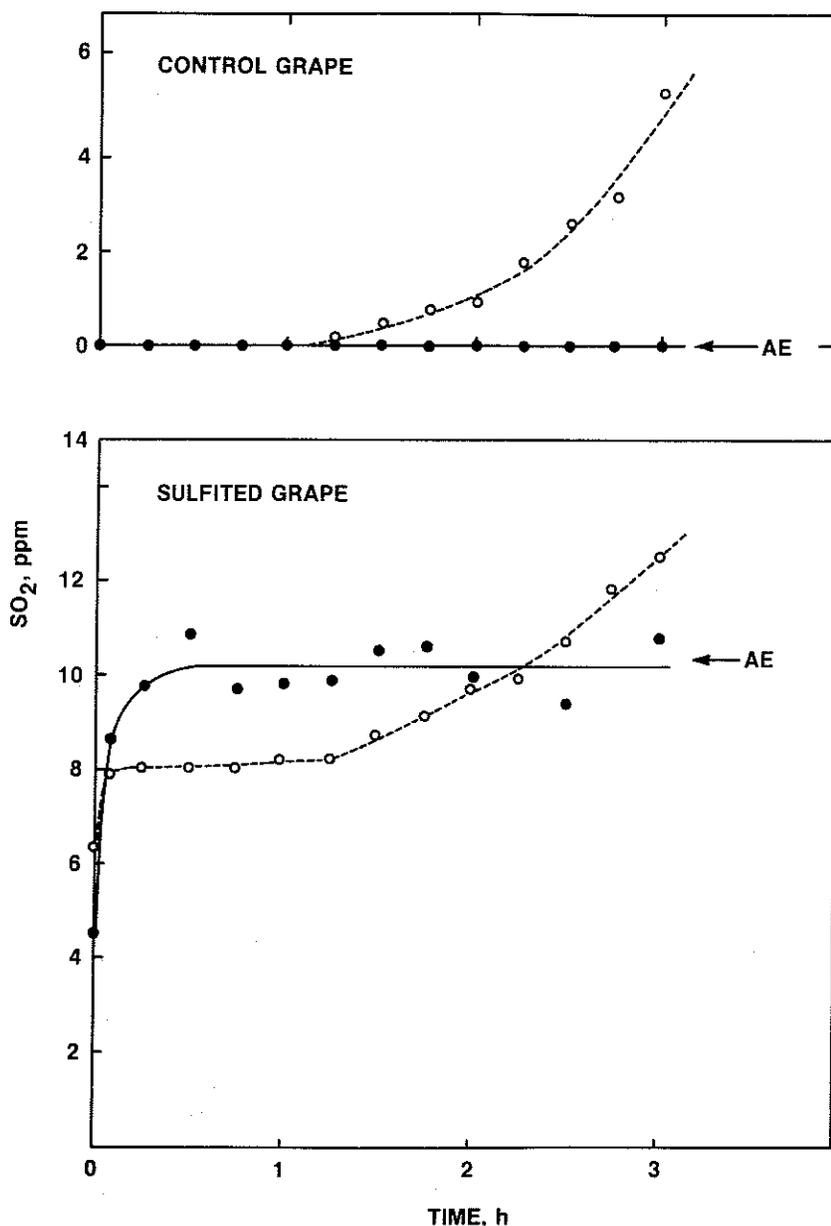


Figure 1. Sulfur dioxide response from control grapes and sulfited grapes determined by the Monier-Williams method (—○—○—) and the AD/IEC-EC method (—●—●—) at different distillation times. Results by the AE/IEC-EC method from the same samples are indicated by an arrow.

suggests that the rapid AE/IEC-EC method is not subject to interference and could be used for determination of residual sulfite in grapes present at low concentrations as shown below.

Sulfited Grapes

Preliminary experiments indicated that cut grapes treated with 200 ppm SO_2 solution consistently yield an initial sulfite concentration around 10 ppm for both red and green grapes. Figure 1, bottom, shows determination by 3 methods of sulfite from red grapes, used for control grape experiments, treated with sulfite. Monier-Williams results represent a cumulative response from 15 min intervals up to the various distillation times. A rapid color change was observed in the trap by Monier-Williams analysis. By the time a steady reflux was obtained (time 0 in Figure 1, bottom), the color change corresponded to 6.3 ppm SO_2 , which represents free sulfite plus some reversibly bound sulfite. Another 1.5 ppm

was released during the next 5 min and there was almost no further increase up to 45 min. The cumulative response after 60 min distillation was 8.2 ppm. If a correction is made for the reported 85–90% recovery (31), the result becomes 9.4 ppm. After 60 min, a gradual increase in the cumulative response was obtained in the same manner as from the control grape. The cumulative response after 105 min distillation without correction was 9.1 ppm and it continued to reach 12.5 ppm after 3 h distillation.

When the sulfited red grapes were subjected to AD/IEC-EC analysis, 4.5 ppm SO_2 (corrected for 94.7% recovery, Figure 2) was observed initially. The free sulfite measured by acid extraction/IEC-EC (14) was 2.2 ppm. The amount of sulfite in the alkali trap quickly increased to 9.6 ppm after 15 min distillation and remained at the average value of 10.2 ppm up to 3 h (Figure 1, bottom). Sulfite in the trap was determined at 15 min intervals during a 3 h distillation, and the data in Figure 1 for each distillation time represent

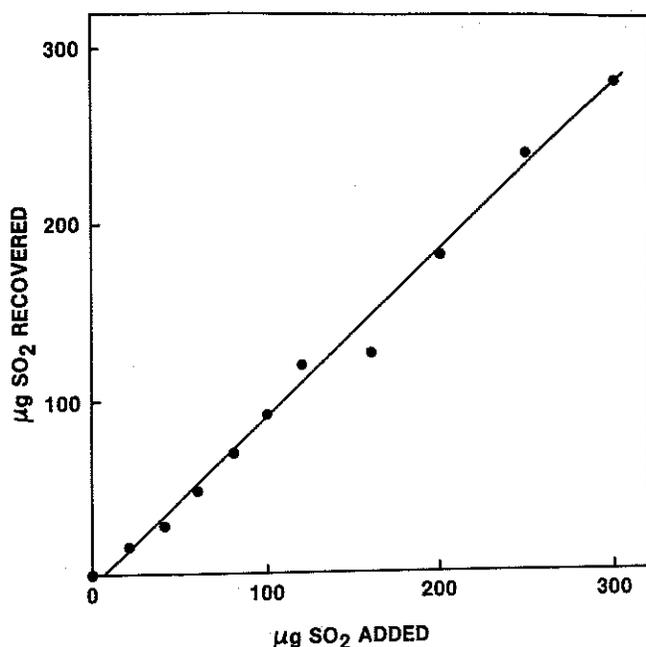


Figure 2. Recovery of authentic sulfite by AD/IEC-EC method. Known amount of sodium sulfite solution was subjected to Monier-Williams distillation and sulfur dioxide trapped in alkali buffer was determined by the IEC-EC method.

average results from duplicate distillations. Unlike Monier-Williams analysis, replacement of the trapping solution was unnecessary because only a small volume was withdrawn for analysis. The steady value obtained by the AD/IEC-EC method was in good agreement with the corrected Monier-Williams result after 60 min distillation (9.4 ppm). This observation clearly indicates that all reversibly bound sulfite is rapidly released by acid distillation and determined accurately by the IEC-EC method. Thus, the AD/IEC-EC method is an extremely sensitive and accurate method for sulfite if acid distillation is preferred for release of sulfite.

The observed discrepancy beyond 60 min between the

Monier-Williams and AD/IEC-EC methods again indicates that the Monier-Williams response beyond 60 min is due to interference. The spectrochromatogram obtained from the alkali trap for sulfited grapes after 105 min distillation was essentially identical with that for the control grapes (Figure 3). It is interesting to note that the uncorrected Monier-Williams result after 105 min distillation, 9.1 ppm, is slightly lower than (but in fairly good agreement with) the true concentration obtained by the AD/IEC-EC method, 10.2 ppm. It appears that incomplete recovery of the Monier-Williams method was compensated for by the false positive response. However, it is unlikely that the uncorrected Monier-Williams results will always be acceptable because the false positive response may vary.

When the rapid AE/IEC-EC method was used, 10.4 ppm was obtained from the same sulfited grapes in good agreement with both the Monier-Williams and AD/IEC-EC methods (see arrow in Figure 1, bottom). This result demonstrates that sulfite in grapes present at the 10 ppm level can be rapidly extracted with alkali and accurately determined by the IEC-EC method. The efficacy of alkali for release of the reversibly bound sulfite was suggested by Monier-Williams himself (33) and recently verified in a number of food matrices (12-17, 21, 22). Sulfite bound to dark pigments or naturally occurring was noted as an exception (23). Because there is no dark pigment in grapes except for the skin of red grapes, sulfite reversibly bound to the components of the grapes was expected to be released by alkali extraction as efficiently as in acid distillation.

It should be pointed out that no correction for recovery is needed for the alkali extraction method, because there is no distillation and trapping involved, which cause incomplete recovery in the Monier-Williams method or the AD/IEC-EC method. Thus, the AE/IEC-EC method is truly rapid and straightforward as well as free from interference. Unlike the Monier-Williams method or the AD/IEC-EC method, in which a distillation set up is needed for each sample, a continuous analysis of multiple samples is feasible by the AE/IEC-EC method. After an extract is injected into the chromatograph, the next sample can be prepared using the same ho-

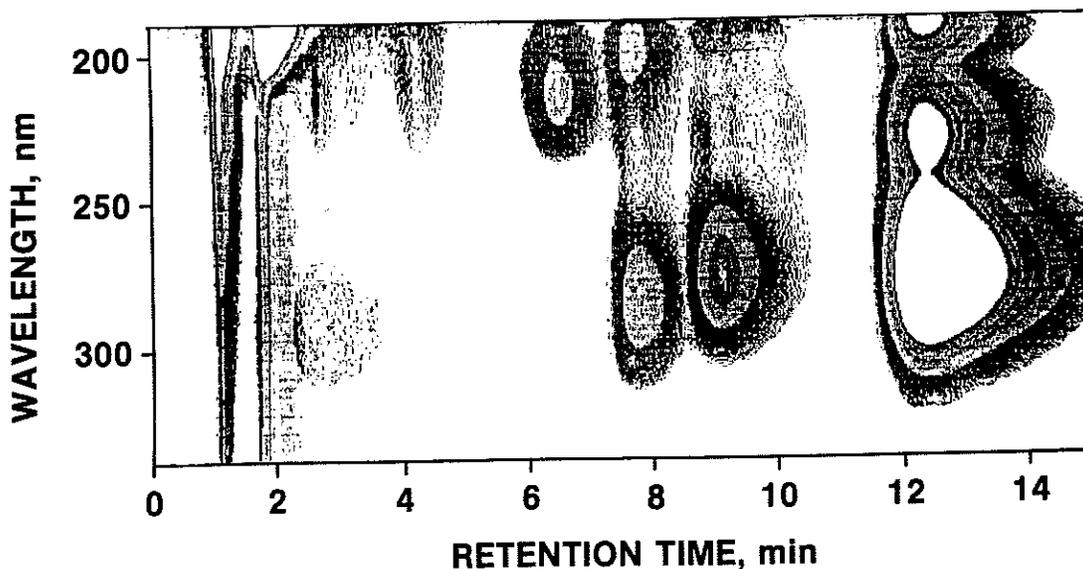


Figure 3. Contour diagram corresponding to a 3-dimensional spectrochromatogram obtained by injecting alkali trapping solution for control grapes after 105 min distillation into the anion exclusion chromatography/photodiode array detection system. The third axis coming out of the plane corresponds to absorbance.

Table 1. Sulfite in grapes by the AE/IEC-EC and Monier-Williams methods

Sample	SO ₂ , ppm	
	AE/IEC-EC ^a	Monier-Williams ^b
Red grape		
Control	<0.2	4.4 (1.8)
Sulfited	12.5 (4.2)	12.2 (2.4)
Green grape		
Control	<0.2	2.9 (0.8)
Sulfited	11.9 (3.8)	11.5 (2.9)

^a Av. of 6 detn. Std dev. in parenthesis.

^b Av. of 4 detn. Std dev. in parenthesis. 105 min distillation. Uncorrected for recovery.

mogenizer while the chromatographic analysis is underway. The typical analysis time is ca 15 min/sample. These considerations strongly support the notion that, even though accurate determination of sulfite in grapes is possible by both the AD/IEC-EC and AE/IEC-EC methods, the official AE/IEC-EC method is the preferred method for routine monitoring of multiple samples.

Variations

To evaluate variation of the false positive response between different lots, the same kind of California red grapes were purchased a week later from the same store and the Monier-Williams analysis (105 min) was performed. The average of 4 determinations from control red grapes was 4.4 ppm, and the standard deviation was 1.8 ppm (Table 1). This result is higher than the 0.8 ppm shown in Figure 1, which might be due to a higher concentration of components responsible for the caramelization reaction or other experimental variables. The average response was 2.9 ppm for green grapes. A similar result was also obtained from seeded red grapes. Overall typical response is considered to be a few ppm.

When the same red and green grapes were analyzed by the AE/IEC-EC method, <0.2 ppm sulfite was observed from both grapes (Table 1). Several different batches of red and green grapes purchased at different stores at different times were also analyzed by the AE/IEC-EC method to determine variation of residual sulfite when grapes reach the consumer. Typically, <0.2 ppm sulfite was observed. Sulfite in excess of 1 ppm has not been detected in any grapes tested. Presumably, the initial sulfite level immediately after fumigation was much higher, but residual sulfite decreased during transportation and distribution.

Results in Table 1 also show that a generally good agreement is obtained, as discussed above, between the AE/IEC-EC method and the Monier-Williams method without correction for treated grapes containing about 12 ppm sulfite. The large standard deviations are probably due to the non-uniform distribution of sulfite throughout grape samples.

Another potential source of variation in the experimental results is loss of sulfite in the extract due to its binding to various components of the grape. When sulfite concentration in the extract was ca 0.4 ppm (10 ppm diluted 25-fold), an approximately 17% decrease was observed 20 min after the initial injection, which was done immediately after extraction and filtration. A decrease of ca 50% was observed after 2 h. Therefore, it is important to inject the extract without delay to avoid errors. On the other hand, because about one-half the sulfite remains after 2 h, one could be assured that

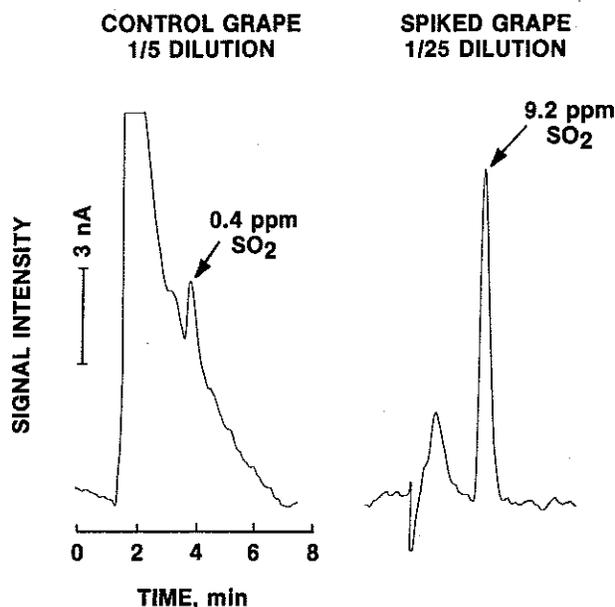


Figure 4. Chromatograms corresponding to 0.4 ppm and 9.2 ppm SO₂ in grapes. Sulfite from control and sulfited grapes was extracted with 4- and 24-fold excess alkali buffer, respectively, and determined by the IEC-EC method.

sulfite concentration in the grapes is below 10 ppm if the result after 2 h shows much less than 5 ppm.

Sensitivity

The extremely high sensitivity of amperometric detection is well known. The chromatogram on the left in Figure 4 shows a sulfite peak of 0.08 ppm in the extract obtained by homogenizing control grapes with 4-fold excess alkali buffer. The Wescan anion exclusion column with a larger diameter (7.8 × 100 mm) and the Waters 460 electrochemical detector with glassy carbon electrode was used. The signal intensity of the peak corresponds to 0.4 ppm sulfite in the grape. The high signal-to-noise ratio of this peak indicates that sulfite in grapes at concentrations well below 10 ppm can be readily detected on the trailing edge of a compound eluting ahead of sulfite.

For routine monitoring of grapes for the 10 ppm tolerance limit, sulfite in the extract after 25-fold dilution can be compared with the 0.4 ppm standard sulfite similar to the 0.37 ppm sulfite peak (9.2 ppm in grapes) shown in Figure 4. The earlier peak does not interfere with sulfite at the higher dilution. Both Wescan and Waters detectors were satisfactory for detecting 10 ppm sulfite in grapes, but the Waters detector equipped with a noise filter was preferred at lower concentrations.

A lower detection limit can be achieved by the AD/IEC-EC method because sulfite in a large amount of sample can be collected in the same weight of trapping solution with no net dilution of sulfite, whereas some dilution is inevitable in the extraction method. However, the extremely high sensitivity of amperometric detection enables determination of <1 ppm sulfite in grapes easily by the AE/IEC-EC method.

Conclusion

Selective determination of sulfite by the IEC-EC method in combination with acid distillation demonstrates that the Monier-Williams response beyond 60 min is a false positive. A good agreement was obtained among the Monier-Williams

method with limited distillation and correction for recovery, the AD/IEC-EC method with correction, and the AE/IEC-EC method. The rapid AE/IEC-EC method is the method of choice. Alternatively, one could carry out the Monier-Williams analysis for 60 min to avoid a false positive response and correct for incomplete recovery.

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