Determination of Total Sulfite in Wine by Ion Chromatography after In-Sample Oxidation

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Sulfur dioxide (SO_2) or sulfites are the most common preservatives used in winemaking. The level of total SO_2 is subject to regulation. Currently, the regulatory determination of total SO_2 (including sulfites) is done by the optimized Monier−Williams (OMW) method, which includes time-consuming distillation and titration steps. This paper describes the development and application of an alternative, rapid, straightforward, and reliable method for the determination of total sulfite in wine. In this method, a simple oxidation step using alkaline hydrogen peroxide (H_2O_2) solution is followed by ion chromatographic (IC) analysis of sulfate coupled with conductometric detection. Thirteen wines were analyzed in order to compare the in-sample oxidation method with the OMW-procedure. A *t*-test revealed satisfying compliance regarding sample preparation, i.e., alkaline H_2O_2 treatment and acidic distillation (OMW method). Comparable results were also obtained between IC analysis and acid/base titration. Our results indicate that the novel method (limit of quantification: 4 mg SO_2 L^{-1}) is well suited for the cost-efficient monitoring of regulatory limits.

KEYWORDS: Sulfur dioxide; wine analysis; hydrogen peroxide oxidation; IC; optimized Monier−Williams method

INTRODUCTION

Addition of sulfur dioxide or sulfites is a practice widely used in the processing of foods like grapes, dried fruits, potato products, fruit juices, and wine. Sulfiting agents contribute to the stabilization and conditioning of foods by preventing oxidation, browning, and microbial reactions. Also, they are cost-efficient and easy to apply. However, sulfites alter the organoleptic profile of food products and are well-known to cause asthma and other allergic reactions in persons hypersensitive to SO_2 (*1−4*). Hence, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has issued an acceptable daily intake (ADI) of sulfur dioxide of 0.7 mg kg^{-1} body weight. In the EU, the maximum amounts of sulfite admitted for addition to wine are regulated (total SO_2: 160 mg L^{-1} for red wines and 210 mg L^{-1} for white and rosé wines) (*5*). This means that the ADI could be exceeded by an intake of only 250 mL of white wine (210 mg L^{-1}) from a person with a body weight of 70 kg.

In wine sulfite is present in free and bound forms (*6*), the latter being hydroxysulfonate adducts formed by the reaction with wine matrix compounds (ketones, aldehydes, sugars, tannins, etc.) (*2,7*). Several analytical methods are known for the determination of either the free sulfite (*8, 9*) or the total sulfite (sum of free and bound sulfite) (*10, 11*).

Two procedures are commonly used by wine industry and authorities for quantifying total sulfite in foods and beverages including wine. One procedure, named the Ripper method, consists of a direct iodometric titration that is prone to interferences from iodine-reactive compounds, thus being heavily criticized (*10, 12*). The official method of the Association of Official Analytical Chemists (AOAC) (*13*), which is also recommended by EU (*6, 14*), is based on the optimized Monier−Williams (OMW) method. This method relies on an indirect determination of total sulfite after distillation, oxidation with hydrogen peroxide, and titration of sulfuric acid with sodium hydroxide solution. However, this sample preparation routine is time-consuming, is labor-intensive, and cannot be used for fast or high-throughput analysis. Furthermore, the OMW method tends to overestimate sulfite levels upon the presence of volatile acidic compounds (*15*). To overcome these limitations, several methodologies have been proposed, e.g., capillary electrophoresis (*6, 11*) and ICP-OES (*4*). One of the most frequently published methods is the flow injection technique combined with different detectors (*10,16,17*).

In order to analyze the total sulfite concentration via the more stable sulfate ion, a dissociation of reversibly bound sulfite-adducts followed by a complete oxidation of sulfite to sulfate is required. Bound sulfites are released either by acid (OMW method) or alkaline treatment at pH > 10 (*7*). In the OMW method, the subsequent oxidation to sulfate is done by hydrogen peroxide (H_2O_2). Such a powerful agent is necessary particularly for an in-sample oxidation, as the ethanol present in wine acts as a free radical scavenger, thus reducing the oxidation rate of sulfite.

Our work aimed to simplify the existing procedures by omitting the distillation and titration steps of the OMW method. An in-sample hydrogen peroxide treatment of an alkalinized wine in combination with ion chromatography (IC) was considered

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Table 1. Analyzed Wine Samples

sample	grape variety	type of wine	origin	ethanol (%)
1	Cabernet/Syrah	red	France	12.5
2	Syrah	red	France	12.0
3	Sangiovese	red	Italia	11.0
4	Shiraz/Cabernet	red	Australia	13.5
5	Dornfelder	red	Germany	11.0
6	Spätburgunder	red	Germany	12.0
7	Merlot	red	France	13.0
8	Gamay	red	France	12.0
9	Cabernet Franc	red	Hungary	13.0
10	Chardonnay/Colombard/Ugni blanc	white	France	11.5
11	Frühburgunder	white	Germany	13.0
12	Chardonnay	white	Australia	13.0
13	Grauburgunder	white	Germany	12.0

suitable for this purpose. IC was chosen, as it was shown to result in a higher selectivity than acid−base titration (*18*). While an in-sample oxidation and IC based approach has been published before (*19*), a comparison to the OMW method was not yet done. Hence, the present paper describes the development and validation of a facile method based on in-sample oxidation of total sulfite in wine and its comparison to the OMW-method.

MATERIALS AND METHODS

Reagents and Chemicals. All reagents were analytical grade and used without further purification. Hydrogen peroxide (30% w/w), sodium hydroxide, sodium hydroxymethanesulfonate (HMS, CH_2OHSO_3Na), and various acidic wine components (acetic, propionic, oxalic, lactic, malonic, maleic, fumaric, tartaric, succinic, malic, octanoic, D-galacturonic, and citric acids) were obtained from Sigma-Aldrich (Taufkirchen, Germany). Ethanol, hydrochloric acid, and sodium sulfate were purchased from Merck (Darmstadt, Germany).

Ultrapure water from a Millipore Water purification system was used for sample preparation, dilution of stock and standard solutions, and IC eluent preparation. Argon and nitrogen were used in 5.0 quality.

For titration of sulfuric acid a sodium hydroxide solution was prepared from a Titrisol ampule (Merck, Darmstadt, Germany) filled to 1 L with water to give a concentration $c(NaOH)$ of 10 mM.

Wine Samples. Nine red and four white wines made from different grape varieties cultivated in different geographical regions (**Table 1**) were purchased in local supermarkets. Each wine sample was transferred to vials prefilled with argon to prevent a contact of oxygen and wine. The vials were closed with sealed screw-caps and stored in darkness at 4 °C until analysis.

In-Sample Oxidation of Wine. Wine samples (1 mL) were diluted to a volume of 50 mL with sodium hydroxide solution (20 mM), thereby raising the pH to 11. By doing so, reversibly bound sulfites are released from the carbonyl adducts. A volume of 10 mL was sampled for measurement of the initial sulfate concentration in the unoxidized wine sample. The residual volume of 40 mL was treated with hydrogen peroxide solution (30% w/w). The amount of oxidizing agent and the reaction time were optimized, resulting in an addition of 100 μL of hydrogen peroxide solution and an incubation time of 1 h at ambient temperature. After conversion, the sulfate concentration was determined by IC.

Ion Chromatography (IC). The instrument used was a DX 500 system (Dionex GmbH, Idstein, Germany) equipped with an AS40 automated sampler, a LC20 chromatographic module, a GP50 gradient pump, an EG40 eluent generator, and a CD20 conductivity cell with a DS3 detection stabilizer. The chromatographic separations were carried out on an IonPac AS15 analytical column (2 mm × 250 mm, particle size 9 μm, stationary phase of ethylvinylbenzene cross-linked with 55% divinylbenzene) fitted with an IonPac AG15 guard column (2 mm × 50 mm), both from Dionex (Idstein, Germany), at a constant column temperature of 30 °C. Potassium hydroxide solution at 22 mM was generated in the EG40 module and applied as eluent with a flow of 0.3 mL min^{-1}. Ion suppression was accomplished by 5 mM sulfuric acid with a flow of 4 mL min^{-1} using an AMMS III 4 mm anion micromembrane suppressor. The injection volume was 25 μL.

Optimized Monier−Williams (OMW) Method. The OMW method was applied according to the German implementation of the EU-recommended method (*20*). The distillation equipment comprised a dropping funnel containing 90 mL of hydrochloric acid (4 M), a 1 L flask filled with 400 mL of water, and a reflux cooler that was attached to a bulb condenser containing 30 mL of a hydrogen peroxide solution (3%, w/w). Prior to analysis the whole system was purged with nitrogen for 15 min. An amount of 10 mL of wine was diluted with 100 mL of ethanol/water 5:95 (v/v) and transferred into the 1 L flask. After addition of the hydrochloric acid the mixture was boiled in order to release sulfur dioxide. The latter was continuously purged out with nitrogen (200 mL min^{-1}) for 105 min and trapped in the bulb condenser, where it was subject to oxidation. Subsequently, the sulfate containing solution was filled up to 50 mL with water. While a volume of 25 mL out of 50 mL was titrated directly against a sodium hydroxide solution, an amount of 10 mL out of 50 mL was diluted with water (1:5, v/v) and analyzed by IC.

Quantification and Quality Parameters. *Ion Chromatography*. Identification of sulfate in wine was done by comparing the retention time against a known standard of sodium sulfate. Additionally, standard addition experiments were performed in order to confirm the identity of sulfate and to calculate its recovery. All standard and calibration solutions were stored at 4 °C. The sulfate content was quantified against an external six point calibration curve established in a range of 1−10 mg SO_4^{2-} L^{-1} ($R^2 = 0.9996$) by preparing each calibration level in duplicate.

For the in-sample oxidation method the total sulfite concentration in wine was determined by subtracting the sulfate concentration of the untreated sample from the one of the oxidized sample.

Titration. The titration of sulfuric acid in the frame of the OMW method is evaluated by means of a color change of the methyl red indicator from red to yellow for at least 20 s. The titer of NaOH solution is stated as 1.000 at a temperature of 20 °C. The blank value obtained by titration of a hydrogen peroxide solution after performing the OMW method without a wine sample was subtracted from all wine analysis results.

RESULTS AND DISCUSSION

In-Sample Oxidation Method. The method development was focused on the optimization of the parameters for the conversion of total sulfite according to eq 1 and the subsequent ion-chromatographic determination of sulfate.

$$SO_3^{2-} + H_2O_2 \rightarrow SO_4^{2-} + H_2O \tag{1}$$
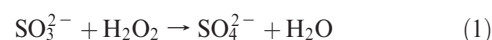
At first, the influence of the pH on the efficiency of bound sulfite release was investigated. For these experiments the sodium salt of HMS was used. HMS is a model substance for bound sulfite, an addition complex of bisulfite and formaldehyde. A red wine sample (sample 7, **Table 1**) spiked with HMS (corresponding to 50 mg SO_2 L^{-1}) was oxidized with hydrogen peroxide in excess between pH 6 and pH 11 (each $n = 3$). While in alkaline solution (pH 11) an average recovery of about 90% was yielded, at pH 6 only < 20% of bound sulfite was recovered. This result corresponds to previously described outcomes (*7*, *11*, *21*).

Authentic red and white wine samples (samples 5, 7, 10, 13; **Table 1**), containing bound sulfite and ethanol, were used for the subsequent optimization step. The ratio of hydrogen peroxide to wine necessary for a maximum oxidation of sulfite (at pH 11) was determined to be at least 1:20 (v/v). For subsequent analyses of wines a ratio of 1:10 was used. Higher amounts of hydrogen peroxide should be avoided to protect the IC separation column.

Conversion experiments showed an almost complete oxidation after 1 h of incubation. Although a slightly higher sulfite conversion rate (recovery) of up to 5% was obtained after 4 h of incubation, 1 h was chosen in view of time efficiency. Ion chromatograms of a real wine analysis using this optimized method are illustrated in **Figure 1**.

The sulfate peak in the wine sample before and after treatment with hydrogen peroxide is well separated from other peaks. However, it should be noted that a broad range of organic acids
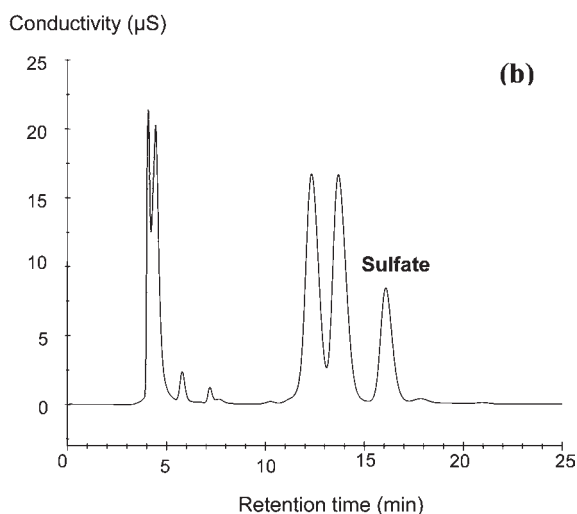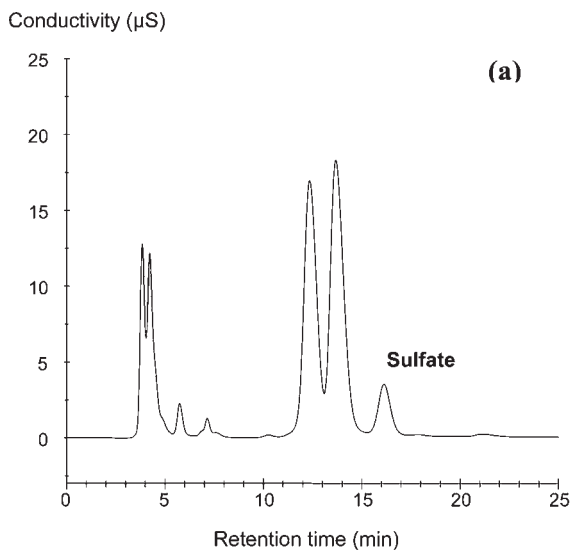
Conductivity (µS)

Conductivity (µS)

Figure 1. Ion chromatograms of a white wine sample (Grauburgunder no.13) by the in-sample oxidation method: (**a**) before treatment with hydrogen peroxide; (**b**) after treatment with hydrogen peroxide.

is present in wine. These acids could be detected with the present method, as an alkaline IC eluent and an anion exchange separation column are used. Tartaric, malic, and citric acids are the dominating natural acids in grapes whereas lactic, succinic, and acetic acids have been found to be major fermentation acids (*22*). Thirteen organic acids were measured before and after oxidation with hydrogen peroxide to verify that no interferences with the sulfate peak do occur. Three of the major organic acids are shown in the chromatogram of a standard solution together with sulfite and sulfate (**Figure 2**).

It can be seen that malic and succinic acids are not separated by this method. The same is true for tartaric acid and sulfite. These compounds represent the main peaks of real wine samples (see **Figure 1**). However, none of the 13 tested compounds coeluted with the sulfate peak, so interferences caused by organic acids could be excluded. Moreover, the conductivity of the organic acids is much lower than that of the sulfate ion.

The recovery of sulfite was determined by standard additions of HMS to a red wine (sample 5) and a white wine (sample 10) in a range of 20–100 mg SO_2 L^{-1} measuring each spiking level of HMS in triplicate. A recovery of (88.5 ± 4.2)% for red wine and (91.6 ± 5.1)% for white wine was calculated from the standard addition regression lines.
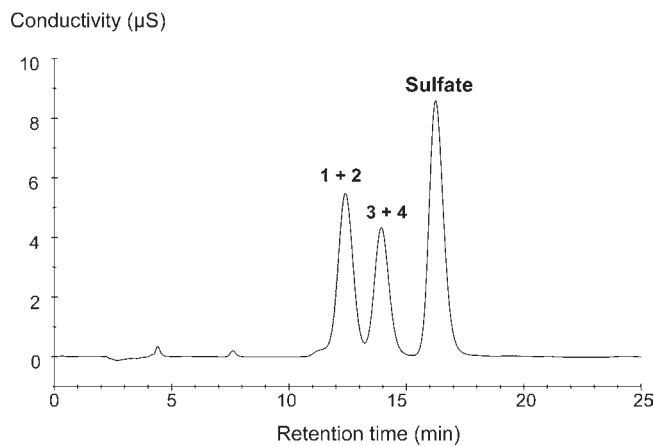
Conductivity (µS)

Figure 2. Ion chromatogram of a standard solution containing malic acid (1), succinic acid (2), and tartaric acid (3) together with sulfite (4) and sulfate.
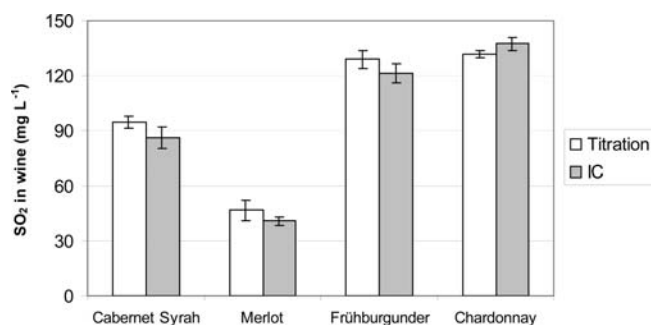

Figure 3. Total sulfur dioxide concentration of four wine samples (mean ± standard deviation, mg L^{-1}) determined by the OMW method after quantification by titration and IC.

Limits of detection (LOD) and quantification (LOQ) were calculated based on the sulfate calibration curve method according to DIN 32645 (*23*) to be 1 mg L^{-1} (SO_2 in wine) and 4 mg L^{-1}, respectively.

Method Comparison. The in-sample oxidation method and the reference OMW method differ in terms of sample preparation and detection. In view of volatile acids in wine, which may interfere in the acid–base titration (*15, 24*), the selectivity of the method is of major importance. Volatile acids are formed by yeast activity during alcoholic fermentation of grapes and by spoilage bacteria during fermentation or aging in concentrations ranging from 0.3 to 1.2 g L^{-1} (expressed as acetic acid). The volatile acid mix usually consists of more than 90% acetic acid and traces of formic, propionic, and butyric acids (*22*). **Figure 3** displays the sulfur dioxide concentration determined by titration and IC analysis of extracts generated by the OMW method (*n* = 3).
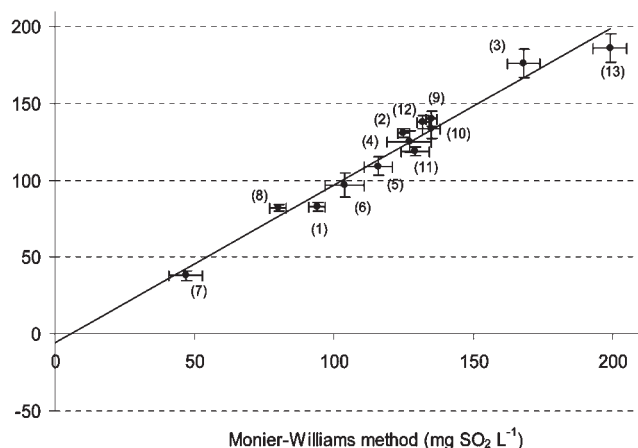
Unexpectedly, the results of titration and IC are in good agreement. Although the findings for titration seem to be slightly higher than those for IC analysis, a significant overestimation of sulfite caused by volatile acids could not be observed when using the titration method.

Further experiments on the oxidation stability of ethanol against hydrogen peroxide showed no acetic acid formation at ambient temperature. However, after thermal treatment (60 °C for 30 min) of the hydrogen peroxide containing solution a significant increase of acetic acid was obtained.

The second part of method comparison was done by IC quantification of sulfate. In this way, the in-sample oxidation and distillation procedure according to Monier–Williams were

Table 2. *t*-Test results for the Method Comparison of In-Sample Oxidation and OMW Method (Quantification by IC) Determining the Total Sulfur Dioxide Concentration of Four Wine Samples (Mean ± Standard Deviation, mg L^{-1})

sample	SO$_2$ in wine by in-sample oxidation method (mg L^{-1}) (*n* = 10)	SO$_2$ in wine by OMW method (mg L^{-1}) (*n* = 10)	|*t*| value	*t*-test result, differences significant?
3	176.0 ± 9.0	173.6 ± 9.0	0.590	no
5	108.6 ± 6.0	116.6 ± 6.0	3.011	yes
10	133.6 ± 6.9	129.0 ± 6.7	1.530	no
13	186.1 ± 9.0	178.8 ± 9.4	1.712	no

Figure 4. Correlation of the in-sample oxidation method to the standard OMW method (using titration) by means of 13 wine samples analyzed for their total sulfur dioxide concentration (mean ± standard deviation, mg L^{-1}). The analyzed wines are indicated in parentheses according to the numbering in **Table 1**.

compared in terms of efficiency, e.g., release and oxidation of total sulfite.

In order to obtain a comprehensive database suited for statistical evaluation (mean value *t*-test), four different wine samples were used, each of them analyzed 10 times by in-sample oxidation and by the OMW method. Prior to *t*-test evaluation the data sets were checked for homogeneity of variances by means of the *F*-test. The *t*-test value \|*t*\| was calculated using the mean values of both methods, the corresponding standard deviations, and the number of measurements. The \|*t*\| values were compared with the quantile of the double sided *t*-distribution $t(1-\alpha/2; f)$ (α, level of significance; *f*, degree of freedom), namely $t(0.975; 18) = 2.101$ as shown in **Table 2**.

The null hypothesis (H_o), method$_A$ = method$_B$, could not be rejected in three out of four cases because the *t*-test value was lower than the *t*-quantile for a level of confidence of 95%. A significant difference between both methods was indicated only for wine sample 5 (Dornfelder).

Finally, 13 wines were analyzed in triplicate by the OMW method (with titration) and the alternative method of in-sample oxidation (with IC) (**Figure 4**).

A correlation of both methods was obtained irrespectively from the type of wine (red/white) indicated by the linear regression line ($y = 1.03x - 6.09$) and a regression coefficient R^2 of R^2: 0.97. As expected, the sulfur dioxide concentration in red wines was found to be lower than in white. Because of more native antioxidant substances contained in red wine, lower amounts of sulfite are necessary to prevent oxidation or browning reactions. One out of 13 wines (sample 3, sangiovese) was found to be close

to the regulatory limit of 160 mg SO$_2$ L^{-1} (confirmed by both methods) taking into account an expanded measurement uncertainty ($k = 2$).

The conclusion that can be drawn from all these aspects is that the presented method of in-sample oxidation fulfills the requirement for a simple and rapid control of the total sulfite concentration in wine. It may therefore be used by responsible authorities as a high throughput method to indicate violations of regulatory limits. Laborious distillation and titration steps can be avoided, and smaller sample intakes for analysis are possible. Fruit juices or beer could be further target matrices for the in-sample oxidation method after testing its suitability. Even the analysis of solid samples like dried fruits or mashed potatoes should be feasible adapting the method with regard to an extraction step.

ABBREVIATIONS USED

ADI, acceptable daily intake; ANOVA, analysis of variance; AOAC, Association of Official Analytical Chemists; HMS, (sodium) hydroxymethanesulfonate; IC, ion chromatography; JECFA, Joint FAO/WHO Expert Committee on Food Additives; OMW, optimized Monier−Williams.

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LITERATURE CITED

(1) Silva, K. R. B.; Raimundo, I. M.; Gimenez, I. F.; Alves, O. L. Optical sensor for sulfur dioxide determination in wines. *J. Agric. Food Chem.* **2006**, *54*, 8697–8701.

(2) Lowinsohn, D.; Bertotti, M. Determination of sulphite in wine by coulometric titration. *Food Addit. Contam.* **2001**, *18*, 773–777.

(3) Araújo, A. N.; Couto, C. M. C. M.; Lima, J. L. F. C.; Montenegro, M. C. B. S. M. Determination of SO$_2$ in wines using a flow injection analysis system with potentiometric detection. *J. Agric. Food Chem.* **1998**, *46*, 168–172.

(4) Cmelik, J.; Machát, J.; Niedobova, E.; Otruba, V.; Kanicky, V. Determination of free and total sulfur dioxide in wine samples by vapour-generation inductively coupled plasma-optical emission spectrometry. *Anal. Bioanal. Chem.* **2005**, *383*, 483–488.

(5) E. C. Council Regulation (EC) No 1493/1999 on the common organisation of the market in wine. *Off. J. Eur. Communities* **1999**, *L179*, 1−84.

(6) Sádecká, J.; Polonský, J. Determination of inorganic ions in food and beverages by capillary electrophoresis. *J. Chromatogr., A* **1999**, *834*, 401–417.

(7) Machado, R. M. D.; Toledo, M. C. F.; Vicente, E. Sulfite content in some Brazilian wines: analytical determination and estimate of dietary exposure. *Eur. Food Res. Technol.* **2009**, *229*, 383–389.

(8) Scampicchio, M.; Lawrence, N. S.; Arecchi, A.; Mannino, S. Determination of sulfite in wine by linear sweep voltammetry. *Electroanalysis* **2008**, *20*, 444–447.

(9) Masár, M.; Danková, M.; Ölvecká, E.; Stachurová, A.; Kaniansky, D.; Stanislawski, B. Determination of free sulfite in wine by zone electrophoresis with isotachophoresis sample pretreatment on a column-coupling chip. *J. Chromatogr., A* **2004**, *1026*, 31–39.

(10) Goncalves, L. M.; Pacheco, J. G.; Magalhaes, P. J.; Rodrigues, J. A.; Barros, A. A. Determination of free and total sulfites in wine using an automatic flow injection analysis system with voltammetric detection. *Food Addit. Contam.* **2010**, *27*, 175–180.

(11) Masár, M.; Danková, M.; Ölvecká, E.; Stachurová, A.; Kaniansky, D.; Stanislawski, B. Determination of total sulfite in wine zone electrophoresis−isotachophoresis quantitation of sulfate on a chip after an in-sample oxidation of total sulfite. *J. Chromatogr., A* **2005**, *1084*, 101–107.

(12) Maquieira, A.; Casamayor, F.; Puchades, R.; Sagrado, S. Determination of total and free sulphur dioxide in wine with a

continuous-flow microdistillation system. *Anal. Chim. Acta* **1993**, *283*, 401–407.

(13) AOAC International. Method 990.28. *Official Methods of Analysis of AOAC International*, 16th ed.; Cunniff, P., Ed.; The Association: Arlington, VA, 1995; Chapter 47, pp 27−29.

(14) E. C. Commission Regulation (EEC) No 2676/90 determining community methods for the analysis of wines. *Off. J. Eur. Communities* **1990**, *L272*, 1.

(15) Pizzoferrato, L.; Di Lullo, G.; Quattrucci, E. Determination of free, bound and total sulphites in foods by indirect photometry−HPLC. *Food Chem.* **1998**, *63*, 275–279.

(16) Oliveira, S. M.; Lopes, T. I. M. S.; Tóth, I. V.; Rangel, A. O. S. S. Development of a gas diffusion multicommuted flow injection system for the determination of sulfur dioxide in wines, comparing malachite green and pararosaniline chemistries. *J. Agric. Food Chem.* **2009**, *57*, 3415–3422.

(17) Mana, H.; Spohn, U. Sensitive and selective flow injection analysis of hydrogen sulfite/sulfur dioxide by fluorescence detection with and without membrane separation by gas diffusion. *Anal. Chem.* **2001**, *73*, 3187–3192.

(18) Pereira, C. F. Application of ion chromatography to the determination of inorganic anions in foodstuffs. *J. Chromatogr., A* **1992**, *624*, 457–470.

(19) Edmond, L. M.; Magee, E. A.; Cummings, J. H. An IEC method for sulphite and sulphate determination in wine without predistillation. *LC-GC Eur.* **2003**, 2–6.

(20) B. V. L. bestimmung von sulfit in lebensmitteln, teil 1: optimiertes monier-williams-verfahren. *ASU 64 LFGB* **1999**, L 00.00-46/1.

(21) Sarudi, I.; Kelemen, J. Determination of sulphur and total sulphur dioxide in wines by an ICP-AES method. *Talanta* **1998**, *45*, 1281–1284.

(22) Amann, R. Säuren in most und wein. *Dtsch. Weinmag.* **2007**, *19* (9).

(23) DIN 32645−Nachweis-, Erfassungs- und Bestimmungsgrenze. German Institute for Standardization (DIN), **1994**.

(24) Kim, H. J. Determination of sulfite in foods and beverages by ion exclusion chromatography with electrochemical detection: collaborative study. *J. Assoc. Off. Anal. Chem.* **1990**, *73*, 216–222.