RESOLUTION OIV-OENO 637-2021

SIMULTANEOUS ANALYSIS OF IRON, COPPER, POTASSIUM, CALCIUM AND MANGANESE IN WINES, USING MICROWAVE-INDUCED PLASMA ATOMIC EMISSION SPECTROMETRY

THE GENERAL ASSEMBLY,

IN VIEW OF the Article 2, paragraph 2 b) iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

AT THE PROPOSAL OF the “Methods of Analysis” Sub-Commission,

DECIDES to add the following method to Annex A of the *Compendium of International Methods of Analysis of Wines and Musts*:

Simultaneous analysis of iron, copper, potassium, calcium and manganese in wines, using MP/AES (microwave-induced plasma atomic emission spectrometry)

Type IV Method

1. Scope of application

This Type IV method, based on nitrogen plasma atomic emission spectrometry, makes it possible to simultaneously determine the following elements in wines.

Copper: 0.05-1 mg·L^{-1}

Iron: 1-10 mg·L^{-1}

Potassium: 15-1200 mg·L^{-1}

Calcium: 1-100 mg·L^{-1}

Manganese: 0.25-4 mg·L^{-1}

Where necessary, it is the responsibility of each laboratory using this method to redefine, and potentially widen, the scope of application via a validation study.

2. References

- ISO 78-2: Chemistry – Layouts for standards.
- ISO 3696: Water for analytical laboratory use.
- Resolution OIV OENO 418-2013.

3. Principle

Microwave-induced plasma atomic emission spectrometry (MP-AES) is a spectroscopic elemental method of analysis that works on the principle of atomic emission, with optical detection.

The sample, in liquid form, is introduced into a concentric nebuliser where an aerosol of the sample is generated via a double-pass cyclonic spray chamber and then introduced into the centre of the plasma using the plasma torch. The plasma is generated using a wave guide that focuses and maintains the microwave energy around the torch. The sample is thus desolvated, atomised and ionised, resulting in excitation of the atoms and ions, which are then transferred into the monochromator optical system.

The CCD detector enables simultaneous analysis of the background and signal for greater precision of analysis.

This apparatus functions with nitrogen plasma generated from compressed air and thus makes it possible to reduce the operational costs compared with other spectroscopy techniques for elemental analysis (ICP or AA).

4. Reagents and solutions

Unless otherwise specified, only reagents of recognised analytical quality should be used.

4.1. Ultra-pure, demineralised water with resistivity ≥ 18 MΩ (ISO standard 3696)

4.2. Mono or multi-elementary solution(s) (at 1000 or 10,000 $mg \cdot L^{-1}$), for the mineral elements analysed (Ca, Cu, Fe, K and Mn) and for the indium (In) used as an internal control. Use certified solutions when possible.

4.3. Internal control (by way of example): prepared mono- or multi-elementary synthetic solutions, and a control wine for which the target values have been obtained under reliable conditions (certified wine, or wine derived from an inter-laboratory comparison programme).

4.4. Nitric acid at over 60% (w/w), for trace analysis (CAS No. 7697-37-2)

4.5. Ethanol at over 99% purity (v/v) (CAS No. 64-17-5)

4.6. Cesium chloride at over 99% purity (w/w) (CAS No. 7647-17-8)

5. Apparatus and equipment

5.1. Atomic emission spectrometer coupled with nitrogen microwave plasma (MP-AES)

Note: The MP-AES can be equipped with a loop for transfer of the sample to increase the life cycle of the consumables (nebuliser and plasma torch). This system carries out rinsing with nitric acid at the sample input channels to the spray chamber. This reduces both the quantity of sample introduced into the nebuliser and the wear level of the equipment.

5.2. Multi-channel micropipettes suitable for taking variable volumes

5.3. Class A volumetric flasks

6. Preparation of the sample

6.1. Example preparation of the calibration range

Quantification is carried out by external calibration using calibration solutions, making it possible to establish 5-point calibration curves.

The calibration solutions are adjusted to 12% v/v ethanol and 0.2% v/v nitric acid.

Example calibration range:

mg·L^{-1}	**S0**	**S1**	**S2**	**S3**	**S4**	**S5**
Cu	0	0.1	0.25	0.5	0.75	1
Fe	0	1	2.5	5	7.5	10
K	0	400	600	800	1000	1200
Ca	0	10	25	50	75	100
Mn	0	0.5	1	2	3	4

6.2. Inline dilution of the samples

The calibration range as well as the samples to be analysed are diluted in line to a dilution factor of 1:2, using a simple Y-shaped device placed at the output of the peristaltic pump. The sample (channel 1) is diluted using nitric acid solution at 0.2% indium (600 mg.L^{-1}) and cesium chloride (0.3 g.L^{-1}) (channel 2). Indium is used as a control of stability throughout the analytical sequence, therefore its intensity is measured for all the analysed solutions.

This dilution makes it possible to limit the effects of saturation, in particular on potassium. Nitric acid produces a minor mineralisation effect, which, though only partial, facilitates the passage of organic compounds into the plasma.

Safety precautions – When handling acids, operators should protect their hands and eyes. Acids must be handled under a suitable hood.

7. Procedure

The parameters used to achieve the performance described in point 8 are as follows:

7.1. Instrumental parameters

The following description refers to an MP-AES instrument and provides example analytical conditions. Changes may be made by the laboratory as needed.

Instrumental parameter	**Specifications**
Nebuliser	OneNeb inert concentric
Spray chamber	Double-pass cyclonic
Tubing for the sample	Black-black (average flow rate 0.25 mL/min)
Tubing for the diluent (HNO_3 at 0.2%)	Black-black (average flow rate 0.25 mL/min)
Outlet tubing	Blue-blue (average flow rate 1 mL/min)
Sampling duration	20 s
Stabilisation duration	15 s
Rinsing duration	15 s

Pump speed	15 rpm
Number of replicates	3
AVS4 valve	**Specifications**
Pump speed	10 rpm
Sampling time	20 s (speed: rapid)
Commutation time	18 s
Flush time	15 s (speed: rapid)
Stabilisation duration	20 s
Number of replicates	3

7.2. Acquisition parameters

	Copper	**Calcium**	**Iron**	**Potassium**	**Manganese**	**Indium**
Reading duration	3 seconds					
Visualisation position	0	10	0	20	0	0
Nebulisation flow rate	0.5 mL/min	0.95 mL/min	0.55 mL/min	0.9 mL/min	0.65 mL/min	0.75 mL/min
Air injection rate	Average					High
Background correction	Automatic					
Calibration adjustment	Rational					/

Analysis wavelength	327.395 nm	445.478 nm	371.993 nm	404.414 nm	403.076 nm	325.600 nm

8. Expression of results

The results are expressed in mg.L^{-1} of element analysed, and the number of decimal places depends on the method performance for the element in question. Therefore, copper and manganese are expressed to 2 decimal places, iron to 1 decimal place, and potassium and calcium to the nearest unit of measurement, in accordance with the measurement uncertainties and limits of quantification of the method.

1. Annex: Example internal validation

Performance evaluation and validation were carried out according to the practical guide for the validation, quality control, and evaluation of a usual oenological method of analysis (Resolution OIV-OENO 418-2013).

1.1. Data acquisition

A total of 7 reference materials (ERM, doped samples and/or synthetic solutions) distributed across the range covering the scope of application of the methods in terms of concentration were used. These materials were analysed in n = 5 series under reproducibility conditions and within the stability time of the material for the parameter considered. For each material and each series, p = 2 repetitions were carried out. In the absence of RM, synthetic solutions composed of 12% ethanol and 0.2% nitric acid may be used.

1.2. Precision results

Copper 327.395 nm

(mg.L-1)

Precision	**Material 1 (rosé wine)**	**Material 2 (red wine)**	**Material 3 (rosé wine)**	**Material 4 (red wine)**	**Material 5 (sparkling white wine)**	**Material 6 (white wine)**

Target value	0.05	0.15	0.25	0.5	0.75	1.0
Sr	0.0008	0	0.0032	0.0095	0.01	0.05
r	0.002	0	0.00885	0.02656	0.029	0.137
s_I	0.0015	0.00548	0.01500	0.01500	0.025	0.051
%CV (k=2)	6.27	7.11	12.88	6.01	6.66	9.96

Where Sr is the repeatability standard deviation, r is the repeatability, S_I the intermediate precision standard deviation and %CV the wider precision coefficient of variation.

Iron 371.993 nm

(mg.L-1)

Precision	**Material 1 (synthetic solution)**	**Material 2 (red wine)**	**Material 3 (sparkling white wine)**	**Material 4 (rosé wine)**	**Material 5 (red wine)**	**Material 6 (red wine)**	**Material 7 (white wine)**
Target value	1	1.5	2	2.3	5	7.5	10
Sr	0.078	0	0.045	0.032	0.109	0.145	0.212
r	0.217	0	0.125	0.088	0.307	0.406	0.255
s*I*	0.106	0.055	0.088	0.122	0.294	0.280	0.415
%CV (k=2)	24.11	6.68	8.63	10.51	11.56	7.19	8.06

Potassium 404.414 nm

(mg.L-1)

Precision	**Material 1 (synthetic solution)**	**Material 2 (rosé wine)**	**Material 3 (sparkling white wine)**	**Material 4 (white wine)**	**Material 5 (red wine)**	**Material 6 (synthetic solution)**	**Material 7 (red wine)**
Target value	15	363	404.5	675	800	1000	1253
Sr	0.4	5.0	2.8	6.0	4.0	5.6	10.8
r	1.2	14.0	7.9	16.8	11.2	15.7	13.0
s*I*	1.0	16.0	19.3	33.5	22.7	41.6	37.1
%CV (k=2)	13.94	8.54	8.79	9.08	5.34	7.87	5.45

Calcium 445.478 nm
(mg.L-1)

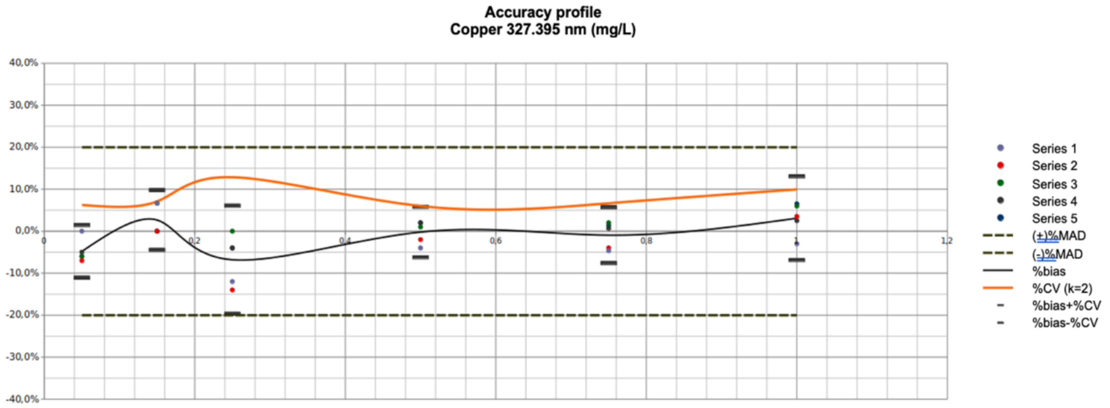
Precision	**Material 1 (synthetic solution)**	**Material 2 (synthetic solution)**	**Material 3 (White wine)**	**Material 4 (sparkling white wine)**	**Material 5 (red wine)**	**Material 6 (white wine)**	**Material 7 (rosé wine)**
Target value	0.5	1	6.8	27.5	54	68	100
Sr	0.08	0.03	0.07	0.32	0.83	0.41	0.86
r	0.23	0.09	0.20	0.90	2.33	1.16	0.15
s*I*	0.14	0.089	0.15	1.49	2.00	2.06	3.28
%CV (k=2)	68.59	15.33	4.81	10.59	6.67	6.01	6.64

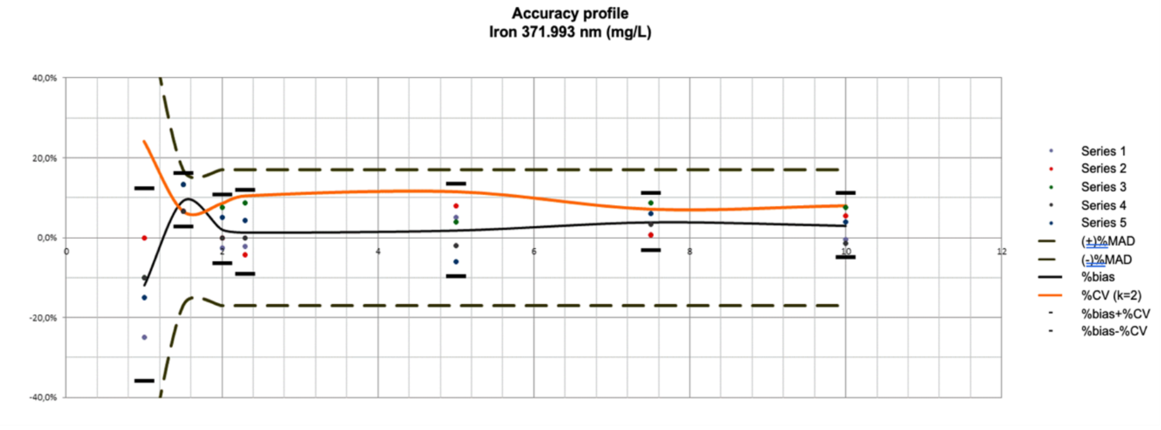
Manganese 403.076 nm
(mg.L-1)

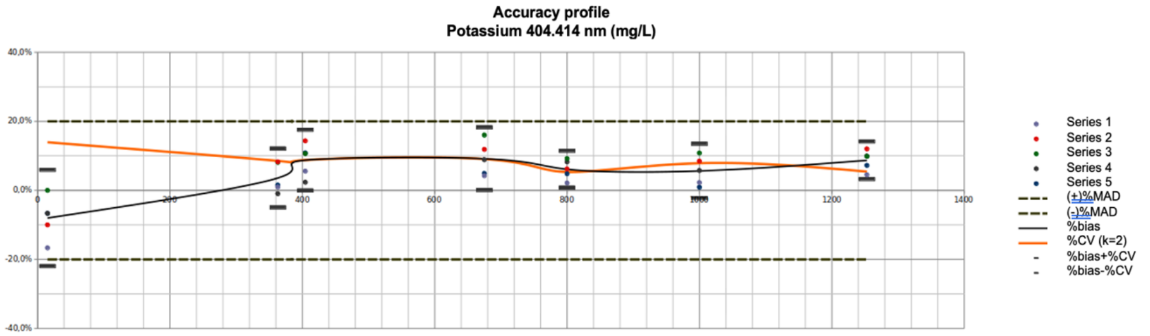
Precision	**Material 1 (synthetic solution)**	**Material 2 (red wine)**	**Material 3 (Rosé wine)**	**Material 4 (red wine)**	**Material 5 (sparkling white wine)**	**Material 6 (rosé wine)**	**Material 7 (white wine)**
Target value	0.25	0.54	0.67	1.34	2	3	4
Sr	0.019	0.008	0.006	0.012	0.015	0.016	0.020
r	0.054	0.023	0.018	0.034	0.042	0.045	0.017
s*I*	0.023	0.011	0.021	0.028	0.051	0.092	0.153
%CV (k=2)	17.46	3.69	5.63	3.67	4.78	5.68	7.22

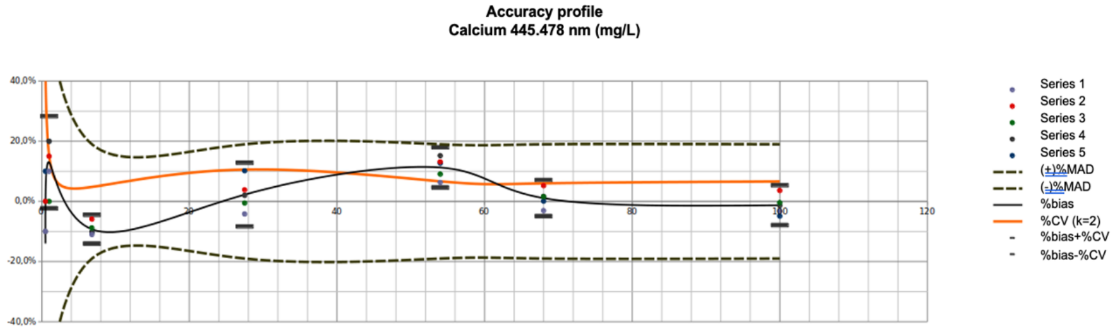
1.3. Trueness of the method

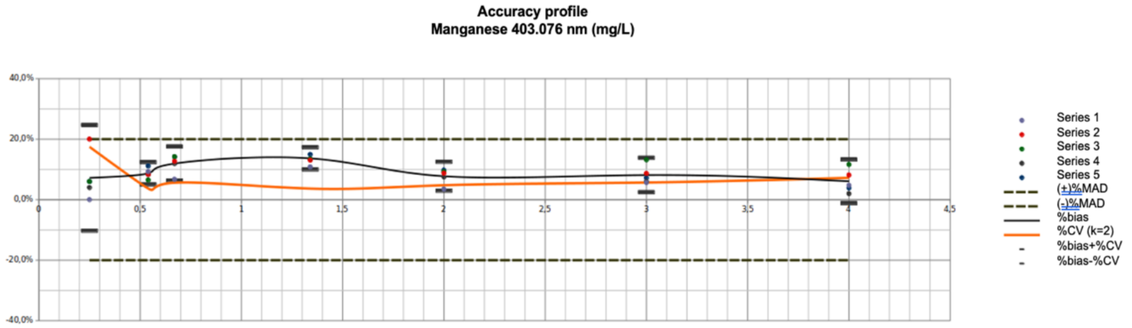
Accuracy profiles are established, graphically.




Accuracy profile
Iron 371.993 nm (mg/L)
40,0%
20,0%
0,0%
-20,0%
-40,0%
0
2
4
6
8
10
12
Series 1
Series 2
Series 3
Series 4
Series 5
(+)%MAD
(-)%MAD
%bias
%CV (k=2)
%bias+%CV
%bias-%CV


Accuracy profile
Potassium 404.414 nm (mg/L)
40,0%
20,0%
0,0%
-20,0%
-40,0%
200
400
600
800
1000
1200
1400
Series 1
Series 2
Series 3
Series 4
Series 5
(+)%MAD
(-)%MAD
%bias
%CV (k=2)
%bias+%CV
%bias-%CV


Accuracy profile
Calcium 445.478 nm (mg/L)
40,0%
20,0%
0,0%
-20,0%
-40,0%
0
20
40
60
80
100
120
Series 1
Series 2
Series 3
Series 4
Series 5
(+)%MAD
(-)%MAD
%bias
%CV (k=2)
%bias+%CV
%bias-%CV



The verification of the trueness is carried out, for each concentration level, by comparing the interval produced by the intermediate precision around the value measured ($CV_{precision}$) with the interval of the MAD (Maximum Acceptable Deviation) around the reference value of the material. The trueness is accepted if the $CV_{precision}$ falls within the MAD. With regard to the MAD, the trueness tests are validated for all of the elements studied.

1.4. Limits of quantification

The limits of quantification (LOQ) are established by studying the range close to the low limit values. The value tested for the LOQ is validated if its %CV (k=2) < 60% (Resolution OIV-OENO 418-2013).

The following LOQ were validated:

mg.L^{-1}	**Method LOQ**
Copper	0.05
Iron	1
Potassium	15
Calcium	1
Manganese	0.25