OIV-MA-AS323-04A1 Free sulphur dioxide

Type IV method

1. **Scope**

This method is for the determination of free sulphur dioxide in wine and must.

2. **Definitions**

Free sulphur dioxide is defined as the sulphur dioxide present in the must or wine in the following forms: H_2SO_3 and HSO_3^-, whose equilibrium is dependent on pH and temperature:

$$H_2SO_3 \leftrightarrow H^+ + HSO_3^-$$

H_2SO_3represents the molecular sulphur dioxide.

3. **Principle**

Sulphur dioxide is entrained by a current of air or nitrogen, and is fixed and oxidised by bubbling through a dilute and neutral solution of hydrogen peroxide. The sulphuric acid formed is determined by titration with a standard solution of sodium hydroxide.

The quantity of sulphur dioxide entrained being strongly temperature dependent, the decision was made to work at room temperature (between 18 and 24°C). This temperature, as for that of the currents of air or nitrogen, should be kept constant throughout the determination.

4. **Reagents and products**

1. Pure phosphoric acid at 85% (ρ_{20} = 1.71 g/mL) (CAS no. 7664-38-2)
2. Diluted phosphoric acid (≈ 25.5%):

By way of example: Dilute 300 mL of phosphoric acid at 85% (4.1) in 1 L of water for analytical use
4.3. Indicator reagent:
Methyl red (CAS no. 493-52-7): 100 mg (± 1 mg)

Methylene blue (CAS no. 7220-79-3) 50 mg (± 0.5 mg)

Ethanol (≥ 95%) (CAS no. 64-17-5) 50 mL

Make up to 100 mL with water for analytical use. Respect the proportions for the volumes that differ from 100 mL.

Commercial indicator reagents with the same composition may be used.

4.4. 1 M Sodium hydroxide (3.84%) or in anhydrous form (pellets) (CAS no. 1310-73-2)

4.5. 0.01 M Sodium hydroxide solution:

By way of example: Dilute 10.0 mL of 1 M sodium hydroxide (4.4) in 1 L of water for analytical use.

If necessary, check the titre of the solution regularly (correction factor to be applied) and keep it away from atmospheric CO_2.

4.6. Hydrogen peroxide solution in 3 volumes (= 9.1 g/L = 0.27 mol/L H_2O_2:), prepared or commercial (e.g. 30% H_2O_2: mixture with CAS no. 7722-84-1)

Note: A solution of 30% by mass corresponds to a titre of 110 volumes ($□_{20} \cong$ 1,11 g/mL), implying the volume of oxygen ideally released per litre of H_2O_2 under standard conditions of temperature and pressure, while a solution of 3% by mass ($□_{20} \cong$ 1 g/mL) corresponds to a titre of 10 volumes (0.89 mol/L). The preparation thus depends on the commercial solution used, considering that in any case the volume used in the method will be in excess.

5. Apparatus

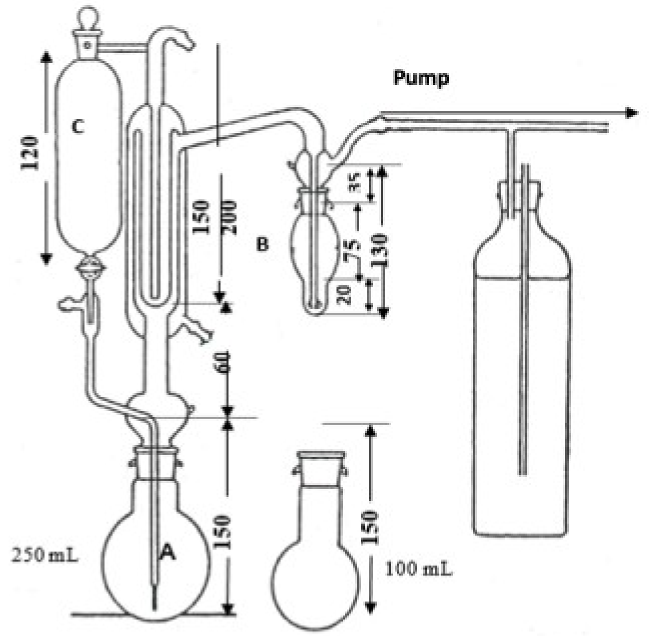
The apparatus to be used should conform to the diagram below, especially with regard to the condenser.

The gas supply tube to bubbler B ends in a small sphere of 1 cm in diameter with 20 holes of 0.2 mm in diameter around its largest horizontal circumference. Alternatively, this tube may end in a sintered glass plate that produces a large number of very small bubbles and thus ensures good contact between the liquid and gaseous phases.

The gas flow through the apparatus should be approximately 40 L/h. The bottle situated on the right of the apparatus is intended to restrict the pressure reduction produced by the water pump to 20-30 cm water. In order to regulate the pressure reduction to achieve the proper flow rate, it is preferable to install a flow meter with a semi-capillary tube between the bubbler and the bottle.

Flask A should be kept at a temperature of between 18°C and 24°C throughout aspiration. Each flask should consequently be temperature-controlled (e.g. using a thermostatic bath) if the room temperature of the laboratory is not within these limits or if 85% phosphoric acid is used, which can significantly increase the temperature in the flask during addition.

Figure 1- The dimensions are indicated in milimetres. The internal diameters of the 4 concentric tubes that make up the condenser are 45,34, 27 and 10 mm



6. Procedure

Air- or nitrogen-rinsing the apparatus before each new determination (e.g. for 5 minutes) is recommended. If a blank test is carried out, the colour of the indicator in the neutralised hydrogen peroxide solution at the exit of the gas-supply tube should not change.

Connect the water from the condenser.

Control the laboratory temperature or stabilise the bath in advance (at between 18 °C and 24 °C).

In bubbler B of the entrainment apparatus, introduce 2-3 mL hydrogen peroxide solution (4.6) and 2 drops of indicator reagent (4.3), and neutralise with the 0.01 M sodium hydroxide solution (4.5); a neutral pH = green colour.

Note: For large sample series, it is also possible to prepare an already neutralised H_2O_2 solution before introducing it into the flask. Adapt the concentrations and volumes accordingly, bearing in mind that the oxidative power of the solution must be maintained (reduced shelf life).

Adapt this bubbler to the apparatus.

Transfer 50 mL of sample to the 250-mL flask A and attach it to the apparatus.

Introduce 15 mL of diluted phosphoric acid (4.2) into bulb C.

Note: If the expected concentration of free sulphur dioxide is higher than 50 mg/L, it is necessary to use phosphoric acid at 85% (4.1). However, ensure that the temperature in flask A does not increase during addition.

Open the tap to add the acid to the sample while simultaneously starting the gas flow and setting the timer to 15 minutes. The entrained free sulphur dioxide is oxidised into sulphuric acid.

After 15 minutes, take bubbler B out and rinse the gas supply tube in water (via the socket).

Titrate the acid formed by the 0.01 M sodium hydroxide solution (4.5) up to the green bend.

The number of millilitres used is expressed by *n*.

7. Calculation and expression of results

The free sulphur dioxide is expressed in milligrams per litre (mg/L), in whole numbers.

Calculation: Free sulphur dioxide in milligrams per litre: 6.4 n

8. Bibliography

Paul, F., *Mitt. Klosterneuburg, Rebe u. Wein,* 1958, ser. A, 821

Collaborative study

Method validation for the determination of free sulphur dioxide

1. Scope of application

An international collaborative study, in accordance with Resolution OIV-OENO 6-2000, for the validation of updates to the methods for the determination of free sulphur dioxide and total sulphur dioxide (OIV-MA-AS323-04A), based on the decision of the OIV “Methods of Analysis” Sub-Commission, April 2018.

2. Standard references

- Update (draft) to the OIV-MA-AS323-04A methods,
- ISO 5725,
- Resolution OIV-OENO 6-2000.

3. Protocol

A total of 20 samples were prepared using homogeneous volumes of 10 wines from various wine regions in France and Portugal. Each sample was made up twice (the second as a blind duplicate), according to the double-blind principle.

The samples were prepared between 18 and 20 June 2018, then shipped without delay to the participating laboratories.

Sample no.	Blind duplicate no.	Nature of sample
A	1-14	Dry white wine
B	2-16	Dry white wine
C	3-19	Dry rosé wine
D	4-12	Dry rosé wine
E	5-20	Dry red wine
F	6-18	Dry red wine
G	7-11	Dry red wine
H	8-15	White liqueur wine
I	9-17	Red liqueur wine
J	10-13	Red liqueur wine

The analyses were carried out simultaneously by all participating laboratories between 16 and 20 July 2018. Samples were kept in refrigerated cabinets by all laboratories between the date of reception and the date of analysis, according to the protocols sent.

The following laboratories provided their results:

Laboratory	City	Country
Estación de Viticultura e Enoloxía de Galicia	Leiro (Ourense)	Spain
Laboratorio arbitral agroalimentario	Madrid	Spain
ASAE	Lisbon	Portugal
SCL Montpellier	Montpellier Cdex 5	France

HBLA und BA für Wein- und Obstbau	Klosterneuburg	Austria
Laboratorio de Salud Pública	Madrid	Spain
Laboratorio Agroambiental de Zaragoza	Zaragoza	Spain
Laboratoire SCL Bordeaux	Pessac Cedex - CS 98080	France
Unione Italiana Vini Servizi	Verona	Italy
Laboratorio Agroalimentario de Valencia	Burjassot (Valencia)	Spain
Agroscope	Nyon	Switzerland
Laboratoires Dubernet	Montredon des Corbières	France
Laboratoire Dioenos Rhône	Orange	France
Laboratoire Natoli	Saint Clément de Rivière	France

NB: The order of laboratories in the table does not correspond with the order in the following tables, in order to preserve the anonymity of results.

4. Free sulphur dioxide

4.1. Free SO_2 data

Free SO_2 (mg/L)	A		B		C		D		E		F		G		H		I		J	
Sample	1	14	2	16	3	19	4	12	5	20	6	18	7	11	8	15	9	17	10	13
Labo 3			31	36	18	18	21	23	20	18	6	6	20	17	5	6				
Labo 5			37	35	21	24	24	25	20	20	8	7	20	20	3	4				
Labo 6	4	1	38	33	21	20	20	26	19	20	7	6	21	19	7	8	1	3	1	1
Labo 7	1	1	37	40	20	22	24	26	20	22	9	8	20	23	8	8	2	1	1	1

Labo 8			31	32	18	19	23	22	22	20	6	7	19	20	5	3	1	1		
Labo 9			35	34	23	19	25	24	21	24			17	17						
Labo 10	2	1	35	34	20	21	24	24	22	21	9	8	21	20	7	7	2	2	1	1
Labo 11	0	0	33	30	17	11	22	16	16	21	6	4	15	19	6	3	1	1	0	0
Labo 15			15	19	15	13	18	20	8	16	6	5	8	15	5	5				
Labo 17	0	0	37	38	24	26	28	28	26	23	8	8	24	22	7	7	1	2	0	0
Labo 18	0	4	33	31	21	11	23	27	15	19	6	4	9	20	3	4	1	1	0	0
Labo 20	0	0	32	32	20	19	21	21	29	21	8	8	20	18	12	4	1	1	0	0
Labo 21	2	1	33	38	19	15	25	22	19	21	6	6	19	20	8	7	2	1	0	0

Results left blank were rendered non-quantifiable (< limit of quantification).

▨ Result removed by the COCHRAN test at 5%

░ Result removed by the GRUBBS test at 5%

4.2. Free SO_2 results

Free SO_2 (mg/L)	A	B	C	D	E	F	G	H	I	J
No. of laboratories selected	7	9	11	10	10	12	11	11	9	8
No. of repetitions	2	2	2	2	2	2	2	2	2	2
Min.	0	31.5	14	19	17	5	17	3.5	1	0
Max.	2.5	38.5	25	28	24.5	8.5	23	8	2	1
Mean	0.9	34.2	19.8	23.4	20.6	6.8	19.6	5.7	1.4	0.4
Standard deviation	0.98	2.67	2.91	2.46	2.04	1.31	1.77	1.72	0.42	0.52
Repeatability variance	0.79	1.67	2.59	1.20	2.60	0.58	2.23	0.82	0.39	0.00
Inter-laboratory standard deviation	0.98	2.67	2.91	2.46	2.04	1.31	1.77	1.72	0.42	0.52

Reproducibility variance	1.35	7.97	9.76	6.64	5.46	2.00	4.25	3.38	0.37	0.27
Repeatability standard deviation	0.89	1.29	1.61	1.10	1.61	0.76	1.49	0.90	0.62	0.00
r limit	2.48	3.61	4.51	3.07	4.51	2.14	4.18	2.53	1.75	0.00
Repeatability %CV (k=2)	191	8	16	9	16	23	15	32	90	0
Reproducibility standard deviation	1.16	2.82	3.12	2.58	2.34	1.41	2.06	1.84	0.61	0.52
R limit	3.25	7.90	8.75	7.22	6.54	3.96	5.78	5.15	1.70	1.45
Reproducibility %CV (k=2)	250	16	32	22	23	42	21	64	87	276
Horwitz PRSD$_R$ (%)	16.18	9.40	10.21	9.95	10.15	12.00	10.22	12.30	15.23	18.55
Horwitz s$_R$	0.15	3.22	2.02	2.33	2.09	0.81	2.00	0.70	0.21	0.07
Horwitz R	0.42	9.10	5.71	6.59	5.91	2.29	5.67	1.99	0.60	0.20
Horwitz Ratio	7.64	0.87	1.53	1.10	1.11	1.73	1.02	2.58	2.84	7.37

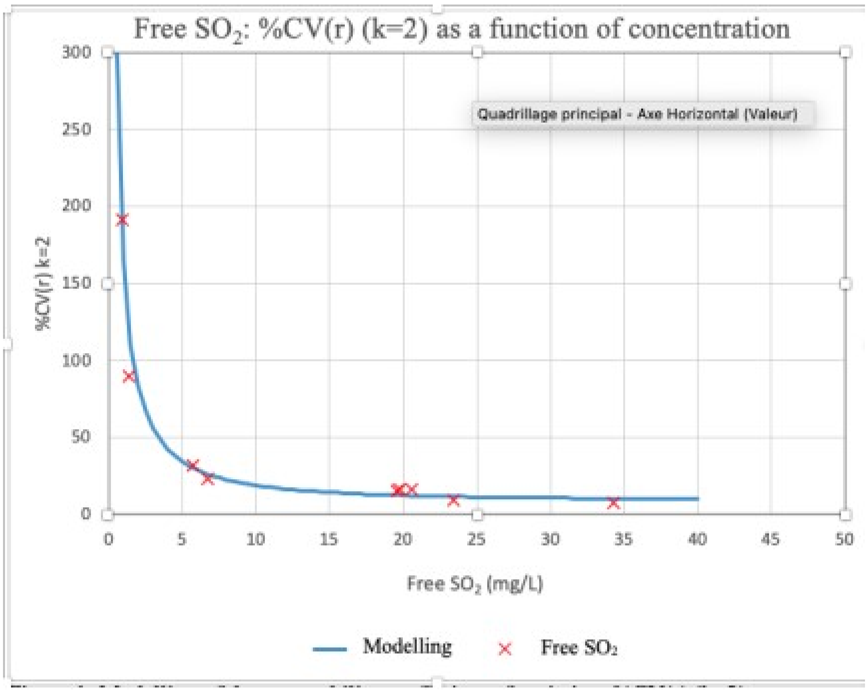


Figure 1: Modelling of the repeatability coefficient of variation, %CV(r) (k=2), as a function of the concentration, C:

$$\%CV(r) = \sqrt{\frac{164.55^2}{C^2} + 9^2}$$

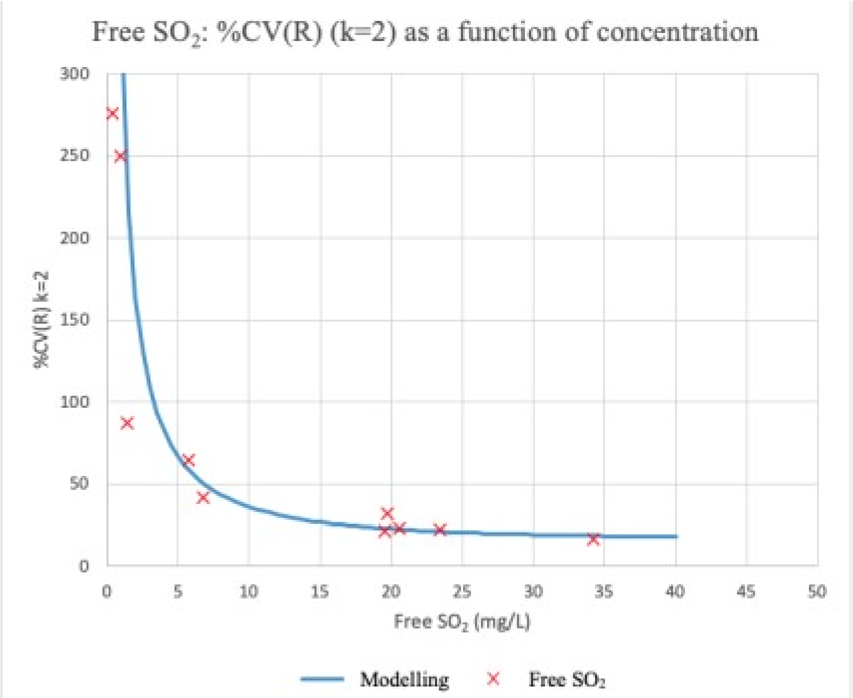


Figure 2: Modelling of the inter-laboratory reproducibility coefficient of variation, %CV(R) (k=2), as a function of concentration, C:

$$\%CV(r) = \sqrt{\frac{323.6^2}{C^2} + 16^2}$$

5. Total sulphur dioxide

1. Total SO_2 data

Total SO_2 (mg/L)	A		B		C		D		E		F		G		H		I		J	
Sample	1	14	2	16	3	19	4	12	5	20	6	18	7	11	8	15	9	17	10	13
Labo 3			128	127	72	73	128	131	61	59	28	28	57	56	102	102	47	45		
Labo 5			122	121	68	71	112	114	42	53	22	22	51	42	102	101	35	34		

Labo 6		1	128	131	72	72	126	131	53	54	22	20	42	49	98	99	31	34	3	1
Labo 7	3	3	131	131	70	74	130	131	54	59	26	23	46	48	106	101	37	40	1	1
Labo 8	2	1	125	127	72	72	129	128	58	57	22	23	46	45	97	99	42	39	1	1
Labo 9			120	128	77	75	132	108	71	59	21	25	44	47	110	99	38	48		
Labo 10	2	2	130	130	74	76	130	130	61	61	28	32	55	56	103	104	43	44	3	4
Labo 11	4	3	119	125	71	74	118	118	39	40	18	21	45	41	89	94	26	38	2	2
Labo 14	3	3	129	128	72	72	127	129	58	58	32	29	50	49	102	101	42	41	3	4
Labo 15			134	136	76	78	134	136	60	58	39	27	52	61	110	106	51	50		
Labo 17	3	3	134	132	82	76	136	133	59	50	24	23	46	44	107	105	35	38	0	0
Labo 18	5	3	130	129	78	73	133	133	62	59	29	32	58	52	105	105	50	48	2	2
Labo 20	1	1	128	131	72	74	130	130	58	56	26	28	48	45	98	93	41	43	0	0
Labo 21		0	124	125	69	72	124	126	45	51	19	20	42	42	97	97	35	34	0	1

Results left blank were rendered non-quantifiable (< limit of quantification).

▨ Result removed by the COCHRAN test at 5%

░ Result removed by the GRUBBS test at 5%

5.2. Total SO_2 results

Total SO_2 (mg/L)	A	B	C	D	E	F	G	H	I	J
No. of laboratories selected	7	12	13	13	8	13	10	13	12	9
No. of repetitions	2	2	2	2	2	2	2	2	2	2
Min.	1	121.5	69.5	113	53.5	19.5	42	91.5	32.5	0
Max.	3.5	135	77	135	61	30.5	56.5	108	50.5	3.5
Mean	2.4	128.8	73.0	128.0	58.3	24.7	47.6	100.9	40.8	1.5
Standard deviation	0.93	3.63	2.20	6.24	2.42	4.04	4.89	4.61	5.80	1.35

Repeatability variance	0.14	1.46	3.27	2.35	1.44	3.04	2.30	3.96	2.21	0.17
Inter-laboratory standard deviation	0.93	3.63	2.20	6.24	2.42	4.04	4.89	4.61	5.80	1.35
Reproducibility variance	0.94	13.93	6.49	40.11	6.57	17.84	25.03	23.28	34.72	1.90
Repeatability standard deviation	0.38	1.21	1.81	1.53	1.20	1.74	1.52	1.99	1.49	0.41
r limit	1.1	3.4	5.1	4.3	3.4	4.9	4.2	5.6	4.2	1.1
Repeatability %CV (k=2)	31	2	5	2	4	14	6	4	7	54
Reproducibility standard deviation	0.97	3.73	2.55	6.33	2.56	4.22	5.00	4.82	5.89	1.38
R limit	2.7	10.5	7.1	17.7	7.2	11.8	14.0	13.5	16.5	3.9
Reproducibility %CV (k=2)	80	6	7	10	9	34	21	10	29	184
Horwitz PRSD$_R$ (%)	14.00	7.70	8.39	7.71	8.68	9.87	8.95	7.99	9.16	15.05
Horwitz s_R	0.34	9.92	6.13	9.86	5.06	2.44	4.26	8.06	3.73	0.23
Horwitz R	0.96	28.05	17.33	27.90	14.31	6.91	12.04	22.80	10.56	0.64
Horwitz Ratio	2.82	0.37	0.41	0.64	0.50	1.71	1.16	0.59	1.56	6.04

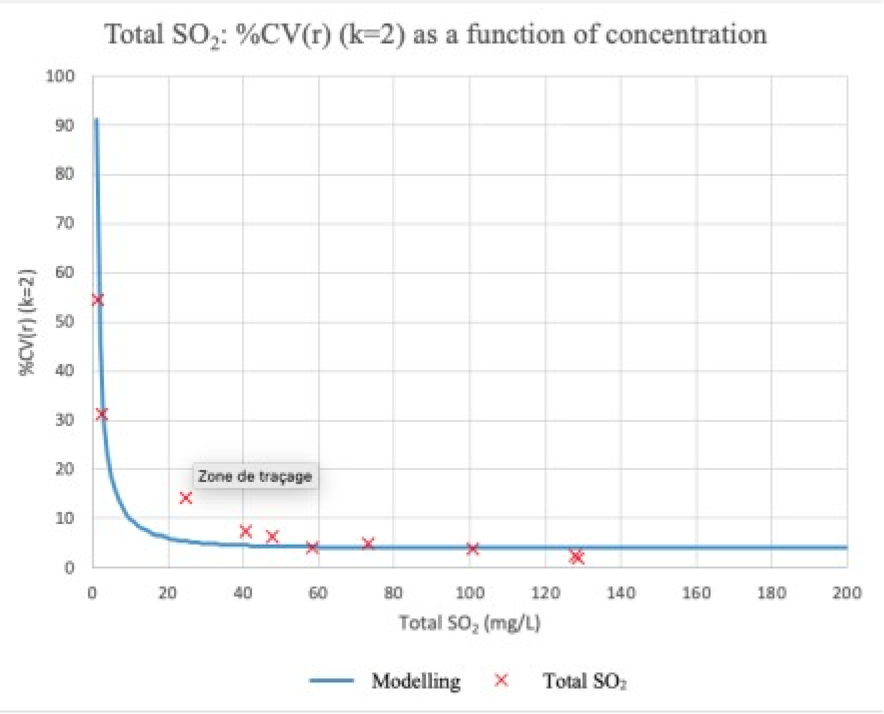


Figure 3: Modelling of the repeatability coefficient of variation, %CV(r) (k=2), as a function of concentration, C:

$$\%CV(r) = \sqrt{\frac{91^2}{C^2} + 4^2}$$

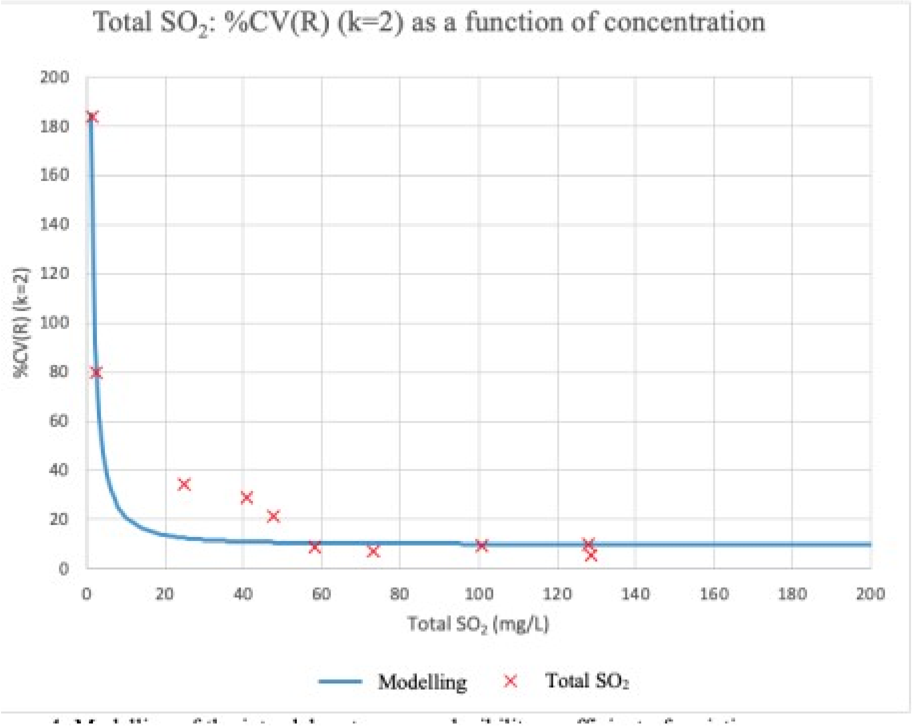


Figure 4: Modelling of the inter-laboratory reproducibility coefficient of variation, $\%CV_R$ (k=2), as a function of concentration, C:

$$\%CV(r) = \sqrt{\frac{184.9^2}{C^2} + 10^2}$$