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*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/1851518> since 2023-02-22T16:34:28Z

*Published version:*

DOI:10.1016/j.measurement.2022.111047

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## Journal Pre-proofs

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PII: S0263-2241(22)00313-X

DOI: <https://doi.org/10.1016/j.measurement.2022.111047>

Reference: MEASUR 111047

To appear in: *Measurement*

Received Date: 27 December 2021

Revised Date: 10 March 2022

Accepted Date: 16 March 2022



Please cite this article as: I. Cagnasso, A. Sacco, M. Petrozziello, F. Bonello, A. Asproudi, S. Giacosa, A.M. Rossi, F. Durbiano, Evidence of a systematic underestimation of free sulfur dioxide by the aeration-oxidation method approved by OIV, *Measurement* (2022), doi: <https://doi.org/10.1016/j.measurement.2022.111047>

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## Evidence of a systematic underestimation of free sulfur dioxide by the aeration-oxidation method approved by OIV

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### Abstract

The aeration-oxidation method stated by the International Organisation of Vine and Wine (OIV) in the Resolution OIV-OENO 591A-2018 is internationally recognized as one of the election technique for free SO<sub>2</sub> quantification in wine.

The present work highlighted that an underestimation of the SO<sub>2</sub> content is obtained by applying this 591A method. A measurement uncertainty evaluation on the basis of the Guide to the Expression of Uncertainty in Measurement and of the Monte Carlo simulations was carried out on a wine simulant solution with a known concentration of free SO<sub>2</sub>, prepared by gravimetry. By comparison between this 591A and the gravimetric methods, the obtained data resulted to be not consistent. To confirm this underestimation, measurement experiments were conducted by nine specialized laboratories with the 591A method on the same sample and none of which estimated the amount of free SO<sub>2</sub> which was present in the wine simulant.

**Keywords:** SO<sub>2</sub>, 591A method, Resolution OIV-OENO 591A-2018, wine simulant, total recovery, measurement uncertainty.

### 1. Introduction

Sulfites represent one of the most used food additives thanks to their preservative properties [1]. The term “sulfites” is generically related to a class of chemical compounds which release sulphur dioxide (SO<sub>2</sub>). In wine, free and bound SO<sub>2</sub> are distinguished. The first form includes the molecular SO<sub>2</sub> fraction, active against microorganisms, and the bisulfite ion HSO<sub>3</sub><sup>-</sup> fraction, mainly known for its antioxidant capabilities. These two fractions are in equilibrium according to wine pH, temperature, and alcohol strength. Free SO<sub>2</sub> becomes volatile under acid conditions. The bound SO<sub>2</sub> refers to the fraction of sulfites that spontaneously react with organic compounds of wine samples creating bonds with the matrix components. These bonds require high temperature and acidic conditions to be broken and to make sulphites volatile. Wine total SO<sub>2</sub> is defined as the sum of free and bound forms.

The Regulation (EU) 2019/934 [2] established legal limits for total SO<sub>2</sub> of 150 mg/l in red wines and 200 mg/l in white wines, given that it is an allergen provoking bronchial spasms, hives and bronchoconstriction in hypersensitive individuals [3,4]. Numerous measurement methods are known for the determination of SO<sub>2</sub> content in wine [5–9].

The International Organisation of Vine and Wine (OIV), an intergovernmental organisation of a scientific and technical nature, drew up a compendium of the official analytical methods for the determination of several parameters and analytes in wine and must [10]. Among these official methods some are devoted to the determination of free SO<sub>2</sub>. One in particular, the aeration-oxidation method described in the Resolution OIV-OENO 591A-2018 [11], is the method of choice for the free SO<sub>2</sub> determination, routinely used in the analysis laboratories and in the centres that perform a control role. In fact, this 591A method is described as simple, robust and reliable. On the other hand, the Resolution OIV-OENO 591B-2018 [12] describes the method for quantifying the bound SO<sub>2</sub> in wine and must.

This work proposes to investigate the 591A method to ascertain whether a complete extraction of the free SO<sub>2</sub> content is obtained. A measurement uncertainty assessment of free SO<sub>2</sub> concentration was carried out to identify the parameters that mostly affect the measurement result and to verify the consistency of the results obtained by the 591A method and a gravimetric method. A wine simulant solution that permits, by an addition of potassium metabisulfite, to calculate the free SO<sub>2</sub> concentration and that meets the requirements of commutability with real wine was prepared. In the used wine simulant no organic components were present. Consequently, the totality of the added SO<sub>2</sub> remains in free form. Free and total SO<sub>2</sub> are equivalent in this case. A concentration of free SO<sub>2</sub> close to the legal limit for total SO<sub>2</sub> in red wine (150 mg/l) was used. Moreover, as a confirmation, measurement experiments by specialized laboratories on the same sample were conducted. In these cases, only the uncertainty due to the repeatability of the result was taken into account, because this is what these laboratories commonly consider in their routine measurements in the wine sector. In fact, an uncertainty assessment of the obtained result is not a mandatory requirement for their analyses.

To the best of the authors' knowledge there have been only a few attempts to calculate in detail the uncertainty of SO<sub>2</sub> determination in food [13], and even fewer to characterize its quantification in wine [14]. A reliable assessment to verify if a complete extraction of SO<sub>2</sub> occurred applying the 591A method improves and strengthens the work carried out by the OIV.

## 2. Materials and methods

### 2.1 Chemicals

The wine simulant solution was prepared aiming to recreate the basic characteristics of an average wine [15,16]. It contained 12 ml of ethanol per 100 ml of water (Carlo Erba) because the alcohol content in wine typically ranges from 10 ml to 14 ml of ethanol per 100 ml of water [17]. 5 g/l of tartaric acid (C<sub>4</sub>H<sub>6</sub>O<sub>6</sub>, Carlo Erba) were added to lower the pH towards the acidic conditions and to mimic the organic composition of the real samples without create bonds with SO<sub>2</sub>. Indeed, tartaric acid is a non-volatile, most abundant acid in wine, with a concentration which ranges from 2 g/l to 6 g/l [17]. The solution was buffered at pH 3.2, typical of wine, using 1 mol/l NaOH solution (Panreac). Lastly, potassium metabisulfite — K<sub>2</sub>S<sub>2</sub>O<sub>5</sub> in powder form, with purity 97 % (Acros Organics), was added to the wine simulant to reach the expected concentrations of SO<sub>2</sub> considering their stoichiometric ratio [17,18]. In the wine simulant solution studied in this work, the free form of SO<sub>2</sub> is the only one present and thus it was taken into account.

For the quantitative determination of the sulfuric acid by titration, a nominal 1×10<sup>-2</sup> mol/l NaOH solution was prepared by NaOH in anhydrous pellets (Panreac) and it was standardized against a 1×10<sup>-2</sup> mol/l potassium

hydrogen phthalate (KHP) —  $C_8H_5KO_4$  (Alfa Aesar) solution. The phenolphthalein indicator reagent for this standardization was prepared adding  $2.5 \times 10^{-1}$  g of indicator in  $5.0 \times 10^{-2}$  l of hydroalcoholic solution with 50 % of ethanol.

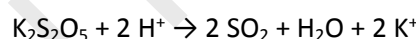
Regarding the measurement method for the determination of free  $SO_2$  close to the legal limit, aliquots of 15 ml of solution containing 85 g of phosphoric acid —  $H_3PO_4$  per 100 g of water (Honeywell Fluka™) were used to acidify the wine simulant solution and to promote the volatility of  $SO_2$ , as reported in the OIV Resolution. The indicator solution, named Flask B solution, was prepared by diluting a hydrogen peroxide —  $H_2O_2$  solution in 10 volumes (3 %, Innovative Naturopathics – Food Grade) to 3 volumes and adding 3 drops of indicator reagent which make the solution violet. The Flask B solution was then neutralized by adding drops of nominal  $1 \times 10^{-2}$  mol/l NaOH solution until the former reached a green colour.

The indicator reagent was prepared by mixing  $1.00 \times 10^{-1}$  g of Methyl Red (Merck),  $5.0 \times 10^{-2}$  g of Methylene Blue (Merck),  $5.0 \times 10^{-2}$  l of a solution containing at least 95 ml of ethanol per 100 ml of water and ultra-pure water (Merck-Milli-Q) to reach a volume of  $1.00 \times 10^{-1}$  l [11,19].

The above described solutions were freshly prepared before measurements.

## 2.2 Measurement procedures

For the gravimetric determination of the concentration of free  $SO_2$ , a wine simulant was prepared. 1.34160 g of potassium metabisulfite ( $K_2S_2O_5$ ) with an expanded uncertainty ( $k = 2$ ) of 0.00031 g were weighed (Denver instrument analytical balance) and put, as the last ingredient, in a calibrated flask already containing the hydroalcoholic solution, the tartaric acid and the NaOH solution, as described in Sec. 2.1. Hydroalcoholic solution was then added to reach the graduation mark of the flask which had the volume of 4.9971 l and an expanded uncertainty of 0.0024 l for a level of confidence 95 % and  $k = 2$ .  $SO_2$  is generated from  $K_2S_2O_5$  as follows:



The gravimetric concentration of free  $SO_2$ ,  $y_{SO_2,g}$ , expressed in mg/l, in a wine simulant solution, was estimated by the following equation [20]:

$$y_{SO_2,g} = \frac{m_{K_2S_2O_5} \cdot P_{K_2S_2O_5} \cdot 10^3}{V} \cdot \frac{MM_{SO_2}}{MM_{K_2S_2O_5}} \cdot 2 \quad (1)$$

where:

- $m_{K_2S_2O_5}$  is the mass, expressed in g, of potassium metabisulfite added to the hydroalcoholic solution,
- $P_{K_2S_2O_5}$  is the purity of potassium metabisulfite,
- $10^3$  is a multiplicative factor for the conversion from g to mg,
- $MM_{SO_2}$  is the molar mass, expressed in g/mol, of  $SO_2$ ,
- 2 is a multiplicative factor due to stoichiometric ratio between potassium metabisulfite and  $SO_2$ ,
- $V$  is the volume, expressed in l, of the prepared wine simulant solution,
- $MM_{K_2S_2O_5}$  is the molar mass, expressed in g/mol, of potassium metabisulfite.

For the determination of free  $SO_2$  concentration with the 591A method, the standardization process of the nominal  $1 \times 10^{-2}$  mol/l NaOH solution was firstly performed by titrimetry. The nominal  $1 \times 10^{-2}$  mol/l NaOH solution was standardised against the KHP following the procedure reported in QUAM [21]. The end-point was determined by the addition of phenolphthalein, as an indicator. The concentration of the KHP solution,  $c_{KHP}$ , was determined on the basis of equation (2):

$$c_{\text{KHP}} = \frac{m_{\text{KHP}} \cdot P_{\text{KHP}}}{MM_{\text{KHP}} \cdot V_{\text{Sol-KHP}}} \quad (2)$$

where:

- $m_{\text{KHP}}$  is the mass, expressed in g, of potassium hydrogen phthalate,
- $P_{\text{KHP}}$  is the purity of potassium hydrogen phthalate,
- $MM_{\text{KHP}}$  is the molar mass, expressed in g/mol, of potassium hydrogen phthalate,
- $V_{\text{Sol-KHP}}$  is the volume, expressed in l, of potassium hydrogen phthalate solution prepared.

$c_{\text{NaOH}}$  was then determined on the basis of equation (3):

$$c_{\text{NaOH}} = \frac{c_{\text{KHP}} \cdot V_{\text{KHP}}}{V_{\text{NaOH.St}}} \quad (3)$$

where:

- $V_{\text{KHP}}$  is the volume, expressed in l, of potassium hydrogen phthalate solution in the flask used for the titration,
- $V_{\text{NaOH.St}}$  is the volume, expressed in l, of NaOH needed for the titration.

After the standardization process was completed, the free  $\text{SO}_2$  concentration by 591A method,  $y_{\text{SO}_2.\text{OIV}}$ , was determined. The 591A method consists of an extraction phase (aeration-oxidation) and a titration phase. A picture of the INRiM measurement system for the extraction phase is shown in Figure 1. A sample of the wine simulant is placed in the Flask A, where  $\text{SO}_2$  is extracted after the acidification of the sample.  $\text{SO}_2$  is entrained by a current of nitrogen into the Flask B, which contains a neutral solution of  $\text{H}_2\text{O}_2$  and the indicator solution. Here,  $\text{SO}_2$  is oxidised and  $\text{H}_2\text{SO}_4$  occurred. In the second phase,  $\text{SO}_2$  is then determined by titration of  $\text{H}_2\text{SO}_4$  with a standard solution of NaOH. For construction details see the OIV Resolution.

The extraction phase of the INRiM measurement system presents the following differences with respect to the scheme reported in the 591A method: *i)* a flow of nitrogen, located upstream of the measurement system, makes the wine simulant gurgle and facilitates the  $\text{SO}_2$  extraction, while a mass flow controller keeps constant the nitrogen flow; *ii)* besides Flask A, for the wine simulant sample, and Flask B, containing the indicator solution (named Flask B solution), for the collection of  $\text{SO}_2$ , the Flask C, containing the same indicator solution, was added in series to the system in order to gather possible  $\text{SO}_2$  gas not transformed in the Flask B; *iii)* Flask A and Flask B are kept dipped in water baths in order to smooth the possible temperature variations.

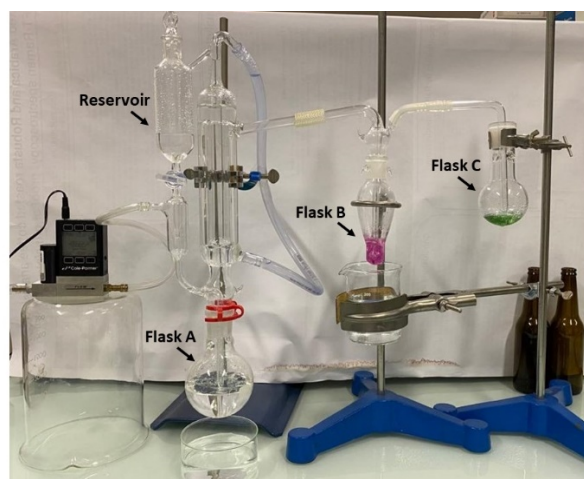
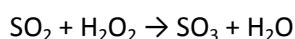
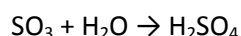


Figure 1: INRiM measurement system for the extraction phase of the determination of free SO<sub>2</sub> by 591A method

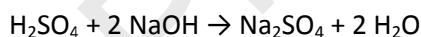
The measurement system was located in a laboratory where temperature was kept constant at  $(21 \pm 3)$  °C. Nominal  $5 \times 10^{-2}$  l of wine simulant were placed into the Flask A of the apparatus. Flask B and Flask C were filled with  $3 \times 10^{-3}$  l of indicator solution each.  $1.5 \times 10^{-2}$  l of H<sub>3</sub>PO<sub>4</sub> were placed into the Reservoir. By adding the H<sub>3</sub>PO<sub>4</sub> into the wine simulant, pH was lowered to less than 1, promoting the volatilization of SO<sub>2</sub>. A flow of N<sub>2</sub> gas kept constant at  $(6.67 \times 10^{-1} \pm 0.067 \times 10^{-1})$  l/min (Cole-Parmer mass flow controller) facilitated the extraction of SO<sub>2</sub> and channelled it into the Flask B solution. Here, SO<sub>2</sub> is oxidized by the H<sub>2</sub>O<sub>2</sub> to sulfuric acid — H<sub>2</sub>SO<sub>4</sub>, resulting in lowering of pH of the Flask B solution and in a colour change from green to violet [19]. The following reactions occurred:



and



According to the 591A method, the extraction phase is concluded after 15 minutes. Nevertheless, in this work, two consecutive cycles of 15 minutes each, with the relative addition of H<sub>3</sub>PO<sub>4</sub>, were adopted in order to ascertain the conclusion of the SO<sub>2</sub> extraction process. After these cycles, the Flask B was removed from the apparatus and the pipes close to it were rinsed with ultra-pure water paying attention to collect it into Flask B solution. The amount of generated H<sub>2</sub>SO<sub>4</sub> was determined by titration with the standardized  $1 \times 10^{-2}$  mol/l NaOH solution. The titration reaction was the following:



Eventually, for the determination of  $y_{\text{SO}_2, \text{OIV}}$ , expressed in mg/l, the following equation (4) was used:

$$y_{\text{SO}_2, \text{OIV}} = MM_{\text{SO}_2} \cdot \frac{V_{\text{NaOH}} \cdot c_{\text{NaOH}}}{2 \cdot V_{\text{wine}}} \cdot 10^3 \quad (4)$$

where:

- $V_{\text{NaOH}}$  is the volume, expressed in l, of NaOH solution used to titrate H<sub>2</sub>SO<sub>4</sub>,
- $c_{\text{NaOH}}$  is the molar concentration of NaOH solution, the nominal value of which was  $1 \times 10^{-2}$  mol/l,
- $V_{\text{wine}}$  is the volume, expressed in l, of the wine sample,
- $10^3$  is a multiplicative factor for the conversion from g to mg,
- 2 is the stoichiometric factor between NaOH and SO<sub>2</sub> (or H<sub>2</sub>SO<sub>4</sub>) moles.

In the equation (4), it is possible to use  $MM_{\text{SO}_2}$  instead of  $MM_{\text{H}_2\text{SO}_4}$  since stoichiometric ratio between SO<sub>2</sub> and H<sub>2</sub>SO<sub>4</sub> is 1 to 1, as reported in the double chemical reaction mentioned above. Equation (4) was applied for the evaluation of the estimate, in place of the approximations provided by the OIV Resolution, and was taken as the model for the uncertainty evaluation of the obtained result.

### 3. Results and discussion

To verify whether a complete determination of free SO<sub>2</sub> concentration in a wine simulant solution by the 591A method was achieved, firstly a rigorous measurement uncertainty of the result obtained at INRiM on a wine simulant solution was assessed.



The values of the uncertainty contributions were reported considering 2 significant digits. The corresponding values of the estimated quantities were reported accordingly, even if the related instruments were equipped with a lower number of digits.

The wine simulant was gravimetrically prepared to be representative of a real wine sample with respect to the free  $\text{SO}_2$  concentration.

Molar masses of  $\text{K}_2\text{S}_2\text{O}_5$ ,  $\text{SO}_2$ , and KHP, with their respective uncertainties, were estimated by the Monte Carlo method [22] as the sum of the masses of the elements constituting them, employing as input the standard atomic weights values recommended by the IUPAC Commission on Isotopic Abundances and Atomic Weights in 2013 [23]. The values of the atomic weights are reported in Table 1. These were reported either as weight intervals or as average values with their uncertainties: their distribution was considered uniform for intervals, and normal for estimated values with their respective uncertainties.

Table 1. Atomic weights values employed in this work. Values and shape of distributions were considered as according to [23]. The potassium distribution is normal: it is reported as an average value with associated an expanded uncertainty considering  $k = 2$ .

Element	Distribution	Atomic weight (g/mol)
H	Uniform	(1.00784, 1.00811)
C	Uniform	(12.0096, 12.0116)
O	Uniform	(15.99903, 15.99977)
S	Uniform	(32.059, 32.076)
K	Normal	39.0983(1)

The number of trials for the Monte Carlo method was  $10^6$  [24]. Calculations were performed with the method recommended in the IUPAC guidelines [25]. In Figure 2, input and output quantities distributions histograms in the calculations of the  $\text{K}_2\text{S}_2\text{O}_5$ ,  $\text{SO}_2$ , and KHP molar masses are shown.

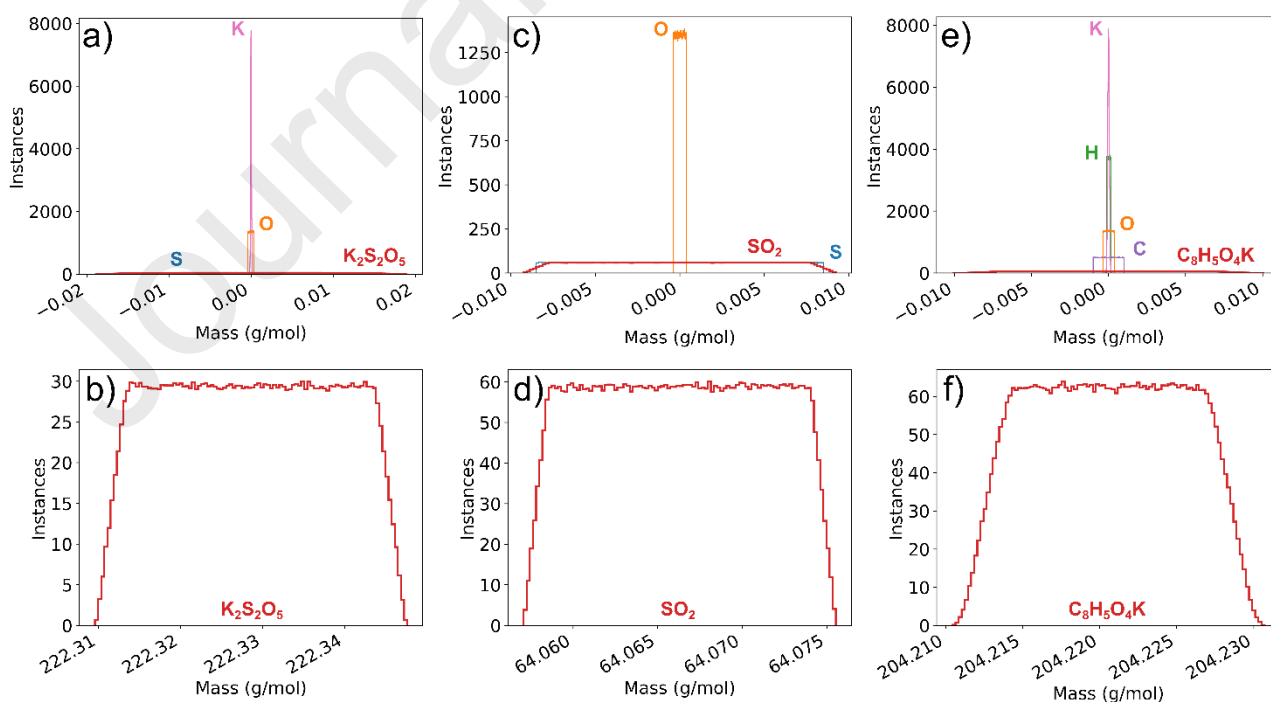




Figure 2. Input and output quantities distributions histograms in the calculations of the molar masses of  $K_2S_2O_5$  (a, b),  $SO_2$  (c, d) and KHP (e, f) with the Monte Carlo method ( $10^6$  trials per molecule, represented with 100 bins per distribution). a), c), e): input and output pdfs, with annotations of their corresponding colour for each (output quantities in red); all distributions are centred to zero for mutual comparison. b), d), f): output quantities, detailed graphs of the calculated mass distributions of the corresponding molecules.

The uniform distribution is very different from the normal one, and while the sum of as few as three independent rectangular distributions of similar variance could be considered approximately normal [26], this is not the case when the variances are diverse, as is the case for  $K_2S_2O_5$  and  $SO_2$ . The Monte Carlo method is the recommended approach, as indicated in the GUM (which is the responsibility of the Joint Committee for Guides in Metrology), for the evaluation of uncertainties of calculated quantities, especially when the conditions for applying the Central Limit Theorem are not met.

The free  $SO_2$  concentration,  $y_{SO_2.g}$ , obtained by a gravimetric method was determined applying equation (1), which was also used as a model for the uncertainty evaluation of  $y_{SO_2.g}$ .

- The molar mass of  $K_2S_2O_5$  was evaluated from the values in Table 1. The resulting distribution exhibits an average molar mass,  $MM_{K_2S_2O_5}$ , equal to 222.3286 g/mol.
- The molar mass of  $SO_2$  was calculated as the sum of three uniform distributions of the elements S and O (see Table 1). This resulted in an average molar mass of  $SO_2$ ,  $MM_{SO_2}$ , equal to 64.0663 g/mol.
- The mass of  $K_2S_2O_5$  was weighed on a balance with a last digit of  $1.0 \times 10^{-4}$  g. For the uncertainty evaluation associated with the balance, only the linearity was considered. The calibration certificate of the balance quoted an expanded uncertainty ( $k = 2$ )  $3.0 \times 10^{-4}$  g.  $u(m_{K_2S_2O_5})$  was calculated considering the certificated value having a normal distribution.
- The volume of the large flask was calibrated in accordance with the EURAMET Guide No. 19 [27].
- $P_{K_2S_2O_5}$  was quoted with a purity of at least 97 % and an expanded uncertainty of 1 %. A uniform distribution was associated.

In Table 2 the uncertainty budget associated with  $y_{SO_2.g}$  is summarized. All input quantities were considered independent.

Table 2. Uncertainty budget associated with  $y_{SO_2.g}$

Quantity, $X_i$	Estimate	Standard uncertainty, $u$	Distribution	Sensitivity coefficient, $c_i$
$m_{K_2S_2O_5}$	1.3416 g	0.00015 g	Normal	111.874 1/l
$V$	4.9969 l	0.0012 l	Normal	-30.037 g/l <sup>2</sup>
$P_{K_2S_2O_5}$	0.97	0.00577	Uniform	154.733 g/l
$MM_{SO_2}$	64.0663 g/mol	0.0049 g/mol	Approx. uniform	2.34274 mol/l
$MM_{K_2S_2O_5}$	222.3286 g/mol	0.0099 g/mol	Approx. triangular	0.67508 mol/l

The combined standard uncertainty of  $y_{SO_2.g}$ ,  $u_c(y_{SO_2.g})$ , was evaluated applying the uncertainty propagation law.  $u_c(y_{SO_2.g})$  resulted normally distributed by:

$$u_c(y_{SO_2.g}) = \sqrt{(c(m_{K_2S_2O_5})^2 \cdot u(m_{K_2S_2O_5})^2 + c(V)^2 \cdot u(V)^2 + c(P_{K_2S_2O_5})^2 \cdot u(P_{K_2S_2O_5})^2 + \dots \\ \dots + c(MM_{SO_2})^2 \cdot u(MM_{SO_2})^2 + c(MM_{K_2S_2O_5})^2 \cdot u(MM_{K_2S_2O_5})^2)}$$

$y_{SO_2.g}$  was evaluated 150.1 mg/l with an expanded uncertainty of 1.8 mg/l for the level of confidence 95 % and  $k = 2$ .

The first step in the determination of free  $\text{SO}_2$  concentration in the wine simulant by the 591A method was devoted to the standardization process, that is the determination of  $c_{\text{NaOH}}$ . The concentration of the KHP solution was determined by equation (2). A mass of KHP of 1.02280 g with an expanded uncertainty  $3.1 \times 10^{-4}$  g for the level of confidence 95 % and  $k = 2$  was weighed and added to  $5.0000 \times 10^{-1}$  l with an expanded uncertainty ( $k = 2$ ) of  $0.0010 \times 10^{-1}$  l of ultra-pure water.  $c_{\text{KHP}}$  was estimated  $1.002 \times 10^{-2}$  mol/l. Successively,  $c_{\text{NaOH}}$  was determined by equation (3). The NaOH solution with nominal concentration  $1 \times 10^{-2}$  mol/l was prepared starting from NaOH pellets. 9 titration repetitions were carried out introducing  $4.000 \times 10^{-3}$  l aliquots of KHP solution,  $V_{\text{KHP}}$ , in a flask and titrating them with the NaOH solution. The repetitions are reported in Table S1 of the Supplementary data. The mean value,  $V_{\text{NaOH.St}}$ , was calculated of  $4.2611 \times 10^{-3}$  l.

The uncertainty budget for the standardization process of  $c_{\text{NaOH}}$  was calculated considering the following contributions:

- The masses of KHP and of NaOH were weighed on a balance with a last digit of  $1.0 \times 10^{-4}$  g. For the uncertainty evaluation associated with the balance, only the linearity was considered. The calibration certificate of the balance quoted an expanded uncertainty of  $\pm 3.0 \times 10^{-4}$  g. The standard uncertainty of  $m_{\text{KHP}}$ ,  $u(m_{\text{KHP}})$ , was calculated considering the certificated value having a normal distribution ( $k = 2$ ).
- $P_{\text{KHP}}$  was quoted to be within the limits of 99.95 % and 100.05 %. Therefore,  $P_{\text{KHP}} = 1.00000 \pm 0.00050$ . The uncertainty  $u(P_{\text{KHP}})$  was taken as having a uniform distribution.
- The molar mass of KHP and its uncertainty were calculated by the Monte Carlo method from the values reported in Table 1 [23,28] and the distributions shown in Figure 2e,f. The resulting molar mass of  $\text{C}_8\text{H}_5\text{O}_4\text{K}$  was calculated as 204.2206 g/mol.
- The uncertainty associated with the volume of KHP solution,  $u(V_{\text{Sol-KHP}})$ , was evaluated considering the limits of accuracy of the volumetric flask, reported by the manufacturer. For the  $5.0000 \times 10^{-1}$  l volumetric flask this value was  $\pm 5.0 \times 10^{-4}$  l.  $u(V_{\text{Sol-KHP}})$  was evaluated assuming a triangular distribution, due to the dominant value in correspondence of the meniscus.
- The uncertainty contribution due to the volume of the KHP solution in the flask used for the titration was determined on the basis of the accuracy of the delivered volume reported by the manufacturer. For a  $1.00 \times 10^{-3}$  l pipette this value was  $\pm 1.0 \times 10^{-4}$  l.  $u(V_{\text{KHP}})$  was evaluated assuming a triangular distribution.
- Five uncertainty sources were associated to  $V_{\text{NaOH.St}}$ . The first was due to the repeatability,  $u(\text{Rep.st})$ , of the 9 titration measurements. The second regarded the uncertainty of the burette calibration,  $u(\text{Cal.st})$ , where the accuracy of the delivered volume was reported by the manufacturer. For a  $2.5000 \times 10^{-2}$  l burette this value was  $\pm 3.0 \times 10^{-5}$  l assuming a triangular distribution. The third source of uncertainty was associated to the difference between the temperature in the laboratory and that of the calibration,  $u(\Delta T.st)$ . It was estimated to be  $\pm 3$  °C with a 95 % confidence ( $k = 2$ ). Using the coefficient of volume expansion for aqueous solution as  $2.1 \times 10^{-4}$  °C<sup>-1</sup> and considering a mean value of  $4.2611 \times 10^{-3}$  l of the NaOH solution required for the titration of the KHP solution. The fourth source of uncertainty was due to the end-point detection,  $u(\text{E-P.st})$ . It was related to the last drop of the nominal  $1 \times 10^{-2}$  mol/l NaOH solution used to reach the end-point detection, considering that potentially not the whole drop was needed for the titration of KHP. A rectangular distribution was associated. The fifth source of uncertainty was related to the use of phenolphthalein as a visual indicator for the end-point of titration. Since there is a difference between the equivalent point and the end-point, the uncertainty due to this indicator,  $u(\text{Ind.st})$ , was evaluated accordingly to what

reported by Forigua & Meija [29,30]. In Table 3 the uncertainty contributions of  $V_{\text{NaOH.st}}$  are summarised.

Table 3. Uncertainty contributions of  $V_{\text{NaOH.st}}$

Quantity, $X_i$	Standard uncertainty, $u$	Distribution
$u(\text{Rep.st})$	$1.1 \times 10^{-5}$ l	Normal
$u(\text{Cal.st})$	$1.2 \times 10^{-5}$ l	Uniform
$u(\Delta T.\text{st})$	$1.4 \times 10^{-6}$ l	Normal
$u(E-P.\text{st})$	$1.4 \times 10^{-5}$ l	Uniform
$u(\text{Ind.st})$	$3.0 \times 10^{-5}$ l	Uniform

By appropriately combining the standard uncertainties of the input estimates, the combined standard uncertainty of  $V_{\text{NaOH.St}}$ ,  $u(V_{\text{NaOH.St}})$ , normally distributed, was evaluated:

$$u(V_{\text{NaOH.St}}) = \sqrt{(u(\text{Cal.st})^2 + u(\Delta T.\text{st})^2 + u(\text{Rep.st})^2 + u(E - P.\text{st})^2 + u(\text{Ind.st})^2)} = 3.7 \times 10^{-5} \text{ l}$$

In Table 4 the uncertainty budget of  $c_{\text{NaOH}}$  is reported.

Table 4. Uncertainty budget associated with  $c_{\text{NaOH}}$

Quantity, $X_i$	Estimate	Standard uncertainty, $u$	Distribution	Sensitivity coefficient, $c_i$
$m_{\text{KHP}}$	1.02280 g	$1.5 \times 10^{-4}$ g	Normal	$9.2002 \times 10^{-3}$ mol/gxl
$P_{\text{KHP}}$	1.00000	$2.9 \times 10^{-4}$	Uniform	$9.41 \times 10^{-3}$ mol/l
$MM_{\text{KHP}}$	204.2206 g/mol	$4.7 \times 10^{-3}$ g/mol	Approx. normal	$-4.60776 \times 10^{-5}$ mol <sup>2</sup> /gxl
$V_{\text{Sol-KHP}}$	$5.0000 \times 10^{-1}$ l	$2.0 \times 10^{-4}$ l	Triangular	$-0.01882$ mol/l <sup>2</sup>
$V_{\text{KHP}}$	$4.000 \times 10^{-3}$ l	$4.1 \times 10^{-5}$ l	Triangular	$2.3525$ mol/l <sup>2</sup>
$V_{\text{NaOH.St}}$	$4.261 \times 10^{-3}$ l	$3.7 \times 10^{-5}$ l	Normal	$-2.20892$ mol/l <sup>2</sup>

$u(c_{\text{NaOH}})$  resulted normally distributed by:

$$u(c_{\text{NaOH}}) = \sqrt{(c(m_{\text{KHP}})^2 \cdot u(m_{\text{KHP}})^2 + c(P_{\text{KHP}})^2 \cdot u(P_{\text{KHP}})^2 + c(MM_{\text{KHP}})^2 \cdot u(MM_{\text{KHP}})^2 + \dots \\ \dots + c(V_{\text{Sol-KHP}})^2 \cdot u(V_{\text{Sol-KHP}})^2 + c(V_{\text{KHP}})^2 \cdot u(V_{\text{KHP}})^2 + c(V_{\text{NaOH.St}})^2 \cdot u(V_{\text{NaOH.St}})^2)}$$

On the basis of equations (2) and (3),  $c_{\text{NaOH}}$  was estimated  $9.40 \times 10^{-3}$  mol/l and the combined uncertainty  $1.3 \times 10^{-4}$  mol/l.

For the determination of free  $\text{SO}_2$  concentration in the wine simulant by the 591A method, the standardized NaOH solution was specially prepared. The wine simulant and the Flask B solution were freshly prepared. Moreover, Flask A and Flask B were kept in containers with water to attenuate their temperature variations. A flow of nitrogen was kept constant during the  $\text{SO}_2$  extraction phase by a mass flow controller and the experiments were performed consecutively in order to avoid the sample degradation. The whole apparatus was washed with ultra-pure water between one measurement and the other to recreate the same starting conditions of the measurements. The indicator solution contained in Flask C did not change colour during the experiments, demonstrating that there was not a loss of  $\text{SO}_2$  gas downstream the Flask B.

For the determination of  $y_{\text{SO}_2.\text{OIV}}$ , on the basis of equation (4), a volumetric pipette was used for the introduction of a volume of  $(4.9800 \times 10^{-2} \pm 5.0 \times 10^{-5})$  l of wine simulant into the Flask A. The mean value of 6

titration measurements was taken into account. The repetitions are reported in Table S2.  $V_{\text{NaOH}}$  was estimated of  $2.3700 \times 10^{-2}$  l.  $c_{\text{NaOH}}$  previously determined was considered.  $y_{\text{SO}_2, \text{OIV}}$  value resulted of 143.3 mg/l, which corresponded to a recovery of free  $\text{SO}_2$  concentration of 95.5 % with respect to  $y_{\text{SO}_2, \text{g}}$ . In this work, percent recovery computes the percentage of the original concentration of  $\text{SO}_2$  retrieved after the 591A method was applied.

Model equation (4) was used for the uncertainty evaluation of  $y_{\text{SO}_2, \text{OIV}}$ .

- The molar mass of  $\text{SO}_2$  was calculated as for  $y_{\text{SO}_2, \text{g}}$ .
- Six uncertainty sources were associated to  $V_{\text{NaOH}}$ . The first source of uncertainty is associated to the repeatability of the 6 titration measurements carried out. The experimental standard deviation of the mean,  $u(\text{Rep})$ , was calculated. The second source of uncertainty was due to the calibration of the burette,  $u(\text{Cal})$ , as reported in the case of  $u(\text{Cal.st})$ . The third source of uncertainty was due to the difference between the temperature at which the measurements were made and that at which the burette calibration was made,  $u(\Delta T)$ , as reported in the case of  $u(\Delta T.st)$ . In this case, a mean value of  $2.3700 \times 10^{-2}$  l of the NaOH solution required for the titration of the Flask B solution was considered. The fourth source of uncertainty regarded the fractionation of the last drop of nominal  $1 \times 10^{-2}$  mol/l NaOH solution used to neutralize the  $\text{H}_2\text{O}_2$  solution present in Flask B before starting the  $\text{SO}_2$  extraction process. In this case, the last drop ( $5.00 \times 10^{-5}$  l) of the NaOH solution was included in the uncertainty calculation. To minimize this contribution,  $1.0000 \times 10^{-1}$  l of Flask B solution were prepared and neutralized with  $1.50 \times 10^{-3}$  l of the nominal  $1 \times 10^{-2}$  mol/l NaOH solution, and  $3.00 \times 10^{-3}$  l of this solution already neutralized were inserted in Flask B. With  $1.0150 \times 10^{-1}$  l of neutral solution it was possible to carry out 33 experiments. A drop fraction with respect to this number of experiments was considered. A rectangular distribution was associated with this contribution. The fifth source of uncertainty was related to the last drop of nominal  $1 \times 10^{-2}$  mol/l NaOH solution used to reach the end point detection. The last drop of  $V_{\text{NaOH}}$  from the burette added to the Flask B solution was included in the uncertainty calculation considering that potentially not the whole drop was needed for the titration of  $\text{H}_2\text{SO}_4$ . In this case a rectangular distribution for  $u(E-P)$  was considered. The sixth source of uncertainty was due to the pH difference of the end-point from the equivalence point,  $u(\text{Ind})$  [31]. This contribution was evaluated considering the resolution of the burette of  $5.0 \times 10^{-5}$  l and a rectangular distribution [29,30].

Table 5 summarized the uncertainty contributions associated with  $V_{\text{NaOH}}$ .

Table 5. Uncertainty contributions associated with  $V_{\text{NaOH}}$

Quantity $X_i$	Standard uncertainty, $u$	Distribution
$u(\text{Rep})$	$2.2 \times 10^{-5}$ l	Normal
$u(\text{Cal})$	$1.2 \times 10^{-5}$ l	Triangular
$u(\Delta T)$	$7.5 \times 10^{-6}$ l	Normal
$u(\text{Flask B})$	$4.3 \times 10^{-7}$ l	Uniform
$u(E-P)$	$1.4 \times 10^{-5}$ l	Uniform
$u(\text{Ind})$	$1.4 \times 10^{-5}$ l	Uniform

The standard uncertainty of  $V_{\text{NaOH}}$ ,  $u(V_{\text{NaOH}})$ , was evaluated normally distributed by combining the six uncertainty contributions:

$$u(V_{\text{NaOH}}) = \sqrt{(u(\text{Rep}))^2 + u(\text{Cal})^2 + u(\Delta T)^2 + u(\text{Flask B})^2 + u(E-P)^2 + u(\text{Ind})^2} = 3.4 \times 10^{-5} \text{ l}$$

- $u(c_{\text{NaOH}})$  was already discussed in the previous uncertainty budget.

- The uncertainty of the volume of wine simulant used in the experiments,  $u(V_{\text{wine}})$ , was evaluated considering the limits of accuracy of the volumetric pipette, reported by the manufacturer. For the volume value of  $4.9800 \times 10^{-2}$  l this value was  $\pm 5.0 \times 10^{-5}$  l, assuming a triangular distribution.

In Table 6, the uncertainty budget associated with  $y_{\text{SO}_2, \text{OIV}}$  was summarized.

Table 6. Uncertainty budget associated with  $y_{\text{SO}_2, \text{OIV}}$

Quantity, $X_i$	Estimate	Standard uncertainty, $u$	Distribution	Sensitivity coefficient, $c_i$
$MM_{\text{SO}_2}$	64.0663 g/mol	$4.9 \times 10^{-3}$ g/mol	Approx. uniform	2.2373 mol/l
$V_{\text{NaOH}}$	$2.3700 \times 10^{-2}$ l	$3.4 \times 10^{-5}$ l	Normal	6048.101266 g/l <sup>2</sup>
$c_{\text{NaOH}}$	$9.40 \times 10^{-3}$ mol/l	$1.3 \times 10^{-4}$ mol/l	Normal	15232.73114 g/mol
$V_{\text{wine}}$	$4.9800 \times 10^{-2}$ l	$2.0 \times 10^{-5}$ l	Triangular	- 2878.313253 g/l <sup>2</sup>

The combined standard uncertainty of  $y_{\text{SO}_2, \text{OIV}}$ ,  $u_c(y_{\text{SO}_2, \text{OIV}})$  was evaluated, normally distributed:

$$u_c(y_{\text{SO}_2, \text{OIV}}) = \sqrt{(c(MM_{\text{SO}_2}))^2 \cdot u(MM_{\text{SO}_2})^2 + c(V_{\text{NaOH}})^2 \cdot u(V_{\text{NaOH}})^2 + c(c_{\text{NaOH}})^2 \cdot u(c_{\text{NaOH}})^2 + \dots + c(V_{\text{wine}})^2 \cdot u(V_{\text{wine}})^2)}$$

$y_{\text{SO}_2, \text{OIV}}$  resulted of **143.3 mg/l** and the expanded uncertainty ( $k = 2$ ) was evaluated of **3.9 mg/l**.

Summarizing the value obtained for the values for  $y_{\text{SO}_2, \text{g}}$  of  $(150.1 \pm 1.8)$  mg/l,  $y_{\text{SO}_2, \text{g}}$  and  $y_{\text{SO}_2, \text{OIV}}$  are not consistent. This statement may show that by 591A method a complete extraction of free  $\text{SO}_2$  concentration is not achieved.

Among the various reasons why a total recovery is not achieved it can be excluded that there is a dispersion of the free  $\text{SO}_2$  after the extraction phase because the Flask C added downstream of INRiM measurement system did not show a colour variation of the indicator solution. Moreover, a residue of  $\text{SO}_2$  dissolved in the solution was unlikely because Flask A with the wine simulant sample was heated and various cycles of 15 minutes were performed with additions of  $\text{H}_3\text{PO}_4$  solution to facilitate the  $\text{SO}_2$  extraction but no further free  $\text{SO}_2$  was observed.

The underestimation of the free  $\text{SO}_2$  by 591A method was also verified through measurement experiments conducted by nine specialized laboratories on the same wine simulant prepared by gravimetric method. From a stability study carried out under repeatability conditions, the wine simulant was found to be stable for 8 days. From homogeneity study no significant variation among the bottles was observed. The skilled laboratories conducted at least 5 measurement repetitions within the established time, with a method attributable to the 591A one. Only the standard deviation of the repetitions, normal distributed, was taken into account for each laboratory as an uncertainty contribution. Each laboratory provided the results as its own usual. For this reason, the collected results were reported with a different number of significant digits, as detailed in Table S3. The obtained values are also shown in Figure 3.



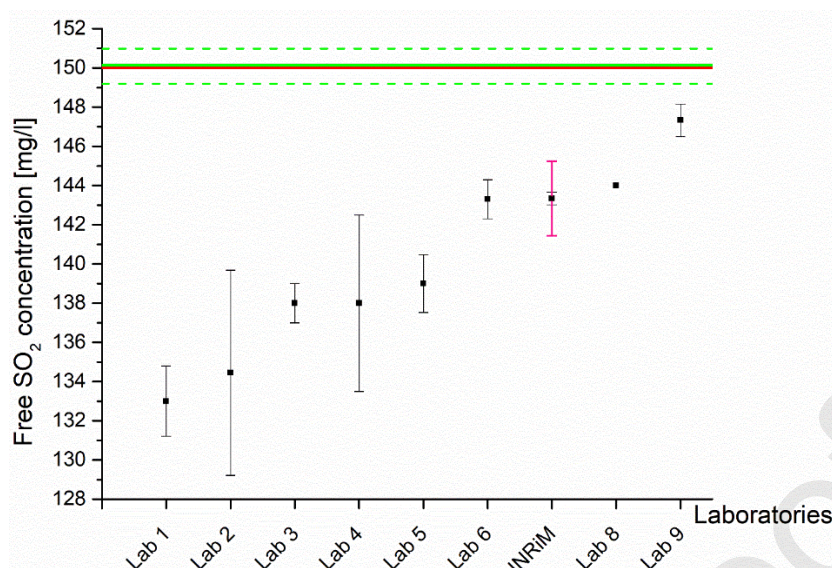


Figure 3. Results of the interlaboratory comparison on the wine simulant. Black squares represent the results obtained by the skilled laboratories. The black bars denote the associated expanded uncertainty ( $k = 2$ ) where the only contribution to the uncertainty is the standard deviations of the result of each laboratory. The pink bars represent the expanded uncertainty for the level of confidence 95 % and coverage factor 2 associated with the INRiM result. The green line and the green dotted lines represent the value of the free SO<sub>2</sub> concentration and the expanded uncertainty for the level of confidence 95 % and coverage factor 2 obtained by gravimetric method, respectively. The red line represents the value of the legal limit for red wines (150 mg/l).

The most important result of these measurements was that none of the laboratories reached the total recovery of free SO<sub>2</sub> present in the wine simulant, the value of  $y_{\text{SO}_2, \text{g}}$ . The average percentage recovery achieved by the laboratories was 93.1 % which corresponded to a concentration of free SO<sub>2</sub> of 139.8 mg/l. This experimental value, so far from the value obtained by the gravimetric method, highlights a criticality of the 591A method. This statement is valid both for wine simulant and for wine, since the two solutions are commutable.

A second output of these measurements was the large variability of the results obtained by the laboratories which is probably due to the different interpretations of 591A method. Greater exchange of information and knowledge transfer among analytical laboratories could lead to a more harmonized procedure for the application of the official 591A method. This would reduce the experimental standard deviation value and the distance from the gravimetric value. Considering that these analyses are routinely performed by laboratories and wineries to assess compliance with legal limits, it is important to delve into this issue in order to provide a reliable correction to be applied to the measurement results obtained on wine.

The evidence of a systematic underestimation of free SO<sub>2</sub> by the 591A method may be useful for further activity to determine whether the requirements specified in the Resolution OIV-OENO 591A-2018 are adequate, also in view of decision rules considering the consumer's and producer's risks.

#### 4. Conclusion

The most important finding is that the official method described in the Resolution OIV-OENO 591A-2018 led to an underestimation of the free SO<sub>2</sub>. By an evaluation of the measurement uncertainty associated with the value obtained with the INRiM measurement system on a wine simulant, an estimate of 143.3 mg/l with

associated an expanded uncertainty of 3.9 mg/l for a level of confidence 95 % and  $k = 2$  was obtained applying the 591A method. Instead, a value of 150.1 mg/l with an expanded uncertainty of 1.8 mg/l for a level of confidence 95 % and  $k = 2$  was obtained by gravimetric method. It follows that the values are not consistent. The calculated measurement uncertainty associated with the 591A method can be considered as the minimum uncertainty to be associated with a result of the free SO<sub>2</sub> concentration in wine. Indeed, the conditions under which the uncertainty contributions are minimized were considered. To the best of the authors' knowledge, this work represents the most complete work for the evaluation of the measurement uncertainty of the free SO<sub>2</sub> determination in wine simulant and in wine to date.

The underestimation of the free SO<sub>2</sub> by the 591A method was also verified through measurement experiments conducted by nine specialized laboratories on the same wine simulant prepared by gravimetric method. The total recovery of free SO<sub>2</sub> was not reached. Another output is that a harmonization of the procedures for the application of the 591A method is needed given that a significant variability of the measurements of free SO<sub>2</sub> concentration in wine simulant was obtained by the specialized laboratories. The used wine simulant was representative of the real wine samples with regards to the free form of SO<sub>2</sub>. The considerations made in this study can be extrapolated to the cases of real wine but exclusively for the free form of SO<sub>2</sub>. In order to have an overall evaluation of the total SO<sub>2</sub> present in real wine samples, further studies on bound SO<sub>2</sub> are necessary. In fact, another official method described in Resolution OIV-OENO 591B-2018 [12] has to be applied for the determination of bound SO<sub>2</sub> and possible interfering substances must be considered.

Moreover, an improved quality of measurement results obtained by the 591A method is relevant for wineries and laboratories that play a control role in the wine sector and since this method is also used for the quantification of free SO<sub>2</sub> in different types of food products.

#### **Declaration of Interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### **Acknowledgment**

The authors thank Dr Loretta Panero and Dr Christos Tsolakis (CREA), Dr Susana Río Segade (Università degli Studi di Torino), Dr Federico Piano (BI.LAB. srl), Dr Paola Manera (Sinergo), Dr Franco Alessandria (Enocontrol S.c.a.r.l.) and Dr Walter Bonetti (CENTRO PER L'ENOLOGIA S.R.L.) for their valuable collaboration for the measurement experiments and Matteo Berruto (INRiM) for his invaluable assistance provided during the measurement campaign.

Funding: This work was supported by Progetto IMPreSA (POR FESR 2014/20 - Asse I, Azione I.1.a.1.5 INFRA-P) from Regione Piemonte.

#### **Appendix A. Supplementary data**

Supplementary data are available.

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## Highlights

- By 591A method an underestimation of free SO<sub>2</sub> content in wine simulant is obtained
- The most complete work for the uncertainty evaluation of SO<sub>2</sub> content in wine to date
- By comparing 591A method and gravimetric method the values resulted not consistent
- A large results variability was obtained by experiments of specialized laboratories

- A harmonization of the procedure for the application of 591A method in wine is needed

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Project administration

### Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: