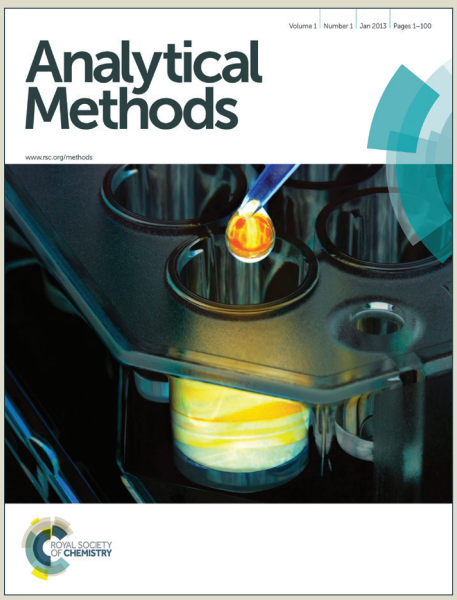
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Journal Name

RSCPublishing

ARTICLE

Direct Quantification of Total Sulfur Dioxide in Wine Using Triple Quadrupole ICP-MS

Cite this: DOI: 10.1039/xoxxoooooox

Xiao-wei Wang,[a] Jing-fu Liu,[b] Xiao-yan Wang,[a] Bing Shao,[a] * Li-ping Liu[a] and Jing Zhang[a]

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

www.rsc.org/

The accurate determination of sulfur dioxide (SO_2) in wine remains a critical analytical task for food safety and in the wine trade. The traditional recommended titration method has been criticized for its weak selectivity, poor precision and long procedure. In this study, an accurate, high-throughput, interference-free method for the absolute determination of SO_2 in wine based on a triple quadrupole ICP-MS instrument (ICP-QQQ) is developed. This novel ICP-QQQ is operated in the MS/MS mode to provide an interference-free measurement. Tested with four different S-containing inorganic ions and organic molecules, the result indicates that the behaviour of S in the plasma is unanimous regardless of the discrepancy between the S-containing compounds, which means the signal intensity of S is merely decided by the concentration of S. The developed method exhibits good linearity ($R > 0.999$) over the concentration range of 0.025–100 mg/L, with a limit of quantification of 10 μg/L for $^{32}SO_4^{2-}$ in wine. The recoveries ranged from 95–105% for the spiked wine samples. The method was successfully applied for the determination of total SO_2 in red wine samples. The concentrations determined using the developed method are in excellent agreement with those obtained using the recommended titration method. This study demonstrates a new approach for quantification of total SO_2 in wine with great convenience and high accuracy.

Introduction

As an essential additive for wine production and preservation, sulfur dioxide (SO_2) is added in wine to inhibit undesirable microbial growth and oxidation processes.[1,2] However, high concentrations of SO_2 impart an unpleasant aroma and negatively affect human health.[2] Therefore, the levels of SO_2 in wine are strictly controlled by legislation in various countries. SO_2 in wine usually exists as either free or bound; however, the total SO_2 is the primary concern for food safety.

As a commonly used method for the determination of total SO_2 in wine, titration has been adopted and recommended through legislation in various countries.[3,4] However, the titration method suffers from poor selectivity and precision, low sample throughput and tedious preparation procedures.[5] As a kind of reliable high-throughput methods, continuous-flow methods, which replaced irreproducible segmented flow analysis, coupled with either optical or electrochemical detection methods are currently the most frequently utilized techniques.[6-8] However, continuous-flow methods involve either a variety of reagents or a complex flow process design; the traditional detection techniques, meanwhile, are susceptibly interfered or failed to detect SO_2 in red wine because of the colour or the particulates from matrix.[9,10]

A more preferred method for the determination of SO_2 is direct analysis using ICP-MS, which either simplifies the sample treatment process or enhances the selectivity, sensitivity, precision and sample throughput.[11-13] Unfortunately, the quantification of SO_2 using a traditional quadrupole-based ICP-MS has been a challenge because of high ionization potential of S and serious polyatomic interferences.[14,15] The recent introduction and application of triple quadrupole-based ICP-MS (ICP-QQQ) allows for ICP-MS operation in the tandem-MS mode (MS/MS mode) and provides a new approach that is not prone to polyatomic interferences. To date, this technique has been successfully applied to the quantification of S-peptides and the S-containing reference material NIST SRM 2773.[15,16]

The presented study aims to develop a highly sensitive, rapid, accurate and reproducible method for the quantitative determination of the total SO_2 in wine using ICP-QQQ without a tedious pre-treatment process. The advantages of ICP-QQQ were exploited to remove polyatomic interferences more controllably and effectively. The analysis of total SO_2 in wine was achieved without complex sample pre-treatments or chromatographic separations, and completed within minutes. Using this approach, the total SO_2 in wine can be accurately and precisely determined in a simple and efficient manner.

Experimental

Reagents and materials

Sulfate stock standard solution (SO_4^{2-}) (10,000 mg/L), sodium sulfite analytical standard (1g), L-cysteine certified reference material (100mg), DL-methionine (99%, 5g) and ethanol (≥99.8%) were purchased from Sigma-Aldrich (St. Louis,

Missouri, USA). A mixed internal standard (100 mg/L) was obtained from Agilent (CA, USA). Nitric Acid (65%) was bought from Merck. Ultra-pure water (18.3 MΩ•cm), used throughout the experiments, was produced using a Milli-Q Gradient system (Millipore, Bedford, USA).

Instrumentation

An Agilent 8800 Triple Quad ICP-MS instrument (ICP-QQQ, Agilent Technologies, Japan) equipped with a quartz spray chamber, a glass ICP torch, a micro mist nebulizer and an X-len ion lense was used. The ICP-QQQ was operated in the MS/MS mode because S suffers serious polyatomic interferences in a single quadrupole ICP-MS system.[15] Armed with the mass selection and ion collision/reaction ability, ICP-QQQ provides interference-free conditions for the precise determination of S. Argon (99.999%) was used as the carrier gas with the addition of optional oxygen (Ar:O2 mixture, 8:2) to eliminate carbon buildup from the wine. Pure oxygen (99.999%) was introduced into the collision/reaction cell to convert S^+ into SO^+ in order to further distinguish S^+ from polyatomic interfering ions after mass selection in Q1. The argon flow rate and the percentage of optional oxygen and pure oxygen were optimized during the method development. The two mass analyser Q1, Q2 were set to Q1→Q2: 32→48, 34→50, 45→45 m/z, with integ time/mass: 0.09 sec, Q2 peak pattern: 3 points, replicates: 3, and sweeps: 10. Nebulizer pump was operated at 0.3 rps for 30 sec for uptake each sample, then lowered to 0.1 rps in the 10 sec stabilization and whole determination processes. All parameters were manually optimized to achieve the best signal intensity and stability, and the critical parameters were listed in Table 1. MassHunter software (Agilent Technologies, USA) was used to control the instrument and to process the data.

Procedures and concentration calculations

Fresh standard solutions were prepared prior to each experiment. Sixteen external standard solutions with sulfate concentrations ranging from 0 to 500 mg/L were diluted from a 10,000 mg/L stock standard solution using ultra-pure water to evaluate the linearity and limit of detection (LOD) of the method. The internal standard method was applied for quantitative analysis. The internal standard solution (1.00 mg/L) was diluted using 1 wt% (w/v) HNO_3 and maintained at 4 °C. ^{45}Sc was selected for internal standardization, since the signal intensity of ^{45}Sc was stable and the decline originated from the influence of O_2 in the collision/reaction cell was negligible.

Bottled red wines were purchased at a local market and stored at 4 °C. Spiked wine samples were mixed 30 min using a shaker at 60 rpm, and both wine samples and spiked wine samples were maintained at 20°C in a full and stoppered flask for 2 days before determination. All samples were directly injected into the ICP-QQQ after dilution. Five different dilution factors and three spike levels were analysed five times in parallel to evaluate the reproducibility and matrix effects of the method. The calibration curves and spiked recovery experiments were carried using SO_4^{2-} standard solution, since the ionization efficiency and signal intensity of S are only determined by the concentration of S, and have not been influenced by the type or the structure of S-containing compounds, which will be discussed in detail in the "Method validation". Therefore, the total SO_2 in the wines was calculated using the formula $C_{SO_2} = \frac{2}{3} \times DF \times C_{SO_4^{2-}}$, where $\frac{2}{3}$ is the ratio of the molecular weights of SO_2 to SO_4^{2-}, DF is the dilution factor, and $C_{SO_4^{2-}}$ is the concentration of SO_4^{2-} measured using ICP-QQQ.

Results and discussion

ICP-QQQ optimization

Table 1. Critical parameter settings for the ICP-QQQ.

Parameter	Value
Scan mode	MS/MS
RF power	1600 W
Carrier gas	1.20 L/min
Optional O_2	5%
Deflect	0 V
Plate bias	-60 V
Reaction O_2	30%
Octp bias	-8.0 V
Octp RF	190 V
KED	-4 V

A spiked wine sample was used to optimize the ICP-QQQ conditions. 1 mL wine sample was spiked with 1 mL SO_4^{2-} standard solution (100 μg/L) and subsequently diluted to 10 mL. The ICP-QQQ was operated in the MS/MS mode. The ICP-QQQ operating parameters were optimized to reduce interferences while maintaining excellent single intensity and sensitivity. The most important ICP-QQQ parameters are summarized in Table 1. Dual O_2 flows were required when measuring the SO_2 in wine to reduce the background level and prevent interference from polyatomic ions: the proportion of optional oxygen, which was added directly in the carrier gas to prevent carbon buildup, was set to 5%; 30% pure O_2 was introduced in the collision/reaction cell to convert S^+ to SO^+ to distinguish S^+ from polyatomic interferences after mass selection of Q1. The Octp Bias and Kinetic Energy Discrimination (KED) values were also optimized when the ICP-QQQ was operated in the MS/MS mode.

Method validation

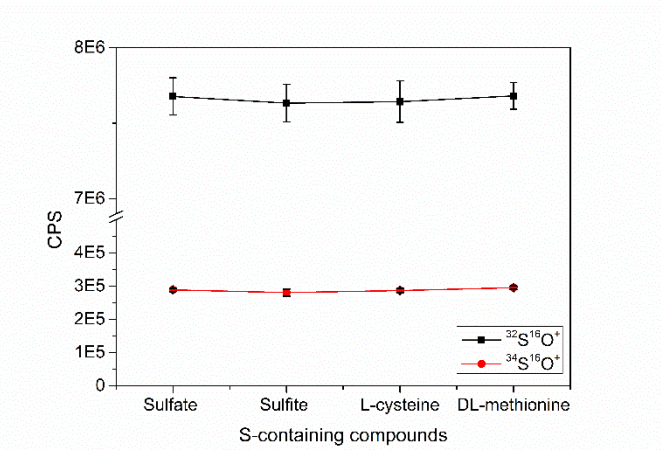


Figure 1. Comparison of the signal intensity of SO^+ in 0.1mM four S-containing compounds.

Since there are various S-containing compounds in real wine, including inorganic ions such as SO_3^{2-}, SO_4^{2-}, and organic compounds that S bonded to carbonyl group, the discrepancy of the ionization efficiency of different S-containing compounds, which is indicated by means of signal intensity of SO^+, has been examined firstly. Fig. 1 indicated the comparison of SO^+ signal

intensity in 0.1 mM SO_4^{2-}, SO_3^{2-}, L-cysteine and DL-methionine. The results manifested clearly that the signal intensity of SO^+ in different ions or molecules are decided by the S concentration merely. The discrepancy between the S-containing compounds have shown negligible influence on the ionization efficiency and signal intensity of SO^+, which is similar to the result which determines Br in various inorganic or organic compounds using ICP-QQQ. [17]

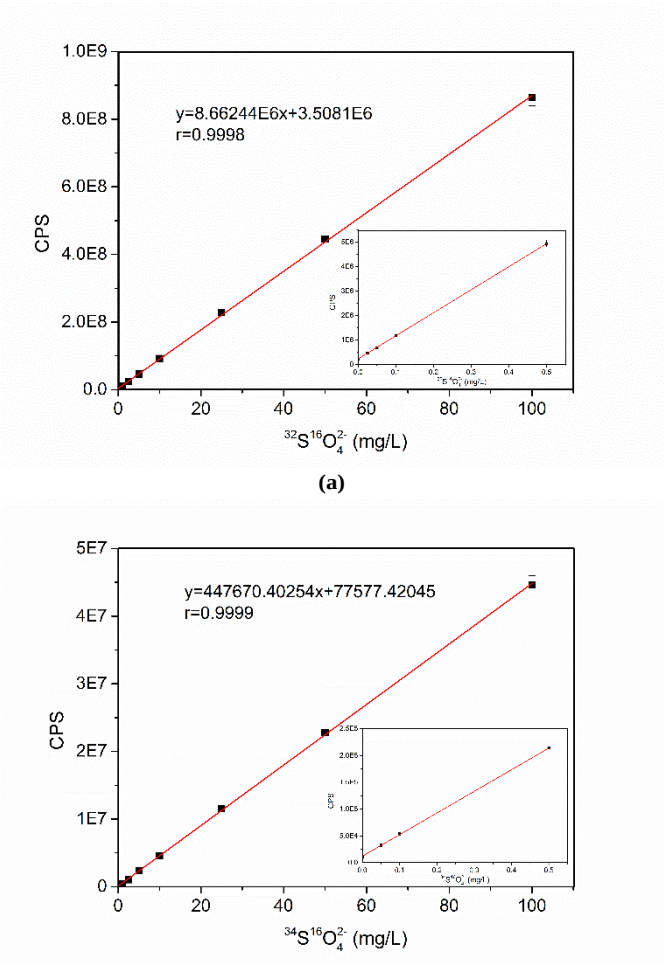


Figure 2. Calibration curves for (a) $^{32}S^{16}O_4^{2-}$ and (b) $^{34}S^{16}O_4^{2-}$ over a concentration range of 0.025–100 mg/L and 0.05–100 mg/L, respectively; the insert indicates the curve in the range of 0.025-0.5 mg/L.

Under these conditions, further assessments were performed to evaluate the practical application of the proposed method using SO_4^{2-}. The linearity was computed using sixteen standard solutions (0.01–500 mg/L) diluted with a 5% ethanol solution. Five measurements were performed for each level. We computed the internal standard calibration curves (Fig. 2) via performing a linear regression analysis of the ratio of the standard solution areas to the internal standard areas vs. the concentration, which confirmed satisfactory linearity in the range of 0.025–100 mg/L for $^{32}SO_4^{2-}$ and 0.05–100 mg/L for $^{34}SO_4^{2-}$, with correlation coefficients greater than 0.999 for both $^{32}SO_4^{2-}$ and $^{34}SO_4^{2-}$. The limit of quantification (LOQ) was 10 μg/L for $^{32}SO_4^{2-}$ and 25 μg/L for $^{32}SO_4^{2-}$. The LODs for the method were estimated according to the formula $\mathrm{LOD} = 3 \times s \times m^{-1}$, where s is the standard deviation of the response, and m is the slope of the calibration curve. Therefore, LOD values of 5 and 13 μg/L for $^{32}SO_4^{2-}$ and $^{34}SO_4^{2-}$ were obtained, which are close to the values reported by Balcaen et al.[15]

In addition, the $^{34}S/^{32}S$ ratios in 5% ethanol, the 50 μg/L standard solution and the 10-fold-diluted wine sample were 0.0491 ± 0.010, 0.0497 ± 0.015 and 0.0494 ± 0.013, respectively; these values are similar to the results reported by Fernández et al.[16] and the theoretical value of 0.0447 ± 0.0025 given by IUPAC.[18] The $^{34}S/^{32}S$ ratio in 5% ethanol indicated contamination from the solvent; the $^{32}SO_4^{2-}$ level in the solvent was approximately 35 μg/L, close to the value published by Fernández et al.[16] Because of its universal existence in water, reagents and instrument accessories,[13,19] further procedures will be needed to avoid contamination and acquire lower LODs.

According to previous studies and the difference between the experimental and theoretical $^{34}S/^{32}S$ values,[13,16,20,21] polyatomic interference was diminished in this study. Because of the addition and function of Q1, $^{32}S^+$ and $^{34}S^+$ were selected and entered the collision/reaction cell sequentially, which means either $^{32}S^{16}O^+$ (m/z = 48) or $^{34}S^{16}O^+$ (m/z = 50) will be generated and selected by Q2 separately. Simultaneously, the conversion of $S^+ \rightarrow SO^+$ occurred in collision/reaction cell will further distinguish the S^+ from the interference of polyatomic ions (such as O_2^+, NO^+, NOH^+ etc.). Therefore, ICP-QQQ proved its excellent ability in interference removal. In this study, $^{32}S^+ \rightarrow {}^{32}S^{16}O^+$ was chosen for quantification, $^{34}S^+ \rightarrow {}^{34}S^{16}O^+$ and $^{34}S/^{32}S$ ratio were monitored for qualitative analysis.

Determination of S in real wine samples

Because of the lack of certified reference materials, the SO_2 concentration in a real red wine sample was measured at different dilutions to investigate and determine the influence of the sample matrix. As shown in Table 2, the SO_2 concentrations determined at five dilutions were consistent; therefore, the influence from the wine matrix was limited and negligible. Since the concentration of total SO_2 were in the range of 50-150 mg/L for majority wine samples,[2] higher than the maxima point of the calibration curves, meant an appropriate dilution is necessity. On the other hand, a higher dilution fold was apt to increase the experiment error or fail to detect samples with low S concentration. Consequently, the subsequent red wine analyses were performed using a 20-fold dilution.

Table 2. Comparison of quantitative results at five dilution factors (mg/L, mean ± SD, n = 5).

Dilution	1:10	1:20	1:50	1:100	1:200
Concentration	150.1 ± 7.2	149.9 ± 4.7	153.9 ± 3.0	152.0 ± 3.4	151.2 ± 1.8

To validate the method and estimate its accuracy, the total SO_2 concentrations in ten red wine samples were measured using the proposed method and the titration method (OIV-MA-AS323-04A) recommended by the International Organisation of Vine and Wine. The recoveries at three different spike levels were evaluated. As shown in Table 3, the two methods showed a great agreement. The total SO_2 concentrations in ten wine samples were in the range of concentrations reported for Spanish wines,[2] and the recoveries ranged from 95 to 105%. The higher results obtained using the titration method may mainly be attributed to matrix effects from the red wine and the deferred judgments of the titration endpoint. In contrast, the matrix effects from red wine were well eliminated utilizing the proposed method.

Table 3. Comparison of the measurement method and spiked recoveries of total SO_2 in real wine samples (mean ± SD, n = 5).

Wine sample	Total SO_2 in wine (mg/L)		Spiked recovery (%)		
	ICP-QQQ	Titration	L*	M	H
1#	155 ± 3	156 ± 3	102	99	98
2#	95 ± 5	98 ± 3	103	102	98
3#	72 ± 1	73 ± 2	101	103	97
4#	76 ± 4	79 ± 4	95	100	101
5#	74 ± 3	77 ± 3	97	98	101
6#	96 ± 3	99 ± 3	103	98	101
7#	148 ± 2	150 ± 3	97	97	96
8#	123 ± 2	124 ± 3	95	105	95
9#	140 ± 3	143 ± 5	99	98	103
10#	109 ± 4	111 ± 5	102	97	104

*L: Low spiked level, 5 mg/L; M: Middle spiked level, 10 mg/L; H: High spiked level, 15 mg/L.

Conclusion

The present study demonstrates that the total SO_2 concentration in red wine can be accurately quantified without a tedious pre-treatment process through the utilizing of ICP-QQQ. The novel ICP-QQQ method provides an interference-free operation and, more important, the ionization efficiency of S-containing compounds is unanimous in the plasma, the signal intensity of SO^+ is merely decided by the concentration of S, regardless of the spices of S-containing compounds. The method exhibits good linearity (R > 0.999) over the range of 0.025–100 mg/L with an LOQ of 10 μg/L $^{32}SO_4^{2-}$ in wine. The total SO_2 concentrations in real red wine samples were quantified. We verified the accuracy of the proposed method by comparing the results with those obtained using the recommended titration method. Excellent agreement was observed between the results obtained using the two methods. An outstanding spiked recovery was achieved, which indicated that the influence from the sample matrix was negligible and well removed. A future study is planned that will focus on the determination of sulfur and sulfate in wine previously separated online using chromatography; these results will be presented elsewhere.

Acknowledgements

The authors gratefully acknowledge the financial support from the National Natural Science Foundation of China for Young Scholars (Grant No. 31301474), the Public Service Subject of Centers for Preventive Medical Research (Grant No. 2014-BJYJ-12), the National Key Technology R & D Program (Grant No. 2012BAK17B01) and the Beijing Municipal Senior Technical Training Plan in the Health System.

Notes and references

[a] Beijing Key Laboratory of Diagnostic and Traceability Technologies for Food Poisoning, Beijing Centers for Diseases Control and Prevention & Centers for Preventive Medical Research, Beijing 100013, China.

[b] State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P. O. Box 2871, Beijing 100085, China.

† Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

1 M. Huang, H. Becker-Ross, S. Florek, U. Heitmann, M. Okruss, *Analytical and Bioanalytical Chemistry*, 2005, **382**, 1877.
2 E. Mataix, M. D. Luque de Castro, *Analyst*, 1998, **123**, 1547.
3 S.K. Williams, J.R. Runyon, A.A. Ashames, *Analytical chemistry*, 2011, **83**, 634.
4 J. Vahl, J. Converse, *Journal-Association of Official Analytical Chemists*, 1980, **63**, 194.
5 J. Sullivan, T. Hollingworth, M. Wekell, V. Meo, H. Saba, A. Etemad-Moghadam, C. Eklund, J. Phillips, B. Gump, *Journal-Association of Official Analytical Chemists*, 1989, **73**, 35.
6 A. Maquieira, F. Casamayor, R. Puchades, S. Sagrado, *Analytica chimica acta*, 1993, **283**, 401.
7 L. Decnop-Weever, J. Kraak, Analytica chimica acta 337 (1997) 125.
8 T.J. Cardwell, R.W. Cattrall, G.N. Chen, G.R. Scollary, I. Hamilton, *Journal of AOAC International*, 1992, **76**, 1389.
9 M.D. Luque de Castro, I. Papaefstathiou, *TrAC Trends in Analytical Chemistry*, 1998, **17**, 41.
10 M.T. Gomes, T.A. Rocha, A.C. Duarte, J.P. Oliveira, *Analytical chemistry*, 1996, **68**, 1561.
11 D.P. Persson, T.H. Hansen, K.H. Laursen, J.K. Schjoerring, S. Husted, *Metallomics*, 2009, **1**, 418.
12 J. Heilmann, S. Boulyga, K. Heumann, *Analytical and Bioanalytical Chemistry*, 2004, **380**, 190.
13 S. Boulyga, J. Heilmann, K. Heumann, *Analytical and Bioanalytical Chemistry*, 2005, **382**, 1808.
14 D.R. Bandura, V.I. Baranov, S.D. Tanner, *Analytical chemistry*, 2002, **74**, 1497.
15 L. Balcaen, G. Woods, M. Resano, F. Vanhaecke, *Journal of Analytical Atomic Spectrometry*, 2013, **28**, 33.
16 S.D. Fernández, N. Sugishama, J.R. Encinar, A. Sanz-Medel, *Analytical Chemistry*, 2012, **84**, 5851.
17 K.Wang, L. Zheng, Y. Peng, J. E. Song, J. Zheng. *Analytical chemistry*, 2014, **86**, 10755.
18 M.E. Wieser, N. Holden, T.B. Coplen, J.K. Böhlke, M. Berglund, W.A. Brand, P. De Bièvre, M. Gröning, R.D. Loss, J. Meija, *Pure & Applied Chemistry*, 2013, **85**, 1407.
19 N. Zinn, R. Krüger, P. Leonhard, J. Bettmer, *Analytical and bioanalytical chemistry*, 2008, **391**, 537.
20 R. Clough, P. Evans, T. Catterick, E.H. Evans, *Analytical chemistry*, 2006, **78**, 6126.
21 J.S. Becker, *Journal of analytical atomic spectrometry*, 2002, **17**, 1172.