RESOLUTION OENO 6/99

VALIDATION PROTOCOL FOR A TYPICAL ANALYTICAL METHOD COMPARED TO THE OIV REFERENCE METHOD

THE GENERAL ASSEMBLY,

GIVEN Article 5, paragraph 4 of the International Unification Convention on Analytical Methods of October 13, 1954, on the proposal of the Sub-Committee on Methods of Analysis and Evaluation of Wine,

DECIDES to add the following chapter, "**Validation Protocol for a Typical Analytical Method Compared to the OIV Reference Method**" to Annex A of the Collection of Analytical Methods:

1. GOAL

This is a protocol which allows a typical oenological analytical method to be evaluated and compared to the OIV reference method so it can be validated.

2. Definitions

2.1. Referential

The referential is composed of the set of methods described in the *OIV International Collection of Methods for Wine and Must Analysis*. When several methods are offered in the Collection, the laboratory will preferably use the methods described as "Reference Methods." However, it could, if justified, used methods described as "Standard Methods."

2.2. Standard Analyses

Standard analyses are analyses currently used in Oenology during wine production and marketing with the exception of specialized analyses which fulfill a need which is limited in number or time.

2.3. Standard Methods

Standard methods are those methods implemented by a laboratory to perform standard analyses. These methods are most often automatic and comparative. The protocol only deals with methods used for large number of determinations.

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2.4. Initial Validation and Permanent Validation Concept

This protocol allows an initial validation of a standard analytical method. It does not suffice, under the framework of quality control rules or good laboratory practices to ensure the total legitimacy of the use of the method. The laboratory must implement additional permanent validation protocols which can be used to produce, for example, control graphs, reference standards and participation in Intralaboratory analyses.

3. Evaluation Protocol for a Standard Analytical Method and Its Link to the OIV Reference Method

This protocol has two distinct parts which will be implemented by the laboratory successively.

1. Evaluation of the standard method
2. Linking to the OIV reference method

The various stages are described and an sample application of an alternative standard method for determining free sulfur dioxide dosage is given as an illustration.

In order to facilitate the reading, the following nomenclature will be used throughout the document:

3.1. Nomenclature

q: number of samples taken

X_i and X'_i: values obtained using the standard method for sample i after double measurements

Y_i and Y'_i: values obtained using the OIV reference method for sample i after double measurements

T_i: reference values used to estimate linearity for sample i

W_i: absolute value of the difference between values obtained for sample i

Sr: standard deviation of repeatability obtained for q samples

r: repeatability

x_i: average of two experimental values, X_i and X'_i

y_i: average of two experimental values, Y_i and Y'_i

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Mx: average of the set of x_i values obtained for q samples

My: average of the set of y_i values obtained for q samples

MT: average of the set of T_i values obtained for q samples

Md: algebraic average of result differences

Sd: standard deviation of result differences

a: ordinate at the origin of the line of regression

b: slope of the line of regression

$x\hat{}_i$: regression value

$y\hat{}_i$: regression value

$s_{x,y}$: residual regression of the line of regression

s_b: standard deviation of slope b

e_i: residual regression for sample i

v_1 and v_2: degrees of freedom

F: Fischer's parameter

S: method sensitivity

□: type 1 risk

□: type 2 risk

S_{blank}: standard deviation of blanks

L_D: limit of detection

L_Q: limit of quantification

3.2. Evaluation of the Standard Method

The laboratory should submit the standard method to several evaluations. The order in which these evaluations are presented in the text indicates the preferred order in which they are to be performed by the operator(s). Certain steps are essential and cannot be changed. Others will only be performed if they are useful due to certain aspects of the standard method in question. The last are optional.

Essential steps:

1. Repeatability
2. Linearity
3. Intralaboratory reproducibility

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Steps to perform as a function of the particular method:

1. Ruggedness
2. Specificity

Optional steps:

1. Sensitivity
2. Detection limit
3. Quantification limit

3.2.1. Repeatability

3.2.1.1. Definitions

Repeatability is the degree of agreement between the results of independent analyses obtained using the same method on the same wine in the same laboratory performed by the same operator using the same equipment over a short period of time.

The repeatability value, r, is the value below which is the absolute difference between the results of two separate analyses obtained under the conditions of repeatability defined above and having a probability of 95%.

The standard deviation of repeatability, Sr, is the standard deviation of the results obtained under the conditions of repeatability. This is a parameter for the dispersion of the results obtained under repeatable conditions.

3.2.1.2. Base Protocol

A single operator should analyze q different samples numbered 1 through q, whose analytical values, for the parameter considered, cover the whole range of measurements within which the laboratory wishes to validate its evaluation. The value of q should be at least 60 for automated methods. For non-automatic methods, it could be reduced to 30.

Each sample shall be analyzed twice. The two sets of analyses will be performed during a short time. However, different samples may be analyzed over a longer period of time.

The absolute value of the difference (W_i) between the results obtained (X_i and X'_i) for a sample with a range of i is calculated.

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Example: *For the standard method of free sulfur dioxide dosage and a measurement range of 0 to 50 mg/liter, the operator will locate 60 samples containing evenly distributed concentrations of between the indicated values.*

The following table shows a portion of the results obtained. The number was limited to 12 for this presentation.

Sample N°	*Xi (in mg/l)*	*X'i (in mg/l)*	*Wi (absolute value)*
1	*14*	*14*	*0*
2	*25*	*24*	*1*
3	*10*	*10*	*0*
4	*2*	*3*	*1*
5	*35*	*35*	*0*
6	*19*	*19*	*0*
7	*23*	*23*	*0*
8	*27*	*27*	*0*
9	*44*	*45*	*1*
10	*30*	*30*	*0*
11	*8*	*8*	*0*
12	*48*	*46*	*2*

3.2.1.3. Results and calculations

The standard deviation of repeatability, Sr, is calculated using the following formula:

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$$S_r = (\frac{1}{2q}\sum_{i=1}^{q} w_i^2)^{1/2}$$

in which:

- Sr = standard deviation of repeatability
- q = number of samples analyzed in pairs
- Wi = absolute difference between pairs

In practice, current software (such as a spreadsheet) allows the calculations to be performed easily. Repeatability, r, is calculated by:

- r = 2.8Sr

The result obtained for repeatability, r, allows confirmation of the fact that at a probability level of 95%, the standard method will have a repeatability of less than r.
In practice, two results from the same wine will be considered suspect if their difference in value is greater than r.
Example: *Taking the values shown in the table, the following results are obtained:*

- q = 12
- Sr = 0.54 mg/l
- r = 1.5 mg/l

This results confirms that at a level of probability of 95%, the results obtained using the method in question will have a repeatability of less than 1.5 mg/l.

3.2.2. Linearity

3.2.2.1. Definition

Linearity is the ability of a method to produce results proportional to the concentration of the compound sought in wine over a given range of measures. This proportionality can be direct or may be obtained after applying a previously defined mathematical formula.

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3.2.2.2. Determination Protocol

To determine a method's linearity, the only variable factor should be the concentration of the compound analyzed. Two methods can be used to ensure these conditions: dilution of the wine into exact, known proportions or additive dosage.

Therefore q samples should be prepared (with q equal to at least 6) which cover the method's range of measurement. Each sample shall be analyzed in pairs using the method described for repeatability. The values obtained from each sample (X_i and X'_i) will serve as the basis for calculations described later.

If one expects a matrix effect (for example, white wine or red wine), the test should be performed for each type of matrix.

3.2.2.2.1. Wine Dilution

When diluting wine to obtain a given concentration of the dosed compound, take care to use a water-ethanol mixture whose alcoholic strength by volume is equal to that of the diluted wine. When they are mixed, the wine and the water-alcohol solution should have the same temperature. Directly calculate the theoretical values of concentration T_i in the diluted wine.

3.2.2.2.2. Additive Dosage

The operator selects a wine with a known concentration , C, of the dosed compound. This wine constitutes the first sample of the experimental series. The operator prepares the other samples of the series by adding a solution with a known concentration, C_1, of the dosed compound. This solution is prepared using a water-ethanol mixture whose alcoholic strength by volume is equal to that of the dilute wine and whose temperature is the same as that of the diluted wine. For a given volume of wine, P, a volume of solution, P_1, is added based on the desired concentration. Thus the final volume is V_1.

Concentration T_i of the compound in the wine to be dosed (to which an additive dose has been added) is calculated as follows:

$$T_i = \frac{C_0 P_0 + C_1 P_1}{V_1}$$

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3.2.2.2.3. Particular Cases

In certain cases, the protocols described above are not applicable. This is the case, for example, when measuring alcoholic strength by volume or when dosing free sulfur dioxide.

For these determinations, the values obtained after dilution or additive dosage cannot be directly established using the simples calculations indicated above. The only workable solution is to let the wine sit after it has been diluted or received an additive dosage for sufficient time to allow it to recover its physical or chemical equilibrium (in the case of free sulfur dioxide), then proceed using OIV reference methods. The values obtained using the reference method will be used as the base concentration value T_i.

3.2.2.3. Interpretation

Record the calculated or measured concentration values T_i and graph them with respect to values X_i and X'_i from the standard method. In this way, the laboratory can directly verify the linearity of the method. In the case of partial non-linearity, the method's field of application should be restricted in use to concentrations corresponding to the linear portion.

Example: *When evaluating free sulfur dioxide dosage, the following values were obtained from a wine which had received increasing sulfur dioxide doses.*

Sample N°	*Value obtained By OIV method (mg/l) Ti*	*Result N°1 (in mg/l) Xi*	*Result N°2 (in mg/l) X'i*
1	*0*	*0*	*0*
2	*18*	*17*	*17*
3	*37*	*34*	*34*
4	*54*	*54*	*53*
5	*68*	*67*	*65*
6	*88*	*90*	*89*

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The following graph shows the X_i and X'_i values measured with respect to reference values T_r. This shows good linearity for the range of our measurements. The dispersion of X_i and X'_i values is limited.

[vertical axis shows X and X' values from the standard method (mg/l) and the horizontal axis shows reference values, T, in mg/l]

3.2.2.3.1.1. Analysis of linearity

To confirm the method's linearity, the laboratory can perform a larger test using traditional methods of analyzing statistical data.

3.2.2.3.1.2. Determining the regression line and calculating its parameters

The first step is to determine the regression line adjusted as closely as possible to the measures taken using the least square method. Its function is:

- X = a + bT

Where:

- b is the slope of the regression line
- a is the intersection of the regression line and the ordinate axis

The steps of the calculation are as follows:

3.2.2.3.1.2.1. Calculate the average of the standard method results

The average of the results obtained using the proposed standard method (Mx) is calculated as follows:

$$Mx = \frac{1}{q}\sum_{i=1}^{q} x_i$$

where:

- q is the total number of samples analyzed numbered 1 through q
- xi is the average of the pairs of sample i taken in a series and calculated by:

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$$x_i = \frac{1}{2}(X_i + X_i)$$

The average of the reference values (MT) is calculated as follows:

$$MT = \frac{1}{q}\sum_{i=1}^{q} T_i$$

3.2.2.3.1.2.2. Calculating the parameters of the regression line

The parameters of the regression line are established by:

$$b = \frac{\sum_{i=1}^{q}(T_i - MT)(x_i - Mx)}{\sum_{i=1}^{q}(T_i - MT)^2}$$

$$a = Mx - bMT$$

3.2.2.3.1.2.3. Calculating the Residual Standard Deviation

The residual standard deviation, $s_{y,x}$ is calculated by:

$$S_{y,x} = \sqrt{\frac{1}{q-2}\sum_{i=1}^{q}(x_i - \hat{x_i})^2}$$

where $\hat{x}_i$ is the regression value obtained for each sample, i, using the function defined by the regression line:

$$\hat{x_i} = a + bT_i$$

3.2.2.3.1.2.4. Calculating the Standard Deviation of the Slope

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The standard deviation, sb, of the slope, b, is calculated as follows:

$$s_b = \frac{s_{y,x}}{\sqrt{\sum_{i=1}^{q}(x_i - Mx)^2}}$$

In practice, current software (such as a spreadsheet) allows the calculations to be performed easily.

Example: *Using the data in the example above, we construct the following table:*

Ti (mg/l)	*Xi (mg/l)*	*X'i (mg/l)*	*Average values xi= (Xi+X'i)/2*	*Regression Values xᵢᵢ*
0	*0*	*0*	*0*	*0,82*
17	*17*	*18*	*17,5*	*17,46*
34	*34*	*37*	*35,5*	*34,12*
54	*53*	*54*	*53,5*	*53,70*
67	*65*	*68*	*66,5*	*66,43*
90	*89*	*88*	*88,5*	*88,95*

- For q=6
- a = 0.821
- b = 0.979
- $s_{x,y}$ = 0.84
- s_b = 0.01

3.2.2.3.1.3. Representation of Residual Regression

A depiction of residual regression can easily be created by graphing the values of e_i

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using the following formula:

$$e_i = x_i - \hat{x_i}$$

In linear cases, the points obtained should be randomly distributed around the regression line:

- x = 0

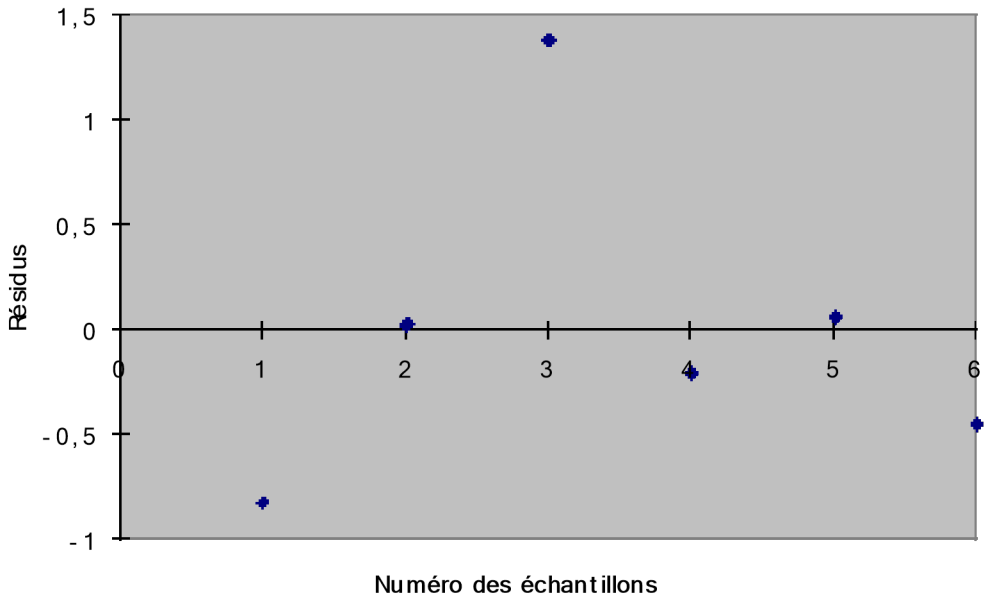
Example: *The following table gives the values calculated in our example:*

Average Values xi	*Regression Values xooo*	*Average Residual ei = x- xo*
0	*0,82*	*-0,82*
17,5	*17,46*	*0,03*
35,5	*34,12*	*1,38*
53,5	*53,70*	*-0,20*
66,5	*66,43*	*0,07*
88,5	*88,95*	*-0,45*

The following graph shows the residual regression:

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[vertical axis is residual, horizontal axis is sample number]

Notice that in this case the residual values are distributed regularly around the line x = 0.

3.2.2.3.1.4. FISHER Test

When in doubt, a FISHER test can be performed to confirm linearity.

Calculate the relationship by:

$$Fobs. \frac{s^2_{y,x}}{Sr^2}$$

Where S_r is the standard deviation of repeatability for the X_i values measured in pairs calculated using the formula described in paragraph 4.2.1.

Use the Snedecor critical value with an □ of 0.05: $F_{1-□}$ is given in the Snedecor's Law table (Annex n°1) as a function of v_2 which is equal to the number of pairs that served to establish S_r.

- $v_2 = q$

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and v_1:

- $v_1 = q-2$

If:

1/ $\mathbf{F_{obs} \geq F_{1-\alpha}}$ non-linearity is confirmed

2/ $\mathbf{F_{obs} < F_{1-\alpha}}$ non-linearity cannot be confirmed

Example: *Using the same example, we observe the following values:*

- sx,y = 0.84
- Sr = 1.32
- Fobs = 0.41
- v2 = 6
- v1 = 4
- F1-□ = 4.53

If the F_{obs} value obtained is less than the value of $F_{1-\alpha}$ then non-linearity cannot be confirmed.

3.2.3. Sensitivity

3.2.3.1. Definition

The method's sensitivity is the smallest variation in concentration of the analyzed compound that can be detected.

3.2.3.2. Determination

Sensitivity, S, is difficult to determine in practice. A mathematical approach can be taken using the residual standard deviation of the regression line ($s_{y,x}$), calculated during the linearity test for the method, with the following formula:

$$S. \frac{t_{1/2}.t_1.S_{y,x}\sqrt{2}}{b}$$

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In which type 1 risk, □, and type 2 risk, □ are equal to 0.05 and where:
1/ $t_{1-□/2}$ is the value given in the Student table (Annex n°2) as a function of the value of P:

- P = 1 - □/2 = 1 – 0,025 = 0,975

and the value of v degrees of freedom is equal to:

- v = q-1

where q represents the number of samples used to determine linearity.
2/ $t_{1-□}$ is the value given in the Student table (Annex n°2) as a function of the value of P:

$$P = 1 - \beta = 1 - 0{,}05 = 0{,}95$$

and the value of v degrees of freedom is equal to:

- □ = q-1

where q represents the number of samples used to determine linearity.
3/ b is the slope of the regression line obtained in the method's linearity study
Example: *Using the same example in which we obtained a residual standard deviation of the regression line of:*

- $s_{x,y}$ = 0.84

we have:

- q = 6
- v = 6-1 = 5
- t1-□/2 = 2.571
- t1-□ = 2.015
- b = 0.979

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The sensitivity value will be:

$$S.\frac{2{,}571\,.\,2{,}015\,.\,0{,}843\,.\,\sqrt{2}}{0{,}979}\,.\,5{,}6\,mg$$

3.2.4. Intralaboratory reproducibility

3.2.4.1. Definition

Intralaboratory reproducibility is the degree of agreement between the results of independent analyses obtained using the same method on the same wine in the same laboratory performed by the same operator or by a different operator using different calibration curves on different days.

In practice, Intralaboratory reproducibility can be accepted as an expression of the total degree of uncertainty inherent in a given method in the laboratory in question.

3.2.4.2. Determination

The operator will select a series of wines which have different values for the parameter under analysis and which are distributed over the range covered by the standard method tested. The standard method should be applied regularly over a period of at least one month and save the results (X_i). The base value, T, will be known for each of the wines. For this, the laboratory can use reference samples, if available, or determine that value by, for example, using the OIV reference method. The operator could also use the average value obtained using the standard method in the short term. The value of T here has only relative significance: it serves as the base of comparison for the values that will be recorded over time. The operator will ensure that the wines used are perfectly preserved.

The protocol described will be used to calculate repeatability using the values of W obtained.

Example: *The table below shows several of the results obtained during the free sulfur dioxide dosage test for which two samples, E_1 and E_2, of known values T_1 = 22 mg/l and T_2 = 55 mg/l where analyzed each day for a period of one month. The values W1 and W2 are the absolute values of the differences between the true value of T and the measured value of X_1 and X_2.*

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Day of analysis	*Result X1 (in mg/l)*	*W1 (absolute value)*	*Result X2 (in mg/l)*	*W2 (absolute value)*
1	*21*	*1*	*55*	*0*
3	*21*	*1*	*54*	*1*
5	*22*	*0*	*53*	*2*
7	*20*	*2*	*55*	*0*
9	*21*	*1*	*54*	*1*
11	*22*	*0*	*55*	*0*
13	*22*	*0*	*52*	*3*
15	*20*	*2*	*53*	*2*
17	*21*	*1*	*51*	*4*
19	*20*	*2*	*53*	*2*
21	*23*	*1*	*56*	*1*
23	*21*	*1*	*52*	*3*
25	*24*	*2*	*56*	*1*
27	*22*	*0*	*55*	*0*
29	*23*	*1*	*56*	*1*
31	*21*	*1*	*52*	*3*

The following values were calculated:

- q = 32
- Sr = 1.14 mg/l

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- r = 3.2 mg/l

This result confirms that with a probability of 95%, the results obtained by the method in question will have an intralaboratory reproducibility level of less than 3.2 mg/l. This value can be used as the uncertainty level for this method.

3.2.5. Limit of detection

3.2.5.1. Definition

The limit of detection is the smallest quantity of the dosed compound which can be detected, but not necessarily exactly quantified. The limit of detection is an experimental limit parameter.

3.2.5.2. Determination

Analyze 20 pairs of blanks then determine the average values for M_{blank} and the standard deviation of repeatability, S_{blank} using the method described for repeatability in paragraph 3.2.1. The limit of detection, L_D is typically defined by:

- $L_D = M_{blank} + (3\ S_{blank})$

Example: *The table below shows several results obtained during the determination of the limit of detection for standard free sulfur dioxide dosage used as an example:*

Sample N°	*X (in mg/l)*	*X' (in mg/l)*	*x = (X+X')/2 (in mg/l)*
1	*0*	*0*	*0*
2	*0*	*1*	*0,5*
3	*0*	*0*	*0*
4	*0*	*0*	*0*
5	*0*	*0*	*0*
6	*1*	*0*	*0,5*

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7	*0*	*0*	*0*
8	*0*	*0*	*0*
9	*0*	*0*	*0*
10	*0*	*0*	*0*
11	*0*	*0*	*0*
12	*0*	*0*	*0*

The following values were calculated:

- q = 12
- Mblank = 0.083
- Sblank = 0.29 mg/l
- LD = 1.7 mg/l

3.2.6. Limit of quantification

3.2.6.1. Definition

The limit of quantification is the smallest quantity of the compound which can be dosed by the method.

3.2.6.2. Determination

Use the average of the values M_{blank} and standard deviation S_{blank} calculated to determine the limit of detection, which will typically be multiplied by 10 to obtain the limit of quantification L_Q.

- $L_Q = M_{blank} + 10 \times S_{blank}$

Example: *Using the same example, the limit of quantification would be:*

- L_Q = 3.7 mg/l

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3.2.7. Ruggedness

3.2.7.1. Definition

The ruggedness of a method is its ability to produce similar results under slight changes in the experimental conditions which may be encountered during the procedure.

3.2.7.2. Determination

If there is a doubt as to the influence of variations in operating parameters, the laboratory shall devise an experimental plan which will manipulate those critical operating parameters likely to be encountered under practical conditions.

The use of these experimental programs is described in detail in bibliographic references (6) and (8).

3.2.8. Specificity

3.2.8.1. Definition

The specificity of a method is its capacity to measure the compound desired.

3.2.8.2. Determination

If a doubt exists as to the specificity of a method, the laboratory shall devise an experimental plan which will determine the effects of interference from compounds other than that tested.

3.3. Adjusting the Standard Method to the OIV Reference Method

Adjusting the standard method to the OIV reference method is done in two stages.. The first stage consists of comparing the repeatability of the two methods. The second stage consists of determining the exactitude of the standard method relative to the reference method.

3.3.1. Comparing repeatabilities

3.3.1.1. Repeatability of the Reference Method

the repeatability value of the OIV reference method is generally given in the *OIV International Collection of Methods for Wine and Must Analysis.* In cases where this value has not been determined, the laboratory shall analyze pairs from 30 samples using the reference method under repeatable conditions and proceeding according to

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the method described in paragraph 4.2.1.

3.3.1.2. Comparing the Repeatability of the Two Methods

The comparison is direct. If the repeatability of the standard method is less than or equal to that of the reference method. The result is favorable. If it is higher, the laboratory should ensure that the result is within the conditions it accepted for the method. In the latter case, it could also apply a FISHER test to determine whether the result is significantly higher than that of the reference method.

3.3.1.2.1. FISHER Test

In the event the laboratory itself determined the repeatability of the reference method, it can use a FISHER test to verify whether the two repeatability values are comparable.

The relationship is calculated by:

$$Fobs\,.\,\frac{Sr^2}{Sréf^2}$$

Use the Snedecor critical value with an □ of 0.05: $F_{1-□}$ is given as a function of the number of pairs used to determine the standard deviation of the standard method's repeatability, Sr:v_1 and that of the number of pairs used to determine the standard deviation of repeatability of the reference method, Sref:v_2. This value is found in the Snedecor's Law table (Annex n°1).

If:

1/ $\mathbf{F_{obs} > F_{1-□}}$ the repeatability value of the standard method is significantly greater than that of the reference method

2/ $\mathbf{F_{obs} < F_{1-□}}$ the repeatability value of the standard method is not confirmed to be significantly greater than that of the reference method

Example: *The standard deviation of repeatability in this example for the free sulfur dioxide dosage method is:*

- Sr = 0.54 mg/l

The laboratory dosed the same samples using the OIV reference method. The standard deviation of repeatability found in this case is:

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- Sref = 0.39 mg/l

$$\text{Fobs} \cdot \frac{0{,}54^2}{0{,}39^2} \cdot \frac{0{,}29}{0{,}15} = 1{,}93$$

- $V_2 = 12$
- $V_1 = 12$
- $\square = 0.05$
- $F_{1-\square} = 2.69$

The resulting F_{obs} value is less than the value of $F_{1-\square}$ so the repeatability value of the standard method is not confirmed to be significantly greater than that of the reference method.

3.3.2. Reliability of the Standard Method Relative to the Reference Method

3.3.2.1. Definition

Reliability is defined as the degree to which the values obtained using the reference method and those obtained using the standard method, independent of errors of accuracy in the methods, are in agreement.

3.3.2.2. Determination

Reliability is determined using a series of q wines which have various concentrations of the analyzed compound covering the range of measures for which validation is sought. In practice, a minimum of 50 wines should be used: the use 100 wines is desirable.

Each sample shall be analyzed in pairs using the two methods over a short period of time.

Calculate the averages x_i of the 2 measures, X_i and X'_i taken using the standard method and the averages y_i of the 2 measures, Y_i and Y'_i taken using the reference method.

3.3.2.3. Calculations

As described above, show each sample using an orthonormal with the values of x_i

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shown on the horizontal axis and the values of y_i shown on the vertical axis.

If the values obtained using the two methods are identical, the points will be perfectly aligned in a line whose function is:

- y = x

This is the theoretically ideal line.

In practice, the points will be distributed in a more random fashion. Using the methods described for estimating linearity, the regression line obtained using the values found shall be represented by the equation:

- y = bx + a

Calculate the parameters of this regression line and compare it to the ideal y = x.

Calculations are done in the following steps:

3.3.2.3.1. Calculating the Average Results of the Two Methods

The average of the results using the proposed method (Mx)and the average of the results using the reference method (My) are calculated as follows:

$$Mx = \frac{1}{q}\sum_{i=1}^{q} x_i$$

$$My = \frac{1}{q}\sum_{i=1}^{q} y_i$$

in which:

- q is the total number of samples analyzed
- xi is the average of the pairs in sample i
- yi is the average of the pairs in sample i

d_i is the algebraic difference of the average results for the two methods for sample i.

Its value is determined using the following formula:

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- $d_i = x_i - y_i$

3.3.2.3.2. Calculating the Algebraic Average of the Differences in Results from the Two Methods

The algebraic difference of the results (Md) from the two methods (average bias) is calculated using the following formula:

$$Md = \frac{1}{q}\sum_{i=1}^{q} d_i = Mx - My$$

3.3.2.3.3. Calculating the Standard Deviation of the Difference in Results from the Two Methods

The standard deviation of the difference in results from the two methods (S_d) is calculated using the following formula:

$$S_d = \sqrt{\frac{1}{q-1}\sum_{i=1}^{q}(d_i - Md)^2}$$

3.3.2.3.4. Calculating the Regression Line Parameters

The parameters of the regression line are calculated as follows:

$$b = \frac{\sum_{i=1}^{q}(x_i - Mx)(y_i - My)}{\sum_{i=1}^{q}(x_i - Mx)^2}$$

$$a = My - bMx$$

3.3.2.3.5. Calculating the Residual Standard Deviation

The residual standard deviation, $s_{y,x}$ is calculated using the following formula:

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$$s_{y,x} = \sqrt{\frac{1}{q-2}\sum_{i=1}^{q}(y_i - \hat{y_i})^2}$$

where $\hat{y}_i$ is the regression value obtained for each sample, i, using the function defined for the regression line:

$$\hat{y_i} = bx_i + a$$

3.3.2.3.6. Calculating the Standard Deviation of the Slope

The standard deviation, s_b, of the slope, b, is calculated using the following formula:

$$s_b = \frac{s_{y,x}}{\sqrt{\sum_{i=1}^{q}(x_i - Mx)^2}}$$

3.3.2.4. Interpretation

If the two methods give identical results, the regression line,

- y = bx + a,

will be very close to the theoretical line,

- y = x.

The equality if these two lines should be verified using the following tests:

3.3.2.4.1. Verifying the Slope

The slope of the regression line should not be statistically different from 1. This can be verified using the following comparisons:

$$b - (t_{1-\alpha/2} * s_b) < 1 < b + (t_{1-\alpha/2} * s_b)$$

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where the value of t is found in the Student table (Annex n°2) as a function of v degrees of liberty where:

- v = q - 2

and the value of P is found in the Student table for an □ equal to 0.05 and:

- P = 1 - $\alpha/2$ = 1 - 0,025 = 0,975

3.3.2.4.2. Verifying the Average of the Differences

The average bias observed, Md, should not be statistically different from 0.
This can be verified using the following comparison:

$$Md - ((t_{1-\alpha/2})s_d/\sqrt{q}) < 0 < Md + ((t_{1-\alpha/2})s_d/\sqrt{q})$$

where the value of t is found in the Student table (Annex n°2) as a function of v degrees of liberty where:

- v = q - 1

and the value of P is found in the Student table for an □ equal to 0.05 and:

- P = 1 - $\alpha/2$ = 1 - 0,025 = 0,975

Example: *The following table shows the results obtained for free sulfur dioxide dosage using the standard method in question and for the OIV reference method. The number of samples analyzed is limited to 28 here to facilitate presentation of the discussion.*

Xi (mg/l)	*X'i (mg/l)*	*Yi (mg/l)*	*Y'i (mg/l)*	*Average xi (mg/l)*	*Average yi (mg/l)*	*di = yi-xi*	*Regres-sion ŷi*	*Average Residual yi- ŷi*
22	*20*	*23*	*21*	*21*	*22*	*-1*	*21,39*	*0,61*

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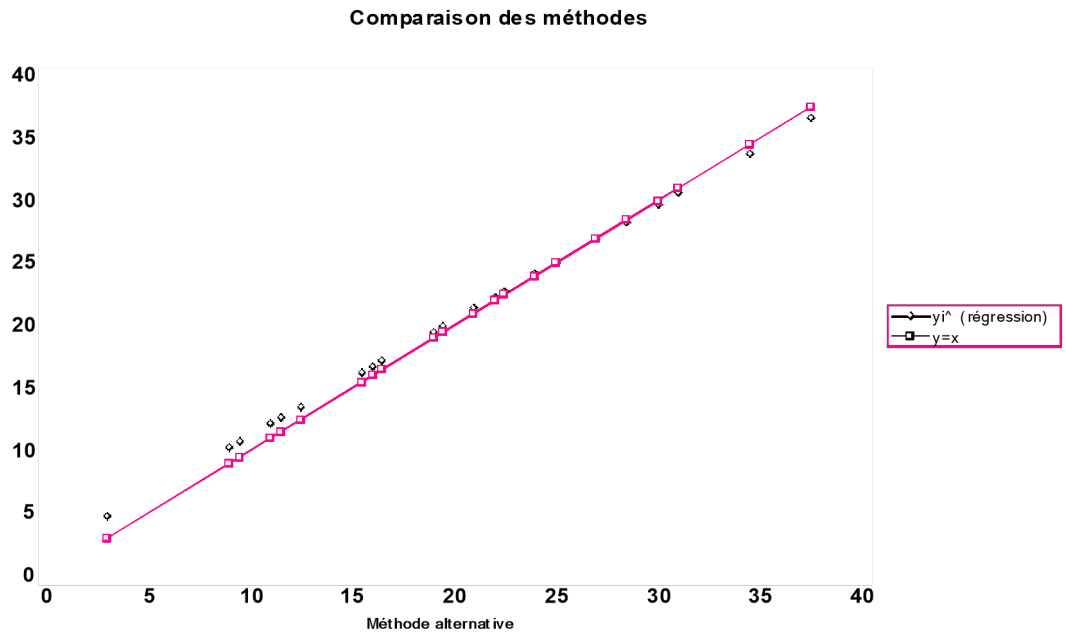
27	27	30	30	27	30	-3	26,91	3,08
29	28	30	31	28,5	30,5	-2	28,30	2,20
15	17	18	18	16	18	-2	16,78	1,22
9	10	11	11	9,5	11	-1,5	10,79	0,21
3	3	6	6	3	6	-3	4,80	1,19
19	20	17	18	19,5	17,5	2	20,00	-2,50
14	11	14	14	12,5	14	-1,5	13,56	0,44
31	29	23	23	30	23	7	29,68	-6,69
32	30	28	27	31	27,5	3,5	30,60	-3,10
18	20	21	20	19	20,5	-1,5	19,54	0,95
16	16	18	19	16	18,5	-2,5	16,78	1,72
31	31	31	31	31	31	0	30,60	0,40
22	22	24	24	22	24	-2	22,31	1,69
25	25	24	25	25	24,5	0,5	25,07	-0,57
24	26	26	26	25	26	-1	25,07	0,93
22	26	26	26	24	26	-2	24,15	1,85
22	23	24	23	22,5	23,5	-1	22,77	0,73
11	11	11	11	11	11	0	12,17	-1,17
10	13	9	9	11,5	9	2,5	12,64	-3,64
16	17	17	17	16,5	17	-0,5	17,24	-0,24
11	11	12	12	11	12	-1	12,17	-0,18

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38	*37*	*37*	*36*	*37,5*	*36,5*	*1*	*36,59*	*-0,09*
35	*34*	*37*	*36*	*34,5*	*36,5*	*-2*	*33,83*	*2,67*
21	*21*	*22*	*21*	*21*	*21,5*	*-0,5*	*21,39*	*0,11*
10	*8*	*10*	*11*	*9*	*10,5*	*-1,5*	*10,33*	*0,17*
30	*30*	*29*	*29*	*30*	*29*	*1*	*29,68*	*-0,68*
16	*15*	*15*	*15*	*15,5*	*15*	*0,5*	*16,32*	*-1,32*

One will copy on a graph the theoretical line y = x and the regression line. Observation will show whether there is a distinct difference.



The following values are calculated:

- q = 28
- a = 2.04

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- b = 0.92
- M_d = -0.41
- S_d = 2.17
- $S_{x,y}$ = 2.09
- S_b = 0.047

It would also be useful to graph the deviation in average x_i values obtained using the standard method in question with respect to the y_i values obtained using the reference method and with respect to the $Y\hat{}_i$ values obtained when calculating the regression line.

Comparison: Deviation (yi-xi) and (yi-y^i) as a function of xi:

The slope is verified using the following formula:

$$b - (t_{1-\alpha/2} * s_b) < 1 < b + (t_{1-\alpha/2} * s_b)$$

- q = 28
- v = q-2 = 26
- b = 0.92
- $t_{1-}\alpha_{/2}$ = 2.056
- S_b = 0.047

$$0{,}92 - (2{,}056 * 0{,}047) < 1 < 0{,}92 + (2{,}056 * 0{,}047)$$

$$0{,}823 < 1 < 1{,}017$$

The resulting values are around 1. The slope is therefore not statistically different from 1.

Verify the average of the difference using the following test:

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$$Md - ((t_{1-\alpha/2})s_d/\sqrt{q}) < 0 < Md + ((t_{1-\alpha/2})s_d/\sqrt{q})$$

- q = 28
- v = q-1 = 27
- $t_{1-\alpha/2}$ = 2.056
- M_d = -0.41
- S_d = 2.17

$$-0{,}41 - ((2{,}052)2{,}17/\sqrt{28}) < 0 < -0{,}41 + ((2{,}052)2{,}17/\sqrt{28})$$

$$-1{,}25 < 0 < 0{,}43$$

The resulting values are around 0. The bias value is therefore not statistically different from 0.

In our example, it is possible to conclude that the differences observed in the results obtained using the standard method and the reference method is not significant.

Annex N° 1

Table A

SNEDECOR'S Law

The table shows the values of F as a function of v_1 and v_2 for an □ of 0.05.

P = 0.950

$v1$ $v2$	1	2	3	4	5	6	7	8	9	10	$v1$ $v2$

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1	161,4	199,5	215,7	224,6	230,2	234,0	236,8	238,9	240,5	241,9	1
2	18,51	19,00	19,16	19,25	19,30	19,33	19,35	19,37	19,38	19,40	2
3	10,13	9,55	9,28	9,12	9,01	8,94	8,89	8,85	8,81	8,79	3
4	7,71	6,94	6,59	6,39	6,26	6,16	6,09	6,04	6,00	5,96	4
5	6,61	5,79	5,41	5,19	5,05	4,95	4,88	4,82	4,77	4,74	5
6	5,99	5,14	4,76	4,53	4,39	4,28	4,21	4,15	4,10	4,06	6
7	5,59	4,74	4,35	4,12	3,97	3,87	3,79	3,73	3,68	3,64	7
8	5,32	4,46	4,07	3,84	3,69	3,58	3,50	3,44	3,39	3,35	8
9	5,12	4,26	3,86	3,63	3,48	3,37	3,29	3,23	3,18	3,14	9
10	4,96	4,10	3,71	3,48	3,33	3,22	3,14	3,07	3,02	2,98	10
11	4,84	3,98	3,59	3,36	3,20	3,09	3,01	2,95	2,90	2,85	11
12	4,75	3,89	3,49	3,26	3,11	3,00	2,91	2,85	2,80	2,75	12
13	4,67	3,81	3,41	3,18	3,03	2,92	2,83	2,77	2,71	2,67	13
14	4,60	3,74	3,34	3,11	2,96	2,85	2,76	2,70	2,65	2,60	14
15	4,54	3,68	3,29	3,06	2,90	2,79	2,71	2,64	2,59	2,54	15
16	4,49	3,63	3,24	3,01	2,85	2,74	2,66	2,59	2,54	2,49	16
17	4,45	3,59	3,20	2,96	2,81	2,70	2,61	2,55	2,49	2,45	17
18	4,41	3,55	3,16	2,93	2,77	2,66	2,58	2,51	2,46	2,41	18
19	4,38	3,52	3,13	2,90	2,74	2,63	2,54	2,48	2,42	2,38	19
20	4,35	3,49	3,10	2,87	2,71	2,60	2,51	2,45	2,39	2,35	20
21	4,32	3,47	3,07	2,84	2,68	2,57	2,49	2,42	2,37	2,32	21

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22	4,30	3,44	3,05	2,82	2,66	2,55	2,46	2,40	2,34	2,30	22
23	4,28	3,42	3,03	2,80	2,64	2,53	2,44	2,37	2,32	2,27	23
24	4,26	3,40	3,01	2,78	2,62	2,51	2,42	2,36	2,30	2,25	24
25	4,24	3,39	2,99	2,76	2,60	2,49	2,40	2,34	2,28	2,24	25
26	4,23	3,37	2,98	2,74	2,59	2,47	2,39	2,32	2,27	2,22	26
27	4,21	3,35	2,96	2,73	2,57	2,46	2,37	2,31	2,25	2,20	27
28	4,20	3,34	2,95	2,71	2,56	2,45	2,36	2,29	2,24	2,19	28
29	4,18	3,33	2,93	2,70	2,55	2,43	2,35	2,28	2,22	2,18	29
30	4,17	3,32	2,92	2,69	2,53	2,42	2,33	2,27	2,21	2,16	30
40	4,08	3,23	2,84	2,61	2,45	2,34	2,25	2,18	2,12	2,08	40
60	4,00	3,15	2,76	2,53	2,37	2,25	2,17	2,10	2,04	1,99	60
120	3,92	3,07	2,68	2,45	2,29	2,17	2,09	2,02	1,96	1,91	120
□	3,84	3,00	2,60	2,37	2,21	2,10	2,01	1,94	1,88	1,83	□
$v2$ $v1$	1	2	3	4	5	6	7	8	9	10	$v2$ $v1$

Annex N° 2

Table B

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STUDENT'S Law

The table shows the values of t as a function of P and v.

P / v	0,55	0,60	0,65	0,70	0,75	0,80	0,85	0,90	0,95	0,975	0,990	0,995	0,9995	P / v
1	0,158	0,325	0,510	0,727	1,000	1,376	1,963	3,078	6,314	12,706	31,821	63,657	636,619	1
2	0,142	0,289	0,445	0,617	0,816	1,061	1,386	1,886	2,920	4,303	6,965	9,925	31,598	2
3	0,137	0,277	0,424	0,584	0,765	0,978	1,250	1,638	2,353	3,182	4,541	5,841	12,929	3
4	0,134	0,271	0,414	0,569	0,741	0,941	1,190	1,533	2,132	2,776	3,747	4,604	8,610	4
5	0,132	0,267	0,408	0,559	0,727	0,920	1,156	1,476	2,015	2,571	3,365	4,032	6,869	5
6	0,131	0,265	0,404	0,553	0,718	0,906	1,134	1,440	1,943	2,447	3,143	3,707	5,959	6
7	0,130	0,263	0,402	0,549	0,711	0,896	1,119	1,415	1,895	2,365	2,998	3,499	5,408	7
8	0,130	0,262	0,399	0,546	0,706	0,889	1,108	1,397	1,860	2,306	2,896	3,355	5,041	8
9	0,129	0,261	0,398	0,543	0,703	0,883	1,100	1,383	1,833	2,262	2,821	3,250	4,781	9
10	0,129	0,260	0,397	0,542	0,700	0,879	1,093	1,372	1,812	2,228	2,764	3,169	4,587	10
11	0,129	0,260	0,396	0,540	0,697	0,876	1,088	1,363	1,796	2,201	2,718	3,106	4,437	11
12	0,128	0,259	0,395	0,539	0,695	0,873	1,083	1,356	1,782	2,179	2,681	3,055	4,318	12
13	0,128	0,259	0,394	0,538	0,694	0,870	1,079	1,350	1,771	2,160	2,650	3,012	4,221	13
14	0,128	0,258	0,393	0,537	0,692	0,868	1,076	1,345	1,761	2,145	2,624	2,977	4,140	14
15	0,128	0,258	0,393	0,536	0,691	0,866	1,074	1,341	1,753	2,131	2,602	2,947	4,073	15
16	0,128	0,258	0,392	0,535	0,690	0,865	1,071	1,337	1,746	2,120	2,583	2,921	4,015	16
17	0,128	0,257	0,392	0,534	0,689	0,863	1,069	1,333	1,740	2,110	2,567	2,898	3,965	17
18	0,127	0,257	0,392	0,534	0,688	0,862	1,067	1,330	1,734	2,101	2,552	2,878	3,922	18
19	0,127	0,257	0,391	0,533	0,688	0,861	1,066	1,328	1,729	2,093	2,539	2,861	3,883	19
20	0,127	0,257	0,391	0,533	0,687	0,860	1,064	1,325	1,725	2,086	2,528	2,845	3,850	20
21	0,127	0,257	0,391	0,532	0,686	0,859	1,063	1,323	1,721	2,080	2,518	2,831	3,819	21
22	0,127	0,256	0,390	0,532	0,686	0,858	1,061	1,321	1,717	2,074	2,508	2,819	3,792	22

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23	0,127	0,256	0,390	0,532	0,685	0,858	1,060	1,319	1,714	2,069	2,500	2,807	3,767	23
24	0,127	0,256	0,390	0,531	0,685	0,857	1,059	1,318	1,711	2,064	2,492	2,797	3,745	24
25	0,127	0,256	0,390	0,531	0,684	0,856	1,058	1,316	1,708	2,060	2,485	2,787	3,725	25
26	0,127	0,256	0,390	0,531	0,884	0,856	1,058	1,315	1,706	2,056	2,479	2,779	3,707	26
27	0,127	0,256	0,389	0,531	0,684	0,855	1,057	1,314	1,703	2,052	2,473	2,771	3,690	27
28	0,127	0,256	0,389	0,530	0,683	0,855	1,056	1,313	1,701	2,048	2,467	2,763	3,674	28
29	0,127	0,256	0,389	0,530	0,683	0,854	1,055	1,311	1,699	2,045	2,462	2,756	3,659	29
30	0,127	0,256	0,389	0,530	0,683	0,854	1,055	1,310	1,697	2,042	2,457	2,750	3,646	30
40	0,126	0,255	0,388	0,529	0,681	0,851	1,050	1,303	1,684	2,021	2,423	2,704	3,551	40
60	0,126	0,254	0,387	0,527	0,679	0,848	1,046	1,296	1,671	2,000	2,390	2,660	3,460	60
120	0,126	0,254	0,386	0,526	0,677	0,845	1,041	1,289	1,658	1,980	2,358	2,617	3,373	120
∞	0,126	0,253	0,385	0,524	0,674	0,842	1,036	1,282	1,645	1,960	2,326	2,576	3,291	∞
$\frac{\upsilon}{P}$	0,55	0,60	0,65	0,70	0,75	0,80	0,85	0,90	0,95	0,975	0,990	0,995	0,9995	$\frac{\upsilon}{P}$

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Certified in conformity Mainz, 9th July 1999
The Director General of the OIV
Secretary of the General Assembly
Georges DUTRUC-ROSSET

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