Wine and its analysis

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The importance of wine and its analysis

Vitiviniculture sector is one of the most important within the world agriculture. The world production in 2014 (excluding juices and musts) was of 271 millions of Hl. Europe is the first wine producer, being the top countries France, Italy and Spain.

The spanish vitiviniculture sector is very important so much for the economical value generated as for the working population and the environmental conservation. The geographical situation, the climatic and edaphic differences, make our country a privileged place for the production of wines of different characteristics.

Historically the wines were meant to be table wines, but nowadays Spain produces very good quality wines and there are 85 Apellations of Origin. They follow the European model of production, with a strict control of the amount produced, the oenological practices and the quality of the wines produced in each zone. The spanish vitivinicultural sector is involved in an important process of update and renewal. It is worth noting the activity and innovation of many wineries which experiment with new varieties of grapes and the use of native grapes, as well as new technologies to produce wines according to consumer’s preferences. All of these changes lead to the necessity of analysis and quality control adapted to satisfy the current market demands.

Standard Operating Procedures in wine analysis

Standard operating procedure (SOP) is an important aspect of a quality system. A SOP for a laboratory can be defined as: A document which describes the regularly recurring operations relevant to the quality of the investigation. The purpose of a SOP is to carry out the operations correctly and always in the same manner. A SOP should be available at the place where the work is done.

This document should be able to answer the following questions:

- What do you want to do? The aim of the SOP
- How do you do it? The basis of the method of the SOP
- When do you have to do it? The chronology of the procedures.
- Which instruments do you need? Equipment and reagents needed, indicating the accuracy of the instruments and the minimal purity of reagents
- Who is going to do it? Working level to achieve the different activities of the SOP
- How do you deal with the results obtained? Rejection or acceptance criteria, uncertainties.

A SOP is a compulsory instruction. If deviations from this instruction are allowed, the conditions for these should be documented, and what exactly the complete procedure will be.

These procedures are followed in many laboratories especially in drinking water analysis but they are not frequent in other food analysis laboratories, such as in wine analysis control.

One of the aims of this project is the information transfer from the university to the wine company in order to facilitate its work. Another aim is to contribute to the students learning, in relation to the procedures used in the food industry.

A SOP is an important training document, which can be studied before beginning the experimental procedure.
**Introduction**

Acidity is one of the characteristics of wine and it contributes to its the taste balance. The acidity level depends on two parameters, fixed acidity due to the organic acids which are present in the grapes, such as tartaric, malic and citric acids, and on the other hand volatile acidity originated during fermentation where certain amount of acetic acid is formed. Malolactic fermentation transforms the malic acid into lactic acid, improving the taste.

Acidity is expressed in concentration of the acids present, tartaric acid (total acidity) or acetic acid (volatile acidity) or in the form of pH.

pH in wines ranges between 3 to 4, in white wines about 3.0 to 3.3 and in red wines about 3.3 to 3.6.

**The concept of pH**

pH (pondus hydrogenii hydrogen’s weight) is the co-logarithm of hydrogen ion activity (concentration) \( \text{pH} = - \log 10 a_{H^+} \)

It is measured by potentiometry with a pH meter, which is a voltmeter of high impedance which measures the difference of potential in mV(voltage) generated between the electrode of pH and the reference electrode in contact with the sample.

This voltage is transformed in pH units following NERNST’s equation

\[
E = E_0 + \frac{2.3RT}{NF} \cdot \log A_H
\]

Where:

- \( E \): Potential measured
- \( E_0 \): Potential of the reference electrode
- \( R \): gas constant
- \( T \): Absolute temperature
- \( N \): number of electrons transferred (H+=1)
- \( F \): Faraday’s constant (96484.5561 C/mol)

---

5
Substituting $S = \frac{2.3RT}{NF}$, the equation is:

$$E = E_0 - S \cdot pH$$

The equation of a line with negative slope where the Potential is the dependent variable (Y) and the pH the independent variable (X), S is the slope of the line. According to Nernst´s equation the theoretical slope is 59.16 mv / pH unit at 25°C.

This slope depends on the temperature according with the following table, thus for the pH determination the temperature must be measured.

**Calibration**

The pH meter must be calibrated every working day using two buffer solutions of pH 7.00 and 4.00 or 10.00, depending on the measure expected. In this case it will be 4.00.

The checking of the isopotential point of the electrode (voltage = ±20mV) is at pH 7.00, then the device shall ask for the second standard.

The calibration curve is constructed with the 4.00 buffer solution.

Depending on the temperature the slope must be the same to the tabulated (Nernst´s slope), if not, the calibration shall be rejected and it is necessary to be repeated. (The value of the theoretical voltage will be approximately 177mv)

Some devices show the percentage of correlation between the theoretical slope and the actual slope. Above 90% is correct.
PROCEDURE PE/BR/01

POTENTIOMETRIC DETERMINATION OF pH IN WINES

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6. RESULTS
   6.1 CÁLCULATION and CRITERIA OF ACCEPTANCE
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1. **PURPOSE**

   Potentiometric measure of pH in wines.

2. **SCOPE**

   This method is valid for all the wines, at room temperature between 10-45ºC.

3. **REFERENCES**

   - PC-BR-01:- General Procedure of elaboration of Procedures in Bromatology laboratory.

4. **GENERAL**

   The determination of pH in musts and wines is a complementary measure of the total acidity. Wine stability, malolactic fermentation, sour taste, colour, REDOX potential and the relationship between free sulphur dioxide and total sulphur dioxide are closely related to the pH of the wine.

5. **DESCRIPTION**

   5.1 **Apparatus and Materials**

   - pHmeter Crisón 2000. (sensitivity ± 0.01U. of pH).
   - Ag/ AgCl electrode
   - 0-50 ºC calibrated thermometer, sensitivity ±0.5 ºC. (or temperature sensor)
   - 100 ml beaker
   - 250 ml beaker
   - Magnetic stirrer with magnet
 PROCEDURE FOR pH DETERMINATION
 IN WINE

- Standard solution (buffer) pH 7.00 ± 0.02 (20 ºC)
- Standard solution (buffer) pH 4.00 ± 0.02 (20 ºC)
- 3 M Potassium chloride solution
- Distilled water
- Clean and dry cloth.

5.2 Preliminary operations
Standard solutions are kept in refrigerator. Once opened the may be kept for up to one month.
Samples and standard solutions should be for at least 15 minutes at room temperature before the measurement and calibration.

5.3 Procedure
  5.3.1 Calibration
The pHmeter has to be calibrated every day, using standard buffer solutions of pH 7.00 and 4.00.

Connect the pHmeter. The display shows

0000  20.0 (pH / temperature)

Press the thermometer button to adjust the temperature measured with the thermometer in the liquid (pressing upper zone the temperature increases and pressing the lower zone the temperature decreases). The electrode of pH usually has a thermometer therefore this adjustment is not always necessary.

Place in a 100 ml beaker the enough volume of the buffer solution to be sure the diafragm of the electrode is immersed in the liquid.
Introduce the magnet and connect the magnetic stirrer at low speed (be careful the magnet does not bounce). Take off the electrode’s rubber cap, rinse the electrode with distilled water, dry it and dip the electrode in 7.02 buffer solution. (this solution must be always the first one).

Stir gently and press the bottle symbol and the pH symbol. pH indicator flashes (it is checking the isopotential point of the electrode) if so, the device will ask for the second standard, the light will get fixed and on the display appears

\[
4 \quad 25.0
\]

Rinse the electrode with distilled water and dry it. Dip the electrode in the 4.00 buffer solution, stir and press the bottle symbol button. The pH indicator flashes, wait until the light is fixed and on the display appears

\[
4.0 \quad 25.0
\]

### 5.3.2 Determination

Dip the electrode into the wine sample to be analyzed and follow the previous instructions. Wait 15 seconds. Carry out two determinations and note down the results on the data sheet H05-01

Remember to rinse the electrode on a 250 ml beaker with distilled water after each determination and dry it with a cellulose cloth.

Once the determination is finished, and the electrode is cleaned, place the cap on the electrode and let it immersed in a NaCl 3M solution or in buffer solution pH 7.00.
6. RESULTS

6.1 Calculation and criteria of acceptance of results

The pH is a direct measurement and is reported to two decimal places. The final result is taken to be the arithmetic mean of two determinations. The difference between the measurements should be less than 0.1 units of pH to comply with the criteria of acceptance. In the event of discrepancies between the data repeat the determinations

\[ \text{Results} = (\text{measure}_1 + \text{measure}_2)/2 \]
DATA SHEET: pH measurement

<table>
<thead>
<tr>
<th>Performer</th>
<th>Date:</th>
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**calibration**

<table>
<thead>
<tr>
<th>Buffer solutions</th>
<th>pH : 7.02</th>
<th>fecha apertura:</th>
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<tbody>
<tr>
<td><strong>pH :</strong></td>
<td></td>
<td>(menor a 1 5 días)</td>
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**Isopotential point**

- [ ] Correcto

**Slope**

- [ ] Correcto

**Lecturas**

<table>
<thead>
<tr>
<th>Sample</th>
<th>T° (^\circ)</th>
<th>pH (^1)</th>
<th>pH (^2)</th>
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**Equipment**

**Observations and incidences**
Introduction

Grapes contain an important amount of organic acids. Tartaric, malic and citric acids are the major organic acids in must. They constitute over 90% of the total acid components of the juice (Amerine and Joslyn 1950). During ripening, the tartrate and malate content of the fruit decrease. This is accompanied by a slight increase in pH. Due to variation in buffer capacity, there is no direct relationship between titratable acidity and pH. However, higher acid levels in fruit are often associated with lower pH values and vice versa. Therefore the acids of the fruit have a significant influence on pH as well as on taste, colour, and microbial stability of the juice. The organic acids in wine are primarily derived from grapes. However, many other acids are formed during the fermentation. The major acids produced during and after the alcoholic fermentation are acetic, lactic, and succinic.

A good knowledge of the organic acid composition of the must is very important for the vintner:

1. To determine the time of vintage.
2. To decide the wine style.
3. To determine must treatment prior to fermentation.
4. To monitor the stability of a wine (e.g. Is a malolactic fermentation occurring when you don’t want it?).
5. To comply with the regulations. The regulations dictate the minimum acid levels of 0.5 percent in table wines if the must or wine is ameliorated. Most of the commercially produced wines contain acid levels in the range of 0.6 to 0.9 percent.

The acid content of the must is determined by titrating a sample (a given volume) with a base such as sodium hydroxide solution to a phenolphthalein end point or alternatively, to a pH of 8.2. The titratable acidity is expressed as grams of tartaric acid per 100 ml.
# PROCEDURE PE/BR/02

**DETERMINATION OF TOTAL ACIDITY IN WINES**

*Edition: 1  Rev. 0*

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1. **PURPOSE**

Measurement of total acidity in all wines.

2. **SCOPE**

This procedure is valid for all wines with total acidity up to 10g tartaric acid/liter. In case of higher values dilute the sample until that value is achieved.

3. **REFERENCES**

- PC-BR-01.- General Procedure of elaboration of Procedures in Bromatology laboratory.
- SOP/BR/02. Procedure of potentiometric determination of pH in wines.
- Instruction’s manual of the pHmeter.

4. **GENERAL**

4.1. **Definition**

The total acidity of the wine is the sum of its titrable acidities when it is titrated to pH 7 against a standard alkaline solution. Carbon dioxide is not included in the total acidity.

4.2. **Principle**

Potentiometric titration with a pH meter to pH = 7.00±0.5 or titration with bromothymol blue as indicator and comparison with and end-point colour standard.

5. **DESCRIPTION**

5.1. **MATERIALS AND REAGENTS**

5.1.2 Materials

- Removal of gases by vacuum: Water vacuum pump connected to a vacuum flask, 500 ml
- 50, 100, 250 and 1000 ml beakers
- 50 ml burette 0.1 ml accuracy
- 10 ml, 5 0ml volumetric pipettes
- Analytical balance 0.005g sensitivity
PROCEDURE FOR TOTAL ACIDITY
DETERMINATION IN WINE

5.1.2. Reactivos

- Buffer solution pH 7.02 and 4.00
- Sodium hydroxide solution, NaOH 0.1M
- Bromothymol blue indicator solution 4g/l

Reagents preparation is described in Annex I

5.2. PRELIMINARY OPERATIONS

5.2.1 Sample preparation: elimination of carbon dioxide in wine
Place 50±1ml of wine, in a vacuum flask; apply vacuum to the flask using a water pump for 1 to 2 minutes, while shaking continuously.

5.2.2 Elimination of carbon dioxide in distilled water:
Boil 100±5 ml of distilled water in a beaker; let it cool at room temperature before using.

5.2.3. Calibration of the pH meter (only when 5.3.2 is carried out).
Follow description (5.2 and 5.3) SOP procedure /BR/02 potentiometric determination of pH in wines.

5.3 PROCEDURE

5.3.1. Titration with bromothymol blue indicator

5.3.1.1 Preliminary test: end-point colour determination
Place 25±1ml of boiled distilled water into a 250 ml porcelain evaporating dish, 1±0.1 ml of bromothymol blue solution, 10±0.1 ml, of wine free of carbon dioxide (as in
5.2.1). Add slowly sodium hydroxide solution 0.1M from a burette until the colour changes to blue-green.
Then add 5 ml of the pH 7 buffer solution.

5.3.1.2 Measurement
Place 30±1ml of boiled distilled water into a 250 ml porcelain evaporating dish, 1±0.1 ml of bromothymol blue solution and 10±0,1 ml, of wine free of carbon dioxide (5.2.1). Add slowly sodium hydroxide solution 0.1M from a burette until the same colour is obtained as in the preliminary test above (5.3.1.1)
Note down the volume (n ml) added on the data sheet HD01-01
Repeat the titration and note down the volume added (n´ ml).

5.3.2. Potentiometric titration
Introduce 25±1ml of boiled distilled water into a 100 ml beaker and 10±0.1 ml of wine, free of carbon dioxide (as in 5.2.1). Place it on a magnetic stirrer and introduce the stirring bar, then the calibrated electrode of the pHmeter and start reading (according to the instruction`s manual), stirring at low speed (do not make bubbles, be careful the bar does not touch the electrode and the lecture scale is within the liquid.)
Add slowly 0.1M sodium hydroxide solution from a burette, verifying the pH increase; finish the titration when pH is 7±0.5.
Note down the volume (n ml) added on the data sheet HD01-01
Repeat the measure and note down the volume (n´ ml) added.

5.3.3 Criteria of acceptance of analytical results
The values are acceptable when they only differ in 0.2 ml or less, if not repeat the analysis a third time until two values comply with this criterium.
Calculate the mean value which will be used in the results (the number of significant figures are the same to n and n´).
6. RESULTS
   a. Calculation of the mean value
      \[(M) \text{ ml} = \frac{n + n'}{2}\]

   b. Calculation of the total acidity in wine:
      Expressed in milli-equivalents per liter: \[AT \text{ mEq/liter} = 10 \cdot M\]
      It is recorded to 1 decimal place
      Expressed in grams of tartaric acid per liter, \[AT \text{ g tartaric acid/liter} = 0.075 \cdot M\]
      The result is quoted to three decimal places.

7. ANNEXES
   Annex 1. Reagent preparation
Annex 1

Reagent preparation

Buffer solution of pH 7.0
Weigh 107.3±0.05 g of monopotassium phosphate in a 1000 ml beaker, add 500±5 ml of a 1M sodium hydroxide commercial solution, dissolve using a glass rod until the liquid becomes transparent and pour through a funnel into a 1000 ml volumetric flask; rinse the flask and the glass rod with distilled water, pour the contents into the flask, finally make up to volume with distilled water.

0.1 M sodium hydroxide solution
Place 50±0.2 ml of a 1N commercial sodium hydroxide in a 500ml volumetric flask and make up to volume with distilled water. Mix thoroughly 2-3 times.

Bromothymol blue solution 4 g/l:
Weigh 4±0.05 g of bromothymol blue in a funnel for solids, introduce the content in a 1000 ml volumetric flask. Wash the funnel with 200±2 ml of ethylic alcohol and pour into the flask. Shake the flask until total dissolution of the colourant, add 200±2 ml of cold distilled water, mix until uniformity and add 7±1 ml 1M NaOH commercial solution up to greenish blue colour. Then make up to volume with distilled water and mix thoroughly.
DATA SHEET: TOTAL ACIDITY

Performe Date:

Procedure

- Bromothymol blue → Colour standard
- pH meter * → Calibration

Sample:

Degassing time: min

Volumes

<table>
<thead>
<tr>
<th>Titration</th>
<th>ml</th>
<th>pH*</th>
<th>Dif. [&lt; 0.2]</th>
<th>ml · factor</th>
<th>Result ml</th>
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NaOH factor:

Calculation

mean ml (∑ results /2)

Total acidity

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<tr>
<th>mEq/liter</th>
<th>(10 • mean)</th>
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<tr>
<td>mg ac tartáric acid/liter</td>
<td>(0.075 • mean)</td>
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Equipment

Observations and incidences
Introducción

The acids present in wine have two origins, primary, those from the must (grape's juice) and secondary, those which appear after fermentation. Malic and tartaric are the principal acids in must, and succinic, lactic, acetic, etc. are the acids in wine, originated during fermentation.

On the other hand different gases such as oxygen, nitrogen, carbon dioxide, and sulphur dioxide are dissolved in wine. Sulphur dioxide and sulphites are wine preservatives, therefore their concentration increases in wine and acidity increases. These gases can be eliminated after boiling, but it is not easy for the sulphur dioxide which in addition to free it may be combined. This combination can be unstable with sugars, acids and anthocyanins, or stable after its addition to acetaldehyde.

Total acidity is the sum of all the acids in wine, however volatile acidity represents the short chain fatty acids belonging to the acetic series (acetic, formic, propionic and butiric), free or in the form of a salt. They are originated during fermentation by Saccharomyces cerevisiae or after bacterial fermentations (Lactobacillus). Lactic and succinic acids, carbon dioxide and free and combined sulphur dioxide are not included in this determination.

Volatile acidity is determined after Julmes method. This method is based on the separation of the volatile acids by fractionate distillation and steam distillation and vapour rectification (fractionated distillation). Steam distillation is used to separate water insoluble organic compounds water and slightly volatile from no volatile substances. The substances immiscible between them follow Dalton’s law on partial pressures (Annex 2). When one of the components is water, a compound with a higher boiling point than water can be separated at a temperature below 100°C at atmospheric pressure.

Previous to distillation the wine is acidified with tartaric acid crystals, thus a higher amount of free
volatile acids is obtained.

Precautions should be taken to prevent the presence of carbon dioxide, since the wine could be acidified.

Phenolphthalein is used as indicator. The acidity of free and combined sulphur dioxide distilled under these conditions should be subtracted from the acidity of the distillate, as well as any sorbic acid present.

Volatile acidity is expressed in g of acetic acid/liter of wine.
DEPARTAMENTO DE NUTRICIÓN Y BROMATOLOGÍA II: BROMATOLOGÍA

PROCEDURE PE/BR/03

DETERMINATION OF VOLATILE ACIDITY IN WINE

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      5.3.1. Sample preparation
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6. RESULTS
   6.1. Calculation of the mean value
   6.2. Calculation of the volatile acidity in wine
7. ANNEXES
1. PURPOSE
Measurement of the volatile acidity in a wine. The volatile acids are previously separated from the wine by steam distillation.

2. SCOPE
This procedure is valid for all the wines, working at a room temperature between 10-45ºC.

3. REFERENCES
- PC-BR-01.- General Procedure of elaboration of Procedures in Bromatology laboratory.
- Instructions manual of the steam distillation equipment.

4. GENERAL
4.1. Definition
Volatile acidity comprises the short chain fatty acids (acetic, formic, propionic and butiric) present in the wine.

4.2. Principle of the method
Volatile acids are separated from the wine by steam distillation. Previously crystals of tartaric acid are added to obtain a higher proportion of free volatile acids. The indicator is phenolphthalein. The acidity of free and combined sulphur dioxide distilled under these conditions should be substracted from the acidity of the distillate.

5. DESCRIPTION
5.1. MATERIALS AND REAGENTS
5.1.1. Apparatus and materials
- Steam distillation apparatus consisting of \((Annex~1)\):
  - A steam generator
  - A flask with steam pipe, (bubbler)
  - A distillation column
5.1.2. Reagents

- Tartaric acid, crystalline (C4H6O6)
- Sodium hydroxide solution N/10
- Phenolphthalein solution
- Hydrochloric acid, concentrate 36.5-38%
- Iodine solution N/100 (I2)
- Potassium iodide, crystalline (KI)
- Starch solution
- Saturated solution of sodium tetraborate
- Sodium hydroxide solution 0.1 M (*)
- Acetic acid, 0.1 M (*)
- Lactic acid solution 1M (*)

The preparation of the reagents is described on Annex 2

(*): Only in the validation of the bubbler

5.2. PRELIMINARY OPERATIONS

5.2.1 Validation of the bubbler

The equipment must pass the following three tests:

1st) The steam must be free of CO₂
Place 20±1 ml of boiled water in the flask; put into operation according to the instructions. Collect 250±0.15 ml of the distillate and add 0.1±0.001 ml of sodium
PROCEDURE FOR VOLATILE ACIDITY
DETERMINATION IN WINE

hydroxide solution 0.1M with a given factor and two drops of phenolphthalein solution. The pink colouration must be stable for at least 10 seconds.

2nd) Determination of the volatile acids flow rate (acetic acid ≥ 99.5 %)
Place 20±0.3 ml acetic acid solution in the flask. Collect 250 ml of distillate. Titrate with the sodium hydroxide solution 0.1M. The volume of the titer must be at least 19.9 ml.

3rd) Determination of the non volatile acids flow rate (lactic acid distilled ≤ 0.5 %)
Place 20±0.03ml lactic acid solution 0.1Mo (with a given factor) flask. Collect 250 ml the distillate and titrate the acid with the sodium hydroxide solution 0.1M (with a given factor). The volume of sodium hydroxide solution added multiplied by the factor must be less than or equal to 1.0 ml.

All the apparatus that comply with these three tests are suitable for this determination.

5.3. PROCEDURE
5.3.1. Sample preparation
Elimination of carbon dioxide. Place about 50±0.5ml of wine in a 250 ml flask and shake for 1-2 minutes.

5.3.2. Steam distillation
Place 20±0.03ml of wine freed from carbon dioxide (5.3.1) into a 250 ml volumetric flask. Add tartaric acid with the tip of the spatula. Introduce the sample into the flask with steam pipe (Annex 1) and collect at least, 250 ml of the distillate.
Repeat at least once more.

5.3.3. Titration
The determination of volatile acidity in wine is carried out after three titrations:
5.3.3.1. First titration
Place all the distillate collected in 5.3.2 into a 500 ml flask and add two drops of
phenolphthalein solution. Titrate, drop by drop from a burette, with a sodium hydroxide solution N/10 (with a given factor).

Note down on the data sheet HD 04-01 the volume added (V ml)

Repeat the titration. Let (V´ ml) be the volume added.

5.3.3.2. Second titration

Continue titration in the same flask (5.3.2.1). Add 1 drop of the concentrate hydrochloric acid, 2-3 drops of the starch solution and potassium iodide with the tip of the spatula. Titrate the free sulphur dioxide with the iodine solution N/100 (with a given factor).

Note down on the data sheet HD 04-01 the volume added. (V1 ml)

Repeat the titration. Let (V1´ ml) be the volume added.

5.3.3.3. Third titration

Add 20±1ml of the saturated sodium tetraborate solution. Titrate the combined sulphur dioxide with the iodine solution N/100.

Note down on the data sheet HD 04-01 the volume added (V2 ml).

Repeat the titration. Let (V2´ ml) be the volume added.

Reactions are shown in Annex 3.

6. TRATAMIENTO DE LOS RESULTADOS

6.1. Calculation of mean value

\[ (M) \text{ ml} = \frac{(V + V')}{2} \]
\[ (M) \text{ ml} = \frac{(V1 + V1')}{2} \]
\[ (M) \text{ ml} = \frac{(V2 + V2')}{2} \]

6.2. Calculation of volatile acidity

\[ (V \times f) - (V1/10 + V2/20) \times f' \]

1 equivalent NaOH = 1 equivalent CH₃COOH

Expressed in acetic (g) / 1 liter wine
\[
\left[ (V \times f) - \left( \frac{V_1}{10} + \frac{V_2}{20} \right) \times f\right] \times \left( \frac{PM\text{actic acid}}{10} \right) = g\text{ acetic/L}
\]

7. ANNEXES

Annex 1. Preparation of reagents

Annex 2.

Appendix 1. Instructions of the steam distillation apparatus
Appendix 2. Figures

Annex 3. Reactions
Annex 1

Preparation of Reagents

Sodium hydroxide solution N/10
Introduce 50±0.1 ml of a commercial sodium hydroxide solution 1N into a 500 ml volumetric flash and bring to volume with distilled water. Shake 2-3 times to mix the solution

Phenolphthalein solution 1%
Weigh 1±0.05 g of the commercial product in a 250 ml beaker. Add 100±0.5 ml of neutral alcohol 96% vol. Mix to dissolve.

Starch solution
Weigh 5±0.1 g of starch in 1000 ml beaker and add 500±0.5 ml of water. Bring to the boil, stirring continuously and boil for 10±0.5 minutes. Add 200±1 g sodium chloride. When cool, place it in a 1000±0.3ml volumetric flask and make up to one liter. Shake 2-3 times.

Saturated solution of sodium tetraborate (Na₂B₄O₇ ·10 H₂O)
Weigh 55±0.05 g of sodium borate in a 2000 ml beaker. Add 1000±5 ml of distilled water at 20 °C. Mix to dissolve. Probably not all the product shall be dissolved.
Annex 2

Appendix 1 - Instructions of the steam distillation apparatus (see appendix 2)

1- Check that bottle beside the apparatus has water. Add 5±1 g NaCl
2- Plug
3- Open the water tap
4- Start (press POWER)
5- Place a tube with distilled water in the bubbler pushing down the inferior bracket
6- Place a flask or beaker in the collector
7- Press the button MANUAL
8- Wait 5 minutes while the distilled water in the tube (4) bubbles
9- Turn off the button MANUAL
10- Introduce the sample in a tube and place as in 4
11- Place a 250 ml volumetric flask in the collector
12- Press MANUAL
13- Once the desired volume is collected turn off MANUAL
14- Alter distilling the simples repeat steps 4 to 6
15- Wait 10 minutes
16- Turn off POWER
17- Close water tap
18- Unplug
19- Open the door behind the apparatus and turn on a small black key to empty the hot water.

When there is no more water turn off the black key and close the door of the apparatus.
Figure 1. Image of a steam distillation equipment
Annex 3
Reactions

First titration:

R-COOH + NaOH \rightarrow R-COONa + H₂O
SO₂ + NaOH \rightarrow NaHSO₃ \rightarrow Na₂SO₃

Second titration:

NaHSO₃
+ HCl \rightarrow SO₂ + Cl⁻ + H₂O
Na₂SO₃

I₂ + 2 e⁻ \rightarrow 2I⁻
SO₂ + H₂O - 2e⁻ \rightarrow SO₃ + 2H⁺

Third titration:

CH₃-CHOH-SO₂⁻ + Na₂B₄O₇ \rightarrow HSO₃⁻ + CH₃⁻

I₂ + 2 e⁻ \rightarrow 2I⁻
SO₂ + H₂O - 2e⁻ \rightarrow SO₃ + 2H⁺
DATA SHEET: VOLATILE ACIDITY

Performer: [Name]
Date: [Date]

Procedure

- □ Volatile acidity
- □ Validation of the bubbler

Validation of the bubbler

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Calculation

Mean ml \( (\text{results/2}) \)

Volatile acidity

\[ g \text{ acetic acid/liter} \quad (0.075 \times \text{mean}) \]

Equipment

Observations and incidences
Introduction

When the term alcohol is mentioned, it refers to ethyl alcohol or ethanol. The presence of alcohol in beverages is a differential aspect, thus they are denominated alcoholic beverages.

Must or grape’s juice contains variable sugar contents. Grapes have 15-25% of glucose and fructose. In ripen grapes both sugars are in the same proportion though fructose is slightly higher than glucose. The relationship glucose/fructose is about 0.95.

Alcoholic fermentation is an anaerobic process carried out by yeasts, Saccharomyces, in spontaneous way or conducted by selected yeasts. During fermentation the yeasts transform the sugars (glucose and fructose) in the must or grape’s juice into ethyl alcohol (ethanol) and carbon dioxide. Moreover, other substances are produced, though in minor quantity but very important for the characteristics of wine. Fermentation decreases the relationship glucose/fructose to 0.3

The temperature control is very important for a good development of fermentation. Yeasts need a temperature of about 20°C; below 13-14°C it is nearly impossible to start the fermentation, and above 32-35°C yeasts cease their activity, causing fermentation stops. A high temperature accelerates the fermentation, but the alcoholic strength of the wine will be low. On the contrary fermentation at low temperature will produce wines with higher alcoholic strength.

The proportion of alcohol in a beverage is named alcoholic strength. Wines have between 7 to 16% alcohol in volume, though most of the bottled wines have 10 to 14%. Sweet wines present an alcoholic strength between 15 and 22%.

Alcoholic beverages can be fermented beverages or distilled beverages.

- A fermented beverage comes from a fruit or cereal (grape, apple, barley) which has undergone an alcoholic fermentation by the action of yeasts. During this process most of the sugars are transformed into alcohol.
- Fermented beverages which later are distilled are known as distilled beverages (brandy, whisky, rhum,…). They present a higher alcohol content than the fermented beverages.
DEPARTAMENTO DE
NUTRICIÓN Y BROMATOLOGÍA II: BROMATOLOGÍA

PROCEDURE PE/BR/04

DETERMINATION OF ALCOHOLIC STRENGTH IN WINES

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   5.2. PRELIMINARY OPERATIONS
      5.2.1. Assembly of the distillator
   5.3. PROCEDURE
6. RESULTS
   6.1. CALCULATION AND CRITERIA OF ACCEPTANCE
7. ANNEXES
1. **PURPOSE**
Determination of alcoholic strength in an alcoholic beverage.

2. **SCOPE**
This procedure is valid for wines with an alcoholic strength ≤25 %.

3. **REFERENCES**
- PC-BR-01: Procedimiento General de elaboración de Procedimientos del Laboratorio de Bromatología
- Compendio de los métodos internacionales de análisis de los vinos y de los mostos. Organización Internacional de la Viña y el Vino (OIV). Ed. 2015

4. **GENERAL**
4.1. **Definition**
The alcoholic strength by volume (%v/v) is the number of liters of ethanol contained in 100 liters of wine, both volumes being measured at 20±0.1°C

4.2. **Principle**
This method is carried out with a direct distillation. A liquid mixture is separated in its components by evaporation followed by condensation. After heating the mixture, the vapor obtained is rich in the most volatile component (that with the lowest boiling point). This method is developed in two steps. First, a simple distillation of the wine made alkaline and then the measurement of the apparent density of the distillate with a pycnometer at 20°C of temperature.
The difference between the weight of a volume of distillate and the weight of the same volume of distilled water enables to know the density of the distillate with the pycnometer. The ratio between both weights is the specific gravity of the distillate, which in Windish tables is related to the alcoholic content, expressed as (%).
5. DESCRIPTION

5.1. MATERIALS AND REAGENTS

5.1.1. Material

- Distillation apparatus consisting of: a Bunsen burner, a round bottomed 1 liter flask, a condenser terminated by a drawn-out tube taking the distillate to the bottom of a volumetric flask (see Annex 2).
- Glass beads.
- 200 ml volumetric flask.
- 10 ml pycnometer
- Thermometer 0-35 ºC, accuracy 0.25 ºC.
- Water bath at 20±0.5ºC
- Analytical balance, sensitivity 0.0005g.

5.1.2. Reagents

- Suspensión de calcium hydroxide 2M

Reagents preparation is described in Annex 1

5.2. PRELIMINARY OPERATIONS

5.2.1. Assembly of the distillator

Place and connect all the pieces of the distillator; connect the entrance and the way out of the condenser with rubber tubes for water flow (see Annex 2)

5.2.2. Cooling bath

Prepare a water bath to cool the distillate at 20ºC±0.5ºC

5.3. REALIZACIÓN DEL ANÁLISIS

5.3.1. Wine distillation

Measure out 200 mL of the wine using a volumetric flask. Record the temperature of the wine.

Transfer the wine to the distillation flask and introduce the steam-pipe of the steam
distillation apparatus. Rinse the volumetric flask four times with successive 5 ml washings of water added to the flask or the steam-pipe. Add 5±1ml of calcium hydroxide 2M and 2-3 glass beads. Open the water flow and light the burner. Collect the distillate in the 200 ml volumetric flask used to measure the wine. Collect about three quarters of the initial volume in a 200 ml volumetric flask. Make up to volume with distilled water.

5.3.2. Measurement of the alcohol in the distillate
Weigh two clean and dry pycnometers, accuracy of 0.1mg at 20±0.5ºC (p1, p2). Fill the pycnometer with distilled water at 20±0.5ºC without bubbles. Note down the weight (p’) on the data sheet HD 04-01. Fill the other one with the distillate at 20±0.5ºC and note down the weight (p’’).

6. RESULTS
6.1. Calculation and criteria of acceptance

\[
\frac{p'' - p1}{p' - p2} \times 1 \, g/ml
\]

D = density of the distillate at 20± 0.5ºC
p1, p2 = weigh expressed in grams of the empty pycnometers
p’ = weigh expressed in grams of the pycnometer filled with distilled water
p” = weigh expressed in grams of the pycnometer filled with the wine distillate

The results must be reported as the mean value. The difference between two determinations should be less than 0.3%, if it exceeds this value the determination must be repeated.

Once the density of the distillate has been calculated, the % of alcohol of the wine can be determined by means of the Windish tables (see Annex 3).

7. ANNEXES

Annex 1. Preparation of reagents
Annex 2. Distillation Apparatus

Annex 3. Windish tables: % alcohol in volume respect to specific gravity at 20º C
Annex I
Preparation of reagents

Calcium hydroxide suspension

Weight 120 ± 0.05 g of calcium oxide in a beaker of 2000 ml, add 1000 ± 5ml of water at 20 °C. Stir until dissolved.
Annex 2
Distillation apparatus
### Annex 3

Windisch Tables: % alcohol in volume respect to specific gravity at 20º C

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**Equipment**

**Observations and incidences**
**Introducción**

SO$_2$ and sulphites have been used during years for their multiple functions. They may be present in food and drinks as food additives with preservative and antioxidant actions. Their mechanism of action is the inhibition of the damage caused by:

- Bacteria, moulds and yeasts.
- Enzymatic and non enzymatic browning reactions which take place during food processing or during food storage.

In Spain sulphur dioxide and sulphites are allowed under certain conditions in a wide variety of food, as in the case of wine. It is regulated by the RD 142/2002 modified by RD 1118/2007, referring to food additives other than colours and sweeteners. The maxima doses allowed depend on the food and range between 10 and 2000 mg/kg of SO$_2$.

In wines: The European Union (UE Regulation nº 1493/1999 y 1622/2000) determines the limits for total SO$_2$ content

- Red wines max. 160 mg/liter
- White and rose wines max. 210 mg/liter.

The presence of sulphites $\geq$ 10 ppm (mg/kg) in foods should appear on the label, even though lower concentrations may produce adverse reactions to certain people.

The mean ADI 0.7 mg/kg body weight determined by WHO, is being exceeded in some groups of population due to the presence of sulphites in higher values to those permitted. A revision of the ADI is recommended.

**Principal adverse reactions:**

- Asmaphthic individuals (5-10 %), specially children, are very sensitive to sulphites. The symptoms appear a few minutes after ingestion
- Allergic reactions such as dermatitis, headache, gastrointestinal tract irritation, urticaria, asthma exacerbation or even anaphylactic shock.

- People with deficit of sulphite oxidase enzyme which takes part on sulphites metabolism may suffer eye injuries and serious brain damage.

- Sulphites are capable of breaking down vitamin B1 or thiamine in its components thiazole and pyrimidine provoking nutritive loss in some foods (i.e. meat)
PROCEDURE PE/BR/05

DETERMINATION OF
SULPHUR DIOXIDE IN WINES

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      5.2.3. Titration of re-combined SO₂
      5.2.4. Valoración de otros reductores
6. RESULTS
   6.1. Calculation of the mean value
   6.2. Calculation of free SO₂
   6.3. Calculation of total SO₂
   6.4. Calculation of combined SO₂
7. ANNEXES
1. PURPOSE

Measurement of total, free and combined sulphur dioxide in all the wines.

2. SCOPE

This procedure is valid for all the wines.

3. REFERENCES

- PC-BR-0.- General Procedure of elaboration of Procedures in Bromatology laboratory.

4. GENERAL

4.1. Definition

Free sulphur dioxide is defined as the sulphur dioxide present in the must or wine in the following forms: H$_2$SO$_3$, HSO$_3^-$, whose equilibrium as a function of pH and temperature is:

$$\text{H}_2\text{SO}_3 \rightleftharpoons \text{H}^+ + \text{HSO}_3^-$$

Total sulphur dioxide is defined as the total of all the various forms of sulphur dioxide present in wine, either in the free state or combined with their constituents.

4.2. Principle

Free sulphur dioxide is determined by direct titration with iodine. The combined sulphur dioxide is subsequently determined by iodometric titration after alkaline hydrolysis. When added to the free sulphur dioxide, it gives the total sulphur dioxide.
Total sulphur dioxide is defined as the total of all the various forms of sulphur dioxide present in wine, either in the free state or combined with their constituents.

5. DESCRIPTION

5.1. MATERIALS AND REAGENTS

5.1.1. Materials

- 50 ml volumetric pipettes
- 5 ml and 10 ml graduated pipettes
- 500 ml flasks
- 250 ml conical flasks
- 25 ml and 50 ml graduated cylinders
- 50 ml burette 0.1 ml accuracy
- Analytical balance 0.005g sensitivity
- 250 ml beaker
- Stopwatch with minutes

5.1.2. Reagents

- Ethylenediaminetetraacetic acid, di-sodium salt (EDTA)
- 4N Sodium hydroxide solution (NaOH)
- 10% (v/v) Sulphuric acid (H₂SO₄)
- Starch solution
- N/20 Iodine solution I₂
- 0.6 or 0.7% Ethanal

The preparation of the reagents is described in Annex 1
5.2. PROCEDURE

5.2.1. Titration of free SO₂

Place in a 500 ml conical flask 50±0.05ml of wine. Add 3±0.5ml de of H₂SO₄10% and 5±1 ml of starch solution. Add 30±1mg of EDTA and mix to dissolve. Titrate with I₂ N/20 until the blue colour persists clearly for 10 to 15 seconds.

Note down on the data sheet HD 05-01 the volume added (V ml).

5.2.2. Titration of combined sulphur dioxide

Add to the same liquid 8±0.5ml of 4N NaOH . Shake the mixture and allow to stand for 5 minutes. Add 10±0.5 ml of 10% H₂SO₄. Titrate with I₂ N/20 until the blue colour persists clearly for 10 to 15 seconds.

Note down on the data sheet HD 05-01 the volume added (V₁ ml).

5.2.3. Titration of re-combined sulphur dioxide

Add to the same liquid 20± 0.5ml of 4N NaOH , shake and allow to stand for 5 minutes. Dilute with 200±1ml of ice-cold water and shake vigorously. Add 30±0.5ml of 10% H₂SO₄. Titrate with I₂ N/20 until the blue colour persists clearly for 10 to15 seconds.

Note down on the data sheet 05-01 the volume added (V₂ ml).

5.2.4. Titration of other reducing substances

Place 50±0.05 ml of wine into a 500 ml conical flask. Add 5±0.05ml of 7% ethanol (p/v). Stopper the flaskand allow to stand for 30 minutes. Add 3±0.5ml of 10% H₂SO₄ and 5±1ml of the starch solution. Titrate with I₂ N/20 until the blue colour persists clearly for 10 to 15 seconds.
Note down on the data sheet HD 05-01 the volume added (V₃ ml).
Repeat the titration to comply with criterium (see 5.2.5).

5.2.5. **Criteria of acceptance of the analytical results**

The values are accepted when they only differ in 0.2 ml or less, if not repeat the analysis a third time until two values comply with this criterium.
Calculate the mean value which will be used in the results (the number of significant figures are the same to V)

6. **RESULTS**

6.1. **Calculation of mean value**

(M) ml = (V + V’)/2
(M₁) ml = (V₁ + V₁’)/2
(M₂) ml = (V₂ + V₂’)/2
(M₃) ml = (V₃ + V₃’)/2

6.2. **Calculation of SO₂ in wine**

Free sulphur dioxide (mg/l) = 32 (M-M₃)
Total sulphur dioxide (mg/l) = 32 ((M + M₁ + M₂)) – M₃
Combined sulphur dioxide (mg/l) = total sulphur dioxide – free sulphur dioxide

The results are expressed in whole numbers.

7. **ANNEXES**

Annex I. Preparation of reagents


**Annex 1**

**Preparation of reagents**

**4N Sodium hydroxide (NaOH)**
Place in a 500ml porcelain evaporating dish 160±0.05 g of sodium hydroxide, add 500±5 ml of distilled water and mix to dissolve stirring with a glass rod. When the liquid is cool place it in a 1000 ml volumetric flask and bring to volume with distilled water. Shake inverting the flask for at least three times.

**10% (v/v) Sulphuric acid (H₂SO₄)**
Dilute 10±0.05ml of H₂SO₄ (ρΤ°20 = 1.84 g/ml) with distilled water in a 100 ml volumetric flask, bring to volume and shake inverting the flask for at least three times.

**Starch solution**
Weigh 5±0.1 g of starch in 1000 ml beaker and add 500±0.5 ml of water. Bring to the boil, stirring continuously and boil for 10±0.5 minutes. Add 200±1g sodium chloride. When cool, place it in a 1000±0.3 ml volumetric flask and make up to one liter. Shake inverting the flask for at least three times.

**I₂ N/20**
Weigh 6.35 ± 0.01 g of iodine and place it in a 1000 ml volumetric flask. Add about 500 ml of distilled water. Mix to dissolve the product and bring to one liter with distilled water. Shake inverting the flask for at least three times.

**0.7% Ethanal**
In a 100 ml volumetric flask weigh with the aid of a graduate pipette 7±0.01g of ethanal. Add distilled water, mix to dissolve and bring to volume with distilled water. Shake inverting the flask for at least three times.
DATA SHEET: Sulphur dioxide in wine  

Performer: Date:

Sample:

Volume of sample:

1st titration. Titration of free SO₂

Factor I₂ N/20

<table>
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<th>Shaking time</th>
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<td>2ª</td>
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2nd Titration of combined SO₂

Allow to stand time

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<th>valoración</th>
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<th>ml ·factor</th>
<th>Dif. [&lt; 0.2]</th>
<th>Media M₁</th>
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3rd Titration of combined SO₂

Allow to stand time:

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4rd Titration of other reducing substances

Allow to stand time:

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<tr>
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CALCULATION

- Free dioxide sulphur (mg/l) = 32 (M-M₃)
  \[ 32 \cdot (\text{M-M₃}) = \text{mg SO₂/l} \]

- Total dioxide sulphur (mg/l) = 32 ((M + M₁ + M₂)) – M₃
  \[ 32 \cdot ((\text{M} + \text{M₁} + \text{M₂})) - \text{M₃} = \text{mg SO₂/l} \]

Observations
Introduction

Polyphenolic or phenolic compounds are a large group of chemical substances with different structures, chemical properties and biological activity; there are more than 8000 different compounds. Polyphenols are chemical compounds with aromatic rings with hydroxyl substituents. Most of them are powerful antioxidants necessary for plant cells. They are present in fruits and vegetables, and in beverages such as tea and wine (Kinsella et al., 1993). The chemical structure of polyphenols is specifically adequate for an antioxidant action.

Assuming the protective action of wine in some chronic diseases due to its antioxidant capacity, different groups of research have dealt with the measurement of this capacity. It has been proved, that the antioxidant properties of wine are due to its polyphenolic components (Abu-amsha et al., 1996). The total polyphenolic content of a wine is directly related to its antioxidant capacity (Rice-Evans et al., 1997; Sato et al., 1998).

Grape’s polyphenolic compounds are in the skin, specially in the epidermal cells and in the seeds; the pulp presents a low concentration. Wine making begins with the obtention of the must from white or red grapes. White and rose wines are obtained after fermentation without the skins, on the contrary red wines are elaborated in contact with the skins, and their colour depend on the time and intensity of the contact (Hidalgo et al., 2004). Therefore the amount of phenolic compounds present in red wine is greater than in white or rose wines. It is worth noting that polyphenols largely contribute to the organoleptic characteristics of wine (colour, astringence, etc.).

The quantity and quality of polyphenols in grapes principally depend on the variety of the vineyard, the climate, the soil and labouring practices. The latter is the easiest factor to be modified.

(FCI). Folin-Ciocalteu Index is one of the principal methods to measure total polyphenols in wine.
DEPARTAMENTO DE
NUTRICIÓN Y BROMATOLOGÍA II: BROMATOLOGÍA

PROCEDURE PE/BR/06

DETERMINATION OF FOLIN-CICICALTEU INDEX IN WINES

Edición: 1  Rev. 0

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1. PURPOSE

Determination of Folin-Ciocalteu index in red and white wines

2. SCOPE

The method is valid for all the wines which once diluted present an absorbance of 0.35 ± 0.05 uA at 750nm.

3. REFERENCES

- PC-BR-01.- General Procedure of elaboration of Procedures in Bromatology laboratory.

4. GENERAL

4.1. Definition

Folin-Ciocalteu Index is the measurement of the amount of polyphenols in wine

4.2. Principle

All phenolic compounds contained in wine are oxidized by Folin-Ciocalteu reagent (phosphotungstic and phosphomolybdic acids) which is reduced to a mixture of blue oxides of tungsten and molybdenum. The blue colouration produced has a maximum absorption in the region of 750 nm and is proportional to the total quantity of phenolic compounds.
5. DESCRIPTION

5.1. APPARATUS, MATERIALS AND REAGENTS

○ **Apparatus and materials**
  - 1ml and 20 ml volumetric pipettes.
  - Analytical balance 0.005g sensitivity
  - Stopwatch with minutes.
  - 50 and 10 0ml graduated cylinders
  - Funnel for solids, glass stirring rod.
  - 100 and 1000 ml volumetric flasks.
  - Spectrophotometer capable of operating at 750±0.1 nm.
  - Cells of 1cm path length for UV-VIS spectrophotometer.

Good precision of results depend on using perfectly clean material (volumetric flasks and spectrophotometer cells)

○ **Reagents**
  - Reagents of analytical quality. Distilled water or similar.
    - Folin-Ciocalteu reagent
    - Anhydrous sodium carbonate,Na₂CO₃ made up into a 20% (m/v) solution.

5.2. PRELIMINARY OPERATIONS

Connect the spectrophotometer at least 15 minutes before the lecture to stabilize the lamp.

Only for red wines place 20±0.02 ml of the wine into a 100 ml volumetric flask. Bring to volume with distilled water, mix thoroughly shaking the flask at least three times.
5.3 PROCEDURE

5.3.1 Red wines

Prepare a volumetric flask for the sample and another one for a blank

- Flask for the sample: In a 100 ml flask introduce strictly in the following order:
  - 1±0.01 ml of the wine previously diluted 1/5
  - 50±0.02 ml of distilled water
  - 5±0.02 ml of Folin-Ciocalteu reagent
  - 20±0.02 ml of sodium carbonate solution

  - Bring to 100 ml with distilled water. Mix thoroughly shaking the flask at least three times.

- Flask for a blank: Prepare a blank with 1±0.01 ml of distilled water in place of the wine. Introduce the other reagents in the same order as in the sample

- Leave both 30±1 minutes for the reaction to stabilize.

Determine the absorbance at 750 nm through a path length of 1 cm with respect to the blank. If the absorbance is not in the region of 0.25 to 0.35 appropriate dilution should be made.

Note down on the data sheet HD 06-01 the absorbance determined (n μA).

Repeat the lecture and note down the absorbance (n’ μA).

5.3.2 White wines

Carry out the same procedure as described in 5.3.1 with 1±0.01 ml of undiluted wine.

Follow the same order and carry out a blank.

Note down on the data sheet HD 06-01 the absorbance determined (n μA).

Repeat the lecture and note down the absorbance (n’ μA).
6. RESULTS

6.1. Criteria of acceptance of the analytical data

The difference between the results of two determinations carried out simultaneously or very quickly one after the other by the same analyst must not be greater than 0.01 uA for red wines and 0.05 uA for white wines. If not repeat the determinations until criteria are accomplished.

The result for the calculation is the mean value.

6.2. Calculation

The result is expressed in the form of an index obtained by:

- Red wine: Multiplying the absorbance by 100 for red wines diluted 1/5 (or by the corresponding factor for other dilutions).
- White wine: Multiplying the absorbance by 20.

7. ANNEXES

Annex 1. Preparation of reagents.
Annex 1

Preparation of reagents

Folin-Ciocalteu reagent

This reagent is available commercially in a form ready for use. It may be prepared as follows: dissolve 100g of sodium tungstate (Na₂WO₄ x 2 H₂O) and 25 g of sodium molybdate (Na₂MoO₄.2 H₂O) in 700 ml of distilled water. Add 50 ml phosphoric acid 85% (δ = 1.71 g/ml) and 100 ml of concentrated hydrochloric (δ = 1.19 g/ml). Bring to boil and reflux for 10 hours. Then add 150g de lithium sulphate (Li₂SO₄. H₂O), a few drops of bromine and boil for 15 minutes. Allow to cool and make up to one liter with distilled water.

Anhydrous sodium carbonate (Na₂CO₃) solution, 20% (m/v).

Weigh in a funnel for solids 200±0.05 g anhydrous sodium carbonate. Introduce the content into a 1000 ml volumetric flask. Make up to one liter with distilled water and mix to dissolve.
# DATA SHEET: FOLIN-CIOCALTEU INDEX IN WINES (PNT/BR/06)

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<th>Wine W/R</th>
<th>Dilution</th>
<th>Waiting time min</th>
<th>1(^{a}) reading</th>
<th>2(^{a}) Reading</th>
<th>Mean (dif &lt;0,01) T / 0,05 B Folin index (20 \cdot \text{dilución})</th>
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**Equipment**

**Observations and incidences**