# RESOLUTION OIV-OENO 419A-2011

# SPECIFIC METHODS FOR THE ANALYSIS OF GRAPE SUGAR (RECTIFIED CONCENTRATED MUSTS)

THE GENERAL ASSEMBLY

CONSIDERING article 2 paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING the works of the Sub-Commission of Methods of Analysis in updating the compendium with specific methods for the analysis of grape sugar (rectified concentrated musts)

CONSIDERING the existing resolution OENO 47/2000 related to the specifications of grape sugar and the related methods and the need to update these specific methods

GIVEN that the following methods are already recognised by international authorities,

DECIDES to create an Annex F entitled “Specific methods for the analysis of grape sugar”

DECIDES to insert, in Annex F of the Compendium of international methods of analysis of wines and musts, the methods described in the following appendix

DECIDES to adapt in consequence the resolution Oeno 47/2000 included in the International Oenological Codex.

# ANNEX B: CONDUCTIVITY

**Type IV**

## 1. Principle

The electrical conductivity of a column of liquid defined by two parallel platinum electrodes at its ends is measured by incorporating it in one arm of a Wheatstone bridge.

The conductivity varies with temperature and it is therefore expressed at 20°C.

## 2. Reagents

Use only reagent grade chemicals

2.1. Purified water for laboratories, with specific conductivity below 2 µS cm-1 at 20°C,



Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2011

for example EN ISO 3696 type II water.

2.2. Reference solution of potassium chloride.

Dissolve 0.581 g of potassium chloride, KCl previously dried to constant mass at a temperature of 105°C, in demineralised water (2.1). Make up to one litre with demineralised water (2.1). This solution has a conductivity of 1 000 µS cm-1 at 20°C. It should not be kept for more than three months.

A commercial preparation can be used.

# 3. Apparatus

3.1. Conductivity meter enabling measurements of conductivity to be made over a range from 1 to 1 000 microsiemens per cm (µS cm-1).

3.2. Water bath for bringing the temperature of samples to be analysed to approximately 20°C (20 ± 2°C).

# 4. Procedure

## 4.1. Preparation of the sample to be analysed

Use a solution with a total sugar concentration of 25 ± 0.5 % (m/m) (25° Brix): weigh a mass equal to 2500/P and make up to 100 g with water (2.1),

P = percentage (m/m) of total sugars in the rectified concentrated must.

## 4.2. Determination of conductivity

Bring the sample to be analysed to 20°C by immersion in a water bath. Maintain the temperature to within ± 0.1°C.

Rinse the conductivity cell twice with the solution to be examined.

Measure the conductivity and express the result in µS cm-1.

# 5. Expression of the Results

The result is expressed in microsiemens per cm (µScm−1) at 20°C to the nearest whole number for the 25% (m/m) (25° Brix) solution of rectified concentrated must.

## 5.1. Calculations

If the apparatus does not have temperature compensation, correct the measured conductivity using Table I. If the temperature is below 20°C, add the correction; if the



Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2011

temperature is above 20°C, subtract the correction.

# 6. Characteristics of the method

Repeatability (r)

- r = 3 µS/cm

Reproducibility (R)

- R = 16 µS/cm

*TABLE I*

*Corrections to be made to the conductivity for temperatures different from 20°C (µS cm−1)*

| Conductivity | Temperature (°C) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 20.2 19.8 | 20.419.6 | 20.619.4 | 20.819.2 | 21.019.0 | 21.218.8 | 21.418.6 | 21.618.4 | 21.818.2 | 22,0([1])18.0([2]) |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 50 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 |
| 100 | 0 | 1 | 1 | 2 | 2 | 3 | 3 | 3 | 4 | 4 |
| 150 | 1 | 1 | 2 | 3 | 3 | 4 | 5 | 5 | 6 | 7 |
| 200 | 1 | 2 | 3 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| 250 | 1 | 2 | 3 | 4 | 6 | 7 | 8 | 9 | 10 | 11 |
| 300 | 1 | 3 | 4 | 5 | 7 | 8 | 9 | 11 | 12 | 13 |
| 350 | 1 | 3 | 5 | 6 | 8 | 9 | 11 | 12 | 14 | 15 |
| 400 | 2 | 3 | 5 | 7 | 9 | 11 | 12 | 14 | 16 | 18 |
| 450 | 2 | 3 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 |
| 500 | 2 | 4 | 7 | 9 | 11 | 13 | 15 | 18 | 20 | 22 |
| 550 | 2 | 5 | 7 | 10 | 12 | 14 | 17 | 19 | 22 | 24 |
| 600 | 3 | 5 | 8 | 11 | 13 | 16 | 18 | 21 | 24 | 26 |



Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2011

(1) Subtract the correction.
(2) Add the correction.

# ANNEX C: HYDROXYMETHYLFURFURAL (HMF) by High-Performance Liquid Chromatography

**Type IV**

## 1. Principle of the Method

High-performance liquid chromatography (HPLC)

Separation through a column by reversed-phase chromatography and determination at 280 nm.

## 2. Reagents

2.1. Purified water for laboratory use and of quality standard EN ISO 3696

2.2. Methanol, $CH_3OH$ , distilled or HPLC quality. – CAS Number 67-59-1

2.3. Acetic acid, $CH_3COOH$ , (□20 = 1.05 g/ml). – CAS Number 64-19-7

2.4. Mobile phase: water (2.1) -methanol (2.2)-acetic acid (2.3) previously filtered through a membrane filter (0.45 µm), (40:9:1 v/v).

This mobile phase must be prepared daily and degassed before use.

2.5. Reference solution of hydroxymethylfurfural, 25 mg/l (m/v).

Into a 100 ml volumetric flask, place 25 mg of hydroxymethylfurfural, $C_6H_3O_6$, accurately weighed, and make up to the mark with methanol ( 2.2). Dilute this solution 1/10 with methanol (2.2) and filter through a membrane filter (0.45 µm).

If kept in a hermetically sealed brown glass bottle in a refrigerator, this solution will keep for two to three months.

(The concentration of the reference solution is given for guidance)

## 3. Equipment

### 3.1. Apparatus

3.1.1. High-performance liquid chromatograph equipped with:

- A loop injector, 5 or 10 µl, (as an example),



Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2011

- Spectrophotometric detector for making measurements at 280 nm,
- Column of octadecyl-bonded silica (e.g.: Bondapak C18 — Corasil, Waters Ass.),
- A recorder and, if required, an integrator
- Flow rate of mobile phase: 1.5 ml/minute (as an example).

3.1.2. Membrane filtration apparatus, pore diameter 0.45 µm.

# 4. Procedure

## 4.1. Preparation of sample

Use the solution obtained by diluting the rectified concentrated must to 40% (m/v) (introduce 200 g of accurately weighed rectified concentrated must into a 500 ml volumetric flask. Make up to the mark with water and homogenise) and filter it through a membrane filter (0.45 µm).

## 4.2. Chromatographic determination

Inject 5 (or 10) µl of the sample prepared as described in paragraph 4.1. and 5 (or 10) µl of the reference hydroxymethylfurfural solution (2.5) into the chromatograph. Record the chromatogram.

The retention time of hydroxymethylfurfural is approximately six to seven minutes.

The volume injected and the sequence are given for guidance. The chromatographic determination can also be done with a calibration curve

# 5. Expression of results

The hydroxymethylfurfural concentration in rectified concentrated musts is expressed in milligrams per kilogram of total sugars.

## 5.1. Method of calculation

Let the hydroxymethylfurfural concentration in the 40% (m/v) solution of the rectified concentrated must be C mg/l.

The hydroxymethylfurfural concentration in milligrams per kilogram of total sugars is given by:

- 250 x C/P



Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2011

P = percentage (m/m) concentration of total sugars in the rectified concentrated must.

## 6. Characteristics of the method

Repeatability (r)

- r = 0.5 mg/kg total sugars

Reproducibility (R)

- R = 3.0 mg/kg total sugars

# ANNEX E: DETERMINATION OF THE ACQUIRED ALCOHOLIC STRENGTH BY VOLUME (ASV) OF CONCENTRATED MUSTS (CM) AND GRAPE SUGAR (OR RECTIFIED CONCENTRATED MUSTS, RCM)

**Type: IV**

## 1. Introduction

Concentrated musts (CM) and grape sugar (RCM) are viscous products with low alcohol contents; to determine their acquired ASV, a method must be used, the characteristics of which (linearity, repeatability, reproducibility, specificity, and detection and quantification limits) must be such that it is possible to measure alcohol contents of less than 1% vol.

## 2. Field of application

The method applies to concentrated musts and grape sugar.

## 3. Principle

A known mass of concentrated must (CM) or grape sugar is made alkaline by a suspension of calcium hydroxide and then distilled. The alcoholic strength by volume of the distillate is determined by electronic densitometry or by densitometry using a hydrostatic balance.



Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2011

# 4. Reagents

- Suspension of 2M calcium hydroxide of analytical quality obtained by carefully pouring one litre of hot water (60°C to 70°C) on to about 120 g of unslaked lime (CaO).
- Antifoam solution obtained by dilution of 2 ml of concentrated silicone antifoam in 100 ml of water.
- Purified water for laboratory use and of quality EN ISO 3696.

# 5. Equipment

- Standard laboratory equipment including volumetric flasks
- Analytic balance capable of weighing to within 0.1 g.
- Any type of distillation or steam distillation apparatus may be used provided that it satisfies the following test:
    - Distil an ethanol water mixture with an alcoholic strength of 10% vol. five times in succession. The distillate should have an alcoholic strength of at least 9.9% vol. after the fifth distillation; i.e. the loss of alcohol during each distillation should not be more than 0.02% vol.
- Electronic density meter or hydrostatic balance.

# 6. Procedure

- Homogenise the test sample by inverting the flask several times.
- In a 500 ml volumetric flask, weigh about 200 g of concentrated must or rectified concentrated must (to within 0.1 g). Note the weight (TS) of this test sample. Fill up to the mark with deionised water. This solution is about 40% m/v in must.

Obtaining the distillate



Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2011

- Transfer 250 ml of the 40% solution to the distillation flask, add to the flask about 10 ml of calcium hydroxide in suspension, about 5 ml of antifoam solution and, where applicable, a boiling regulator (e.g. pieces of porcelain).
- Gently bring to the boil.
- Recover the distillate in a 100 ml volumetric flask (about 90 ml).
- Leave the distillate to return to ambient temperature, then fill up to the mark with deionised water.

Measurement of ASV

This is performed by electronic densitometry or by hydrostatic balance.

# 7. Calculation

$$Acquired\ alcoholic\ strength\ by\ volume = \frac{ASV\ measured\ \times\ 200\ \times\ MV}{TS}$$

ASV measured = alcohol content given by the density meter, as % vol.

TS = test sample of concentrated must or grape sugar, in weight.

MV= density of concentrated must or rectified concentrated must, in g/ml

The results are expressed to 2 decimal places and rounded to within 0.05 %vol.

# 8. Characteristics of the method

## 8.1. Linearity of response

The linearity of the density meter for low ASV values is one of the critical parameters of this method. A standard range of 10 aqueous-alcoholic solutions of ASV ranging between 0 and 5%vol. was prepared. Each solution was analysed 3 times.

The response of the density meter is perfectly linear within this range as shown by the calibration line in Figure 1.



Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2011

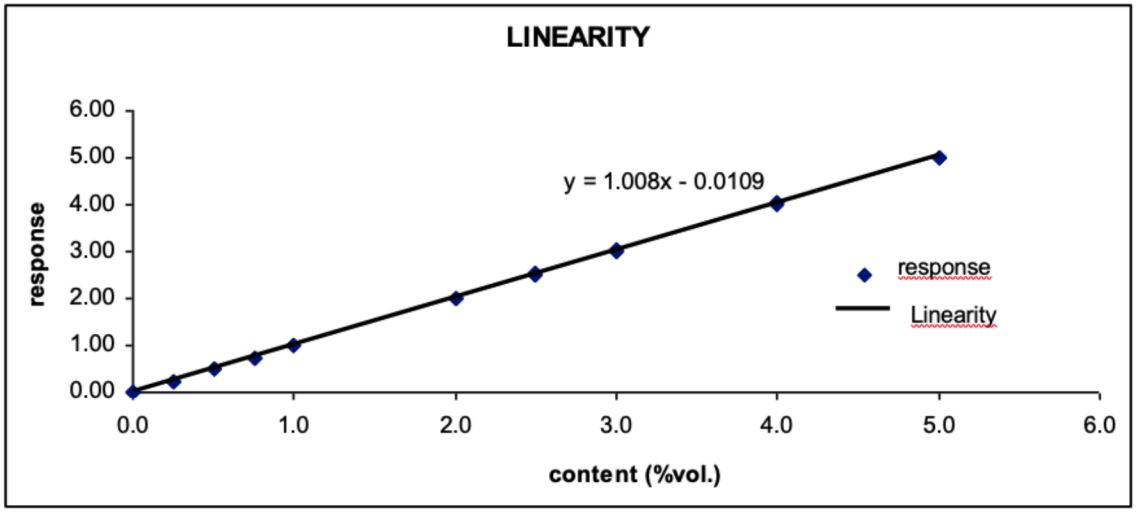



*Figure 1: Linearity of determination of the ASV by electronic densitometry between 0 and 5%vol.*

## 8.2. Specificity of the method

The second critical point of this method is the distillation of viscous musts containing small quantities of alcohol. In order to verify the specificity, known quantities of ethanol (from 0.25% vol to 5% vol) were added to CMs and grape sugar. The supplemented test specimens were distilled in the conditions defined earlier, then the distillates were analysed by electronic densitometry or by hydrostatic balance.

The results are shown in Table 1. The recovery rate is satisfactory, ranging between 88% and 99%. As shown by the line in Figure 2, the method is specific (slope in the vicinity of 1, intercept point in the vicinity of 0).

| Test specimen | Initial content (%vol.) | Added content (%vol.) | Recovered content (%vol.) | Recovery rate (%) |
|---|---|---|---|---|
| CM 1 | 0.00 | 0.25 | 0.22 | 88 |
| CM 1 | 0.00 | 1.00 | 0.98 | 98 |
| Grape Sugar (RCM) 1 | 0.00 | 1.00 | 0.94 | 94 |

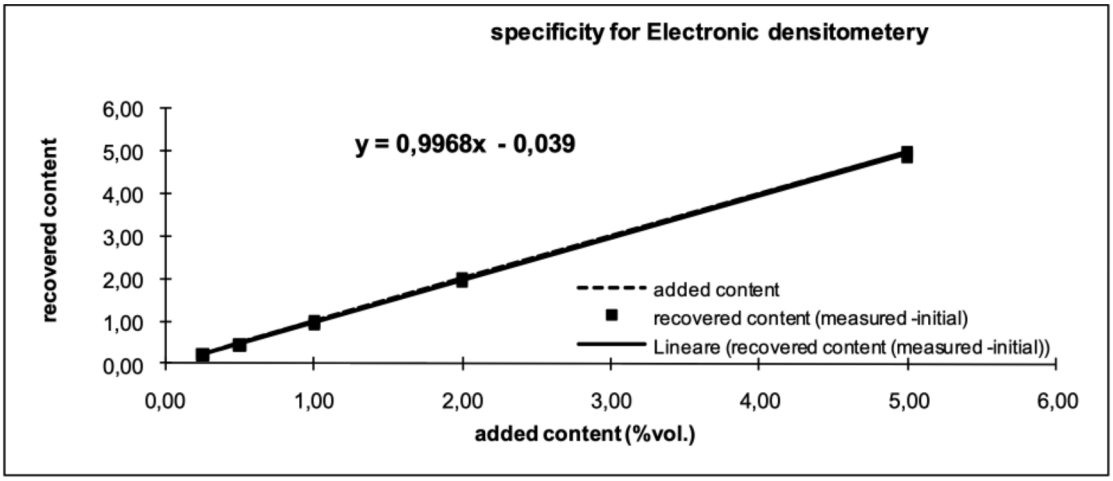


Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2011

| | | | | |
|---|---|---|---|---|
| Grape Sugar (RCM) 1 | 0.00 | 2.00 | 1.97 | 99 |
| CM 2 | 0.00 | 0.50 | 0.44 | 88 |
| Grape Sugar (RCM)2 | 0.00 | 5.00 | 4.94 | 99 |

*Table 1: Recovery rate for determination of the acquired ASV of CMs and Grape Sugar*



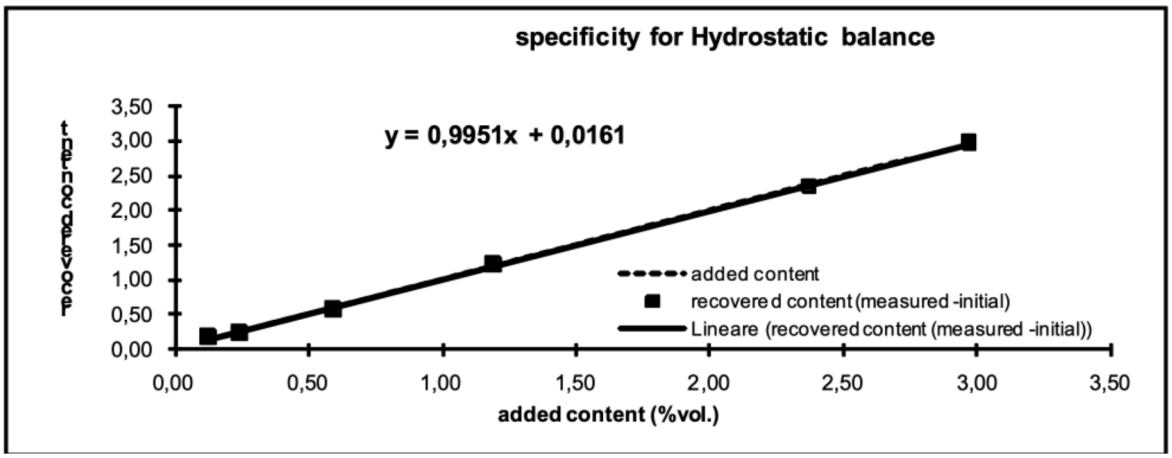




*Figure 2: Specificity of determination of the acquired ASV of CMs and Grape Sugar*



Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2011

## 8.3. Repeatability

The repeatability of the method was determined using 20 test specimens of CM or grape sugar supplemented with alcohol or not. Each CM or RCM test specimen was analysed 3 times, in order to ensure identical conditions. The repeatability limits obtained are as follows:

| Repeatability for electronic densitometry | Calculated value |
|---|---|
| Standard deviation | 0.009 |
| CV or RSD as % | 0.9% |
| r limit | 0.024 %vol. |
| r limit as % | 3% |
| **Repeatability for Hydrostatic balance** | **Calculated value** |
| Standard deviation | 0.013 |
| CV or RSD as % | 1.7% |
| r limit | 0.038 %vol. |
| r limit as % | 5,3% |

*Table 2: Repeatability of determination of the acquired ASV of CMs and Grape Sugar*

## 8.4. Reproducibility

The reproducibility of the results is determined by analysing the same must twice, at different dates during a given period of time. The results are given in Table 3.

| Reproducibility for electronic densitometry | Calculated value |
|---|---|
| Standard deviation | 0.043 |
| CV or RSD as % | 3% |
| R limit | 0.12%vol. |
| R limit as % | 9% |



Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2011

| Reproducibility for Hydrostatic balance | Calculated value |
|---|---|
| Standard deviation | 0.026 |
| CV or RSD as % | 3.4% |
| R limit | 0.076%vol. |
| R limit as % | 10.6% |

*Table 3 - Reproducibility of determination of the acquired ASV of CMs and grape sugar*

### 8.5. Detection and quantification limits

The limits of detection (LD) and quantification (LQ) estimated based on the linearity study are as follows:

- LD = 0.01%vol.
- LQ = 0.05%vol.

The quantification limit was verified by analysis of musts having an ASV at a concentration level of 0.05%vol.

### 8.6. Uncertainty

Uncertainty, evaluated based on the reproducibility standard deviation, is 0.10%vol.

**ANNEX G: SUCROSE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY**

**Type IV**

## 1. Principle

For testing and determination by high-performance liquid chromatography: the sucrose is separated in a column of alkylamine-bonded silica and detected by refractometry. The result is quantified by reference to an external standard analysed under the same conditions.

Note: Authentication of a must or of a wine may be checked by the method using NMR of deuterium described for detecting the enrichment of musts, rectified concentrated musts and wines.

The chromatographic conditions are given for guidance.



© OIV 2011

Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

# 2. Reagents

2.1. Purified water for laboratory use and of quality EN ISO 3696..

2.2. HPLC quality acetonitrile ($CH_3CN$) – CAS Number 75-05-8

2.3. Sucrose – CAS Number 57-50-1

2.4. Mobile phase: acetonitrile-water (80:20 v/v)., previously subjected to membrane filtration (0.45 µm); the composition of the mobile phase is given as an example.

This mobile phase must be degassed before being used.

2.5. Standard solution: 1.2 g/l aqueous sucrose solution. Filter using a 0.45 µm membrane filter. (The concentration of the standard solution is given as an example.)

# 3. Equipment

3.1. High-performance liquid chromatograph equipped with:

1. 10 µl loop injector (as an example)
2. a detector: a differential refractometer or an interferometer refractometer
3. an alkylamine-bonded silica column, length 25 cm, internal diameter 4 mm (as an example)
4. a guard column filled with the same phase (as an example)
5. an arrangement for insulating the guard column and analytical columns or for maintaining their temperature (30 ° C),
6. a recorder and, if required, an integrator,
7. mobile phase flow rate: 1 ml/min (as an example).

3.2. Equipment for membrane filtration (0.45 µm).

# 4. Procedure

## 4.1. Preparation of sample:

Use the solution obtained by diluting the rectified concentrated must to 40 % (m/v) as described in Annex H 'Total acidity', section 5.1., and filtering it using a 0.45 µm membrane filter.



Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2011

### 4.2. Chromatographic determination

Inject in turn into the chromatograph 10 µl of the standard solution and 10 µl of the sample prepared as described in 4.1.

Repeat these injections in the same order.

Record the chromatogram.

The retention time of the sucrose is approximately 10 minutes.

The sample volume and sequence are given for guidance. The chromatographic determination can also be done with a calibration curve

## 5. Calculations

For the calculation, use the average of two results for the standard solution and the sample.

Let C be the sucrose concentration in g/l of the 40 % (m/v) solution of rectified concentrated must.

The sucrose concentration in g/kg of the rectified concentrated must is then:

- 2.5 x C

## 6. Expression of results

The sucrose concentration is expressed in grams per kilogram, to one decimal place.

## 7. Characteristics of the method

Repeatability (r)

- r = 1.1 g/kg must

# ANNEX H: TOTAL ACIDITY

**Type IV**



Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2011

# 1. Definition

The total acidity of the rectified concentrated must 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.

# 2. Principle of the method

2.1. Potentiometric titration or titration with bromothymol blue as an indicator and comparison with an end-point colour standard.

# 3. Reagents

3.1. Buffer solutions

3.1.1. pH 7.0:

| | |
|---|---|
| monopotassium phosphate, ($KH_2PO_4$) | 107.3 g |
| 1 M sodium hydroxide (NaOH) solution | 500 ml |
| water to | 1000 ml |

3.1.2. pH 4.0

Solution of potassium hydrogen phthalate, 0.05 M, containing 10.211 g of potassium hydrogen phthalate ($C_8H_5KO_4$) per litre at 20 °C.

Note. Commercial reference buffer solutions traceable to the SI may be used.

For example:

- pH 1.679 ± 0.01 at 25°C
- pH 4.005 ± 0.01 at 25°C
- pH 7.000 ± 0.01 at 25°C

3.2. 0,1 M sodium hydroxide (NaOH) solution.

3.3. 4 g/l bromothymol blue indicator solution:



Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2011

| | |
|---|---|
| Bromothymol blue $C_{27}H_{28}Br_2O_5S$ | 4 g |
| Neutral ethanol 96 % vol | 200 ml |

Dissolve and add:

| | |
|---|---|
| Water free of $CO_2$ | 200 ml |
| 1 M sodium hydroxide solution sufficient to produce blue-green colour (pH 7) approximately | 7.5 ml |
| Water to: | 1000 ml |

# 4. Apparatus

4.1. Potentiometer with scale graduated in pH values, and electrodes.

As a reminder, the glass electrode must be kept in distilled water. The calomel/saturated potassium chloride electrode must be kept in a saturated potassium chloride solution. A combined electrode is most frequently used: it should be kept in distilled water.

4.2. Conical flask 100 ml.

# 5. Procedure

## 5.1. Preparation of sample:

Introduce 200 g of accurately weighed rectified concentrated must. Make up to the mark with 500 ml water. Homogenize.

## 5.2. Potentiometric titration

### 5.2.1. Zeroing of the apparatus

Zeroing is carried out before any measurement is made, according to the instructions provided with the apparatus used.



Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2011

#### 5.2.2. Calibration of the pH meter

The pH meter must be calibrated at 20°C using standard buffer solutions traceable to the SI. The pH values selected must encompass the range of values that may be encountered in musts. If the pH meter used is not compatible with calibration at sufficiently low values, a verification using a standard buffer solution linked to the SI and which has a pH value close to the values encountered in the musts may be used.

#### 5.2.3. Method of measurement

Into a conical flask (4.4), introduce a 50 ml of the sample, prepared as described in 5.1.

Add about 10 ml of distilled water and then add the 0.1 M sodium hydroxide solution (3.2) from the burette until the pH is equal to 7 at 20 °C. The sodium hydroxide must be added slowly and the solution stirred continuously.

Let n ml be the volume of 0.1 M NaOH added.

### 5.3. Titration with indicator (bromothymol blue)

#### 5.3.1. Preliminary test: end-point colour determination.

Into a conical flask (4.4) place 25 ml of boiled distilled water, 1 ml of bromothymol blue solution (3.3) and 50 ml of the sample prepared as in (5.1).

Add the 0.1 M sodium hydroxide solution (3.2) until the colour changes to blue-green.

Then add 5 ml of the pH 7 buffer solution (3.1)

#### 5.3.2. Measurement

Into a conical flask (4.4) place 30 ml of boiled distilled water, 1 ml of bromothymol blue solution (3.3) and 50 ml of the sample, prepared as described in 5.1.

Add 0.1 M sodium hydroxide solution (3.2) until the same colour is obtained as in the preliminary test above (5.3.1).

Let n ml be the volume of 0.1 M sodium hydroxide added.

## 6. Expression of results

### 6.1. Method of calculation

- The total acidity expressed in milliequivalents per kilogram of rectified concentrated must is given by: $A = 5 \times n$



Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2011

- The total acidity expressed in milliequivalents per kilogram of total sugars is given by:
    - A = (500 x n)/P
    - P = % concentration (m/m) of total sugars.

It is recorded to one decimal place.

# 7. Characteristics of the method

Repeatability (r)

- r = 0.4 meq /kg total sugars

Reproducibility (R)

- R = 2.4 meq /kg total sugars

# ANNEX I: pH

**Type IV**

# 1. Principle

The difference in potential between two electrodes immersed in the liquid under test is measured. One of these two electrodes has a potential which is a function of the pH of the liquid, while the other has a fixed and known potential and constitutes the reference electrode.

# 2. Reagents

## 2.1. Buffer solutions

2.1.1. Saturated solution of potassium hydrogen tartrate, containing at least 5.7 g of potassium hydrogen tartrate per litre ($C_4H_5KO_6$) at 20 °C. (This solution may be kept for up to two months by adding 0.1 g of thymol per 200 ml.)

pH/temperature



© OIV 2011

Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

———————————

- 3.57 at 20 ºC
- 3.56 at 25 ºC
- 3.55 at 30 ºC

2.1.2. Solution of potassium hydrogen phthalate, 0.05 M, containing 10.211 g of potassium hydrogen phthalate ($C_8H_5KO_4$) per litre at 20 °C.
(Maximum keeping period, two months.)
pH/temperature

———————————

- 3.999 at 15 ºC
- 4.003 at 20 ºC
- 4.008 at 25 ºC
- 4.015 at 30 ºC

2.1.3. Solution containing:

| | |
|---|---|
| monopotassium phosphate, $KH_2PO_4$ | 3.402 g |
| dipotassium phosphate, $K_2HPO_4$ | 4.354 g |
| water to | 1 000 ml |

(maximum keeping period, two months)
pH/temperature

———————————

- 6.90 at 15 ºC
- 6.88 at 20 ºC
- 6.86 at 25 ºC
- 6.85 at 30 ºC



Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2011

Note. Commercial reference buffer solutions traceable to the SI may be used.
For example: pH 1.679 ±0.01 at 25°C

- pH 4.005 ± 0.01 at 25°C
- pH 7.000 ± 0.01 at 25°C

# 3. Apparatus

3.1. pH meter with a scale calibrated in pH units and enabling measurements to be made to at least ±0.01 .

3.2. Electrodes:

3.2.1. Glass electrode.

3.2.2. Calomel-saturated potassium chloride reference electrode

3.2.3. Or a combined electrode.

# 4. Procedure

## 4.1. Preparation of the sample for analysis

Dilute the rectified concentrated must with water to produce a concentration of 25 ± 0.5 % (m/m) of total sugars (25 ° Brix).

If P is the percentage concentration (m/m) of total sugars in the rectified concentrated must, weigh a mass of:

- 2500/P

and make up to 100 g with water.

The water used must have a conductivity below 2 microsiemens per cm.

## 4.2. Zeroing of the apparatus

Zeroing is carried out before any measurement is made, according to the instructions provided with the apparatus used.

## 4.3. Calibration of the pH meter

The pH meter must be calibrated at 20°C using standard buffer solutions traceable to



Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2011

the SI. The pH values selected must encompass the range of values that may be encountered in musts . If the pH meter used is not compatible with calibration at sufficiently low values, a verification using a standard buffer solution linked to the SI and which has a pH value close to the values encountered in the musts may be used.

### 4.4. Determination

Dip the electrode into the sample to be analysed, the temperature of which should be between 20 and 25 °C and as close as possible to 20 °C.

Read the pH value directly off the scale.

Carry out at least two determinations on the same sample.

The final result is taken to be the arithmetic mean of two determinations.

## 5. Expression of results

The pH of the 25 % (m/m) (25 ° Brix) solution of rectified concentrated must is quoted to two decimal places.

## 6. Characteristics of the method

Repeatability (r)

- r = 0.07

Reproducibility (R)

- R = 0.07

# ANNEX J: SULPHUR DIOXIDE

**Type IV**

## 1. Definitions

Free sulphur dioxide is defined as the sulphur dioxide present in the must in the following forms: : $H_2SO_3$, $HSO_3^-$

The equilibrium between these forms is a function of pH and temperature:



© OIV 2011

Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI



$H_2SO_3$represents molecular sulphur dioxide.

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

# 2. Materials

Total sulphur dioxide is extracted from the previously diluted rectified concentrated must by entrainment at high temperature (approximately 100 °C).

## 2.1. Reagents

2.1.1. Phosphoric acid, 85 % (H3PO4) (□20 = 1.71 g/ml) .

2.1.2. Hydrogen peroxide solution, 9.1 g $H_2O_2$/litre (three volumes).

2.1.3. Indicator reagent:

| | |
|---|---|
| methyl red | 100 mg |
| methylene blue | 50 mg |
| alcohol 50 % vol. | 100 ml |

2.1.4. Sodium hydroxide solution (NaOH), 0.01 M.

## 2.2. Apparatus

2.2.1. The apparatus used should conform to the diagram shown below, particularly with regard to the condenser.



Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2011

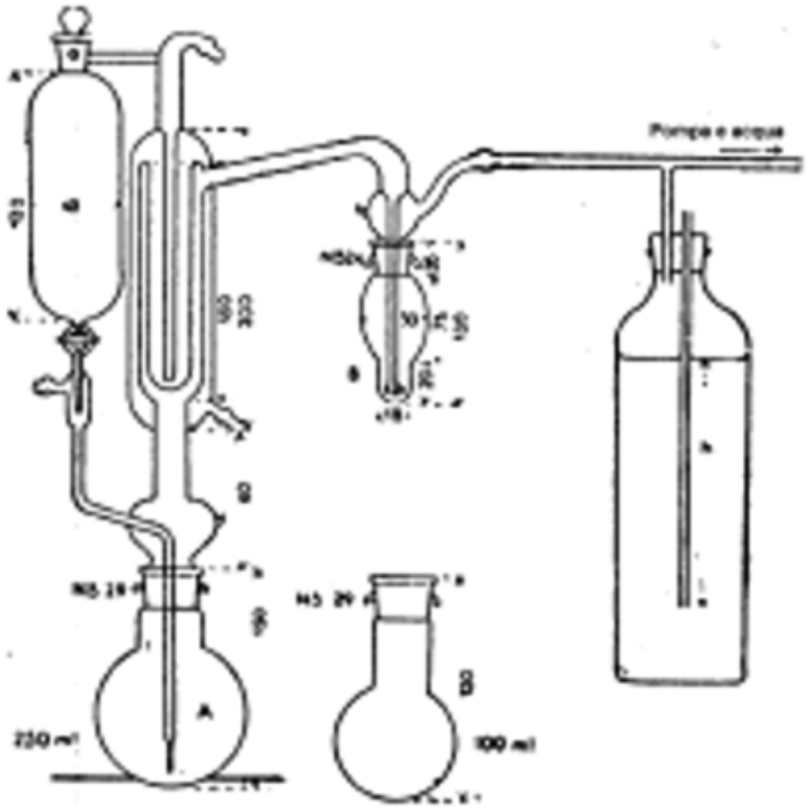


*Fig. 1 The dimensions given are in millimetres. The internal diameters of the four concentric tubes forming the condenser are 45, 34, 27 and 10 mm.*

The gas feed tube to the bubbler B ends in a small sphere of 1 cm diameter with 20 0.2-mm diameter holes around its largest horizontal circumference. Alternatively, this tube may end in a frit glass plate which produces a large number of very small bubbles and thus ensures good contact between the liquid and gaseous phases.

The gas flow through the apparatus should be approximately 40 litres per hour. The bottle on the right of the diagram is intended to restrict the pressure reduction produced by the water pump to 20 to 30 cm of water. To regulate the vacuum to its correct value, a flowmeter with a semi-capillary tube should be installed between the bubbler and the bottle.

2.2.2. A microburette.



Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2011

## 3. Procedure

3.1. For rectified concentrated musts, use the solution obtained by diluting the sample to be analysed to 40 % (m/v) as indicated in the chapter 'Total acidity', section 5.1. Introduce 50 ml of this solution and 5 ml of phosphoric acid (2.2.1) into the 250 ml flask A of the entrainment apparatus. Connect the flask to the apparatus.

3.2. Place 2 to 3 ml of hydrogen peroxide solution (2.2.2) in the bubbler B, neutralize with the 0.01 M sodium hydroxide solution (2.2.4) and bring the must in the flask A to the boil using a small flame of 4 to 5 cm height which should directly touch the bottom of the flask. Do not put the flask on a metal plate but on a disc with a hole of approximately 30 mm diameter in it. This is to avoid overheating substances extracted from the sample that are deposited on the walls of the flask.

Maintain boiling while passing a current of air (or nitrogen). Within 15 minutes the total sulphur dioxide has been carried over and oxidized. Determine the sulphuric acid which has formed by titration with the 0.01 M sodium hydroxide solution (2.2.4).

Let n ml be the volume used.

## 4. Calculation

Total sulphur dioxide in milligrams per kilogram of total sugars (50 ml prepared test sample (3.1):

- (1600 x n)/P

where P = percentage concentration (m/m) of total sugars

## 5. Expression of results

Total sulphur dioxide is expressed in mg/kg of total sugars.

## 6. Characteristics of the method

Repeatability (r)

- 50 ml test sample < 50 mg/l; r = 1x 250/P mg/kg of total sugars

Reproducibility (R)



Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2011

- 50 ml test sample < 50 mg/l; R = 9x250/P mg/kg of total sugars

**ENCLOSED K: CHROMATIC PROPERTIES**

**Type IV**

# 1. Principle of the method

The absorbance of the rectified concentrated must is measured at 425 nm through a pathlength of 1 cm after dilution to bring the sugar concentration to 25 % (m/m) (25° Brix)

# 2. Apparatus

2.1. Spectrophotometer enabling measurements to be made between 300 and 700 nm.

2.2. Glass cells with optical paths of 1 cm.

2.3. Membrane filter of pore diameter 0.45 µm.

# 3. Procedure

## 3.1. Preparation of the sample

Use the solution with a sugar concentration of 25 % (m/m) (25° Brix) prepared as described in the chapter 'pH', section 4.1. Filter through a membrane filter of pore diameter 0.45 µm.

## 3.2. Determination of absorbance

Zero the absorbance scale at a wavelength of 425 nm using a cell with an optical path of 1 cm containing distilled water.

Measure the absorbance A at the same wavelength of the solution containing 25 % sugar (25° Brix) prepared as in 3.1 and placed in a cell with an optical path of 1 cm.

# 4. Expression of results

The absorbance at 425 nm of the rectified concentrated must in a solution with 25 % sugar (25° Brix) is quoted to two decimal places.

Repeatability (r)



Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2011

- r = 0.01 AU at 25°Brix



Certified in conformity Porto, 24th June 2011
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

© OIV 2011