Technical paper

Determination of Free and Total Sulfite in Red Globe Grape by Ion Chromatography

Jiangli LIN¹, Yajuan ZHU¹, Wenhui CHENG¹, Jinxia WANG¹, Bin WU² and Jide WANG^{1*}

¹Key Laboratory of Oil & Gas Fine Chemicals, Ministry of Education & Xinjiang Uyghur Autonomous Region, College of Chemistry and Chemical Engineering, Xinjiang University, Urumqi, Xinjiang 830046, China

²Farm Product Storage and Freshening Institute, Xinjiang Academy of agricultural sciences, Urumqi 830091, China

Received January 24, 2014 ; Accepted May 18, 2014

Sulfiting agents have been used wide-spread for prolonging shelf life of berries, excessive of sulfur dioxide would cause food allergies to humans. Conventional methods mainly focus on the determination of free or total sulfite in liquid samples, and are not suitable to analyze berries with low level of sulfite. Here formaldehyde method and ion chromatography were coupled to determine the residues of the free and total sulfites in Red Globe grape. Both standard calibration curves showed good correlation between anion peak area and concentration. To free and total sulfite, limits of detection were 0.002 and 0.05 mg/L and recoveries ranged from 88 – 93% and 87 – 98%, respectively. Relative standard deviation values of repeatability and intraday precision for standard anions with the same sample were less than 3%. The method developed was well applicable to be used to determine free and total sulfite in barriers, especially to those with color.

Keywords: red globe grape, free sulfite, total sulfite, formaldehyde, ion chromatography

Introduction

Addition of sulfites is a practice widely used in the processing of storage foods like grapes, dried fruits, fruit juices and wine because of their effects in improving the appearance and flavour of food during the process of preparation, storage and distribution by stabilizing the product color and inhibiting discoloration (Mcfeeters and Barish, 2003; Falcone and Maxwell, 1992; Iammarino *et al.*, 2010; Jackowet and Orduña, 2012). However, the over-ingestion of sulfite had shown hazardous to humans, and when the daily sulfite consumption is more than 50 mg/kg body weight it might result in headaches, nausea and diarrhea (Suh *et al.*, 2007). In recent years, the sulfite contents in foods and beverages had been strictly controlled and limited world widely (Zhong *et al.*, 2012). The United States Food and Drug Administration (FDA) require sulfite warning on the label of foods in concentrations of 10 mg/kg sulfite or more (Claudia and Francisco, 2009).

When added into food and beverages, parts of sulphite would react with aldehydes, proteins, ketones, sugars, etc., and parts without changes, accordingly presenting as the bound and free sulphite (Adams, 1997). The former are hydroxymethanesulfonate formed by reaction of carbonyl groups with HSO_3^- , no more effective as bleaching agent, antioxidant, sterilizing agent or preservative, but contributing as the flavouring agent by inactivating the disagreeable odor of aldehyde, and the latter refers to all the species (SO_2 , H_2SO_3 , HSO_3^- and SO_3^{2-}) that may rapidly and quantitatively be converted to SO_2 (Zhao *et al.*, 2006). The sum of them is the total sulfite (Sarudi and Kelemen, 1998; Koch *et al.*, 2010; Brychkovaa *et al.*, 2012). In the quality control of foods, both contents of free and total sulfite are important analytical parameters.

Till to now, many methods have been developed for sulfite determination, and can be classified according to the analytical

*To whom correspondence should be addressed.

E-mail: xjuwangjd@163.com

methods (Filik and Çetintaş, 2012). The first is based on chemical reaction, in which the total sulfite is determined by an acid-base titration of released sulfur dioxide through acidify the sample. A typical example of the titration approach was the Monier-Williams method, which was recommended by the Association of Official Analytical Chemists (AOAC), but this method is an indirect method, and cannot be used for fast or high-throughput analysis (Koch *et al.*, 2010). Furthermore, the method tends to overestimate sulfite levels upon the presence of volatile acidic compounds (Pizzoferrato *et al.*, 1998). The second is based on instruments, in which free and total sulfite are determined directly, including GC (Hamano, 1979; Mitsuhashi *et al.*, 1979), HPLC (Mcfeeters, 2003), electrochemistry (Zhao *et al.*, 2006), and flow injection technique (Claudia, 2009; Navarro *et al.*, 2010), but all of them are seldom applicable in practice. Spectrophotometry (Segundo and Rangel, 2001; Li and zhao, 2006) is rather widely applied, even if it requires the complicated sample pretreatment and reagent preparation processes. But it cannot meet the more and more critical requirement of food industry for its bad sensitivity limited by the nature of spectrophotometry method. Recent years, ion chromatography (Iammarino *et al.*, 2010; Brychkova *et al.*, 2012; Zhong *et al.*, 2012) was recommended to test free and total sulfite in liquid samples such as wine, fruit juice for its high sensitivity and accuracy.

The sulfite is low-level presence in complex matrices and can be rapidly oxidized to sulfate in aqueous solution, which lead to very hard to accurate quantification of them. To over this problem, various reagents were added as stabilizers to decrease the oxidation of the sulfite, including formaldehyde (Massom and Townshend, 1986; Michigami and Ueda, 1994; Masár *et al.*, 2004), acetone, ethanol, mannitol, EDTA, glycerol and tetrachloromercurate (Iammarino *et al.*, 2010). It has been proved the content of the free sulfite would decrease about 25–30% after exaction from wine without adding stabilizers.

In this work, formaldehyde was introduced into the homogenized grape as the stabilizer to decrease the loss of the free sulfite, and ion chromatography (IC) was utilized for direct determination of the residues of sulfite in Red Globe grape. This combination was well applicable to determine free and total sulfite in barriers with high accuracy and sensitivity.

Materials and Methods

Reagents and standards Hydrogen peroxide, sodium hydroxide, sodium sulfate, sodium sulfite, sodium carbonate, sodium bicarbonate, mercuric sodium tetrachloride, ammonium sulfamate and formaldehyde were purchased from Tianjin Chemical Reagent Co. (Tianjin, China). Ultrapure water (specific resistivity $18.2 \text{ M}\Omega \cdot \text{cm}^{-1}$) obtained from a CLASSIC UF Water Purification system (PALL, USA) was used for sample preparation, dilution of samples, standard solutions, and IC eluent preparation. Eluent solution was prepared by dissolving appropriate amount of

sodium carbonate and sodium bicarbonate in ultrapure water and degassing in an ultrasonic bath for 15 min. The standard solutions and eluent solution were prepared fresh daily, the standard solutions were diluted as appropriated just before use.

Red Globe grape sample treatment Red Globe grape was purchased from Sanping Farm (Xinjiang, China), and quickly transported to the laboratory on the day of the harvest and stored at 0°C overnight before processing. And then, grapes were stored by SO_2 preservative paper obtained from Green Doctor Fresh-Keeping Science & Technology Co. Ltd of Urumqi (Xinjiang, China). The grapes with different storage time were selected for uniform size, maturity and appearance, freed from defects and mechanical damage, and frozen using liquid nitrogen and stored at -80°C for analysis.

Extraction of free sulfite using formaldehyde as stabilizer The extraction of free sulfite was based on the method described previously with few modifications (Masár *et al.*, 2005; Koch *et al.*, 2010). 20 g portion of homogenized sample were dispersed in freshly ultrapure water to make a 50 mL sample solution containing formaldehyde (corresponding to a 10 mmol/L final concentration of formaldehyde), and then the solution was ultrasonic oscillator for 15 min at 0°C . To guarantee a full conversion of free sulfite to hydroxymethanesulfonate (HMS), the solution was further incubated for another 60 min, then the mixture centrifuged at 10,000 r/min for 10 min at ambient temperature, the supernatant was carefully removed and adjusted to 50 mL with ultrapure water. Finally the mixture was diluted 10 times and purified with SPE C18 column (50 mg/1 mL, Dikma, USA) and analyzed by IC as soon as possible. A control sample (without formaldehyde) was prepared in the same way, in parallel.

Determination of total sulfite through hydrogen peroxide oxidation Homogenized samples (20 g) were dispersed into 30 mL sodium hydroxide solution (10 mM). Subsequently the mixture was treated with hydrogen peroxide solution (30% w/w) in ultrasonic oscillator for 15 min, and incubated for another 2 h at ambient temperature to guaranty a full conversion of sulfite to sulfate. Finally the mixture centrifuged at 10,000 r/min for 10 min at ambient temperature, the supernatant was carefully removed and adjusted to 50 mL with ultrapure water. Finally the mixture was diluted 10 times and purified with SPE C18 column and analyzed by IC as soon as possible. A control sample (without H_2O_2) was also prepared in the same way to deduct the amount of sulfate in grape, in parallel.

Ion chromatography The instrument used was an ICS-900 system (Dionex, USA) equipped with an ASRS300 4-mm suppressor, a DS5 conductivity detector. Ion chromatographic condition was used according to our previous study with few modifications (Zhong *et al.*, 2006). The chromatographic separations were carried out on an IonPac AS23 analytical column ($4 \times 250 \text{ mm}$, particle size $6 \mu\text{m}$, stationary phase of supermacroporous resin cross-linked with 55% divinyl- benzene) fitted with an IonPac AG23 guard col-

umn (4×50 mm, particle size $11 \mu\text{m}$, stationary phase of microporous resin cross-linked with 55% divinylbenzene), both from Dionex (Dionex, USA), at room temperature. The mobile phase was $3.84 \text{ mM Na}_2\text{CO}_3 - 6.00 \text{ mM NaHCO}_3$ with isocratic elution at the flow rate of 1.0 mL/min . A self-regenerating suppressor operating at 40 mA was used for suppressed conductivity detection. The injection volume was $10 \mu\text{L}$.

PHS Method According to the China National Standard GB/T 5009.34-2003, 20 g portion of homogenized sample were dispersed in freshly ultrapure water, and then $20 \text{ mL } 0.05 \text{ M Na}_2(\text{HgCl}_4)$ solution was added, after incubation for 4 h to completely release bound sulfite from carbonyl adducts, the solution was filtered into a 100 mL volumetric flask. A volume of 5 mL solution was sampled, and then mixed with $5 \text{ mL Na}_2(\text{HgCl}_4)$ solution, $1 \text{ mL NH}_4\text{SO}_3\text{NH}_2$ solution (12 g/L), 1 mL formaldehyde solution (2 g/L) and 1 mL pararosaniline hydrochloride solution in test tube. After incubation 20 min , UV (UV-2450, SHIMADZU, Japan) analysis was performed at 550 nm .

Results and Discussion

Optimized conditions of sample treatment

a) Determination of free sulfite

Sulfites were rather labile and can be quickly degraded during extraction procedure. Masár groups (Masár *et al.*, 2004) introduced formaldehyde as stabilizer to transfer sulfite into HMS by following reaction:

Here, formaldehyde was also used as stabilizer to analyze grape samples. The amount of formaldehyde and the reaction time were investigated to obtain optimized conditions for the accurate

determination of free sulfite in grapes. It was found when the concentration of formaldehyde was in the range $5 - 10 \text{ mmol}$, the peak area of sulfite was increased sharply, and when the adding amount above 10 mmol , the peak area of sulfite kept constantly (Fig. 1 (A)). The reaction time also significantly affected the peak area of free sulfite, and when it reached 60 min , the biggest peak area was gotten (Fig. 1 (B)).

Red Globe grape samples just after the harvest or after storage of 0 day and 5 days by SO_2 preservative paper were analyzed with/without formaldehyde treatment in parallel. To the grapes just after harvesting, the results with/without formaldehyde treatment were consistent, the free sulfite were not detected. To samples after storage of 5 days by SO_2 preservative paper, an average amount of free sulfite were 8.62 mg/kg and 7.12 mg/kg (a decrease of 17.4%) with/without formaldehyde treatment, respectively. This result testified formaldehyde treatment can largely avoid the loss of sulfite in the procedure of sample treatment. This result testified the addition of formaldehyde can efficiently avoid the loss of free sulfite.

b) Determination of total sulfite

When the sample was alkalinized, the reversibly bound sulfite can be released, and sulfite can be subsequently oxidized to sulfate by hydrogen peroxide (Edmond *et al.*, 2003; Koch *et al.*, 2010). But there are lots of solid in berries, such as peel, pulp etc., which meant the matrix of grapes was much more complex than those of wine and other liquid samples, so the reaction time and amount of oxidant should be optimized to guaranty a full oxidization of sulfite to sulfate by hydrogen peroxide, and the results were summarized in Fig. 2. It showed that the optimized conditions were at $\text{pH } 11$ (Fig. 2 (A)), 120 min of reaction time (Fig. 2 (B)), and 5 mL of H_2O_2 (Fig. 2 (C)). The optimized pH value and the volume of H_2O_2

Fig. 1. (A) Effect of the amount of formaldehyde, and (B) reaction time to the peak area of SO_3^{2-} .

Fig. 2. (A) Effect of pH value, (B) oxidation time, and (C) H₂O₂ volume to the peak area of SO₄²⁻

Fig. 3. Typical ion chromatogram.
a Red Globe grape sample; b standard solution of sulfate and sulfite. Identified peak anions: 1 sulfate; 2 sulfite

Table 1. Regression equation and correlation coefficient, linear range, LOD, RSD and recoveries of IC and PHS

Method	Anions	Regression equation	Correlation coefficient	Linear range (mg/L)	LOD (mg/L)	RSD (%)	Recoveries (%)		
IC	SO ₃ ²⁻	Y = 3.532 × 10 ⁻² X + 0.5320 × 10 ⁻²	0.9998	0.1 – 40	0.05	2.34	88 ^a	93 ^b	89 ^c
	SO ₄ ²⁻	Y = 4.981 × 10 ⁻² X + 5.398 × 10 ⁻²	0.9997	0.01 – 40	0.002	1.67	87 ^a	98 ^b	96 ^c
PHS	SO ₃ ²⁻	Y = 6.009 × 10 ⁻² X – 9.112 × 10 ⁻⁴	0.9996	0.2 – 4.0	0.1	2.20	81 ^d	96 ^e	94 ^f

The amount of added standards: a, 4 mg/L; b, 10 mg/L; c, 20 mg/L; d, 0.6 mg/L; e, 2.0 mg/L; f, 4.0 mg/L.

Fig. 4. amount of free sulfite, total sulfite and bound sulfite in Red Globe grape during different storage time at 0°C

Table 2. IC method and pararosaniline hydrochloride 51 spectrophotometry method determining the total sulfite

Storage Time (d)	IC		PHS	
	(mg/kg, n=3)	RSD (%)	(mg/kg, n=3)	RSD (%)
0	1.67 ± 0.13	7.78	1.83 ± 0.22	12.0
5	14.28 ± 0.33	2.31	13.42 ± 0.25	1.86
7	17.22 ± 0.32	1.86	16.18 ± 0.18	1.11
15	18.99 ± 0.41	2.16	17.62 ± 0.36	2.04
30	23.14 ± 0.46	1.99	22.24 ± 0.24	1.08
60	23.94 ± 0.54	2.26	23.14 ± 0.76	3.28
110	27.34 ± 0.72	2.63	27.23 ± 0.28	1.03

(Mean ± Standard Deviation, mg/kg)

were similar with previous reports (Masár *et al.*, 2005; Koch *et al.*, 2010), but the reaction time was almost doubled in this work.

Chromatographic separation Chromatographic separation was performed under optimized condition (data not showed). The typical chromatogram was shown in Fig. 3. The retention time of sulfate and sulphite were 21.5 min and 22.7 min, respectively. The linearities of the IC method to sulfite and sulfate were evaluated and results were listed in Table 1. The calibration graphs were rectilinear for 0.1 – 40 mg/L (correlation coefficient, $r = 0.9998$) and 0.01 – 40 mg/L ($r = 0.9997$) to sulphite and sulphate, respectively. RSD (%) of intraday repeatability (with 3 replicates) were below 2.3%, which testified the accuracy of the method. The LODs were as low as 0.002 and 0.050 mg/L to sulfate and sulfite, respectively (calculated as the concentration corresponding to three times of signal-to-noise ratio). To testify the accuracy of method, the recoveries were analyzed by spiking sulfite and sulfate

standards to reach the end concentration of 4, 10, 20 mg/L of into real grape sample, and amounted to 88 – 93% to sulfite and 87 – 98% to sulfate (the data was also shown in Table 1).

Comparison of PHS and IC method As a comparison, a series of grape samples with different storage time were analyzed using the method proposed here and China National Standard GB/T 5009.34 – 2003, PHS method, used to determine the total sulfite in Red Globe grape; the results were listed in Table 1. By IC method, limits of detection to free sulfite and total sulfite (sulfate) were 0.05 mg/L and 0.002 mg/L, respectively. As comparison, PHS method only can be used to detect total sulfite with the limit of detection of 0.1 mg/L. It should be pointed that the complicated sample pretreatment and reagent preparation processes of PHS are much more time consuming, which almost double than that of IC method.

Changes of the free and total sulfite accumulation in grapes during storage The Red Globe grape samples with different storage time were analyzed using the method proposed in this study, and changes of the free and total sulfite accumulation were shown in Fig. 4. PHS method was also used to determine the amount of total sulfite. Table 2 illustrated the quantitative results of total sulfite derived from two methods. It was found that reliabilities of IC method were acceptable. Except o day, the relative standard deviation (RSD) of IC method was less than 3.3%, meanwhile RSD of PHS method was less < 2.6%. This result testified the high reliability of IC method.

From Fig.4, it can be seen that the accumulation of both free and total sulfites were increased sharply at the first week, and then increased slowly down, the accumulation of free and total sulfites reached 19.97 mg/kg and 27.18 mg/kg till 110 days, respectively. It

shows there existed big distance between the results of the accumulation between free and total sulfites, which meant there were parts of bound forms of sulfite existing. Since there were many components, including sugar, aldehydes and ketons, would react with SO₂, and transferred into binding compounds, which may have a significant effect on bound sulfites level (Jackowetz, 2012).

Conclusion In this work, an accurate and reliable analytical method had been developed and validated for the suitable and quantitative determination of free and total sulfites in Red Globe grape. Relative standard deviation values of repeatability were less than 3%. Most importantly, LOD of IC increased 1 order of magnitude than that of the spectrophotometry to total sulfite, the time consumption was only half of the time PHS needed. The proposed method in this article can be applied to the determination of low-level sulfite in the barriers with color, and can meet the critical requirement of the increasingly stringent food safety standards around the world.

Acknowledgements The authors gratefully acknowledge national natural science foundation of China, grants no. 31160341 and 31060227.

References

- Adams, J. B. (1997). Food additive-additive interactions involving sulphur dioxide and ascorbic and nitrous acids: A review. *Food Chem.*, **59**, 401-409.
- Brychkova, G., Yarmolinskaya, D., Fluhr, R., and Sagia, M. (2012). The determination of sulfite levels and its oxidation in plant leaves. *Plant Sci.*, **190**, 123-130.
- Claudia, R.C. and Francisco, J.C. (2009). Application of flow injection analysis for determining sulphites in food and beverages: A review. *Food Chem.*, **112**, 487-493.
- Edmond, L.M., Magee, E.A., and Cummings, J.H. (2003). An IEC Method for Sulphite and Sulphate Determination in Wine without Predistillation. *LC•GC Europe*, 1-5.
- Falcone, F. and Maxwell, C.K. (1992). Simultaneous Continuous Flow Analysis of Free and Total Sulfur Dioxide in Wine. *J. Agric. Food Chem.*, **40**, 1355-1357.
- Filik, H. and Çetintaş, G. (2012). Determination of Sulfite in Water and Dried Fruit Samples by Dispersive Liquid-Liquid Microextraction Combined with UV-Vis Fiber Optic Linear Array Spectrophotometry. *Food Anal. Methods*, **5**, 1362-1367.
- Hamano, T., Mitsunashi, Y., Matsuki, Y., Ikuzawa, M., Fujita, K., Izumi, T., Adachi, T., Nonogi, H., Fuke, T., Suzuki, H., Toyoda, M., Ito, Y., and Iwaida, M. (1979). Application of Gas Chromatography for the Separate Determination of Free and Combined Sulphites in Foods. I. *Z. Lebensm. Unters. Forsch.*, **168**, 195-199.
- Iammarino, M., Taranto, A.D., Muscarella, M., Nardiello, D., Palermob, C., and Centonze, D. (2010). Development of a new analytical method for the determination of sulfites in fresh meats and shrimps by ion-exchange chromatography with conductivity detection. *Anal. Chim. Acta*, **672**, 61-65.
- Jackowetz, J.N. and Orduña, R.M. (2012). Metabolism of SO₂ binding compounds by *Oenococcus oeni* during and after malolactic fermentation in white wine. *Int. J. Food Microbiol.*, **155**, 153-157.
- Koch, M., Köppen, R., Siegel, D., Witt, A., and Nehls, I. (2010). Determination of Total Sulfite in Wine by Ion Chromatography after In-Sample Oxidation. *J. Agric. Food Chem.*, **58**, 9463-9467.
- Li, Y.J. and Zhao, M.P. (2006). Simple methods for rapid determination of sulfite in food products. *Food Control*, **17**, 975-980.
- Masár, M., Danková, M., Ůlvecká, E., Stachurová, A., Kaniánsky, D., and Stanislawski, B. (2004). Determination of free sulfite in wine by zone electrophoresis with isotachopheresis sample pretreatment on a column-coupling chip. *J. Chromatogr. A*, **1026**, 31-39.
- Masár, M., Danková, M., Ůlvecká, E., Stachurová, A., Kaniánsky, D., and Stanislawski, B. (2005). Determination of total sulfite in wine Zone electrophoresis-isotachopheresis quantitation of sulfate on a chip after an in-sample oxidation of total sulfite. *J. Chromatogr. A*, **1084**, 101-107.
- Massom, M. and Townshend, A. (1986). Flow-injection determination of sulphite and assay of sulphite oxidase. *Anal Chim Acta*, **179**, 399-405.
- Mcfeeters, R.F. and Barish, A.O. (2003). Sulfite Analysis of Fruits and Vegetables by High-Performance Liquid Chromatography (HPLC) with Ultraviolet Spectrophotometric Detection. *J. Agric. Food Chem.*, **51**, 1513-1517.
- Michigami, Y. and Ueda, K. (1994). Sulphite stabilizer in ion chromatography. *J Chromatogr A*, **663**, 255-258.
- Mitsunashi, Y., Hamano, T., Hasegawa, A., Tanaka, K., Matsuki, Y., Adachi, T., Obara, K., Nonogi, H., Fuke, T., Sudo, M., Ikuzawa, M., Fujita, K., Izumi, T., Ogaw, S., Toyod, M., Ito, Y., and Iwaida, M. (1979). Comparative Determination of Free and Combined Sulphites in Foods by the Modified Rankine Method and Flame Photometric Detection Gas Chromatography. V.*. *Z. Lebensm. Unters. Forsch.*, **168**, 299-304.
- Navarro, M.V., Payán, M.R., López, M.A.B., Torres, R.F., and Mochón, M.C. (2010). Rapid flow injection method for the determination of sulfite in wine using the permanganate-luminol luminescence system. *Talanta*, **82**, 2003-2006.
- Pizzoferrato, L., Lullo, G.D., and Quattrucci, E. (1998). Determination of free, bound and total sulphites in foods by indirect photometry-HPLC. *Food Chem.*, **63**, 275-279.
- Sarudi, I. and Kelemen, J. (1998). Determination of sulphur and total sulphur dioxide in wines by an ICP-AES method. *Talanta*, **45**, 1281-1284.
- Segundo, M.A. and Rangel, A.O. (2001). A gas diffusion sequential injection system for the determination of sulphur dioxide in wines. *Anal. Chim. Acta*, **427**, 279-286.
- Suh, H.J., Cho, Y.H., Chung, M.S., and Kim, B.H. (2007). Preliminary data on sulphite intake from the Korean diet. *J Food Compos Anal.*, **20**, 212-219.
- Wu, J.M., Zhong, M., Wu, B., and Wang, J.D. (2008). Study on SO₂ Accumulation on Table Grapes in Storage, *Food Sci.*, **29**, 486-489.

Zhao, M., Hibbert, D.B., and Gooding, J.J. (2006). Determination of sulfite in beer samples using an amperometric fill and flow channel biosensor employing sulfite oxidase. *Anal. Chim. Acta*, **556**, 195-200.

Zhong, Z.X., Li, G.K., Zhu, B.H., Luo, Z.B., Huang, L., and Wu, X.M.

(2012). A rapid distillation method coupled with ion chromatography for the determination of total sulphur dioxide in foods. *Food Chem.*, **131**, 1044-1050.