Cite this: *Anal. Methods*, 2015, 7, 3224

Direct quantification of total sulfur dioxide in wine using triple quadrupole ICP-MS

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The accurate determination of sulfur dioxide (SO₂) in wine remains a critical analytical task for food safety and in 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 SO₂ 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⁻¹, with a limit of quantification of 10 µg L⁻¹ for ³²S¹⁶O₄²⁻ in wine. The recoveries ranged from 95–105% for the spiked wine samples. The method was successfully applied for the determination of total SO₂ 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 the quantification of total SO₂ in wine with great convenience and high accuracy.

Received 3rd December 2014
Accepted 17th February 2015

DOI: 10.1039/c4ay02863e

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Introduction

As an essential additive for wine production and preservation, sulfur dioxide (SO₂) is added in wine to inhibit undesirable microbial growth and oxidation processes.^{1,2} However, high concentrations of SO₂ impart an unpleasant aroma and negatively affect human health.³ Therefore, the levels of SO₂ in wine are strictly controlled by legislation in various countries. SO₂ in wine usually exists as either free or bound; however, the total SO₂ is the primary concern for food safety.

As a commonly used method for the determination of total SO₂ 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 method, 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 susceptible interfered or failed to detect SO₂ in red wine because of the colour or the particulates from the matrix.^{9,10}

A more preferred method for the determination of SO₂ 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 SO₂ using a traditional quadrupole-based ICP-MS has been a challenge because of the high ionization potential of S and serious polyatomic interference.^{14,15} The recent introduction and application of the 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 interference. 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 present study aims to develop a highly sensitive, rapid, accurate and reproducible method for the quantitative determination of the total SO₂ in wine using ICP-QQQ without a tedious pre-treatment process. The advantages of ICP-QQQ were exploited to remove polyatomic interference more controllably and effectively. The analysis of the total SO₂ in wine was achieved without complex sample pre-treatments or chromatographic separations, and completed within minutes. Using this

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approach, the total SO₂ in wine can be accurately and precisely determined in a simple and efficient manner.

Experimental

Reagents and materials

Sulfate stock standard solution (SO₄^{2−}) (10 000 mg L^{−1}), sodium sulfite analytical standard (1 g), the L-cysteine certified reference material (100 mg), DL-methionine (99%, 5 g) and ethanol (≥99.8%) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). A mixed internal standard (100 mg L^{−1}) 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-lens ion lens was used. The ICP-QQQ was operated in the MS/MS mode because S suffers serious polyatomic interference 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 analysers Q1 and Q2 were set to Q1 → Q2: 32 → 48, 34 → 50, 45 → 45 *m/z*, with the integ. time/mass: 0.09 s, Q2 peak pattern: 3 points, replicates: 3, and sweeps: 10. The nebulizer pump was operated at 0.3 rps for 30 s for the uptake of each sample, then lowered to 0.1 rps in the 10 s stabilization and whole determination processes. All parameters were manually optimized to achieve the best signal intensity and stability, and the critical parameters are listed in Table 1. MassHunter software (Agilent Technologies, USA) was used to control the instrument and to process the data.

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 ^{−1}
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

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^{−1} were diluted from a 10 000 mg L^{−1} stock standard solution using 5% ethanol solution 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^{−1}) was diluted using 1 wt% (w/v) HNO₃ and maintained at 4 °C. ⁴⁵Sc was selected for internal standardization, since the signal intensity of ⁴⁵Sc was stable and the decline originating from the influence of O₂ 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 for 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 SO₄^{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 SO₂ in the wines was calculated using the formula $C_{\text{SO}_2} = \frac{2}{3} \times \text{DF} \times C_{\text{SO}_4^{2-}}$, where $\frac{2}{3}$ is the ratio of the molecular weights of SO₂ to SO₄^{2−}, DF is the dilution factor, and $C_{\text{SO}_4^{2-}}$ is the concentration of SO₄^{2−} measured using ICP-QQQ.

Results and discussion

ICP-QQQ optimization

A spiked wine sample was used to optimize the ICP-QQQ conditions. 1 mL of the wine sample was spiked with 1 mL of SO₄^{2−} standard solution (100 μg L^{−1}) 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 interference while maintaining excellent single intensity and sensitivity. The most important ICP-QQQ parameters are summarized in Table 1. Dual O₂ flows were required when measuring the SO₂ in wine to reduce the background level and to 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 O₂ was introduced in the collision/reaction cell to convert S⁺ to SO⁺ to distinguish S⁺ from polyatomic interference after the 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

Since there are various S-containing compounds in real wine, including inorganic ions such as SO₃^{2−}, SO₄^{2−}, and organic

compounds which are S bonded to the carbonyl group, the discrepancy of the ionization efficiency of different S-containing compounds, which is indicated by means of the signal intensity of SO^+ , has been examined firstly. Fig. 1 indicated the comparison of the SO^+ signal intensity in 0.1 mM SO_4^{2-} , SO_3^{2-} , L-cysteine and DL-methionine. The results clearly manifested that the signal intensity of SO^+ in different ions or molecules are merely decided by the S concentration. 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

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 ($0.01\text{--}500\text{ mg L}^{-1}$) 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 $0.025\text{--}100\text{ mg L}^{-1}$ for $^{32}\text{S}^{16}\text{O}_4^{2-}$ and $0.05\text{--}100\text{ mg L}^{-1}$ for $^{34}\text{S}^{16}\text{O}_4^{2-}$, with correlation coefficients greater than 0.999 for both $^{32}\text{S}^{16}\text{O}_4^{2-}$ and $^{34}\text{S}^{16}\text{O}_4^{2-}$. The limit of quantification (LOQ) was $10\text{ }\mu\text{g L}^{-1}$ for $^{32}\text{S}^{16}\text{O}_4^{2-}$ and $25\text{ }\mu\text{g L}^{-1}$ for $^{34}\text{S}^{16}\text{O}_4^{2-}$. The LODs for the method were estimated according to the formula $\text{LOD} = 3 \times s \times m^{-1}$, where s is the standard deviation of the response, and m is the slope of the calibration curve. Therefore, the LOD values of 5 and $13\text{ }\mu\text{g L}^{-1}$ for $^{32}\text{S}^{16}\text{O}_4^{2-}$ and $^{34}\text{S}^{16}\text{O}_4^{2-}$ were obtained, which are close to the values reported by Balcaen *et al.*¹⁵

In addition, the $^{34}\text{S}/^{32}\text{S}$ ratios in 5% ethanol, the $50\text{ }\mu\text{g L}^{-1}$ 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 $^{34}\text{S}/^{32}\text{S}$ ratio in 5% ethanol indicated contamination from the solvent; the $^{32}\text{S}^{16}\text{O}_4^{2-}$ level in the solvent was approximately $35\text{ }\mu\text{g L}^{-1}$, close to the value published by Fernández *et al.*¹⁶ Because of its universal existence in water,

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

Fig. 2 Calibration curves of (a) $^{32}\text{S}^{16}\text{O}_4^{2-}$ and (b) $^{34}\text{S}^{16}\text{O}_4^{2-}$ over a concentration range of $0.025\text{--}100\text{ mg L}^{-1}$ and $0.05\text{--}100\text{ mg L}^{-1}$, respectively; the inset indicates the curve in the range of $0.025\text{--}0.5\text{ mg L}^{-1}$.

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 $^{34}\text{S}/^{32}\text{S}$ values,^{13,16,20,21} polyatomic interference was diminished in this study. Because of the addition and function of Q1, $^{32}\text{S}^+$ and $^{34}\text{S}^+$ were selected and entered the collision/reaction cell sequentially, which means either $^{32}\text{S}^{16}\text{O}^+$ ($m/z = 48$) or $^{34}\text{S}^{16}\text{O}^+$ ($m/z = 50$) will be generated and selected by Q2 separately. Simultaneously, the conversion of $\text{S}^+ \rightarrow \text{SO}^+$ that occurred in the 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, $^{32}\text{S}^+ \rightarrow ^{32}\text{S}^{16}\text{O}^+$ was chosen for quantification, $^{34}\text{S}^+ \rightarrow ^{34}\text{S}^{16}\text{O}^+$ and $^{34}\text{S}/^{32}\text{S}$ ratios 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

Table 2 Comparison of quantitative results at five dilution factors (mg L^{-1} , mean $\pm$ SD, and $n = 5$)

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

Table 3 Comparison of the measurement method and spiked recoveries of total SO_2 in real wine samples (mean $\pm$ SD and $n = 5$)

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

^a L: the low spiked level, 5 mg L^{-1} ; M: the middle spiked level, 10 mg L^{-1} ; H: the high spiked level, 15 mg L^{-1} .

determined at five dilutions were consistent; therefore, the influence from the wine matrix was limited and negligible. Since the concentrations of the total SO_2 were in the range of 50–150 mg L^{-1} for the majority of wine samples,² higher than the maxima point of the calibration curves meant that an appropriate dilution is necessary. 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.

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 the 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.

Conclusions

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 utilization 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^{-1} with an LOQ of 10 $\mu\text{g L}^{-1}$ $^{32}\text{S}^{16}\text{O}_4^{2-}$ 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 these 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 that will focus on the determination of sulfur and sulfate in wine previously separated online using chromatography is planned; these results will be presented elsewhere.

Acknowledgements

The authors gratefully acknowledge 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.

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