

Free, Total and Double SO₂ in Wine

DOC316.52.93088

Endpoint potentiometric titration with imposed current
Scope and application: Wines

1. Introduction

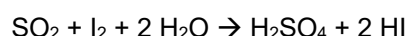
This working procedure refers to the titration of sulfur dioxide in wines. A double platinum electrode is used: an alternating current is imposed and the resulting potential is measured.

The table that follows shows the configuration to analyze the different forms of SO₂ in wines.

Measured parameter	Application name (download to AT1000)	Configuration 1: AT1000 only	Configuration 2: AT1000 + AS1000 sample changer	Details
Free SO ₂	Free SO ₂	X	X	4.3.1 Free SO₂ application
Total SO ₂	Total SO ₂	X		4.3.2 Total SO₂ and Total SO₂ with Sample preparation Refer to <i>Sample preparation</i> application (DOC316.52.93121) for more information.
	Sample Preparation Total SO ₂ + Total SO ₂ with Sample preparation		X	
Double SO ₂ (Free SO ₂ + Combined SO ₂ = Total SO ₂)	Double SO ₂	X		4.3.3 "Double SO₂" application and "Double SO₂ 1 Free TM1000" + "Double SO₂ 2 Combined TM1000" applications.
	Double SO ₂ 1–Free TM1000 + Double SO ₂ 2–Combined TM1000		X	

2. Principle

The protocol rests on the redox reaction between sulfur dioxide and iodine:



According to the Ripper method, free SO₂ is directly titrated in acidic media (sulfuric acid is added to the sample). Total SO₂ is the sum of free SO₂ and combined SO₂. Hydrolysis releases the latter in alkaline media (sodium hydroxide is added to the sample before sulfuric acid). Then, iodine solution is added until the potential measured drops abruptly, which corresponds to the equivalence. The SO₂ concentration is expressed in mg/L.

3. Electrode and reagents

Electrodes: Intellical double platinum electrode with integrated temperature sensor, MTC695

Titrant: Iodine I₂ 0.0282 eq/L (part number 2333353)

Acid reagent: Sulfuric acid H₂SO₄ 10 eq/L (part number 93153); install this reagent on **PUMP 1**

Alkali reagent: Sodium hydroxide NaOH 5 eq/L (part number 245053); install this reagent on **PUMP 2**
Note: Only required for Total SO₂ and Double SO₂ analyses.

Solid KI: With dispenser (part number 2834100)

Standard for titrant calibration: Sodium thiosulfate Na₂S₂O₃ 0.00564 eq/L (part number 2408853)

Deionized water

4. Ranges and settings

4.1. Default parameters

The working procedure is described using the following parameters:

- V sample = 20 mL
- Syringe volume = 10 mL

4.2. Working range

For most samples, 1 syringe (10 mL) of titrant should be sufficient to reach the equivalent point. It provides the following range:

Veq (mL)	0.1	9.5
SO₂ (mg/L)	4.5	430

For low SO₂ concentrations below 45 mg/L, (equivalent volumes under 1 mL) the titration typically lasts less than 1 minute.

For SO₂ concentrations up to 110 mg/L (equivalent volume around 2.5 mL), the titration typically lasts then than 2 minutes.

For SO₂ concentrations between 110 and 430 mg/L, it is recommended to add a predose to keep titration duration to around 2-3 minutes. Orders of magnitude are given in the table below:

Expected SO₂ concentration (mg/L)	Expected equivalent volume (mL)	Recommended predose (mL)
150	3.3	0.8
200	4.4	1.9
250	5.5	3.0
300	6.6	4.1
350	7.7	5.2
400	8.9	6.4

4.3. Settings

Name	Default parameter	Unit
Sample		
Name	Wine	
Amount	20	mL
Amount min	0	mL
Amount max	22	mL
Titrant		
Name	I2	
Titrant concentration	0.0282	eq/L
Syringe	Syringe 1	
Probe		
Recommended probe	MTC695	
Leveling		
Active	No	
Time	30	seconds

4.3.1. Free SO₂ application

The sample is acidified with a concentrated sulfuric acid solution. Then, the free SO₂ is titrated with an iodine solution.

Name	Default parameter	Unit
Step 1: Acid addition		
Duration	1	seconds
Stirrer speed	20	%
Step 2: Reaction		
Duration	15	seconds
Stirrer speed	0	%
Step 3: Titration		
Stirring speed	50	%
Measured parameter		mV
Predose	0	mL
Max volume stop point	10	mL
Stop on last EQP	True	
Delay	0	seconds
Addition mode	Monotonic	
Increment size	0.08	mL
EP Ordinates	160	mV
Result 1 name	Free SO ₂	
R1 resolution	Integer	
R1 min	5	mg/L
R1 max	430	mg/L
R1 QC min	5	mg/L
R1 QC max	430	mg/L

4.3.2. Total SO₂ and Total SO₂ with Sample preparation applications

Use the *Total SO₂ with sample preparation* application together with the *Sample Preparation Total SO₂* application to prepare a batch of samples. The system adds alkali solution on several samples before the titration. Refer to the *Sample Preparation* (DOC316.52.93121) application note for more information. If the preparation of a batch of samples is not necessary, use the *Total SO₂* application.

Name	Default parameter		Unit
	Total SO ₂	Total SO ₂ with Sample preparation	
Step 1: Alkali addition			
Duration	3	Refer to <i>Sample Preparation Total SO₂</i> application	seconds
Stirrer speed	20		%
Step 2: Reaction			
Duration	5	Refer to <i>Sample Preparation Total SO₂</i> application	minutes
Stirrer speed	0		%
Step 3: Acid addition			
Duration	3	3	seconds
Stirrer speed	20	20	%
Step 4: Reaction			
Duration	15	15	seconds
Stirrer speed	0	0	%
Step 5: Titration			
Stirring speed	50	50	%
Measured parameter	mV	mV	mV
Predose	0	0	mL
Max volume stop point	10	10	mL
Stop on last EQP	True	True	
Delay	0	0	seconds
Addition mode	Monotonic	Monotonic	
Increment size	0.08	0.08	mL

Name	Default parameter		Unit
	Total SO ₂	Total SO ₂ with Sample preparation	
EP Ordinates	140	140	mV
Result 1 name	Total SO ₂	Total SO ₂	
R1 resolution	Integer	Integer	
R1 min	5	5	mg/L
R1 max	430	430	mg/L
R1 QC min	5	5	mg/L
R1 QC max	430	430	mg/L

4.3.3. Double SO₂ application and Double SO₂ 1–Free TM1000 + Double SO₂ 2 Combined TM1000 applications

The measurement of double SO₂ consists of a first measurement of free SO₂ followed directly by a measurement of combined SO₂. The sum of the 2 results gives the total SO₂.

The applications *Double SO₂ 1–Free TM1000* and *Double SO₂ 2–Combined TM1000* must be used with TM1000 PC software and AS1000 sample changer. First, the *Double SO₂ 1–Free TM1000* application is used to determine the free SO₂ in the sample batch (with Alkali addition at the end of each titration). Next, the *Double SO₂ 2–Combined TM1000* application is used to determine the combined SO₂ in the same sample batch. The system shows the Free and Combined SO₂ results for each sample and calculates the Total SO₂ by adding the two results.

Refer to section 5.3 [Double SO₂ 1–Free TM1000 and Double SO₂ 2–Combined TM1000 applications](#) to prepare a sample stack with TM1000 PC software and start an analysis with the two applications.

Note: Do not change the result sections of the Double SO₂ 1–Free TM1000 and Double SO₂ 2–Combined TM1000 applications. The system uses these results to calculate the Combined SO₂ and Total SO₂ on TM1000 software. If the double SO₂ is determined with the AT1000 instrument only, then use the Double SO₂ application. The instrument does the two titrations, for Free and Combined SO₂, in the same beaker one after the other.

Name	Default parameter		Unit
	Double SO ₂	Double SO ₂ 1–Free TM1000	
Step 1: Acid addition			
Duration	1	1	seconds
Stirrer speed	20	20	%
Step 2: Reaction			
Duration	15	15	seconds
Stirrer speed	0	0	%
Step 3 : Free SO₂ titration			
Stirring speed	50	50	%
Measured parameter	mV	mV	mV
Predose	0	0	mL
Max volume stop point	10	10	mL
Stop on last EQP	True	True	
Delay	0	0	seconds
Addition mode	Monotonic	Monotonic	
Increment size	0.08	0.08	
EP Ordinates	160	160	mL
Result 1 name	Free SO ₂	Free SO ₂	mV
R1 resolution	Integer	Integer	
R1 min	5	5	mg/L
R1 max	430	430	mg/L
R1 QC min	5	5	mg/L
R1 QC max	430	430	mg/L
Result 2 name	Veq1	Veq1	
Hide	True	True	
- / Result 3 name	-	Vf1	
- / Hide	-	True	
- / Result 4 name	-	Titration concentration	
- / Hide	-	True	

Name	Default parameter		Unit
	Double SO ₂	Double SO ₂ 1–Free TM1000	
- / R4 equation	-	TC x FX	
- / R4 user value (FX)	-	1	
- / R4 unit	-	eq/L	
- / Result 5 name	-	Sample volume	
- / Hide	-	True	
Step 4: Alkali addition			
Duration	4	4	seconds
Stirrer speed	20	20	%
		Double SO₂ 2–Combined TM1000	
Step 5: Reaction			
Duration	5	5 by default Managed on TM1000	minutes
Stirrer speed	0	-	%
Step 6: Acid addition			
Duration	3	3	seconds
Stirrer speed	20	20	%
Step 7: Reaction			
Duration	15	15	seconds
Stirrer speed	0	0	%
Step 8: Combined SO₂ titration			
Stirring speed	50	50	%
Measured parameter	mV	mV	mV
Predose	0	0	mL
Max volume stop point	10	10	mL
Stop on last EQP	True	True	
Delay	0	0	seconds
Addition mode	Monotonic	Monotonic	
Increment size	0.08	0.08	mL
EP Ordinates	140	140	mV
Result 3 name / Result 1 name	Ve _{q2}	Ve _{q2}	
Hide	True	True	
Equation result name	Combined SO ₂	-	
Equation result resolution	Integer	-	
Equation result min	5	-	mg/L
Equation result max	430	-	mg/L
Equation result QC min	5	-	mg/L
Equation result QC max	430	-	mg/L

4.4. Modification of the settings

The parameters are defined to have the best compromise between accuracy and titration time.

For higher concentration with a high titrant volume, titration time can be reduced with an addition of titrant (predose) at the beginning of the titration. Enter the predose volume (in mL) and the stirring time after the addition in the application edit window.

5. Titration procedure

5.1. Leveling

To use this method, an external pump is required. All elements (probes, tubes from the titrator and the tube from the external pump) have to be well installed on the probe holder. The beaker has to contain a level of sample higher than the position of the tube of the external pump. When the beaker is attached to the probe holder, this method allows the system to automatically remove the excess sample by a defined pump working time, and always keep the same sample volume before launching the analysis.

To define this volume, autoleveling calibration sequence has to be previously executed. Refer to section [8.2 Autoleveling calibration](#).

When this option is active, the working time of the external pump must be set (default 30 s). The minimum working time must allow the pump to be removing air during the last few seconds of the external pump activation.

Note: Do not forget to re-edit the sample amount with the expected value when deactivating the leveling method.

5.2. Titration

Rinse the probe with deionized water. If leveling is disabled, use a pipette to collect precisely 20 mL of sample.

Pour the sample into the 50-mL polypropylene beaker, put in a magnetic stir bar, dip the probe and the delivery tip in the solution and then start the application.

At the end of the titration, a first window displays the result. A second window displays the titration curve and the equivalent point coordinates.

After the titration, there are two possibilities:

- Replicate the sample. This is used to study the repeatability by analyzing several samples successively. At the end of each titration, a window displays the average value, the standard deviation (SD) and the relative standard deviation (RSD in %).
- Analyze a new sample. Another titration can be started but no Standard Deviation and RSD value will be made.

5.3. Double SO₂ 1–Free TM1000 and Double SO₂ 2–Combined TM1000 applications

5.3.1. AT1000 instrument

To analyze Double SO₂ with a system (AT1000 instrument, TM1000 PC software and AS1000 sampler changer), download the applications *Double SO₂ 1–Free TM1000* and *Double SO₂ 2–Combined TM1000* to the AT1000 instrument from the supplied USB key.

Note: *Double SO₂ 1–Free TM1000* and *Double SO₂ 2–Combined TM1000* applications are only applicable to TM1000 PC software.

5.3.2. TM1000 PC software

Do the steps that follow to start the analysis in the TM1000 PC software:

1. Click the SETTINGS tab. Click on ANALYSIS. Select ENABLE SECOND ANALYSIS ON SAME SAMPLE.
2. Select the applicable settings based on the expected results: Combined SO₂ or Total SO₂. Select “DISPLAY TIME TO START SECOND APPLICATION” to show the time to start the second application on the display.
3. Click the ADD ANALYSIS tab. One or two new columns show based on the previous selected settings. At sample stack creation, select *Double SO₂ 1–Free TM1000* as first application and *Double SO₂ 2–Combined TM1000* as second application.

Note: *Titration calibration and autoleveling calibration are done based on the settings in the first application. If the sample amount or titrant concentration is manually changed, make sure to change the amount in both applications, mainly for display purposes.*

6. Results

6.1. Result calculation

The generic equation is:

$$SO_2 \text{ (mg/L)} = \frac{n_{e- \text{ titrant}}}{n_{e- SO_2}} \times \frac{C_{\text{titrant}} \text{ (eq/L)} \times V_{\text{titrant}} \text{ (mL)}}{n_{e- \text{ titrant}} \times V_{\text{sample}} \text{ (mL)}} \times M_{SO_2} \text{ (g/mol)} \times 1000$$

For Free SO₂:

$$\text{Free } SO_2 \text{ (mg/L)} = \frac{2}{2} \times \frac{0.0282 \text{ (eq/L)} \times V_{\text{eq}} \text{ (mL)}}{2 \times 20 \text{ (mL)}} \times 64.1 \text{ (g/mol)} \times 1000$$

For Total SO₂:

$$\text{Total } SO_2 \text{ (mg/L)} = \frac{2}{2} \times \frac{0.0282 \text{ (eq/L)} \times V_{\text{eq}} \text{ (mL)}}{2 \times 20 \text{ (mL)}} \times 64.1 \text{ (g/mol)} \times 1000$$

For Double SO₂:

$$\text{Free } SO_2 \text{ (mg/L)} = \frac{2}{2} \times \frac{0.0282 \text{ (eq/L)} \times V_{\text{eq1}} \text{ (mL)}}{2 \times 20 \text{ (mL)}} \times 64.1 \text{ (g/mol)} \times 1000$$

$$\text{Combined } SO_2 \text{ (mg/L)} = \frac{2}{2} \times \frac{0.0282 \text{ (eq/L)} \times [V_{\text{eq2}} + V'] \text{ (mL)}}{2 \times 20 \text{ (mL)}} \times 64.1 \text{ (g/mol)} \times 1000 \times \text{FX}$$

Where **V'** is the volume of excess titrant added after V_{eq1} during **Free SO₂** titration.

For **Combined SO₂**, an equation is used since the result cannot be calculated directly from the equivalent volume. Therefore, the factor FX (1 by default) has to be changed if the titrant unit is changed, for example:

Titration titer unit	eq/L	meq/L
Factor FX	1	0.001

Change the factor FX in the **Combined SO₂** section of the *Double SO₂* application or in the **Titration concentration** section of the *Double SO₂ 1–Free TM1000* application.

For **Total SO₂** result from the Double SO₂ analysis, the value can be rounded up to the next highest number because the system calculates the value with all decimals.

Note: Do not change the result sections of the applications Double SO₂ 1–Free TM1000 and Double SO₂ 2–Combined TM1000. The system uses these results to calculate the Combined SO₂ and Total SO₂ on TM1000 software.

6.2. Experimental results

These results are indicative and have been obtained for given samples, for 8 to 10 successive determinations.

6.2.1 Free SO₂

For 10 determinations on red wine:

Measurement	Parameter	Unit
Mean concentration	25	mg/L
Standard deviation	0.4	mg/L
Relative standard deviation	1.7	%
Mean titration duration	45	seconds

For 10 determinations on white wine:

Measurement	Parameter	Unit
Mean concentration	4	mg/L
Standard deviation	0.1	mg/L
Relative standard deviation	1.8	%
Mean titration duration	19	seconds

6.2.2. Total SO₂

For 10 determinations on red wine:

Measurement	Parameter	Unit
Mean concentration	80	mg/L
Standard deviation	2.8	mg/L
Relative standard deviation	3.5	%
Mean titration duration	108	seconds

For 10 determinations on white wine:

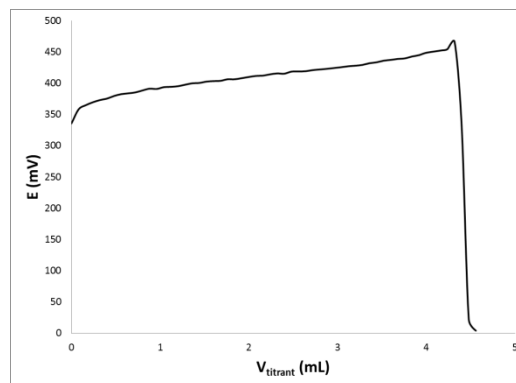
Measurement	Parameter	Unit
Mean concentration	72	mg/L
Standard deviation	1.2	mg/L
Relative standard deviation	1.7	%
Mean titration duration	99	seconds

6.2.3. Double SO₂

For 8 determinations on red wine:

Measurement	Parameter	Unit
Free SO₂		
Mean concentration	9	mg/L
Standard deviation	0.1	mg/L
Relative standard deviation	0.9	%
Mean titration duration	14	seconds
Combined SO₂		
Mean concentration	48	mg/L
Standard deviation	0.9	mg/L
Relative standard deviation	1.8	%
Mean titration duration	48	seconds

6.3. Example of a titration curve



7. Recommendations

Always rinse the probe and the delivery tip between measurements.

When AS1000 sampler is used, it is recommended to set the stirring speed of the titrations as high as possible to ensure the best sample homogenization.

8. Appendices

8.1. Titrant calibration

The iodine solution can be calibrated: its exact concentration can be determined from a titration using a sodium thiosulfate standard solution.

Use a pipette to collect precisely 20 mL of 0.00564 N sodium thiosulfate solution. Pour the standard in the 50 mL propylene beaker and add a stir bar. Dip the probe and the delivery tip into the solution and launch the titrant calibration sequence.

At the end of the titrant calibration, titer (eq/L) is displayed and the user can reject, replicate, or save the result.

Default settings for titrant calibration

Name	Default parameter	Unit
Titrant		
Name	I2	
Titrant concentration	0.0282	eq/L
Syringe	Syringe 1	
Standard		
Name	Na2S2O3	
Amount	20	mL
EP titration		
Stirring speed	15	%
Measured parameter		mV
Predose	2	mL
Max volume stop point	8	mL
Stop on last EQP	True	
Delay	0	seconds
Increment size	0.04	mL
EP Ordinates	400	mV
Result name	Titer	
Result resolution	5 decimals	
Result min	0.02538	eq/L
Result max	0.03102	eq/L

Note: When the titer is saved, it is used for all applications where this titrant is necessary.

8.2. Autoleveling calibration

The aim of this method is to calibrate the volume of sample by leveling. The result of this calibration will be used as the sample volume for the following titrations, but only with the application that was used to do the autoleveling calibration.

This option is **ONLY** available from the calibration menu if **Method Leveling** is set to Active (**Yes**). Refer to the documentation delivered with the external pump for a correct installation, paying particular attention to the suction tube from the pump.

Use a 0.00564 eq/L sodium thiosulfate standard solution and pour a sufficient amount of the solution into a beaker allowing the external pump tube to be immersed in the liquid. In the calibration menu select **Autoleveling calibration** and then **Free SO₂**, **Total SO₂** or **Double SO₂**.

The result in mL is compared to minimum and maximum amounts defined for the sample volume. The calculation used is:

$$V_{\text{sample}} = \frac{V_{\text{titrant}} (\text{mL}) \times n_{\text{e- titrant}} \times C_{\text{titrant}} (\text{eq/L})}{n_{\text{e- sodium thiosulfate}} \times C_{\text{sodium thiosulfate solution}} (\text{eq/L})}$$
$$= \frac{V_{\text{titrant}} (\text{mL}) \times 2 \times 0.0282 (\text{eq/L})}{2 \times 0.00564 (\text{eq/L})}$$

Autoleveling calibration uses the same settings as for a titrant calibration (refer to section [8.1 Titrant calibration](#)).

Default settings for autoleveling calibration

Name	Default parameter	Unit
Sample		
Amount min	0	mL
Amount max	22	mL
Autoleveling calibration		
Solution name	Na2S2O3	
Concentration	0.00564	eq/L
Resolution	3 decimals	

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