RESOLUTION OENO 7/2006

CRITERIA FOR THE METHODS OF QUANTIFICATION OF LEAD IN WINE

THE GENERAL ASSEMBLY,

CONSIDERING Article 2 paragraph 2 iv of the agreement establishing the International Organisation of Vine and Wine,

UPON THE PROPOSAL of the Sub-commission of Methods of Analysis and Appraisal of Wine,

DECIDES: to delete the method for the detection of lead in wine in Annex C of the Compendium of International Methods of Analysis of Musts and Wines

POINTS OUT that these criteria should be reconsidered taking into account decisions of other international bodies, notably the Codex Alimentarius committee on methods of analysis and sampling

DECIDES: to adopt the criteria below for the methods for the quantification of lead in wine. As examples, the two following Type III methods (Example 1 and Example 2) respond to the mentioned criteria performance:

CRITERIA FOR THE METHODS OF QUANTIFICATION OF LEAD IN WINE

1.1. Method Criteria Definitions

Trueness: the closeness of agreement between the average value obtained from a large series of test results and an accepted reference value

r = Repeatability limit, the value below which the absolute difference between 2 single test results obtained under repeatability conditions (i.e., same sample, same operator, same apparatus, same laboratory, and short interval of time) may be expected to lie within a specific probability (typically 95%) and hence r = 2.8 x s_r.

S_r = Standard deviation, calculated from results generated under repeatability conditions.

RSD_r= Relative standard deviation, calculated from results generated under repeatability conditions [($S_r/\bar{x}$) x 100], where $\bar{x}$ is the average of results over all laboratories and samples.

R = Reproducibility limit, the value below which the absolute difference between single test results obtained under reproducibility conditions (i.e., on identical material obtained by operators in different laboratories, using the standardised test method),

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may be expected to lie within a certain probability (typically 95%); R = 2.8 x s_R.

S_R = Standard deviation, calculated from results under reproducibility conditions.

RSD_R = Relative standard deviation calculated from results generated under reproducibility conditions [($S_R/\bar{x}$ x 100]

Ho_R = HORRAT value: the observed RSD_R value divided by the RSD_R value calculated from the Horwitz equation [1].

2. Method of analysis to be used by the laboratory and laboratory control requirements

2.1. Requirements

Specific methods for the determination of lead in wine are not prescribed. Laboratories shall use a method validated to OIV requirements [2] that fulfils the performance criteria indicated in Table 1 e.g. GFAA or ICP-MS methods are applicable provided they meet the performance criteria outlined below. Wherever possible, the validation shall include a certified reference material in the collaborative trial test materials. If not an alternative estimation of trueness should be used. Examples of suitably validated methods for the determination of lead in wine are provided in Appendices 1 & 2.

2.2. General considerations

All apparatus which comes into contact with the sample shall be made of an inert material (e.g. polypropylene, polytetrafluoroethylene [PTFE], etc.). The use of ceramic materials is not advisable because of the possibility that lead might be present. If it is not certain that the materials available are free from the analytes in question, their use shall be assessed by means of *ad hoc* studies, which should be considered as an integral part of the validation of the method of analysis. All plastic ware including sample containers shall be acid cleaned. If possible, equipment used for preparing samples should be reserved for lead analyses only.

Table 1: Performance criteria for methods of analyses for lead in wine

Parameter	Value/Comment
Applicability	Suitable for determining lead in wine for official purposes.

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Detection limit	No more than one tenth of the value of the OIV limit (expressed in □g/L)
Limit of quantification	No more than one fifth of the value of the OIV limit (expressed in □g/L) except if the value of the limit for lead is less than 100 □g/L. For the latter, no more than two fifth of the value of the specification
Precision	HORRAT values of less or equal to 2 in the validation collaborative trial
Recovery	80% - 105% (as indicated in the collaborative trial)
Specificity	Free from matrix or spectral interferences
Trueness	$\lvert\bar{x} - m\rvert < 1{,}96 * \sqrt{S_{R(lab)^2} - S_{r(lab)^2} * (1 - 1/n)}$ where m is the certified value of the wine reference material and $\bar{x}$ is the average of n measurements of lead content in this wine, within the same laboratory. $S_R(lab)$ and $S_r(lab)$ are standard deviations, calculated from results within the same laboratory under reproducibility and repeatability conditions.

2.3. Estimation of the analytical trueness and recovery calculations

Wherever possible the trueness of the analyses shall be estimated [3] by including suitable certified reference materials in the analytical run. The analyst shall also take due note of the 'Harmonised Guidelines for the Use of Recovery Information in Analytical Measurement' [4] developed under the auspices of IUPAC/ISO/AOAC. The recovery should be approximately 100 % in which case recovery calculations are of minor importance.

References

1. W Horwitz, "Evaluation of Analytical Methods for Regulation of Foods and Drugs", Anal. Chem., 1982, **54**, 67A - 76A
2. Protocol for the design, conduct and interpretation of method-performance studies,

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Secretary of the General Assembly
Frederico CASTELLUCCI

FV 1061, OIV, 1998

3. ISO 5725-6:1994, 4.2.3. International Organisation for Standardisation, case Postal 56, CH-1211, Genève 20, Switzerland.

4. ISO/AOAC/IUPAC Harmonised Guidelines for the Use of Recovery Information in Analytical Measurement. *Edited* Michael Thompson, Steven L R Ellison, Ales Fajgelj, Paul Willetts and Roger Wood, Pure Appl. Chem., 1999, 71, 337 – 348

EXAMPLE 1

DETERMINATION OF LEAD IN WINE BY ATOMIC ABSORPTION SPECTROMETRY (TYPE III)

1. SCOPE AND FIELD OF APPLICATION

The method can be used for red, white, still, sparkling and fortified wines.

2. DEFINITION

The lead content of wine: the content of lead determined by this procedure expressed as mg/L.

3. PRINCIPLE

Wine is diluted by a matrix matching cocktail and the lead concentration measured directly by graphite furnace atomic absorption spectrometry (GFAAS). A matrix matching mixture is added to both the wine to be determined and the lead calibration standard solutions. This mixture contains both GFAAS 'matrix modifiers' and wine simulating components. Their purpose is to 'modify' the matrices so that the same shape absorption vs. time profile is obtained from both standard solutions and sample solutions during the graphite furnace atomisation stage.

A delayed atomisation mechanism is required e.g. L'vov platform.

The exact composition of the diluent may need to be adjusted to suit particular models of graphite furnace instruments. Before the method is applied experiments must be conducted to check the absorbance vs. time profiles produced by standards and samples and necessary adjustments made to the diluent. The instrument used

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Secretary of the General Assembly
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must be capable of monitoring the absorbance vs. time profile during atomisation. The profile should be such that standards and samples perform alike and that the lead atomisation peak precedes the bulk of the background non-specific absorption enabling the background correction mechanism employed to operate effectively. Examples of matched profiles are given in Annex 2.

4. REAGENTS

Chemicals should be of the highest quality available in terms of being free of lead. Deionised distilled water, or water of equivalent purity, is to be used. Unless otherwise indicated all solutions are prepared fresh daily.

4.1. Diluent solution

NOTE 1: The exact composition of the diluent used may need adjustment to suit the specific model of instrument and graphite furnace employed. If problems are experienced with the suggested modifier composition adjust the phosphate and nitrate concentrations to give:

i. a stable element signal at the optimum ashing temperature and

ii. atomisation with a single reproducible analyte peak which is time separated from the background signal.

Equipment with VDU facilities will allow analysts to confirm time separation of the sample and background peaks (See Annex). The following is an example of a technique for determining the absorbance versus time profile:

Measure the full peak width at half maximum height (FWHM) of a sample peak and compare it to the FWHM of a calibration standard with a similar maximum absorbance. If the peak shapes are visibly different then the composition of the matrix modification modifier needs to be adjusted.

The following are examples of diluents utilised for:

(a) a Perkin-Elmer 3030 equipped with deuterium arc background corrector with an HGA 500 furnace; and (b) a Thermo-Electron Video 12E equipped with Smith-Hieftje background corrector, a CTF 188 furnace and a FASTAC sample deposition system.

4.1.1. Perkin-Elmer 3030 diluent:

To 187 g of water in a 250 ml plastic bottle (5.1) add 11 g ethanol (4.1.3.), 1.1 g of glucose (4.1.4.), 1.1 g of fructose (4.1.5.) and 0.28 g of sodium chloride (4.1.6.). Shake to dissolve

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the solids. Then add 22 ml nitric acid (4.1.7.) and 4.4 g ammonium dihydrogen orthophosphate (4.1.8.). Shake until all the phosphate has dissolved. Finally add 0.88 g magnesium nitrate (4.1.9.) and shake again until no undissolved solid remains.

4.1.2. Thermo-Electron Video 12E diluent:

As above but only 0.66 g of ammonium dihydrogen orthophosphate (4.1.8.) and 0.44 g magnesium nitrate (4.1.9.) are used.

4.1.3. *Ethanol (absolute)*

4.1.4. *D-glucose*

4.1.5. *D(-)fructose*

4.1.6. *Sodium chloride*

4.1.7. *Nitric acid (concentrated)*

4.1.8. *Ammonium dihydrogen orthophosphate*

4.1.9. *Magnesium nitrate hexahydrate*

4.2. 10% ethanol (v/v)

To 180 ml water in a 250 ml plastic bottle (5.1.) add using a pipette 20 ml of ethanol (4.1.3.) and shake to mix.

4.3. Lead standard solutions

4.3.1. *Lead standard solution (1000 mg/l)*

4.3.2 *Lead standard solution (10.00 mg/l)*

Into a 100 ml volumetric flask (5.2.) pipette (5.7.) 1.00 ml of lead standard solution (4.3.1.). Dilute to volume with water and mix thoroughly.

NOTE 2 : Check calibration of pipette immediately prior to use.

4.3.3. *Lead working standard (1.00 mg/l)*

Into a 100 ml volumetric flask (5.2.) weigh out 10.00 g of the lead stock solution (4.3.2.) using a Pasteur pipette (5.3.). Wash the inside neck of the volumetric flask with water, add 1 ml of nitric acid (4.1.7.) and make up to the mark with water. Shake to mix thoroughly.

4.3.4. *Lead calibration solutions.*

The eight calibration standards are made up in universal containers (5.4.). A range of 0 to 50 □g/l is covered by the standards. They are 0.0, 2.5, 5.0, 10.0, 20.0, 30.0, 40.0 and 50.0 □g/l. A ninth container is used to prepare a reagent blank.

Rinse out the inside of each container three times with water and shake dry; rinse the caps three times and shake dry. Leave the capped containers standing upright for 5-10

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The Director General of the OIV
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minutes and then shake out residual liquid. Pipette (5.8) into the 9 containers, in order: 5.00, 5.00, 4.95, 4.90, 4.80, 4.60, 4.40, 4.20 and 4.00 ml of water. Into each of the containers pipette (5.8.) 5.00 ml of 10 % ethanol (4.2) followed by two 5.00 ml aliquots of diluent (4.1).

Into the 9 containers pipette (5.6) (5.7) in order: 0 (reagent blank), 0, 50, 100, 200, 400, 600, 800 and 1000 µl of working standard (4.3.3). Cap the containers and shake to mix the contents. Prepare fresh for each batch of samples.

4.4. 1 % (v/v) nitric acid.

5. APPARATUS

All glass and plastic ware used must be acid cleaned (soaked in 20 % nitric acid for at least 24 hours), rinsed thoroughly with distilled water prior to use and kept covered (with cling-film if appropriate) to prevent aerial contamination.

5.1. 250 plastic bottles, with caps (for example: Nalgene or equivalent).

5.2. Volumetric flasks, 100 ml (Grade A).

5.3. Pasteur pipettes, with teats

5.4. Universal containers, 30 ml (Nunc, Sterilin or equivalent).

5.5. Glass beakers, 600 ml.

5.6. Pipette*, 40 - 200µl (Labsystems Finnpipette or equivalent).

5.7. Pipette*, 200 - 1000µl (Labsystems Finnpipette or equivalent).

5.8. Pipette*, 0.5 - 5.0 ml (Labsystems Finnpipette or equivalent).

5.9. Pipette*, 2.0 - 10.0 ml (Labsystems Finnpipette or equivalent).

**NOTE 3: pipettes should be calibrated each day (of use).*

5.10. Analytical balance, (+ or - 1 mg, Mettler PC440 or equivalent).

5.11. Vortex type mixer or equivalent.

5.12. Test tubes, 20 ml capacity.

5.13. Test tube racks, suitable for use with 5.12.

5.14. Container racks, suitable for use with 5.4.

5.15. Magnetic stirrer.

5.16. Magnetic follower, PTFE coated.

5.17. Pipette tips, suitable for use with 5.6, 5.7, 5.8 and 5.9

5.18. Atomic absorption spectrometer,

Atomic absorption spectrophotometer equipped with a graphite furnace, atomisation

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The Director General of the OIV
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Frederico CASTELLUCCI

delay cuvette, auto-injector, background corrector, and absorbance vs. time profile monitoring facility equivalent to the following. Instrumental conditions should be adjusted appropriately for the model used. **The following are given as examples**:

(a). Atomic absorption spectrophotometer, Perkin-Elmer 3030 equipped with deuterium arc background corrector for non-specific absorption. Lead hollow cathode lamp operated at 12 mA. Monitor the 283.3 nm line; slit width 0.7 nm. Graphite furnace, HGA 500 fitted with pyrolytically coated graphite tube with a solid pyrolytic graphite L'vov platform resting inside. Use argon as the purge gas. The furnace conditions for the HGA 500 are as follows:

Step	1	2	3	4	5	6
Temperature (□C)	200	1100	1100	1800	2400	20
Ramp (s)	5	20	1	0	1	1
Hold (s)	60	20	2	3	6	25
Gas	Ar	Ar	Ar	Ar	Ar	Ar
Gas flow (mL/min)	50	50	0	0	300	300
Read (2.5s integration)				X		

Auto-sampler/injector, AS 40. 20 □l injection volume, 3 injections per tray position.

(b) Thermo-electron Video 12E Atomic absorption spectrophotometer used with a CTF 188 Graphite Furnace and a FASTAC sample deposition system with the following conditions:

Step	1	2	3	4	5
Temperature (□C)	150	350	650	1000	2400
Ramp (s)	0	30	15	1	
Hold (s)	2	0	5	4	10

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The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

Gas	Ar	Ar	Ar	Ar	Ar
Gas flow (mL/min)	50	50	0	0	300
Read (2.5s integration)				X	

Sample deposition 5 s, FASTAC delay time 10 s, with 3 injections per tray position. Monitor the 283.3 nm line.

6. PROCEDURE

6.1. Preparation of wine

Shake the wine container to thoroughly mix the contents before sub-sampling. Sparkling wines should be transferred to a clean beaker and placed in an ultrasonic bath until gas is no longer evolved prior to use.

6.2. Measurement solutions

6.2.1. Wine samples

Into a 20 ml test tube (5.12) pipette (5.8) 2.00 ml of water, 4.00 ml of diluent (4.1) and 2.00 ml of the sample wine. Mix thoroughly using the vortex mixer (5.11).

6.2.2. Recovery estimates

For recovery estimate purposes pipette (5.8) into a 20 ml test tube (5.12) 1.80 ml of water, 4.00 ml of diluent (4.1), 2.00 ml of the sample wine and add using a pipette (5.7) 0.200 ml of lead working standard (4.3.3). Mix thoroughly using the vortex mixer (5.11).

NOTE 4: Any sample that exceeds the highest calibration standard will have to be re-analysed using a smaller sample aliquot. Add extra 10% ethanol (4.2) to the sample volume.

6.3. Measurement

Determinations are carried out in batches. Each batch is to contain at least four replicates of the reagent blank and three spiked replicates of samples for recovery estimate purposes. The lead calibration solutions are distributed evenly amongst the unknowns on the auto-sampler tray. Transfer the samples and standards to the auto-sampler sample containers using a Pasteur pipette (5.3). Discard the first filling of the

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The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

container and measure the second filling (if there is not enough sample solution, care will have to be taken to ensure that the sample containers are scrupulously clean). Wash the Pasteur pipette four or five times with 1% nitric acid (4.4.) between each standard and sample transfer.

6.4. Quantification of lead

The mean absorbance from 3 injections is used in all cases.Construct a calibration graph from the mean responses given by the in-batch standards. Note the absorbances recorded by the instrument for each sample. The lead concentration of the sample solutions are determined by comparison with the calibration graph.

NOTE 5: It is recommended that the furnace tube and platform be replaced every two batches or sooner if there is a marked decrease in the measured absorbance of the standards.

7. EXPRESSION OF RESULTS

Correct the results for the average in-batch recovery.

7.1. Calculation

Obtain from the calibration graph, the lead content of all the measurement solutions. Calculate the lead content of the wine samples and spiked wine samples using the following calculation:

$$Pb\ concentration\ (mg/l) = \frac{(C_m - C_b) \times V_t}{V_m}$$

where:

C_m is the mean lead concentration of the measurement solution (mg/l).

C_b is the mean measured lead concentration of the reagent blank solutions (mg/l).

V_t. is the final total volume of the measurement solution (ml).

V_m is the volume of the wine sample taken (ml).

7.2. Calculation of recovery estimates

$$Recovery\ (\%) = \frac{(C_s - C_a) \times V_s \times 100}{S}$$

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The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

Where:
C_s is the calculated mean lead concentration of the spiked wine sample (mg/l).
C_a is the calculated mean lead concentration of the unspiked wine (mg/l).
V_s is the volume of wine to which the spike is added (ml).
S is the amount of spike added (□g).

7.3. Calculation of recovery corrected results

$$Corerected\ Pb\ concentration\ (mg/L) = \frac{C_w \times 100}{R_a}$$

where:
C_w is the calculated lead concentration of the wine sample (mg/l).
R_a is the average in-batch recovery (%).

ANNEX: VALIDATION STUDY

The following study was carried out to internationally procedures (1)(2).
TABLE 1: SAMPLE SCHEME

Sample Code	Sample Description
5 & 9	Bordeaux (Sweet White)
3 & 11	Italian Chardonnay (White)
7 & 8	Spanish Red fortified at 260 □g/l
6 & 10	Romanian Pinot Noir
2 & 12	Romanian Pinot Noir fortified with 150 □g/l
1	Sample 3/11 fortified with 124 □g/l
4	Sample 3/11 fortified with 134 □g/l

TABLE II SUMMARY OF STATISTICAL PARAMETERS FOR LEAD IN WINE

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Frederico CASTELLUCCI

COLLABORATIVE TRIAL (The results from one laboratory were assessed as being inappropriate for inclusion in the statistical analysis)

Sample	A	B	C	D	E	F	F1
Code	5, 9	3, 11	7, 8	6, 10	2, 12	1	4
n	16	15*	16	16	16	16	
n (-outl)	16	15	14	16	15	16	
Targ.	56	24	279	67	192	143	153
Mean	50.8	27.2	298	70.6	189	143	149
r	23	15	24	32	51	38	
Sr	8.1	5.3	8.7	11.8	18.2	13.6	
RSDr	16	19	3	17	10	9	
Hor	**1.0**	**1.1**	**0.2**	**1.1**	**0.7**	**0.7**	
R	42	25	83	57	154	79	
SR	15.1	8.8	29.8	20.3	55.2	28.2	
RSDR	**30**	**28**	**10**	**29**	**29**	**19**	
HoR	**1.2**	**1.2**	**0.5**	**1.2**	**1.4**	**0.9**	

KEY TO TABLES I-II

n : Initial number of laboratories

n (-outl) : Number of laboratories after removal of outliers

(*): Laboratory 17 reported <20 µg/l for test material 11. Their results have not been included in the statistical analysis for this sample (B).

Mean: The observed mean, the mean obtained from the collaborative trial data after removal of outliers.

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Targ.: The mean observed value obtained "in-house" using ICP-MS

r: Repeatability limit, the value below which the absolute difference between 2 single test results obtained under repeatability conditions (i.e., same sample, same operator, same apparatus, same laboratory, and short interval of time) may be expected to lie within a specific probability (typically 95%) and hence r = 2.8 x sr.

S_r: The standard deviation of the repeatability.

RSD^r: The relative standard deviation of the repeatability (Sr x 100/MEAN).

Ho^r: The observed RSDr divided by the RSDr value estimated from the Horwitz equation using the assumption r=0.66R.

R: Reproducibility limit, the value below which the absolute difference between single test results obtained under reproducibility conditions (i.e., on identical material obtained by operators in different laboratories, using the standardised test method), may be expected to lie within a certain probability (typically 95%); R = 2.8 x sR.

S^R: The standard deviation of the reproducibility (between laboratory variation).

RSD^R: The relative standard deviation of the reproducibility (SR x 100/MEAN).

Ho^R: The observed RSDR value divided by the RSDR value calculated from the Horwitz equation.

- $RSD_R = 2(1-0.5\log_{10}C)$
- (where C = concentration expressed as a decimal)

HORRAT[4] values are:

For repeatability, the observed RSDr divided by the RSDr value estimated from the Horwitz equation using the assumption r = 0.66R.

For reproducibility, the observed RSDR divided by the RSDR value estimated from the Horwitz equation.

8. REFERENCES

1. Paul A. Brereton, Paul Robb, Christine M Sargent, Helen M. Crews and Roger Wood. Determination of Lead in Wine by Graphite Furnace Atomic Absorption Spectrometry: Interlaboratory Study. *JAOAC Int.*, 1997, **80**, No 6, 1287-1297.

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Secretary of the General Assembly
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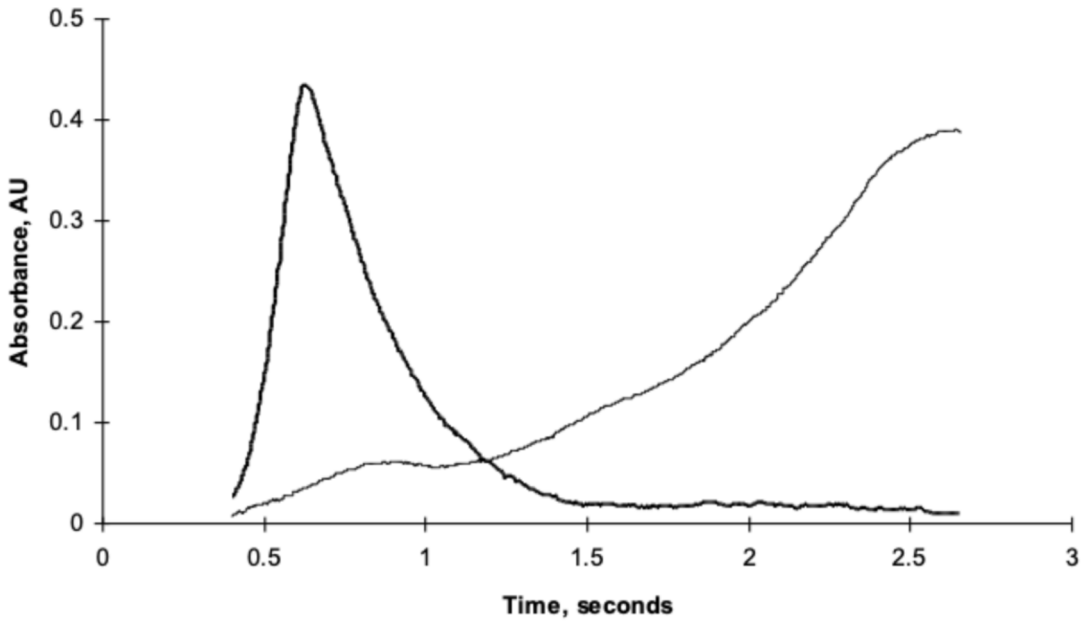
2. "Protocol for the Design, Conduct and Interpretation of Collaborative Studies." Editor W Horwitz, Pure & Appl. Chem., Vol. 67, No., 2, pp.331-343, 1995

3. Horwitz W, Evaluation of Methods Used for Regulation of Foods and Drugs, *Analytical Chemistry*, 1982, **57**, 67A-76A

4. Peeler J T, Horwitz W and Albert R, Precision Parameters of Standard Methods of Analysis for Dairy Products, *JAOAC*, 1989, 72, No 5, 784-806.

Annex

Absorbance vs. Time profiles for the measurement of lead in wine using a Perkin□Elmer 3030 atomic absorbtion spectrometer with deuterium arc background correction.



(i) 30 ng/l wine standard

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Secretary of the General Assembly
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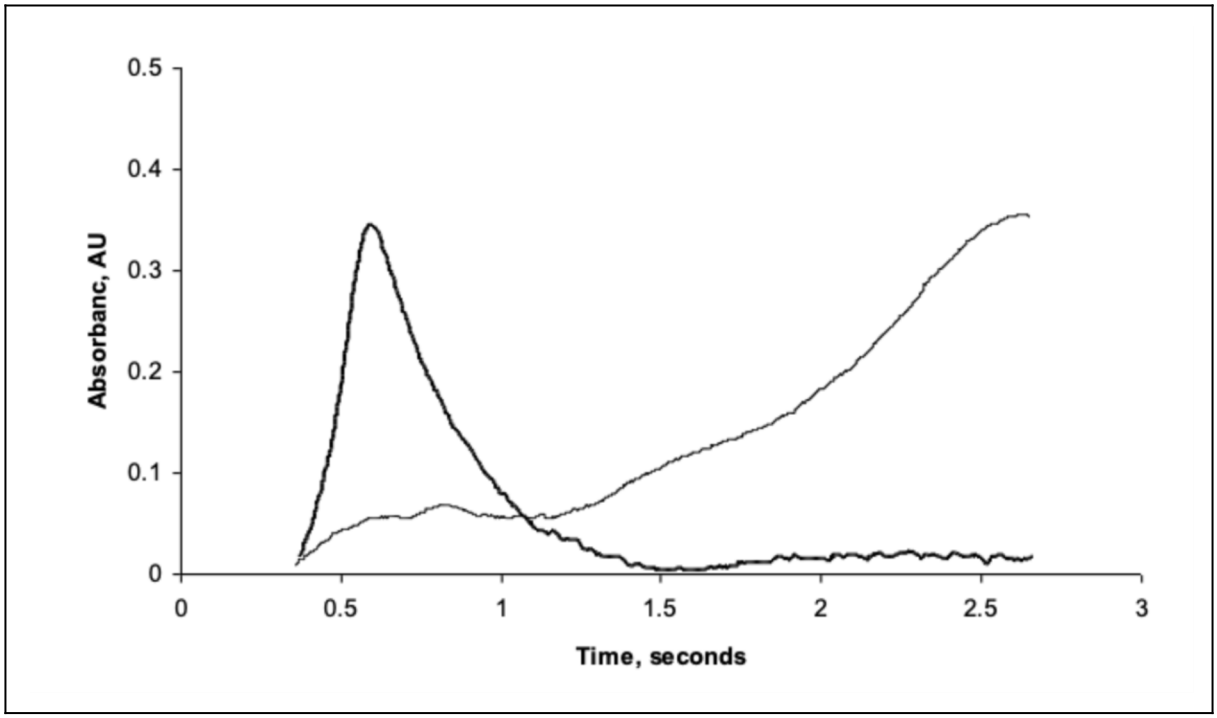

(ii) wine sample

Key: þþþþþþþþþþ corrected absorbance, □□ background absorbance

EXAMPLE 2

DETERMINATION OF LEAD IN WINE BY ATOMIC ABSORPTION SPECTROMETRY (TYPE III)

1. Field of application :

This analysis method can be applied to all types of wine, given the maximum limit set by the O.I.V.

2. References:

1. Journal Officiel des Communautés Européennes (3 octobre 1990). *Méthode de dosage du plomb dans le vin* (p. 152 et 153).

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Frederico CASTELLUCCI

2. Teissèdre P.L., Brun S., Médina B. (1992). *Dosage du plomb dans les vins / Proposition de modifications à la méthode du Recueil*. Feuillet Vert de l'O.I.V., n°928, 1997/151292.

3. Moreira Balio da Silva M., Gaye J., Médina B. (1996). *Comparaison de six méthodes de dosage du plomb dans les vins par absorption atomique en four graphite*. Feuillet Vert de l'O.I.V. n° 1013, 2310/190196.

4. Brereton P., Robb P., Sargent C., Crews H., Wood R. (1996). *Validation of a graphite furnace atomic absorption spectrometry method for the detection of lead in wine*. Feuillet Vert de l'O.I.V. n° 1016, 2913/230196.

5. Bourguignon J.B., Douet Ch., Gaye J., Médina B. (1997). *Dosage du plomb dans le vin / Interprétation des résultats de l'essai interlaboratoire*. Feuillet Vert 1055 de l'O.I.V. n° 2456/190397.

3. Principle:

The wine will undergo no preparations, except dilution in the case of white sweet wines.

Adding ammonium dihydrogeno-phosphate enables the lead contained in wine to be stable at high temperatures, which leads to eliminating interferences – and to acting in an identical manner to the standard solution.

The atomizer is a pyrolytic graphite equipped with a platform heated by the Joule effect.

The wavelength of the ray used is 283.3 nanometres.

The non specific absorption correction can be done by the Zeeman effect or by using a deuterium discharge lamp.

The type of lead determination in wine is a direct dosage method with external calibration.

4. Reagents

4.1. Demineralised water: ultra pure; with resistivity above 18 MΩ/cm.

4.2. Nitric acid: 65 % ; « suprapur » quality acid.

4.3. Ammonium dihydrogeno-phosphate $NH_4H_2PO_4$ for analysis.

4.4. Lead standard solution: at 1000 µg/ml (or 1 g/l) in 2% nitric acid (commercial

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solution, ready to use).

5. Apparatus

5.1. Analytic balance (e = 1 mg).

5.2. Glass ware:

5.2.1. Volumetric flask 50, 100 ml (class A),

5.2.2. Volumetric pipette 1, 10 ml (class A),

5.2.3. Decontamination of glassware used: rinse in demineralised water; soak at least 24 hours in a basin of 10% nitric acid; rinse two times in demineralised water.

5.3. Atomic absorption spectrophotometer equipped with a graphite tube atomizer for non-specific absorption correction and an auto-sampler (rinse the sampler buckets with 10% nitric acid).

5.3.1. Pyrolytically coated graphite furnace containing an L'Vov platform **possibly** tantalite (reference 9.1 – see below:

5.3.1.1. Tantalum solution: place 3 g of tantalum powder (metal tantalum with a purity above 99.7%) in a 100 ml teflon cylindrical flask; add 10 ml of diluted fluorhydric acid (1 + 1), 3 g of dehydrated oxalic acid and 0.5 ml of 30% hydrogen peroxide solution; heat together carefully to dissolve metal; add hydrogen peroxide when r reaction slows down. Add 4 g of dehydrated oxalic acid and approximately 30 ml of demineralised water when completely dissolved. Dissolve acid. Fill the solution up to 50 ml. This solution is stored in a plastic flask.

5.3.1.2. Tantalisation of a platform: the platform is placed inside the graphite tube. These items are placed together on a spectrophotometer atomization unit. 10 µl tantalum solution is injected on a platform using an auto-sampler. The temperature cycle is set according to the following program: drying at 150°C for 40 s; mineralization at 900°C for 60 s; atomization at 2600°C for 2.5 s. Argon is used as an inert gas.

6. Procedure

6.1. Test portion: The neck of the wine bottle with a tinned lead capsule must be carefully cleaned before uncorking.

6.2. Sample preparation: In general, no preparation of wine is necessary; samples are placed directly in the automatic sampler buckets. Cloudy wine needs to be filtered. To prolong the utilisation period of the platforms, sweet white wines are diluted for sugar contents between 10 to 50 g/L, dilute by 1/2; for contents above 50 g/l, dilute by 1/4.

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Secretary of the General Assembly
Frederico CASTELLUCCI

6.3. Preparation of solutions:

6.3.1. *White dilution:*

The solution is used as an additional volume to be injected and is made up of demineralised water containing 1 % nitric acid (4.2.).

6.3.2. Matrix modifying agent:

Into a 50 ml flask (5.2.1) introduce 3 g of ammonium dihydrogeno-phosphate (4.3.); dissolve and fill with demineralised water (4.1.).

6.3.3. 10 mg/ of lead solution:

Into a 100 ml flask (5.2.1) place 1 ml of 1 g/l (4.4.) solution; add 1 % nitric acid (4.2.); fill to volume with demineralised water (4.1.). This solution can be kept one month at a temperature + 4°C.

6.3.4. *100 µg/L lead solution:*

Into a 100 ml flask (5.2.1) place 1 ml of 10 mg/l (6.3.3.) lead solution; fill to volume with demineralised water (4.1.). This solution must be prepared every analysis day.

6.3.5. *Calibration scale* (for information purposes): 0 ; 16.7 ; 33.3 et 50 µg/l (see Table II).

6.4. Calibration and determination:

6.4.1. *Spectrometric measurement:*

6.4.1.1. wavelength: 283.3 nm;

6.4.1.2. slot with: 0.5 nm;

6.4.1.3. hollow cathode lamp intensity: 5 mA ;

6.4.1.4. correction continum: by Zeeman or deuterium effect;

6.4.1.5. introduction of standards heated and samples in a graphite furnace using an automatic sampler. The flushing liquid is made up of 500 ml of demineralised water containing a drop of Triton X 100.

Note: in order to inject at 90°C on a platform, the furnace temperature should be regulated to approximately 150°C.

6.4.1.6. signal measurement: peak height;

6.4.1.7. Duration of measurement: 3 seconds;

6.4.1.8. Number of measurements by standard or sample: 2

Note: the average of these two determinations constitutes the trial result. If the variation coefficient for the two determinations is greater than 15 %, the two other determinations must be re-done.

6.4.1.9. Furnace parameters (for information purpose): see Table I.

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Secretary of the General Assembly
Frederico CASTELLUCCI

Table I – Furnace parameters For determination of lead in wine				
Temperature (in °C)	Duration (in s)	Gas type	Gas flow (in l/mn)	lecture du signal
150	60	argon	3.0	
750	10	argon	3.0	
750	30	argon	3.0	
750	2	argon	0	
2400	1	argon	0	oui
2400	2	argon	0	oui
2400	2	argon	3.0	
40	20	argon	3.0	

6.4.1.10. Automatic sampler parameters (for information purposes): see Table II.

Table II – Sampler parameters for the dosage of lead in wine				
Analysis:	volumes injected in µl			
	sample	Pb solution 100 µg/l	"white" dilution	Matrix modifier
Calibration blank	0	0	5	1
Standard 1	0	1	4	1

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Secretary of the General Assembly
Frederico CASTELLUCCI

Standard 2	0	2	3	1
Standard 3	0	3	2	1
Sample	2	0	3	1

6.4.2. Tracing of calibration curve: the automatic distributor cycle enables the preparation of standards from 100 µg/l (Table II) lead solutions. The calibration graph is drawn up: absorbency according to lead concentration in micrograms per litre.

7. EXPRESSION OF RESULTS

7.1. Concentration of lead in injected solution: This is obtained from calibration curve (6.4.2.).

7.2. Concentration of lead in wine: This is calculated by multiplying by 3 the result given in 7.1. (2 µl of solution injected for a final volume of 6 µl on the platform). Take into account the possible dilution of wines (in the case of sweet white wines).

7.3. Result: is expressed in milligrams of lead per liter of wine (mg/l), to two digits.

8. INTER-LABORATORY TRIALS

A "double-blind" trial was carried out on 8 different wines obtained from mixtures of Bordeaux wines: two red wines (R1 and R2), two rosé wines (Ro1 and Ro2), two dry white wines (Bs1 and Bs2) and two sweet white wines (D1 and D2). Eleven Spanish, Portuguese, Moroccan and French laboratories participated by determining lead in 16 samples received.

8.1. Presentation of 8 wine samples:

Table III: Characteristics of wine used in interlaboratory trials

Wine	Type	T.A.V. (% Vol.)	Total acidity (g/l H_2SO_4)	Volatile acidity (g/l H_2SO_4)	Reducing sugar (g/l)
R1	Red	11,86	4,43	1,57	1,2
R2	Red	12,54	3,77	0,34	1,5

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The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

Ro1	Rosé	12,23	5,30	0,44	1,2
Ro2	Rosé	11,43	4,88	0,45	1,1
Bs1	Dry white	11,65	4,62	0,37	2,2
Bs2	Dry white	12,32	4,57	0,31	0,9
D1	Sweet white	12,94	3,72	0,67	76,4
D2	Sweet white	12,66	4,70	0,45	62,8

8.2. Statistics of results:

Table IV: Statistical analysis of inter-laboratory trial results

Wine sample	R1	R2	Ro1	Ro2	Bs1	Bs2	D1	D2
Double-blind repetitions	C & K	F & I	D & G	J & L	B & H	P & N	A & E	M & 0
Initial number of laboratories	11	11	11	11	11	11	11	11
Number of laboratories After elimination of large variances	11	10	11	11	10	10	11	10
Average (µg/l)	44	162	28	145	52	138	60	145
Repeatability limit								

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Secretary of the General Assembly
Frederico CASTELLUCCI

r	18	12	7	17	6	13	28	7
Standard deviation of repeatability S_r	6,4	4,3	2,5	6,1	2,1	4,6	10	2,5
Relative standard deviation of reproducibility RSDr (en %)	14,5	2,8	9,2	4,2	4,2	3,4	16,5	1,8
Horrat value(Ho_r): Observed RSDr / RSD_r Horwitz	0,6	0,1	0,3	0,2	0,2	0,2	0,7	0,1
Reproducibility limit R	34	105	23	86	30	101	86	144
Standard deviation of reproducibility S_R	12,3	37,5	8,2	30,8	10,7	35,9	30,6	51,6
Relative standard deviation of reproducibility RSD_R (en %)	28	23,1	29,3	21,2	20,6	26	51	35,6
Horrat values (Ho_R): Observed RSD_R / RSD_R Horwitz	1,1	1,1	1,1	1	0,8	1,2	2,1	1,7

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Secretary of the General Assembly
Frederico CASTELLUCCI

Out of the 11 laboratories which participated in the trial, 7 declared that they had followed the proposed method and 4 modified some of the parameters.

9. Method performances and quality control

9.1. Detection limit

This is determined from a series of 20 blank analytical repetitions and is equal to 3 standard deviations. In the case of the proposed method a series of 20 blank analytical measurements resulted in: average = 1,29 µg/l ; standard deviation = 0,44 µg/l ; detection limit = 1,3 µg/l .

9.2. Limit of quantification:

This is equal to 3 times the detection limit. In the case of the proposed method, the limit of quantification is 4 µg/l (3 * 1,32 = 3,96).

9.3. Trueness

The confidence interval for the average of a series of results is compared to the reference material data.

Three reference materials are used including: red wine, dry white wine, sweet white wine for which lead concentrations are certified by the B.C.R. (Bureau Communautaire de Référence) in 1992.

Table V. Trueness of the method

		Red wine BCR E	Dry white wine BCR C	Sweet white wine BCR D
Lead concentration (µg/l)	Certified value (B.C.R. 1992)	36,1 ± 4,9	65,1 ± 9,1	132,4 ± 32
	Average value (series: 10 results)	41,0 ± 3,8	66,0 ± 4,4	128,3 ± 14,1

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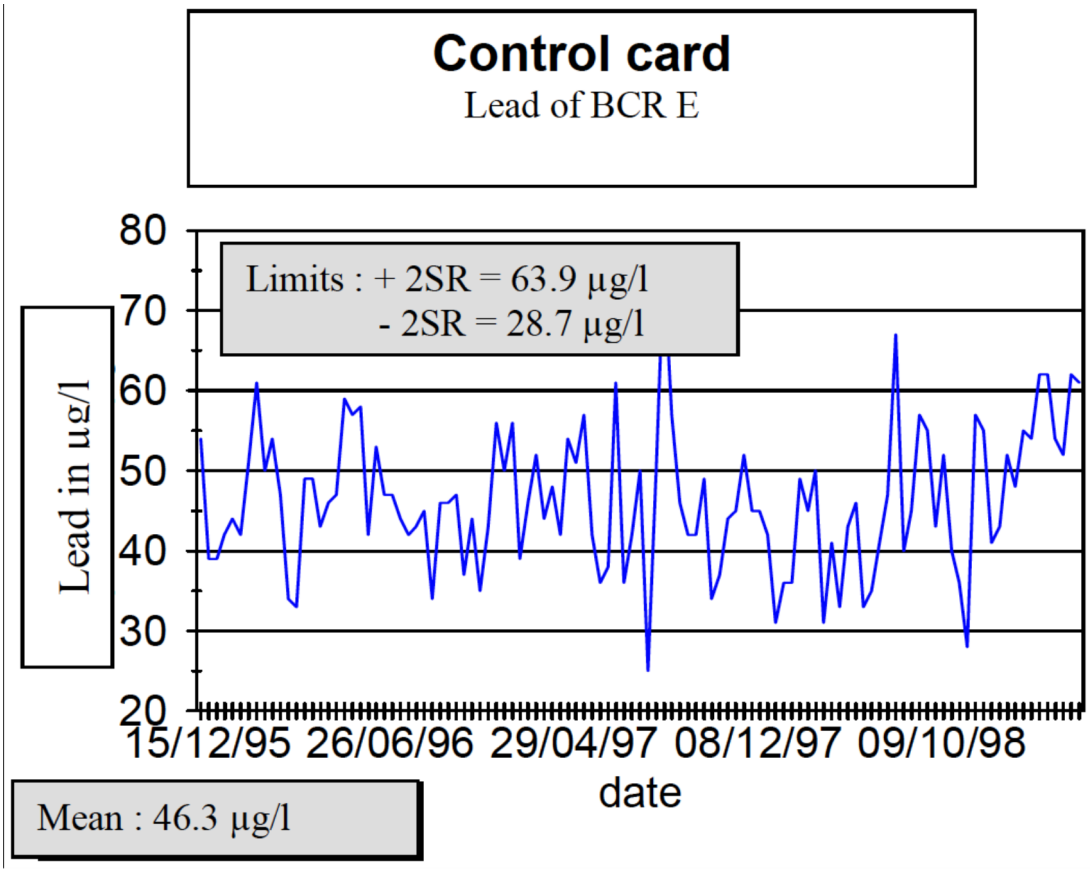
9.4. Control card

A control card can be drawn up for each reference material used. Control limits are equal to: +/- 2 $S_R$intra (S_R intra: reproductibility standard deviation).



10. BIBLIOGRAPHY

1. Zatka V. (1978). Treated graphite atomizer tubes for atomic absorption spectrometry. Analytical Chemistry, vol. 50, n°3.
2. US Bureau of Alcohol, Tobacco and Firearms (1991). *Analysis of lead in wines and related products by graphite furnace atomic absorption spectrometry*. Note d'information de l'O.I.V. du 21 août 1991 : « Plomb dans les vins aux U.S.A. ».
3. Mindak W.R. (1994). *Determination of lead in table wines by graphite furnace*

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atomic absorption spectrometry. Journal of A.O.A.C. International, vol. 77, n°4, p. 1023-1030.

4. Médina B. (1994). *Apport de nouvelles techniques au dosage des métaux dans les vins*. Congrès des Œnologues de France à Bordeaux.
5. Norme française NF ISO 5725-2 (1994). *Application de la statistique : Exactitude (justesse et fidélité) des résultats et méthodes de mesure*.
6. Jorhem L., Sundström B. (1995). *Direct determination of lead in wine using graphite furnace AAS*. Atomic Spectroscopy, September/October 1995.

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