OIV-MA-F1-10 Specific methods for the analysis of grape sugar

Type IV method

Annex D: Heavy metals

D.1 Determination of lead level by ETAAS

Heavy metals are described in detail in the steps for preparing grape sugar samples (concentrated musts and MCR rectified concentrated musts) for determining lead content.

The instrumentation and computer tools used in the testing laboratories vary and change.

As a result, general criteria for calibration and metrics are given.

1. Warning

Security precautions – Operators must protect their hands and eyes when handling acids. Acids must be handled under an appropriate fume hood.

2. Scope

This method specifies an electrothermal atomic absorption spectrometry method (ETAAS) for determining the lead content of rectified concentrated musts between 10 µg/kg to 200 µg/kg.

3. Normative references

ISO 3696, Water for analytical laboratory use - Specification and test methods

4. Principle

In Electrothermal Atomic Absorption Spectrometry (ETAAS), a sample volume is inserted into a graphite tube which may be heated to over 2800°C. When the temperature is gradually increased, the sample matrix dries and thermal decomposition and dissociation occur. For most elements, the peak height is proportional to the concentration of the element in the solution, although in a very large number of cases, it is preferable to work with the peak area.

5. Reagents and solutions

Unless otherwise indicated, only use lead-free reagents of recognised analytical quality.

5.1. Demineralised, ultra-pure water with resistivity above 18 MΩ, following ISO 3696.

5.2. Nitric acid of a concentration above 60% (Normapur quality).

5.3. Ammonium dihydrogen phosphate $NH_4H_2PO_4$

5.4. Matrix modifier: $NH_4H_2PO_4$, 6% solution

Pour 3g of $NH_4H_2PO_4$ into a 50 ml volumetric flask. Dissolve and bring to volume with demineralised water.

5.5. Magnesium nitrate Mg(NO_3)$_2$ to 6 molecules of water

5.6. 0.5% magnesium nitrate solution (keep refrigerated).

Pour 0.5g of magnesium nitrate into a 100 ml volumetric flask. Dissolve and bring to volume with demineralised water. Keep this solution refrigerated for a maximum of 15-20 days.

5.7. Certified mono element lead solution(s) (at 1000 mg/l)

5.8. 10 mg/l lead solution

Place 1 ml of the stock solution (5.7) and 10 ml of nitric acid (5.2) in a 100 ml flask. Bring to volume with ultra-pure water (5.1).

5.9. 100 µg/l lead solution

Place 1 ml of the stock solution (5.8) in a 100 ml flask. Add 10 ml of nitric acid (5.2). Bring to volume with ultra-pure water (5.1).

5.10. Triton X-100 (1% v/v)

5.11. Blank test: 10% nitric acid

Preparation of the calibration range

The number of calibration solutions depends on the required precision. At least five standards are required. The precision and accuracy of the results may be verified by analysing a control sample.

It should be stressed that the linearity of the calibration curve is often limited.

Correct the absorbance values of the calibration solutions by subtracting the absorbance value of the blank test. To plot a calibration curve or calculate the calibration function, use the values obtained as well as the analyte concentration of the calibration solutions.

Depending on the type of apparatus, it is possible to work with an autosampler to direct inject predetermined volumes from the 100 µg/l solution in order to have a calibration range of 0 to 100 µg/l (e.g. 0, 10, 25, 50, 100 µg/l). It is also possible to prepare calibration solutions separately.

Note 1*: A smaller volume of test sample may be used to determine lead content greater than 100* µ*g/l.*

6. Apparatus and equipment

6.1. Atomic absorption spectrometer equipped with an electrothermal atomiser, a hollow-cathode lamp adapted for lead functioning with the manufacturer-recommended current, an automatic correction device for background noise and a computer reading system or high-speed graphic recorder. A correction of background noise should be used with electrothermal atomic absorption spectrometry. The minimum acceptable technical specification is based on deuterium. A correction of the Zeeman background noise is preferable if the signal from background noise is high. To increase the analyte signal-to-noise ratio, the use of a graphite tube with a pyrolytic platform is recommended.

Note 2: *a wavelength of 217.0 nm can be used for lead. The sensitivity is about two times higher than that obtained at 283.3 nm. However, since the noise and risk of interference are greater, it is necessary to work with a Zeeman background noise correction system.*

6.2. Precision balance accurate to 0.1 mg

6.3. Class A graduated pipettes: 0.5 ml, 1 ml, 5 ml

6.4. Class A volumetric flasks: 50 ml and 100 ml

Note 3: *the material in contact with the sample must remain in a 10% nitric acid solution (5.2) for at least 12 hours and must be subsequently rinsed several times with demineralised water (5.1).*

7. Sampling

Preparation of the sample for testing

In a 50 ml flask (6.4), pour 10 g of the sample accurate to 0.1 mg, 5 ml of nitric acid (5.2), and 0.5 ml of triton X-100 (1% v/v) (5.10). Bring to 50 ml with the demineralised water (5.1) and homogenise.

8. Procedure

Adjust the instrumental parameters and align the electrothermal atomiser following the manufacturer's instructions in order to derive maximum benefit from the background noise correction system. Adjust the sampler in the same way. Determine the optimal parameters for the electrothermal atomiser for the particular type of atomiser and sample volume, as recommended by the apparatus manufacturer, in order to cover the optimal measuring range. Adjust the baseline of the apparatus to

zero. Verify the stability of zero in the atomisation system by executing the predefined temperature programme for the white heating of the graphite atomiser. The following furnace settings may be used:

phase	temperature	level-off	rise (ramp)	type of gas	gas speed	reading
	(°C)	(s)	(°C/s)		(l/mn)	
1	130	15	10	argon	0.2	no
2	350	5	25	argon	0.2	no
3	500	5	50	argon	0.2	no
4	750	10	100	argon	0.2	no
5	1900	3	0	argon	stopped	yes
6	2500	3	0	argon	0.2	no
7	100	10	0	argon	0.2	no

Using an autosampler, inject a set volume of the solution. Add a set volume of modifier solution and atomise the blank test (5.11), the calibration solutions and the diluted test sample solutions by order of increasing response of the apparatus. If the peak height (or the peak area) of the test sample is greater than the value of the calibration solution with the highest concentration, a lower test sample volume must be used.

External calibration

The following programme for the sampler is given as an example (volume in µl) to determine the lead content through external calibration.

	blank test	sample	standard 1	standard 2	standard 3	standard 4
			10 µg/l	25 µg/l	50 µg/l	100 µg/l

blank (HNO_3 10%)	5.0					
thinner (HNO, 10%)			4.5	3.7	2.5	0
sample		5.0				
stock solution (Pb 100 µg/l)			0.5	1.3	2.5	5.0
$NH_4H_2PO_4$ 6%	4.0	4.0	4.0	4.0	4.0	4.0
Mg(NO_3)$_2$ 0.5%	2.0	2.0	2.0	2.0	2.0	2.0
total volume	11.0	11.0	11.0	11.0	11.0	11.0

Atomise each solution at least two times and, if the reproducibility is acceptable in compliance with the quality control system used in the laboratory, calculate the average of readings. If necessary, reset the baseline to zero.

Addition method

It is also possible to use the addition method to reduce the effect of non-spectral interferences between the standards and the samples, as long as the calibration curve is linear in the absorbance range used.

Transfer identical volumes of the testing sample in three receptacles (for example, autosampler cups). Add a small quantity of standard solution to two of the receptacles, calculated in order to obtain a concentration for the samples, respectively, of between 100% and 200% higher than the concentration in the original sample. Add the same quantity of water in the third receptacle. Carefully mix the solutions. Measure the integrated absorbance of each solution and plot a curve with the added concentration on the *x*-axis and the measured absorbance on the *y*-axis. Determine the analyte concentration of the reagent blank solution or the test blank solution in the same manner.

The following programme for the sampler is given as an example (volume in µl) to

determine the lead content using the addition method.

	blank test	sample	addition 1	addition 2
			25 µg/l	50 µg/l
blank (HNO_3 10%)	5.0			
thinner (HNO, 10%)	2.5	2.5	1.2	0
sample		5.0	5.0	5.0
stock solution (Pb 100 µg/l)			1.3	2.5
$NH_4H_2PO_4$ 6 %	1.0	1.0	1.0	1.0
Mg(NO_3)$_2$ 0.5%	2.0	2.0	2.0	2.0
total volume	10.5	10.5	10.5	10.5

9. Calculation

The apparatus software establishes the calibration graph (absorbance as a function of lead concentration in µg/l). It gives the lead concentration of the samples. Take into account any dilution, if applicable.

10. Precision parameters

For a lead concentration inferior to 150 µG/kg:

r (Repetability) = 15 µg/kg

R (Reproducibility) = 25 µg/kg

11. Bibliography

- Laboratoire SCL33. *Détermination du plomb dans le vin par atomic absorption spectrometry (four-graphite)*. Manuel d'instructions, 2010.
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- Rodriguez Garcia J.C. *Desarrollo de metodologías para la determinación de metales en miel mediante ETAAS y estudio quimiométrico de su empleo como bioindicador*. Universidad de Santiago de Compostela, Facultad de Ciencias, Campus de Lugo, 2006.