►B

COMMISSION REGULATION(EEC) No 000/90
of 17 September 1990
determining Community methods for the analysis of wines

(OJ L 272, 3.10.1990, p. 1)

Amended by:

		Official Journal		
		No	page	date
►M1	Commission Regulation (EEC) No 2645/92 of 11 September 1992	L 266	10	12.9.1992
►M2	Commission Regulation (EC) No 60/95 of 16 January 1995	L 11	19	17.1.1995
►M3	Commission Regulation (EC) No 69/96 of 18 January 1996	L 14	13	19.1.1996
►M4	Commission Regulation (EC) No 822/97 of 6 May 1997	L 117	10	7.5.1997
►M5	Commission Regulation (EEC) No 2348/91 of 29 July 1991	L 214	39	2.8.1991
►M6	amended by Commission Regulation (EC) No 1932/97 of 3 October 1997	L 272	10	4.10.1997
►M7	Commission Regulation (EC) No 761/1999 of 12 April 1999	L 99	4	14.4.1999
►M8	Commission Regulation (EC) No 440/2003 of 10 March 2003	L 66	15	11.3.2003
►M9	Commission Regulation (EC) No 1622/2000 of 24 July 2000	L 194	1	31.7.2000
►M10	amended by Commission Regulation (EC) No 1609/2001 of 6 August 2001	L 212	9	7.8.2001

▼B

COMMISSION REGULATION(EEC) No 000/90

of 17 September 1990

determining Community methods for the analysis of wines

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Regulation (EEC) No 822/87 of 16 March 1987 on the common organization of the market in wine (1), as amended by Regulation (EEC) No 1325/90 (2), and in particular Article 74 thereof,

Whereas Article 74 (1) of Regulation (EEC) No 822/87 prescribes the adoption of methods of analysis for establishing the composition of the products indicated in Article 1 of that Regulation and of rules for checking whether these products have been subjected to treatments in violation of authorized oenological pratice;

Whereas, inasmuch as the Community has not yet laid down maximum levels for substances whose presence indicates that certain oenological practices have been used and has not yet adopted tables enabling analysis data to be compared, there is good reason to authorize Member States to determine such maximum levels;

Whereas Article 13 (1) of Regulation (EEC) No 822/87 provides for an analytical test, including at the least an assessment of the characteristics, as listed in the Annex to that Regulation, of the quality wine psr in question;

Whereas the verification of the particulars on documents concerning the products in question calls for the introduction of uniform methods of analysis to ensure that accurate and comparable information is obtained; whereas, consequently, these methods should be compulsory for all commercial transactions and all verification procedures; whereas, however, in view of the trade's limited facilities, a restricted number of usual procedures should be admitted enabling the requisite factors to be determined rapidly and with reasonable accuracy;

Whereas, as far as is possible, generally recognized methods such as those developed under the 1954 International Convention for the Unification of Methods of Analysis and Appraisal of Wines, which are published in the *Recueil des méthodes internationales d'analyse des vins* (Compendium of international methods for the analysis of wines) by the International Office of Vine and Wine, may usefully be retained;

Whereas the Community methods of analysis applicable to wine were laid down in Commission Regulation (EEC) No 1108/82 (3); whereas scientific progress has made it necessary to replace certain of the methods by some that are more suitable, to modify others, and to introduce new methods, particularly those approved since the aforesaid Regulation by the International Office of Vine and Wine; whereas, because of the profusion and complexity of these changes, all the analyses should be reassembled in a new Regulation, and Regulation (EEC) No 1108/82 should be repealed;

Whereas, in order to ensure the comparability of the results obtained by applying the analytical methods referred to in Article 74 of Regulation (EEC) No 822/87, steps should be taken to refer, in regard to the accuracy, repeatability and reproducibility of these results, to the definitions laid down by the International Office of Vine and Wine;

Whereas, in order to recognize the scientific advances on the one hand and the technical equipment of official laboratories on the other, and with the aim of increasing the efficiency and profitability of these

---

(1) OJ No L 84, 27. 3. 1987, p. 1.

(2) OJ No L 132, 23. 5. 1990, p. 19.

(3) OJ No L 133, 14. 5. 1982, p. 1.

▼B

laboratories, there is good reason to allow automated analytical methods to be applied under certain conditions; whereas it is important to specify that, where a dispute arises, the automated methods may not replace the reference methods and the usual methods;

Whereas the results of a density measurement using the automated method based on the principle of the frequency oscillator are, in respect of their accuracy, repeatability and reproducibility, at least as good as the results obtained by the methods listed in section 1 of the Annex to the present Regulation for measuring the density or specific gravity; whereas it is therefore indicated, by virtue of Article 74 (3) of Regulation (EEC) No 822/87, that this automated method shall be considered as equivalent to the said methods listed in the Annex to the present Regulation;

Whereas the procedure described in Chapter 25 under point 2.2.3.3.2 in the Annex hereto for analysing the total sulphur dioxide content of wines and grape musts of a presumed content of less than 50 mg/l results in better extraction of that substance compared to the methods described in Chapter 13 under point 13.4 of the Annex to Regulation (EEC) No 1108/82; whereas the result is higher total sulphur dioxide contents of the products analysed, which may exceed, in particular, in the case of certain grape juices, the maximum limit laid down; whereas, in order to avoid difficulties in the disposal of grape juice already prepared at the time of entry into force of this Regulation and until such time as the production processes are adapted to result in more complete de-sulphiting of grape musts with fermentation arrested by the addition of alcohol, the procedure described in the abovementioned Regulation should be allowed to be used during a transitional period;

Whereas the measures provided for in this Regulation are in accordance with the opinion of the Management Committee for Wine,

HAS ADOPTED THIS REGULATION:

Article 1

1. The Community methods for the analysis of wine making it possible, in the context of commercial transactions and all control operations, to:

— establish the composition of the products listed in Article 1 of Regulation (EEC) No 822/87,

— check whether these products have been subjected to treatments in violation of authorized oenological practice,

are those set out in the Annex to this Regulation.

2. For substances for which reference methods and usual methods are prescribed, the results obtained by the use of the reference methods shall prevail.

Article 2

For the purposes of applying this Regulation:

(a) the repeatability shall be the value below which the absolute difference between the two single test results obtained on identical test material, under the same conditions (same operator, same apparatus, same laboratory and a short interval of time), may be expected to lie with a specified probability;

(b) the reproducibility shall be the value below which the absolute difference between two single test results obtained identical test material, under different conditions (different operators, different apparatus and/or different laboratories and/or different time), may be expected to lie with a specified probability.

The term 'single test result' shall be the value obtained when the standardized test method is applied fully and once to a single sample.

Unless otherwise stated, the probability shall be 95 %.

▼**B**

Article 3

1. Automated analytical methods shall be acceptable, under the responsibility of the Director of a laboratory on condition that the accuracy, repeatability and reproducibility of the results are at least equivalent to those of the results obtained by the analytical methods listed in the Annex.

Where a dispute arises, the methods listed in the Annex may not be replaced by automated methods.

2. The automated method for measuring density based on the principle of the frequency oscilliator shall be considered as equivalent to the methods listed in section 1 of the Annex to the present Regulation.

Article 4

Wherever mention is made of water for solution, dilution, or washing purposes, this shall mean distilled water or demineralized water of equivalent purity. All chemicals shall be of analytical reagent quality except where otherwise specified.

Article 5

Regulation (EEC) No 1108/82 is repealed.

However Article 1 (4) of that Regulation shall apply until 31 December 1990.

Article 6

This Regulation shall enter into force on the day of its publication in the *Official Journal of the European Communities*.

It shall apply with effect from 1 October 1990.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

▼B

ANNEX

1. DENSITY AND SPECIFIC GRAVITY AT 20 ºC

1. DEFINITIONS

The density is the mass per unit volume of wine or must at 20 ºC. It is expressed in grams per millilitre and denoted by the symbol ρ 20 ºC.

The specific gravity at 20 ºC (or the 20 ºC/20 ºC relative density) is the ratio, expressed as a decimal number, of the density of a certain volume of the wine or must at 20 ºC to the density of same volume water at the same temperature. It is denoted by the symbol $d_{20\,°C}^{20\,°C}$.

2. PRINCIPLE OF METHODS

The density and specific gravity at 20 ºC are measured on a test sample:

- either by pigmentary: reference method,
- or by hydrometry or densimetry using a hydrostatic balance: usual methods.

Note:

For very accurate measurement, the density must be corrected for the effect of sulphur dioxide by using the formula:

$$\rho_{20\,°C} = \rho'_{20\,°C} - 0{,}0006 \times S$$

where $\rho_{20\,°C}$ = corrected density

$\rho'_{20\,°C}$ = observed density

S = total quantity of sulphur dioxide in grams per litre

3. PRELIMINARY TREATMENT OF SAMPLE

If the wine or must contains appreciable quantities of carbon dioxide, remove most of it by stirring 250 ml of the wine in a 1-litre flask or by filtration under reduced pressure through 2 g of cotton wool placed in an extension tube.

4. REFERENCE METHOD

4.1. **Apparatus:**

Normal laboratory equipment, and in particular:

4.1.1. A Pyrex pycnometer (1), of approximately 100 ml capacity, with a removable ground-glass jointed thermometer calibrated in tenths of a degree from 10 to 30 ºC (Figure 1). The thermometer must be standardized.

---

(1) Any pycnometer with equivalent characteristics may be used.

▼B

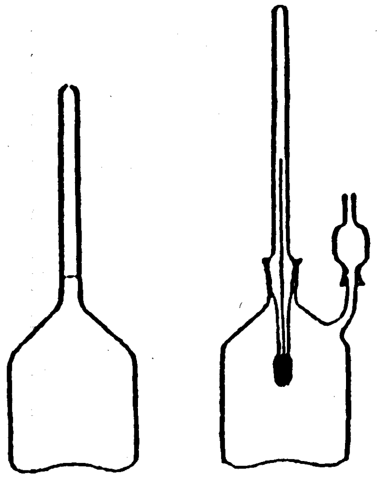


Figure 1

The pycnometer and its tare

The pycnometer has a side tube 25 mm in length and 1 mm (maximum) in internal diameter, ending in a conical ground joint. This side tube may be capped by a 'reservoir stopper' consisting of a conical ground-glass joint tube and terminating in a drawn-out section. This stopper serves as an expansion chamber.

The two ground joints of the apparatus should be prepared with great care.

4.1.2. A tare bottle, consisting of a vessel with the same outside volume (to within at least 1 ml) as the pycnometer and with a mass equal to the mass of the pycnometer filled with a liquid of specific gravity 1,01 (sodium chloride solution 2,0 % m/v).

A thermally insulated container, exactly fitting the body of the pycnometer.

4.1.3. A twin-pan balance with a range of at least 300 g and a sensitivity of 0,1 mg,

or

a single-pan balance with a range of at least 200 g and a sensitivity of 0,1 mg.

4.2. Calibration of pycnometer

Calibration of the pycnometer involves a determination of the following quantities:

- tare of the empty pycnometer,
- volume of the pycnometer at 20 °C,
- mass of the water-filled pycnometer at 20 °C.

4.2.1. Method using a twin-pan balance

Place the tare bottle on the left-hand pan of the balance and the clean and dry pycnometer, fitted with its 'reservoir stopper', on the right-hand pan. Add weights to the pan holding the pycnometer and record the weight required to establish equilibrium: let it be p grams.

Carefully fill the pycnometer with distilled water at the ambient temperature and fit the thermometer; carefully wipe the pycnometer dry and place it in the thermally insulated container. Mix it by inverting the container until the temperature reading on the thermometer is constant. Accurately adjust the level to the upper rim of the side tube. Wipe the side tube dry and put the reservoir stopper on; read the temperature t °C carefully, possibly correcting it for the inaccuracy in the temperature scale. Weigh the water-filled pycnometer against the tare and record the weight p' in grams required to establish equilibrium.

▼**B**

Calculation (¹)*:*

Taring of empty pycnometer:

tare of empty pycnometer = $p + m$,

where m = mass of air contained in the pycnometer,

m = $0{,}0012\ (p - p')$.

Volume at 20 ºC:

$V_{20\ ^\circ C} = (p + m - p') \cdot F_t$,

where F_t = a factor taken from Table I for the temperature t ºC.

$V_{20\ ^\circ C}$ should be known to within ± 0,001 ml.

Mass of water at 20 ºC:

$M_{20\ ^\circ C} = 0{,}998203\ V_{20\ ^\circ C}$,

where 0,998203 is the density of water at 20 ºC.

4.2.2. *Method using a single-pan balance*

Determine:

- the mass of the clean and dry pycnometer: let this be P,
- the mass of the pycnometer filled with water at t ºC, following the procedure described in 4.2.1 above: let this be P_1,
- the mass of the tare: T_0.

Calculation (¹):

Taring of empty pycnometer:

tare of empty pycnometer = $P - m$,

where m = mass of air contained in the pycnometer,

m = $0{,}0012\ (P_1 - P)$.

Volume at 20 ºC:

$V_{20\ ^\circ C} = [P_1 - (P - \mathrm{m})] \times F_t$

where F_t = a factor taken from Table I for the temperature t ºC.

The volume at 20 ºC should be known to within ± 0,001 ml.

Mass of water at 20 ºC:

$M_{20\ ^\circ C} = 0{,}998203\ V_{20\ ^\circ C}$,

where 0,998203 is the density of water at 20 ºC.

4.3. **Method of measurement** (¹)

4.3.1. *Method using twin-pan balance*

Fill the pycnometer with the prepared test sample, following the procedure described in 4.2.1 above.

Let p'' be the weight in grams required to establish equilibrium at t ºC.

Mass of liquid contained in the pycnometer = $p + m - p''$.

Apparent density at t ºC:

$$\rho_{t\ ^\circ C} = \frac{p + m - p''}{V_{20\ ^\circ C}}$$

Calculate the density at 20 ºC using one of the correction tables given later, in accordance with the nature of the liquid being measured: dry wine (Table II), natural or concentrated must (Table III), sweet wine (Table IV).

The 20 ºC/20 ºC specific gravity of the wine is calculated by dividing its density at 20 ºC by 0,998203.

(¹) A numeric example is given in section 6 of this chapter.

▼B

4.3.2. *Method using a single-pan balance* (1)

Weigh the tare bottle and let its mass be T.

Calculate $dT = T_1 - T_0$.

Mass of the empty pycnometer at the time of measurement = $P - m + dT$.

Weigh the pycnometer filled with the prepared test sample, following the procedure described in 4.2.1 above. Let its mass at t ºC be P_2.

Mass of liquid contained in pycnometer at t ºC = $P_2 - (P - m + dT)$

Apparent density at t ºC:

$$\rho_{t\,ºC} = \frac{P_2 - (P - m + dT)}{V_{20\,ºC}}$$

Calculate the density at 20 ºC of the liquid under test (dry wine, natural or concentrated must, or sweet wine) as indicated in 4.3.1 above.

The 20 ºC/20 ºC specific gravity is calculated by dividing the density at 20 ºC by 0,998203.

4.3.3. The repeatability of the density measurements

for dry and semi-sweet wines: $r = 0{,}00010$,

and for sweet wines: $r = 0{,}00018$.

4.3.4. The reproducibility of the density measurements:

for dry and semi-sweet wines: $R = 0{,}00037$,

and for sweet wines: $R = 0{,}00045$.

▼M9

▼B

6. EXAMPLE OF THE CALCULATION OF THE DENSITY AT 20 ºC AND THE 20 ºC/20 ºC SPECIFIC GRAVITY (REFERENCE METHOD)

6.1. Pycnometry using a twin-pan balance

6.1.1. *Standardization of the pycnometer:*

1. Weighing of clean dry pycnometer:

   Tare = pycnometer + p
   p = 104,9454 g

2. Weighing of pycnometer filled with water at t ºC:

   Tare = pycnometer + water + p'
   p' = 1,2396 g at t = 20,5 ºC

3. Calculation of mass of air contained in the pycnometer:

   $m = 0{,}0012\ (p - p')$
   $m = 0{,}0012\ (104{,}9454 - 1{,}2396)$
   $m = 0{,}1244$ g

4. Characteristic values to be retained:

   Tare of empty pycnometer, $p + m$:

   $p + m = 104{,}9454 + 0{,}1244$
   $p + m = 105{,}0698$ g

   Volume at 20 ºC = $F(p + m - p')_{t ºC}$

   $F_{20{,}5\,ºC} = 1{,}001900$
   $V_{20\,ºC} = (105{,}0698 - 1{,}2396) \times 1{,}001900$

---

(1) A numeric example is given in section 6 of this chapter.

▼**B**

$V_{20\,°C}$ = 104,0275 ml

Mass of water at 20 °C =

$M_{20\,°C}$ = $V_{20\,°C} \cdot 0{,}998203$

$M_{20\,°C}$ = 103,8405 g

6.1.2. *Determination of the density and specific gravity at 20 °C/20 °C of a dry wine*

ρ'' = 1,2622 at 17,80 °C

$$\rho_{17{,}80\,°C} = \frac{105{,}0698 - 1{,}2622}{104{,}0275}$$

$\rho_{17{,}80\,°C}$ = 0,99788 g/ml

Table II enables $\rho_{20\,°C}$ to be calculated from $\rho_{t°C}$ using the relationship:

$$\rho_{20\,°C} = \rho_{t\,°C} \pm \frac{c}{1000}$$

For t = 17,80 °C and for an alcoholic strength of 11 % vol. c = 0,54.

$$\rho_{20\,°C} = 0{,}99788 - \frac{0{,}54}{1000}$$

$\rho_{20\,°C}$ = 0,99734 g/ml

$$d^{20\,°C}_{20\,°C} = \frac{0{,}99734}{0{,}998203} = 0{,}99913$$

6.2. **Pycnometry using a single-pan balance**

6.2.1. *Standardization of the pycnometer:*

1. Weight of clean dry pycnometer:

   P = 67,7913 g

2. Weight of pycnometer filled with water at t °C:

   P_1 = 169,2715 at 21,65 °C

3. Mass of air contained in the pycnometer:

   m = 0,0012 ($P_1 - P$)
   m = 0,0012 × 101,4802
   m = 0,1218 g

4. Characteristic values to be retained:

   Tare of empty pycnometer, $P - m$:

   $P - m$ = 67,7913 − 0,1218
   $P - m$ = 67,6695 g

   Volume at 20 °C = $[P_1 - (P - m)]\, F_{t°C}$

   $F_{21{,}65\,°C}$ = 1,002140
   $V_{20\,°C}$ = 1,002140 (169,2715 − 67,6695)
   $V_{20\,°C}$ = 101,8194 ml

   Mass of water at 20 °C:

   $M_{20\,°C}$ = $V_{20\,°C} \times 0{,}998203$
   $M_{20\,°C}$ = 101,6364 g

   Mass of tare bottle, T_o:

   T_o = 171,9160 g

▼**B**

6.2.2. *Determination of the density and specific gravity at 20º C of a dry wine*

T_1 = 171,9178 g

dT = 171,9178 − 171,9160 = 0,0018 g

$P - m + dT$ = 67,6695 + 0,0018 = 67,6713 g

P_2 = 169,2799 at 18 ºC

$$\rho_{18\,°C} = \frac{169,2799 - 67,6713}{101,8194}$$

$\rho_{18\,°C}$ = 0,99793 g/ml

Table II enables $\rho_{20\,°C}$ to be calculated from $\rho_{t\,°C}$ using the relationship:

$$\rho_{20\,°C} = \rho_{t\,°C} \pm \frac{c}{1000}$$

For t = 18 ºC and an alcoholic strength of 11 % vol. c = 0,49.

$$\rho_{20\,°C} = 0,99793 - \frac{0,49}{1000}$$

$\rho_{20\,°C}$ = 0,99744 g/ml

$$d^{20\,°C}_{20\,°C} = \frac{0,99744}{0,998203} = 0,99923$$

▼B

TABLE I

F factors by which the mass of water contained in the Pyrex pycnometer at t ºC has to be multiplied in order to calculate the pycnometer volume at 20 ºC

t ºC	F	*t* ºC	F	*t* ºC	F	*t* ºC	F	*t* ºC	F	*t* ºC	F	*t* ºC	F
10,0	1,000398	13,0	1,000691	16,0	1,001097	19,0	1,001608	22,0	1,002215	25,0	1,002916	28,0	1,003704
,1	1,000406	,1	1,000703	,1	1,001113	,1	1,001627	,1	1,002238	,1	1,002941	,1	1,003731
,2	1,000414	,2	1,000714	,2	1,001128	,2	1,001646	,2	1,002260	,2	1,002966	,2	1,003759
,3	1,000422	,3	1,000726	,3	1,001144	,3	1,001665	,3	1,002282	,3	1,002990	,3	1,003787
,4	1,000430	,4	1,000738	,4	1,001159	,4	1,001684	,4	1,002304	,4	1,003015	,4	1,003815
10,5	1,000439	13,5	1,000752	16,5	1,001175	19,5	1,001703	22,5	1,002326	25,5	1,003041	28,5	1,003843
,6	1,000447	,6	1,000764	,6	1,001191	,6	1,001722	,6	1,002349	,6	1,003066	,6	1,003871
,7	1,000456	,7	1,000777	,7	1,001207	,7	1,001741	,7	1,002372	,7	1,003092	,7	1,003899
,8	1,000465	,8	1,000789	,8	1,001223	,8	1,001761	,8	1,002394	,8	1,003117	,8	1,003928
,9	1,000474	,9	1,000803	,9	1,001239	,9	1,001780	,9	1,002417	,9	1,003143	,9	1,003956
11,0	1,000483	14,0	1,000816	17,0	1,001257	20,0	1,001800	23,0	1,002439	26,0	1,003168	29,0	1,003984
,1	1,000492	,1	1,000829	,1	1,001273	,1	1,001819	,1	1,002462	,1	1,003194	,1	1,004013
,2	1,000501	,2	1,000842	,2	1,001290	,2	1,001839	,2	1,002485	,2	1,003222	,2	1,004042
,3	1,000511	,3	1,000855	,3	1,001306	,3	1,001859	,3	1,002508	,3	1,003247	,3	1,004071
,4	1,000520	,4	1,000868	,4	1,001323	,4	1,001880	,4	1,002531	,4	1,003273	,4	1,004099
11,5	1,000530	14,5	1,000882	17,5	1,001340	20,5	1,001900	23,5	1,002555	26,5	1,003299	29,5	1,004128
,6	1,000540	,6	1,000895	,6	1,001357	,6	1,001920	,6	1,002578	,6	1,003326	,6	1,004158
,7	1,000550	,7	1,000909	,7	1,001374	,7	1,001941	,7	1,002602	,7	1,003352	,7	1,004187
,8	1,000560	,8	1,000923	,8	1,001391	,8	1,001961	,8	1,002625	,8	1,003379	,8	1,004216
,9	1,000570	,9	1,000937	,9	1,001409	,9	1,001982	,9	1,002649	,9	1,003405	,9	1,004245
12,0	1,000580	15,0	1,000951	18,0	1,001427	21,0	1,002002	24,0	1,002672	27,0	1,003432	30,0	1,004275
,1	1,000591	,1	1,000965	,1	1,001445	,1	1,002023	,1	1,002696	,1	1,003458		
,2	1,000601	,2	1,000979	,2	1,001462	,2	1,002044	,2	1,002720	,2	1,003485		
,3	1,000612	,3	1,000993	,3	1,001480	,3	1,002065	,3	1,002745	,3	1,003513		
,4	1,000623	,4	1,001008	,4	1,001498	,4	1,002086	,4	1,002769	,4	1,003540		
12,5	1,000634	15,5	1,001022	18,5	1,001516	21,5	1,002107	24,5	1,002793	27,5	1,003567		
,6	1,000645	,6	1,001037	,6	1,001534	,6	1,002129	,6	1,002817	,6	1,003594		
,7	1,000656	,7	1,001052	,7	1,001552	,7	1,002151	,7	1,002842	,7	1,003621		
,8	1,000668	,8	1,001067	,8	1,001570	,8	1,002172	,8	1,002866	,8	1,003649		
,9	1,000679	,9	1,001082	,9	1,001589	,9	1,002194	,9	1,002891	,9	1,003676		

▼**B**

TABLE II

Temperature corrections *c* to the density of alcohol-free dry wines measured with a Pyrex glass pycnometer at *t* ºC to relate the result to 20 ºC

$$\rho_{20} = \rho_t \pm \frac{c}{1000} \begin{cases} - \text{ if } t \text{ ºC is lower than 20 ºC} \\ + \text{ if } t \text{ ºC is higher than 20 ºC} \end{cases}$$

		Alcoholic strengths																							
		0	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Temperature (ºC)	10	1,59	1,64	1,67	1,71	1,77	1,84	1,91	2,01	2,11	2,22	2,34	2,46	2,60	2,73	2,88	3,03	3,19	3,35	3,52	3,70	3,87	4,06	4,25	4,44
	11	1,48	1,53	1,56	1,60	1,64	1,70	1,77	1,86	1,95	2,05	2,16	2,27	2,38	2,51	2,63	2,77	2,91	3,06	3,21	3,36	3,53	3,69	3,86	4,03
	12	1,36	1,40	1,43	1,46	1,50	1,56	1,62	1,69	1,78	1,86	1,96	2,05	2,16	2,27	2,38	2,50	2,62	2,75	2,88	3,02	3,16	3,31	3,46	3,61
	13	1,22	1,26	1,28	1,32	1,35	1,40	1,45	1,52	1,59	1,67	1,75	1,83	1,92	2,01	2,11	2,22	2,32	2,44	2,55	2,67	2,79	2,92	3,05	3,18
	14	1,08	1,11	1,13	1,16	1,19	1,23	1,27	1,33	1,39	1,46	1,52	1,60	1,67	1,75	1,84	1,93	2,03	2,11	2,21	2,31	2,42	2,52	2,63	2,74
	15	0,92	0,96	0,97	0,99	1,02	1,05	1,09	1,13	1,19	1,24	1,30	1,36	1,42	1,48	1,55	1,63	1,70	1,78	1,86	1,95	2,03	2,12	2,21	2,30
	16	0,76	0,79	0,80	0,81	0,84	0,86	0,89	0,93	0,97	1,01	1,06	1,10	1,16	1,21	1,26	1,32	1,38	1,44	1,51	1,57	1,64	1,71	1,78	1,85
	17	0,59	0,61	0,62	0,63	0,65	0,67	0,69	0,72	0,75	0,78	0,81	0,85	0,88	0,95	0,96	1,01	1,05	1,11	1,15	1,20	1,25	1,30	1,35	1,40
	18	0,40	0,42	0,42	0,43	0,44	0,46	0,47	0,49	0,51	0,53	0,55	0,57	0,60	0,63	0,65	0,68	0,71	0,74	0,77	0,81	0,84	0,87	0,91	0,94
	19	0,21	0,21	0,22	0,22	0,23	0,23	0,24	0,25	0,26	0,27	0,28	0,29	0,30	0,32	0,33	0,34	0,36	0,37	0,39	0,41	0,42	0,44	0,46	0,47
	20																								
	21	0,21	0,22	0,22	0,23	0,23	0,24	0,25	0,26	0,27	0,28	0,29	0,30	0,31	0,32	0,34	0,36	0,37	0,38	0,40	0,41	0,43	0,44	0,46	0,48
	22	0,44	0,45	0,46	0,47	0,48	0,49	0,51	0,52	0,54	0,56	0,59	0,61	0,63	0,66	0,69	0,71	0,74	0,77	0,80	0,83	0,87	0,90	0,93	0,97
	23	0,68	0,70	0,71	0,72	0,74	0,76	0,78	0,80	0,83	0,86	0,90	0,93	0,96	1,00	1,03	1,08	1,13	1,17	1,22	1,26	1,31	1,37	1,41	1,46
	24	0,93	0,96	0,97	0,99	1,01	1,03	1,06	1,10	1,13	1,18	1,22	1,26	1,31	1,36	1,41	1,47	1,52	1,58	1,64	1,71	1,77	1,84	1,90	1,97
	25	1,19	1,23	1,25	1,27	1,29	1,32	1,36	1,40	1,45	1,50	1,55	1,61	1,67	1,73	1,80	1,86	1,93	2,00	2,08	2,16	2,24	2,32	2,40	2,48
	26	1,47	1,51	1,53	1,56	1,59	1,62	1,67	1,72	1,77	1,83	1,90	1,96	2,03	2,11	2,19	2,27	2,35	2,44	2,53	2,62	2,72	2,81	2,91	3,01
	27	1,75	1,80	1,82	1,85	1,89	1,93	1,98	2,04	2,11	2,18	2,25	2,33	2,41	2,50	2,59	2,68	2,78	2,88	2,98	3,09	3,20	3,31	3,42	3,53
	28	2,04	2,10	2,13	2,16	2,20	2,25	2,31	2,38	2,45	2,53	2,62	2,70	2,80	2,89	3,00	3,10	3,21	3,32	3,45	3,57	3,69	3,82	3,94	4,07
	29	2,34	2,41	2,44	2,48	2,53	2,58	2,65	2,72	2,81	2,89	2,99	3,09	3,19	3,30	3,42	3,53	3,65	3,78	3,92	4,05	4,19	4,33	4,47	4,61
	30	2,66	2,73	2,77	2,81	2,86	2,92	3,00	3,08	3,17	3,27	3,37	3,48	3,59	3,72	3,84	3,97	4,11	4,25	4,40	4,55	4,70	4,85	4,92	5,17

Note: This table may be used to convert the specific gravity d_{20}^{t} to the specific gravity d_{20}^{20}.

TABLE III

Temperature corrections *c* to the density of natural musts and of concentrated musts measured with a Pyrex glass pycnometer at *t* ºC to relate the result to 20 ºC

$$\rho_{20} = \rho_t \pm \frac{c}{1000} \quad \begin{matrix} - \text{ if } t \text{ ºC is lower than 20 ºC} \\ + \text{ if } t \text{ ºC is higher than 20 ºC} \end{matrix}$$

		Densities																					
		1,05	1,06	1,07	1,08	1,09	1,10	1,11	1,12	1,13	1,14	1,15	1,16	1,18	1,20	1,22	1,24	1,26	1,28	1,30	1,32	1,34	1,36
Temperature (ºC)	10º	2,31	2,48	2,66	2,82	2,99	3,13	3,30	3,44	3,59	3,73	3,88	4,01	4,28	4,52	4,76	4,98	5,18	5,42	5,56	5,73	5,90	6,05
	11º	2,12	2,28	2,42	2,57	2,72	2,86	2,99	3,12	3,25	3,37	3,50	3,62	3,85	4,08	4,29	4,48	4,67	4,84	5,00	5,16	5,31	5,45
	12º	1,92	2,06	2,19	2,32	2,45	2,58	2,70	2,82	2,94	3,04	3,15	3,26	3,47	3,67	3,85	4,03	4,20	4,36	4,51	4,65	4,78	4,91
	13º	1,72	1,84	1,95	2,06	2,17	2,27	2,38	2,48	2,58	2,69	2,78	2,88	3,05	3,22	3,39	3,55	3,65	3,84	3,98	4,11	4,24	4,36
	14º	1,52	1,62	1,72	1,81	1,90	2,00	2,09	2,17	2,26	2,34	2,43	2,51	2,66	2,82	2,96	3,09	3,22	3,34	3,45	3,56	3,67	3,76
	15º	1,28	1,36	1,44	1,52	1,60	1,67	1,75	1,82	1,89	1,96	2,04	2,11	2,24	2,36	2,48	2,59	2,69	2,79	2,88	2,97	3,03	3,10
	16º	1,05	1,12	1,18	1,25	1,31	1,37	1,43	1,49	1,55	1,60	1,66	1,71	1,81	1,90	2,00	2,08	2,16	2,24	2,30	2,37	2,43	2,49
	17º	0,80	0,86	0,90	0,95	1,00	1,04	1,09	1,13	1,18	1,22	1,26	1,30	1,37	1,44	1,51	1,57	1,62	1,68	1,72	1,76	1,80	1,84
	18º	0,56	0,59	0,62	0,66	0,68	0,72	0,75	0,77	0,80	0,83	0,85	0,88	0,93	0,98	1,02	1,05	1,09	1,12	1,16	1,19	1,21	1,24
	19º	0,29	0,31	0,32	0,34	0,36	0,37	0,39	0,40	0,42	0,43	0,44	0,45	0,48	0,50	0,52	0,54	0,56	0,57	0,59	0,60	0,61	0,62
	20º																						
	21º	0,29	0,30	0,32	0,34	0,35	0,37	0,38	0,40	0,41	0,42	0,44	0,46	0,48	0,50	0,53	0,56	0,58	0,59	0,60	0,61	0,62	0,62
	22º	0,58	0,61	0,64	0,67	0,70	0,73	0,76	0,79	0,81	0,84	0,87	0,90	0,96	1,00	1,05	1,09	1,12	1,15	1,18	1,20	1,22	1,23
	23º	0,89	0,94	0,99	1,03	1,08	1,12	1,16	1,20	1,25	1,29	1,33	1,37	1,44	1,51	1,57	1,63	1,67	1,73	1,77	1,80	1,82	1,84
	24º	1,20	1,25	1,31	1,37	1,43	1,49	1,54	1,60	1,66	1,71	1,77	1,82	1,92	2,01	2,10	2,17	2,24	2,30	2,36	2,40	2,42	2,44
	25º	1,51	1,59	1,66	1,74	1,81	1,88	1,95	2,02	2,09	2,16	2,23	2,30	2,42	2,53	2,63	2,72	2,82	2,89	2,95	2,99	3,01	3,05
	26º	1,84	1,92	2,01	2,10	2,18	2,26	2,34	2,42	2,50	2,58	2,65	2,73	2,87	3,00	3,13	3,25	3,36	3,47	3,57	3,65	3,72	3,79
	27º	2,17	2,26	2,36	2,46	2,56	2,66	2,75	2,84	2,93	3,01	3,10	3,18	3,35	3,50	3,66	3,80	3,93	4,06	4,16	4,26	4,35	4,42
	28º	2,50	2,62	2,74	2,85	2,96	3,07	3,18	3,28	3,40	3,50	3,60	3,69	3,87	4,04	4,21	4,36	4,50	4,64	4,75	4,86	4,94	5,00
	29º	2,86	2,98	3,10	3,22	3,35	3,47	3,59	3,70	3,82	3,93	4,03	4,14	4,34	4,53	4,72	4,89	5,05	5,20	5,34	5,46	5,56	5,64
	30º	3,20	3,35	3,49	3,64	3,77	3,91	4,05	4,17	4,30	4,43	4,55	4,67	4,90	5,12	5,39	5,51	5,68	5,84	5,96	6,08	6,16	6,22

Note: This table may be used to convert the specific gravity d_{20}^{t} to the specific gravity d_{20}^{20}.

▼**B**

TABLE IV

Temperature corrections *c* to the density of wines of 13 % vol and above containing residual sugar measured with a Pyrex glass pycnometer at *t* ºC to relate the result to 20 ºC

$$\rho_{20} = \rho_t \pm \frac{c}{1000} \left\{ \begin{array}{l} - \text{ if } t \text{ ºC is lower than 20 ºC} \\ + \text{ if } t \text{ ºC is higher than 20 ºC} \end{array} \right.$$

		Wines of 13 % vol							Wines of 15 % vol							Wines of 17 % vol						
		Densities							Densities							Densities						
		1,000	1,020	1,040	1,060	1,080	1,100	1,120	1,000	1,020	1,040	1,060	1,080	1,100	1,120	1,000	1,020	1,040	1,060	1,080	1,100	1,120
Temperature (ºC)	10º	2,36	2,71	3,06	3,42	3,72	3,96	4,32	2,64	2,99	3,36	3,68	3,99	4,30	4,59	2,94	3,29	3,64	3,98	4,29	4,60	4,89
	11º	2,17	2,49	2,80	2,99	3,39	3,65	3,90	2,42	2,73	3,05	3,34	3,63	3,89	4,15	2,69	3,00	3,32	3,61	3,90	4,16	4,41
	12º	1,97	2,25	2,53	2,79	3,05	3,29	3,52	2,19	2,47	2,75	3,01	3,27	3,51	3,73	2,42	2,70	2,98	3,24	3,50	3,74	3,96
	13º	1,78	2,02	2,25	2,47	2,69	2,89	3,09	1,97	2,21	2,44	2,66	2,87	3,08	3,29	2,18	2,42	2,64	2,87	3,08	3,29	3,49
	14º	1,57	1,78	1,98	2,16	2,35	2,53	2,70	1,74	1,94	2,14	2,32	2,52	2,69	2,86	1,91	2,11	2,31	2,50	2,69	2,86	3,03
	15º	1,32	1,49	1,66	1,82	1,97	2,12	2,26	1,46	1,63	1,79	1,95	2,10	2,25	2,39	1,60	1,77	1,93	2,09	2,24	2,39	2,53
	16º	1,08	1,22	1,36	1,48	1,61	1,73	1,84	1,18	1,32	1,46	1,59	1,71	1,83	1,94	1,30	1,44	1,58	1,71	1,83	1,95	2,06
	17º	0,83	0,94	1,04	1,13	1,22	1,31	1,40	0,91	1,02	1,12	1,21	1,30	1,39	1,48	1,00	1,10	1,20	1,30	1,39	1,48	1,56
	18º	0,58	0,64	0,71	0,78	0,84	0,89	0,95	0,63	0,69	0,76	0,83	0,89	0,94	1,00	0,69	0,75	0,82	0,89	0,95	1,00	1,06
	19º	0,30	0,34	0,37	0,40	0,43	0,46	0,49	0,33	0,37	0,40	0,43	0,46	0,49	0,52	0,36	0,39	0,42	0,46	0,49	0,52	0,54
	20º																					
	21º	0,30	0,33	0,36	0,40	0,43	0,46	0,49	0,33	0,36	0,39	0,43	0,46	0,49	0,51	0,35	0,39	0,42	0,45	0,48	0,51	0,54
	22º	0,60	0,67	0,73	0,80	0,85	0,91	0,98	0,65	0,72	0,78	0,84	0,90	0,96	1,01	0,71	0,78	0,84	0,90	0,96	1,01	1,07
	23º	0,93	1,02	1,12	1,22	1,30	1,39	1,49	1,01	1,10	1,20	1,29	1,38	1,46	1,55	1,10	1,19	1,29	1,38	1,46	1,55	1,63
	24º	1,27	1,39	1,50	1,61	1,74	1,84	1,95	1,37	1,49	1,59	1,72	1,84	1,95	2,06	1,48	1,60	1,71	1,83	1,95	2,06	2,17
	25º	1,61	1,75	1,90	2,05	2,19	2,33	2,47	1,73	1,87	2,02	2,17	2,31	2,45	2,59	1,87	2,01	2,16	2,31	2,45	2,59	2,73
	26º	1,94	2,12	2,29	2,47	2,63	2,79	2,95	2,09	2,27	2,44	2,62	2,78	2,94	3,10	2,26	2,44	2,61	2,79	2,95	3,11	3,26
	27º	2,30	2,51	2,70	2,90	3,09	3,27	3,44	2,48	2,68	2,87	3,07	3,27	3,45	3,62	2,67	2,88	3,07	3,27	3,46	3,64	3,81
	28º	2,66	2,90	3,13	3,35	3,57	3,86	4,00	2,86	3,10	3,23	3,55	3,77	3,99	4,20	3,08	3,31	3,55	3,76	3,99	4,21	4,41
	29º	3,05	3,31	3,56	3,79	4,04	4,27	4,49	3,28	3,53	3,77	4,02	4,26	4,49	4,71	3,52	3,77	4,01	4,26	4,50	4,73	4,95
	30º	3,44	3,70	3,99	4,28	4,54	4,80	5,06	3,68	3,94	4,23	4,52	4,79	5,05	5,30	3,95	4,22	4,51	4,79	5,07	5,32	5,57

		Wines of 19 % vol							Wines of 21 % vol						
		Densities							Densities						
		1,000	1,020	1,040	1,060	1,080	1,100	1,120	1,000	1,020	1,040	1,060	1,080	1,100	1,120
Temperature (ºC)	10º	3,27	3,62	3,97	4,30	4,62	4,92	5,21	3,62	3,97	4,32	4,66	4,97	5,27	5,56
	11º	2,99	3,30	3,61	3,90	4,19	4,45	4,70	3,28	3,61	3,92	4,22	4,50	4,76	5,01
	12º	2,68	2,96	3,24	3,50	3,76	4,00	4,21	2,96	3,24	3,52	3,78	4,03	4,27	4,49
	13º	2,40	2,64	2,87	3,09	3,30	3,51	3,71	2,64	2,88	3,11	3,33	3,54	3,74	3,95
	14º	2,11	2,31	2,51	2,69	2,88	3,05	3,22	2,31	2,51	2,71	2,89	3,08	3,25	3,43
	15º	1,76	1,93	2,09	2,25	2,40	2,55	2,69	1,93	2,10	2,26	2,42	2,57	2,72	2,86
	16º	1,43	1,57	1,70	1,83	1,95	2,08	2,18	1,56	1,70	1,84	1,97	2,09	2,21	2,32
	17º	1,09	1,20	1,30	1,39	1,48	1,57	1,65	1,20	1,31	1,41	1,50	1,59	1,68	1,77
	18º	0,76	0,82	0,88	0,95	1,01	1,06	1,12	0,82	0,88	0,95	1,01	1,08	1,13	1,18
	19º	0,39	0,42	0,45	0,49	0,52	0,55	0,57	0,42	0,46	0,49	0,52	0,55	0,58	0,61
	20º														
	21º	0,38	0,42	0,45	0,48	0,51	0,54	0,57	0,41	0,45	0,48	0,51	0,54	0,57	0,60
	22º	0,78	0,84	0,90	0,96	1,02	1,07	1,13	0,84	0,90	0,96	1,02	1,08	1,14	1,19
	23º	1,19	1,28	1,38	1,47	1,55	1,64	1,72	1,29	1,39	1,48	1,57	1,65	1,74	1,82
	24º	1,60	1,72	1,83	1,95	2,06	2,18	2,29	1,73	1,85	1,96	2,08	2,19	2,31	2,42
	25º	2,02	2,16	2,31	2,46	2,60	2,74	2,88	2,18	2,32	2,47	2,62	2,76	2,90	3,04
	26º	2,44	2,62	2,79	2,96	3,12	3,28	3,43	2,53	2,81	2,97	3,15	3,31	3,47	3,62
	27º	2,88	3,08	3,27	3,42	3,66	3,84	4,01	3,10	3,30	3,47	3,69	3,88	4,06	4,23
	28º	3,31	3,54	3,78	4,00	4,22	4,44	4,64	3,56	3,79	4,03	4,25	4,47	4,69	4,89
	29º	3,78	4,03	4,27	4,52	4,76	4,99	5,21	4,06	4,31	4,55	4,80	5,04	5,27	5,48
	30º	4,24	4,51	4,80	5,08	5,36	5,61	5,86	4,54	4,82	5,11	5,39	5,66	5,91	6,16

▼**B**

TABLE V

Temperature corrections *c* to the density of dry wines and alcohol-free dry wines measured with an ordinary glass pycnometer or hydrometer at *t* ºC to relate the result to 20 ºC

$$\rho_{20} = \rho_t \pm \frac{c}{1000} \left\{ \begin{array}{l} - \text{ if } t \text{ ºC is lower than 20 ºC} \\ + \text{ if } t \text{ ºC is higher than 20 ºC} \end{array} \right.$$

		Alcoholic strength																							
		0	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Temperature (ºC)	10º	1,45	1,51	1,55	1,58	1,64	1,70	1,78	1,88	1,98	2,09	2,21	2,34	2,47	2,60	2,75	2,90	3,06	3,22	3,39	3,57	3,75	3,93	4,12	4,31
	11º	1,35	1,40	1,43	1,47	1,52	1,58	1,65	1,73	1,83	1,93	2,03	2,15	2,26	2,38	2,51	2,65	2,78	2,93	3,08	3,24	3,40	3,57	3,73	3,90
	12º	1,24	1,28	1,31	1,34	1,39	1,44	1,50	1,58	1,66	1,75	1,84	1,94	2,04	2,15	2,26	2,38	2,51	2,63	2,77	2,91	3,05	3,19	3,34	3,49
	13º	1,12	1,16	1,18	1,21	1,25	1,30	1,35	1,42	1,49	1,56	1,64	1,73	1,82	1,91	2,01	2,11	2,22	2,33	2,45	2,57	2,69	2,81	2,95	3,07
	14º	0,99	1,03	1,05	1,07	1,11	1,14	1,19	1,24	1,31	1,37	1,44	1,52	1,59	1,67	1,75	1,84	1,93	2,03	2,13	2,23	2,33	2,44	2,55	2,66
	15º	0,86	0,89	0,90	0,92	0,95	0,98	1,02	1,07	1,12	1,17	1,23	1,29	1,35	1,42	1,49	1,56	1,63	1,71	1,80	1,88	1,96	2,05	2,14	2,23
	16º	0,71	0,73	0,74	0,76	0,78	0,81	0,84	0,87	0,91	0,96	0,99	1,05	1,10	1,15	1,21	1,27	1,33	1,39	1,45	1,52	1,59	1,66	1,73	1,80
	17º	0,55	0,57	0,57	0,59	0,60	0,62	0,65	0,67	0,70	0,74	0,77	0,81	0,84	0,88	0,92	0,96	1,01	1,05	1,11	1,15	1,20	1,26	1,31	1,36
	18º	0,38	0,39	0,39	0,40	0,41	0,43	0,44	0,46	0,48	0,50	0,52	0,55	0,57	0,60	0,62	0,65	0,68	0,71	0,74	0,78	0,81	0,85	0,88	0,91
	19º	0,19	0,20	0,20	0,21	0,21	0,22	0,23	0,24	0,25	0,26	0,27	0,28	0,29	0,30	0,32	0,33	0,35	0,36	0,38	0,39	0,41	0,43	0,44	0,46
	20º																								
	21º	0,21	0,22	0,22	0,23	0,23	0,24	0,25	0,25	0,26	0,27	0,29	0,29	0,31	0,32	0,34	0,35	0,36	0,38	0,39	0,41	0,43	0,44	0,46	0,48
	22º	0,43	0,45	0,45	0,46	0,47	0,49	0,50	0,52	0,54	0,56	0,58	0,60	0,63	0,65	0,68	0,71	0,73	0,77	0,80	0,83	0,86	0,89	0,93	0,96
	23º	0,67	0,69	0,70	0,71	0,72	0,74	0,77	0,79	0,82	0,85	0,88	0,91	0,95	0,99	1,03	1,07	1,12	1,16	1,21	1,25	1,30	1,35	1,40	1,45
	24º	0,91	0,93	0,95	0,97	0,99	1,01	1,04	1,07	1,11	1,15	1,20	1,24	1,29	1,34	1,39	1,45	1,50	1,56	1,62	1,69	1,76	1,82	1,88	1,95
	25º	1,16	1,19	1,21	1,23	1,26	1,29	1,33	1,37	1,42	1,47	1,52	1,57	1,63	1,70	1,76	1,83	1,90	1,97	2,05	2,13	2,21	2,29	2,37	2,45
	26º	1,42	1,46	1,49	1,51	1,54	1,58	1,62	1,67	1,73	1,79	1,85	1,92	1,99	2,07	2,14	2,22	2,31	2,40	2,49	2,58	2,67	2,77	2,86	2,96
	27º	1,69	1,74	1,77	1,80	1,83	1,88	1,93	1,98	2,05	2,12	2,20	2,27	2,35	2,44	2,53	2,63	2,72	2,82	2,93	3,04	3,14	3,25	3,37	3,48
	28º	1,97	2,03	2,06	2,09	2,14	2,19	2,24	2,31	2,38	2,46	2,55	2,63	2,73	2,83	2,93	3,03	3,14	3,26	3,38	3,50	3,62	3,75	3,85	4,00
	29º	2,26	2,33	2,37	2,40	2,45	2,50	2,57	2,64	2,73	2,82	2,91	2,99	3,11	3,22	3,34	3,45	3,58	3,70	3,84	3,97	4,11	4,25	4,39	4,54
	30º	2,56	2,64	2,67	2,72	2,77	2,83	2,90	2,98	3,08	3,18	3,28	3,38	3,50	3,62	3,75	3,88	4,02	4,16	4,30	4,46	4,61	4,76	4,92	5,07

Note: This table may be used to convert the specific gravity d_{20}^{t} to the specific gravity d_{20}^{20}.

TABLE VI

Temperature corrections *c* to the density of natural musts and of concentrated musts measured with a pycnometer or hydrometer of ordinary glass at *t* ºC to relate the result to 20 ºC

$$\rho_{20} = \rho_t \pm \frac{c}{1000} \left\{ \begin{array}{l} - \text{ if } t \text{ ºC is lower than 20 ºC} \\ + \text{ if } t \text{ ºC is higher than 20 ºC} \end{array} \right.$$

		Density																							
		1,05	1,06	1,07	1,08	1,09	1,10	1,11	1,12	1,13	1,14	1,15	1,16	1,18	1,20	1,22	1,24	1,26	1,28	1,30	1,32	1,34	1,36
Temperature (ºC)	10º	2,17	2,34	2,52	2,68	2,85	2,99	3,16	3,29	3,44	3,58	3,73	3,86	4,13	4,36	4,60	4,82	5,02	5,25	5,39	5,56	5,73	5,87
	11º	2,00	2,16	2,29	2,44	2,59	2,73	2,86	2,99	3,12	3,24	3,37	3,48	3,71	3,94	4,15	4,33	4,52	4,69	4,85	5,01	5,15	5,29
	12º	1,81	1,95	2,08	2,21	2,34	2,47	2,58	2,70	2,82	2,92	3,03	3,14	3,35	3,55	3,72	3,90	4,07	4,23	4,37	4,52	4,64	4,77
	13º	1,62	1,74	1,85	1,96	2,07	2,17	2,28	2,38	2,48	2,59	2,68	2,77	2,94	3,11	3,28	3,44	3,54	3,72	3,86	3,99	4,12	4,24
	14º	1,44	1,54	1,64	1,73	1,82	1,92	2,00	2,08	2,17	2,25	2,34	2,42	2,57	2,73	2,86	2,99	3,12	3,24	3,35	3,46	3,57	3,65
	15º	1,21	1,29	1,37	1,45	1,53	1,60	1,68	1,75	1,82	1,89	1,97	2,03	2,16	2,28	2,40	2,51	2,61	2,71	2,80	2,89	2,94	3,01
	16º	1,00	1,06	1,12	1,19	1,25	1,31	1,37	1,43	1,49	1,54	1,60	1,65	1,75	1,84	1,94	2,02	2,09	2,17	2,23	2,30	2,36	2,42
	17º	0,76	0,82	0,86	0,91	0,96	1,00	1,05	1,09	1,14	1,18	1,22	1,25	1,32	1,39	1,46	1,52	1,57	1,63	1,67	1,71	1,75	1,79
	18º	0,53	0,56	0,59	0,63	0,65	0,69	0,72	0,74	0,77	0,80	0,82	0,85	0,90	0,95	0,99	1,02	1,06	1,09	1,13	1,16	1,18	1,20
	19º	0,28	0,30	0,31	0,33	0,35	0,36	0,38	0,39	0,41	0,42	0,43	0,43	0,46	0,48	0,50	0,52	0,54	0,55	0,57	0,58	0,59	0,60
	20º																						
	21º	0,28	0,29	0,31	0,33	0,34	0,36	0,37	0,39	0,40	0,41	0,43	0,44	0,46	0,48	0,51	0,54	0,56	0,57	0,58	0,59	0,60	0,60
	22º	0,55	0,58	0,61	0,64	0,67	0,70	0,73	0,76	0,78	0,81	0,84	0,87	0,93	0,97	1,02	1,06	1,09	1,12	1,15	1,17	1,19	1,19
	23º	0,85	0,90	0,95	0,99	1,04	1,08	1,12	1,16	1,21	1,25	1,29	1,32	1,39	1,46	1,52	1,58	1,62	1,68	1,72	1,75	1,77	1,79
	24º	1,15	1,19	1,25	1,31	1,37	1,43	1,48	1,54	1,60	1,65	1,71	1,76	1,86	1,95	2,04	2,11	2,17	2,23	2,29	2,33	2,35	2,37
	25º	1,44	1,52	1,59	1,67	1,74	1,81	1,88	1,95	2,02	2,09	2,16	2,22	2,34	2,45	2,55	2,64	2,74	2,81	2,87	2,90	2,92	2,96
	26º	1,76	1,84	1,93	2,02	2,10	2,18	2,25	2,33	2,41	2,49	2,56	2,64	2,78	2,91	3,03	3,15	3,26	3,37	3,47	3,55	3,62	3,60
	27º	2,07	2,16	2,26	2,36	2,46	2,56	2,65	2,74	2,83	2,91	3,00	3,07	3,24	3,39	3,55	3,69	3,82	3,94	4,04	4,14	4,23	4,30
	28º	2,39	2,51	2,63	2,74	2,85	2,96	3,06	3,16	3,28	3,38	3,48	3,57	3,75	3,92	4,08	4,23	4,37	4,51	4,62	4,73	4,80	4,86
	29º	2,74	2,86	2,97	3,09	3,22	3,34	3,46	3,57	3,69	3,80	3,90	4,00	4,20	4,39	4,58	4,74	4,90	5,05	5,19	5,31	5,40	5,48
	30º	3,06	3,21	3,35	3,50	3,63	3,77	3,91	4,02	4,15	4,28	4,40	4,52	4,75	4,96	5,16	5,35	5,52	5,67	5,79	5,91	5,99	6,04

Note: This table may be used to convert the specific gravity d_{20}^{t} to the specific gravity d_{20}^{20}.

▼**B**

TABLE VII

Temperature corrections *c* to the density of wines of 13 % vol and above containing residual sugar measured with a hydrometer or pycnometer of ordinary glass at *t* ºC to relate the result to 20 ºC

$$\rho_{20} = \rho_t \pm \frac{c}{1000} \begin{cases} - \text{ if } t \text{ ºC is lower than 20 ºC} \\ + \text{ if } t \text{ ºC is higher than 20 ºC} \end{cases}$$

		Wines of 13 % vol							Wines of 15 % vol							Wines of 17 % vol						
		Densities							Densities							Densities						
		1,000	1,020	1,040	1,060	1,080	1,100	1,120	1,000	1,020	1,040	1,060	1,080	1,100	1,120	1,000	1,020	1,040	1,060	1,080	1,100	1,120
Temperature (ºC)	10º	2,24	2,58	2,93	3,27	3,59	3,89	4,18	2,51	2,85	3,20	3,54	3,85	4,02	4,46	2,81	3,15	3,50	3,84	4,15	4,45	4,74
	11º	2,06	2,37	2,69	2,97	3,26	3,53	3,78	2,31	2,61	2,93	3,21	3,51	3,64	4,02	2,57	2,89	3,20	3,49	3,77	4,03	4,28
	12º	1,87	2,14	2,42	2,67	2,94	3,17	3,40	2,09	2,36	2,64	2,90	3,16	3,27	3,61	2,32	2,60	2,87	3,13	3,39	3,63	3,84
	13º	1,69	1,93	2,14	2,37	2,59	2,80	3,00	1,88	2,12	2,34	2,56	2,78	2,88	3,19	2,09	2,33	2,55	2,77	2,98	3,19	3,39
	14º	1,49	1,70	1,90	2,09	2,27	2,44	2,61	1,67	1,86	2,06	2,25	2,45	2,51	2,77	1,83	2,03	2,23	2,42	2,61	2,77	2,94
	15º	1,25	1,42	1,59	1,75	1,90	2,05	2,19	1,39	1,56	1,72	1,88	2,03	2,11	2,32	1,54	1,71	1,87	2,03	2,18	2,32	2,47
	16º	1,03	1,17	1,30	1,43	1,55	1,67	1,78	1,06	1,27	1,40	1,53	1,65	1,77	1,88	1,25	1,39	1,52	1,65	1,77	1,89	2,00
	17º	0,80	0,90	1,00	1,09	1,17	1,27	1,36	0,87	0,98	1,08	1,17	1,26	1,35	1,44	0,96	1,06	1,16	1,26	1,35	1,44	1,52
	18º	0,54	0,61	0,68	0,75	0,81	0,86	0,92	0,60	0,66	0,73	0,80	0,85	0,91	0,97	0,66	0,72	0,79	0,86	0,92	0,97	1,03
	19º	0,29	0,33	0,36	0,39	0,42	0,45	0,48	0,32	0,36	0,39	0,42	0,45	0,48	0,51	0,35	0,38	0,41	0,45	0,48	0,51	0,53
	20º																					
	21º	0,29	0,32	0,35	0,39	0,42	0,45	0,47	0,32	0,35	0,38	0,42	0,45	0,48	0,50	0,34	0,38	0,41	0,44	0,47	0,50	0,53
	22º	0,57	0,64	0,70	0,76	0,82	0,88	0,93	0,63	0,69	0,75	0,81	0,87	0,93	0,98	0,68	0,75	0,81	0,87	0,93	0,99	1,04
	23º	0,89	0,98	1,08	1,17	1,26	1,34	1,43	0,97	1,06	1,16	1,25	1,34	1,42	1,51	1,06	1,15	1,25	1,34	1,42	1,51	1,59
	24º	1,22	1,34	1,44	1,56	1,68	1,79	1,90	1,32	1,44	1,54	1,66	1,78	1,89	2,00	1,43	1,56	1,65	1,77	1,89	2,00	2,11
	25º	1,61	1,68	1,83	1,98	2,12	2,26	2,40	1,66	1,81	1,96	2,11	2,25	2,39	2,52	1,80	1,94	2,09	2,24	2,39	2,52	2,66
	26º	1,87	2,05	2,22	2,40	2,56	2,71	2,87	2,02	2,20	2,37	2,54	2,70	2,85	3,01	2,18	2,36	2,53	2,71	2,86	3,02	3,17
	27º	2,21	2,42	2,60	2,80	3,00	3,18	3,35	2,39	2,59	2,78	2,98	3,17	3,35	3,52	2,58	2,78	2,97	3,17	3,36	3,54	3,71
	28º	2,56	2,80	3,02	3,25	3,47	3,67	3,89	2,75	2,89	3,22	3,44	3,66	3,86	4,07	2,97	3,21	3,44	3,66	3,88	4,09	4,30
	29º	2,93	3,19	3,43	3,66	3,91	4,14	4,37	3,16	3,41	3,65	3,89	4,13	4,36	4,59	3,40	3,66	3,89	4,13	4,38	4,61	4,82
	30º	3,31	3,57	3,86	4,15	4,41	4,66	4,92	3,55	3,81	4,10	4,38	4,66	4,90	5,16	3,82	4,08	4,37	4,65	4,93	5,17	5,42

		Wines of 19 % vol							Wines of 21 % vol						
		Densities							Densities						
		1,000	1,020	1,040	1,060	1,080	1,100	1,120	1,000	1,020	1,040	1,060	1,080	1,100	1,120
Temperature (ºC)	10º	3,14	3,48	3,83	4,17	4,48	4,78	5,07	3,50	3,84	4,19	4,52	4,83	5,12	5,41
	11º	2,87	3,18	3,49	3,78	4,06	4,32	4,57	3,18	3,49	3,80	4,09	4,34	4,63	4,88
	12º	2,58	2,86	3,13	3,39	3,65	3,88	4,10	2,86	3,13	3,41	3,67	3,92	4,15	4,37
	13º	2,31	2,55	2,77	2,99	3,20	3,41	3,61	2,56	2,79	3,01	3,23	3,44	3,65	3,85
	14º	2,03	2,23	2,43	2,61	2,80	2,96	3,13	2,23	2,43	2,63	2,81	3,00	3,16	3,33
	15º	1,69	1,86	2,02	2,18	2,33	2,48	2,62	1,86	2,03	2,19	2,35	2,50	2,65	2,80
	16º	1,38	1,52	1,65	1,78	1,90	2,02	2,13	1,51	1,65	1,78	1,91	2,03	2,15	2,26
	17º	1,06	1,16	1,26	1,35	1,44	1,53	1,62	1,15	1,25	1,35	1,45	1,54	1,63	1,71
	18º	0,73	0,79	0,85	0,92	0,98	1,03	1,09	0,79	0,85	0,92	0,98	1,05	1,10	1,15
	19º	0,38	0,41	0,44	0,48	0,51	0,52	0,56	0,41	0,44	0,47	0,51	0,54	0,57	0,59
	20º														
	21º	0,37	0,41	0,44	0,47	0,50	0,53	0,56	0,41	0,44	0,47	0,51	0,54	0,57	0,59
	22º	0,75	0,81	0,87	0,93	0,99	1,04	1,10	0,81	0,88	0,94	1,00	1,06	1,10	1,17
	23º	1,15	1,30	1,34	1,43	1,51	1,60	1,68	1,25	1,34	1,44	1,63	1,61	1,70	1,78
	24º	1,55	1,67	1,77	1,89	2,00	2,11	2,23	1,68	1,80	1,90	2,02	2,13	2,25	2,36
	25º	1,95	2,09	2,24	2,39	2,53	2,67	2,71	2,11	2,25	2,40	2,55	2,69	2,83	2,97
	26º	2,36	2,54	2,71	2,89	3,04	3,20	3,35	2,55	2,73	2,90	3,07	3,22	3,38	3,54
	27º	2,79	2,99	3,18	3,38	3,57	3,75	3,92	3,01	3,20	3,40	3,59	3,78	3,96	4,13
	28º	3,20	3,44	3,66	3,89	4,11	4,32	4,53	3,46	3,69	3,93	4,15	4,36	4,58	4,77
	29º	3,66	3,92	4,15	4,40	4,64	4,87	5,08	3,95	4,20	4,43	4,68	4,92	5,15	5,36
	30º	4,11	4,37	4,66	4,94	5,22	5,46	5,71	4,42	4,68	4,97	5,25	5,53	5,77	6,02

▼B

2. EVALUATION BY REFRACTOMETRY OF THE SUGAR CONCENTRATION IN GRAPE MUSTS, CONCENTRATED GRAPE MUSTS AND RECTIFIED CONCENTRATED GRAPE MUSTS

1. PRINCIPLE OF THE METHOD

The refractive index at 20 ºC, expressed either as an absolute value or as a percentage by mass of sucrose, is given in the appropriate table to provide a means of obtaining the sugar concentration in grams per litre and in grams per kilogram for grape musts, concentrated grape musts and rectified concentrated grape musts.

2. APPARATUS

2.1. **Abbé refractometer**

The refractometer used must be fitted with a scale giving:

- either percentage by mass of sucrose to 0,1 %,
- or refractive indices to four decimal places.

The refractometer must be equipped with a thermometer having a scale extending at least from + 15 ºC to + 25 ºC and with an arrangement for circulating water enabling measurements to be made at a temperature of 20 ± 5 ºC.

The operating instructions for this instrument must be strictly adhered to, particularly with regard to calibration and the light source.

3. PREPARATION OF THE SAMPLE

3.1. **Must and concentrated must**

Pass the must, if necessary, through a dry gauze folded into four and, after discarding the first drops of the filtrate, carry out the determination on the filtered product.

3.2. **Rectified concentrated must**

Depending on the concentration, use either the rectified concentrated must itself or a solution obtained by making up 200 g of rectified concentrated must to 500 g with water, all weighings being carried out accurately.

4. PROCEDURE

Bring the sample to a temperature close to 20 ºC. Place a small test sample on the lower prism of the refractometer, taking care (because the prisms are pressed firmly against each other) that this test sample covers the glass surface uniformly. Carry out the measurement in accordance with the operating instructions of the instrument used.

Read off the percentage by mass of sucrose to within 0,1 % or read the refractive index to four decimal places.

Carry out at least two determinations on the same prepared sample.

Note the temperature *t* ºC.

5. CALCULATION

5.1. **Temperature correction**

5.1.1. Instruments graduated in percentage by mass of sucrose: use Table I to obtain the temperature correction.

5.1.2. Instruments graduated in refractive index: find the index measured at *t* ºC in Table II to obtain (column 1) the corresponding value of the percentage by mass of sucrose at *t* ºC. This value is corrected for temperature and expressed as a concentration at 20 ºC by means of Table I.

5.2. **Sugar concentration in must and concentrated must**

Find the percentage by mass of sucrose at 20 ºC in Table II and read from the same row the sugar concentration in grams per litre and

▼B

grams per kilogram. The sugar concentration is expressed in terms of invert sugar to one decimal place.

5.3. Sugar concentration in rectified concentrated must

Find the percentage by mass of sucrose at 20 ºC in Table III and read from the same row the sugar concentration in grams per litre and grams per kilogram. The sugar concentration is expressed in terms of invert sugar to one decimal place.

If the measurement was made on diluted rectified concentrated must, multiply the result by the dilution factor.

5.4. Refractive index of must, concentrated must and rectified concentrated must

Find the percentage by mass of sucrose at 20 ºC in Table II and read from the same row the refractive index at 20 ºC. This index is expressed to four decimal places.

TABLE I

Correction to be made when the percentage by mass of sucrose has been determined at a temperature different from 20 ºC

Temperature ºC	Sucrose in grams per 100 grams of product									
	5	10	15	20	30	40	50	60	70	75
	Subtract									
15	0,25	0,27	0,31	0,31	0,34	0,35	0,36	0,37	0,36	0,36
16	0,21	0,23	0,27	0,27	0,29	0,31	0,31	0,32	0,31	0,23
17	0,16	0,18	0,20	0,20	0,22	0,23	0,23	0,23	0,20	0,17
18	0,11	0,12	0,14	0,15	0,16	0,16	0,15	0,12	0,12	0,09
19	0,06	0,07	0,08	0,08	0,08	0,09	0,09	0,08	0,07	0,05
	Add									
21	0,06	0,07	0,07	0,07	0,07	0,07	0,07	0,07	0,07	0,07
22	0,12	0,14	0,14	0,14	0,14	0,14	0,14	0,14	0,14	0,14
23	0,18	0,20	0,20	0,21	0,21	0,21	0,21	0,22	0,22	0,22
24	0,24	0,26	0,26	0,27	0,28	0,28	0,28	0,28	0,29	0,29
25	0,30	0,32	0,32	0,34	0,36	0,36	0,36	0,36	0,36	0,37

Temperatures must not differ from 20 ºC by more than ± 5 ºC.

▼B

TABLE II

Table giving the sugar concentration (¹) in must and concentrated must in grams per litre and grams per kilogram, determined by means of a refractometer graduated either in percentage by mass of sucrose at 20 ºC or in refractive index at 20 ºC. The density at 20 ºC is also given

Sucrose % (m/m)	Refractive index at 20 ºC	Density at 20 ºC	Sugar in g/l	Sugar in g/kg	Alcoholic strength % vol at 20 ºC
10.0	1.34781	1.0390	82.3	79.2	4,89
10.1	1.34798	1.0394	83.4	80.2	4,95
10.2	1.34814	1.0398	84.5	81.3	5,02
10.3	1.34830	1.0402	85.6	82.2	5,09
10.4	1.34845	1.0406	86.6	83.2	5,14
10.5	1.34860	1.0410	87.6	84.1	5,20
10.6	1.34875	1.0414	88.6	85.1	5,26
10.7	1.34890	1.0419	89.7	86.1	5,33
10.8	1.34906	1.0423	90.8	87.1	5,39
10.9	1.34921	1.0427	91.8	88.1	5,45
11.0	1.34936	1.0431	92.9	89.1	5,52
11.1	1.34952	1.0435	94.0	90.0	5,58
11.2	1.34968	1.0439	95.0	91.0	5,64
11.3	1.34984	1.0443	96.1	92.0	5,71
11.4	1.34999	1.0447	97.1	92.9	5,77
11.5	1.35015	1.0452	98.2	94.0	5,83
11.6	1.35031	1.0456	99.3	95.0	5,90
11.7	1.35046	1.0460	100.3	95.9	5,96
11.8	1.35062	1.0464	101.4	96.9	6,02
11.9	1.35077	1.0468	102.5	97.9	6,09
12.0	1.35092	1.0473	103.6	98.9	6,15
12.1	1.35108	1.0477	104.7	99.9	6,22
12.2	1.35124	1.0481	105.7	100.8	6,28
12.3	1.35140	1.0485	106.8	101.9	6,35
12.4	1.35156	1.0489	107.9	102.9	6,41
12.5	1.35172	1.0494	109.0	103.8	6,47
12.6	1.35187	1.0498	110.0	104.8	6,53
12.7	1.35203	1.0502	111.1	105.8	6,60
12.8	1.35219	1.0506	112.2	106.8	6,66
12.9	1.35234	1.0510	113.2	107.8	6,73
13.0	1.35249	1.0514	114.3	108.7	6,79
13.1	1.35266	1.0519	115.4	109.7	6,86
13.2	1.35282	1.0523	116.5	110.7	6,92
13.3	1.35298	1.0527	117.6	111.7	6,99
13.4	1.35313	1.0531	118.6	112.6	7,05
13.5	1.35329	1.0536	119.7	113.6	7,11
13.6	1.35345	1.0540	120.8	114.6	7,18
13.7	1.35360	1.0544	121.8	115.6	7,24
13.8	1.35376	1.0548	122.9	116.5	7,30
13.9	1.35391	1.0552	124.0	117.5	7,37
14.0	1.35407	1.0557	125.1	118.5	7,43
14.1	1.35424	1.0561	126.2	119.5	7,50
14.2	1.35440	1.0565	127.3	120.5	7,56
14.3	1.35456	1.0569	128.4	121.5	7,63
14.4	1.35472	1.0574	129.5	122.5	7,69
14.5	1.35488	1.0578	130.6	123.4	7,76
14.6	1.35503	1.0582	131.6	124.4	7,82
14.7	1.35519	1.0586	132.7	125.4	7,88
14.8	1.35535	1.0591	133.8	126.3	7,95
14.9	1.35551	1.0595	134.9	127.3	8,01

(¹) The sugar concentration is expressed in terms of invert sugar.

▼**B**

Sucrose % (m/m)	Refractive index at 20 °C	Density at 20 °C	Sugar in g/l	Sugar in g/kg	Alcoholic strength % vol at 20 °C
15.0	1.35567	1.0599	136.0	128.3	8,08
15.1	1.35583	1.0603	137.1	129.3	8,15
15.2	1.35599	1.0608	138.2	130.3	8,21
15.3	1.35615	1.0612	139.3	131.3	8,27
15.4	1.35631	1.0616	140.4	132.3	8,34
15.5	1.35648	1.0621	141.5	133.2	8,41
15.6	1.35664	1.0625	142.6	134.2	8,47
15.7	1.35680	1.0629	143.7	135.2	8,54
15.8	1.35696	1.0633	144.8	136.2	8,60
15.9	1.35712	1.0638	145.9	137.2	8,67
16.0	1.35728	1.0642	147.0	138.1	8,73
16.1	1.35744	1.0646	148.1	139.1	8,80
16.2	1.35760	1.0651	149.2	140.1	8,86
16.3	1.35776	1.0655	150.3	141.1	8,93
16.4	1.35793	1.0660	151.5	142.1	9,00
16.5	1.35809	1.0664	152.6	143.1	9,06
16.6	1.35825	1.0668	153.7	144.1	9,13
16.7	1.35842	1.0672	154.8	145.0	9,20
16.8	1.35858	1.0677	155.9	146.0	9,26
16.9	1.35874	1.0681	157.0	147.0	9,33
17.0	1.35890	1.0685	158.1	148.0	9,39
17.1	1.35907	1.0690	159.3	149.0	9,46
17.2	1.35923	1.0694	160.4	150.0	9,53
17.3	1.35939	1.0699	161.5	151.0	9,59
17.4	1.35955	1.0703	162.6	151.9	9,66
17.5	1.35972	1.0707	163.7	152.9	9,73
17.6	1.35988	1.0711	164.8	153.9	9,79
17.7	1.36004	1.0716	165.9	154.8	9,86
17.8	1.36020	1.0720	167.0	155.8	9,92
17.9	1.36036	1.0724	168.1	156.8	9,99
18.0	1.36053	1.0729	169.3	157.8	10,06
18.1	1.36070	1.0733	170.4	158.8	10,12
18.2	1.36086	1.0738	171.5	159.7	10,19
18.3	1.36102	1.0742	172.6	160.7	10,25
18.4	1.36119	1.0746	173.7	161.6	10,32
18.5	1.36136	1.0751	174.9	162.6	10,39
18.6	1.36152	1.0755	176.0	163.6	10,46
18.7	1.36169	1.0760	177.2	164.6	10,53
18.8	1.36185	1.0764	178.3	165.6	10,59
18.9	1.36201	1.0768	179.4	166.6	10,66
19.0	1.36217	1.0773	180.5	167.6	10,72
19.1	1.36234	1.0777	181.7	168.6	10,80
19.2	1.36251	1.0782	182.8	169.5	10,86
19.3	1.36267	1.0786	183.9	170.5	10,93
19.4	1.36284	1.0791	185.1	171.5	11,00
19.5	1.36301	1.0795	186.3	172.5	11,07
19.6	1.36318	1.0800	187.4	173.5	11,13
19.7	1.36335	1.0804	188.6	174.5	11,21
19.8	1.36351	1.0809	189.7	175.5	11,27
19.9	1.36367	1.0813	190.8	176.5	11,34

▼**B**

Sucrose % (m/m)	Refractive index at 20 ºC	Density at 20 ºC	Sugar in g/l	Sugar in g/kg	Alcoholic strength % vol at 20 ºC
20.0	1.36383	1.0817	191.9	177.4	11,40
20.1	1.36400	1.0822	193.1	178.4	11,47
20.2	1.36417	1.0826	194.2	179.4	11,54
20.3	1.36434	1.0831	195.3	180.4	11,60
20.4	1.36451	1.0835	196.5	181.4	11,67
20.5	1.36468	1.0840	197.7	182.3	11,75
20.6	1.36484	1.0844	198.8	183.3	11,81
20.7	1.36501	1.0849	200.0	184.3	11,88
20.8	1.36518	1.0853	201.1	185.3	11,96
20.9	1.36534	1.0857	202.2	186.2	12,01
21.0	1.36550	1.0862	203.3	187.2	12,08
21.1	1.36568	1.0866	204.5	188.2	12,15
21.2	1.36585	1.0871	205.7	189.2	12,22
21.3	1.36601	1.0875	206.8	190.2	12,29
21.4	1.36618	1.0880	207.9	191.1	12,35
21.5	1.36635	1.0884	209.1	192.1	12,42
21.6	1.36652	1.0889	210.3	193.1	12,49
21.7	1.36669	1.0893	211.4	194.1	12,56
21.8	1.36685	1.0897	212.5	195.0	12,63
21.9	1.36702	1.0902	213.6	196.0	12,69
22.0	1.36719	1.0906	214.8	196.9	12,76
22.1	1.36736	1.0911	216.0	198.0	12,83
22.2	1.36753	1.0916	217.2	199.0	12,90
22.3	1.36770	1.0920	218.3	199.9	12,97
22.4	1.36787	1.0925	219.5	200.9	13,04
22.5	1.36804	1.0929	220.6	201.8	13,11
22.6	1.36820	1.0933	221.7	202.8	13,17
22.7	1.36837	1.0938	222.9	203.8	13,24
22.8	1.36854	1.0943	224.1	204.8	13,31
22.9	1.36871	1.0947	225.2	205.8	13,38
23.0	1.36888	1.0952	226.4	206.7	13,45
23.1	1.36905	1.0956	227.6	207.7	13,52
23.2	1.36922	1.0961	228.7	208.7	13,59
23.3	1.36939	1.0965	229.9	209.7	13,66
23.4	1.36956	1.0970	231.1	210.7	13,73
23.5	1.36973	1.0975	232.3	211.6	13,80
23.6	1.36991	1.0979	233.4	212.6	13,87
23.7	1.37008	1.0984	234.6	213.6	13,94
23.8	1.37025	1.0988	235.8	214.6	14,01
23.9	1.37042	1.0993	237.0	215.6	14,08
24.0	1.37059	1.0998	238.2	216.6	14,15
24.1	1.37076	1.1007	239.3	217.4	14,22
24.2	1.37093	1.1011	240.3	218.2	14,28
24.3	1.37110	1.1016	241.6	219.4	14,35
24.4	1.37128	1.1022	243.0	220.5	14,44
24.5	1.37145	1.1026	244.0	221.3	14,50
24.6	1.37162	1.1030	245.0	222.1	14,56
24.7	1.37180	1.1035	246.4	223.2	14,64
24.8	1.37197	1.1041	247.7	224.4	14,72
24.9	1.37214	1.1045	248.7	225.2	14,78

▼B

Sucrose % (m/m)	Refractive index at 20 ºC	Density at 20 ºC	Sugar in g/l	Sugar in g/kg	Alcoholic strength % vol at 20 ºC
25.0	1.37232	1.1049	249.7	226.0	14,84
25.1	1.37249	1.1053	250.7	226.8	14,90
25.2	1.37266	1.1057	251.7	227.6	14,96
25.3	1.37283	1.1062	253.0	228.7	15,03
25.4	1.37300	1.1068	254.4	229.9	15,11
25.5	1.37317	1.1072	255.4	230.7	15,17
25.6	1.37335	1.1076	256.4	231.5	15,23
25.7	1.37353	1.1081	257.8	232.6	15,32
25.8	1.37370	1.1087	259.1	233.7	15,39
25.9	1.37387	1.1091	260.1	234.5	15,45
26.0	1.37405	1.1095	261.1	235.3	15,51
26.1	1.37423	1.1100	262.5	236.4	15,60
26.2	1.37440	1.1106	263.8	237.5	15,67
26.3	1.37457	1.1110	264.8	238.3	15,73
26.4	1.37475	1.1114	265.8	239.2	15,79
26.5	1.37493	1.1119	267.2	240.3	15,88
26.6	1.37510	1.1125	268.5	241.4	15,95
26.7	1.37528	1.1129	269.5	242.2	16,01
26.8	1.37545	1.1133	270.5	243.0	16,07
26.9	1.37562	1.1138	271.8	244.1	16,15
27.0	1.37580	1.1144	273.2	245.2	16,23
27.1	1.37598	1.1148	274.2	246.0	16,29
27.2	1.37615	1.1152	275.2	246.8	16,35
27.3	1.37632	1.1157	276.5	247.9	16,43
27.4	1.37650	1.1163	277.9	249.0	16,51
27.5	1.37667	1.1167	278.9	249.8	16,57
27.6	1.37685	1.1171	279.9	250.6	16,63
27.7	1.37703	1.1176	281.3	251.6	16,71
27.8	1.37721	1.1182	282.6	252.7	16,79
27.9	1.37739	1.1186	283.6	253.5	16,85
28.0	1.37757	1.1190	284.6	254.3	16,91
28.1	1.37775	1.1195	286.0	255.4	16,99
28.2	1.37793	1.1201	287.3	256.5	17,07
28.3	1.37810	1.1205	288.3	257.3	17,13
28.4	1.37828	1.1209	289.3	258.1	17,19
28.5	1.37846	1.1214	290.7	259.2	17,27
28.6	1.37863	1.1220	292.0	260.3	17,35
28.7	1.37881	1.1224	293.0	261.0	17,41
28.8	1.37899	1.1228	294.0	261.8	17,47
28.9	1.37917	1.1233	295.3	262.9	17,55
29.0	1.37935	1.1239	296.7	264.0	17,63
29.1	1.37953	1.1244	298.1	265.1	17,71
29.2	1.37971	1.1250	299.4	266.1	17,79
29.3	1.37988	1.1254	300.4	266.9	17,85
29.4	1.38006	1.1258	301.4	267.7	17,91
29.5	1.38024	1.1263	302.8	268.8	17,99
29.6	1.38042	1.1269	304.1	269.9	18,07
29.7	1.38060	1.1273	305.1	270.6	18,13
29.8	1.38078	1.1277	306.1	271.4	18,19
29.9	1.38096	1.1282	307.4	272.5	18,26

▼**B**

Sucrose % (m/m)	Refractive index at 20 °C	Density at 20 °C	Sugar in g/l	Sugar in g/kg	Alcoholic strength % vol at 20 °C
30.0	1.38114	1.1288	308.8	273.6	18,35
30.1	1.38132	1.1293	310.0	274.5	18,42
30.2	1.38150	1.1298	311.2	275.5	18,49
30.3	1.38168	1.1302	312.4	276.4	18,56
30.4	1.38186	1.1307	313.6	277.3	18,63
30.5	1.38204	1.1312	314.8	278.3	18,70
30.6	1.38222	1.1317	316.0	279.2	18,77
30.7	1.38240	1.1322	317.2	280.2	18,85
30.8	1.38258	1.1327	318.4	281.1	18,92
30.9	1.38276	1.1332	319.6	282.0	18,99
31.0	1.38294	1.1336	320.8	283.0	19,06
31.1	1.38312	1.1341	322.0	283.9	19,13
31.2	1.38330	1.1346	323.2	284.9	19,20
31.3	1.38349	1.1351	324.4	285.8	19,27
31.4	1.38367	1.1356	325.6	286.8	19,35
31.5	1.38385	1.1361	326.8	287.7	19,42
31.6	1.38403	1.1366	328.1	288.6	19,49
31.7	1.38421	1.1371	329.3	289.6	19,56
31.8	1.38440	1.1376	330.5	290.5	19,64
31.9	1.38458	1.1380	331.7	291.5	19,71
32.0	1.38476	1.1385	332.9	292.4	19,78
32.1	1.38494	1.1391	334.2	293.4	19,86
32.2	1.38513	1.1396	335.5	294.4	19,93
32.3	1.38531	1.1401	336.7	295.4	20,00
32.4	1.38550	1.1406	338.0	296.4	20,08
32.5	1.38568	1.1411	339.3	297.3	20,16
32.6	1.38586	1.1416	340.6	298.3	20,24
32.7	1.38605	1.1422	341.9	299.3	20,31
32.8	1.38623	1.1427	343.1	300.3	20,38
32.9	1.38642	1.1432	344.4	301.3	20,46
33.0	1.38660	1.1437	345.7	302.3	20,54
33.1	1.38678	1.1442	346.9	303.2	20,61
33.2	1.38697	1.1447	348.1	304.1	20,68
33.3	1.38715	1.1452	349.3	305.0	20,75
33.4	1.38734	1.1457	350.5	305.9	20,82
33.5	1.38753	1.1461	351.7	306.9	20,90
33.6	1.38771	1.1466	352.9	307.8	20,97
33.7	1.38790	1.1471	354.1	308.7	21,04
33.8	1.38808	1.1476	355.3	309.6	21,11
33.9	1.38827	1.1481	356.5	310.5	21,18
34.0	1.38845	1.1486	357.7	311.4	21,25
34.1	1.38864	1.1491	359.0	312.4	21,33
34.2	1.38882	1.1496	360.3	313.4	21,41
34.3	1.38901	1.1501	361.5	314.3	21,48
34.4	1.38919	1.1506	362.8	315.3	21,55
34.5	1.38938	1.1512	364.1	316.3	21,63
34.6	1.38957	1.1517	365.4	317.3	21,71
34.7	1.38975	1.1522	366.7	318.2	21,79
34.8	1.38994	1.1527	367.9	319.2	21,86
34.9	1.39012	1.1532	369.2	320.2	21,94

▼B

Sucrose % (m/m)	Refractive index at 20 ºC	Density at 20 ºC	Sugar in g/l	Sugar in g/kg	Alcoholic strength % vol at 20 ºC
35.0	1.39031	1.1537	370.5	321.1	22,01
35.1	1.39050	1.1543	371.8	322.1	22,09
35.2	1.39069	1.1548	373.0	323.0	22,16
35.3	1.39087	1.1553	374.3	324.0	22,24
35.4	1.39106	1.1558	375.6	325.0	22,32
35.5	1.39125	1.1563	376,9	325.9	22,39
35.6	1.39144	1.1568	378.1	326.9	22,45
35.7	1.39163	1.1573	379.4	327.8	22,54
35.8	1.39181	1.1579	380.7	328.8	22,62
35.9	1.39200	1.1584	381.9	329.7	22,69
36.0	1.39219	1.1589	383.2	330.7	22,77
36.1	1.39238	1.1594	384.5	331.6	22,85
36.2	1.39257	1.1599	385.8	332.6	22,92
36.3	1.39276	1.1604	387.0	333.5	22,99
36.4	1.39295	1.1610	388.3	334.5	23,07
36.5	1.39314	1.1615	389.6	335.4	23,15
36.6	1.39332	1.1620	390.9	336.4	23,22
36.7	1.39351	1.1625	392.2	337.3	23,30
36.8	1.39370	1.1630	393.4	338.3	23,37
36.9	1.39389	1.1635	394.7	339.2	23,45
37.0	1.39408	1.1641	396.0	340.2	23,53
37.1	1.39427	1.1646	397.3	341.1	23,60
37.2	1.39446	1.1651	398.6	342.1	23,68
37.3	1.39465	1.1656	399.8	343.0	23,75
37.4	1.39484	1.1661	401.1	344.0	23,83
37.5	1.39504	1.1666	402.4	344.9	23,91
37.6	1.39523	1.1672	403.7	345.9	23,99
37.7	1.39542	1.1677	405.0	346.8	24,06
37.8	1.39561	1.1682	406.2	347.7	24,13
37.9	1.39580	1.1687	407.5	348.7	24,21
38.0	1.39599	1.1692	408.8	349.6	24,29
38.1	1.39618	1.1698	410.1	350.6	24,37
38.2	1.39637	1.1703	411.3	351.5	24,44
38.3	1.39657	1.1708	412.6	352.4	24,51
38.4	1.39676	1.1713	413.9	353.4	24,59
38.5	1.39695	1.1718	415.2	354.3	24,67
38.6	1.39714	1.1723	416.4	355.2	24,74
38.7	1.39733	1.1728	417.7	356.1	24,82
38.8	1.39753	1.1733	419.0	357.1	24,90
38.9	1.39772	1.1739	420.2	358.0	24,97
39.0	1.39791	1.1744	421.5	358.9	25,04
39.1	1.39810	1.1749	422.8	359.8	25,12
39.2	1.39830	1.1754	424.1	360.8	25,20
39.3	1.39849	1.1759	425.3	361.7	25,27
39.4	1.39869	1.1764	426.6	362.6	25,35
39.5	1.39888	1.1770	427.9	363.6	25,42
39.6	1.39907	1.1775	429.2	364.5	25,50
39.7	1.39927	1.1780	430.5	365.4	25,58
39.8	1.39946	1.1785	431.7	366.3	25,65
39.9	1.39966	1.1790	433.0	367.3	25,73

▼**B**

Sucrose % (m/m)	Refractive index at 20 °C	Density at 20 °C	Sugar in g/l	Sugar in g/kg	Alcoholic strength % vol at 20 °C
40.0	1.39985	1.1796	434.3	368.2	25,80
40.1	1.40004	1.1801	435.6	369.2	25,88
40.2	1.40024	1.1806	437.0	370.1	25,96
40.3	1.40043	1.1812	438.3	371.1	26,04
40.4	1.40063	1.1817	439.7	372.1	26,12
40.5	1.40083	1.1823	441.0	373.0	26,20
40.6	1.40102	1.1828	442.3	374.0	26,28
40.7	1.40122	1.1833	443.7	374.9	26,36
40.8	1.40141	1.1839	445.0	375.9	26,44
40.9	1.40161	1.1844	446.4	376.9	26,52
41.0	1.40180	1.1850	447.7	377.8	26,60
41.1	1.40200	1.1855	449.0	378.7	26,68
41.2	1.40219	1.1860	450.2	379.6	26,75
41.3	1.40239	1.1865	451.5	380.5	26,83
41.4	1.40259	1.1870	452.8	381.4	26,90
41.5	1.40279	1.1875	454.1	382.3	26,98
41.6	1.40298	1.1881	455.3	383.2	27,05
41.7	1.40318	1.1886	456.6	384.2	27,13
41.8	1.40338	1.1891	457.9	385.1	27,21
41.9	1.40357	1.1896	459.1	386.0	27,28
42.0	1.40377	1.1901	460.4	386.9	27,35
42.1	1.40397	1.1907	461.7	387.8	27,43
42.2	1.40417	1.1912	463.1	388.8	27,52
42.3	1.40436	1.1917	464.4	389.7	27,59
42.4	1.40456	1.1923	465.8	390.7	27,68
42.5	1.40476	1.1928	467.2	391.6	27,76
42.6	1.40496	1.1934	468.5	392.6	27,84
42.7	1.40516	1.1939	469.9	393.5	27,92
42.8	1.40535	1.1945	471.2	394.5	28,00
42.9	1.40555	1.1950	472.6	395.4	28,08
43.0	1.40575	1.1956	473.9	396.4	28,16
43.1	1.40595	1.1961	475.2	397.3	28,23
43.2	1.40615	1.1967	476.6	398.3	28,32
43.3	1.40635	1.1972	477.9	399.2	28,40
43.4	1.40655	1.1977	479.3	400.1	28,48
43.5	1.40675	1.1983	480.6	401.1	28,56
43.6	1.40695	1.1988	481.9	402.0	28,63
43.7	1.40715	1.1994	483.3	402.9	28,72
43.8	1.40735	1.1999	484.6	403.9	28,79
43.9	1.40755	1.2005	486.0	404.8	28,88
44.0	1.40775	1.2010	487.3	405.7	28,95
44.1	1.40795	1.2015	488.6	406.7	29,03
44.2	1.40815	1.2021	490.0	407.6	29,11
44.3	1.40836	1.2026	491.3	408.5	29,19
44.4	1.40856	1.2032	492.7	409.5	29,27
44.5	1.40876	1.2037	494.0	410.4	29,35
44.6	1.40896	1.2042	495.3	411.3	29,43
44.7	1.40916	1.2048	496.7	412.3	29,51
44.8	1.40937	1.2053	498.0	413.2	29,59
44.9	1.40957	1.2059	499.4	414.1	29,67

▼**B**

Sucrose % (m/m)	Refractive index at 20 ºC	Density at 20 ºC	Sugar in g/l	Sugar in g/kg	Alcoholic strength % vol at 20 ºC
45.0	1.40977	1.2064	500.7	415.0	29,75
45.1	1.40997	1.2070	502.1	416.0	29,83
45.2	1.41018	1.2076	503.5	417.0	29,92
45.3	1.41038	1.2081	504.9	417.9	30,00
45.4	1.41058	1.2087	506.3	418.9	30,08
45.5	1.41079	1.2093	507.8	419.9	30,17
45.6	1.41099	1.2098	509.2	420.9	30,25
45.7	1.41119	1.2104	510.6	421.8	30,34
45.8	1.41139	1.2110	512.0	422.8	30,42
45.9	1.41160	1.2115	513.4	423.7	30,50
46.0	1.41180	1.2121	514.8	424.7	30,59
46.1	1.41200	1.2127	516.1	425.6	30,66
46.2	1.41221	1.2132	517.5	426.5	30,75
46.3	1.41241	1.2137	518.8	427.5	30,82
46.4	1.41262	1.2143	520.2	428.4	30,91
46.5	1.41282	1.2148	521.5	429.3	30,99
46.6	1.41302	1.2154	522.8	430.2	31,06
46.7	1.41323	1.2159	524.2	431.1	31,15
46.8	1.41343	1.2165	525.5	432.0	31,22
46.9	1.41364	1.2170	526.9	432.9	31,31
47.0	1.41384	1.2175	528.2	433.8	31,38
47.1	1.41405	1.2181	529.6	434.8	31,47
47.2	1.41425	1.2187	531.0	435.7	31,55
47.3	1.41446	1.2192	532.4	436.7	31,63
47.4	1.41466	1.2198	533.8	437.6	31,72
47.5	1.41487	1.2204	535.3	438.6	31,81
47.6	1.41508	1.2210	536.7	439.5	31,89
47.7	1.41528	1.2215	538.1	440.5	31,97
47.8	1.41549	1.2221	539.5	441.4	32,05
47.9	1.41569	1.2227	540.9	442.4	32,14
48.0	1.41590	1.2232	542.3	443.3	32,22
48.1	1.41611	1.2238	543.6	444.2	32,30
48.2	1.41632	1.2243	545.0	445.1	32,38
48.3	1.41652	1.2249	546.3	446.0	32,46
48.4	1.41673	1.2254	547.7	446.9	32,59
48.5	1.41694	1.2260	549.1	447.8	32,63
48.6	1.41715	1.2265	550.4	448.7	32,70
48.7	1.41736	1.2271	551.8	449.7	32,79
48.8	1.41756	1.2276	553.1	450.6	32,86
48.9	1.41777	1.2282	554.5	451.4	32,95
49.0	1.41798	1.2287	555.8	452.3	33,02
49.1	1.41819	1.2293	557.2	453.3	33,11
49.2	1.41840	1.2298	558.6	454.2	33,19
49.3	1.41861	1.2304	560.0	455.1	33,27
49.4	1.41882	1.2310	561.4	456.1	33,36
49.5	1.41903	1.2315	562.8	457.0	33,44
49.6	1.41924	1.2321	564.2	457.9	33,52
49.7	1.41945	1.2327	565.6	458.8	33,61
49.8	1.41966	1.2332	567.0	459.8	33,69
49.9	1.41987	1.2338	568.4	460.7	33,77

▼B

Sucrose % (m/m)	Refractive index at 20 °C	Density at 20 °C	Sugar in g/l	Sugar in g/kg	Alcoholic strength % vol at 20 °C
50.0	1.42008	1.2344	569.8	461.6	33,86
50.1	1.42029	1.2349	571.2	462.5	33,94
50.2	1.42050	1.2355	572.6	463.5	34,02
50.3	1.42071	1.2361	574.0	464.4	34,10
50.4	1.42092	1.2366	575.4	465.3	34,19
50.5	1.42114	1.2372	576.9	466.2	34,28
50.6	1.42135	1.2378	578.3	467.2	34,36
50.7	1.42156	1.2384	579.7	468.1	34,44
50.8	1.42177	1.2389	581.1	469.0	34,53
50.9	1.42198	1.2395	582.5	469.9	34,61
51.0	1.42219	1.2401	583.9	470.9	34,69
51.1	1.42240	1.2407	585.4	471.8	34,78
51.2	1.42261	1.2413	586.9	472.8	34,87
51.3	1.42283	1.2419	588.3	473.8	34,95
51.4	1.42304	1.2425	589.8	474.7	35,04
51.5	1.42325	1.2431	591.3	475.7	35,13
51.6	1.42346	1.2437	592.8	476.6	35,22
51.7	1.42367	1.2443	594.3	477.6	35,31
51.8	1.42389	1.2449	595.7	478.6	35,39
51.9	1.42410	1.2455	597.2	479.5	35,48
52.0	1.42431	1.2461	598.7	480.5	35,57
52.1	1.42452	1.2466	600.1	481.4	35,65
52.2	1.42474	1.2472	601.5	482.3	35,74
52.3	1.42495	1.2478	602.9	483.2	35,82
52.4	1.42517	1.2483	604.3	484.1	35,91
52.5	1.42538	1.2489	605.8	485.0	35,99
52.6	1.42559	1.2495	607.2	485.9	36,08
52.7	1.42581	1.2500	608.6	486.8	36,16
52.8	1.42602	1.2506	610.0	487.7	36,24
52.9	1.42624	1.2512	611.4	488.6	36,33
53.0	1.42645	1.2518	612.8	489.6	36,41
53.1	1.42666	1.2524	614.3	490.5	36,50
53.2	1.42686	1.2530	615.8	491.4	36,59
53.3	1.42707	1.2536	617.2	492.4	36,67
53.4	1.42727	1.2542	618.7	493.3	36,76
53.5	1.42748	1.2548	620.2	494.3	36,85
53.6	1.42769	1.2554	621.7	495.2	36,94
53.7	1.42789	1.2560	623.2	496.2	37,03
53.8	1.42810	1.2566	624.6	497.1	37,11
53.9	1.42830	1.2571	626.1	498.0	37,20
54.0	1.42851	1.2577	627.6	499.0	37,29
54.1	1.42874	1.2583	629.0	499.9	37,37
54.2	1.42897	1.2589	630.4	500.8	37,45
54.3	1.42919	1.2595	631.8	501.7	37,54
54.4	1.42942	1.2600	633.2	502.6	37,62
54.5	1.42965	1.2606	634.7	503.5	37,71
54.6	1.42988	1.2612	636.1	504.3	37,79
54.7	1.43011	1.2617	637.5	505.2	37,88
54.8	1.43033	1.2623	638.9	506.1	37,96
54.9	1.43056	1.2629	640.3	507.0	38,04

▼**B**

Sucrose % (m/m)	Refractive index at 20 °C	Density at 20 °C	Sugar in g/l	Sugar in g/kg	Alcoholic strength % vol at 20 °C
55.0	1.43079	1.2635	641.7	507.9	38,11
55.1	1.43101	1.2640	643.2	508.8	38,22
55.2	1.43123	1.2646	644.6	509.7	38,30
55.3	1.43145	1.2652	646.1	510.7	38,39
55.4	1.43167	1.2658	647.6	511.6	38,48
55.5	1.43189	1.2664	649.1	512.5	38,57
55.6	1.43210	1.2670	650.5	513.4	38,65
55.7	1.43232	1.2676	652.0	514.3	38,74
55.8	1.43254	1.2682	653.5	515.3	38,83
55.9	1.43276	1.2688	654.9	516.2	38,91
56.0	1.43298	1.2694	656.4	517.1	39,00
56.1	1.43320	1.2700	657.9	518.0	39,09
56.2	1.43342	1.2706	659.4	518.9	39,18
56.3	1.43364	1.2712	660.8	519.9	39,26
56.4	1.43386	1.2718	662.3	520.8	39,35
56.5	1.43409	1.2724	663.8	521.7	39,44
56.6	1.43431	1.2730	665.3	522.6	39,53
56.7	1.43453	1.2736	666.8	523.5	39,62
56.8	1.43475	1.2742	668.2	524.4	39,70
56.9	1.43497	1.2748	669.7	525.4	39,79
57.0	1.43519	1.2754	671.2	526.3	39,88
57.1	1.43541	1.2760	672.7	527.2	39,97
57.2	1.43563	1.2766	674.3	528.2	40,06
57.3	1.43586	1.2773	675.8	529.1	40,15
57.4	1.43608	1.2779	677.4	530.1	40,25
57.5	1.43630	1.2785	678.9	531.0	40,34
57.6	1.43652	1.2791	680.4	532.0	40,43
57.7	1.43674	1.2797	682.0	532.9	40,52
57.8	1.43697	1.2804	683.5	533.8	40,61
57.9	1.43719	1.2810	685.1	534.8	40,70
58.0	1.43741	1.2816	686.6	535.7	40,80
58.1	1.43763	1.2822	688.1	536.6	40,88
58.2	1.43786	1.2828	689.6	537.5	40,97
58.3	1.43808	1.2834	691.0	538.4	41,06
58.4	1.43831	1.2840	692.5	539.3	41,14
58.5	1.43854	1.2846	694.0	540.2	41,23
58.6	1.43876	1.2852	695.5	541.1	41,32
58.7	1.43899	1.2858	697.0	542.0	41,41
58.8	1.43921	1.2864	698.4	542.9	41,50
58.9	1.43944	1.2870	699.9	543.8	41,58
59.0	1.43966	1.2876	701.4	544.7	41,67
59.1	1.43989	1.2882	702.9	545.7	41,76
59.2	1.44011	1.2888	704.5	546.6	41,86
59.3	1.44034	1.2895	706.0	547.5	41,95
59.4	1.44056	1.2901	707.6	548.5	42,04
59.5	1.44079	1.2907	709.1	549.4	42,13
59.6	1.44102	1.2913	710.6	550.3	42,22
59.7	1.44124	1.2920	712.2	551.2	42,32
59.8	1.44147	1.2926	713.7	552.2	42,41
59.9	1.44169	1.2932	715.3	553.1	42,50

▼**B**

Sucrose % (m/m)	Refractive index at 20 ºC	Density at 20 ºC	Sugar in g/l	Sugar in g/kg	Alcoholic strength % vol at 20 ºC
60.0	1.44192	1.2938	716.8	554.0	42,59
60.1	1.44215	1.2944	718.3	554.9	42,68
60.2	1.44237	1.2950	719.8	555.8	42,77
60.3	1.44260	1.2956	721.2	556.7	42,85
60.4	1.44283	1.2962	722.7	557.6	42,94
60.5	1.44306	1.2968	724.2	558.4	43,03
60.6	1.44328	1.2974	725.7	559.3	43,12
60.7	1.44351	1.2980	727.2	560.2	43,21
60.8	1.44374	1.2986	728.6	561.1	43,29
60.9	1.44396	1.2992	730.1	562.0	43,38
61.0	1.44419	1.2998	731.6	562.8	43,47
61.1	1.44442	1.3004	733.1	563.8	43,56
61.2	1.44465	1.3011	734.7	564.7	43,65
61.3	1.44488	1.3017	736.2	565.6	43,74
61.4	1.44511	1.3023	737.8	566.5	43,84
61.5	1.44533	1.3030	739.4	567.4	43,93
61.6	1.44556	1.3036	740.9	568.4	44,02
61.7	1.44579	1.3042	742.5	569.3	44,12
61.8	1.44602	1.3048	744.0	570.2	44,21
61.9	1.44625	1.3055	745.6	571.1	44,30
62.0	1.44648	1.3061	747.1	572.0	44,39
62.1	1.44671	1.3067	748.6	572.9	44,48
62.2	1.44694	1.3073	750.2	573.8	44,57
62.3	1.44717	1.3080	751.7	574.7	44,66
62.4	1.44740	1.3086	753.3	575.6	44,76
62.5	1.44764	1.3092	754.8	576.5	44,85
62.6	1.44787	1.3098	756.3	577.4	44,94
62.7	1.44810	1.3104	757.9	578.3	45,03
62.8	1.44833	1.3111	759.4	579.2	45,12
62.9	1.44856	1.3117	761.0	580.1	45,21
63.0	1.44879	1.3123	762.5	581.0	45,31
63.1	1.44902	1.3130	764.1	582.0	45,40
63.2	1.44926	1.3136	765.7	582.9	45,49
63.3	1.44949	1.3143	767.3	583.8	45,59
63.4	1.44972	1.3149	768.9	584.8	45,69
63.5	1.44996	1.3156	770.6	585.7	45,79
63.6	1.45019	1.3162	772.2	586.6	45,88
63.7	1.45042	1.3169	773.8	587.6	45,98
63.8	1.45065	1.3175	775.4	588.5	46,07
63.9	1.45089	1.3182	777.0	589.4	46,17
64.0	1.45112	1.3188	778.6	590.4	46,26
64.1	1.45135	1.3195	780.1	591.3	46,35
64.2	1.45159	1.3201	781.7	592.1	46,45
64.3	1.45183	1.3207	783.2	593.0	46,53
64.4	1.45206	1.3213	784.8	593.9	46,63
64.5	1.45230	1.3219	786.3	594.8	46,72
64.6	1.45253	1.3226	787.8	595.7	46,81
64.7	1.45276	1.3232	789.4	596.6	46,90
64.8	1.45300	1.3238	790.9	597.5	46,99
64.9	1.45324	1.3244	792.5	598.3	47,09

▼**B**

Sucrose % (m/m)	Refractive index at 20 °C	Density at 20 °C	Sugar in g/l	Sugar in g/kg	Alcoholic strength % vol at 20 °C
65.0	1.45347	1.3251	794.0	599.2	47,18
65.1	1.45371	1.3257	795.6	600.1	47,27
65.2	1.45394	1.3264	797.2	601.1	47,37
65.3	1.45418	1.3270	798.8	602.0	47,46
65.4	1.45441	1.3277	800.4	602.9	47,56
65.5	1.45465	1.3283	802.1	603.8	47,66
65.6	1.45489	1.3290	803.7	604.7	47,75
65.7	1.45512	1.3296	805.3	605.6	47,85
65.8	1.45536	1.3303	806.9	606.6	47,94
65.9	1.45559	1.3309	808.5	607.5	48,04
66.0	1.45583	1.3316	810.1	608.4	48,13
66.1	1.45607	1.3322	811.6	609.3	48,22
66.2	1.45630	1.3328	813.2	610.1	48,32
66.3	1.45654	1.3335	814.8	611.0	48,41
66.4	1.45678	1.3341	816.3	611.9	48,50
66.5	1.45702	1.3347	817.9	612.8	48,60
66.6	1.45725	1.3353	819.4	613.6	48,69
66.7	1.45749	1.3360	820.9	614.5	48,77
66.8	1.45773	1.3366	822.5	615.4	48,87
66.9	1.45796	1.3372	824.1	616.2	48,97
67.0	1.45820	1.3378	825.6	617.1	49,05
67.1	1.45844	1.3385	827.2	618.0	49,15
67.2	1.45868	1.3391	828.8	618.9	49,24
67.3	1.45892	1.3398	830.4	619.8	49,34
67.4	1.45916	1.3404	832.0	620.7	49,43
67.5	1.45940	1.3411	833.7	621.6	49,53
67.6	1.45964	1.3418	835.3	622.5	49,63
67.7	1.45988	1.3424	836.9	623.4	49,73
67.8	1.46012	1.3431	838.5	624.3	49,82
67.9	1.46036	1.3437	840.1	625.2	49,92
68.0	1.46060	1.3444	841.7	626.1	50,01
68.1	1.46084	1.3450	843.4	627.0	50,11
68.2	1.46108	1.3457	845.1	628.0	50,21
68.3	1.46132	1.3464	846.7	628.9	50,31
68.4	1.46156	1.3471	848.4	629.8	50,41
68.5	1.46181	1.3478	850.1	630.8	50,51
68.6	1.46205	1.3484	851.8	631.7	50,61
68.7	1.46229	1.3491	853.5	632.6	50,71
68.8	1.46253	1.3498	855.1	633.5	50,81
68.9	1.46277	1.3505	856.8	634.5	50,91
69.0	1.46301	1.3512	858.5	635.4	51,01
69.1	1.46325	1.3518	860.1	636.3	51,10
69.2	1.46350	1.3525	861.7	637.2	51,20
69.3	1.46374	1.3531	863.3	638.0	51,29
69.4	1.46398	1.3538	864.9	638.9	51,39
69.5	1.46423	1.3544	866.6	639.8	51,49
69.6	1.46447	1.3551	868.2	640.7	51,58
69.7	1.46471	1.3557	869.8	641.6	51,68
69.8	1.46495	1.3564	871.4	642.4	51,78
69.9	1.46520	1.3570	873.0	643.3	51,87

▼**B**

Sucrose % (m/m)	Refractive index at 20 ºC	Density at 20 ºC	Sugar in g/l	Sugar in g/kg	Alcoholic strength % vol at 20 ºC
70.0	1.46544	1.3577	874.6	644.2	51,97
70.1	1.46568	1.3583	876.2	645.1	52,06
70.2	1.46593	1.3590	877.8	645.9	52,15
70.3	1.46618	1.3596	879.4	646.8	52,25
70.4	1.46642	1.3603	881.0	647.7	52,35
70.5	1.46667	1.3609	882.7	648.6	52,45
70.6	1.46691	1.3616	884.3	649.4	52,54
70.7	1.46715	1.2622	885.9	650.3	52,64
70.8	1.46740	1.3629	887.5	651.2	52,73
70.9	1.46765	1.3635	889.1	652.1	52,83
71.0	1.46789	1.3642	890.7	652.9	52,92
71.1	1.46814	1.3649	892.4	653.8	53,02
71.2	1.46838	1.3655	894.1	654.7	53,12
71.3	1.46863	1.3662	895.7	655.6	53,22
71.4	1.46888	1.3669	897.4	656.5	53,32
71.5	1.46913	1.3676	899.1	657.4	53,42
71.6	1.46937	1.3683	900.8	658.3	53,52
71.7	1.46962	1.3689	902.5	659.2	53,62
71.8	1.46987	1.3696	904.1	660.1	53,72
71.9	1.47011	1.3703	905.8	661.0	53,82
72.0	1.47036	1.3710	907.5	661.9	53,92
72.1	1.47061	1.3717	909.2	662.8	54,02
72.2	1.47086	1.3723	910.8	663.7	54,12
72.3	1.47110	1.3730	912.5	664.6	54,22
72.4	1.47135	1.3737	914.2	665.5	54,32
72.5	1.47160	1.3744	915.9	666.4	54,42
72.6	1.47185	1.3750	917.5	667.3	54,51
72.7	1.47210	1.3757	919.2	668.2	54,62
72.8	1.47234	1.3764	920.9	669.0	54,72
72.9	1.47259	1.3771	922.5	669.9	54,81
73.0	1.47284	1.3777	924.2	670.8	54,91
73.1	1.47309	1.3784	925.9	671.7	55,01
73.2	1.47334	1.3791	927.6	672.6	55,11
73.3	1.47359	1.3798	929.2	673.5	55,21
73.4	1.47384	1.3804	930.9	674.4	55,31
73.5	1.47409	1.3811	932.6	675.2	55,41
73.6	1.47434	1.3818	934.3	676.1	55,51
73.7	1.47459	1.3825	936.0	677.0	55,61
73.8	1.47484	1.3832	937.6	677.9	55,71
73.9	1.47509	1.3838	939.3	678.8	55,81
74.0	1.47534	1.3845	941.0	679.7	55,91
74.1	1.47559	1.3852	942.7	680.5	56,01
74.2	1.47584	1.3859	944.4	681.4	56,11
74.3	1.47609	1.3866	946.0	682.3	56,21
74.4	1.47634	1.3872	947.7	683.2	56,31
74.5	1.47660	1.3879	949.4	684.0	56,41
74.6	1.47685	1.3886	951.1	684.9	56,51
74.7	1.47710	1.3893	952.8	685.8	56,61
74.8	1.47735	1.3900	954.4	686.7	56,71
74.9	1.47760	1.3906	956.1	687.5	56,81

▼B

TABLE III

Table giving the sugar (1) concentration in rectified concentrated must in grams per litre and grams per kilogram, determined by means of a refractometer graduated either in percentage by mass of sucrose at 20 ºC or in refractive index at 20 ºC. The density at 20 ºC is also given

Sucrose % (m/m)	Refractive index at 20 ºC	Density at 20 ºC	Sugar in g/l	Sugar in g/kg	Alcoholic strength % vol at 20 ºC
50.0	1.42008	1.2342	627.6	508.5	37,28
50.1	1.42029	1.2348	629.3	509.6	37,38
50.2	1.42050	1.2355	630.9	510.6	37,48
50.3	1.42071	1.2362	632.4	511.6	37,56
50.4	1.42092	1.2367	634.1	512.7	37,66
50.5	1.42113	1.2374	635.7	513.7	37,76
50.6	1.42135	1.2381	637.3	514.7	37,85
50.7	1.42156	1.2386	638.7	515.7	37,94
50.8	1.42177	1.2391	640.4	516.8	38,04
50.9	1.42198	1.2396	641.9	517.8	38,13
51.0	1.42219	1.2401	643.4	518.8	38,22
51.1	1.42240	1.2406	645.0	519.9	38,31
51.2	1.42261	1.2411	646.5	520.9	38,40
51.3	1.42282	1.2416	648.1	522.0	38,50
51.4	1.42304	1.2421	649.6	523.0	38,59
51.5	1.42325	1.2427	651.2	524.0	38,68
51.6	1.42347	1.2434	652.9	525.1	38,78
51.7	1.42368	1.2441	654.5	526.1	38,88
51.8	1.42389	1.2447	656.1	527.1	38,97
51.9	1.42410	1.2454	657.8	528.2	39,07
52.0	1.42432	1.2461	659.4	529.2	39,17
52.1	1.42453	1.2466	661.0	530.2	39,26
52.2	1.42475	1.2470	662.5	531.3	39,35
52.3	1.42496	1.2475	664.1	532.3	39,45
52.4	1.42517	1.2480	665.6	533.3	39,54
52.5	1.42538	1.2486	667.2	534.4	39,63
52.6	1.42560	1.2493	668.9	535.4	39,73
52.7	1.42581	1.2500	670.5	536.4	39,83
52.8	1.42603	1.2506	672.2	537.5	39,93
52.9	1.42624	1.2513	673.8	538.5	40,02
53.0	1.42645	1.2520	675.5	539.5	40,12
53.1	1.42667	1.2525	677.1	540.6	40,22
53.2	1.42689	1.2530	678.5	541.5	40,30
53.3	1.42711	1.2535	680.2	542.6	40,40
53.4	1.42733	1.2540	681.8	543.7	40,50
53.5	1.42754	1.2546	683.4	544.7	40,59
53.6	1.42776	1.2553	685.1	545.8	40,69
53.7	1.42797	1.2560	686.7	546.7	40,79
53.8	1.42819	1.2566	688.4	547.8	40,89
53.9	1.42840	1.2573	690.1	548.9	40,99
54.0	1.42861	1.2580	691.7	549.8	41,09
54.1	1.42884	1.2585	693.3	550.9	41,18
54.2	1.42906	1.2590	694.9	551.9	41,28
54.3	1.42927	1.2595	696.5	553.0	41,37
54.4	1.42949	1.2600	698.1	554.0	41,47
54.5	1.42971	1.2606	699.7	555.1	41,56
54.6	1.42993	1.2613	701.4	556.1	41,66
54.7	1.43014	1.2620	703.1	557.1	41,76
54.8	1.43036	1.2625	704.7	558.2	41,86
54.9	1.43058	1.2630	706.2	559.1	41,95

---

(1) The sugar concentration is expressed in terms of invert sugar.

▼**B**

Sucrose % (m/m)	Refractive index at 20 °C	Density at 20 °C	Sugar in g/l	Sugar in g/kg	Alcoholic strength % vol at 20 °C
55.0	1.43079	1.2635	707.8	560.2	42,04
55.1	1.43102	1.2639	709.4	561.3	42,14
55.2	1.43124	1.2645	711.0	562.3	42,23
55.3	1.43146	1.2652	712.7	563.3	42,33
55.4	1.43168	1.2659	714.4	564.3	42,44
55.5	1.43189	1.2665	716.1	565.4	42,54
55.6	1.43211	1.2672	717.8	566.4	42,64
55.7	1.43233	1.2679	719.5	567.5	42,74
55.8	1.43255	1.2685	721.1	568.5	42,83
55.9	1.43277	1.2692	722.8	569.5	42,93
56.0	1.43298	1.2699	724.5	570.5	43,04
56.1	1.43321	1.2703	726.1	571.6	43,13
56.2	1.43343	1.2708	727.7	572.6	43,23
56.3	1.43365	1.2713	729.3	573.7	43,32
56.4	1.43387	1.2718	730.9	574.7	43,42
56.5	1.43409	1.2724	732.6	575.8	43,52
56.6	1.43431	1.2731	734.3	576.8	43,62
56.7	1.43454	1.2738	736.0	577.8	43,72
56.8	1.43476	1.2744	737.6	578.8	43,81
56.9	1.43498	1.2751	739.4	579.9	43,92
57.0	1.43519	1.2758	741.1	580.9	44,02
57.1	1.43542	1.2763	742.8	582.0	44,12
57.2	1.43564	1.2768	744.4	583.0	44,22
57.3	1.43586	1.2773	745.9	584.0	44,31
57.4	1.43609	1.2778	747.6	585.1	44,41
57.5	1.43631	1.2784	749.3	586.1	44,51
57.6	1.43653	1.2791	751.0	587.1	44,61
57.7	1.43675	1.2798	752.7	588.1	44,71
57.8	1.43698	1.2804	754.4	589.2	44,81
57.9	1.43720	1.2810	756.1	590.2	44,91
58.0	1.43741	1.2818	757.8	591.2	45,01
58.1	1.43764	1.2822	759.5	592.3	45,11
58.2	1.43784	1.2827	761.1	593.4	45,21
58.3	1.43809	1.2832	762.6	594.3	45,30
58.4	1.43832	1.2837	764.3	595.4	45,40
58.5	1.43854	1.2843	766.0	596.4	45,50
58.6	1.43877	1.2850	767.8	597.5	45,61
58.7	1.43899	1.2857	769.5	598.5	45,71
58.8	1.43922	1.2863	771.1	599.5	45,80
58.9	1.43944	1.2869	772.9	600.6	45,91
59.0	1.43966	1.2876	774.6	601.6	46,01
59.1	1.43988	1.2882	776.3	602.6	46,11
59.2	1.44011	1.2889	778.1	603.7	46,22
59.3	1.44034	1.2896	779.8	604.7	46,32
59.4	1.44057	1.2902	781.6	605.8	46,43
59.5	1.44079	1.2909	783.3	606.8	46,53
59.6	1.44102	1.2916	785.2	607.9	46,64
59.7	1.44124	1.2921	786.8	608.9	46,74
59.8	1.44147	1.2926	788.4	609.9	46,83
59.9	1.44169	1.2931	790.0	610.9	46,93

▼**B**

Sucrose % (m/m)	Refractive index at 20 ºC	Density at 20 ºC	Sugar in g/l	Sugar in g/kg	Alcoholic strength % vol at 20 ºC
60.0	1.44192	1.2936	791.7	612.0	47,03
60.1	1.44215	1.2942	793.3	613.0	47,12
60.2	1.44238	1.2949	795.2	614.1	47,23
60.3	1.44260	1.2956	796.9	615.1	47,34
60.4	1.44283	1.2962	798.6	616.1	47,44
60.5	1.44305	1.2969	800.5	617.2	47,55
60.6	1.44328	1.2976	802.2	618.2	47,65
60.7	1.44351	1.2981	803.9	619.3	47,75
60.8	1.44374	1.2986	805.5	620.3	47,85
60.9	1.44397	1.2991	807.1	621.3	47,94
61.0	1.44419	1.2996	808.7	622.3	48,04
61.1	1.44442	1.3002	810.5	623.4	48,14
61.2	1.44465	1.3009	812.3	624.4	48,25
61.3	1.44488	1.3016	814.2	625.5	48,36
61.4	1.44511	1.3022	815.8	626.5	48,46
61.5	1.44534	1.3029	817.7	627.6	48,57
61.6	1.44557	1.3036	819.4	628.6	48,67
61.7	1.44580	1.3042	821.3	629.7	48,79
61.8	1.44603	1.3049	823.0	630.7	48,89
61.9	1.44626	1.3056	824.8	631.7	48,99
62.0	1.44648	1.3062	826.6	632.8	49,10
62.1	1.44672	1.3068	828.3	633.8	49,20
62.2	1.44695	1.3075	830.0	634.8	49,30
62.3	1.44718	1.3080	831.8	635.9	49,40
62.4	1.44741	1.3085	833.4	636.9	49,50
62.5	1.44764	1.3090	835.1	638.0	49,60
62.6	1.44787	1.3095	836.8	639.0	49,71
62.7	1.44810	1.3101	838.5	640.0	49,81
62.8	1.44833	1.3108	840.2	641.0	49,91
62.9	1.44856	1.3115	842.1	642.1	50,02
63.0	1.44879	1.3121	843.8	643.1	50,12
63.1	1.44902	1.3128	845.7	644.2	50,23
63.2	1.44926	1.3135	847.5	645.2	50,34
63.3	1.44949	1.3141	849.3	646.3	50,45
63.4	1.44972	1.3148	851.1	647.3	50,56
63.5	1.44995	1.3155	853.0	648.4	50,67
63.6	1.45019	1.3161	854.7	649.4	50,77
63.7	1.45042	1.3168	856.5	650.4	50,88
63.8	1.45065	1.3175	858.4	651.5	50,99
63.9	1.45088	1.3180	860.0	652.5	51,08
64.0	1.45112	1.3185	861.6	653.5	51,18
64.1	1.45135	1.3190	863.4	654.6	51,29
64.2	1.45158	1.3195	865.1	655.6	51,39
64.3	1.45181	1.3201	866.9	656.7	51,49
64.4	1.45205	1.3208	868.7	657.7	51,60
64.5	1.45228	1.3215	870.6	658.8	51,71
64.6	1.45252	1.3221	872.3	659.8	51,81
64.7	1.45275	1.3228	874.1	660.8	51,92
64.8	1.45299	1.3235	876.0	661.9	52,03
64.9	1.45322	1.3241	877.8	662.9	52,14

▼**B**

Sucrose % (m/m)	Refractive index at 20 ºC	Density at 20 ºC	Sugar in g/l	Sugar in g/kg	Alcoholic strength % vol at 20 ºC
65.0	1.45347	1.3248	879.7	664.0	52,25
65.1	1.45369	1.3255	881.5	665.0	52,36
65.2	1.45393	1.3261	883.2	666.0	52,46
65.3	1.45416	1.3268	885.0	667.0	52,57
65.4	1.45440	1.3275	886.9	668.1	52,68
65.5	1.45463	1.3281	888.8	669.2	52,79
65.6	1.45487	1.3288	890.6	670.2	52,90
65.7	1.45510	1.3295	892.4	671.2	53,01
65.8	1.45534	1.3301	894.2	672.3	53,12
65.9	1.45557	1.3308	896.0	673.3	53,22
66.0	1.45583	1.3315	898.0	674.4	53,34
66.1	1.45605	1.3320	899.6	675.4	53,44
66.2	1.45629	1.3325	901.3	676.4	53,54
66.3	1.45652	1.3330	903.1	677.5	53,64
66.4	1.45676	1.3335	904.8	678.5	53,75
66.5	1.45700	1.3341	906.7	679.6	53,86
66.6	1.45724	1.3348	908.5	680.6	53,96
66.7	1.45747	1.3355	910.4	681.7	54,08
66.8	1.45771	1.3361	912.2	682.7	54,18
66.9	1.45795	1.3367	913.9	683.7	54,29
67.0	1.45820	1.3374	915.9	684.8	54,40
67.1	1.45843	1.3380	917.6	685.8	54,51
67.2	1.45867	1.3387	919.6	686.9	54,62
67.3	1.45890	1.3395	921.4	687.9	54,73
67.4	1.45914	1.3400	923.1	688.9	54,83
67.5	1.45938	1.3407	925.1	690.0	54,95
67.6	1.45962	1.3415	927.0	691.0	55,06
67.7	1.45986	1.3420	928.8	692.1	55,17
67.8	1.46010	1.3427	930.6	693.1	55,28
67.9	1.46034	1.3434	932.6	694.2	55,40
68.0	1.46060	1.3440	934.4	695.2	55,50
68.1	1.46082	1.3447	936.2	696.2	55,61
68.2	1.46106	1.3454	938.0	697.2	55,72
68.3	1.46130	1.3460	939.9	698.3	55,83
68.4	1.46154	1.3466	941.8	699.4	55,94
68.5	1.46178	1.3473	943.7	700.4	56,06
68.6	1.46202	1.3479	945.4	701.4	56,16
68.7	1.46226	1.3486	947.4	702.5	56,28
68.8	1.46251	1.3493	949.2	703.5	56,38
68.9	1.46275	1.3499	951.1	704.6	56,50
69.0	1.46301	1.3506	953.0	705.6	56,61
69.1	1.46323	1.3513	954.8	706.6	56,72
69.2	1.46347	1.3519	956.7	707.7	56,83
69.3	1.46371	1.3526	958.6	708.7	56,94
69.4	1.46396	1.3533	960.6	709.8	57,06
69.5	1.46420	1.3539	962.4	710.8	57,17
69.6	1.46444	1.3546	964.3	711.9	57,28
69.7	1.46468	1.3553	966.2	712.9	57,39
69.8	1.46493	1.3560	968.2	714.0	57,51
69.9	1.46517	1.3566	970.0	715.0	57,62

▼B

Sucrose % (m/m)	Refractive index at 20 ºC	Density at 20 ºC	Sugar in g/l	Sugar in g/kg	Alcoholic strength % vol at 20 ºC
70.0	1.46544	1.3573	971.8	716.0	57,72
70.1	1.46565	1.3579	973.8	717.1	57,84
70.2	1.46590	1.3586	975.6	718.1	57,95
70.3	1.46614	1.3593	977.6	719.2	58,07
70.4	1.46639	1.3599	979.4	720.2	58,18
70.5	1.46663	1.3606	981.3	721.2	58,29
70.6	1.46688	1.3613	983.3	722.3	58,41
70.7	1.46712	1.3619	985.2	723.4	58,52
70.8	1.46737	1.3626	987.1	724.4	58,63
70.9	1.46761	1.3633	988.9	725.4	58,74
71.0	1.46789	1.3639	990.9	726.5	58,86
71.1	1.46810	1.3646	992.8	727.5	58,97
71.2	1.46835	1.3653	994.8	728.6	59,09
71.3	1.46859	1.3659	996.6	729.6	59,20
71.4	1.46884	1.3665	998.5	730.7	59,31
71.5	1.46908	1.3672	1 000.4	731.7	59,42
71.6	1.46933	1.3678	1 002.2	732.7	59,53
71.7	1.46957	1.3685	1 004.2	733.8	59,65
71.8	1.46982	1.3692	1 006.1	734.8	59,76
71.9	1.47007	1.3698	1 008.0	735.9	59,88
72.0	1.47036	1.3705	1 009.9	736.9	59,99
72.1	1.47056	1.3712	1 012.0	738.0	60,11
72.2	1.47081	1.3718	1 013.8	739.0	60,22
72.3	1.47106	1.3725	1 015.7	740.0	60,33
72.4	1.47131	1.3732	1 017.7	741.1	60,45
72.5	1.47155	1.3738	1 019.5	742.1	60,56
72.6	1.47180	1.3745	1 021.5	743.2	60,68
72.7	1.47205	1.3752	1 023.4	744.2	60,79
72.8	1.47230	1.3758	1 025.4	745.3	60,91
72.9	1.47254	1.3765	1 027.3	746.3	61,02
73.0	1.47284	1.3772	1 029.3	747.4	61,14
73.1	1.47304	1.3778	1 031.2	748.4	61,25
73.2	1.47329	1.3785	1 033.2	749.5	61,37
73.3	1.47354	1.3792	1 035.1	750.5	61,48
73.4	1.47379	1.3798	1 037.1	751.6	61,60
73.5	1.47404	1.3805	1 039.0	752.6	61,72
73.6	1.47429	1.3812	1 040.9	753.6	61,83
73.7	1.47454	1.3818	1 042.8	754.7	61,94
73.8	1.47479	1.3825	1 044.8	755.7	62,06
73.9	1.47504	1.3832	1 046.8	756.8	62,18
74.0	1.47534	1.3838	1 048.6	757.8	62,28
74.1	1.47554	1.3845	1 050.7	758.9	62,41
74.2	1.47579	1.3852	1 052.6	759.9	62,52
74.3	1.47604	1.3858	1 054.6	761.0	62,64
74.4	1.47629	1.3865	1 056.5	762.0	62,76
74.5	1.47654	1.3871	1 058.5	763.1	62,87
74.6	1.47679	1.3878	1 060.4	764.1	62,99
74.7	1.47704	1.3885	1 062.3	765.1	63,10
74.8	1.47730	1.3892	1 064.4	766.2	63,23
74.9	1.47755	1.3898	1 066.3	767.2	63,33
75.0	1.47785	1.3905	1 068.3	768.3	63,46

▼B

3. ALCOHOLIC STRENGTH BY VOLUME

1. DEFINITION

The alcoholic strength by volume is the number of litres of ethanol contained in 100 litres of wine, both volumes being measured at a temperature of 20 ºC. It is expressed by the symbol '% vol'.

Note:

Homologues of ethanol, together with the ethanol and ethanol homologues in ethyl esters, are included in the alcoholic strength since they occur in the distillate.

2. PRINCIPLE OF METHODS

2.1. Distillation of wine made alkaline by a suspension of calcium hydroxide. Measurement of the alcoholic strength of the distillate.

2.2. **Reference method:** measurement of the density of the distillate with a pycnometer.

2.3. **Usual methods:**

2.3.1. Measurement of the alcoholic strength of the distillate with a hydrometer.

2.3.2. Measurement of the alcoholic strength of the distillate by densimetry using a hydrostatic balance.

2.3.3. Measurement of the alcoholic strength of the distillate by refractometry.

Note:

To obtain the alcoholic strength from the density of the distillate, use Tables I, II and III in Appendix II to this section of the Annex. These have been calculated from the *International Tables of Alcoholic Strength* published in 1972 by the International Legal Metrology Organization in its Recommendation 22 and adopted by the OIV (General Assembly, 1974).

Table I gives the general formula relating the alcoholic strength by volume and the density of alcohol-water mixtures as a function of temperature.

3. METHOD OF OBTAINING DISTILLATE

3.1. **Apparatus**

3.1.1. Distillation apparatus, consisting of:

- a round-bottomed 1-litre flask with ground-glass joints,
- a rectifying column about 20 cm in height or any apparatus to prevent splashing,
- a source of heat; any pyrolysis of extracted matter must be prevented by a suitable arrangement,
- a condenser terminated by a drawn-out tube taking the distillate to the bottom of a graduated receiving flask containing several ml of water.

3.1.2. Steam-distillation apparatus consisting of:

1. a steam-generator;
2. a steam pipe;
3. a rectifying column;
4. a condenser.

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.

▼B

3.2. Reagents

3.2.1. A 2 M suspension of calcium hydroxide, obtained by carefully pouring 1 litre of water at 60 to 70 ºC on to 120 g of quicklime (CaO).

3.3. Preparation of sample

Remove the bulk of any carbon dioxide from young and sparkling wines by stirring 250 to 300 ml of the wine in a 500-ml flask.

3.4. Procedure

Measure out 200 ml of the wine using a graduated 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 graduated flask four times with successive 5-ml washings of water added to the flask or the steam-pipe. Add 10 ml of (3.2.1) calcium hydroxide and several pieces of inert porous material (pumice, etc).

Collect the distillate in the 200-ml graduated flask used to measure the wine.

Collect a volume of about three-quarters of the initial volume if distillation is used and a volume of 198 to 199 ml of distillate if steam distillation is used. Make up to 200 ml with distilled water, keeping the distillate at a temperature within 2 ºC of the initial temperatures.

Mix with great care, using a circular motion.

Note:

In the case of wines containing particularly large concentrations of ammonium ions, the distillate may be redistilled under the conditions described above, but replacing the suspension of calcium hydroxide with 1 M sulphuric acid diluted to 10 parts in 100 (vol/vol).

4. REFERENCE METHOD

Measurement of the alcoholic strength of the distillate using a pycnometer.

4.1. Apparatus

4.1.1. Use the standardized pycnometer as described in the chapter ‘Density and specific gravity’ (Annex, chapter 1).

4.2. Procedure

Measure the apparent density of the distillate (3.4) at t ºC as described in the chapter ‘Density and specific gravity’ (Annex, chapter 1, sections 4.3.1 and 4.3.2). Let this density be ρ_t.

4.3. Expression of results

4.3.1. *Method of calculation*

Find the alcoholic strength at 20 ºC using Table I. In the horizontal line of this table corresponding to the temperature T (expressed as a whole number) immediately below t ºC,find the smallest density greater than ρ_t. Use the tabular difference just below this density to calculate the density ρ at this temperature T.

On the line of the temperature T, find the density ρ immediately above ρ' and calculate the difference between the densities ρ and ρ'. Divide this difference by the tabular difference just to the right of the density ρ'. The quotient gives the decimal part of the alcoholic strength, while the whole number part of this strength is shown at the head of the column in which the density ρ' is located.

An example of the calculation of an alcoholic strength is given in Appendix I to this chapter of the Annex.

▼B

Note:

This temperature correction has been incorporated in a computer program and might possibly be carried out automatically.

4.3.2. Repeatability, *r*: r = 0,10 % vol.

4.3.3. Reproducibility, *R*: R = 0,19 % vol.

▼M9

▼B

6. EXAMPLE OF THE CALCULATION OF THE ALCOHOLIC STRENGTH OF A WINE

6.1. Measurement by pycnometer on a twin-pan balance

6.1.1. The constants of the pycnometer have been determined and calculated as described in chapter 1, ‘Density and specific gravity’, section 6.1.1.

6.1.2. *Weighing of pycnometer filled with distillate*

Numeric example

$$\text{Tare} = \text{pycnometer} + \text{distillate at } t\ ^\circ\text{C} + p'' \begin{cases} t\ ^\circ\text{C} & = 18{,}90\ ^\circ\text{C} \\ t\ ^\circ\text{C corrected} & = 18{,}70\ ^\circ\text{C} \\ p'' & = 2{,}8074\ \text{g} \end{cases}$$

$p + m - p''$ = mass of distillate at t °C   105,0698 − 2,8074 = 102,2624 g

Apparent density at t °C

$$\rho_t = \frac{\mathrm{p + m - p''}}{\text{volume of pycnometer at 20 °C}} \quad \left\{ \rho_{18,70\ ^\circ\mathrm{C}} = \frac{102,2624}{104,0229} = 0,983076 \right.$$

6.1.3. *Calculation of alcoholic strength*

Refer to the table of apparent densities of water-alcohol mixtures at different temperatures, as indicated above

On the line 18 °C of the table of apparent densities, the smallest density greater than the observed density of 0,983076 is 0,98398 in column 11 % vol

The density at 18 °C is: $(98307{,}6 + 0{,}7 \times 22)\ 10^{-5} = 0{,}98323$

0,98398 − 0,98323 = 0,00075

The decimal portion of the % vol of alcoholic strength is 75/114 = 0,65

The alcoholic strength is 11,65 % vol

6.2. Measurement by pycnometer on a single pan balance

6.2.1. The constants of the pycnometer have been determined and calculated as described in chapter 1, ‘Density and specific gravity’, section 6.2.1.

6.2.2. *Weighing of the pycnometer filled with distillate*

Weight of tare bottle at the time of measurement in grams:	T_1	= 171,9178
Pycnometer filled with distillate at 20,50 °C in grams:	P_2	= 167,8438
Variation in the buoyancy of air:	dT	= 171,9178 − 171,9160 = + 0,0018
Mass of the distillate at 20,50 °C:	L_t	= 167,8438 − (67,6695 + 0,0018) = 100,1725
Apparent density of the distillate:		$\rho_{20,50\ ^\circ\mathrm{C}} = \frac{100,1725}{100,8194} = 0,983825$

▼**B**

6.2.3. *Calculation of alcoholic strength*

Refer to the table of apparent densities of water-alcohol mixtures at different temperatures, as indicated above

On the line 20 ºC of the table of apparent densities, the smallest density greater than observed density of 0,983825 is 0,98471 in column 10 % vol

The density at 20 ºC is: $(98382{,}5 + 0{,}5 \times 24)\ 10^{-5} = 0{,}983945$

$0{,}98471 - 0{,}983945 = 0{,}000765$

The decimal portion of the % vol of alcoholic strength is 76,5/119 = 0,64

The alcoholic strength is 10,64 % vol

▼**B**

FORMULA FROM WHICH TABLES OF ALCOHOLIC STRENGHTS OF ETHANOL-WATER MIXTURES ARE CALCULATED

The density p in kilograms per metre cubed (kg/m^3) of an ethanol-water mixture at temperature t in degrees Celsius is given by the formula below as a function of:

- — the alcoholic strength by weight p expressed as a decimal ([1]),
- — the temperature t in ºC (EIPT 68),
- — the numerical coefficients below.

The formula is valid for temperatures between − 20 and +40 ºC.

$$\varrho = A_1 + \sum_{k=2}^{12} A_k\, p^{k-1} + \sum_{k=1}^{6} B_k (t - 20\ ^{\circ}\mathrm{C})^k$$

$$+ \sum_{i=1}^{n} \sum_{k=1}^{m} C_{i,k}\, p^{k(t - 20\ ^{\circ}\mathrm{C})^i} \qquad \begin{array}{ll} n = 5 & m_3 = 9 \\ m_1 = 11 & m_4 = 4 \\ m_2 = 10 & m_5 = 2 \end{array}$$

Numerical coefficients in the formula

k	A_k	B_k
	kg/m^3	
1	9,982 012 300 · 10^{2}	− 2,061 851 3 · 10^{-1} kg/(m^3 · ºC)
2	− 1,929 769 495 · 10^{2}	− 5,268 254 2 · 10^{-3} kg/(m^3 · ºC^2)
3	3,891 238 958 · 10^{2}	3,613 001 3 · 10^{-5} kg/(m^3 · ºC^3)
4	− 1,668 103 923 · 10^{3}	− 3,895 770 2 · 10^{-7} kg/(m^3 · ºC^4)
5	1,352 215 441 · 10^{4}	7,169 354 0 · 10^{-9} kg/(m^3 · ºC^5)
6	− 8,829 278 388 · 10^{4}	− 9,973 923 1 · 10^{-11} kg/(m^3 · ºC^6)
7	3,062 874 042 · 10^{5}	
8	− 6,138 381 234 · 10^{5}	
9	7,470 172 998 · 10^{5}	
10	− 5,478 461 354 · 10^{5}	
11	2,234 460 334 · 10^{5}	
12	− 3,903 285 426 · 10^{4}	

k	$C_{1,k}$	$C_{2,k}$
	kg/(m^3 · ºC)	kg/(m^3 · ºC^2)
1	1,693 443 461 530 087 · 10^{-1}	− 1,193 013 005 057 010 · 10^{-2}
2	− 1,046 914 743 455 169 · 10^{1}	2,517 399 633 803 461 · 10^{-1}
3	7,196 353 469 546 523 · 10^{1}	− 2,170 575 700 536 993
4	− 7,047 478 054 272 792 · 10^{2}	1,353 034 988 843 029 · 10^{1}
5	3,924 090 430 035 045 · 10^{3}	− 5,029 988 758 547 014 · 10^{1}
6	− 1,210 164 659 068 747 · 10^{4}	1,096 355 666 577 570 · 10^{2}
7	2,248 646 550 400 788 · 10^{4}	− 1,422 753 946 421 155 · 10^{2}
8	− 2,605 562 982 188 164 · 10^{4}	1,080 435 942 856 230 · 10^{2}
9	1,852 373 922 069 467 · 10^{4}	− 4,414 153 236 817 392 · 10^{1}
10	− 7,420 201 433 430 137 · 10^{3}	7,442 971 530 188 783
11	1,285 617 841 998 974 · 10^{3}	

k	$C_{3,k}$	$C_{4,k}$	$C_{5,k}$
	kg/(m^3 · ºC^3)	kg/(m^3 · ºC^4)	kg/(m^3 · ºC^5)
1	− 6,802 995 733 503 803 · 10^{-4}	4,075 376 675 622 027 · 10^{-6}	− 2,788 074 354 782 409 · 10^{-8}
2	1,876 837 790 289 664 · 10^{-2}	− 8,763 058 573 471 110 · 10^{-6}	1,345 612 883 493 354 · 10^{-8}
3	− 2,002 561 813 734 156 · 10^{-1}	6,515 031 360 099 368 · 10^{-6}	
4	1,022 992 966 719 220	− 1,515 784 836 987 210 · 10^{-6}	
5	− 2,895 696 483 903 638		
6	4,810 060 584 300 675		
7	− 4,672 147 440 794 683		
8	2,458 043 105 903 461		
9	− 5,411 227 621 436 812 · 10^{-1}		

---

[1] For example, for an alcoholic strength of 12 % by weight, p = 0,12.

▼B

TABLE I

INTERNATIONAL ALCOHOLIC STRENGTH AT 20 °C

Table of apparent densities of ethanol-water mixtures — Pyrex pycnometer

Densities at *t* °C corrected for air buoyancy

t°	Alcoholic strength by % vol																							
	0		**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**		**9**		**10**		**11**	
0°	999,64	1,50	998,14	1,44	996,70	1,40	995,30	1,35	993,95	1,30	992,65	1,24	991,41	1,19	990,22	1,14	989,08	1,10	987,98	1,05	986,93	1,00	985,93	0,95
	−0,07		−0,06		−0,06		−0,06		−0,06		−0,06		−0,06		−0,05		−0,04		−0,03		−0,02		−0,01	
1°	999,71	1,51	998,20	1,44	996,76	1,40	995,36	1,35	994,01	1,30	992,71	1,24	991,47	1,20	990,27	1,15	989,12	1,11	988,01	1,06	986,95	1,01	985,94	0,97
	−0,05		−0,05		−0,04		−0,04		−0,04		−0,04		−0,03		−0,03		−0,02		−0,02		−0,01		0,00	
2°	999,76	1,51	998,25	1,45	996,80	1,40	995,40	1,35	994,05	1,30	992,75	1,25	991,50	1,20	990,30	1,16	989,14	1,11	988,03	1,07	986,96	1,02	985,94	0,98
	−0,03		−0,03		−0,03		−0,02		−0,02		−0,02		−0,02		−0,01		−0,01		0,00		0,01		0,02	
3°	999,79	1,51	998,28	1,45	996,83	1,41	995,42	1,35	994,07	1,30	992,77	1,25	991,52	1,21	990,31	1,16	989,15	1,12	988,03	1,08	986,95	1,03	985,92	1,00
	−0,02		−0,02		−0,01		−0,02		−0,01		−0,01		0,00		0,00		0,01		0,02		0,03		0,04	
4°	999,81	1,51	998,30	1,46	996,84	1,40	995,44	1,36	994,08	1,30	992,78	1,26	991,52	1,21	990,31	1,17	989,14	1,13	988,01	1,09	986,92	1,04	985,88	1,00
	0,00		0,00		0,00		0,00		0,01		0,02		0,02		0,02		0,02		0,03		0,04		0,05	
5°	999,81	1,51	998,30	1,46	996,84	1,40	995,44	1,37	994,07	1,31	992,76	1,26	991,50	1,21	990,29	1,17	989,12	1,14	987,98	1,10	986,88	1,05	985,83	1,01
	0,01		0,01		0,01		0,02		0,01		0,02		0,03		0,04		0,05		0,05		0,05		0,06	
6°	999,80	1,51	998,29	1,46	996,83	1,41	995,42	1,36	994,06	1,32	992,74	1,27	991,47	1,22	990,25	1,18	989,07	1,14	987,93	1,10	986,83	1,06	985,77	1,03
	0,03		0,03		0,03		0,03		0,04		0,04		0,04		0,05		0,06		0,07		0,08		0,09	
7°	999,77	1,51	998,26	1,46	996,80	1,41	995,39	1,37	994,02	1,32	992,70	1,27	991,43	1,23	990,20	1,19	989,01	1,15	987,86	1,11	986,75	1,07	985,68	1,03
	0,05		0,04		0,04		0,05		0,05		0,05		0,05		0,06		0,06		0,07		0,08		0,09	
8°	999,72	1,50	998,22	1,46	996,76	1,42	995,34	1,37	993,97	1,32	992,65	1,27	991,38	1,24	990,14	1,19	988,95	1,16	987,79	1,12	986,67	1,08	985,59	1,05
	0,05		0,06		0,06		0,06		0,06		0,06		0,07		0,07		0,08		0,09		0,10		0,11	
9°	999,67	1,51	998,16	1,46	996,70	1,42	995,28	1,37	993,91	1,32	992,59	1,28	991,31	1,24	990,07	1,20	988,87	1,17	987,70	1,13	986,57	1,09	985,48	1,06
	0,07		0,07		0,07		0,07		0,07		0,08		0,08		0,09		0,09		0,10		0,11		0,12	
10°	999,60	1,51	998,09	1,46	996,63	1,42	995,21	1,37	993,84	1,33	992,51	1,28	991,23	1,25	989,98	1,20	988,78	1,17	987,60	1,14	986,46	1,10	985,36	1,06
	0,09		0,09		0,09		0,08		0,09		0,09		0,10		0,10		0,11		0,11		0,12		0,13	
11°	999,51	1,51	998,00	1,46	996,54	1,41	995,13	1,38	993,75	1,33	992,42	1,29	991,13	1,25	989,88	1,21	988,67	1,18	987,49	1,15	986,34	1,11	985,23	1,07
	0,10		0,09		0,09		0,10		0,10		0,11		0,11		0,11		0,12		0,13		0,13		0,14	
12°	999,41	1,50	997,91	1,46	996,45	1,42	995,03	1,38	993,65	1,34	992,31	1,29	991,02	1,25	989,77	1,22	988,55	1,19	987,36	1,15	986,21	1,12	985,09	1,09
	0,11		0,11		0,11		0,11		0,11		0,11		0,12		0,12		0,13		0,14		0,15		0,16	
13°	999,30	1,50	997,80	1,46	996,34	1,42	994,92	1,38	993,54	1,34	992,20	1,30	990,90	1,25	989,65	1,23	988,42	1,20	987,22	1,16	986,06	1,13	984,93	1,09
	0,12		0,12		0,12		0,13		0,13		0,13		0,13		0,14		0,14		0,15		0,16		0,16	
14°	999,18	1,50	997,68	1,46	996,22	1,43	994,79	1,38	993,41	1,34	992,07	1,30	990,77	1,26	989,51	1,23	988,28	1,21	987,07	1,17	985,90	1,13	984,77	1,11
	0,13		0,14		0,14		0,13		0,13		0,14		0,14		0,15		0,16		0,16		0,17		0,18	
15°	999,05	1,51	997,54	1,46	996,08	1,42	994,66	1,38	993,28	1,35	991,93	1,30	990,63	1,27	989,36	1,24	988,12	1,21	986,91	1,18	985,73	1,14	984,59	1,12
	0,15		0,14		0,14		0,15		0,15		0,15		0,16		0,16		0,17		0,17		0,18		0,19	
16°	998,90	1,50	997,40	1,46	995,94	1,43	994,51	1,38	993,13	1,35	991,78	1,31	990,47	1,27	989,20	1,25	987,95	1,21	986,74	1,19	985,55	1,15	984,40	1,13
	0,16		0,16		0,16		0,16		0,16		0,17		0,17		0,18		0,18		0,19		0,19		0,20	
17°	998,74	1,50	997,24	1,46	995,78	1,43	994,35	1,38	992,97	1,36	991,61	1,31	990,30	1,28	989,02	1,25	987,77	1,22	986,55	1,19	985,36	1,16	984,20	1,14
	0,17		0,17		0,17		0,16		0,17		0,17		0,18		0,18		0,19		0,20		0,21		0,22	
18°	998,57	1,50	997,07	1,46	995,61	1,42	994,19	1,39	992,80	1,36	991,44	1,32	990,12	1,28	988,84	1,26	987,58	1,23	986,35	1,20	985,15	1,17	983,98	1,14
	0,18		0,18		0,18		0,19		0,19		0,19		0,19		0,20		0,20		0,20		0,21		0,22	
19°	998,39	1,50	996,89	1,46	995,43	1,43	994,00	1,39	992,61	1,36	991,25	1,32	989,93	1,29	988,64	1,26	987,38	1,23	986,15	1,21	984,94	1,10	983,76	1,16
	0,19		0,19		0,19		0,19		0,19		0,19		0,20		0,20		0,21		0,22		0,23		0,24	
20°	998,20	1,50	996,70	1,46	995,24	1,43	993,81	1,39	992,42	1,36	991,06	1,33	989,73	1,29	988,44	1,27	987,17	1,24	985,93	1,22	984,71	1,19	983,52	1,16

t°	Alcoholic strength by % vol																							
	0		**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**		**9**		**10**		**11**	
20°	998,20	1,50	996,70	1,46	995,24	1,43	993,81	1,39	992,42	1,36	991,06	1,33	989,73	1,29	988,44	1,27	987,17	1,24	985,93	1,22	984,71	1,19	983,52	1,16
	0,20		0,20		0,20		0,20		0,21		0,21		0,21		0,22		0,22		0,23		0,24		0,24	
21°	998,00	1,50	996,50	1,46	995,04	1,43	993,61	1,40	992,21	1,36	990,85	1,33	989,52	1,30	988,22	1,27	986,95	1,25	985,70	1,23	984,47	1,19	983,28	1,18
	0,21		0,21		0,21		0,21		0,21		0,22		0,22		0,23		0,24		0,24		0,24		0,26	
22°	997,79	1,50	996,29	1,46	994,83	1,43	993,40	1,40	992,00	1,37	990,63	1,33	989,30	1,31	987,99	1,28	986,71	1,25	985,46	1,23	984,23	1,21	983,02	1,18
	0,22		0,22		0,23		0,23		0,23		0,23		0,24		0,24		0,24		0,25		0,26		0,25	
23°	997,57	1,50	996,07	1,47	994,60	1,43	993,17	1,40	991,77	1,37	990,40	1,34	989,06	1,31	987,75	1,28	986,47	1,26	985,21	1,24	983,97	1,20	982,77	1,20
	0,24		0,23		0,23		0,23		0,24		0,24		0,24		0,25		0,26		0,26		0,27		0,29	
24°	997,33	1,49	995,84	1,47	994,37	1,43	992,94	1,41	991,53	1,37	990,16	1,34	988,82	1,32	987,50	1,29	986,21	1,26	984,95	1,25	983,70	1,22	982,48	1,20
	0,24		0,25		0,24		0,25		0,24		0,25		0,26		0,26		0,26		0,27		0,28		0,28	
25°	997,09	1,50	995,59	1,46	994,13	1,44	992,69	1,40	991,29	1,38	989,91	1,35	988,56	1,32	987,24	1,29	985,95	1,27	984,68	1,26	983,42	1,22	982,20	1,21
	0,25		0,25		0,26		0,25		0,26		0,26		0,26		0,26		0,28		0,28		0,28		0,30	
26°	996,84	1,50	995,34	1,47	993,87	1,43	992,44	1,41	991,03	1,38	989,65	1,35	988,30	1,32	986,98	1,31	985,67	1,27	984,40	1,26	983,14	1,24	981,90	1,22
	0,26		0,26		0,26		0,27		0,27		0,27		0,27		0,28		0,28		0,29		0,30		0,30	
27°	996,58	1,50	995,08	1,47	993,61	1,44	992,17	1,41	990,76	1,38	989,38	1,35	988,03	1,33	986,70	1,31	985,39	1,28	984,11	1,27	982,84	1,24	981,60	1,23
	0,27		0,27		0,27		0,27		0,28		0,28		0,29		0,29		0,29		0,30		0,31		0,32	
28°	996,31	1,50	994,81	1,47	993,34	1,44	991,90	1,42	990,48	1,38	989,10	1,36	987,74	1,33	986,41	1,31	985,10	1,29	983,81	1,28	982,53	1,25	981,28	1,23
	0,28		0,28		0,28		0,29		0,28		0,29		0,29		0,30		0,31		0,31		0,31		0,32	
29°	996,03	1,50	994,53	1,47	993,06	1,45	991,61	1,41	990,20	1,39	988,81	1,36	987,45	1,34	986,11	1,32	984,79	1,29	983,50	1,28	982,22	1,26	980,96	1,24
	0,28		0,29		0,29		0,29		0,30		0,30		0,31		0,31		0,31		0,32		0,32		0,33	
30°	995,75	1,51	994,24	1,47	992,77	1,45	991,32	1,42	989,90	1,39	988,51	1,37	987,14	1,34	985,80	1,32	984,48	1,30	983,18	1,28	981,90	1,27	980,63	1,25
	0,30		0,30		0,30		0,30		0,31		0,31		0,31		0,31		0,32		0,33		0,34		0,34	
31°	995,45	1,51	993,94	1,47	992,47	1,45	991,02	1,43	989,59	1,39	988,20	1,37	986,83	1,34	985,49	1,33	984,16	1,31	982,85	1,29	981,56	1,27	980,29	1,26
	0,31		0,31		0,31		0,32		0,31		0,32		0,32		0,33		0,33		0,34		0,35		0,36	
32°	995,14	1,51	993,63	1,47	992,16	1,46	990,70	1,42	989,28	1,40	987,88	1,37	986,51	1,35	985,16	1,33	983,83	1,32	982,51	1,30	981,21	1,28	979,93	1,26
	0,31		0,31		0,32		0,32		0,32		0,33		0,33		0,34		0,35		0,35		0,35		0,35	
33°	994,83	1,51	993,32	1,48	991,84	1,46	990,38	1,42	988,96	1,41	987,55	1,37	986,18	1,36	984,82	1,34	983,48	1,32	982,16	1,30	980,86	1,28	979,58	1,28
	0,32		0,33		0,33		0,33		0,35		0,34		0,35		0,35		0,34		0,35		0,36		0,37	
34°	994,51	1,52	992,99	1,48	991,51	1,46	990,05	1,44	988,61	1,40	987,21	1,38	985,83	1,36	984,47	1,33	983,14	1,33	981,81	1,31	980,50	1,29	979,21	1,28
	0,33		0,33		0,34		0,35		0,34		0,35		0,35		0,35		0,36		0,36		0,36		0,37	
35°	994,18	1,52	992,66	1,49	991,17	1,47	989,70	1,43	988,27	1,41	986,86	1,38	985,48	1,36	984,12	1,34	982,78	1,33	981,45	1,31	980,14	1,30	978,84	1,29
	0,34		0,35		0,35		0,35		0,35		0,35		0,35		0,36		0,36		0,37		0,37		0,38	
36°	993,84	1,53	992,31	1,49	990,82	1,47	989,35	1,43	987,92	1,41	986,51	1,38	985,13	1,37	983,76	1,34	982,42	1,34	981,08	1,31	979,77	1,31	978,46	1,29
	0,35		0,35		0,36		0,35		0,36		0,36		0,37		0,37		0,38		0,37		0,39		0,39	
37°	993,49	1,53	991,96	1,50	990,46	1,46	989,00	1,44	987,56	1,41	986,15	1,39	984,76	1,37	983,39	1,35	982,04	1,33	980,71	1,33	979,38	1,31	978,07	1,30
	0,36		0,36		0,36		0,37		0,37		0,37		0,37		0,37		0,38		0,39		0,38		0,39	
38°	993,13	1,53	991,60	1,50	990,10	1,47	988,63	1,44	987,19	1,41	985,78	1,39	984,39	1,37	983,02	1,36	981,66	1,34	980,32	1,32	979,00	1,32	977,68	1,31
	0,36		0,37		0,37		0,37		0,38		0,38		0,38		0,39		0,38		0,39		0,40		0,40	
39°	992,77	1,54	991,23	1,50	989,73	1,47	988,26	1,45	986,81	1,41	985,40	1,39	984,01	1,38	982,63	1,35	981,28	1,35	979,93	1,33	978,60	1,32	977,28	1,32
	0,37		0,37		0,38		0,39		0,38		0,39		0,39		0,39		0,40		0,39		0,40		0,41	
40°	992,40	1,54	990,86	1,51	989,35	1,48	987,87	1,44	986,43	1,42	985,01	1,39	983,62	1,38	982,24	1,36	980,88	1,34	979,54	1,34	978,20	1,33	976,87	1,32

▼B

t°	Alcoholic strength by % vol																							
	10		**11**		**12**		**13**		**14**		**15**		**16**		**17**		**18**		**19**		**20**		**21**	
0	986,93	1,00	985,93	0,95	984,98	0,92	984,06	0,88	983,18	0,84	982,34	0,80	981,54	0,78	980,76	0,75	980,01	0,73	979,28	0,72	978,56	0,70	977,86	0,70
	−0,02		−0,01		0,01		0,01		0,03		0,04		0,07		0,08		0,10		0,12		0,14		0,17	
1	986,95	1,01	985,94	0,97	984,97	0,92	984,05	0,90	983,15	0,85	982,30	0,83	981,47	0,79	980,68	0,77	979,91	0,75	979,16	0,74	978,42	0,73	977,69	0,72
	−0,01		0,00		0,01		0,03		0,04		0,07		0,08		0,10		0,12		0,14		0,16		0,18	
2	986,96	1,02	985,94	0,98	984,96	0,94	984,02	0,91	983,11	0,88	982,23	0,84	981,39	0,81	980,58	0,79	979,79	0,77	979,02	0,76	978,26	0,75	977,51	0,74
	0,01		0,02		0,04		0,05		0,06		0,07		0,09		0,11		0,13		0,15		0,17		0,19	
3	986,95	1,03	985,92	1,00	984,92	0,95	983,97	0,92	983,05	0,89	982,16	0,86	981,30	0,83	980,47	0,81	979,66	0,79	978,87	0,78	978,09	0,77	977,32	0,77
	0,03		0,04		0,04		0,06		0,07		0,09		0,10		0,12		0,14		0,16		0,18		0,20	
4	986,92	1,04	985,88	1,00	984,88	0,97	983,91	0,93	982,98	0,91	982,07	0,87	981,20	0,85	980,35	0,83	979,52	0,81	978,71	0,80	977,91	0,79	977,12	0,79
	0,04		0,05		0,06		0,07		0,09		0,10		0,12		0,14		0,15		0,17		0,19		0,22	
5	986,88	1,05	985,83	1,01	984,82	0,98	983,84	0,95	982,89	0,92	981,97	0,89	981,08	0,87	980,21	0,84	979,37	0,83	978,54	0,82	977,72	0,82	976,90	0,80
	0,05		0,06		0,08		0,09		0,10		0,12		0,13		0,14		0,17		0,19		0,21		0,22	
6	986,83	1,06	985,77	1,03	984,74	0,99	983,75	0,96	982,79	0,94	981,85	0,90	980,95	0,88	980,07	0,87	979,20	0,85	978,35	0,84	977,51	0,83	976,68	0,83
	0,08		0,09		0,09		0,10		0,12		0,13		0,15		0,16		0,18		0,19		0,21		0,23	
7	986,75	1,07	985,68	1,03	984,65	1,00	983,65	0,98	982,67	0,95	981,72	0,92	980,80	0,89	979,91	0,89	979,02	0,86	978,16	0,86	977,30	0,85	976,45	0,85
	0,08		0,09		0,11		0,13		0,13		0,14		0,15		0,18		0,19		0,21		0,23		0,25	
8	986,67	1,08	985,59	1,05	984,54	1,02	983,52	0,98	982,54	0,96	981,58	0,93	980,65	0,92	979,73	0,90	978,83	0,88	977,95	0,88	977,07	0,87	976,20	0,87
	0,10		0,11		0,12		0,12		0,14		0,16		0,18		0,19		0,21		0,22		0,24		0,26	
9	986,57	1,09	985,48	1,06	984,42	1,02	983,40	1,00	982,40	0,98	981,42	0,95	980,47	0,93	979,54	0,92	978,62	0,89	977,73	0,90	976,83	0,89	975,94	0,89
	0,11		0,12		0,12		0,14		0,16		0,17		0,18		0,20		0,20		0,23		0,24		0,26	
10	986,46	1,10	985,36	1,06	984,30	1,04	983,26	1,02	982,24	0,99	981,25	0,96	980,29	0,95	979,34	0,92	978,42	0,92	977,50	0,91	976,59	0,91	975,68	0,91
	0,12		0,13		0,14		0,16		0,16		0,17		0,19		0,20		0,23		0,25		0,27		0,29	
11	986,34	1,11	985,23	1,07	984,16	1,06	983,10	1,02	982,08	1,00	981,08	0,98	980,10	0,96	979,14	0,95	978,19	0,94	977,25	0,93	976,32	0,93	975,39	0,92
	0,13		0,14		0,16		0,16		0,18		0,19		0,21		0,22		0,24		0,25		0,27		0,28	
12	986,21	1,12	985,09	1,09	984,00	1,06	982,94	1,04	981,90	1,01	980,89	1,00	979,89	0,97	978,92	0,97	977,95	0,95	977,00	0,95	976,05	0,94	975,11	0,95
	0,15		0,16		0,16		0,18		0,19		0,20		0,21		0,23		0,24		0,26		0,28		0,30	
13	986,06	1,13	984,93	1,09	983,84	1,08	982,76	1,05	981,71	1,02	980,69	1,01	979,68	0,99	978,69	0,98	977,71	0,97	976,74	0,97	975,77	0,96	974,81	0,96
	0,16		0,16		0,18		0,18		0,20		0,22		0,23		0,24		0,26		0,27		0,28		0,30	
14	985,90	1,13	984,77	1,11	983,66	1,08	982,58	1,07	981,51	1,04	980,47	1,02	979,45	1,00	978,45	1,00	977,45	0,98	976,47	0,98	975,49	0,98	975,51	0,98
	0,17		0,18		0,19		0,20		0,21		0,22		0,24		0,25		0,26		0,28		0,30		0,32	
15	985,73	1,14	984,59	1,12	983,47	1,09	982,38	1,08	981,30	1,05	960,25	1,04	979,21	1,01	978,20	1,01	977,19	1,00	976,19	1,00	975,19	1,00	974,19	1,00
	0,18		0,19		0,20		0,22		0,22		0,24		0,24		0,27		0,28		0,30		0,31		0,32	
16	985,55	1,15	984,40	1,13	983,27	1,11	982,16	1,08	981,08	1,07	980,01	1,04	978,97	1,04	977,93	1,02	976,91	1,02	975,89	1,01	974,88	1,01	973,87	1,02
	0,19		0,20		0,21		0,22		0,23		0,24		0,26		0,27		0,29		0,30		0,32		0,33	
17	985,36	1,16	984,20	1,14	983,06	1,12	981,94	1,09	980,85	1,08	979,77	1,06	978,71	1,05	977,66	1,04	976,62	1,03	975,59	1,03	974,56	1,02	973,54	1,04
	0,21		0,22		0,22		0,23		0,25		0,26		0,27		0,28		0,29		0,31		0,32		0,35	
18	985,15	1,17	983,98	1,14	982,84	1,13	981,71	1,11	980,60	1,09	979,51	1,07	978,44	1,06	977,38	1,05	976,33	1,05	975,28	1,04	974,24	1,05	973,19	1,05
	0,21		0,22		0,24		0,24		0,25		0,26		0,28		0,29		0,31		0,32		0,34		0,35	
19	984,94	1,18	983,76	1,16	982,60	1,13	981,47	1,12	980,35	1,10	979,25	1,09	978,16	1,07	977,09	1,07	976,02	1,06	974,96	1,06	973,90	1,06	972,84	1,06
	0,23		0,24		0,24		0,26		0,27		0,28		0,29		0,30		0,31		0,33		0,34		0,36	
20	984,71	1,19	983,52	1,16	982,36	1,15	981,21	1,13	980,08	1,11	978,97	1,10	977,87	1,08	976,79	1,08	975,71	1,08	974,63	1,07	973,56	1,08	972,48	1,08

t°	Alcoholic strength by % vol																							
	10		**11**		**12**		**13**		**14**		**15**		**16**		**17**		**18**		**19**		**20**		**21**	
20°	984,71	1,19	983,52	1,16	982,36	1,15	981,21	1,13	980,08	1,11	978,97	1,10	977,87	1,08	976,79	1,08	975,71	1,08	974,63	1,07	973,56	1,08	972,48	1,08
	0,24		0,24		0,26		0,26		0,27		0,28		0,29		0,31		0,33		0,34		0,36		0,37	
21°	984,47	1,19	983,28	1,18	982,10	1,15	980,95	1,14	978,81	1,12	978,69	1,11	977,58	1,10	976,48	1,10	975,38	1,09	974,29	1,09	973,20	1,09	972,11	1,09
	0,24		0,26		0,28		0,29		0,30		0,31		0,33		0,33		0,35		0,35		0,36		0,37	
22°	984,23	1,21	983,02	1,18	981,84	1,17	980,67	1,15	979,52	1,13	978,39	1,12	977,27	1,12	976,15	1,10	975,05	1,11	973,94	1,10	972,84	1,10	971,74	1,12
	0,26		0,26		0,27		0,28		0,29		0,31		0,32		0,33		0,35		0,35		0,37		0,39	
23°	983,97	1,20	982,77	1,20	981,57	1,18	980,39	1,16	979,23	1,15	978,08	1,13	976,95	1,13	975,82	1,12	974,70	1,11	973,59	1,12	972,47	1,12	972,47	1,12
	0,27		0,29		0,29		0,29		0,30		0,31		0,33		0,33		0,35		0,37		0,38		0,40	
24°	983,70	1,22	982,48	1,20	981,28	1,18	980,10	1,17	978,93	1,16	977,77	1,15	976,62	1,13	975,49	1,14	974,35	1,13	973,22	1,13	972,09	1,14	970,95	1,14
	0,28		0,28		0,29		0,31		0,32		0,33		0,33		0,35		0,36		0,37		0,39		0,40	
25°	983,42	1,22	982,20	1,21	980,99	1,20	979,79	1,18	978,61	1,17	977,44	1,15	976,29	1,15	975,14	1,15	973,99	1,14	972,85	1,15	971,70	1,15	970,55	1,16
	0,28		0,30		0,31		0,31		0,32		0,33		0,35		0,36		0,37		0,39		0,40		0,41	
26°	983,14	1,24	981,90	1,22	980,68	1,20	979,48	1,19	978,29	1,18	977,11	1,17	975,94	1,16	974,78	1,16	973,62	1,16	972,46	1,16	971,30	1,16	970,14	1,17
	0,30		0,30		0,31		0,32		0,33		0,34		0,35		0,36		0,38		0,39		0,40		0,42	
27°	982,84	1,24	981,60	1,23	980,37	1,21	979,16	1,20	977,96	1,19	976,77	1,18	975,59	1,17	974,42	1,18	973,24	1,17	972,07	1,17	970,90	1,18	969,72	1,18
	0,31		0,32		0,32		0,33		0,34		0,35		0,36		0,38		0,38		0,40		0,41		0,43	
28°	982,53	1,25	981,28	1,23	980,05	1,22	978,83	1,21	977,62	1,20	976,42	1,19	975,23	1,19	974,04	1,18	972,86	1,19	971,67	1,18	970,49	1,20	969,29	1,20
	0,31		0,32		0,33		0,34		0,35		0,36		0,37		0,38		0,40		0,40		0,42		0,43	
29°	982,22	1,26	980,96	1,24	979,72	1,23	978,49	1,22	977,27	1,21	976,06	1,20	974,86	1,20	973,66	1,20	972,46	1,19	971,27	1,20	970,07	1,21	968,86	1,22
	0,32		0,33		0,34		0,35		0,36		0,37		0,38		0,40		0,41		0,43		0,44		0,45	
30°	981,90	1,27	980,63	1,25	979,38	1,24	978,14	1,23	976,91	1,22	975,69	1,21	974,48	1,22	973,26	1,21	972,05	1,21	970,84	1,21	969,63	1,22	968,41	1,23
	0,34		0,34		0,35		0,36		0,37		0,38		0,40		0,40		0,41		0,42		0,44		0,45	
31°	981,56	1,27	980,29	1,26	979,03	1,25	977,78	1,24	976,54	1,23	975,31	1,23	974,08	1,22	972,86	1,22	971,64	1,22	970,42	1,23	969,19	1,23	967,96	1,24
	0,35		0,36		0,36		0,37		0,38		0,39		0,39		0,40		0,42		0,43		0,44		0,46	
32°	981,21	1,28	979,93	1,26	978,67	1,26	977,41	1,25	976,16	1,24	974,92	1,23	973,69	1,23	972,46	1,24	971,22	1,23	969,99	1,24	968,75	1,25	967,50	1,25
	0,35		0,35		0,37		0,37		0,38		0,39		0,40		0,42		0,42		0,44		0,45		0,46	
33°	980,86	1,28	979,58	1,28	978,30	1,26	977,04	1,26	975,78	1,25	974,53	1,24	973,29	1,25	972,04	1,24	970,80	1,25	969,55	1,25	968,30	1,26	967,04	1,27
	0,36		0,37		0,37		0,38		0,39		0,40		0,41		0,42		0,43		0,44		0,46		0,47	
34°	980,50	1,29	979,21	1,28	977,93	1,27	976,66	1,27	975,39	1,26	974,13	1,25	972,88	1,26	971,62	1,25	970,37	1,26	969,11	1,27	967,84	1,27	966,57	1,29
	0,36		0,37		0,38		0,39		0,39		0,40		0,42		0,42		0,44		0,46		0,46		0,48	
35°	980,14	1,30	978,84	1,29	977,55	1,28	976,27	1,27	975,00	1,27	973,73	1,27	972,46	1,26	971,20	1,27	969,93	1,28	968,65	1,27	967,38	1,29	966,09	1,30
	0,37		0,38		0,38		0,39		0,40		0,41		0,42		0,44		0,45		0,45		0,47		0,48	
36°	979,77	1,31	978,46	1,29	977,17	1,29	975,88	1,28	974,60	1,28	973,32	1,28	972,04	1,28	970,76	1,28	969,48	1,28	968,20	1,29	966,91	1,30	965,61	1,32
	0,39		0,39		0,40		0,40		0,41		0,42		0,43		0,44		0,45		0,47		0,48		0,49	
37°	978,38	1,31	978,07	1,30	976,77	1,29	975,48	1,29	974,19	1,29	972,90	1,29	971,61	1,29	970,32	1,29	969,03	1,30	967,73	1,30	966,43	1,31	965,12	1,33
	0,38		0,39		0,40		0,41		0,42		0,43		0,44		0,45		0,46		0,47		0,49		0,50	
38°	979,00	1,32	977,68	1,31	976,37	1,30	975,07	1,30	973,77	1,30	972,47	1,30	971,17	1,30	969,87	1,30	968,57	1,31	967,26	1,32	965,94	1,32	964,62	1,34
	0,40		0,40		0,41		0,42		0,42		0,43		0,44		0,45		0,47		0,48		0,49		0,50	
39°	978,60	1,32	977,28	1,32	975,96	1,31	974,65	1,30	973,35	1,31	972,04	1,31	970,73	1,31	969,42	1,32	968,10	1,32	966,78	1,33	965,45	1,33	964,12	1,36
	0,40		0,41		0,41		0,42		0,43		0,44		0,45		0,46		0,47		0,48		0,49		0,51	
40°	978,20	1,33	976,87	1,32	975,55	1,32	974,23	1,31	972,92	1,32	971,60	1,32	970,28	1,32	968,96	1,33	967,63	1,33	966,30	1,34	964,96	1,35	963,61	1,37

▼B

t°	Alcoholic strength by % vol																							
	20		**21**		**22**		**23**		**24**		**25**		**26**		**27**		**28**		**29**		**30**		**31**	
0	978,56	0,70	977,86	0,70	977,16	0,69	976,47	0,71	975,76	0,71	975,05	0,72	974,33	0,75	973,58	0,77	972,81	0,80	972,01	0,83	971,18	0,87	970,31	0,90
	0,14		0,17		0,19		0,22		0,24		0,26		0,29		0,31		0,34		0,36		0,39		0,41	
1	978,42	0,73	977,69	0,72	976,97	0,72	976,25	0,73	975,52	0,73	974,79	0,75	974,04	0,77	973,27	0,80	972,47	0,82	971,65	0,86	970,79	0,89	969,90	0,92
	0,16		0,18		0,20		0,23		0,25		0,28		0,30		0,32		0,34		0,37		0,39		0,41	
2	978,26	0,75	977,51	0,74	976,77	0,75	976,02	0,75	975,27	0,76	974,51	0,77	973,74	0,79	972,95	0,82	972,13	0,85	971,28	0,88	970,40	0,91	969,49	0,95
	0,17		0,19		0,22		0,23		0,26		0,28		0,31		0,33		0,36		0,38		0,40		0,42	
3	978,09	0,77	977,32	0,77	976,55	0,76	975,79	0,78	975,01	0,78	974,23	0,80	973,43	0,81	972,62	0,85	971,77	0,87	970,90	0,90	970,00	0,93	969,07	0,98
	0,18		0,20		0,22		0,25		0,27		0,29		0,31		0,34		0,36		0,38		0,40		0,43	
4	977,91	0,79	977,12	0,79	976,33	0,79	975,54	0,80	974,94	0,80	973,94	0,82	973,12	0,84	972,28	0,87	971,41	0,89	970,52	0,92	969,60	0,96	968,64	1,00
	0,19		0,22		0,23		0,26		0,27		0,30		0,33		0,35		0,37		0,39		0,42		0,44	
5	977,72	0,82	976,90	0,80	976,10	0,82	975,28	0,81	974,47	0,83	973,64	0,85	972,79	0,86	971,93	0,89	971,04	0,91	970,13	0,95	969,18	0,98	968,20	1,01
	0,21		0,22		0,25		0,26		0,29		0,31		0,33		0,35		0,37		0,40		0,42		0,44	
6	977,51	0,83	976,68	0,83	975,85	0,83	975,02	0,84	974,18	0,85	973,33	0,87	972,46	0,86	971,58	0,91	970,67	0,94	969,73	0,97	968,76	1,00	967,76	1,03
	0,21		0,23		0,25		0,28		0,30		0,32		0,34		0,36		0,38		0,40		0,42		0,44	
7	977,30	0,85	976,45	0,85	975,60	0,86	974,74	0,86	973,88	0,87	973,01	0,89	972,12	0,90	971,22	0,93	970,29	0,96	969,33	0,99	968,34	1,02	967,32	1,06
	0,23		0,25		0,27		0,28		0,31		0,33		0,35		0,37		0,40		0,42		0,43		0,46	
8	977,07	0,87	976,20	0,87	975,33	0,87	974,46	0,89	973,57	0,89	972,68	0,91	971,77	0,92	970,85	0,96	969,89	0,98	968,91	1,00	967,91	1,05	966,86	1,07
	0,24		0,26		0,28		0,30		0,31		0,34		0,35		0,38		0,39		0,41		0,44		0,46	
9	976,83	0,89	975,94	0,89	975,05	0,89	974,16	0,90	973,26	0,92	972,34	0,92	971,42	0,95	970,47	0,97	969,50	1,00	968,50	1,03	967,47	1,07	966,40	1,09
	0,24		0,26		0,28		0,30		0,33		0,34		0,37		0,39		0,41		0,43		0,45		0,46	
10	976,59	0,91	975,68	0,91	974,77	0,91	973,86	0,93	972,93	0,93	972,00	0,95	971,05	0,97	970,08	0,99	969,09	1,02	968,07	1,05	967,02	1,08	965,94	1,12
	0,27		0,29		0,30		0,33		0,34		0,36		0,38		0,40		0,42		0,44		0,46		0,47	
11	976,32	0,93	975,39	0,92	974,47	0,94	973,53	0,94	972,59	0,95	971,64	0,97	970,67	0,99	969,68	1,01	968,67	1,04	967,63	1,07	966,56	1,09	965,47	1,13
	0,27		0,28		0,31		0,32		0,34		0,36		0,38		0,40		0,42		0,44		0,45		0,48	
12	976,05	0,94	975,11	0,95	974,16	0,95	973,21	0,96	972,25	0,97	971,28	0,99	970,29	1,01	969,28	1,03	968,25	1,06	967,19	1,08	966,11	1,12	964,99	1,15
	0,28		0,30		0,31		0,33		0,35		0,37		0,39		0,41		0,43		0,45		0,47		0,49	
13	975,77	0,96	974,81	0,96	973,85	0,97	972,88	0,98	971,90	0,99	970,91	1,01	969,90	1,03	968,87	1,05	967,82	1,08	966,74	1,10	965,64	1,14	964,50	1,17
	0,28		0,30		0,32		0,34		0,36		0,38		0,40		0,41		0,43		0,45		0,47		0,49	
14	975,49	0,98	974,51	0,98	973,53	0,99	972,54	1,00	971,54	1,01	970,53	1,03	969,50	1,04	968,46	1,07	967,39	1,10	966,29	1,12	965,17	1,16	964,01	1,19
	0,30		0,32		0,34		0,35		0,37		0,39		0,40		0,42		0,44		0,46		0,48		0,49	
15	975,19	1,00	974,19	1,00	973,19	1,00	972,19	1,02	971,17	1,03	970,14	1,04	969,10	1,06	968,04	1,09	966,95	1,12	965,83	1,14	964,69	1,17	963,52	1,21
	0,31		0,32		0,34		0,36		0,37		0,39		0,41		0,43		0,45		0,46		0,48		0,51	
16	974,88	1,01	973,87	1,02	972,85	1,02	971,83	1,03	970,80	1,05	969,75	1,06	968,69	1,08	967,61	1,11	966,50	1,13	965,37	1,16	964,21	1,20	963,01	1,22
	0,32		0,33		0,35		0,37		0,39		0,40		0,42		0,44		0,45		0,48		0,50		0,50	
17	974,56	1,02	973,54	1,04	972,50	1,04	971,46	1,05	970,41	1,06	969,35	1,08	968,27	1,10	967,17	1,12	966,05	1,16	964,89	1,18	963,71	1,20	962,51	1,24
	0,32		0,35		0,36		0,37		0,39		0,41		0,43		0,45		0,47		0,48		0,49		0,52	
18	974,24	1,05	973,19	1,05	972,14	1,05	971,09	1,07	970,02	1,08	968,94	1,10	967,84	1,12	966,72	1,14	965,58	1,17	964,41	1,19	963,22	1,23	961,99	1,25
	0,34		0,35		0,36		0,39		0,40		0,42		0,43		0,45		0,47		0,48		0,50		0,52	
19	973,90	1,06	972,84	1,06	971,78	1,08	970,70	1,08	969,62	1,10	968,52	1,11	967,41	1,14	966,27	1,16	965,11	1,18	963,93	1,21	962,72	1,25	961,47	1,27
	0,34		0,36		0,38		0,39		0,41		0,42		0,45		0,46		0,47		0,49		0,51		0,52	
20	973,56	1,08	972,48	1,08	971,40	1,09	970,31	1,10	969,21	1,11	968,10	1,14	966,96	1,15	965,81	1,17	964,64	1,20	963,44	1,23	962,21	1,26	960,95	1,29

t°	Alcoholic strength by % vol																							
	20		**21**		**22**		**23**		**24**		**25**		**26**		**27**		**28**		**29**		**30**		**31**	
20	973,56	1,08	972,48	1,08	971,40	1,09	970,31	1,10	969,21	1,11	968,10	1,14	966,96	1,15	965,81	1,17	964,64	1,20	963,44	1,23	962,21	1,26	960,95	1,29
	0,36		0,37		0,38		0,40		0,42		0,44		0,45		0,46		0,49		0,50		0,52		0,53	
21	973,20	1,09	972,11	1,09	971,02	1,11	969,91	1,12	968,79	1,13	967,66	1,15	966,51	1,16	965,35	1,20	964,15	1,21	962,94	1,25	961,69	1,27	960,42	1,31
	0,36		0,37		0,40		0,41		0,42		0,44		0,45		0,48		0,49		0,51		0,52		0,54	
22	972,84	1,10	971,74	1,12	970,62	1,12	969,50	1,13	968,37	1,15	967,22	1,16	966,06	1,19	964,87	1,21	963,66	1,23	962,43	1,26	961,17	1,29	959,88	1,32
	0,37		0,39		0,40		0,42		0,43		0,45		0,47		0,48		0,49		0,51		0,53		0,55	
23	972,47	1,12	971,35	1,13	970,22	1,14	969,08	1,14	967,94	1,17	966,77	1,18	965,59	1,20	964,39	1,22	963,17	1,25	961,92	1,28	960,64	1,31	959,33	1,33
	0,38		0,40		0,41		0,42		0,44		0,45		0,47		0,49		0,51		0,52		0,54		0,55	
24	972,09	1,14	970,95	1,14	969,81	1,15	968,66	1,16	967,50	1,18	966,32	1,20	965,12	1,22	963,90	1,24	962,66	1,26	961,40	1,30	960,10	1,32	958,78	1,35
	0,39		0,40		0,42		0,43		0,45		0,47		0,48		0,49		0,51		0,53		0,54		0,55	
25	971,70	1,15	970,55	1,16	969,39	1,16	968,23	1,18	967,05	1,20	965,85	1,21	964,64	1,23	963,41	1,26	962,15	1,28	960,87	1,31	959,56	1,33	958,23	1,37
	0,40		0,41		0,42		0,44		0,46		0,47		0,49		0,50		0,51		0,53		0,54		0,57	
26	971,30	1,16	970,14	1,17	968,97	1,18	967,79	1,20	966,59	1,21	965,38	1,23	964,15	1,24	962,91	1,27	961,64	1,30	960,34	1,32	959,02	1,36	957,66	1,38
	0,40		0,42		0,43		0,45		0,46		0,48		0,49		0,51		0,53		0,54		0,56		0,56	
27	970,90	1,18	969,72	1,18	968,54	1,20	967,34	1,21	966,13	1,23	964,90	1,24	963,66	1,26	962,40	1,29	961,11	1,31	959,80	1,34	958,46	1,36	957,10	1,40
	0,41		0,43		0,45		0,46		0,47		0,48		0,50		0,52		0,54		0,56		0,57		0,59	
28	970,49	1,20	969,29	1,20	968,09	1,21	966,88	1,22	965,66	1,24	964,42	1,26	963,16	1,28	961,88	1,31	960,57	1,33	959,24	1,35	957,89	1,38	956,51	1,41
	0,42		0,43		0,45		0,47		0,49		0,50		0,52		0,53		0,53		0,55		0,56		0,58	
29	970,07	1,21	968,86	1,22	967,64	1,23	966,41	1,24	965,17	1,25	963,92	1,28	962,64	1,29	961,35	1,31	960,04	1,35	958,69	1,36	957,33	1,40	955,93	1,42
	0,44		0,45		0,46		0,47		0,49		0,50		0,51		0,53		0,55		0,55		0,58		0,58	
30	969,63	1,22	968,41	1,23	967,18	1,24	965,94	1,26	964,68	1,26	963,42	1,29	962,13	1,31	960,82	1,33	959,49	1,35	958,14	1,39	956,75	1,40	955,35	1,44
	0,44		0,45		0,46		0,48		0,49		0,51		0,52		0,53		0,55		0,57		0,58		0,60	
31	969,19	1,23	967,96	1,24	966,72	1,26	965,46	1,27	964,19	1,28	962,91	1,30	961,61	1,32	960,29	1,35	958,94	1,37	957,57	1,40	956,17	1,42	954,75	1,44
	0,44		0,46		0,47		0,48		0,50		0,51		0,53		0,54		0,55		0,57		0,58		0,59	
32	968,75	1,25	967,50	1,25	966,25	1,27	964,98	1,29	963,69	1,29	962,40	1,32	961,08	1,33	959,75	1,36	958,39	1,39	957,00	1,41	955,59	1,43	954,16	1,46
	0,45		0,46		0,48		0,49		0,50		0,52		0,53		0,55		0,57		0,57		0,59		0,61	
33	968,30	1,26	967,04	1,27	965,77	1,28	964,49	1,30	963,19	1,31	961,88	1,33	960,55	1,35	959,20	1,38	957,82	1,39	956,43	1,43	955,00	1,45	953,55	1,47
	0,46		0,47		0,49		0,50		0,51		0,53		0,54		0,56		0,56		0,59		0,59		0,60	
34	967,84	1,27	966,57	1,29	965,28	1,29	963,99	1,31	962,68	1,33	961,35	1,34	960,01	1,37	958,64	1,38	957,26	1,42	955,84	1,43	954,41	1,46	952,95	1,49
	0,46		0,48		0,49		0,51		0,52		0,53		0,55		0,56		0,58		0,58		0,60		0,62	
35	967,38	1,29	966,09	1,30	964,79	1,31	963,48	1,32	962,16	1,34	960,82	1,36	959,46	1,38	958,08	1,40	956,68	1,42	955,26	1,45	953,81	1,48	952,33	1,50
	0,47		0,48		0,50		0,51		0,53		0,54		0,55		0,57		0,58		0,60		0,61		0,62	
36	966,91	1,30	965,61	1,32	964,29	1,32	962,97	1,34	961,63	1,35	960,28	1,37	958,91	1,40	957,51	1,41	956,10	1,44	954,66	1,46	953,20	1,49	951,71	1,51
	0,48		0,49		0,50		0,52		0,53		0,55		0,56		0,57		0,59		0,60		0,61		0,62	
37	966,43	1,31	965,12	1,33	963,79	1,34	962,45	1,35	961,10	1,37	959,73	1,38	958,35	1,41	956,94	1,43	955,51	1,45	954,06	1,47	952,59	1,50	951,09	1,53
	0,49		0,50		0,51		0,52		0,54		0,55		0,57		0,58		0,59		0,60		0,62		0,63	
38	965,94	1,32	964,62	1,34	963,28	1,35	961,93	1,37	960,56	1,38	959,18	1,40	957,78	1,42	956,36	1,44	954,92	1,46	953,46	1,49	951,97	1,51	950,46	1,54
	0,49		0,50		0,52		0,53		0,54		0,56		0,57		0,58		0,60		0,61		0,62		0,64	
39	965,45	1,33	964,12	1,36	962,76	1,36	961,40	1,38	960,02	1,40	958,62	1,41	957,21	1,43	955,78	1,46	954,32	1,47	952,85	1,50	951,35	1,53	949,82	1,55
	0,49		0,51		0,52		0,54		0,55		0,56		0,58		0,59		0,60		0,62		0,63		0,64	
40	964,96	1,35	963,61	1,37	962,24	1,38	960,86	1,39	959,47	1,41	958,06	1,43	956,63	1,44	955,19	1,47	953,72	1,49	952,23	1,51	950,72	1,54	949,18	1,57

▼B

TABLE II

INTERNATIONAL ALCOHOLIC STRENGTH AT 20 ºC

Table of corrections to be applied to the apparent alcoholic strength to correct for the effect of temperature

Add or subtract from the apparent alcoholic strength at *t* ºC (ordinary glass alcoholmeter) the correction indicated below

			Apparent alcoholic strength at *t* ºC																
			0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Temperature (ºC)	0º	Add	0,76	0,77	0,82	0,87	0,95	1,04	1,16	1,31	1,49	1,70	1,95	2,26	2,62	3,03	3,49	4,02	4,56
	1º		0,81	0,83	0,87	0,92	1,00	1,09	1,20	1,35	1,52	1,73	1,97	2,26	2,59	2,97	3,40	3,87	4,36
	2º		0,85	0,87	0,92	0,97	1,04	1,13	1,24	1,38	1,54	1,74	1,97	2,24	2,54	2,89	3,29	3,72	4,17
	3º		0,88	0,91	0,95	1,00	1,07	1,15	1,26	1,39	1,55	1,73	1,95	2,20	2,48	2,80	3,16	3,55	3,95
	4º		0,90	0,92	0,97	1,02	1,09	1,17	1,27	1,40	1,55	1,72	1,92	2,15	2,41	2,71	3,03	3,38	3,75
	5º		0,91	0,93	0,98	1,03	1,10	1,17	1,27	1,39	1,53	1,69	1,87	2,08	2,33	2,60	2,89	3,21	3,54
	6º		0,92	0,94	0,98	1,02	1,09	1,16	1,25	1,37	1,50	1,65	1,82	2,01	2,23	2,47	2,74	3,02	3,32
	7º		0,91	0,93	0,97	1,01	1,07	1,14	1,23	1,33	1,45	1,59	1,75	1,92	2,12	2,34	2,58	2,83	3,10
	8º		0,89	0,91	0,94	0,98	1,04	1,11	1,19	1,28	1,39	1,52	1,66	1,82	2,00	2,20	2,42	2,65	2,88
	9º		0,86	0,88	0,91	0,95	1,01	1,07	1,14	1,23	1,33	1,44	1,57	1,71	1,87	2,05	2,24	2,44	2,65
	10º		0,82	0,84	0,87	0,91	0,96	1,01	1,08	1,16	1,25	1,35	1,47	1,60	1,74	1,89	2,06	2,24	2,43
	11º		0,78	0,79	0,82	0,86	0,90	0,95	1,01	1,08	1,16	1,25	1,36	1,47	1,60	1,73	1,88	2,03	2,20
	12º		0,72	0,74	0,76	0,79	0,83	0,88	0,93	0,99	1,07	1,15	1,24	1,34	1,44	1,56	1,69	1,82	1,96
	13º		0,66	0,67	0,69	0,72	0,76	0,80	0,84	0,90	0,96	1,03	1,11	1,19	1,28	1,38	1,49	1,61	1,73
	14º		0,59	0,60	0,62	0,64	0,67	0,71	0,74	0,79	0,85	0,91	0,97	1,04	1,12	1,20	1,29	1,39	1,49
	15º		0,51	0,52	0,53	0,55	0,58	0,61	0,64	0,68	0,73	0,77	0,83	0,89	0,95	1,02	1,09	1,16	1,24
	16º		0,42	0,43	0,44	0,46	0,48	0,50	0,53	0,56	0,60	0,63	0,67	0,72	0,77	0,82	0,88	0,94	1,00
	17º		0,33	0,33	0,34	0,35	0,37	0,39	0,41	0,43	0,46	0,48	0,51	0,55	0,59	0,62	0,67	0,71	0,75
	18º		0,23	0,23	0,23	0,24	0,25	0,26	0,27	0,29	0,31	0,33	0,35	0,37	0,40	0,42	0,45	0,48	0,51
	19º		0,12	0,12	0,12	0,12	0,13	0,13	0,14	0,15	0,16	0,17	0,18	0,19	0,20	0,21	0,23	0,24	0,25

			Apparent alcoholic strength at *t* ºC																
			0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Temperature (º)	21º	Subtract		0,13	0,13	0,13	0,14	0,14	0,15	0,16	0,17	0,18	0,19	0,19	0,20	0,22	0,23	0,25	0,26
	22º			0,26	0,27	0,28	0,29	0,30	0,31	0,32	0,34	0,36	0,37	0,39	0,41	0,44	0,47	0,49	0,52
	23º			0,40	0,41	0,42	0,44	0,45	0,47	0,49	0,51	0,54	0,57	0,60	0,63	0,66	0,70	0,74	0,78
	24º			0,55	0,56	0,58	0,60	0,62	0,64	0,67	0,70	0,73	0,77	0,81	0,85	0,89	0,94	0,99	1,04
	25º			0,69	0,71	0,73	0,76	0,79	0,82	0,85	0,89	0,93	0,97	1,02	1,07	1,13	1,19	1,25	1,31
	26º			0,85	0,87	0,90	0,93	0,96	1,00	1,04	1,08	1,13	1,18	1,24	1,30	1,36	1,43	1,50	1,57
	27º				1,03	1,07	1,11	1,15	1,19	1,23	1,28	1,34	1,40	1,46	1,53	1,60	1,68	1,76	1,84
	28º				1,21	1,25	1,29	1,33	1,38	1,43	1,49	1,55	1,62	1,69	1,77	1,85	1,93	2,02	2,11
	29º				1,39	1,43	1,47	1,52	1,58	1,63	1,70	1,76	1,84	1,92	2,01	2,10	2,19	2,29	2,39
	30º				1,57	1,61	1,66	1,72	1,78	1,84	1,91	1,98	2,07	2,15	2,25	2,35	2,45	2,56	2,67
	31º				1,75	1,80	1,86	1,92	1,98	2,05	2,13	2,21	2,30	2,39	2,49	2,60	2,71	2,83	2,94
	32º				1,94	2,00	2,06	2,13	2,20	2,27	2,35	2,44	2,53	2,63	2,74	2,86	2,97	3,09	3,22
	33º					2,20	2,27	2,34	2,42	2,50	2,58	2,67	2,77	2,88	2,99	3,12	3,24	3,37	3,51
	34º					2,41	2,48	2,56	2,64	2,72	2,81	2,91	3,02	3,13	3,25	3,38	3,51	3,65	3,79
	35º					2,62	2,70	2,78	2,86	2,95	3,05	3,16	3,27	3,39	3,51	3,64	3,78	3,93	4,08
	36º					2,83	2,91	3,00	3,09	3,19	3,29	3,41	3,53	3,65	3,78	3,91	4,05	4,21	4,37
	37º						3,13	3,23	3,33	3,43	3,54	3,65	3,78	3,91	4,04	4,18	4,33	4,49	4,65
	38º						3,36	3,47	3,57	3,68	3,79	3,91	4,03	4,17	4,31	4,46	4,61	4,77	4,94
	39º						3,59	3,70	3,81	3,93	4,05	4,17	4,30	4,44	4,58	4,74	4,90	5,06	5,23
	40º						3,82	3,94	4,06	4,18	4,31	4,44	4,57	4,71	4,86	5,02	5,19	5,36	5,53

▼**B**

			Apparent alcoholic strength at *t* ºC																
			14	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Temperature (ºC)	0º	Add	3,49	4,02	4,56	5,11	5,65	6,16	6,63	7,05	7,39	7,67	7,91	8,07	8,20	8,30	8,36	8,39	8,40
	1º		3,40	3,87	4,36	4,86	5,35	5,82	6,26	6,64	6,96	7,23	7,45	7,62	7,75	7,85	7,91	7,95	7,96
	2º		3,29	3,72	4,17	4,61	5,05	5,49	5,89	6,25	6,55	6,81	7,02	7,18	7,31	7,40	7,47	7,51	7,53
	3º		3,16	3,55	3,95	4,36	4,77	5,17	5,53	5,85	6,14	6,39	6,59	6,74	6,86	6,97	7,03	7,07	7,09
	4º		3,03	3,38	3,75	4,11	4,48	4,84	5,17	5,48	5,74	5,97	6,16	6,31	6,43	6,53	6,59	6,63	6,66
	5º		2,89	3,21	3,54	3,86	4,20	4,52	4,83	5,11	5,35	5,56	5,74	5,89	6,00	6,10	6,16	6,20	6,23
	6º		2,74	3,02	3,32	3,61	3,91	4,21	4,49	4,74	4,96	5,16	5,33	5,47	5,58	5,67	5,73	5,77	5,80
	7º		2,58	2,83	3,10	3,36	3,63	3,90	4,15	4,38	4,58	4,77	4,92	5,05	5,15	5,24	5,30	5,34	5,37
	8º		2,42	2,65	2,88	3,11	3,35	3,59	3,81	4,02	4,21	4,38	4,52	4,64	4,74	4,81	4,87	4,92	4,95
	9º		2,24	2,44	2,65	2,86	3,07	3,28	3,48	3,67	3,84	3,99	4,12	4,23	4,32	4,39	4,45	4,50	4,53
	10º		2,06	2,24	2,43	2,61	2,80	2,98	3,16	3,33	3,48	3,61	3,73	3,83	3,91	3,98	4,03	4,08	4,11
	11º		1,88	2,03	2,20	2,36	2,52	2,68	2,83	2,98	3,12	3,24	3,34	3,43	3,50	3,57	3,62	3,66	3,69
	12º		1,69	1,82	1,96	2,10	2,24	2,38	2,51	2,64	2,76	2,87	2,96	3,04	3,10	3,16	3,21	3,25	3,27
	13º		1,49	1,61	1,73	1,84	1,96	2,08	2,20	2,31	2,41	2,50	2,58	2,65	2,71	2,76	2,80	2,83	2,85
	14º		1,29	1,39	1,49	1,58	1,68	1,78	1,88	1,97	2,06	2,13	2,20	2,26	2,31	2,36	2,39	2,42	2,44
	15º		1,09	1,16	1,24	1,32	1,40	1,48	1,56	1,64	1,71	1,77	1,83	1,88	1,92	1,96	1,98	2,01	2,03
	16º		0,88	0,94	1,00	1,06	1,12	1,19	1,25	1,31	1,36	1,41	1,46	1,50	1,53	1,56	1,58	1,60	1,62
	17º		0,67	0,71	0,75	0,80	0,84	0,89	0,94	0,98	1,02	1,05	1,09	1,12	1,14	1,17	1,18	1,20	1,21
	18º		0,45	0,48	0,51	0,53	0,56	0,59	0,62	0,65	0,68	0,70	0,72	0,74	0,76	0,78	0,79	0,80	0,81
	19º		0,23	0,24	0,25	0,27	0,28	0,30	0,31	0,33	0,34	0,35	0,36	0,37	0,38	0,39	0,40	0,40	0,41

			Apparent alcoholic strength at *t* ºC																
			14	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Temperature (ºC)	21º	Subtract	0,23	0,25	0,26	0,28	0,29	0,30	0,31	0,33	0,34	0,35	0,35	0,37	0,38	0,38	0,39	0,39	0,40
	22º		0,47	0,49	0,52	0,55	0,57	0,60	0,62	0,65	0,67	0,70	0,72	0,74	0,75	0,76	0,78	0,79	0,80
	23º		0,70	0,74	0,78	0,82	0,86	0,90	0,93	0,97	1,01	1,04	1,07	1,10	1,12	1,15	1,17	1,18	1,19
	24º		0,94	0,99	1,04	1,10	1,15	1,20	1,25	1,29	1,34	1,39	1,43	1,46	1,50	1,53	1,55	1,57	1,59
	25º		1,19	1,25	1,31	1,37	1,43	1,49	1,56	1,62	1,68	1,73	1,78	1,83	1,87	1,90	1,94	1,97	1,99
	26º		1,43	1,50	1,57	1,65	1,73	1,80	1,87	1,94	2,01	2,07	2,13	2,19	2,24	2,28	2,32	2,35	2,38
	27º		1,68	1,76	1,84	1,93	2,01	2,10	2,18	2,26	2,34	2,41	2,48	2,55	2,61	2,66	2,70	2,74	2,77
	28º		1,93	2,02	2,11	2,21	2,31	2,40	2,49	2,58	2,67	2,76	2,83	2,90	2,98	3,03	3,08	3,13	3,17
	29º		2,19	2,29	2,39	2,50	2,60	2,70	2,81	2,91	3,00	3,09	3,18	3,26	3,34	3,40	3,46	3,51	3,55
	30º		2,45	2,56	2,67	2,78	2,90	3,01	3,12	3,23	3,34	3,44	3,53	3,62	3,70	3,77	3,84	3,90	3,95
	31º		2,71	2,83	2,94	3,07	3,19	3,31	3,43	3,55	3,67	3,78	3,88	3,98	4,07	4,15	4,22	4,28	4,33
	32º		2,97	3,09	3,22	3,36	3,49	3,62	3,74	3,87	4,00	4,11	4,22	4,33	4,43	4,51	4,59	4,66	4,72
	33º		3,24	3,37	3,51	3,65	3,79	3,92	4,06	4,20	4,33	4,45	4,57	4,68	4,79	4,88	4,97	5,04	5,10
	34º		3,51	3,65	3,79	3,94	4,09	4,23	4,37	4,52	4,66	4,79	4,91	5,03	5,15	5,25	5,34	5,42	5,49
	35º		3,78	3,93	4,08	4,23	4,38	4,53	4,69	4,84	4,98	5,12	5,26	5,38	5,50	5,61	5,71	5,80	5,87
	36º		4,05	4,21	4,37	4,52	4,68	4,84	5,00	5,16	5,31	5,46	5,60	5,73	5,86	5,97	6,08	6,17	6,25
	37º		4,33	4,49	4,65	4,82	4,98	5,15	5,31	5,48	5,64	5,80	5,95	6,09	6,22	6,33	6,44	6,54	6,63
	38º		4,61	4,77	4,94	5,12	5,29	5,46	5,63	5,80	5,97	6,13	6,29	6,43	6,57	6,69	6,81	6,92	7,01
	39º		4,90	5,06	5,23	5,41	5,59	5,77	5,94	6,12	6,30	6,47	6,63	6,78	6,93	7,06	7,18	7,29	7,39
	40º		5,19	5,36	5,53	5,71	5,90	6,08	6,26	6,44	6,62	6,80	6,97	7,13	7,28	7,41	7,54	7,66	7,76

▼B

TABLE III

INTERNATIONAL ALCOHOLIC STRENGTH AT 20 °C

Table of apparent densities of ethanol-water mixtures — Ordinary glass apparatus

Densities at *t* °C corrected for air buoyancy

	Alcoholic strength by % vol																							
t°	**0**		**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**		**9**		**10**		**11**	
0	999,34	1,52	997,82	1,45	996,37	1,39	994,98	1,35	993,63	1,29	992,34	1,24	991,10	1,18	989,92	1,15	988,77	1,09	987,68	1,05	986,63	1,00	985,63	0,96
	−0,09		−0,09		−0,09		−0,08		−0,08		−0,08		−0,07		−0,05		−0,05		−0,04		−0,03		−0,02	
1	999,43	1,52	997,91	1,45	996,46	1,40	995,06	1,35	993,71	1,29	992,42	1,25	991,17	1,20	989,97	1,15	988,82	1,10	987,72	1,06	986,66	1,01	985,65	0,97
	−0,06		−0,06		−0,06		−0,06		−0,06		−0,05		−0,05		−0,04		−0,03		−0,02		−0,02		−0,01	
2	999,49	1,52	997,97	1,40	996,52	1,40	995,12	1,35	993,77	1,30	992,47	1,25	991,22	1,21	990,01	1,16	988,85	1,11	987,74	1,06	986,68	1,02	985,66	0,98
	−0,05		−0,05		−0,04		−0,04		−0,04		−0,04		−0,03		−0,03		−0,03		−0,02		0,00		0,01	
3	999,54	1,52	998,02	1,46	996,56	1,40	995,16	1,35	993,81	1,30	992,51	1,26	991,25	1,21	990,04	1,16	988,88	1,12	987,76	1,08	986,68	1,03	985,65	0,99
	−0,03		−0,03		−0,03		−0,03		−0,02		−0,02		−0,02		−0,01		0,00		0,01		0,01		0,02	
4	999,57	1,52	998,05	1,46	996,59	1,40	995,19	1,36	993,83	1,30	992,53	1,26	991,27	1,22	990,05	1,17	988,88	1,13	987,75	1,08	986,67	1,04	985,63	1,00
	−0,02		−0,02		−0,02		−0,02		−0,02		−0,01		0,00		0,00		0,00		0,01		0,02		0,03	
5	999,59	1,52	998,07	1,46	996,61	1,40	995,21	1,36	993,85	1,31	992,54	1,27	991,27	1,22	990,05	1,17	988,88	1,14	987,74	1,09	986,65	1,05	985,60	1,02
	0,00		0,00		0,00		0,01		0,01		0,01		0,01		0,02		0,03		0,03		0,04		0,06	
6	999,59	1,52	998,07	1,46	996,61	1,41	995,20	1,36	993,84	1,31	992,53	1,27	991,26	1,23	990,03	1,18	988,85	1,14	987,71	1,10	986,61	1,07	985,54	1,02
	0,01		0,01		0,01		0,01		0,01		0,02		0,02		0,02		0,03		0,04		0,05		0,06	
7	999,58	1,52	998,06	1,46	996,60	1,41	995,19	1,36	993,83	1,32	992,51	1,27	991,24	1,23	990,01	1,19	988,82	1,15	987,67	1,11	986,56	1,08	985,48	1,04
	0,03		0,03		0,03		0,03		0,04		0,04		0,05		0,05		0,06		0,07		0,07		0,08	
8	999,55	1,52	998,03	1,46	996,57	1,41	995,16	1,37	993,79	1,32	992,47	1,28	991,19	1,23	989,96	1,20	988,76	1,16	987,60	1,11	986,49	1,09	985,40	1,05
	0,04		0,04		0,04		0,04		0,04		0,04		0,05		0,06		0,06		0,06		0,08		0,08	
9	999,51	1,52	997,99	1,46	996,53	1,41	995,12	1,37	993,75	1,32	992,43	1,29	991,14	1,24	989,90	1,20	988,70	1,16	987,54	1,13	986,41	1,09	985,32	1,06
	0,06		0,06		0,06		0,06		0,06		0,07		0,07		0,07		0,08		0,09		0,10		0,11	
10	999,45	1,52	997,93	1,46	996,47	1,41	995,06	1,37	993,69	1,33	992,36	1,29	991,07	1,24	989,83	1,21	988,62	1,17	987,45	1,14	986,31	1,10	985,21	1,07
	0,07		0,06		0,06		0,07		0,07		0,07		0,07		0,08		0,09		0,10		0,10		0,11	
11	999,38	1,51	997,87	1,46	996,41	1,42	994,99	1,37	993,62	1,33	992,29	1,29	991,00	1,25	989,75	1,22	988,53	1,18	987,35	1,14	986,21	1,11	985,10	1,08
	0,09		0,09		0,09		0,09		0,09		0,09		0,10		0,11		0,11		0,11		0,12		0,13	
12	999,29	1,51	997,78	1,46	996,32	1,42	994,90	1,37	993,53	1,33	992,20	1,30	990,90	1,26	989,64	1,22	988,42	1,18	987,24	1,15	986,09	1,12	984,97	1,09
	0,09		0,09		0,09		0,09		0,10		0,10		0,10		0,10		0,11		0,12		0,13		0,14	
13	999,20	1,51	997,69	1,46	996,23	1,42	994,81	1,38	993,43	1,33	992,10	1,30	990,80	1,26	989,54	1,23	988,31	1,19	987,12	1,16	985,96	1,13	984,83	1,10
	0,11		0,11		0,11		0,11		0,11		0,12		0,12		0,13		0,13		0,14		0,15		0,16	
14	999,09	1,51	997,58	1,46	996,12	1,42	994,70	1,38	993,32	1,34	991,98	1,30	990,68	1,27	989,41	1,23	988,18	1,20	986,98	1,17	985,81	1,14	984,67	1,11
	0,12		0,12		0,12		0,12		0,12		0,12		0,13		0,13		0,14		0,14		0,15		0,16	
15	998,97	1,51	997,46	1,46	996,00	1,42	994,58	1,38	993,20	1,34	991,86	1,31	990,55	1,27	989,28	1,24	988,04	1,20	986,84	1,18	985,66	1,15	984,51	1,12
	0,13		0,13		0,13		0,13		0,14		0,14		0,14		0,15		0,15		0,17		0,17		0,18	
16	998,84	1,51	997,33	1,46	995,87	1,42	994,45	1,39	993,06	1,34	991,72	1,31	990,41	1,28	989,13	1,24	987,89	1,22	986,67	1,18	985,49	1,16	984,33	1,13
	0,14		0,14		0,14		0,14		0,14		0,15		0,15		0,15		0,16		0,17		0,17		0,18	
17	998,70	1,51	997,19	1,46	995,73	1,42	994,31	1,39	992,92	1,35	991,57	1,31	990,26	1,28	988,98	1,25	987,73	1,22	986,50	1,18	985,32	1,17	984,15	1,14
	0,15		0,15		0,16		0,16		0,16		0,16		0,17		0,17		0,18		0,18		0,19		0,19	
18	998,55	1,51	997,04	1,47	995,57	1,42	994,15	1,39	992,76	1,35	991,41	1,32	990,09	1,28	988,81	1,26	987,55	1,23	986,32	1,19	985,13	1,17	983,96	1,15
	0,17		0,16		0,16		0,16		0,16		0,16		0,17		0,18		0,18		0,19		0,20		0,21	
19	998,38	1,50	996,88	1,47	995,41	1,42	993,99	1,39	992,60	1,35	991,25	1,33	989,92	1,29	988,63	1,26	987,37	1,24	986,13	1,20	984,93	1,18	983,75	1,16
	0,18		0,18		0,18		0,18		0,19		0,19		0,19		0,20		0,21		0,22		0,22		0,23	
20	998,20	1,50	996,70	1,47	995,23	1,42	993,81	1,40	992,41	1,35	991,06	1,33	989,73	1,30	988,43	1,27	987,16	1,24	985,92	1,21	984,71	1,19	983,52	1,17

	Alcoholic strength by % vol																							
t°	**0**		**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**		**9**		**10**		**11**	
20	998,20	1,50	996,70	1,47	995,23	1,42	993,81	1,40	992,41	1,35	991,06	1,33	989,73	1,30	988,43	1,27	987,16	1,24	985,92	1,21	984,71	1,19	983,52	1,17
	0,19		0,19		0,19		0,19		0,19		0,20		0,20		0,21		0,21		0,22		0,23		0,23	
21	998,01	1,50	996,51	1,47	995,04	1,42	993,62	1,40	992,22	1,36	990,86	1,33	989,53	1,31	988,22	1,27	986,95	1,25	985,70	1,22	984,48	1,19	983,29	1,17
	0,20		0,20		0,19		0,20		0,20		0,20		0,21		0,21		0,22		0,22		0,23		0,24	
22	987,81	1,50	996,31	1,46	994,85	1,43	993,42	1,40	992,02	1,36	990,66	1,34	989,32	1,31	988,01	1,28	986,73	1,25	985,48	1,23	984,25	1,20	983,05	1,18
	0,21		0,21		0,21		0,21		0,21		0,22		0,22		0,22		0,23		0,24		0,24		0,25	
23	997,60	1,50	996,10	1,46	994,64	1,43	993,21	1,40	991,81	1,37	990,44	1,34	989,10	1,31	987,79	1,29	986,50	1,26	985,24	1,23	984,01	1,21	982,80	1,19
	0,21		0,21		0,22		0,22		0,22		0,22		0,23		0,23		0,23		0,24		0,25		0,26	
24	997,39	1,50	995,89	1,47	994,42	1,43	992,99	1,40	991,59	1,37	990,22	1,35	988,87	1,31	987,56	1,29	986,27	1,27	985,00	1,24	983,76	1,22	982,54	1,20
	0,23		0,23		0,23		0,23		0,24		0,24		0,24		0,25		0,25		0,25		0,26		0,27	
25	997,16	1,50	995,66	1,47	994,19	1,43	992,76	1,41	991,35	1,37	989,98	1,35	988,63	1,32	987,31	1,29	986,02	1,27	984,75	1,25	983,50	1,23	982,27	1,21
	0,23		0,23		0,23		0,24		0,24		0,24		0,24		0,25		0,26		0,27		0,27		0,28	
26	996,93	1,50	995,43	1,47	993,96	1,44	992,52	1,41	991,11	1,37	989,74	1,35	988,39	1,33	987,06	1,30	985,76	1,28	984,48	1,25	983,23	1,24	981,99	1,22
	0,25		0,25		0,25		0,25		0,25		0,26		0,26		0,26		0,27		0,28		0,29		0,29	
27	996,68	1,50	995,18	1,47	993,71	1,44	992,27	1,41	990,86	1,38	989,48	1,35	988,13	1,33	986,80	1,31	985,49	1,29	984,20	1,26	982,94	1,24	981,70	1,23
	0,25		0,25		0,26		0,26		0,26		0,26		0,27		0,28		0,28		0,28		0,29		0,30	
28	996,43	1,50	994,93	1,48	993,45	1,44	992,01	1,41	990,60	1,38	989,22	1,36	987,86	1,34	986,52	1,31	985,21	1,29	983,92	1,27	982,65	1,25	981,40	1,23
	0,26		0,27		0,27		0,27		0,27		0,28		0,28		0,28		0,29		0,29		0,30		0,31	
29	996,17	1,51	994,66	1,48	993,18	1,44	991,74	1,41	990,33	1,39	988,94	1,36	987,58	1,34	986,24	1,32	984,92	1,29	983,63	1,28	982,35	1,26	981,09	1,24
	0,27		0,27		0,27		0,28		0,28		0,28		0,28		0,29		0,29		0,30		0,31		0,32	
30	995,90	1,51	994,39	1,48	992,91	1,45	991,46	1,41	990,05	1,39	988,66	1,37	987,29	1,34	985,95	1,32	984,63	1,30	983,33	1,29	982,04	1,27	980,77	1,25
	0,29		0,29		0,29		0,29		0,30		0,30		0,30		0,31		0,31		0,32		0,32		0,32	
31	995,61	1,51	994,10	1,48	992,62	1,45	991,17	1,42	989,75	1,39	988,36	1,37	986,99	1,35	985,64	1,33	984,31	1,30	983,01	1,29	981,72	1,27	980,45	1,26
	0,29		0,29		0,29		0,29		0,30		0,31		0,31		0,31		0,31		0,32		0,33		0,34	
32	995,32	1,51	993,81	1,48	992,33	1,45	990,88	1,42	989,45	1,40	988,05	1,37	986,68	1,35	985,33	1,33	984,00	1,31	982,69	1,30	981,39	1,28	980,11	1,26
	0,30		0,31		0,31		0,31		0,31		0,31		0,31		0,32		0,33		0,33		0,34		0,34	
33	995,02	1,52	993,50	1,48	992,02	1,45	990,57	1,43	989,14	1,40	987,74	1,37	986,37	1,36	985,01	1,34	983,67	1,31	982,36	1,31	981,05	1,28	979,77	1,27
	0,30		0,31		0,31		0,31		0,31		0,32		0,33		0,33		0,33		0,34		0,34		0,35	
34	994,72	1,53	993,19	1,48	991,71	1,45	990,26	1,43	988,83	1,41	987,42	1,38	986,04	1,36	984,68	1,34	983,34	1,32	982,02	1,31	980,71	1,29	979,42	1,28
	0,32		0,32		0,32		0,33		0,33		0,33		0,33		0,33		0,33		0,34		0,34		0,35	
35	994,40	1,53	992,87	1,48	991,39	1,46	989,93	1,43	988,50	1,41	987,09	1,38	985,71	1,36	984,35	1,34	983,01	1,33	981,68	1,31	980,37	1,30	979,07	1,29
	0,32		0,32		0,33		0,33		0,33		0,33		0,34		0,34		0,35		0,35		0,36		0,37	
36	994,08	1,53	992,55	1,49	991,06	1,46	989,60	1,43	988,17	1,41	986,76	1,39	985,37	1,36	984,01	1,35	982,66	1,33	981,33	1,32	980,01	1,31	978,70	1,29
	0,33		0,34		0,34		0,34		0,35		0,35		0,35		0,35		0,36		0,36		0,36		0,37	
37	993,75	1,54	992,21	1,49	990,72	1,46	989,26	1,44	987,82	1,41	986,41	1,39	985,02	1,37	983,65	1,35	982,30	1,33	980,97	1,32	979,65	1,32	978,33	1,30
	0,34		0,34		0,35		0,36		0,36		0,36		0,36		0,36		0,37		0,38		0,38		0,38	
38	993,41	1,54	991,87	1,50	990,37	1,47	988,90	1,44	987,46	1,41	986,05	1,39	984,66	1,37	983,29	1,36	981,93	1,34	980,59	1,32	979,27	1,32	977,95	1,31
	0,35		0,35		0,36		0,36		0,36		0,37		0,37		0,37		0,37		0,38		0,38		0,39	
39	993,06	1,54	991,52	1,51	990,01	1,47	988,54	1,44	987,10	1,41	985,68	1,39	984,29	1,37	982,92	1,36	981,56	1,34	980,22	1,33	978,89	1,33	977,56	1,31
	0,35		0,36		0,36		0,37		0,38		0,38		0,38		0,38		0,38		0,39		0,39		0,39	
40	992,71	1,55	991,16	1,51	989,65	1,48	988,17	1,45	986,72	1,42	985,30	1,39	983,91	1,37	982,54	1,36	981,18	1,35	979,83	1,33	978,50	1,33	977,17	1,32

▼**B**

t°	Alcoholic strength by % vol																							
	10		11		12		13		14		15		16		17		18		19		20		21	
0	986,63	1,00	985,63	0,96	984,67	0,92	983,75	0,87	982,88	0,84	982,04	0,81	981,23	0,77	980,46	0,75	979,71	0,73	978,98	0,72	978,26	0,70	977,56	0,70
	−0,03		−0,02		−0,01		0,00		0,02		0,04		0,05		0,07		0,09		0,11		0,13		0,15	
1	986,66	1,01	985,65	0,97	984,68	0,93	983,75	0,89	982,86	0,86	982,00	0,82	981,18	0,79	980,39	0,77	979,62	0,75	978,87	0,74	978,13	0,72	977,41	0,72
	−0,02		−0,01		0,00		0,01		0,03		0,04		0,06		0,08		0,10		0,12		0,14		0,17	
2	986,68	1,02	985,66	0,98	984,68	0,94	983,74	0,91	982,83	0,87	981,96	0,84	981,12	0,81	980,31	0,79	979,52	0,77	978,75	0,76	977,99	0,75	977,24	0,74
	0,00		0,01		0,02		0,04		0,05		0,06		0,08		0,10		0,12		0,14		0,16		0,18	
3	986,68	1,03	985,65	0,99	984,66	0,96	983,70	0,92	982,78	0,88	981,90	0,86	981,04	0,83	980,21	0,81	979,40	0,79	978,61	0,78	977,83	0,77	977,06	0,76
	0,01		0,02		0,03		0,04		0,05		0,07		0,08		0,10		0,12		0,14		0,16		0,18	
4	986,67	1,04	985,63	1,00	984,63	0,97	983,66	0,93	982,73	0,90	981,83	0,87	980,96	0,85	980,11	0,83	979,28	0,81	978,47	0,80	977,67	0,79	976,88	0,79
	0,02		0,03		0,05		0,06		0,08		0,09		0,11		0,13		0,14		0,16		0,18		0,20	
5	986,65	1,05	985,60	1,02	984,58	0,98	983,60	0,95	982,65	0,91	981,74	0,89	980,85	0,87	979,98	0,84	979,14	0,83	978,31	0,82	977,49	0,81	976,68	0,81
	0,04		0,06		0,06		0,07		0,08		0,10		0,11		0,13		0,15		0,17		0,19		0,21	
6	986,61	1,07	985,54	1,02	984,52	0,99	983,53	0,96	982,57	0,93	981,64	0,90	980,74	0,89	979,85	0,86	978,99	0,85	978,14	0,84	977,30	0,83	976,47	0,83
	0,05		0,06		0,08		0,09		0,10		0,12		0,14		0,15		0,17		0,19		0,20		0,22	
7	986,56	1,08	985,48	1,04	984,44	1,00	983,44	0,97	982,47	0,95	981,52	0,92	980,60	0,90	979,70	0,88	978,82	0,87	977,95	0,85	977,10	0,85	976,25	0,85
	0,07		0,08		0,09		0,10		0,11		0,12		0,14		0,16		0,18		0,19		0,21		0,23	
8	986,49	1,09	985,40	1,05	984,35	1,01	983,34	0,98	982,36	0,96	981,40	0,94	980,46	0,92	979,54	0,90	978,64	0,88	977,76	0,87	976,89	0,87	976,02	0,87
	0,08		0,08		0,09		0,11		0,13		0,14		0,15		0,16		0,18		0,20		0,22		0,24	
9	986,41	1,09	985,32	1,06	984,26	1,03	983,23	1,00	982,23	0,97	981,26	0,95	980,31	0,93	979,38	0,92	978,48	0,90	977,56	0,89	976,67	0,89	975,78	0,89
	0,10		0,11		0,12		0,13		0,14		0,16		0,17		0,18		0,19		0,21		0,23		0,25	
10	986,31	1,10	985,21	1,07	984,14	1,04	983,10	1,01	982,09	0,99	981,10	0,96	980,14	0,94	979,20	0,93	978,27	0,92	977,35	0,91	976,44	0,91	975,53	0,91
	0,10		0,11		0,12		0,13		0,15		0,16		0,17		0,19		0,21		0,23		0,25		0,27	
11	986,21	1,11	985,10	1,08	984,02	1,05	982,97	1,03	981,94	1,00	980,94	0,97	979,97	0,96	979,01	0,95	978,06	0,94	977,12	0,93	976,19	0,93	975,26	0,92
	0,12		0,13		0,14		0,15		0,16		0,17		0,19		0,21		0,22		0,24		0,26		0,27	
12	986,09	1,12	984,97	1,09	983,88	1,06	982,82	1,04	981,78	1,01	980,77	0,99	979,78	0,98	978,80	0,96	977,84	0,96	976,88	0,95	975,93	0,94	974,99	0,94
	0,13		0,14		0,15		0,16		0,17		0,19		0,20		0,21		0,23		0,24		0,26		0,28	
13	985,96	1,13	984,83	1,10	983,73	1,07	982,66	1,05	981,61	1,03	980,58	1,00	979,58	0,99	978,59	0,98	977,61	0,97	976,64	0,97	975,67	0,96	974,71	0,96
	0,15		0,16		0,17		0,18		0,19		0,20		0,22		0,23		0,24		0,26		0,27		0,29	
14	985,81	1,14	984,67	1,11	983,56	1,08	982,48	1,06	981,42	1,04	980,38	1,02	979,36	1,00	978,36	0,99	977,37	0,99	976,38	0,98	975,40	0,98	974,42	0,98
	0,15		0,16		0,17		0,18		0,19		0,20		0,22		0,24		0,26		0,27		0,28		0,30	
15	985,66	1,15	984,51	1,12	983,39	1,09	982,30	1,07	981,23	1,05	980,18	1,04	979,14	1,02	978,12	1,01	977,11	1,00	976,11	0,99	975,12	1,00	974,12	1,00
	0,17		0,18		0,19		0,20		0,21		0,22		0,23		0,25		0,26		0,28		0,30		0,31	
16	985,49	1,16	984,33	1,13	983,20	1,10	982,10	1,08	981,02	1,06	979,96	1,05	978,91	1,04	977,87	1,02	976,85	1,02	975,83	1,01	974,82	1,01	973,81	1,02
	0,17		0,18		0,19		0,20		0,21		0,23		0,24		0,25		0,27		0,29		0,30		0,31	
17	985,32	1,17	984,15	1,14	983,01	1,11	981,90	1,09	980,81	1,08	979,73	1,06	978,67	1,05	977,62	1,04	976,58	1,04	975,54	1,02	974,52	1,02	973,50	1,04
	0,19		0,19		0,20		0,22		0,24		0,25		0,26		0,27		0,28		0,29		0,31		0,33	
18	985,13	1,17	983,96	1,15	982,81	1,13	981,68	1,11	980,57	1,09	979,48	1,07	978,41	1,06	977,35	1,05	976,30	1,05	975,25	1,04	974,21	1,04	973,17	1,05
	0,20		0,21		0,22		0,23		0,24		0,25		0,26		0,27		0,29		0,30		0,32		0,34	
19	984,93	1,18	983,75	1,16	982,59	1,14	981,45	1,12	980,33	1,10	979,23	1,08	978,15	1,07	977,08	1,07	976,01	1,06	974,94	1,05	973,89	1,06	972,83	1,06
	0,22		0,23		0,24		0,24		0,25		0,26		0,28		0,29		0,30		0,31		0,33		0,35	
20	984,71	1,19	983,52	1,17	982,35	1,14	981,21	1,13	980,08	1,11	978,97	1,10	977,87	1,08	976,79	1,08	975,71	1,08	974,63	1,07	973,56	1,08	972,48	1,08

t°	Alcoholic strength by % vol																							
	10		11		12		13		14		15		16		17		18		19		20		21	
20°	984,71	1,19	983,52	1,17	982,35	1,14	981,21	1,13	980,08	1,11	978,97	1,10	977,87	1,08	976,79	1,08	975,71	1,08	974,63	1,07	973,56	1,08	972,48	1,08
	0,23		0,23		0,23		0,25		0,26		0,28		0,29		0,31		0,32		0,33		0,35		0,36	
21°	984,48	1,19	983,29	1,17	982,12	1,16	980,96	1,14	979,82	1,13	978,69	1,11	977,58	1,10	976,48	1,09	975,39	1,09	974,30	1,09	973,21	1,09	972,12	1,09
	0,23		0,24		0,25		0,26		0,27		0,28		0,29		0,31		0,32		0,33		0,35		0,36	
22	984,25	1,20	983,05	1,18	981,97	1,17	980,70	1,15	979,55	1,14	978,41	1,12	977,29	1,12	976,17	1,10	975,07	1,10	973,97	1,10	972,86	1,10	971,76	1,11
	0,24		0,25		0,26		0,27		0,28		0,29		0,30		0,31		0,33		0,34		0,35		0,37	
23	984,01	1,21	982,80	1,19	981,61	1,18	980,43	1,16	979,27	1,15	978,12	1,13	976,99	1,13	975,86	1,12	974,74	1,11	973,63	1,12	972,51	1,12	971,39	1,13
	0,25		0,26		0,27		0,28		0,29		0,30		0,31		0,32		0,33		0,35		0,36		0,38	
24	983,76	1,22	982,54	1,20	981,34	1,19	980,15	1,17	978,98	1,16	977,82	1,14	976,68	1,14	975,54	1,13	974,41	1,13	973,28	1,13	972,15	1,14	971,01	1,14
	0,26		0,27		0,28		0,29		0,30		0,31		0,32		0,33		0,35		0,36		0,38		0,39	
25	983,50	1,23	982,27	1,21	981,06	1,20	979,86	1,18	978,68	1,17	977,51	1,16	976,36	1,15	975,21	1,15	974,06	1,14	972,92	1,15	971,77	1,15	970,62	1,15
	0,27		0,28		0,29		0,29		0,30		0,31		0,33		0,34		0,35		0,37		0,38		0,39	
26	983,23	1,24	981,99	1,22	980,77	1,20	979,57	1,19	978,38	1,18	977,20	1,17	976,03	1,16	974,87	1,16	973,71	1,16	972,55	1,16	971,39	1,16	970,23	1,17
	0,29		0,29		0,30		0,31		0,32		0,33		0,34		0,36		0,37		0,38		0,39		0,41	
27	982,94	1,24	981,70	1,23	980,47	1,21	979,26	1,20	978,06	1,19	976,87	1,18	975,69	1,18	974,51	1,17	973,34	1,17	972,17	1,17	921,00	1,18	969,82	1,18
	0,29		0,30		0,30		0,31		0,32		0,33		0,35		0,36		0,38		0,39		0,40		0,41	
28	982,65	1,25	981,40	1,23	980,17	1,22	978,95	1,21	977,74	1,20	976,54	1,20	975,34	1,19	974,15	1,19	972,96	1,18	971,78	1,18	970,60	1,19	969,41	1,20
	0,30		0,31		0,32		0,33		0,34		0,35		0,36		0,37		0,38		0,39		0,40		0,42	
29	982,35	1,26	981,09	1,24	979,85	1,23	978,62	1,22	977,40	1,21	976,19	1,21	974,98	1,20	973,78	1,20	972,58	1,19	971,39	1,19	970,20	1,21	968,99	1,21
	0,31		0,32		0,33		0,34		0,35		0,36		0,37		0,38		0,38		0,40		0,42		0,43	
30	982,04	1,27	980,77	1,25	979,52	1,24	978,28	1,23	977,05	1,22	975,83	1,21	974,62	1,21	973,41	1,21	972,20	1,21	970,99	1,21	969,78	1,22	968,56	1,23
	0,32		0,32		0,33		0,34		0,35		0,36		0,37		0,38		0,39		0,40		0,42		0,43	
31	981,72	1,27	980,45	1,26	979,19	1,25	977,94	1,24	976,70	1,23	975,47	1,22	974,25	1,22	973,03	1,22	971,81	1,22	970,59	1,23	969,36	1,23	968,13	1,24
	0,33		0,34		0,34		0,35		0,36		0,37		0,38		0,39		0,40		0,42		0,43		0,45	
32	981,39	1,28	980,11	1,26	978,85	1,26	977,59	1,25	976,34	1,24	975,10	1,23	973,87	1,23	972,64	1,23	971,41	1,24	970,17	1,24	968,93	1,25	967,68	1,26
	0,34		0,34		0,35		0,35		0,36		0,37		0,39		0,40		0,41		0,42		0,43		0,45	
33	981,05	1,28	979,77	1,27	978,50	1,26	977,24	1,26	975,78	1,25	974,73	1,25	973,48	1,24	972,24	1,24	971,00	1,25	969,75	1,25	968,50	1,27	967,23	1,27
	0,34		0,35		0,36		0,37		0,38		0,39		0,40		0,41		0,42		0,43		0,45		0,45	
34	980,71	1,29	979,42	1,28	978,14	1,27	976,87	1,27	975,60	1,26	974,34	1,26	973,08	1,25	971,83	1,25	970,58	1,26	969,32	1,27	968,05	1,27	966,78	1,29
	0,34		0,35		0,36		0,37		0,38		0,39		0,40		0,41		0,43		0,44		0,45		0,47	
35	980,37	1,30	979,07	1,29	977,78	1,28	976,50	1,28	975,22	1,27	973,95	1,27	972,68	1,26	971,42	1,27	970,15	1,27	968,88	1,28	967,60	1,29	966,31	1,30
	0,36		0,37		0,37		0,38		0,38		0,39		0,40		0,42		0,43		0,44		0,45		0,47	
36	980,01	1,31	978,70	1,29	977,41	1,29	976,12	1,28	974,84	1,28	973,56	1,28	972,28	1,28	971,00	1,28	969,72	1,28	968,44	1,29	967,15	1,31	965,84	1,31
	0,36		0,37		0,38		0,39		0,40		0,41		0,42		0,43		0,44		0,45		0,46		0,47	
37	979,65	1,32	978,33	1,30	977,03	1,30	975,73	1,29	974,44	1,29	973,15	1,29	971,86	1,29	970,57	1,29	969,28	1,29	967,99	1,30	966,69	1,32	965,37	1,32
	0,38		0,38		0,39		0,39		0,40		0,41		0,42		0,43		0,44		0,46		0,47		0,48	
38	979,27	1,32	977,95	1,31	976,64	1,30	975,34	1,30	974,04	1,30	972,74	1,30	971,44	1,30	970,14	1,30	968,84	1,31	967,53	1,31	966,22	1,33	964,89	1,34
	0,38		0,39		0,39		0,40		0,41		0,42		0,43		0,44		0,45		0,46		0,48		0,49	
39	978,89	1,33	977,56	1,31	976,25	1,31	974,94	1,31	973,63	1,31	972,32	1,31	971,01	1,31	969,70	1,31	968,39	1,32	967,07	1,33	965,74	1,34	964,40	1,36
	0,39		0,39		0,40		0,41		0,42		0,42		0,43		0,45		0,47		0,48		0,49		0,50	
40	978,50	1,33	977,17	1,32	975,85	1,32	974,53	1,32	973,21	1,31	971,90	1,32	970,58	1,33	969,25	1,33	967,92	1,33	966,59	1,34	965,25	1,35	963,90	1,37

▼B

t°	Alcoholic strength by % vol																							
	20		**21**		**22**		**23**		**24**		**25**		**26**		**27**		**28**		**29**		**30**		**31**	
0	978,26	0,70	977,56	0,70	976,86	0,69	976,17	0,70	975,47	0,72	974,75	0,72	974,03	0,74	973,29	0,77	972,52	0,80	971,72	0,83	970,89	0,87	970,02	0,90
	0,13		0,15		0,17		0,20		0,22		0,24		0,27		0,30		0,32		0,35		0,37		0,39	
1	978,13	0,72	977,41	0,72	976,69	0,72	975,97	0,72	975,25	0,74	974,51	0,75	973,76	0,77	972,99	0,79	972,20	0,83	971,37	0,85	970,52	0,89	969,63	0,93
	0,14		0,17		0,19		0,21		0,24		0,26		0,29		0,31		0,34		0,36		0,38		0,41	
2	977,99	0,75	977,24	0,74	976,50	0,74	975,76	0,75	975,01	0,76	974,25	0,78	973,47	0,79	972,68	0,82	971,86	0,85	971,01	0,87	970,14	0,92	960,22	0,96
	0,16		0,18		0,20		0,23		0,25		0,27		0,29		0,32		0,34		0,36		0,38		0,40	
3	977,83	0,77	977,06	0,76	976,30	0,77	975,53	0,77	974,76	0,78	973,98	0,80	973,18	0,82	972,36	0,84	971,52	0,87	970,65	0,89	969,76	0,94	968,82	0,98
	0,16		0,18		0,21		0,23		0,25		0,28		0,30		0,32		0,34		0,36		0,39		0,42	
4	977,67	0,79	976,88	0,79	976,09	0,79	975,30	0,79	974,51	0,81	973,70	0,82	972,88	0,84	972,04	0,86	971,18	0,89	970,29	0,92	969,37	0,96	968,40	1,00
	0,18		0,20		0,22		0,24		0,26		0,28		0,30		0,33		0,35		0,38		0,40		0,41	
5	977,49	0,81	976,68	0,81	975,87	0,81	975,06	0,81	974,25	0,83	973,42	0,84	972,58	0,86	971,71	0,88	970,83	0,92	969,91	0,94	968,97	0,98	967,99	1,02
	0,19		0,21		0,23		0,25		0,27		0,30		0,33		0,34		0,37		0,39		0,41		0,43	
6	977,30	0,83	976,47	0,83	975,64	0,83	974,81	0,84	973,97	0,85	973,12	0,87	972,25	0,88	971,37	0,91	970,46	0,94	969,52	0,96	968,56	1,00	967,56	1,04
	0,20		0,22		0,24		0,26		0,28		0,30		0,32		0,35		0,37		0,39		0,41		0,43	
7	976,10	0,85	976,25	0,85	975,40	0,85	974,55	0,86	973,69	0,87	972,82	0,89	971,93	0,91	971,02	0,93	970,09	0,96	969,13	0,98	968,15	1,02	967,13	1,06
	0,21		0,23		0,25		0,27		0,29		0,31		0,33		0,35		0,37		0,39		0,42		0,44	
8	976,89	0,87	976,02	0,87	975,15	0,87	974,28	0,88	973,40	0,89	972,51	0,91	971,60	0,93	970,67	0,95	969,72	0,98	968,74	1,01	967,73	1,04	966,69	1,08
	0,22		0,24		0,26		0,28		0,30		0,32		0,34		0,36		0,39		0,41		0,43		0,45	
9	976,67	0,89	975,78	0,89	974,89	0,89	974,00	0,90	973,10	0,91	972,19	0,93	971,26	0,95	970,31	0,98	969,33	1,00	968,33	1,03	967,30	1,06	966,24	1,09
	0,23		0,25		0,27		0,29		0,31		0,33		0,35		0,37		0,39		0,41		0,43		0,45	
10	976,44	0,91	975,53	0,91	974,62	0,91	973,71	0,92	972,79	0,93	971,86	0,95	970,91	0,97	969,94	1,00	968,94	1,02	967,92	1,05	966,87	1,08	965,79	1,11
	0,25		0,27		0,28		0,30		0,32		0,34		0,36		0,38		0,40		0,42		0,44		0,45	
11	976,11	0,93	975,26	0,92	974,34	0,93	973,41	0,94	972,47	0,95	971,52	0,97	970,55	0,99	969,56	1,02	968,54	1,04	967,50	1,07	966,43	1,09	965,34	1,13
	0,26		0,27		0,29		0,31		0,33		0,35		0,37		0,39		0,40		0,42		0,44		0,46	
12	975,93	0,94	974,99	0,94	974,05	0,95	973,10	0,96	972,14	0,97	971,17	0,99	970,18	1,01	969,17	1,03	968,14	1,06	967,08	1,09	965,99	1,11	964,88	1,15
	0,26		0,28		0,30		0,32		0,34		0,36		0,38		0,39		0,41		0,43		0,45		0,47	
13	975,67	0,96	974,71	0,96	973,75	0,97	972,78	0,98	971,80	0,99	970,81	1,01	969,80	1,02	968,78	1,05	967,73	1,08	966,65	1,11	965,54	1,13	964,41	1,17
	0,27		0,29		0,31		0,33		0,35		0,37		0,38		0,40		0,42		0,44		0,45		0,47	
14	975,40	0,98	974,42	0,98	973,44	0,99	972,45	1,00	971,45	1,01	970,44	1,02	969,42	1,04	968,38	1,07	967,31	1,10	966,21	1,12	965,09	1,15	963,94	1,19
	0,28		0,30		0,32		0,33		0,35		0,37		0,39		0,41		0,43		0,45		0,47		0,49	
15	975,12	1,00	974,12	1,00	973,12	1,00	972,12	1,02	971,10	1,03	970,07	1,04	969,03	1,06	967,97	1,09	966,88	1,12	965,76	1,14	964,62	1,17	963,45	1,20
	0,30		0,31		0,33		0,35		0,36		0,38		0,40		0,42		0,44		0,45		0,47		0,49	
16	974,82	1,01	973,81	1,02	972,79	1,02	971,77	1,03	970,74	1,05	969,69	1,06	968,63	1,08	967,55	1,11	966,44	1,13	965,31	1,16	964,15	1,19	962,96	1,22
	0,30		0,31		0,33		0,35		0,37		0,38		0,40		0,42		0,43		0,45		0,47		0,49	
17	974,52	1,02	973,50	1,04	972,46	1,04	971,42	1,05	970,37	1,06	969,31	1,08	968,23	1,10	967,13	1,12	966,01	1,15	964,86	1,18	963,68	1,21	962,47	1,24
	0,31		0,33		0,34		0,36		0,38		0,40		0,42		0,43		0,45		0,47		0,48		0,50	
18	974,21	1,04	973,17	1,05	972,12	1,06	971,06	1,07	969,99	1,08	968,91	1,10	967,81	1,11	966,70	1,14	965,56	1,17	964,39	1,19	963,20	1,23	961,97	1,26
	0,32		0,34		0,35		0,36		0,38		0,40		0,42		0,44		0,46		0,47		0,49		0,50	
19	973,89	1,06	972,83	1,06	971,77	1,07	970,70	1,09	969,61	1,10	968,51	1,11	967,39	1,13	966,26	1,16	965,10	1,18	963,92	1,21	962,71	1,24	961,47	1,28
	0,33		0,35		0,37		0,39		0,40		0,41		0,42		0,45		0,46		0,48		0,50		0,52	
20	973,56	1,08	972,48	1,08	971,40	1,09	970,31	1,10	969,21	1,11	968,10	1,13	966,97	1,14	965,81	1,17	964,64	1,20	963,44	1,23	962,21	1,26	960,95	1,29

t°	Alcoholic strength by % vol																							
	20		**21**		**22**		**23**		**24**		**25**		**26**		**27**		**28**		**29**		**30**		**31**	
20°	973,56	1,08	972,48	1,08	971,40	1,09	970,31	1,10	969,21	1,11	968,10	1,13	966,97	1,16	965,81	1,17	964,64	1,20	963,44	1,23	962,21	1,26	960,95	1,29
	0,35		0,36		0,37		0,39		0,40		0,42		0,44		0,45		0,47		0,49		0,50		0,52	
21°	973,21	1,09	972,12	1,09	971,03	1,11	969,92	1,11	968,81	1,13	967,68	1,15	966,53	1,17	965,36	1,19	964,17	1,22	962,95	1,24	961,71	1,28	960,43	1,31
	0,35		0,36		0,38		0,39		0,41		0,43		0,44		0,46		0,48		0,49		0,51		0,52	
22	972,86	1,10	971,76	1,11	970,65	1,12	969,53	1,13	968,40	1,15	967,25	1,16	966,09	1,19	964,90	1,21	963,69	1,23	962,46	1,26	961,20	1,29	959,91	1,32
	0,35		0,37		0,39		0,40		0,42		0,43		0,45		0,46		0,48		0,50		0,52		0,53	
23	972,51	1,12	971,39	1,13	970,26	1,13	969,13	1,15	967,98	1,16	966,82	1,18	965,64	1,20	964,44	1,23	963,21	1,25	961,96	1,28	960,68	1,30	959,38	1,33
	0,36		0,38		0,39		0,41		0,42		0,44		0,46		0,48		0,49		0,51		0,53		0,54	
24	972,15	1,14	971,01	1,14	969,87	1,15	968,72	1,16	967,56	1,18	966,38	1,20	965,18	1,22	963,96	1,24	962,72	1,27	961,45	1,29	960,16	1,32	958,84	1,34
	0,38		0,39		0,40		0,42		0,44		0,45		0,46		0,48		0,50		0,51		0,53		0,54	
25	971,77	1,15	970,62	1,15	969,47	1,17	968,30	1,18	967,12	1,19	965,93	1,21	964,72	1,24	963,48	1,26	962,22	1,28	960,94	1,31	959,63	1,33	958,30	1,36
	0,38		0,39		0,41		0,42		0,44		0,46		0,48		0,49		0,50		0,52		0,53		0,55	
26	971,39	1,16	970,23	1,17	969,06	1,18	967,88	1,20	966,68	1,21	965,47	1,23	964,24	1,25	962,99	1,27	961,72	1,30	960,42	1,32	959,10	1,35	957,75	1,38
	0,39		0,41		0,42		0,44		0,45		0,46		0,48		0,50		0,51		0,52		0,53		0,55	
27	971,00	1,18	969,82	1,18	968,64	1,20	967,44	1,21	966,23	1,22	965,01	1,25	963,76	1,27	962,49	1,28	961,21	1,31	959,90	1,33	958,57	1,37	957,20	1,40
	0,40		0,41		0,43		0,44		0,46		0,48		0,49		0,50		0,52		0,53		0,55		0,56	
28	970,60	1,19	969,41	1,20	968,21	1,21	967,00	1,23	965,77	1,24	964,53	1,26	963,27	1,28	961,99	1,30	960,69	1,32	959,37	1,35	958,02	1,38	956,64	1,41
	0,40		0,42		0,43		0,45		0,46		0,48		0,49		0,50		0,52		0,54		0,55		0,56	
29	970,20	1,21	968,99	1,21	967,78	1,23	966,55	1,24	965,31	1,26	964,05	1,27	962,78	1,29	961,49	1,32	960,17	1,34	958,83	1,36	957,47	1,39	956,08	1,43
	0,42		0,43		0,45		0,46		0,47		0,48		0,50		0,52		0,53		0,54		0,56		0,58	
30	969,78	1,22	968,56	1,23	967,33	1,24	966,09	1,25	964,84	1,27	963,57	1,29	962,28	1,31	960,97	1,33	959,64	1,35	958,29	1,38	956,91	1,41	955,50	1,44
	0,42		0,43		0,44		0,45		0,47		0,49		0,51		0,52		0,53		0,55		0,56		0,58	
31	969,36	1,23	968,13	1,24	966,89	1,25	965,64	1,27	964,37	1,29	963,08	1,31	961,77	1,32	960,45	1,34	959,11	1,37	957,74	1,39	956,35	1,43	954,92	1,45
	0,43		0,45		0,46		0,48		0,49		0,50		0,51		0,52		0,54		0,56		0,57		0,58	
32	968,93	1,25	967,68	1,25	966,43	1,27	965,16	1,28	963,88	1,30	962,58	1,32	961,26	1,33	959,93	1,36	958,57	1,39	957,18	1,40	955,78	1,44	954,34	1,47
	0,43		0,45		0,47		0,48		0,50		0,51		0,52		0,54		0,55		0,56		0,58		0,59	
33	968,50	1,27	967,23	1,27	965,96	1,28	964,68	1,30	963,38	1,31	962,07	1,33	960,74	1,35	959,39	1,37	958,02	1,40	956,62	1,42	955,20	1,45	953,75	1,48
	0,45		0,45		0,47		0,49		0,50		0,51		0,52		0,54		0,55		0,56		0,58		0,60	
34	968,05	1,27	966,78	1,29	965,49	1,30	964,19	1,31	962,88	1,32	961,56	1,34	960,22	1,37	958,85	1,38	957,47	1,41	956,06	1,44	954,62	1,47	953,15	1,49
	0,45		0,47		0,48		0,49		0,50		0,52		0,54		0,55		0,57		0,58		0,59		0,60	
35	967,60	1,29	996,31	1,30	965,01	1,31	963,70	1,32	962,38	1,34	961,04	1,36	959,68	1,38	958,30	1,40	956,90	1,42	955,48	1,45	954,03	1,48	952,55	1,50
	0,45		0,47		0,48		0,49		0,51		0,53		0,54		0,55		0,57		0,59		0,60		0,61	
36	967,15	1,31	965,84	1,31	964,53	1,32	963,21	1,34	961,87	1,36	960,51	1,37	959,14	1,39	957,75	1,42	956,33	1,44	954,89	1,46	953,43	1,49	951,94	1,51
	0,46		0,47		0,48		0,50		0,52		0,53		0,55		0,56		0,57		0,58		0,60		0,61	
37	966,69	1,32	965,37	1,32	964,05	1,34	962,71	1,36	961,35	1,37	959,98	1,39	958,59	1,40	957,19	1,43	955,76	1,45	954,31	1,48	952,83	1,50	951,33	1,52
	0,47		0,48		0,50		0,51		0,52		0,54		0,55		0,57		0,58		0,59		0,60		0,61	
38	966,22	1,33	964,89	1,34	963,55	1,35	962,20	1,37	960,83	1,39	959,44	1,40	958,04	1,42	956,62	1,44	955,18	1,46	953,72	1,49	952,23	1,51	950,72	1,54
	0,48		0,49		0,51		0,52		0,53		0,54		0,56		0,57		0,58		0,60		0,61		0,62	
39	965,74	1,34	964,40	1,36	963,04	1,36	961,68	1,38	960,30	1,40	958,90	1,42	957,48	1,43	956,05	1,45	954,60	1,48	953,12	1,50	951,62	1,52	950,10	1,55
	0,49		0,50		0,51		0,53		0,54		0,55		0,56		0,58		0,60		0,61		0,62		0,64	
40	965,25	1,35	963,90	1,37	962,53	1,38	961,15	1,39	959,76	1,41	958,35	1,43	956,92	1,45	955,47	1,47	954,00	1,49	952,51	1,51	951,00	1,54	949,49	1,56

▼B

TABLE IV

Table giving the refractive indices of pure ethanol-water mixtures and distillates at 20 °C and the corresponding alcoholic strengths at 20 °C

Refractive index at 20 °C	Alcoholic strength at 20 °C			
	Ethanol-water mixtures		Distillates	
1,33628	6,54	0,25	6,48	0,26
1,33642	6,79	0,26	6,74	0,26
1,33656	7,05	0,25	7,00	0,27
1,33670	7,30	0,28	7,27	0,27
1,33685	7,58	0,25	7,54	0,25
1,33699	7,83	0,26	7,79	0,26
1,33713	8,09	0,25	8,05	0,25
1,33727	8,34	0,28	8,30	0,26
1,33742	8,62	0,25	8,56	0,25
1,33756	8,87	0,25	8,81	0,25
1,33770	9,12	0,24	9,06	0,24
1,33784	9,36	0,27	9,30	0,25
1,33799	9,63	0,24	9,55	0,26
1,33813	9,87	0,25	9,81	0,24
1,33827	10,12	0,23	10,05	0,24
1,33841	10,35	0,26	10,29	0,25
1,33856	10,61	0,25	10,54	0,24
1,33870	10,86	0,24	10,78	0,24
1,33884	11,10	0,23	11,02	0,24
1,33898	11,33	0,24	11,26	0,24
1,33912	11,47	0,24	11,50	0,24
1,33926	11,81	0,24	11,74	0,24
1,33940	12,05	0,25	11,98	0,24
1,33955	12,30	0,23	12,22	0,24
1,33969	12,53	0,23	12,46	0,23
1,33983	12,76	0,24	12,69	0,23
1,33997	13,00	0,23	12,92	0,23
1,34011	13,23	0,24	13,15	0,25
1,34025	13,47	0,23	13,40	0,22
1,34039	13,70	0,23	13,62	0,24
1,34053	13,93	0,23	13,86	0,23
1,34067	14,16	0,25	14,09	0,23
1,34081	14,41	0,25	14,32	0,25
1,34096	14,66	0,23	14,57	0,24
1,34110	14,89	0,24	14,81	0,25
1,34124	15,13	0,23	15,06	0,22
1,34138	15,36	0,23	15,28	0,22
1,34152	15,59	0,24	15,50	0,24
1,34166	15,83	0,23	15,74	0,22
1,34180	16,06	0,23	15,96	0,23
1,34194	16,29	0,23	16,19	0,22
1,34208	16,52	0,24	16,41	0,24
1,34222	16,76	0,23	16,65	0,23
1,34236	16,99	0,23	16,88	0,24
1,34250	17,22	0,22	17,12	0,22
1,34264	17,44	0,24	17,34	0,22
1,34278	17,68	0,21	17,56	0,22
1,34291	17,89	0,23	17,78	0,23
1,34305	18,12	0,24	18,01	0,22
1,34319	18,36	0,23	18,23	0,23
1,34333	18,59	0,23	18,46	0,24
1,34347	18,82	0,23	18,70	0,22
1,34361	19,05	0,23	18,92	0,25
1,34375	19,28	0,23	19,17	0,23
1,34389	19,51	0,24	19,40	0,22
1,34403	19,75	0,23	19,62	0,24
1,34417	19,98	0,24	19,86	0,23
1,34431	20,22	0,22	20,09	0,24
1,34445	20,44	0,21	20,33	0,21
1,34458	20,65	0,24	20,54	0,22
1,34472	20,89	0,22	20,76	0,23
1,34486	21,11	0,23	20,99	0,22
1,34500	21,34	0,21	21,21	0,23
1,34513	21,55	0,23	21,44	0,21
1,34527	21,78	0,22	21,65	0,22
1,34541	22,00	0,23	21,87	0,23
1,34555	22,23	0,21	22,10	0,21
1,34568	22,44	0,23	22,31	0,23
1,34582	22,67	0,23	22,54	0,21
1,34596	22,90	0,23	22,75	0,21
1,34610	23,13	0,20	22,96	0,21
1,34623	23,33	0,24	23,17	0,23
1,34637	23,57	0,24	23,40	0,21
1,34651	23,81	0,23	23,61	0,24
1,34665	24,04	0,22	23,85	0,24
1,34678	24,26	0,22	24,09	0,22
1,34692	24,48	0,24	24,31	0,25
1,34706	24,72	0,23	24,56	0,22
1,34720	24,95	0,21	24,78	0,22
1,34733	25,16	0,24	25,00	0,23
1,34747	25,40	0,22	25,23	0,22
1,34760	25,62	0,24	25,45	0,25
1,34774	25,86	0,24	25,70	0,23
1,34788	26,10	0,22	25,93	0,22

▼B

4. TOTAL DRY EXTRACT

Total dry matter

1. DEFINITION

The total dry extract or the total dry matter includes all matter which is non-volatile under specified physical conditions. These physical conditions must be such that the matter forming the extract undergoes as little alteration as possible while the test is being carried out.

The sugar-free extract is the difference between the total dry extract and the total sugars.

The reduced extract is the difference between the total dry extract and the total sugars in excess of 1 g/l, potassium sulphate in excess of 1 g/l, any mannitol present and any other chemical substances which may have been added to the wine.

The residual extract is the sugar-free extract less the fixed acidity expressed as tartaric acid.

The extract is expressed in grams per litre and it should be determined to within the nearest 0,5 g.

2. PRINCIPLE OF THE METHOD

Single method: measurement by a densimeter

The total dry extract is calculated indirectly from the specific gravity of the must and, for wine, from the specific gravity of the alcohol-free wine.

This dry extract is expressed in terms of the quantity of sucrose which, when dissolved in water and made up to a volume of one litre, gives a solution of the same specific gravity as the must or the alcohol-free wine. This quantity is shown in Table I.

3. METHOD OF CALCULATION

The 20/20 specific gravity d_r of the 'alcohol-free wine' is calculated using the following formula:

$$d_r = d_v - d_a + 1{,}000$$

where:

d_v = specific gravity of the wine at 20 ºC (corrected for volatile acidity) (1),

d_a = specific gravity at 20 ºC of a water-alcohol mixture of the same alcoholic strength as the wine.

d_r may also be calculated from the densities at 20 ºC, ρ_v of the wine and ρ_a of the water-alcohol mixture of the same alcoholic strength by the formula:

$$d_r = 1{,}0018\ (\rho_v - \rho_a) + 1{,}000$$

where the coefficient 1,0018 approximates to 1 when ρ_v is below 1,05, which is most often the case.

4. EXPRESSION OF RESULTS

Table I should be used for calculating the total dry extract in g/l from the 20/20 specific gravity d_r of the alcohol-free wine or from the specific gravity $d_{20^\circ C}^{20^\circ C}$ of the must.

The total dry extract is expressed in g/l to one decimal place.

---

(1) Before carrying out this calculation, the specific gravity (or the density) of the wine measured as specified above should be corrected for the effect of the volatile acidity using the formula:

$d_v = d_{20^\circ C}^{20^\circ C} - 0{,}0000086a$ or $\rho_v = \rho_{20} - 0{,}0000086a$

where a is the volatile acidity expressed in milliequivalents per litre.

▼B

TABLE I

for the calculation of the content total dry extract (g/l)

Specific gravity to two decimal places	Third decimal place of the specific gravity									
	0	1	2	3	4	5	6	7	8	9
	Grams of extract per litre									
1,00	0	2,6	5,1	7,7	10,3	12,9	15,4	18,0	20,6	23,2
1,01	25,8	28,4	31,0	33,6	36,2	38,8	41,3	43,9	46,5	49,1
1,02	51,7	54,3	56,9	59,5	62,1	64,7	67,3	69,9	72,5	75,1
1,03	77,7	80,3	82,9	85,5	88,1	90,7	93,3	95,9	98,5	101,1
1,04	103,7	106,3	109,0	111,6	114,2	116,8	119,4	122,0	124,6	127,2
1,05	129,8	132,4	135,0	137,6	140,3	142,9	145,5	148,1	150,7	153,3
1,06	155,9	158,6	161,2	163,8	166,4	169,0	171,6	174,3	176,9	179,5
1,07	182,1	184,8	187,4	190,0	192,6	195,2	197,8	200,5	203,1	205,8
1,08	208,4	211,0	213,6	216,2	218,9	221,5	224,1	226,8	229,4	232,0
1,09	234,7	237,3	239,9	242,5	245,2	247,8	250,4	253,1	255,7	258,4
1,10	261,0	263,6	266,3	268,9	271,5	274,2	276,8	279,5	282,1	284,8
1,11	287,4	290,0	292,7	295,3	298,0	300,6	303,3	305,9	308,6	311,2
1,12	313,9	316,5	319,2	321,8	324,5	327,1	329,8	332,4	335,1	337,8
1,13	340,4	343,0	345,7	348,3	351,0	353,7	356,3	359,0	361,6	364,3
1,14	366,9	369,6	372,3	375,0	377,6	380,3	382,9	385,6	388,3	390,9
1,15	393,6	396,2	398,9	401,6	404,3	406,9	409,6	412,3	415,0	417,6
1,16	420,3	423,0	425,7	428,3	431,0	433,7	436,4	439,0	441,7	444,4
1,17	447,1	449,8	452,4	455,2	457,8	460,5	463,2	465,9	468,6	471,3
1,18	473,9	476,6	479,3	482,0	484,7	487,4	490,1	492,8	495,5	498,2
1,19	500,9	503,5	506,2	508,9	511,6	514,3	517,0	519,7	522,4	525,1
1,20	527,8	—	—	—	—	—	—	—	—	—

Interpolation table

Fourth decimal place of the specific gravity	Grams of extract per litre	Fourth decimal place of the specific gravity	Grams of extract per litre	Fourth decimal place of the specific gravity	Grams of extract per litre
1	0,3	4	1,0	7	1,8
2	0,5	5	1,3	8	2,1
3	0,8	6	1,6	9	2,3

▼B

5. **REDUCING SUGARS**

1. DEFINITION

Reducing sugars comprise all the sugars exhibiting ketonic and aldehydic functions and are determined by their reducing action on an alkaline solution of a copper salt.

2. PRINCIPLE OF THE METHODS

2.1. **Clarification**

2.1.1. *Reference method:* after neutralization and removal of alcohol, the wine is passed through an ion exchange column in which its anions are exchanged for acetate ions, followed by clarification with neutral lead acetate.

2.1.2. *Usual methods:* the wine is treated with one of the following reagents:

2.1.2.1. Neutral lead acetate;

2.1.2.2. Zinc 2-hexacyanoferrate.

2.2. Determination

2.2.1. *Single method:* the clarified wine or must is reacted with a specific quantity of an alkaline copper salt solution and the excess copper ions are then determined iodometrically.

3. CLARIFICATION

The sugar content of the liquid in which sugar is to be determined must lie between 0,5 and 5 g/l.

Dry wines should not be diluted during clarification; sweet wines should be diluted during clarification in order to bring the sugar level to within the limits prescribed in the following table:

Description	Sugar content (g/l)	Density	Dilution (%)
Musts and mistelles	> 125	> 1,038	1
Sweet wines, whether fortified or not	25 to 125	1,005 to 1,038	4
Semi-sweet wines	5 to 25	0,997 to 1,005	20
Dry wines	< 5	< 0,997	No dilution

3.1. **Reference method**

3.1.1. *Reagents*

3.1.1.1. 1 M solution hydrochloric acid (HCl);

3.1.1.2. 1 M solution sodium hydroxide (NaOH);

3.1.1.3. 4 M solution acetic acid (CH_3COOH);

3.1.1.4. 2 M solution sodium hydroxide (NaOH);

3.1.1.5. Anion exchange resin (Dowex 3 (20-50 mesh) or equivalent resin).

Preparation of the anion exchange resin column

Place a small plug of glass wool and 15 ml of the anion exchange resin (3.1.1.5) in the bottom of the burette.

Before the resin is used, subject it to two complete cycles of regeneration by passing alternately the 1 M solutions of hydrochloric acid (3.1.1.1) and sodium hydroxide (3.1.1.2) through it. After rinsing with 50 ml of distilled water, transfer the resin to a beaker, add 50 ml of the 4 M solution of acetic acid (3.1.1.3) and stir for five minutes. Refill the burette with resin and pour 100 ml of the 4 M acetic acid solution (3.1.1.3) through the column. (It is preferable to have a stock of the resin stored in a bottle filled with this 4 M acetic acid solution.) Wash the column with distilled water until the effluent is neutral.

▼**B**

Regeneration of the resin

Pour 150 ml of a 2 M sodium hydroxide solution through the resin to remove acids and most of the pigments fixed to the resin. Rinse with 100 ml of water, and then pour 100 ml of 4 M acetic acid solution through it. Wash the column until the effluent is neutral.

3.1.1.6. Neutral lead acetate solution (approximately saturated)

Neutral lead acetate [$Pb(CH_3COO)_2 \cdot 3H_2O$], 250 g;

very hot water to 500 ml;

stir until dissolved.

3.1.1.7. Calcium carbonate ($CaCO_3$)

3.1.2. *Procedure*

3.1.2.1. Dry wines

Place 50 ml of the wine in a beaker having a diameter of about 10 to 12 cm together with ½ ($n - 0,5$) ml of 1 M sodium hydroxide solution (3.1.1.2) (n being the volume of 0,1 M sodium hydroxide solution used for determining total acidity in 10 ml of wine), and evaporate over a boiling water bath in a stream of hot air until the liquid is reduced to about 20 ml.

Pour this liquid through an anion exchange resin column in acetate form (3.1.1.5) at a rate of 3 ml every two minutes. Collect the effluent in a 100 ml volumetric flask. Wash the vessel and column six times using 10 ml of distilled water each time. Stirring all the time, add 2,5 ml of saturated lead acetate solution (3.1.1.6) and 0,5 g of calcium carbonate (3.1.1.7) to the effluent: shake several times and allow to stand for at least 15 minutes. Make up to the mark with water. Filter.

1 ml of this filtrate corresponds to 0,5 ml of wine.

3.1.2.2. Musts, mistelles, sweet and semi-sweet wines:

The dilutions below are given for guidance.

1. *Musts and mistelles:* prepare a 10 % solution of the liquid to be analysed and take 10 ml of the diluted sample.
2. *Sweet wines, whether fortified or not,* having a density between 1,005 and 1,038: prepare a 20 % solution of the liquid to be analysed and take 20 ml of the diluted sample.
3. *Semi-sweet wines* having a density at 20 °C between 0,997 and 1,005: take 20 ml of the undiluted wine.

Allow the abovementioned volume of wine or must to flow through an anion exchange column in acetate form at a rate of 3 ml every two minutes. Collect the effluent in a 100 ml volumetric flask, and rinse the column with water until about 90 ml of the effluent is obtained. Add 0,5 g calcium carbonate and 1 ml saturated lead acetate solution to the effluent. Stir and allow to stand for 15 minutes, stirring occasionally. Make up to the mark with water. Filter.

In case:

1. 1 ml of filtrate corresponds to 0,01 ml of must or mistelle.
2. 1 ml of filtrate corresponds to 0,04 ml of sweet wine.
3. 1 ml of filtrate corresponds to 0,20 ml of semi-sweet wine.

▼**M9**

▼**B**

4. DETERMINATION OF SUGARS

4.1. **Reagents**

4.1.1 *Alkaline copper salt solution:*

copper sulphate, pure, $CuSO_4 \cdot 5H_2O$	25 g
citric acid ($C_6H_8O_7 \cdot H_2O$)	50 g
crystalline sodium carbonate, $Na_2CO_3 \cdot 10H_2O$	388 g
water to	1 000 ml

▼**B**

Dissolve the copper sulphate in 100 ml of water, the citric acid in 300 ml of water and the sodium carbonate in 300 to 400 ml of hot water. Mix the citric acid and sodium carbonate solutions. Add the copper sulphate solution and make up to one litre.

4.1.2. *30 % potassium iodide solution:*

potassium iodide (KI)	30 g
water to	100 ml

Store in a coloured glass bottle.

4.1.3. *25 % sulphuric acid:*

concentrated sulphuric acid, (H_2SO_4) ρ_{20} = 1,84 g/ml	25 g
water to	100 ml

Add the acid slowly to the water, allow to cool and make up to 100 ml with water.

4.1.4. *5 g/l starch solution:*

Mix 5 g of starch in with about 500 ml of water. Bring to the boil, stirring all the time, and boil for 10 minutes. Add 200 g of sodium chloride (NaCl). Allow to cool and then make up to one litre with water.

Sodium thiosulphate, 0,1 M solution

Invert sugar solution, 5 g/l, to be used for checking the method of determination:

Place the following into a 200 ml volumetric flask:

pure dry sucrose ($C_{12}H_{22}O_{11}$)	4,75 g
water, approximately	100 ml
concentrated hydrochloric acid (HCl) (ρ_{20} = 1,16 − 1,19 g/ml)	5 ml

Heat the flask in a water-bath maintained at 60 ºC until the temperature of the solution reaches 50 ºC; then keep the flask and solution at 50 ºC for 15 minutes. Allow the flask to cool naturally for 30 minutes and then immerse it in a cold water-bath. Transfer the solution to a one-litre volumetric flask and make up to one litre. This solution keeps satisfactorily for a month. When it is to be used, neutralize the test sample (the solution being approximately 0,06 M acid) with sodium hydroxide solution.

4.2. **Procedure**

Mix 25 ml of the alkaline copper salt solution, 15 ml water and 10 ml of the clarified solution in a 300 ml conical flask. This volume of sugar solution must not contain more than 60 mg of invert sugar.

Add a few small pieces of pumice stone. Fit a reflux condenser to the flask and bring the mixture to the boil within two minutes. Keep the mixture boiling for exactly 10 minutes.

Cool the flask immediately in cold running water. When completely cool, add 10 ml of 30 % potassium iodide solution (4.1.2), 25 ml of 25 % sulphuric acid (4.1.3) and 2 ml of starch solution (4.1.4).

Titrate with 0,1 M sodium thiosulphate solution (4.1.5) Let n be the number of ml used.

Also carry out a blank titration in which the 10 ml of sugar solution is replaced by 10 ml of distilled water. Let n' be the number of ml of sodium thiosulphate used.

4.3. **Expression of results**

4.3.1. *Calculations*

The quantity of sugar, expressed as invert sugar, contained in the test sample is given in the table below as a function of the number ($n' - n$) of ml of sodium thiosulphate used.

▼**B**

The sugar content of the wine is to be expressed in grams of invert sugar per litre to one decimal place, account being taken of the dilution made during clarification and of the volume of the test sample.

4.3.2. *Repeatability*

$r = 0{,}015\ x_i$

x_i = concentration of inverted sugar in g/l per sample

4.3.3. *Reproducibility*

$R = 0{,}058\ x_i$

x_i = concentration of inverted sugar in g/l per sample

Relation between the volume of 0,1 M sodium thiosulphate solution, *(n′ − n)* ml, and the quantity of reducing sugars in mg

$Na_2S_2O_3$ (ml 0,1 M)	Reducing sugars (mg)	Difference	$Na_2S_2O_3$ (ml 0,1 M)	Reducing sugars (mg)	Difference
1	2,4	2,4	13	33,0	2,7
2	4,8	2,4	14	35,7	2,8
3	7,2	2,5	15	38,5	2,8
4	9,7	2,5	16	41,3	2,9
5	12,2	2,5	17	44,2	2,9
6	14,7	2,6	18	47,2	2,9
7	17,2	2,6	19	50,0	3,0
8	19,8	2,6	20	53,0	3,0
9	22,4	2,6	21	56,0	3,1
10	25,0	2,6	22	59,1	3,1
11	27,6	2,7	23	62,2	
12	30,3	2,7			

▼B

6. **SUCROSE**

1. PRINCIPLE OF METHODS

I. For qualitative testing by thin-layer chromatography: sucrose is separated from other sugars using thin-layer chromatography on plate coated with cellulose. The developing agent is urea-hydrochloric acid at 105 º C.

II. 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.

For testing and determination of sucrose, chromatography in gaseous phase may also be used, as described in chapter 42, point (f).

2. QUALITATIVE TESTING BY THIN-LAYER CHROMATOGRAPHY

2.1. **Equipment**

2.1.1. Chromatograhic plates covered with a desired thickness of cellulose powder (e.g. MN 300) (20 × 20).

2.1.2. Chromatography tank.

2.1.3. Micrometric syringe or micropipette.

2.1.4. Oven with regulation to 105 ± 2 ºC.

2.2. **Reagents**

2.2.1. Decolourizing charcoal.

2.2.2. *Mobile phase:* Dichloromethane — glacial acetic acid (p20 — 1,05 g/ml) — ethanol — methanol — water (50:25:9:6:10).

2.2.3. *Developing agent*

Urea	5	g
Hydrochloric acid 2 M	20	ml
Ethanol	100	ml

2.2.4. *Reference solutions*

Glucose	35	g
Fructose	35	g
Sucrose	0,5	g
Distilled water	1 000	ml

2.3. **Procedure**

2.3.1. *Preparation of sample*

When the must or wine is strongly coloured, decolourize it by treating it with activated charcoal.

For rectified concentrated musts, use the solution with a sugar concentration of 25 % by mass (25 º Brix) prepared as described in the chapter ‘pH of wine and must’, section 4.1.2,and dilute it with water to a quarter of its concentration by making 25 ml up to 100 ml in a volumetric flask.

2.3.2. *Obtaining the chromatogram*

Place on a parallel line 2,5 cm from the bottom edge of the plate:

— 10 µl of sample

— 10 µl of standard.

▼**B**

Place the plate in the tank, previously saturated with the vapour from the mobile phase. Allow the mobile phase to migrate to within 1 cm of the top of the plate. Remove the plate and dry it in a current of warm air. Repeat the migration two more times, drying the plate each time. Spray the plate uniformly with 15 ml of colouring agent and place in the oven at 105 ºC for approximately five minutes.

2.4. Results

Saccharose and fructose appear as a deep blue spot on a white background: glucose gives a less intense green spot.

3. TESTING AND DETERMINATION BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

The chromatographic conditions are given for guidance

3.1. Equipment

3.1.1. High-performance liquid chromatograph equipped with:

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

3.1.2. Arrangement for membrane filtration (0,45 µm).

3.2. Reagents

3.2.1. Doubly distilled water.

3.2.2. HPLC quality acetonitrile (CH_3CN).

3.2.3. *Mobile phase:* acetonitrile-water, previously subjected to membrane filtration (0,45 µm), (80:20 v/v).

This mobile phase must be outgassed before being used.

3.2.4. *Standard solution:* 1,2 g/l aqueous sucrose solution. Filter using a 0,45 µm membrane filter.

3.3. Procedure

3.3.1. *Preparation of sample:*

- For wines and musts: filter using a 0,45 µm membrane filter.
- For rectified concentrated musts: use the solution obtained by diluting the rectified concentrated must to 40 % (m/v) as described in the chapter 'Total acidity', section 5.1.2., and filtering it using a 0,45 µm membrane filter.

3.3.2. *Chromatographic determination*

Inject in turn into the chromatograph 10 µl of the standard solution and 10 µm of the sample prepared as described in 3.3.1. Repeat these injections in the same order.

Record the chromatogram.

The retention time of the sucrose is approximately 10 minutes.

3.4. Calculations

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

3.4.1. *For wines and musts:* calculate the concentration in g/l.

3.4.2. *For rectified concentrated musts:* let *C* be the sucrose concentration in g/l of the 40 % (m/v) solution of rectified concentrated must. The

▼**B**

sucrose concentration in g/kg of the rectified concentrated must is then: 2,5*C*.

3.5. Expression of results

The sucrose concentration in wines, musts and rectified concentrated musts is expressed in grams per litre for wines and musts and in grams per kilogram for rectified concentrated musts, each to one place of decimals.

▼B

7. GLUCOSE AND FRUCTOSE

1. DEFINITION

Glucose and fructose may be determined individually by an enzymatic method, with the sole aim of calculating the glucose/fructose ratio.

2. PRINCIPLE OF THE METHOD

Glucose and fructose are phosphorylated by adenosine triphosphate (ATP) during an enzymatic reaction catalysed by hexokinase (HK), and produce glucose 6-phosphate (G6P) and fructose 6-phosphate (F6P):

$$\text{glucose} + \text{ATP} \xrightleftharpoons{\text{HK}} \text{G6P} + \text{ADP}$$

$$\text{fructose} + \text{ATP} \xrightleftharpoons{\text{HK}} \text{F6P} + \text{ADP}$$

The glucose 6-phosphate is first oxidized to gluconate 6-phosphate by nicotinamide adenine dinucleotide phosphate (NADP) in the presence of the enzyme glucose 6-phosphate dehydrogenase (G6PDH). The quantity of reduced nicotinamide adenine dinucleotide phosphate (NADPH) produced corresponds to that of glucose 6-phosphate and thus to that of glucose.

$$\text{G6P} + \text{NADP}^+ \xrightleftharpoons{\text{G6PDH}} \text{gluconate 6-phosphate} + \text{NADPH} + \text{H}^+$$

The reduced nicotinamide adenine dinucleotide phosphate is determined from its absorption at 340 nm.

At the end of this reaction, the fructose 6-phosphate is converted into glucose 6-phosphate by the action of phosphoglucose isomerase (PGI):

$$\text{F6P} \xrightleftharpoons{\text{PGI}} \text{G6P}$$

The glucose 6-phosphate again reacts with the nicotinamide adenine dinucleotide phosphate to give gluconate 6-phosphate and reduced nicotinamide adenine dinucleotide phosphate, and the latter is then determined.

3. APPARATUS

- A spectrophotometer enabling measurements to be made at 340 nm, the wavelength at which absorption by NADPH is at a maximum. Absolute measurements are involved (i.e. calibration plots are not used but standardization is made using the extinction coefficient of NADPH), so that the wavelength scales of and absorbence values obtained from the apparatus must be checked.

  If not available, a spectrophotometer using a source with a discontinuous spectrum which enables measurements to be made at 334 nm or at 365 nm may be used.
- Glass cells with optical path lengths of 1 cm or single-use cells.
- Pipettes for use with enzymatic test solutions, 0,02, 0,05, 0,1, 0,2 ml.

4. REAGENTS

4.1. **Solution 1:** buffer solution (0,3 M triethanolamine, pH 7,6, 4×10^{-3} M in Mg^{2+}): dissolve 11,2 g triethanolamine hydrochloride ($(C_2H_5)_3N \cdot HCl$) and 0,2 g $MgSO_4 \cdot 7H_2O$ in 150 ml of doubly distilled water, add about 4 ml of 5 M sodium hydroxide (NaOH) solution to obtain a pH value of 7,6 and make up to 200 ml.

This buffer solution may be kept for four weeks at + 4 °C.

4.2. **Solution 2:** nicotinamide adenine dinucleotide phosphate solution (about $11{,}5 \times 10^{-3}$ M): dissolve 50 mg disodium nicotinamide adenine dinucleotide phosphate in 5 ml of doubly distilled water.

This solution may be kept for four weeks at +4 °C.

▼B

4.3. **Solution 3:** adenosine 5′-triphosphate solution (about 81×10^{-3} M): dissolve 250 mg disodium adenosine 5′-triphosphate and 250 mg sodium hydrogencarbonate ($NaHCO_3$) in 5 ml of doubly distilled water.

This solution may be kept for four weeks at +4 ºC.

4.4. **Solution 4:** hexokinase/glucose 6-phosphate dehydrogenase: mix 0,5 ml hexokinase (2 mg of protein/ml or 280 U/ml) with 0,5 ml glucose 6-phosphate dehydrogenase (1 mg of protein/ml).

This mixture may be kept for a year at about +4 ºC.

4.5. **Solution 5:** phosphoglucose isomerase (2 mg of protein/ml or 700 U/ml). The suspension is used undiluted.

This may be kept for a year at about +4 ºC.

Note:

All solutions used above are available commercially.

5. PROCEDURE

5.1. **Preparation of sample**

Depending on the estimated amount of glucose + fructose per litre, dilute the sample as follows:

Measurement at 340 and 334 nm	Measurement at 365 nm	Dilution with water	Dilution factor *F*
up to 0,4 g/l	0,8 g/l	—	—
up to 4,0 g/l	8,0 g/l	1 + 9	10
up to 10,0 g/l	20,0 g/l	1 + 24	25
up to 20,0 g/l	40,0 g/l	1 + 49	50
up to 40,0 g/l	80,0 g/l	1 + 99	100
above 40,0 g/l	80,0 g/l	1 + 999	1 000

5.2. **Determination**

With the spectrophotometer adjusted to the 340 nm wavelength, make measurements using air (no cell in the optical path) or water as reference.

Temperature between 20 and 25 ºC.

Into two cells with 1 cm optical paths, place the following:

	Reference cell	Sample cell
Solution 1 (4.1) (taken to 20 ºC)	2,50 ml	2,50 ml
Solution 2 (4.2)	0,10 ml	0,10 ml
Solution 3 (4.3)	0,10 ml	0,10 ml
Sample to be measured		0,20 ml
Doubly distilled water	0,20 ml	

Mix, and after about three minutes read off the absorbence of the solutions (A_1). Start the reaction by adding:

Solution 4 (4.4)	0,02 ml	0,02 ml

Mix; wait 15 minutes; read off the absorbence and check that the reaction has stopped after a further two minutes (A_2).

Add immediately:

Solution 5 (4.5)	0,02 ml	0,02 ml

Mix; read off the absorbence after 10 minutes and check that the reaction has stopped after a further two minutes (A_3).

Calculate the differences in the absorbences:

$\mathbf{A}_2 - A_1$ corresponding to glucose,

▼B

$\mathbf{A}_3 - A_2$ corresponding to fructose,

for the reference and sample cells.

Calculate the differences in absorbence for the reference cell (ΔA_R) and the sample cell (ΔA_S) and then obtain:

for glucose: $\Delta A_G = \Delta A_S - \Delta A_R$

for fructose: $\Delta A_F = \Delta A_S - \Delta A_R$

Note:

The time needed for the completion of enzyme activity may vary from one batch to another. The above value is given only for guidance and it is recommended that it be determined for each batch.

5.3. Expression of results

5.3.1. *Calculation*

The general formula for calculating the concentrations is:

$$C\ (\text{g/l}) = \frac{V \times M}{\varepsilon \times d \times v \times 1\,000}\ \Delta A$$

where

V = volume of the test solution (ml)

v = volume of the sample (ml)

M = molecular mass of the substance to be determined

d = optical path in the cell (cm)

ε = absorption coefficient of NADPH at 340 nm (= 6,3 $\text{mM}^{-1} \times \text{l} \times \text{cm}^{-1}$)

and

V = 2,92 ml for the determination of glucose

V = 2,94 ml for the determination of fructose

v = 0,20 ml

M = 180

d = 1

so that:

For glucose: C (g/l) = 0,417 ΔA_G

For fructose: C (g/l) = 0,420 ΔA_F

If the sample was diluted during its preparation, multiply the result by the dilution factor *F*.

Note:

If the measurements are made at 334 or 365 nm, then the following expressions are obtained:

- measurement at 334 nm: $\varepsilon = 6{,}2\ (\text{mmole}^{-1} \times \text{l} \times \text{cm}^{-1})$
  For glucose: C (g/l) = 0,425 ΔA_G
  For fructose: C (g/l) = 0,428 ΔA_F
- measurement at 365 nm: $\varepsilon = 3{,}4\ (\text{mmole} \times \text{l}^{-1} \times \text{cm}^{-1})$
  For glucose: C (g/l) = 0,773 ΔA_G
  For fructose: C (g/l) = 0,778 ΔA_F

5.3.2. *Repeatability (r)*

r = 0,056 x_i

5.3.3. *Reproducibility (R)*

R = 0,12 + 0,076 x_i

x_i = concentration of glucose or fructose in g/l.

▼B

8. DETECTING ENRICHMENT OF GRAPE MUSTS, CONCENTRATED GRAPE MUSTS, RECTIFIED CONCENTRATED GRAPE MUSTS AND WINES BY APPLICATION OF NUCLEAR MAGNETIC RESONANCE OF DEUTERIUM (SNIF-NMR/RMN-FINS)

1. DEFINITION

The deuterium contained in the sugars and the water in grape must will be redistributed after fermentation in molecules I, II, III and IV of the wine:

$CH_2D\ CH_2\ OH$ I	$CH_3\ CHD\ OH$ II
$CH_3\ CH_2\ OD$ III	HOD IV

The addition of exogenous sugar (sugaring in the dry) before the must ferments will have an effect on the distribution of the deuterium.

As compared with the figures for parameters for a natural control wine from the same region, the enrichment of an exogenous sugar will lead to the following variations:

Parameters / Wine	$(D/H)_I$	$(D/H)_{II}$	$(D/H)_W^Q$	*R*
— Natural	→	→	→	→
— Enriched:				
— beet sugar	↘	↗	↗	↗
— cane sugar — maize sugar	↗	↗	↗	↘

$(D/H)_I$: Isotope ratio associated with molecule I

$(D/H)_{II}$: Isotope ratio associated with molecule II

$(D/H)_W^Q$: Isotope ratio of the water in the wine.

R = $2(D/H)_{II}/(D/H)_I$ expresses the relative distribution of deuterium in molecules I and II; *R* is measured directly from the h-intensities of the signals and then $R = 3h_{II}/h_I$.

$(D/H)_I$ mainly characterizes the vegetable species which synthesized the sugar and to a lesser extent the geographical location of the place of harvest (type of water used during photosynthesis).

$(D/H)_{II}$ represents the climatology of the place of production of the grapes (type of rainwater and weather conditions) and to a lesser extent the sugar concentration of the original must.

$(D/H)_W^Q$ represents the climatology of the place of production and the sugar content of the original must.

2. PRINCIPLE

The parameters defined above (*R*, $(D/H)_I$, $(D/H)_{II}$) are determined by nuclear magnetic resonance of the deuterium in the ethanol extracted from the wine or from the fermentation products of the must, the concentrated must or the rectified concentrated must obtained under given conditions; they may be supplemented by determining the isotope ratio of the water extracted from the wine, $(D/H)_W^Q$ and by determining the ratio $^{13}C/\ ^{12}C$ in the ethanol.

▼M5

▼**B**

3. PREPARATION OF THE SAMPLE FOR ANALYSIS

3.1. **Extraction of ethanol and water in the wine**

Note:

Any method for ethanol extraction can be used as long as 98 to 98,5 % of the total alcohol in the wine is recovered in a distillate which contains 92 to 93 % mas (95 % vol).

3.1.1. *Apparatus and reagents*

Apparatus for extracting ethanol (Figure 1) comprising:

- electric heating mantle with voltage regulator,
- one-litre round-bottom flask with ground glass neck joint,
- Cadiot column with rotating band (moving part in Teflon),
- 125 ml conical flasks with ground glass neck joints,
- 125 and 60 ml bottles with plastic stoppers.

Reagents for the determination of water by the Karl Fischer method (e.g. Merck 9241 and 9243).

3.1.2. *Procedure*

3.1.2.1. Determine the alcoholic strength of the wine (t^v) to better than the nearest 0,05 % vol.

3.1.2.2. Extraction of the ethanol

Introduce a homogeneous sample of 500 ml of wine of alcoholic strength t^v into the flask in the distillation apparatus with a constant reflux ratio of about 0,9. Place a 125 ml ground conical flask, calibrated beforehand, to receive the distillate. Collect the boiling liquid between 78,0 and 78,2 °C, i.e. approximately 40 to 60 ml. If the temperature exceeds 78,5 °C, discontinue collection for five minutes.

When the temperature returns to 78 °C, recommence collecting the distillate until 78,5 °C and repeat this operation until the temperature, after discontinuing collection and operating within a closed circuit, remains constant. Complete distillation lasts approximately five hours. This procedure enables between 98 and 98,5 % of the total alcohol in the wine to be recovered in a distillate with a strength of between 92 and 93 % mas (95 % vol), a strength for which the NMR conditions described in section 4 have been established.

The ethanol collected is weighed.

A homogeneous 60 ml sample of the residues is kept in a 60 ml flask and represents the water in the wine. Its isotope ratio may be determined if required.

Note:

If a spectrometer fitted with a 10 mm probe is available (cf. section 4), a homogeneous test sample of 300 ml of wine is sufficient.

3.1.2.3. Determination of the alcoholic strength of the alcohol extracted

The water content (p' g) is determined by the Karl Fischer method using a sample of about 0,5 ml of alcohol of exactly known mass p.

▼B

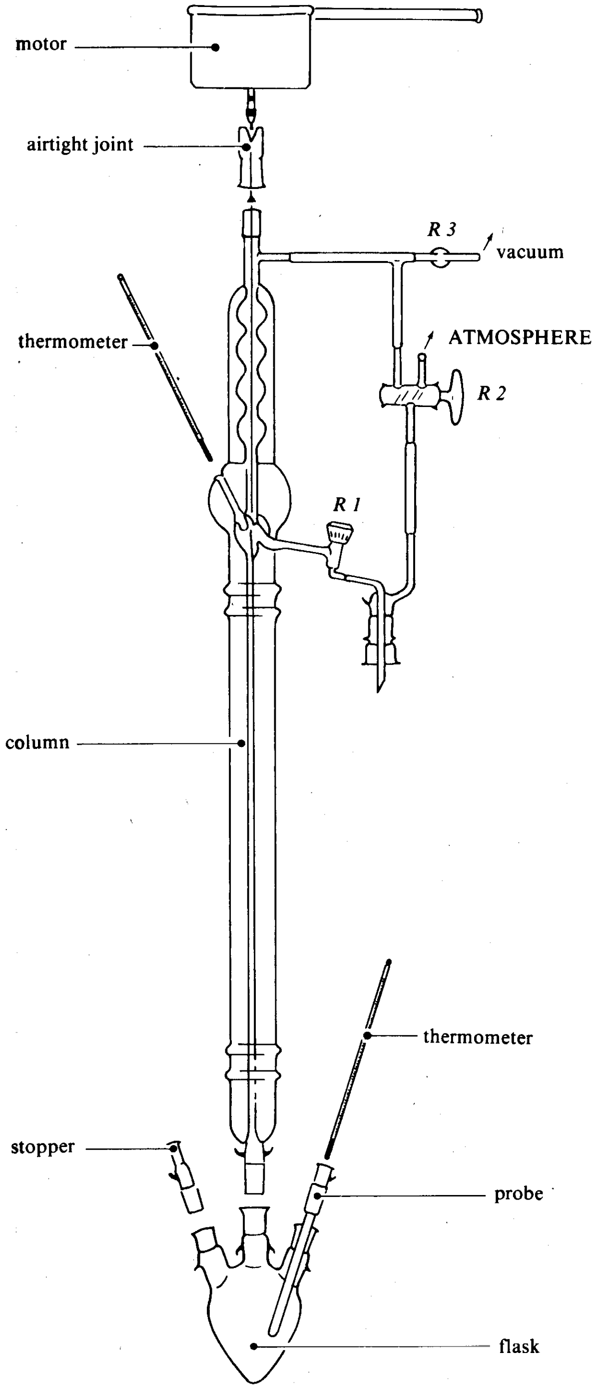


Figure 1

Distillation apparatus for the extraction of ethanol

The strength by mass of the alcohol is given by

$$t_m^D = \frac{p - p'}{p} \times 100$$

3.2. Fermentation of musts, concentrated musts and rectified concentrated musts

3.2.1. *Apparatus and reagents*

Tartaric acid

DIFCO Bacto Yeast Nitrogen Base without amino acids

Active dry yeasts *(Saccharomyces cerevisæ).*

▼**B**

If the isotope ratio of the must is known the yeast can be reactivated prior to use for 15 minutes in a minimum amount of lukewarm non-distilled water, so that the isotope ratio is similar to that of the must.

If the isotope ratio of the must is not known it is better to use fresh/direct.

Fermentation vessel of a capacity of 1,5 litres fitted with a device to keep it airtight and to condense alcohol vapour, since no loss of ethanol during fermentation must be tolerated. The rate of conversion of fermentable sugars into ethanol should be greater than 98 %.

3.2.2. *Procedure*

3.2.2.1. Musts

- Fresh musts

  Place one litre of must, whose concentration of fermentable sugars has been previously determined, in the fermentation vessel. Add 1 g of dry yeast reactivated beforehand. Insert device to keep it airtight. Allow fermentation to proceed at around 20 ºC until the sugar is used up. After determining the alcoholic strength of the fermentation product and calculating the rate of conversion of sugars into alcohol, the fermented liquid is centrifuged and distilled to extract the ethanol.
- Musts with fermentation prevented by the addition of sulphur dioxide

  De-sulphite a quantity of must slightly in excess of one litre (i.e. 1,2 litre) by bubbling nitrogen through the must in a water bath at 70 to 80 ºC under reflux until the total sulphur dioxide content is less than 200 mg/l. Take care to see that the must is not concentrated through evaporation of water by using effective cooling. Place 1 litre of de-sulphited must in the fermentation vessel and continue as described for fresh must.

  Note:

  If potassium metabisulphite is used to sulphite the must, 0,25 ml of sulphuric acid (ρ_{20} = 1,84 g/ml) per gram of metabisulphite used per litre of must should be added to the must before de-sulphiting.

3.2.2.2. Concentrated musts

Place *V* ml of concentrated must containing a known amount of sugar (approximately 170 g) into the fermentation vessel. Top up to one litre with (1 000 − *V*) ml of water from the normal water supply of same isotope ratio as natural must samples. Add (3.2.1) dry yeasts (1 g) and 3 g of DIFCO Bacto Yeast Nitrogen Base without amino acids. Homogenize and proceed as before.

3.2.2.3. Rectified concentrated musts

Proceed as described in 3.2.2.2, topping up to one litre with (1 000 − *V*) ml of water from the normal water supply of same isotope ratio but also containing 3 g dissolved tartaric acid.

Note:

Retain 50 ml of sample of must or sulphur dioxide treated must or concentrated must or rectified concentrated must with a view to the possible extraction of the water and the determination of its isotope ratio $(D/H)_W^Q$. The extraction of the water contained in the must may be very simply carried out by azotropic distillation using toluene.

3.3. **Preparation of alcohol sample for NMR measurement**

3.3.1. *Reagents*

N, N-tetramethyl urea (TMU); use a sample of standard TMU with a given, monitored isotope ratio D/H. This sample may be supplied by:

Directorate-General for Science, Research and Development,
Community Bureau of References,
200 rue de la Loi, B-1049 Brussels.

3.3.2. *Procedure*

- 15 mm diameter NMR probe:

  in a previously weighed bottle, collect 7 ml alcohol obtained as in 3.1.2 and weigh it to the nearest 0,1 mg (m_A); then take a 3 ml

▼B

sample of the internal standard (TMU) and weigh to the nearest 0,1 mg (m_{st}). Homogenize by shaking.

- 10 mm diameter NMR probe:

  3,2 ml of alcohol and 1,3 ml TMU are sufficient.

  Depending on the type of spectrometer and probe used (cf. section 4), add a sufficient quantity of hexafluorobenzene as a field-frequency stabilization substance (lock):

Spectrometer	10 mm probe	15 mm probe
7,05 T	150 µl	200 µl
9,4 T	35 µl	50 µl

3.4. Preparation of a water sample for the NMR measurement, for the purpose of a possible determination of its isotope ratio

3.4.1. *Reagents*

N, N-tetramethyl urea (TMU): see 3.3.1.

3.4.2. *Procedure*

Place 3 ml of water obtained as in 3.1.2 or 3.2 (note) into a tared flask and weigh to the nearest 0,1 mg (m'_E). Place 4 ml of internal standard (TMU) and weigh to the nearest 0,1 mg (m'_{st}). Homogenize by shaking.

Note:

If the laboratory has a mass spectrometer for determining isotope ratios, the measurement may be carried out on this instrument to reduce the load on the NMR spectrometer. It is necessary to standardize the ratio Trv (5.2) for each series of wines examined.

4. RECORDING OF ^{2}H NMR SPECTRA OF THE ALCOHOL AND THE WATER

Determination of isotope parameters.

4.1. Apparatus

- NMR spectrometer fitted with a specific ‘deuterium’ probe tuned to the characteristic frequency V_o of the field Bo (e.g. for B_o = 7,05 T, V_o = 46,05 MHz and for B_o = 9,4 T, V_o = 61,4 MHz) having a proton decoupling channel (B_2) and field-frequency stabilization channel (lock) at the fluorine frequency.

  The resolution measured on the spectrum, transformed without exponential multiplication (i.e. LB = 0) (Figure 2b) and expressed by the half-width of the methyl and methylene signals of ethanol and the methyl signal of TMU, must be less than 0,5 Hz. The sensitivity, measured with an exponential multiplying factor LB equal to 2 (Figure 2a) must be greater than or equal to 150 for the methyl signal of ethanol of alcoholic strength 95 % vol (93,5 % mas).

  Under these conditions, the confidence interval for the measurement of the signal height, calculated for a 97,5 % probability (one-sided test) and 10 repetitions of the spectrum, is 0,35 %.
- Automatic sample changer (possibly)
- Data-processing software
- 15 mm or 10 mm sample tubes according to spectrometer performance.

4.2. Standardization of spectrometer and checks

4.2.1. *Standardization*

Carry out customary standardization for homogeneity and sensitivity according to the manufacturer's specifications.

▼B

4.2.2. *Checking the validity of the standardization*

Use standard ethanols, designated by the letters C, V and B, having isotope concentrations that are different but accurately standardized. They carry the following meaning:

- — C: alcohol from cane sugar or maize,
- — V: wine spirit,
- — B: beet alcohol.

These samples are supplied by the Community Bureau of References.

Following the procedure described in 4.3, determine the isotope values of these alcohols, denoting them C_{meas}, V_{meas}, B_{meas} (see 5.3).

Compare them with the given corresponding standard values, denoted by a superscript C_{st}, B_{st}, V_{st} (see 5.3).

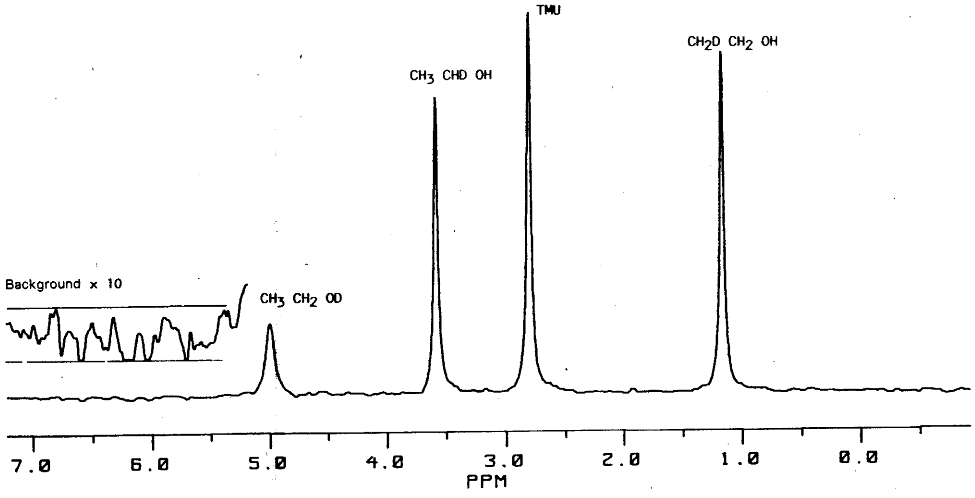


Figure 2a

^2H NMR spectrum of an ethanol from wine with an internal standard (TMU: N, N-tetramethylurea)

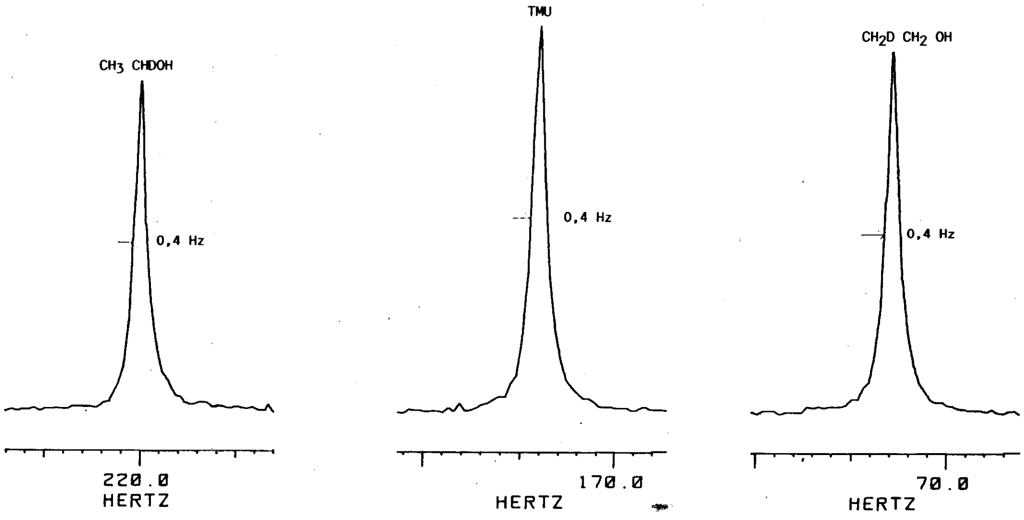


Figure 2b

^2H spectrum of ethanol taken under the same conditions as those of Figure 2a, but without exponential multiplication (LB = 0)

The standard deviation for repeatability obtained on an average of 10 repetitions of each spectrum must be less than 0,01 for the ratio R and less than 0,3 ppm for $(D/H)_I$ and $(D/H)_{II}$.

The average values obtained for the various isotopic parameters (R, $(D/H)_I$, $(D/H)_{II}$) must be within the corresponding standard deviation of repeatability given for those parameters for the three standard

▼B

alcohols by the Community Bureau of References. If they are not, carry out the checks again.

4.3. Conditions for obtaining NMR spectra

Place a sample of alcohol prepared as in 3.3 (or the water sample, prepared as in 3.4) in a 15-mm or 10-mm tube and introduce it into the probe.

The conditions for obtaining NMR spectra are as follows:

- a constant probe temperature (e.g. 302 K);
- acquisition time of at least 6,8 s for 1 200 Hz spectral width (16K memory) (i.e. about 20 ppm at 61,4 MHz or 27 ppm at 46,1 MHz);
- 90° pulse;
- adjustment of acquisition time: its value must be of the same order as the dwell time;
- parabolic detection: fix the offset 01 between the OD and CHD reference signals for ethanol and between the HOD and TMU reference signals for water;
- determine the value of the decoupling offset 02 from the proton spectrum measured by the decoupling coil on the same tube. Good decoupling is obtained when 02 is located in the middle of the frequency interval existing between the CH_3- and CH_2-groups. Use the wide band decoupling mode.

For each spectrum, carry out a number of accumulations NS sufficient to obtain the signal-to-noise ratio given in 4.1 and repeat this set of NS accumulations NE = 10 times. The values of NS depend on the types of spectrometer and probe used (cf. section 4). Examples of the possible choices are:

Spectrometer	10 mm probe	15 mm probe
7,05 T	NS = 304	NS = 200
9,4 T	NS = 200	NS = 128

5. EXPRESSION OF RESULTS

5.1. Ethanol

For each of the 10 spectra (see NMR spectrum for ethanol, Figure 2a), determine:

- $R = \frac{3h_{II}}{h_I} = 3 \times \frac{\text{height of signal II } (CH_3\ CHD\ OH)}{\text{height of signal I } (CH_2D\ CH_2OH)}$
- $(D/H)_I = 1,5866 \times T_I \times \frac{m_{st}}{m_A} \times \frac{(D/H)_{st}}{t_m^D}$
- $(D/H)_{II} = 2,3799 \times T_{II} \times \frac{m_{st}}{m_A} \times \frac{(D/H)_{st}}{t_m^D}$

with

- $T_I = \frac{\text{height of signal I } (CH_2D\ CH_2\ OH)}{\text{height of signal of internal standard (TMU)}}$
- $T_{II} = \frac{\text{height of signal II } (CH_3CHD\ OH)}{\text{height of signal of internal standard (TMU)}}$
- m_{st} and m_A, see 3.3.2.
- t^D, see 3.1.2.3.
- $(D/H)_{st}$ = isotope ratio of internal standard (TMU) indicated on the bottle supplied by the Community Bureau of References.

The use of peak heights instead of peak area, which is less precise, supposes that peak width at half height is identical and is a reasonable approximation if applicable (Figure 2b).

▼**B**

5.2. Water

When the isotope ratio of water is determined by NMR from the water-TMU mixture, the following relationship is used:

— $(D/H)_W^Q = 0,9306 \times T_{IV} \times \frac{m'_{st}}{m'_E} \times (D/H)_{st}$

with

— $T_{IV} = \frac{\text{Area of (HOD) signal of the water extracted from the wine}}{\text{Area of the signal from the internal standard (TMU)}}$

— m'_{st} and m'_E, see 3.4.2.

— $(D/H)_{st}$ = isotope ratio of the internal standard (TMU) indicated on the bottle supplied by the Community Bureau of References.

5.3. For each of the isotope parameters, calculate the average of 10 determinations and the confidence interval.

Optional software (e.g. SNIF-NMR) suitable for the spectrometer computer enables such calculations to be carried out on-line.

Note:

If, after standardization of the spectrometer, there is a systematic difference between the average values obtained for the characteristic isotopes of the standard alcohols (4.2.2) and the values indicated by the Community Bureau of References, to within the standard deviation, the following corrections may be applied to obtain the true value for any sample X.

The interpolation will be made by taking the values for the standard sample which straddle that of the sample X.

Let $(D/H)_{i\,\mathrm{meas}}^{X}$ be the measured value and $(D/H)_{i\,\mathrm{corr}}^{X}$ be the corrected value. This will give:

$(D/H)_{i\,\mathrm{corr}}^{X} = (D/H)_{i\,\mathrm{st}}^{B} + \alpha\,[(D/H)_{i\,\mathrm{meas}}^{X} - (D/H)_{i\,\mathrm{meas}}^{B}]$

where

$$\alpha = \frac{(D/H)_i^{V_{st}} - (D/H)_i^{B_{st}}}{(D/H)_i^{V_{meas}} - (D/H)_i^{B_{meas}}}$$

Example:

Standard samples supplied and standardized by the Community Bureau of References:

$(D/H)_{I\,\mathrm{st}}^{V}$ = 102,0 ppm $(D/H)_{I\,\mathrm{st}}^{B}$ = 91,95 ppm

Standard samples measured by the laboratory:

$(D/H)_{I\,\mathrm{meas}}^{V}$ = 102,8 ppm $(D/H)_{I\,\mathrm{meas}}^{B}$ = 93,0 ppm

Suspect uncorrected sample: $(D/H)_{I\,\mathrm{meas}}^{X}$ = 100,2 ppm

α = 1,0255 and $(D/H)_{I\,\mathrm{corr}}^{X}$ = 99,3 ppm are calculated.

6. INTERPRETATION OF RESULTS

Compare the value R^X obtained for the R ratio of the suspect sample with the ratios obtained for the control wines. If R^X differs by more than two standard deviations from the average R^T value obtained for the control wine, adulteration may be assumed.

6.1. Addition of beet sugar, cane sugar or maize glucose

6.1.1. *Wines*

R^X higher than R^T: beet sugar is assumed to have been added.

R^X less than R^T: cane sugar or maize sugar is assumed to have been added.

Note that $(D/H)_I^X$ and $(D/H)_W^{QX}$ are increased.

Consider $(D/H)_I^X$:

— — Beet sugar is assumed to have been added: $(D/H)_I^X$ of the suspect sample is lower than $(D/H)_I^T$, the average value obtained from the control samples, by more than one standard deviation

— Cane sugar or maize sugar is assumed to have been added:

▼B

$(D/H)_I^X$ is greater than $(D/H)_I^T$ by more than one standard deviation

— Calculation of enrichment E expressed in % vol of ethanol:

— Addition of beet sugar:

$$E\ \%\ \text{vol} = t^V \frac{(D/H)_I^T - (D/H)_I^X}{(D/H)_I^T - (D/H)_I^B}$$

where

$(D/H)_I^B$ = isotope ratio for the location I of the beet alcohol;

$(D/H)_I^B$ = 92,5 (1)

t^V = alcoholic strength of the analysed wine (X).

— Addition of cane sugar or maize sugar:

$$E\ \%\ \text{vol} = t^V \frac{(D/H)_I^X - (D/H)_I^T}{(D/H)_I^C - (D/H)_I^T}$$

where

$(D/H)_I^C$ = isotope ratio for the location I of the cane sugar or maize sugar;

$(D/H)_I^C$ = 110,5 (1)

t^V = alcoholic strength of the analysed wine (X)

6.1.2. *Musts, concentrated musts and rectified concentrated masts*

The values of the isotopic parameters for the alcohol extracted as described in 3.1 from the fermented product obtained (3.2) from must, concentrated must and rectified concentrated must are examined according to the instructions given in 6 under ‘Interpretation of results’ (6.1.1) and compared with the alcohol extracted from the fermentation product of musts.

The enrichment, E % vol, expresses the volume of alcohol added to the fermented product. Knowing the dilution that may have been carried out prior to fermentation (concentrated musts and rectified concentrated musts), assuming that 16,83 g of sugar yield 1 % vol of alcohol, calculate the amount of sugar (mass) added per litre of must, concentrated must or rectified concentrated must.

6.2. Addition of a mixture of beet sugar and cane sugar or maize glucose

The isotope ratios $(D/H)_I$ and *R* are changed less than when only one type of sugar is added.

$(D/H)_{II}$ is higher, as is $(D/H)_W^Q$.

These additions may be confirmed by determining the $^{13}C/^{12}C$ ratio of the ethanol by mass spectrometry; in that case the ratio is higher.

---

(1) These values are given pending the creation of a Community data bank of such values.

▼B

9. ASH CONTENT

1. DEFINITION

The ash content is defined to be all those products remaining after igniting the residue left from evaporation of the wine. The ignition is carried out in such a way that all the cations (excluding the ammonium cation) are converted into carbonates or other anhydrous inorganic salts.

2. PRINCIPLE OF THE METHOD

The wine extract is ignited at a temperature between 500 and 550 ºC until complete combustion (oxidation) of organic material has been achieved.

3. APPARATUS

3.1. boiling water-bath;

3.2. balance sensitive to 0,1 mg;

3.3. hot-plate or infra-red evaporator;

3.4. temperature-controlled electric muffle furnace;

3.5. desiccator;

3.6. flat-bottomed platinum dish 70 mm in diameter and 25 mm in height.

4. PROCEDURE

Pipette 20 ml of wine into the previously tared platinum dish (original weight P^o g). Evaporate on the boiling water-bath, and heat the residue on the hot-plate at 200 ºC or under the infra-red evaporator until carbonization begins. When no more fumes are produced, place the dish in the electric muffle furnace maintained at 525 ± 25 ºC. After 15 minutes of carbonization, remove the dish from the furnace, add 5 ml of distilled water, evaporate on the water-bath or under the infra-red evaporator, and again heat the residue to 525 ºC for 10 minutes.

If combustion (oxidation) of the carbonized particles is not complete, repeat the operations of washing the carbonized particles, evaporation of water and ignition.

For wines with a high sugar content, it is advantageous to add a few drops of pure vegetable oil to the extract before the first ashing to prevent excessive foaming.

After cooling in the desiccator, the dish is weighed (P_1 g).

The weight of the ash in the sample (20 ml) is then $P = (P_1 - P_o)$ g.

5. EXPRESSION OF RESULTS

5.1. Method of calculation

The weight P of the ash in grams per litre will be given to two decimal places by the expression: $P = 50p$

▼**B**

10. **ALKALINITY OF THE ASH**

1. DEFINITION

The alkalinity of the ash is defined as the sum of cations, other than the ammonium ion, combined with the organic acids in the wine.

2. PRINCIPLE OF THE METHOD

The ash is dissolved in a known (excess) amount of a hot standardized acid solution; the excess is determined by titration using methyl orange as an indicator.

3. REAGENTS AND APPARATUS

3.1. 0,05 M sulphuric acid solution (H_2SO_4);

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

3.3. methyl orange, 0,1 % solution in distilled water;

3.4. boiling water-bath.

4. PROCEDURE

Add 10 ml of the 0,05 M sulphuric acid solution (3.1) to the ash from 20 ml of wine contained in the platinum dish. Place the dish on the boiling water-bath for about 15 minutes, breaking up and agitating the residue with a glass rod to speed up the dissolution. Add two drops of methyl orange solution and titrate the excess sulphuric acid against 0,1 M sodium hydroxide (3.2) until the colour of the indicator changes to yellow.

5. EXPRESSION OF RESULTS

Method of calculation

The alkalinity of the ash, expressed in milliequivalents per litre to one decimal place, is given by

$A = 5\ (10 - n)$

where n ml is the volume of 0,1 M sodium hydroxide used.

▼**B**

11. **CHLORIDES**

1. PRINCIPLE

Chlorides are determined directly in the wine by potentiometry using an Ag/AgCl electrode.

2. APPARATUS

2.1. pH/mV meter graduated at intervals of at least 2 mV.

2.2. Magnetic stirrer.

2.3. Ag/AcCl electrode with a saturated solution of nitrate potassium as electrolyte.

2.4. Microburette graduated in 1/100 ml.

2.5. Chronometer.

3. REAGENTS

3.1. Standard chloride solution: 2,1027 g of potassium chloride, KCl (max. 0,005 % Br), dried before use by leaving in a desiccator for several days, are diluted in distilled water and made up to one litre. 1 ml of this solution contains 1 mg Cl^-.

3.2. Silver nitrate titrating solution: 4,7912 g of analytical grade silver nitrate, $AgNO_3$ are diluted in a 10 % (v/v) alcohol solution and made up to one litre. 1 ml of this solution corresponds to 1 mg Cl^-.

3.3. Nitric acid, of at least 65 % purity (ρ_{20} = 1,40 g/ml).

4. PROCEDURE

4.1. 5,0 ml of standard chloride solution are measured into a 150 ml cylindrical vessel placed on a magnetic stirrer, diluted with distilled water to approximately 100 ml and acidified with 1,0 ml of nitric acid (at least 65 %). After immersing the electrode, titrate by adding the silver nitrate titrating solution with the microburette, with moderate stirring. Begin by adding 1,00 ml for the first 4 ml and read the corresponding millivolt values. Add the next 2 ml in fractions of 0,20 ml. Finally, continue the addition in fractions of 1 ml until a total of 10 ml has been added. After each addition, wait for approximately 30 seconds before reading the corresponding millivolts. Transfer the values thus obtained onto graph paper against the corresponding millilitres of titrating solution and determine the potential of the equivalence point on the basis of the singular point on the curve obtained.

4.2. 5 ml of the standard chloride solution are measured into a 150 ml cylindrical vessel with 95 ml of distilled water and 1 ml of nitric acid (at least 65 %). Immerse the electrode and titre, whilst stirring, until the potential of the equivalence point is obtained. This determination is repeated until a good degree of agreement in the results is obtained. This check must be carried out before each series of measurements of chlorides in the samples.

4.3. 50 ml of wine for analysis are measured into a 150 ml cylindrical vessel. Add 50 ml of distilled water and 1 ml of nitric acid (at least 65 %) and titrate using the procedure described in 4.2.

5. EXPRESSION OF RESULTS

5.1. **Calculations**

If *n* represents the number of millilitres of silver nitrate titrating solution, the chloride content in the tested liquid is:

$20 \times n$	expressed as milligrams of Cl per litre,
$0,5633 \times n$	expressed as milliequivalents per litre,
$32,9 \times n$	expressed as milligrams of sodium chloride per litre.

5.2. **Repeatability (r):**

r = 1,2 mg Cl per litre

r = 0,03 meg per litre

r = 2,0 mg NaCl per litre

5.3. **Reproducibility (R):**

R = 4,1 mg Cl per litre

R = 0,12 meg per litre

R = 6,8 mg NaCl per litre

6. *Note:*

For very precise determination.

Refer to the complete titration curve obtained during determination of the test liquid with the silver nitrate solution.

(a) Measure 50 ml of the wine to be analysed into a 150 ml cylindrical vessel. Add 50 ml of distilled water and 1 ml of nitric acid (at least 65 %). Titrate using the silver nitrate solution, adding 0,5 ml at a time and recording the corresponding potential in millivolts. Derive from this first titration the approximate volume of silver nitrate solution required.

(b) Recommence determination in the same conditions. Begin by adding 0,5 ml of titrating solution at a time until the volume added is 1,5 to 2 ml less that the volume determined in (a). Hereafter add 0,2 ml at a time. Continue to add the solution beyond the approximately located equivalence point in a symmetrical manner, i.e. by adding 0,2 ml and then 0,5 ml at a time.

The end point of the measurement and the exact volume of silver nitrate consumed are obtained:

— either by drawing the curve and determining the equivalence point,

— or by the following calculation:

$$V = V' + \Delta V_i \frac{\Delta\Delta E_1}{\Delta\Delta E_1 + \Delta\Delta E_2}$$

Where:

V = volume of titrating solution at equivalence point;

V' = volume of titrating solution before the largest potential change;

ΔV_i = constant volume of the increments of titrating solution, i.e. 0,2 ml;

$\Delta\Delta E_1$ = second difference in potential before the largest potential change;

$\Delta\Delta E_2$ = second difference in potential after the largest potential change.

▼**B**

Example:

Volume of $AgNO_3$ titrating solution	E potential in mV	Difference Δ E	Second difference ΔΔ E
0	204		
		4	
0,2	208		0
		4	
0,4	212		2
		6	
0,6	218		0
		6	
0,8	224		0
		6	
1,0	230		2
		8	
1,2	238		4
		12	
1,4	250		10
		22	
1,6	272		22
		44	
1,8	316		10
		34	
2,0	350		8
		26	
2,2	376		6
		20	
2,4	396		

In this example, the end point of the titration is between 1,6 and 1,8 ml: the largest potential change (Δ *E* = 44 mV) occurs in this interval. The volume of silver nitrate titrating solution consumed to measure the chlorides in the test sample is:

$$V = 1,6 + 0,2 \, \frac{22}{22 + 10} = 1,74 \text{ ml}$$

▼B

12. SULPHATES

1. PRINCIPLE

1.1. **Reference method**

Precipitation of barium sulphate and weighing. The barium phosphate precipitated in the same conditions is eliminated by washing the precipitate in hydrochloric acid.

In the case of musts or wine rich in sulphur dioxide, prior desulphiting by boiling in an airtight vessel is recommended.

1.2. **Quick test method**

Wines are classified into several categories using the so-called limits method, based on the precipitation of barium sulphate using a barium ion titrant.

2. REFERENCE METHOD

2.1. **Reagents**

2.1.1. 2 M solution of hydrochloric acid.

2.1.2. Barium chloride solution of 200 g/l of $BaCl_2 \cdot 2H_2O$.

2.2. **Procedure**

2.2.1. *General procedure:*

Measure 40 ml of the analysis sample into a 50 ml centrifuge tube; add 2 ml of 2 M hydrochloric acid and 2 ml of barium chloride solution at 200 g/l. Stir with a glass stirrer; rinse the stirrer with a little distilled water and leave to stand for five minutes. Centrifuge for five minutes, then carefully decant the supernatant liquid.

Next wash the barium sulphate precipitate as follows: add 10 ml of 2 M hydrochloric acid, place the precipitate in suspension and centrifuge for five minutes, then carefully decant the supernatant liquid. Repeat the washing procedure twice in the same conditions using 15 ml distilled water each time.

Quantitatively transfer the precipitate, by rinsing with distilled water, into a tared platinum capsule and place over a water bath at 100 ºC until fully evaporated. The dried precipitate is calcined several times briefly over a flame until a white residue is obtained. Leave to cool in a desiccator and weigh.

Let m = the mass in milligrams of barium sulphate obtained.

2.2.2. *Special procedure:* sulphited must and wine with a high sulphur dioxide content.

Beforehand, eliminate the sulphur dioxide.

Measure 25 ml of water and 1 ml of pure hydrochloric acid (ρ_{20} = 1,15 to 1,18 g/ml) into a 500 ml conical flask equipped with a dropping funnel and an outlet tube. Boil the solution to remove the air and introduce 100 ml of wine through the dropping funnel. Continue boiling until the volume of liquid in the flask has been reduced to about 75 ml and quantitatively transfer it, after cooling, to a 100 ml volumetric flask. Make up to mark with water. Determine the sulphates in a 40 ml sample as indicated in 2.2.1.

2.3. **Expression of results**

2.3.1. *Calculations:*

The sulphate content, expressed in milligrams per litre of potassium sulphate, K_2SO_4 is:

$18{,}67 \times m$

The sulphate content in musts or wine is expressed in milligrams per litre of potassium sulphate, with no decimal point.

2.3.2. *Repeatability*

up to 1 000 mg/l: r = 27 mg/l

▼**B**

about 1 500 mg/l: $r = 41$ mg/l

2.3.3. *Reproducibility*

up to 1 000 mg/l: $R = 51$ mg/l

about 1 500 mg/l: $R = 81$ mg/l

▼**M9**

▼B

13. **TOTAL ACIDITY**

1. DEFINITION

The total acidity of the wine is the sum of its titratable 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

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

3. REAGENTS

3.1. Buffer solution pH 7,0:

— monopotassium phosphate, (KH_2PO_4)	107,3	g
— 1 M sodium hydroxide (NaOH) solution	500	ml
— water to	1 000	ml

Alternatively, ready made buffer solutions are available commercially.

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

3.3. 4 g/l bromothymol blue indicator solution:

— 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)	7,5	ml approximately
— water to	1 000	ml

4. APPARATUS

4.1. Water vacuum pump.

4.2. 500 ml vacuum flask.

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

4.4. Measuring cylinders 50 ml (wine), 100 ml (rectified concentrated must).

5. PROCEDURE

5.1. **Preparation of sample:**

5.1.1. *Wines*

Elimination of carbon dioxide. Place about 50 ml of wine in a vacuum flask; apply vacuum to the flask with the water pump for one to two minutes, whilst shaking continuously.

5.1.2. *Rectified concentrated musts*

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. *Calibration of pH meter*

The pH meter is now calibrated for use at 20 ºC, according to the manufacturer's instructions, with the pH 7,00 buffer solution at 20 ºC.

5.2.2. *Method of measurement*

Into a measuring cylinder (4.4), introduce a volume of the sample, prepared as described in 5.1, equal to 10 ml in the case of wine and 50 ml in the case of rectified concentrated must. 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 ºC. 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 measuring cylinder (4.4) place 25 ml of boiled distilled water, 1 ml of bromothymol blue solution (3.3) and a volume prepared as in (5.1) equal to 10 ml in the case of wine and 50 ml in the case of rectified concentrated must. 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 (3.7).

5.3.2. *Measurement*

Into a measuring cylinder (4.4) place 30 ml of boiled distilled water, 1 ml of bromothymol blue solution (3.3) and a volume of the sample, prepared as described in 5.1, equal to 10 ml in the case of wine and 50 ml in the case of rectified concentrated must. 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**

6.1.1. *Wines*

The total acidity expressed in milliequivalents per litre is given by:

$A = 10n$.

It is recorded to one decimal place

The total acidity expressed in grams of tartaric acid per litre is given by:

$A' = 0{,}075A$

It is recorded to one decimal place.

6.1.2. *Rectified concentrated musts*

- The total acidity expressed in milliequivalents per kilogram of rectified concentrated must is given by $a = 5n$.
- The total acidity expressed in milliequivalents per kilogram of total sugars is given by

$$A = \frac{500 \times n}{P}$$

P = % concentration (m/m) of total sugars.

It is recorded to one decimal place.

6.2. **Repeatability (r)** for titration with the indicator:

r = 0,9 meq/litre

r = 0,07 g tartaric acid/litre

for white, rosé and red wines.

▼**B**

6.3. **Reproducibility (R)** for titration with the indicator (5.3):

For white and rosé wines:

R = 3,6 meq/litre
R = 0,3 g tartaric acid/litre

For red wines:

R = 5,1 meq/litre
R = 0,4 g tartaric acid/litre

▼B

14. **VOLATILE ACIDITY**

1. DEFINITION

The volatile acidity is formed from the acids of the acetic series present in wine in the free state and combined as a salt.

2. PRINCIPLE OF THE METHOD

Titration of the volatile acids separated from the wine by steam distillation and titration of the distillate.

Carbon dioxide is first removed from the wine.

The acidity of free and combined sulphur dioxide distilled under these conditions should be deducted from the acidity of the distillate.

The acidity of any sorbic acid which may have been added to the wine must also be deducted.

Note:

Part of the salicylic acid used in some countries to stabilize the wines before analysis is present in the distillate. This must be determined and deducted from the acidity. The method of determination is given in section 7 of this chapter.

3. REAGENTS

3.1. Crystalline tartaric acid ($C_4H_6O_6$).

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

3.3. 1 % phenolphthalein solution in 96 % vol neutral alcohol.

3.4. Hydrochloric acid (ρ_{20} = 1,18 to 1,19 g/ml) diluted 1/4 (v/v).

3.5. 0,005 M iodine (I_2) solution.

3.6. Crystalline potassium iodide (KI).

3.7. 5 g/l starch solution.

Mix 5 g of starch with about 500 ml of water. Bring to the boil, stirring continuously and boil for 10 minutes. Add 200 g sodium chloride. When cool, make up to one litre.

3.8. Saturated solution of sodium borate ($Na_2B_4O_7 \cdot 10H_2O$), i.e. about 55 g/l at 20 ºC.

4. APPARATUS

4.1. Steam distillation apparatus consisting of:

1. a steam generator; the steam must be free of carbon dioxide;
2. a flask with steam pipe;
3. a distillation column;
4. a condenser.

   This equipment must pass the following three tests:

   (a) Place 20 ml of boiled water in the flask. Collect 250 ml of the distillate and add to it 0,1 ml of 0,1 M sodium hydroxide solution (3.2) and two drops of the phenolphthalein solution (3.3). The pink colouration must be stable for at least 10 seconds (i.e. steam to be free of carbon dioxide).

   (b) Place 20 ml of a 0,1 M acetic acid solution in the flask. Collect 250 ml of the distillate. Titrate with the 0,1 M sodium hydroxide solution (3.2): the volume ofthis used must be at least 19,9 ml (i.e. at least 99,5 % of the acetic acid entrained with the steam).

   (c) Place 20 ml of 1 M lactic acid solution in the flask. Collect 250 ml of the distillate and titrate the acid with the 0,1 M sodium hydroxide solution (3.2).

   The volume of sodium hydroxide solution added must be less than or equal to 1,0 ml (i.e. not more than 0,5 % of lactic acid is distilled).

   Any apparatus or procedure which passes these tests satisfactorily fulfils the requirements of official international apparatus or procedures.

▼B

4.2. Water pump.

4.3. Vacuum flask.

5. PROCEDURE

5.1. **Preparation of sample:** elimination of carbon dioxide. Place about 50 ml of wine in a vacuum flask; apply vacuum to the flask with the water pump for one to two minutes, shaking continuously.

5.2. Steam distillation

Place 20 ml of wine, freed from carbon dioxide as in 5.1, in the flask. Add about 0,5 g of tartaric acid (3.1). Collect at least 250 ml of the distillate.

5.3. Titration

Titrate with the 0,1 M sodium hydroxide solution (3.2) using two drops of phenolphthalein (3.3) as indicator. Let n ml be the volume of sodium hydroxide used.

Add four drops of 1/4 dilute hydrochloric acid (3.4), 2 ml starch solution (3.3) and a few crystals of potassium iodide (3.6). Titrate the free sulphur dioxide with the 0,005 M iodine solution (3.5). Let n'' ml be the volume used.

Add the saturated sodium borate solution (3.8) until the pink coloration reappears. Titrate the combined sulphur dioxide with the 0,005 M iodine solution (3.5). Let n'' ml be the volume used.

6. EXPRESSION OF RESULTS

6.1. Method of calculation

The volatile acidity, expressed in milliequivalents per litre to one decimal place, is given by:

$\mathbf{A} = 5\ (n - 0{,}1\ n' - 0{,}05\ n'')$.

The volatile acidity, expressed in grams of acetic acid per litre to two decimal places, is given by:

$0{,}300\ (n - 0{,}1\ n' - 0{,}05\ n'')$.

6.2. Repeatability (r)

r = 0,7 meq/litre

r = 0,04 g acetic acid/litre.

6.3. Reproducibility (R)

R = 1,3 meq/litre

R = 0,08 g acetic acid/litre.

6.4. Wine with sorbic acid present

Since 96 % of sorbic acid is steam distilled with a distillate volume of 250 ml, its acidity must be deducted from the volatile acidity, knowing that 100 mg of sorbic acid corresponds to an acidity of 0,89 milliequivalents or 0,053 g of acetic acid and knowing the concentration of sorbic acid in mg/l as determined by other methods.

7. DETERMINATION OF SALICYLIC ACID ENTRAINED IN THE DISTILLATE FROM THE VOLATILE ACIDITY

7.1. Principle

After the determination of the volatile acidity and the correction for sulphur dioxide, the presence of salicylic acid is indicated, after acidification, by the violet colouration that appears when an iron (III) salt is added.

The determination of the salicylic acid entrained in the distillate with the volatile acidity is carried out on a second distillate having the same volume as that on which the determination of volatile acidity was carried out. In this distillate, the salicylic acid is determined by

▼**B**

a comparative colorimetric method. It is deducted from the acidity of the volatile acidity distillate.

7.2. Reagents

7.2.1. Hydrochloric acid (HCl) (ρ_{20} = 1,18 to 1,19 g/l).

7.2.2. Sodium thiosulphate, ($Na_2S_2O_3 \cdot 5H_2O$) in a 0,1 M solution.

7.2.3. 10 % (m/v) solution of iron (III) ammonium sulphate ($Fe_2(SO_4)_3 \cdot (NH_4)_2SO_4 \cdot 24H_2O$).

7.2.4. 0,01 M solution of sodium salicylate.

Solution containing 1,60 g/l of sodium salicylate ($NaC_7H_5O_3$).

7.3. Procedure

7.3.1. *Identification of salicylic acid in the volatile acidity distillate.*

Immediately after the determination of the volatile acidity and the correction for free and combined sulphur dioxide, introduce into a conical flask 0,5 ml hydrochloric acid (7.2.1), 3 ml of the 0,1 M sodium thiosulphate solution (7.2.2) and 1 ml of the iron (III) ammonium sulphate solution (7.2.3).

If salicylic acid is present, a violet coloration appears.

7.3.2. *Determination of salicylic acid*

On the above conical flask, indicate the volume of the distillate by a reference mark. Empty and rinse the flask.

Subject a new test sample of 20 ml wine to steam distillation and collect the distillate in the conical flask up to the reference mark. Add 0,3 ml pure hydrochloric acid (7.2.1), and 1 ml of the iron (III) ammonium sulphate solution (7.2.3). The contents of the conical flask turn violet.

Into a conical flask identical to that carrying the reference mark, introduce distilled water up to the same level as that of the distillate. Add 0,3 ml pure hydrochloric acid (7.2.1) and 1 ml of the iron (III) ammonium sulphate solution (7.2.3). From the burette run in the 0,01 M sodium salicylate solution (7.2.4) until the violet coloration obtained has the same intensity as that of the conical flask containing the wine distillate.

Let n'' ml be the volume of solution added from the burette.

7.3.3. *Correction to the volatile acidity*

Subtract the volume 0,1 × n''' ml from the volume n ml of 0,1 M sodium hydroxide solution used to titrate the acidity of the distillate during the determination of volatile acidity.

▼**B**

15. **FIXED ACIDITY**

1. PRINCIPLE

The fixed acidity is calculated from the difference between total acidity and volatile acidity.

2. EXPRESSION OF RESULTS

The fixed acidity is expressed in:

— milliequivalents per litre,
— grams of tartaric acid per litre.

▼B

16. **TARTARIC ACID**

1. PRINCIPLE OF METHODS

1.1. **Reference method**

Tartaric acid is precipitated in the form of calcium (±)tartrate and determined gravimetrically. This determination may be completed by a volumetric procedure for comparison. The conditions for precipitation (pH, total volume used, concentrations of precipitating ions) are such that precipitation of the calcium (±)tartrate is complete whereas the calcium D(−) tartrate remains in solution.

When mesotartaric acid has been added to the wine, which causes the precipitation of the calcium (±)tartrate to be incomplete, it must first be hydrolysed.

1.2. **Usual method**

The tartaric acid, separated using an ion exchange column, is determined colorimetrically in the eluate by measurement of the red colour produced on reaction with vanadic acid. The eluate also contains lactic and malic acids which do not interfere.

2. REFERENCE METHOD

2.1. **Gravimetric method**

2.1.1. *Reagents*

2.1.1.1. Calcium acetate solution containing 10 g of calcium per litre:

calcium carbonate ($CaCO_3$)	25 g
acetic acid glacial (CH_3COOH) (ρ_{20} = 1,05 g/ml)	40 ml
water to	1 litre

2.1.1.2. Calcium (±)tartrate, crystallized: $CaC_4O_6H_4 \cdot 4H_2O$:

Place 20 ml of L(+) tartaric acid solution (5 g/l) into a 400 ml beaker. Add 20 ml of ammonium D(−) tartrate solution (6,126 g/l) and 6 ml of calcium acetate solution containing 10 g of calcium per litre (2.1.1.1).

Allow to stand for two hours to precipitate. Collect the precipitate in a sintered glass crucible of porosity No 4, and wash it three times with about 30 ml of distilled water. Dry to constant weight in the oven at 70 ºC. Using the quantities of reagent indicated above, about 340 mg of crystallized calcium (±)tartrate is obtained.

Store in a stoppered bottle.

2.1.1.3. Precipitation solution (pH 4,75):

— D(−) tartaric acid	122 mg
— 25 % (v/v) ammonium hydroxide solution (ρ_{20} = 0,97 g/ml)	0,3 ml
— calcium acetate solution (10 g calcium/litre)	8,8 ml
— water to	1 000 ml

Dissolve the D(−) tartaric acid, add the ammonium hydroxide and make up to about 900 ml; add 8,8 ml of calcium acetate solution (2.1.1), make up to a litre and adjust the pH to 4,75 with acetic acid. Since calcium (±)tartrate is slightly soluble in this solution, add 5 mg of calcium (±)tartrate per litre, stir for 12 hours and filter.

2.1.2. *Procedure*

2.1.2.1. Wines with no added mesotartaric acid

Place 500 ml of precipitation solution and 10 ml of wine into a 600 ml beaker. Mix and initiate precipitation by rubbing the sides of the vessel with the tip of a glass rod. Leave to precipitate for 12 hours (overnight).

▼B

Filter the liquid and precipitate through a weighed sintered glass crucible of porosity No 4 fitted on a clean vacuum flask. Rinse the vessel in which precipitation took place with the filtrate to ensure that all precipitate is transferred.

Dry to constant weight in an oven at 70 ºC. Weigh. Let *p* be the weight of crystallized calcium (±)tartrate ($CaC_4O_6H_4 \cdot 4H_2O$) obtained.

2.1.2.2. Wines to which mesotartaric acid has been added

When analysing wines to which mesotartaric acid has been or is suspected of having been added, proceed by first hydrolysing this acid as follows:

Place 10 ml of wine and 0,4 ml of glacial acetic acid (CH_3COOH, ρ_{20} = 1,05 g/ml) into a 50-ml conical flask. Place a reflux condenser on top of the flask and boil for 30 minutes. Allow to cool and then transfer the solution in the conical flask to a 600-ml beaker. Rinse the flask twice using 5 ml of water each time and then continue as described above.

Mesotartaric acid is calculated and included as tartaric acid in the final result.

2.1.3. *Expression of results*

One molecule of calcium (±)tartrate corresponds to half a molecule of L(+) tartaric acid in the wine.

The quantity of tartaric acid per litre of wine, expressed in milliequivalents is equal to 384,5 *p*.

It is quoted to one decimal place.

The quantity of tartaric acid per litre of wine, expressed in grams of tartaric acid is equal to 28,84 *p*.

It is quoted to one decimal place.

The quantity of tartaric acid per litre of wine, expressed in grams of potassium acid tartrate is equal to 36,15 *p*.

It is quoted to one decimal place.

2.2. **Comparative volumetric analysis**

2.2.1. *Reagents*

2.2.1.1. Hydrochloric acid (HCl) (1:5 v/v) (ρ_{20} = 1,18 to 1,19 g/ml)

2.2.1.2. EDTA solution, 0,05 M:

EDTA (ethylenediaminetetraacetic acid disodium salt:		
($C_{10}H_{14}N_2O_8Na_2 \cdot 2H_2O$)	18,61	g
distilled water to	1 000	ml

2.2.1.3. Sodium hydroxide solution, 40 % (m/v):

sodium hydroxide (NaOH)	40	g
distilled water to	100	ml

2.2.1.4. Complexometric indicator: 1 % (m/m)

2-hydroxy-1-(2-hydroxy-4-sulpho-1-naphthylazo)-3-naphthoic acid ($C_{21}H_{14}N_2O_7S \cdot 3H_2O$)	1	g
anhydrous sodium sulphate (Na_2SO_4)	100	g

2.2.2. *Procedure*

After weighing, replace the sintered glass crucible containing the precipitate of calcium (±)tartrate on the vacuum flask and dissolve the precipitate with 10 ml of dilute hydrochloric acid (2.2.1.1). Wash the sintered glass crucible with 50 ml of distilled water.

▼**B**

Add 5 ml of 40 % sodium hydroxide solution (2.2.1.3) and about 30 mg of indicator (2.2.1.4). Titrate with 0,05 M EDTA (2.2.1.2). Let the number of ml used be *n*.

2.2.3. *Expression of results*

The quantity of tartaric acid per litre of wine, expressed in milliequivalents is equal to 5 *n*.

It is quoted to one decimal place.

The quantity of tartaric acid per litre of wine, expressed in grams of tartaric acid is equal to 0,375 *n*.

It is quoted to one decimal place.

The quantity of tartaric acid per litre of wine, expressed in grams of potassium acid tartrate is equal to 0,470 *n*.

It is quoted to one decimal place.

▼**M9**

▼B

17. **CITRIC ACID**

1. PRINCIPLE OF THE METHOD

Citric acid is converted into oxaloacetate and acetate in a reaction catalysed by citrate-lyase (CL):

$$\text{citrate} \xrightleftharpoons{\text{CL}} \text{oxaloacetate} + \text{acetate}$$

In the presence of malate dehydrogenase (MDH) and lactate dehydrogenase (LDH), the oxaloacetate and its decarboxylation derivative, pyruvate, are reduced to L-malate and L-lactate by reduced nicotinamide adenine dinucleotide (NADH):

$$\text{oxaloacetate} + \text{NADH} + \text{H}^+ \xrightleftharpoons{\text{MDH}} \text{L-malate} + \text{NAD}^+$$

$$\text{pyruvate} + \text{NADH} + \text{H}^+ \xrightleftharpoons{\text{LDH}} \text{L-lactate} + \text{NAD}^+$$

The amount of NADH oxidized to NAD^+ in these reactions is proportional to the amount of citrate present. The oxidation of NADH is measured by the resultant decrease in absorbence at a wavelength of 340 nm.

2. REAGENTS

2.1. Buffer solution pH 7,8.

(0,51 M glycylglycine; pH 7,8; Zn^{2+} ($0{,}6 \times 10^{-3}$M):

dissolve 7,13 g of glycylglycine in approximately 70 ml of doubly distilled water.

Adjust the pH to 7,8 with approximately 13 ml of 5 M sodium hydroxide solution, add 10 ml of zinc chloride ($ZnCl_2$ 80 mg in 100 ml H_2O) solution and make up to 100 ml with doubly distilled water.

2.2. Reduced nicotinamide adenine dinucleotide (NADH) solution (approximately 6×10^{-3} M): dissolve 30 mg NADH and 60 mg $NaHCO_3$ in 6 ml of doubly distilled water.

2.3. Malate dehydrogenase/lactate dehydrogenase solution (MDH/LDH, 0,5 mg MDH/ml, 2,5 mg LDH/ml): mix together 0,1 ml MDH (5 mg MDH/ml), 0,4 ml ammonium sulphate solution (3,2 M and 0,5 ml LDH (5 mg/ml). This suspension remains stable for at least a year at 4 °C.

2.4. Citrate-lyase (CL, 5 mg protein/ml): dissolve 168 mg lyophilisate in 1 ml ice-cold water. This solution remains stable for at least a week at 4 °C and for at least four weeks if frozen.

It is recommended that, prior to the determination, the enzyme activity should be checked.

2.5. Polyvinylpolypyrrolidone (PVPP)

Note:

All the reagents above are available commercially.

3. APPARATUS

3.1. A spectrophotometer permitting measurement to be made at 340 nm, the wavelength at which absorption by NADH is at a maximum.

Failing that, a spectrophotometer, with a discontinuous spectrum source permitting measurements to be made at 334 nm or 365 nm, may be used.

Since absolute absorbence measurements are involved (i.e. calibration curves are not used but standardization is made by consideration of the extinction coefficient of NADH), the wavelength scales and spectral absorbence of the apparatus must be checked.

3.2. Glass cells with optical path lengths of 1 cm or single-use cells.

3.3. Micropipettes for pipetting volumes in the range 0,02 to 2 ml.

▼B

4. PREPARATION OF THE SAMPLE

Citrate determination is normally carried out directly on the wine, without preliminary removal of pigmentation (colouration) and without dilution provided that the citric acid content is less than 400 mg/l. If this is not so, dilute the wine until the citrate concentration lies between 20 and 400 mg/l (i.e. between 5 and 80 µg of citrate in the test sample).

With red wines that are rich in phenolic compounds, preliminary treatment with PVPP is recommended:

Form a suspension of about 0,2 g of PVPP in water and allow to stand for 15 minutes. Filter using a fluted filter.

Place 10 ml of wine in a 50 ml conical flask, add the moist PVPP removed from the filter with a spatula. Shake for two to three minutes. Filter.

5. PROCEDURE

With the spectrophotometer adjusted to a wavelength of 340 nm, determine the absorbence using the 1 cm cells, using air as the zero absorbence (reference) standard (no cell in the optical path). Place the following in the 1 cm cells:

	Reference cell (ml)	Sample cell (ml)
Solution 2.1	1,00	1,00
Solution 2.2	0,10	0,10
Sample to be measured	-	0,20
Doubly distilled water	2,00	1,80
Solution 2.3	0,02	0,02

Mix, and after about five minutes read the absorbence of the solutions in the reference and sample cells (A_1).

Add:

Solution 2.4	0,02 ml	0,02 ml

Mix; wait until the reaction is completed (about five minutes) and read the absorbences of the solutions in the reference and sample cells (A_2).

Calculate the absorbence difference ($A_2 - A_1$) for the reference and sample cells, ΔA_R and ΔA_S.

Finally, calculate the difference between those differences:

$\Delta A = \Delta A_s - \Delta A_R$

Note:

The time needed for the completion of enzyme activity can vary from one batch to another. The above value is given only for guidance and it is recommended that it be determined for each batch.

6. EXPRESSION OF RESULTS

Citric acid concentration is given in milligrams per litre to the nearest whole number.

6.1. **Method of calculation**

The general formula for calculating the concentration in mg/l is:

$$C = \frac{V \times M}{\varepsilon \times d \times v} \times \Delta A$$

where V = volume of test solution in ml (here 3,14 ml)

v = volume of the sample in ml (here 0,2 ml)

M = molecular mass of the substance to be determined

▼**B**

(here, for anhydrous citric acid, $M = 192,1$)

d = optical path in the cell in cm (here, 1 cm)

ε = absorption coefficient of NADH, (at 340 nm, $\varepsilon = 6,3 \text{ mmol}^{-1} \times 1 \times \text{cm}^{-1}$),

so that

$C = 479 \times \Delta A$

If the sample was diluted during its preparation, multiply the result by the dilution factor.

Note:

At 334 nm: $C = 488 \times \Delta A$ (= $6,2 \text{ m mol}^{-1} \times 1 \times \text{cm}^{-1}$).

At 365 nm: $C = 887 \times \Delta A$ (= $3,4 \text{ m mol}^{-1} \times 1 \times \text{cm}^{-1}$).

6.2. Repeatability (r)

Citric acid concentration less than 400 mg/l: $r = 14$ mg/l.

Citric acid concentration greater than 400 mg/l: $r = 28$ mg/l.

6.3. Reproducibility (R)

Citric acid concentration less than 400 mg/l: $R = 39$ mg/l.

Citric acid concentration greater than 400 mg/l: $R = 65$ mg/l.

▼B

18. **LACTIC ACID**

1. PRINCIPLE OF THE METHOD

Total lactic acid (L-lactate and D-lactate) is oxidized by nicotinamide adenine dinucleotide (NAD) to pyruvate in a reaction catalysed by L-lactate dehydrogenase (L-LDH) and D-lactate dehydrogenase (D-LDH).

The equilibrium of the reaction normally lies more strongly in favour of the lactate. Removal of the pyruvate from the reaction mixture displaces the equilibrium towards the formation of pyruvate.

In the presence of L-glutamate, the pyruvate is transformed into L-alanine in a reaction catalysed by glutamate pyruvate transaminase (GPT):

(1) $\text{L-lactate} + \text{NAD}^+ \xrightleftharpoons{\text{L-LDH}} \text{pyruvate} + \text{NADH} + \text{H}^+$

(2) $\text{D-lactate} + \text{NAD}^+ \xrightleftharpoons{\text{D-LDH}} \text{pyruvate} + \text{NADH} + \text{H}^+$

(3) $\text{Pyruvate} + \text{L-glutamate} \xrightleftharpoons{\text{GPT}} \text{L-alanine} + \alpha\text{-ketoglutarate}$

The amount of NADH formed, measured by the increase in absorbence at the wavelength of 340 nm, is proportional to the quantity of lactate originally present.

Note:

L-lactic acid may be determined independently by using reactions (1) and (3), while D-lactic acid may be similarly determined by using reactions (2) and (3).

1.2. **Usual method**

The lactic acid, separated by passage through an ion exchange resin column, is oxidized to ethanal and determined by colorimetry after reacting with sodium nitroprusside and piperidine.

2. REFERENCE METHOD

2.1. **Reagents**

2.1.1. Buffer solution, pH 10 (glycylglycine 0,6 mol/l; L-glutamate 0,1 mol/l):

dissolve 4,75 g of glycylglycine and 0,88 g of L-glutamic acid in approximately 50 ml of doubly distilled water; adjust the pH to 10 with a few millilitres of 10 M sodium hydroxide and make up to 60 ml with doubly distilled water.

This solution will remain stable for at least 12 weeks at 4 °C.

2.1.2. Nicotinamide adenine dinucleotide (NAD) solution, approximately 40×10^{-3} M: dissolve 900 mg of NAD in 30 ml of doubly distilled water. This solution will remain stable for at least four weeks at 4 °C.

2.1.3. Glutamate pyruvate transaminase (GPT) suspension, 20 mg/ml. The suspension remains stable for at least a year at 4 °C.

2.1.4. L-lactate dehydrogenase (L-LDH) suspension, 5 mg/ml. This suspension remains stable for at least a year at 4 °C.

2.1.5. D-lactate dehydrogenase (D-LDH) suspension, 5 mg/ml. This suspension remains stable for at least a year at 4 °C.

It is recommended that, prior to the determination, the enzyme activity should be checked.

Note:

All the reagents are available commercially.

2.2. **Apparatus**

2.2.1. A spectrophotometer permitting measurements to be made at 340 nm, the wavelength at which absorption by NADH is at a maximum.

▼B

Failing that, a spectrophotometer with a discontinuous spectrum source permitting measurements to be made at 334 or 365 nm may be used.

Since absolute absorbence measurements are involved (i.e. calibration curves are not used, but standardization is made by consideration of the extinction coefficient of NADH), the wavelength scales and spectral absorbence of the apparatus must be checked.

2.2.2. Glass cells with optical path lengths of 1 cm or single-use cells.

2.2.3. Micropipettes for pipetting sample volumes in the range 0,02 to 2 ml.

2.3. Preparation of the sample

Preliminary note: No part of the glassware that comes into contact with the reaction mixture should be touched with the fingers, since this could introduce L-lactic acid and thus give erroneous results.

Lactate determination is normally carried out directly on the wine, without prior removal of pigmentation (colouration) and without dilution provided that the lactic acid concentration is less than 100 mg/l. If, however, the lactic acid concentration lies between:

— 100 mg/l and 1 g/l, dilute 1/10 with doubly distilled water,
— 1 g/l and 2,5 g/l, dilute 1/25 with doubly distilled water,
— 2,5 g/l and 5 g/l, dilute 1/50 with doubly distilled water.

2.4. Procedure

2.4.1. *Determination of total lactic acid*

The buffer solution must be at a temperature between 20 and 25 ºC before proceeding to the measurement.

With the spectrophotometer adjusted to a wavelength of 340 nm, determine the absorbence using the cells having optical paths of 1 cm, with air as the zero absorbence (reference) standard (no cell in the optical path) or with water as the standard.

Place the following in the cells having 1 cm optical paths:

	Reference cell (ml)	Sample cell (ml)
Solution 2.1.1	1,00	1,00
Solution 2.1.2	0,20	0,20
Doubly distilled water	1,00	0,80
Suspension 2.1.3	0,02	0,02
Sample to be measured	—	0,20

Mix using a glass stirrer or a rod of synthetic material with a flattened end; after about five minutes, measure the absorbences of the solutions in the reference and sample cells (A_1).

Add 0,02 ml of solution 2.1.4 and 0,05 ml of solution 2.1.5, homogenize, wait for the reaction to be completed (about 30 minutes) and measure the absorbences of the solutions in the reference and sample cells (A_2).

Calculate the differences ($A_2 - A_1$) in the absorbences of the solutions in the reference and sample cells, ΔA_R and ΔA_S.

Finally, calculate the difference between those differences:

$\Delta A = \Delta A_S - \Delta A_R$

2.4.2. *Determination of L-lactic acid and D-lactic acid*

Determinations of the L-lactic acid or D-lactic acid can be carried out independently by applying the procedure for total lactic acid up to the determination of A_1 and then continuing as follows:

Add 0,02 ml of solution 2.1.4, homogenize, wait until the reaction is complete (about 20 minutes) and measure the absorbences of the solutions in the reference and sample cells (A_2).

▼B

Add 0,05 ml of solution 2.1.5, homogenize, wait until the reaction is complete (about 30 minutes) and measure the absorbences of the solutions in the reference and sample cells (A_3).

Calculate the differences ($A_2 - A_1$) for L-lactic acid and ($A_3 - A_2$) for D-lactic acid between the absorbences of the solutions in the reference and sample cells, ΔA_R and ΔA_S.

Finally, calculate the difference between those differences:

$\Delta A = \Delta A_S - \Delta A_R$

Note:

The time needed for the completion of enzyme activity can vary from one batch to another. The above value is given only for guidance and it is recommended that it be determined for each batch. When determining the L-lactic acid alone, the incubation time may be reduced to 10 minutes.

2.5. **Expression of results**

Lactic acid concentration is given in grams per litre to one decimal place.

2.5.1. *Method of calculation*

The general formula for calculating the concentration in g/l is:

$$C = \frac{V \times M}{\varepsilon \times d \times v \times 1\,000} \times \Delta A$$

where

V = volume of test solution in ml (V = 2,24 ml for L-lactic acid, V = 2,29 ml for D-lactic acid and total lactic acid)

v = volume of the sample in ml (here 0,2 ml)

M = molecular mass of the substance to be determined (here, for DL-lactic acid, $M = 90{,}08$)

d = optical path in the cell in cm (here, 1 cm)

ε = absorption coefficient of NADH, (at 340 nm, $\varepsilon = 6{,}3$ mmol $^{-1}$ × l × cm $^{-1}$).

2.5.1.1. Total lactic acid and D-lactic acid

$C = 0{,}164 \times \Delta A$

If the sample was diluted during its preparation, multiply the result by the dilution factor.

Note:

Measurement at 334 nm: $C = 0{,}167 \times \Delta A$, ($\varepsilon = 6{,}2$ m mol $^{-1}$ × l × cm $^{-1}$).

Measurement at 365 nm: $C = 0{,}303 \times \Delta A$, ($\varepsilon = 3{,}4$ m mol $^{-1}$ × l × cm $^{-1}$).

2.5.1.2. L-lactic acid

$C = 0{,}160 \times \Delta A$

If the sample was diluted during its preparation, multiply the result by the dilution factor.

Note:

Measurement at 334 nm: $C = 0{,}163\ \Delta A$, ($\varepsilon = 6{,}2$ m mol $^{-1}$ × l × cm $^{-1}$).

Measurement at 365 nm: $C = 0{,}297\ \Delta A$, ($\varepsilon = 3{,}4$ m mol $^{-1}$ × l × cm $^{-1}$).

2.5.2. *Repeatability (r)*

$r = 0{,}02 + 0{,}07x_i$ g/l

x_i is the lactic acid concentration in the sample in g/l.

▼**B**

2.5.3. *Reproducibility (R)*

$R = 0{,}05 + 0{,}125x_i$ g/l

x_i is the lactic acid concentration in the sample in g/l.

▼**M9**

▼B

19. L-MALIC ACID

1. PRINCIPLE OF THE METHOD

L-malic acid (L-malate) is oxidized by nicotinamide adenine dinucleotide (NAD) to oxaloacetate in a reaction catalysed by L-malate dehydrogenase (L-MDH).

The equilibrium of the reaction normally lies more strongly in favour of the malate. Removal of the oxaloacetate from the reaction mixture displaces the equilibrium towards the formation of oxaloacetate. In the presence of L-glutamate, the oxaloacetate is transformed into L-aspartate in a reaction catalysed by glutamate oxaloacetate transaminase (GOT):

(1) $\text{L-malate} + \text{NAD}^{+} \xrightleftharpoons{\text{L-MDH}} \text{oxaloacetate} + \text{NADH} + \text{H}^{+}$

(2) $\text{Oxaloacetate} + \text{L-glutamate} \xrightleftharpoons{\text{GOT}} \text{L-aspartate} + \alpha\text{-ketoglutarate}$

The amount of NADH formed, measured by the increase in absorbence at the wavelength of 340 nm, is proportional to the quantity of L-malate originally present.

2. REAGENTS

2.1. Buffer solution, pH 10

(glycylglycine 0,6 M; L-glutamate 0,1 M):

dissolve 4,75 g of glycylglycine and 0,88 g of L-glutamic acid in approximately 50 ml of doubly distilled water; adjust the pH to 10 with about 4,6 ml of 10 M sodium hydroxide and make up to 60 ml with doubly distilled water.

This solution will remain stable for at least 12 weeks at 4 °C.

2.2. Nicotinamide adenine dinucleotide (NAD) solution, approximately 47×10^{-3} M:dissolve 420 mg of NAD in 12 ml of doubly distilled water. This solution will remain stable for at least four weeks at 4 °C.

2.3. Glutamate oxaloacetate transaminase (GOT) suspension, 2 mg/ml. The suspension remains stable for at least a year at 4 °C.

2.4. L-malate dehydrogenase (L-MDH) solution, 5 mg/ml. This solution remains stable for at least a year at 4 °C.

Note:

All the reagents above are available commercially.

3. APPARATUS

3.1. A spectrophotometer permitting measurement to be made at 340 nm, the wavelength at which absorption by NADH is at a maximum.

Failing that, a spectrophotometer, with a discontinuous spectrum source permitting measurements to be made at 334 or 365 nm, may be used.

Since absolute measurements of absorbence are involved (i.e. calibration curves are not used, but standardization is made by consideration of the extinction coefficient of NADH), the wavelength scales and spectral absorbence of the apparatus must be checked.

3.2. Glass cells with optical path lengths of 1 cm or single-use cells.

3.3. Micropipettes for pipetting sample volumes in the range 0,01 to 2 ml.

4. PREPARATION OF THE SAMPLE

L-malate determination is normally carried out directly on the wine, without prior removal of pigmentation (colouration) and without dilution provided that the L-malic acid concentrationis less than 350 mg/l (measured at 365 mg/l). If this is not so, dilute the wine with doubly distilled water until the L-malate concentration lies between 30 and 350 mg/l (i.e. amount of L-malate in the test sample lies between 3 and 35 µg).

▼B

If the malate concentration in the wine is less than 30 mg/l, the volume of the test sample may be increased up to 1 ml. In this case, the volume of water to be added is reduced in such a way that the total volumes in the two cells are equal.

5. PROCEDURE

With the spectrophotometer adjusted to a wavelength of 340 nm, determine the absorbence using the cells having optical paths of 1 cm, with air as the zero absorbence (reference) standard (no cell in the optical path) or with water as the standard.

Place the following in the cells having 1 cm optical paths:

	Reference cell (ml)	Sample cell (ml)
Solution 2.1	1,00	1,00
Solution 2.2	0,20	0,20
Doubly distilled water	1,00	0,90
Suspension 2.3	0,01	0,01
Sample to be measured	-	0,10

Mix; after about three minutes, measure the absorbences of the solutions in the reference and sample cells (A_1).

Add:

Solution 2.4	0,01 ml	0,01 ml

Mix; wait for the reaction to be completed (about 5 to 10 minutes) and measure the absorbences of the solutions in the reference and sample cells (A_2).

Calculate the differences ($A_2 - A_1$) in the absorbences of the solutions in the reference and sample cells, ΔA_R and ΔA_S.

Finally, calculate the difference between those differences: $\Delta A = \Delta A_S - \Delta A_R$

Note:

The time needed for the completion of enzyme activity can vary from one batch to another. The above value is given only for guidance and it is recommended that it be determined for each batch.

6. EXPRESSION OF RESULTS

L-malic acid concentration is given in grams per litre to one decimal place.

6.1. **Method of calculation**

The general formula for calculating the concentration in g/l is:

$$C = \frac{V \times M}{\varepsilon \times d \times v \times 1\,000} \times \Delta A$$

where

V = volume of test solution in ml (here 2,22 ml)

v = volume of the sample in ml (here 0,1 ml)

M = molecular mass of the substance to be determined (here, for L-malic acid, $M = 134{,}09$)

d = optical path in the cell in cm (here, 1 cm)

ε = absorption coefficient of NADH, (at 340 nm, $\varepsilon = 6{,}3$ m mol^{-1} × 1 × cm^{-1}),

so that for L-malate:

$C = 0{,}473 \times \Delta A$ g/l

▼**B**

If the sample was diluted during its preparation, multiply the result by the dilution factor.

Note:

Measurement at 334 nm: $C = 0{,}482 \times \Delta A$

Measurement at 365 nm: $C = 0{,}876 \times \Delta A$

6.2. **Repeatability (r)**

$r = 0{,}03 + 0{,}034x_i$

x_i is the malic acid concentration in the sample in g/l.

6.3. **Reproducibility (R)**

$R = 0{,}05 + 0{,}071x_i$

x_i is the malic acid concentration in the sample in g/l.

▼**M7**

20. **D-MALIC ACID**

(enzymatic method)

1. PRINCIPLE

In the presence of D-malate dehydrogenase (D-MDH), D-malic acid (D-malate) is oxidised by nicotinamide adenine dinucleotide (NAD) to oxaloacetate. The oxaloacetate formed is split into pyruvate and carbon dioxide.

$$\text{D-malate} + \text{NAD}^+ \overset{\text{D-MDH}}{\rightleftharpoons} \text{pyruvate} + \text{CO}_2 + \text{NADH} + \text{H}^+$$

The quantity of NADH formed is proportional to the concentration of D-malic acid and is measured at a wavelength of 334, 340 or 365 nm.

2. REAGENTS

Test combination for approximately 30 determinations:

(a) Bottle 1 with about 30 ml of solution consisting of Hepes buffer [N-(2-hydroxyethyl)piperazine-N′-2-ethanesulfonic acid] pH = 9,0 and stabilisers;

(b) Bottle 2 with about 210 mg of NAD lyophilisate;

(c) Three bottles 3 with D-MDH lyophilisate, about 8 U each.

Preparation of the solutions

1. Use content of bottle 1 undiluted. Before using bring solution to 20 to 25 ºC.

2. Dissolve content of bottle 2 in 4 ml double-distilled water.

3. Dissolve content of one of bottles 3 in 0,6 ml double-distilled water. Before using bring solution to 20 to 25 ºC.

Stability of the solutions

The content of bottle 1 is stable for at least one year if stored at +4 ºC; solution 2 is stable for three weeks if stored at +4 ºC, and for two months if stored at − 20 ºC; solution 3 is stable for five days if stored at +4 ºC.

3. APPARATUS

3.1. A spectrophotometer permitting measurement to be made at 340 nm, the wavelength at which NADH absorption is at a maximum. Failing that, a spectrophotometer with a discontinuous spectrum source permitting measurements to be made at 334 or 365 nm may be used. Since absolute absorbance measurements are involved (i.e. no set of calibration solutions but reference to the extinction coefficient of NADH), the wavelength scales and spectral absorbance of the apparatus must be checked.

3.2. Glass cuvettes with optical path lengths of 1 cm (if preferred disposable cuvettes may be used).

3.3. Micropipettes for pipetting volumes in the range 0,01 to 2 ml.

4. PREPARATION OF THE SAMPLE

D-malate analysis is normally carried out directly on the wine, without preliminary decolorisation.

The amount of D-malate in the cuvette should be between 2 and 50 µg. The wine therefore must be diluted to yield a D-malate concentration between 0,02 and 0,5 g/l or 0,02 and 0,3 g/l, respectively (depending on the apparatus used).

▼**M7**

Dilution table:

Estimated quantity of D-malate/litre		Dilution with water	Dilution factor F
Measured at:			
340 or 334 nm	365 nm		
< 0,3 g	< 0,5 g	—	1
0,3 - 3,0 g	0,5 - 5,0 g	1 + 9	10

5. PROCEDURE

With the spectrophotometer adjusted to a wavelength of 340 nm, determine the absorbance using the 1 cm cuvettes, either using air to set zero absorbance (no cuvette in the optical path) or using water.

Pipette into the cuvettes:

	Reference	Test
Solution 1	1,00 ml	1,00 ml
Solution 2	0,10 ml	0,10 ml
Double-distilled water	1,80 ml	1,70 ml
Sample for measurement	—	0,10 ml

Mix, and after about six minutes measure the absorbance of the reference and test solutions (A_1).

Add:

	Reference	Test
Solution 3	0,05 ml	0,05 ml

Mix; wait until the reaction is completed (about 20 minutes) and measure the absorbances of the reference and test solutions (A_2).

Calculate the absorbance difference ($A_2 - A_1$) for the reference (ΔA_T) and test (ΔA_E) solutions. Finally, calculate the difference between those differences: $\Delta A = \Delta A_E - \Delta A_T$.

Note:

The time needed for the completion of enzyme activity can vary from one batch to another. The above time is given only for guidance and it is recommended that it be determined for each batch.

D-malic acid reacts rapidly. The enzyme also transforms L-tartaric acid, although very much more slowly. This explains the slight side reaction, which can be corrected by means of extrapolation (see Appendix A).

6. EXPRESSION OF THE RESULTS

The general formula for calculating the concentration in mg/l is:

$$C = \frac{V \times PM}{\varepsilon \times d \times \nu \times \Delta A}$$

where:

V = volume of test solution in ml (2,95 ml)

ν = volume of the sample in ml (0,1 ml)

PM = molecular mass of the substance to be determined (for D-malic acid, PM = 134,09)

▼**M7**

d = optical path of the cuvette in cm (1 cm)

ε = absorption coefficient of NADH:

at 340 nm = 6,3 (1 $mmol^{-1}$ cm^{-1})

at 365 nm = 3,4 (1 $mmol^{-1}$ cm^{-1})

at 334 nm = 6,18 (1 $mmol^{-1}$ cm^{-1}).

If the sample was diluted during its preparation, multiply the result by the dilution factor.

The D-malic acid concentration is given in milligrams per litre (mg/l), with no decimal places.

7. ACCURACY

Details of the interlaboratory trial on the accuracy of the method are summarised in Appendix B. The values derived from the interlaboratory trial may not be applicable to ranges of analyte concentration and matrices other than those in Appendix B.

7.1. **Repeatability**

The absolute difference between two individual results obtained on identical matter submitted to a trial by an operator using the same apparatus, within the shortest time interval, will not exceed repeatability value r in more than 5 % of cases.

r = 11 mg/l.

7.2. **Reproducibility**

The absolute difference between two individual results obtained on identical matter submitted to a trial in two different laboratories will not exceed reproducibility value R in more than 5 % of cases.

R = 20 mg/l.

▼**M8**

8. DOSAGE OF D-MALIC ACID (D(+)-MALIC ACID) IN WINES WITH LOW LEVELS

8.1. **Field of application**

The method described is applied to the dosage, by enzymatic means, of D-malic acid of wines with levels under 50 mg/l.

8.2. **Principle**

The principle of the method is described in point 1. The formation of NADH after the introduction into the cuvette of 50 mg/l of D-malic acid is proportional to the quantity of D-malate present and is measured on the basis of the increase in absorbance at a wavelength of 340 nm.

8.3. **Reagents**

A 0,199 g/l D-malic acid solution plus the reagents indicated in point 2.

8.4. **Apparatus**

Apparatus indicated in point 3.

8.5. **Preparation of the sample**

As indicated in point 4.

8.6. **Procedure**

The procedure is as described in point 5, but with the introduction into the measuring cuvette of 50 mg/l of D-malic acid. (Introduction of 0,025 ml of 0,199 g/l D-malic acid solution, displacing the equivalent volume of water); the values obtained are decreased by 50 mg/l.

▼**M8**

8.7. Internal validation

The table below summarises the internal validation file on the method for determining the dosage of D(+)-malic acid after the addition of 50 mg/l of the isomer.

Working range	0 mg to 70 mg of D-malic acid per litre. Within these limits, the method is linear with a correlation coefficient of between 0,990 and 0,994
Limit of quantification	24,4 mg/l
Limit of detection	8,3 mg/l
Sensitivity	0,0015 abs/mg/l
Recovery ratio	87,5 to 115,0 % for white wines and 75 to 105 % for red wines
Repeatability	= 12,4 mg/l for white wines (according to the OIV method = 12,5 mg/l) = 12,6 mg/l for red wines (according to OIV method = 12,7 mg/l)
Coefficient of variation	4,2 % to 7,6 % (white wines and red wines)
Intralaboratory variability	CV=7,4 % (s = 4,4 mg/l; mean = 59,3 mg/l)

▼M7

Appendix A

How to deal with side reactions

Side reactions are generally due to secondary reactions of the enzyme, to the presence of other enzymes in the sample matrix, or to interaction of one or more elements of the matrix with a co-factor in the enzymatic reaction.

With a normal reaction, absorbance reaches a constant value after a certain time, generally 10 to 20 minutes, depending on the speed of the specific enzymatic reaction. However, when secondary reactions occur, absorbance does not reach a constant value, but increases regularly with time. This type of process is commonly called a 'side reaction'.

When side reaction occurs, the absorbance of the solution should be measured at regular intervals (every two to five minutes) after the required time for the standard solution to reach its final absorbance has elapsed. If the absorbance increases regularly, five or six measurements should be made, and extrapolated back by means of a graph or of calculation, to determine the absorbance that would have been observed when the final enzyme was added (T0). The substrate concentration is calculated on the basis of the difference in absorbance extrapolated at that time (Af − Ai).

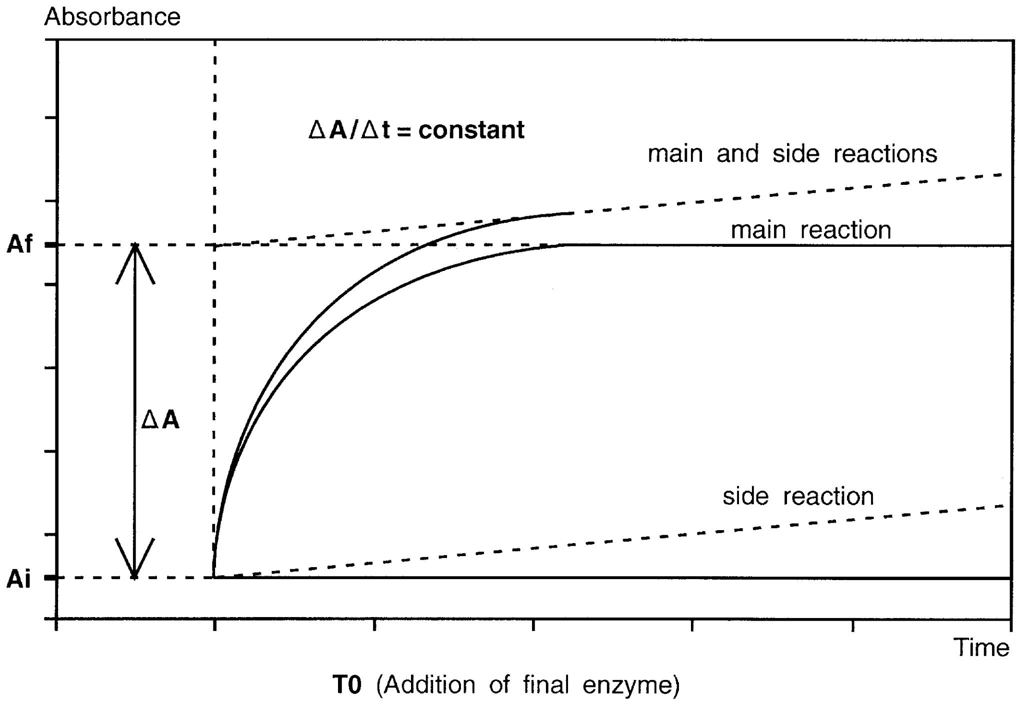


Figure 1. Side reaction

▼**M7**

Appendix B

Statistical results of interlaboratory trial

Year of the interlaboratory trial: 1995
Number of laboratories: 8
Number of samples: 5 with addition of D-malic acid

Sample	A	B	C	D	E
Number of laboratories retained after elimination of laboratories presenting aberrant results	7	8	7	8	7
Number of laboratories presenting aberrant results	1	—	1	—	1
Number of results accepted	35	41	35	41	36
Average value ($\bar{\chi}$) (mg/l)	161,7	65,9	33,1	106,9	111,0
Standard deviation of repeatability (s_r) (mg/l)	4,53	4,24	1,93	4,36	4,47
Relative standard deviation of repeatability (RSD_r) (%)	2,8	6,4	5,8	4,1	4,00
Repeatability limit (r) (mg/l)	12,7	11,9	5,4	12,2	12,5
Standard deviation of reproducibility (s_R) (mg/l)	9,26	7,24	5,89	6,36	6,08
Relative standard deviation of reproducibility (RSD_R) (%)	5,7	11	17,8	5,9	5,5
Reproducibility limit (R) (mg/l)	25,9	20,3	16,5	17,8	17,0

Types of samples:
A: red wine; B: red wine; C: white wine; D: white wine; E: white wine;

▼B

21. TOTAL MALIC ACID

1. PRINCIPLE

Malic acid, separated by means of an anion exchange column, is determined colorimetrically in the eluent by measuring the yellow coloration it forms with chromotropic acid in the presence of concentrated sulphuric acid. A correction for interfering substances is made by subtracting the absorbence, obtained using 86 % sulphuric and chromotropic acid respectively (malic acid does not react at these acid concentrations), from the absorbence obtained from using 96 % strength acids.

2. APPARATUS

2.1. Glass column approximately 250 mm in length and 35 mm internal diameter, fitted with drain tap.

2.2. Glass column approximately 300 mm in length and 10 to 11 mm internal diameter, fitted with drain tap.

2.3. Thermostatically controlled water bath at 100 ºC.

2.4. Spectrophotometer set to measure absorbence at 420 nm using 10 mm cells.

3. REAGENTS

3.1. A strongly basic ion exchange resin (e.g. Merck III).

3.2. Sodium hydroxide 5 % (m/v).

3.3. Acetic acid 30 % (m/v).

3.4. Acetic acid 0,5 % (m/v).

3.5. Sodium sulphate solution 10 % (m/v).

3.6. Concentrated sulphuric acid 95 to 97 % (m/m).

3.7. Sulphuric acid 86 % (m/m).

3.8. Chromotropic acid 5 % (m/v)

Prepare fresh solution before each determination by dissolving 500 mg sodium chromotropate, ($C_{10}H_6Na_2O_8S_2.2H_{20}$) in 10 ml distilled water.

3.9. DL-Malic acid solution 0,5 g/l.

Dissolve 250 g malic acid ($C_4H_6O_5$) in sodium sulphate solution (10 %), make up to 500 ml with sodium sulphate solution (10 %) (3.5).

4. PROCEDURE

4.1. **Preparation of ion exchange resin**

Place a plug of cotton wool impregnated with distilled water at the bottom of the column (35 × 250 mm) above the tap. Pour a suspension of the anion exchange resin into the glasscolumn. The level of the liquid should be 50 mm above the top of the resin. Rinse with 1 000 ml of distilled water. Wash the column with sodium hydroxide solution (5 %), allow to drain to within 2 to 3 mm of the top of the resin and repeat with two further washings of sodium hydroxide 5 % and leave for one hour. Wash the column with 1 000 ml of distilled water. Refill the column with acetic (30 %) acid, allow to drain to within 2 to 3 mm from the top of the column and repeat with two further washings of acetic acid (30 %). Leave for at least 24 hours before use. Keep the ion exchange resin in acetic acid (30 %) for the subsequent analyses.

4.2. **Preparation of ion exchange column**

Place a plug of cotton wool at the bottom of the column (11 × 300 mm) above the tap. Pour in the suspension of ion exchange resin (prepared in 4.1) to a height of 10 cm. Open the tap and allow the acetic acid solution (30 %) to drain to within 2 to 3 mm of the top of the resin. Wash with a 50 ml portion of acetic acid (0,5 %).

▼B

4.3. Separation of DL-malic acid

Pour onto the column (prepared in 4.2) 10 ml of wine or must. Allow to drain one drop at a time (average rate of one drop per second) and stop the flow 2 to 3 mm from the top of the resin. Wash the column with 50 ml acetic acid (0,5 %) then with 50 ml of distilled water and allow to drain at the same rate as previously, stopping the flow 2 to 3 mm from the top of the resin.

Elute the acids absorbed on the exchange resin with a 10 % sodium sulphate solution (3.5). Collect the eluate in a 100 ml volumetric flask.

The column can be regenerated using the procedure desribed in (4.1).

4.4. Determination of malic acid

Label two wide necked 30-ml tubes (fitted with ground glass stoppers) A and B. Into each tube add 1,0 ml of the eluent (4.3) and 1 ml of chromotropic acid (5 %). Add 10 ml sulphuric acid (86 %) (reference) to tube A and 10 ml (96 %) sulphuric acid to tube B (sample). Stopper and shake to homogenize, taking care not to wet the glass stopper. Immerse the tubes in a boiling water bath for exactly 10 minutes. Cool the tubes in the dark at 20 ºC for exactly 90 minutes. Immediately measure the absorbence relative to the control at 420 nm in a 10 mm cell.

4.5. Plotting the calibration curve

Pipette 5,0, 10,0, 15,0 and 20 ml aliquots respectively into 4 × 50 ml volumetric flasks. Make up to the mark with sodium sulphate solution (10 %).

These solutions correspond to eluates obtained from the wine containing 0,5, 1,0, 1,5 and 2,0 g/l of malic acid.

Continue as in 4.4.

The graph of the absorbences of these solutions is a function of their malic acid concentration represented as a straight line passing through the origin.

The intensity of the colour produced depends to a large extent on the strength of the sulphuric acid used, it is necessary to check the calibration curve with at least one point per series of readings to check if the concentration of the sulphuric acid has changed.

5. EXPRESSION OF RESULTS

The concentration of the eluent is found using the calibration graph by extrapolation of the measured absorbence value to give the corresponding malic acid concentration in g/l. The result is expressed to one decimal place.

Repeatability:

Contents < 2 g/l: $r = 0,1$ g/l.

Contents> 2 g/l: $r = 0,2$ g/l.

Reproducibility:

$R = 0,3$ g/l.

▼B

22. SORBIC ACID

1. PRINCIPLE OF METHODS

1.1. Determination by ultraviolet absorption spectrophotometry

Sorbic acid (*trans, trans,* 2,4-hexadienoic acid) extracted by steam distillation is determined in the wine distillate by ultraviolet absorption spectrophotometry. Substances that interfere in the ultraviolet are removed by evaporation to dryness using a lightly alkali, calcium hydroxide. Thin layer chromatography is used for confirmation of levels (1 mg/l) less than 20 mg/l.

1.2. Determination by gas chromatography

Sorbic acid extracted in ethyl ether is determined by gas chromatography with an internal standard.

1.3. Identification of traces by thin-layer chromatography

Sorbic acid extracted in ethyl ether is separated by thin layer chromatography and its concentration is evaluated semi-quantitatively.

2. DETERMINATION BY ULTRAVIOLET ABSORPTION SPECTROPHOTOMETRY

2.1. Reagents

2.1.1. Crystalline tartaric acid, $C_4H_6O_6$.

2.1.2. Calcium hydroxide, $Ca(OH)_2$, solution, approximately 0,02 M.

2.1.3. Reference sorbic acid solution, 20 mg/l:

Dissolve 20 mg of sorbic acid, $C_6H_8O_2$, in approximately 2 ml of 0,1 M sodium hydroxide solution. Pour into a 1 000 ml volumetric flask, and make up to the mark with water. It is also possible to dissolve 26,8 mg of potassium sorbate, $C_6H_7KO_2$, in water and make up to 1 000 ml with water.

2.2. Apparatus

2.2.1. Steam distillation apparatus (see chapter ‘Volatile acidity’).

2.2.2. Water bath at 100 °C.

2.2.3. Spectrophotometer enabling absorbence measurements to be made at a wavelength of 256 nm and having a quartz cell with a 1 cm optical path.

2.3. Procedure

2.3.1. *Distillation*

Place in the flask of the steam distillation apparatus 10 ml of wine and add 1 to 2 g tartaric acid (2.1.1). Collect 250 ml of the distillate.

2.3.2. *Preparation of the calibration curve*

Prepare, by dilution of the reference solution (2.1.3), four dilute reference solutions with 0,5, 1,0, 2,5 and 5 mg of sorbic acid per litre. Measure their absorbences with the spectrophotometerat 256 nm using that of distilled water as a blank. Plot a curve showing the variation of absorbence as a function of concentration. The variation is linear.

2.3.3. *Determination*

Place 5 ml of the distillate in an evaporating dish of 55 mm diameter, add 1 ml of calcium hydroxide solution (2.1.2). Evaporate to dryness on a water bath.

Dissolve the residue in several ml of distilled water, transfer completely to a 20 ml volumetric flask and make up to the mark with rinsing water. Measure the absorbence at 256 nm using the spectrophotometer against a blank consisting of a solution obtained by diluting 1 ml of calcium hydroxide solution (2.1.2) to 20 ml with water.

Plot the value of the measured absorbence on the calibration curve and from this find the concentration *C* of sorbic acid in the solution.

Note:

In this method the loss due to evaporation can be neglected and the absorbence measured on the treated distillate diluted ¼ with distilled water.

2.4. **Expression of results**

2.4.1. *Calculation*

The sorbic acid concentration in the wine expressed in mg per litre is given by 100 × *C*

where

C = concentration of sorbic acid in the solution analysed by spectrophotometry expressed in mg per litre.

3. DETERMINATION BY GAS CHROMATOGRAPHY

3.1. **Reagents**

3.1.1. Ethyl ether, $(C_2H_5)_2O$, distilled just before use.

3.1.2. Internal reference solution: solution of undecanoic acid, $C_{11}H_{22}O_2$, in 95 % vol ethanol at a strength of 1 g/l.

3.1.3. Aqueous solution of sulphuric acid, H_2SO_4 (ρ_{20} = 1,84 g/ml) diluted 1:3 (v/v).

3.2. **Apparatus**

3.2.1. Gas chromatograph fitted with a flame ionization detector and a stainless steel column (4 m × ⅛ inch) previously treated with dimethyldichlorosilane and packed with a stationary phase consisting of a mixture of diethyleneglycol succinate (5 %) and phosphoric acid (1 %) (DEGS — H_3PO_4) or of a mixture of diethyleneglycol adipate (7 %) and phosphoric acid (1 %) (DEGA — H_3PO_4) bonded on Gaschrom Q 80 — 100 mesh.

Treatment of column with DMDCS — pass through the column a solution containing 2 to 3 g of DMDCS in toluene. Immediately wash with methanol, followed by nitrogen and then wash with hexane followed by more nitrogen. It is now ready to be packed.

Operating conditions:

Oven temperature: 175 ºC.

Temperature of the injector and detector: 230 ºC.

Carrier gas: nitrogen (flow rate = 200 ml/min).

3.2.2. Microsyringe, 10 µl capacity graduated in 0,1 µl.

Note:

Other types of columns that give a good separation can be used, particularly capillary columns (e.g. FFAP). The working method described is given as an example.

3.3. **Procedure**

3.3.1. *Preparation of sample to be analysed*

Into a glass test tube of approximately 40 ml capacity and fitted with a ground glass stopper, introduce 20 ml of wine, add 2 ml of the internal reference solution (3.1.2) and 1 ml of dilute sulphuric acid (3.1.3).

After mixing the solution by repeatedly turning the tube over, add to its contents 10 ml of ethyl ether (3.1.1). Extract the sorbic acid in the organic phase by shaking the tube for five minutes. Leave to settle.

3.3.2. *Preparation of the reference solution*

Select a wine for which the chromatogram of the ether extract shows no peak corresponding to the elution of sorbic acid. Overload this wine with sorbic acid at a concentration of 100 mg per litre. Treat

▼B

20 ml of the sample prepared in this way according to the procedure described in 3.3.1.

3.3.3. *Chromatography*

Using a microsyringe, inject into the chromatograph in turn 2 µl of the ether-extract phase obtained in 3.3.2 and 2 µl of the ether-extracted phase obtained in 3.3.1.

Record the respective chromatograms: check the identity of the respective retention times of the sorbic acid and the internal standard. Measure the height (or area) of each of the recorded peaks.

3.4. **Expression of results**

3.4.1. *Calculation*

The concentration of sorbic acid in the analysed wine, expressed in mg per litre, is given by:

$$100 \times \frac{h}{H} \times \frac{I}{i}$$

where

H = height of the sorbic acid peak in the reference solution

h = height of the sorbic acid peak in the sample for analysis

I = height of the internal standard peak in the reference solution

i = height of the internal standard peak in the sample for analysis

Note:

The sorbic acid concentration may be determined in the same way from measurements of the areas under the respective peaks.

4. IDENTIFICATION OF TRACES OF SORBIC ACID BY THIN LAYER CHROMATOGRAPHY

4.1. **Reagents**

4.1.1. Ethyl ether, $(C_2H_5)_2O$.

4.1.2. Aqueous sulphuric acid solution, H_2SO_4 (ρ_{20} = 1,84 g/ml), diluted 1:3 (v/v).

4.1.3. Reference solution of sorbic acid in an approximately 10 % vol ethanol/water mixture containing 20 mg per litre.

4.1.4. Mobile phase: hexane-pentane-acetic acid (20:20:3) ($C_6H_{14}/C_5H_{12}/CH_3COOH$, ρ_{20} = 1,05 g/ml).

4.2. **Apparatus**

4.2.1. Precoated 20 × 20 cm plates for thin layer chromatography coated with polyamide gel (0,15 mm thick) with the addition of a fluorescent indicator.

4.2.2. Cell for thin layer chromatography.

4.2.3. Micropipette or microsyringe for delivering volumes of 5 µl to within ± 0,1 µl.

4.2.4. Ultraviolet lamp (254 nm).

4.3. **Procedure**

4.3.1. *Preparation of sample to be analysed*

Into a glass test tube of approximately 25 ml capacity and fitted with a ground glass stopper, place 10 ml of wine, add 1 ml of dilute sulphuric acid (4.1.2) and 5 ml of ethyl ether (4.1.2). Mix by repeatedly turning the tube over. Leave to settle.

4.3.2. *Preparation of dilute reference solutions*

Prepare five dilute reference solutions from the solution in 4.1.3 containing 2, 4, 6, 8 and 10 mg sorbic acid per litre.

▼B

4.3.3. *Chromatography*

Using a microsyringe or micropipette, deposit 5 µl of the ether-extracted phase obtained in 4.3.1 and 5 µl of each of the dilute reference solutions (4.3.2) at points 2 cm from the lower edge of the plate and 2 cm apart from each other.

Place the mobile phase (4.1.4) in the chromatograph tank to a height of about 0,5 cm and allow the atmosphere in the tank to become saturated with solvent vapours. Place the plate in the tank. Allow the chromatogram to develop over 12 to 15 cm (development time approximately 30 minutes). Dry the plate in a current of cool air. Examine the chromatogram under a 254 nm ultraviolet lamp.

The spots indicating the presence of sorbic acid will appear to be dark violet against the yellow fluorescent background of the plate.

4.4. **Expression of results**

A comparison of the intensities of the spots produced by the sample to be analysed and by the reference solutions will enable a semi-quantitative assessment to be made of the sorbic acid concentration between 2 and 10 mg per litre. A concentration of 1 mg per litre could be determined with the deposition of 10 µl of the sample solution to be analysed.

Concentrations above 10 mg per litre could be determined with the deposition of less than 5 µl of the solution to be analysed (measured out using a microsyringe).

▼B

23. L-ASCORBIC ACID

1. PRINCIPLE OF METHODS

The methods proposed enable the L-ascorbic acid and dehydroascorbic acid present in wines or musts to be determined.

1.1. Reference method (fluorimetry)

The L-ascorbic acid is oxidized on activated carbon into dehydroascorbic acid. The latter forms a fluorescent compound by reacting with orthophenylenediamine (OPDA). A control test in the presence of boric acid enables spurious fluorescence to be determined (by the formation of a boric acid/dehydroascorbic acid complex) and the fluorimetric determination to be deduced.

1.2. Usual method (colorimetry)

The L-ascorbic acid is oxidized by iodine to dehydroascorbic acid which is then precipitated using 2,4-dinitrophenylhydrazine to produce bis (2,4-dinitrophenylhydrazone). After separation by thin layer chromatography and dissolution in acetic acid medium the red-coloured derivative is determined by spectrophotometry at 500 nm.

2. REFERENCE METHOD (fluorimetric method)

2.1. Reagents

2.1.1. Orthophenylenediamine dihydrochloride solution, $C_6H_{10}Cl_2N_2$, 0,02 g per 100 ml; prepared just before use.

2.1.2. Sodium acetate trihydrate solution, $CH_3COONa \cdot 3H_2O$, 500 g/litre.

2.1.3. Mixed solution of boric acid and sodium acetate:

dissolve 3 g of boric acid, H_3BO_3, in 100 ml of sodium acetate solution (2.1.2). This solution must be prepared just before use.

2.1.4. Glacial acetic acid solution, CH_3COOH (ρ_{20} = 1,05 g/ml), diluted to 56 % (v/v) with pH near to 1,2.

2.1.5. Reference solution of L-ascorbic acid, 1 g/litre:

Just before use, dissolve 50 mg of L-ascorbic acid, $C_6H_8O_6$, previously dehydrated in a desiccator protected against light, in 50 ml of acetic acid solution (2.1.4).

2.1.6. Very pure analytical grade activated carbon (1)

Into a 2-litre conical flask, place 100 g of activated carbon and add 500 ml of 10 % (v/v) hydrochloric acid (HCl) solution (ρ_{20} = 1,19 g/ml). Bring to the boil, filter using a sintered glass filter of porosity 3. Collect the carbon treated in this way in a 2-litre conical flask, add 1 litre of water, shake and filter using a sintered glass filter of porosity 3. Repeat this operation two more times. Place the residue in an oven controlled to 115 ± 5 ºC for 12 hours (overnight).

2.2. Apparatus

2.2.1. Fluorimeter. Use a spectrofluorimeter equipped with a lamp giving a continuous spectrum by using it at minimum power. The optimum excitation and emission wavelengths for the test will be determined beforehand and depend on the equipment used. As a guide, the excitation wavelength will be approximately 350 nm and the emission wavelength approximately 430 nm. Cells of 1 cm path length.

2.2.2. Sintered glass filter of porosity 3.

2.2.3. Test tubes (diameter approximately 10 mm).

2.2.4. Stirring rods for test tubes.

---

(1) One of the trade names is ‘Norite’.

▼B

2.3. Procedure

2.3.1. *Preparation of the sample of wine or must*

Take a volume of the wine or must and dilute to 100 ml in a graduated flask with the 56 % acetic acid solution (2.1.4) in order to obtain a solution with an L-ascorbic acid concentration between 0 and 60 mg/litre. Homogenize the contents of the flask by stirring. Add 2 g of activated carbon (2.1.6) and allow to stand for 15 minutes, stirring occasionally. Filter using ordinary filter paper, discarding the first few millilitres of filtrate.

Into two 100 ml graduated flasks, introduce 5 ml of the filtrate and, in the first, 5 ml of the mixed solution of boric acid and sodium acetate solution (2.1.3) (sample blank) and, in the second, 5 ml of the sodium acetate solution (2.1.2) (sample). Allow to stand for 15 minutes, stirring occasionally. Make up to 100 ml with distilled water.

Take 2 ml from the contents of each flask and add 5 ml of orthophenylenediamine solution (2.1.1), stir; leave the reaction to proceed for 30 minutes until the solution darkens and then make the spectrofluorimetric measurements.

2.3.2. *Preparation of the calibration curve*

Into three 100 ml graduated flask place 2, 4 and 6 ml respectively of the reference L-ascorbic acid solution (2.1.5), make up to 100 ml with acetic acid solution (2.1.4) and homogenize by stirring. The reference solutions prepared in this way contain 2, 4 and 6 mg per 100 ml.

Add 2 g of activated carbon (2.1.6) to each of the flasks and allow to stand for 15 minutes, stirring occasionally. Filter through ordinary filter paper, discarding the first few millilitres. Introduce 5 ml of each filtrate collected into three 100-ml graduated flasks (first series). Repeat the operation and obtain a second series of three graduated flasks. To each of the flasks in the first series (corresponding to the blank test) add 5 ml of the mixed solution of boric acid and sodium acetate (2.1.5), and to each of the flasks in the second series add 5 ml of the sodium acetate solution (2.1.2).

Allow to stand for 15 minutes, stirring occasionally. Make up to 100 ml with distilled water. Take 2 ml of the contents of each flask, add 5 ml of orthophenylenediamine solution (2.1.1), stir, leave the reaction to proceed for 30 minutes until the solution darkens and then make the spectrofluorimetric measurements.

2.3.3. *Fluorimetric determination*

For each solution contributing to the calibration curve and for the solution to be determined set the zero on the scale of measurements using the corresponding control test sample. Then measure the intensity of the fluorescence for each solution over the calibration range and for the solution to be determined.

Plot the calibration curve, which should be a straight line passing through the origin. On this line, find the value relative to the determination and thus deduce the concentration C L-ascorbic acid + dehydroascorbic acid in the solution to be analysed.

2.3.4. *Expression of results*

The concentration of L-ascorbic acid and dehydroascorbic acid in the wine in milligrams per litre is given by $C \times F$, where F is the dilution factor.

▼M9

▼B

24. **pH**

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. APPARATUS

2.1. pH meter with a scale calibrated in pH units and enabling measurements to be made to at least ±0,05 pH unit.

2.2. **Electrodes:**

2.2.1. Glass electrode, kept in distilled water.

2.2.2. Calomel-saturated potassium chloride reference electrode, kept in a saturated solution of potassium chloride.

2.2.3. Or a combined electrode, kept in distilled water.

3. REAGENTS

3.1. **Buffer solutions**

3.1.1. Saturated solution of potassium hydrogen tartrate, containing at least 5,7 g of potassium hydrogen tartrate per litre ($C_4H_5KO_6$) at 20 ºC. (This solution may be kept for up to two months by adding 0,1 g of thymol per 200 ml.)

$$\mathrm{pH} \begin{cases} 3{,}57 \text{ at } 20\ ^\circ\mathrm{C} \\ 3{,}56 \text{ at } 25\ ^\circ\mathrm{C} \\ 3{,}55 \text{ at } 30\ ^\circ\mathrm{C} \end{cases}$$

3.1.2. Solution of potassium hydrogen phthalate, 0,05 M, containing 10,211 g of potassium hydrogen phthalate ($C_8H_5KO_4$) per litre at 20 ºC. (Maximum keeping period, two months.)

$$\mathrm{pH} \begin{cases} 3{,}999 \text{ at } 15\ ^\circ\mathrm{C} \\ 4{,}003 \text{ at } 20\ ^\circ\mathrm{C} \\ 4{,}008 \text{ at } 25\ ^\circ\mathrm{C} \\ 4{,}015 \text{ at } 30\ ^\circ\mathrm{C} \end{cases}$$

3.1.3. 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)

$$\mathrm{pH} \begin{cases} 6{,}90 \text{ at } 15\ ^\circ\mathrm{C} \\ 6{,}88 \text{ at } 20^\circ\mathrm{C} \\ 6{,}86 \text{ at } 25\ ^\circ\mathrm{C} \\ 6{,}85 \text{ at } 30\ ^\circ\mathrm{C} \end{cases}$$

Note:

Commerical reference buffer solutions may also be used.

▼**B**

4. PROCEDURE

4.1. **Preparation of the sample for analysis**

4.1.1. For must and wine: use the must or wine directly.

4.1.2. For rectified concentrated must: 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

$$\frac{2500}{P}$$

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**

Calibrate the pH meter at 20 ºC using buffer solutions of pH 6,88 and 3,57 at 20 ºC.

Use the buffer solution of pH 4,00 at 20 ºC to check the calibration of the scale.

4.4. **Determination**

Dip the electrode into the sample to be analysed, the temperature of which should be between 20 and 25 ºC and as close as possible to 20 ºC. 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 must, the wine or the 25 % (m/m) (25 ° Brix) solution of rectified concentrated must is quoted to two decimal places.

▼B

25. **SULPHUR DIOXIDE**

1. DEFINITIONS

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

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

$$H_2SO_3 \rightleftharpoons H^+ + HSO_3^-$$

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 wine, either in the free state or combined with its constituents.

2. FREE AND TOTAL SULPHUR DIOXIDE

2.1. **Principle of the methods**

2.1.1. *Reference method*

2.1.1.1. For wines and musts

The sulphur dioxide is carried over by a current of air or nitrogen; it is fixed and oxidized by being bubbled through a dilute and neutral hydrogen peroxide solution. The sulphuric acid formed is determined by titration with a standard solution of sodium hydroxide. Free sulphur dioxide is purged from the wine by entrainment at low temperature (10 ºC).

Total sulphur dioxide is purged from the wine by entrainment at high temperature (approximately 100 ºC).

2.1.1.2. For rectified concentrated musts

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

2.1.2. *Rapid method of determination (for wines and musts)*

Free sulphur dioxide is determined by direct iodometric titration.

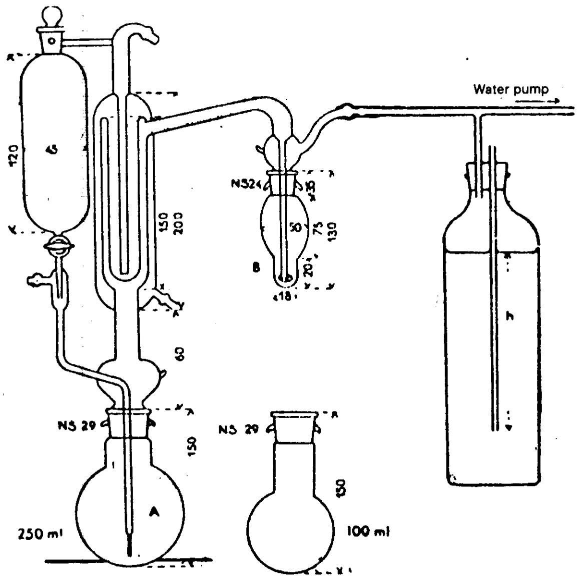
Combined sulphur dioxide is subsequently determined by iodometric titration after alkaline hydrolysis. When added to the free sulphur dioxide, it gives the total sulphur dioxide.

2.2. **Reference method**

2.2.1. *Apparatus*

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

▼B



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.

2.2.1.2. A microburette.

2.2.2. *Reagents*

2.2.2.1. Phosphoric acid, 85 % (H_3PO_4, ρ_{20} = 1,71 g/ml).

2.2.2.2. Hydrogen peroxide solution, 9,1 g H_2O_2/litre (three volumes).

2.2.2.3. Indicator reagent:

methyl red	100 mg
methylene blue	50 mg
alcohol, 50 % vol	100 ml

2.2.2.4. Sodium hydroxide solution, NaOH, 0,01 M

2.2.3. *Procedure*

2.2.3.1. Determination of free sulphur dioxide

The wine must be kept in a full and stoppered bottle at 20 °C for two days before the determination.

Place 2 to 3 ml of hydrogen peroxide solution (2.2.2.2) and two drops of the indicator reagent in the bubbler B and neutralize the hydrogen peroxide solution with the 0,01 M sodium hydroxide solution (2.2.2.4). Connect the bubbler to the apparatus.

Introduce 50 ml of the sample and 15 ml of phosphoric acid (2.2.2.1) into the flask A of the entrainment apparatus. Connect the flask into the apparatus.

Bubble air (or nitrogen) through it for 15 minutes. The free sulphur dioxide carried over is oxidized to sulphuric acid. Remove the bubbler from the apparatus and titrate the acid which has formed against the 0,01 M sodium hydroxide solution (2.2.2.4). Let *n* ml be the volume used.

▼**B**

2.2.3.2. Expression of results

The liberated sulphur dioxide is expressed in mg/l to the nearest whole number.

2.2.3.2.1. Calculation

The free sulphur dioxide in milligrams per litre is 6,4 *n*.

2.2.3.3. Determination of total sulphur dioxide

2.2.3.3.1. 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 5.1.2. Introduce 50 ml of this solution and 5 ml of phosphoric acid (2.2.2.1) into the 250 ml flask A of the entrainment apparatus. Connect the flask into the apparatus.

2.2.3.3.2. Wines and musts

If the estimated concentration in the sample is no greater than 50 mg of total SO_2 per litre, place 50 ml of the sample and 15 ml of phosphoric acid (2.2.2.1) in the 250-ml flask A of the entrainment apparatus. Connect the flask to the apparatus.

However, until ►**M3** 31 August 1996 ◄ at the latest, to analyse the sulphur dioxide content of grape juice, 5 ml of a 25 % solution (m/v) of phosphoric acid (2.2.2.1) shall be used.

If the estimated concentration in the sample is greater than 50 mg of total SO_2 per litre, place 20 ml of the sample and 5 ml of phosphoric acid (2.2.2.1) in the 100 ml flask A of the entrainment apparatus. Connect the flask to the apparatus.

Place 2 to 3 ml of hydrogen peroxide solution (2.2.2.2) in the bubbler B, neutralized as before, and bring the wine 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 wine 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 (2.2.2.4).

Let *n* ml be the volume used.

2.2.3.4. Expression of results

Musts and wines: Total sulphur dioxide is expressed in mg/l.

Rectified concentrated must: Total sulphur dioxide is expressed in mg/kg of total sugar.

2.2.3.4.1. Calculation

For wines:

Total sulphur dioxide in milligrams per litre:

- samples low in sulphur dioxide (50 ml test sample): 6,4 *n*
- other samples (20 ml test sample): 16 *n*

For rectified concentrated musts:

Total sulphur dioxide in milligrams per kilogram of total sugars (50 ml prepared test sample (2.2.3.3.1)):

$$\frac{1\,600 \times n}{P}$$

where

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

2.2.3.4.2. Repeatability (*r*)

50 ml test sample < 50 mg/l; *r* = 1 mg/l.

20ml test sample > 50 mg/l; *r* = 6 mg/l.

2.2.3.4.3. Reproducibility (*R*)

50ml test sample < 50 mg/l; *R* = 9 mg/l.

▼B

20ml test sample > 50 mg/l; R = 15 mg/l.

▼M9

▼B

3. MOLECULAR SULPHUR DIOXIDE

3.1. Principle of the method

The percentage of molecular sulphur dioxide, H_2SO_3, in free sulphur dioxide is calculated as a function of pH, alcoholic strength and temperature.

For a given temperature and alcoholic strength:

$$H_2SO_3 \rightleftharpoons H^+ + HSO_3^-,$$

$$[H_2SO_3] = \frac{L}{10^{(pH - pK_M)+1}} \qquad (1)$$

with

$$pK_M = pK_T - \frac{A\sqrt{I}}{1 + B\sqrt{I}}$$

$$L = [H_2SO_3] + [HSO_3^-]$$

where

I = ionic strength,

A and B = coefficients varying with temperature and alcoholic strength,

K_T = thermodynamic dissociation constant: values of pK_T are given in Table 1 for various alcoholic strengths and temperatures,

K_M = mixed dissociation constant.

Taking a mean value of 0,038 for the ionic strength I, Table 2 gives values of pK_M for various temperatures and alcoholic strengths.

The molecular sulphur dioxide content calculated using expression (1) is given in Table 3 for various values of pH, temperature and alcoholic strength.

3.2. Calculation

From a knowledge of the pH of the wine and its alcoholic strength, the percentage of molecular sulphur dioxide is given in Table 3 for a temperature T °C. Let this be X %.

The molecular sulphur dioxide content in mg/l is:

$$X \times C$$

where

C = the free sulphur dioxide content in mg/l.

▼**B**

TABLE 1

Values of the thermodynamic dissociation constant pK_T

Alcohol strength(% vol)	Temperature (ºC)				
	20	25	30	35	40
0	1,798	2,000	2,219	2,334	2,493
5	1,897	2,098	2,299	2,397	2,527
10	1,997	2,198	2,394	2,488	2,606
15	2,099	2,301	2,503	2,607	2,728
20	2,203	2,406	2,628	2,754	2,895

TABLE 2

Values of the mixed dissociation constant pK_M (I = 0,038)

Alcohol strength(% vol)	Temperature (ºC)				
	20	25	30	35	40
0	1,723	1,925	2,143	2,257	2,416
5	1,819	2,020	2,220	2,317	2,446
10	1,916	2,116	2,311	2,405	2,522
15	2,014	2,216	2,417	2,520	2,640
20	2,114	2,317	2,538	2,663	2,803

TABLE 3

Molecular sulphur dioxide as a percentage of free sulphur dioxide

Molecular SO_2/ free SO_2 (%)	T = 20 ºC					I = 0,038
pH	Alcoholic strength (% vol)					
	0	5	10	15	20	
2,8	7,73	9,46	11,55	14,07	17,09	
2,9	6,24	7,66	9,40	11,51	14,07	
3,0	5,02	6,18	7,61	9,36	11,51	
3,1	4,03	4,98	6,14	7,58	9,36	
3,2	3,22	3,99	4,94	6,12	7,58	
3,3	2,58	3,20	3,98	4,92	6,12	
3,4	2,06	2,56	3,18	3,95	4,92	
3,5	1,64	2,04	2,54	3,16	3,95	
3,6	1,31	1,63	2,03	2,53	3,16	
3,7	1,04	1,30	1,62	2,02	2,53	
3,8	0,83	1,03	1,29	1,61	2,02	
T = 25 ºC						
2,8	11,47	14,23	17,15	20,67	24,75	
2,9	9,58	11,65	14,12	17,15	22,71	
3,0	7,76	9,48	11,55	14,12	17,18	
3,1	6,27	7,68	9,40	11,55	14,15	
3,2	5,04	6,20	7,61	9,40	11,58	
3,3	4,05	4,99	6,14	7,61	9,42	
3,4	3,24	4,00	4,94	6,14	7,63	
3,5	2,60	3,20	3,97	4,94	6,16	
3,6	2,07	2,56	3,18	3,97	4,55	
3,7	1,65	2,05	2,54	3,18	3,98	
3,8	1,32	1,63	2,03	2,54	3,18	

▼**B**

Molecular SO_2/ free SO_2 (%)	*T* = 20 ºC					*I* = 0,038
pH	Alcoholic strength (% vol)					
	0	5	10	15	20	
T = 30 ºC						
2,8	18,05	20,83	24,49	29,28	35,36	
2,9	14,89	17,28	20,48	24,75	30,29	
3,0	12,20	14,23	16,98	20,71	25,66	
3,1	9,94	11,65	13,98	17,18	21,52	
3,2	8,06	9,48	11,44	14,15	17,88	
3,3	6,51	7,68	9,30	11,58	14,75	
3,4	5,24	6,20	7,53	9,42	12,08	
3,5	4,21	4,99	6,08	7,63	9,84	
3,6	3,37	4,00	4,89	6,16	7,98	
3,7	2,69	3,21	3,92	4,95	6,44	
3,8	2,16	2,56	3,14	3,98	5,19	
T = 35 ºC						
2,8	22,27	24,75	28,71	34,42	42,18	
2,9	18,53	20,71	24,24	29,42	36,69	
3,0	15,31	17,18	20,26	24,88	31,52	
3,1	12,55	14,15	16,79	20,83	26,77	
3,2	10,24	11,58	13,82	17,28	22,51	
3,3	8,31	9,42	11,30	14,23	18,74	
3,4	6,71	7,63	9,19	11,65	15,49	
3,5	5,44	6,16	7,44	9,48	12,71	
3,6	4,34	4,95	6,00	7,68	10,36	
3,7	3,48	3,98	4,88	6,20	8,41	
3,8	2,78	3,18	3,87	4,99	6,80	
T = 40 ºC						
2,8	29,23	30,68	34,52	40,89	50,14	
2,9	24,70	26,01	29,52	35,47	44,74	
3,0	20,67	21,83	24,96	30,39	38,85	
3,1	17,15	18,16	20,90	25,75	33,54	
3,2	14,12	14,98	17,35	21,60	28,62	
3,3	11,55	12,28	14,29	17,96	24,15	
3,4	9,40	10,00	11,70	14,81	20,19	
3,5	7,61	8,11	9,52	12,13	16,73	
3,6	6,14	6,56	7,71	9,88	13,77	
3,7	4,94	5,28	6,22	8,01	11,25	
3,8	3,97	4,24	5,01	6,47	9,15	

▼B

26. **SODIUM**

1. PRINCIPLE OF THE METHODS

1.1. **Reference method: atomic absorption spectrophotometry**

Sodium is determined directly in the wine by atomic absorption spectrophotometry after the addition of an ionization suppression agent (caesium chloride) to prevent ionization of sodium.

1.2. **Usual method: flame photometry**

Sodium is determined directly in diluted wine (at least 1:10) by flame photometry.

2. REFERENCE METHOD

2.1. **Reagents**

2.1.1. *Solution containing 1 g of sodium per litre:*

Use a standard commercial solution containing 1 g of sodium per litre. This solution may be prepared by dissolving 2,542 g of anhydrous sodium chloride, NaCl, in distilled water and making up to a volume of 1 litre.

Keep this solution in a polyethylene bottle.

2.1.2. *Matrix (model) solution:*

citric acid, $C_6H_8O_7 \cdot H_2O$	3,5	g
sucrose, $C_{12}H_{22}O_{11}$	1,5	g
glycerol, $C_3H_8O_3$	5,0	g
anhydrous calcium chloride, $CaCl_2$	50	mg
anhydrous magnesium chloride, $MgCl_2$	50	mg
absolute alcohol, C_2H_5OH	50	ml
de-ionized water to	500	ml

2.1.3. *Caesium chloride solution containing 5 % caesium:*

dissolve 6,330 g of caesium chloride, CsCl, in 100 ml of distilled water.

2.2. **Apparatus**

2.2.1. Atomic absorption spectrophotometer equipped with an air-acetylene burner.

2.2.2. Sodium hollow cathode lamp.

2.3. **Procedure**

2.3.1. *Preparation of sample*

Pipette 2,5 ml of wine into a 50 ml volumetric flask, add 1 ml of the caesium chloride solution (2.1.3) and make up to the mark with distilled water.

2.3.2. *Calibration*

Place 5,0 ml of the matrix solution in each one of a set of 100 ml volumetric flasks and add 0, 2,5, 5,0, 7,5 and 10 ml respectively of the 1 g/l sodium solution (2.1.1) previously diluted by 1:100. Add 2 ml of the caesium chloride solution (2.1.3) to each flask and make up to 100 ml with distilled water.

The standard solutions prepared in this way contain 0, 0,25, 0,50, 0,75 and 1,00 mg of sodium per litre respectively and each contains 1 g of caesium per litre. Keep these solutions in polyethylene bottles.

2.3.3. *Determination*

Set the wavelength to 589,0 nm. Zero the absorbence scale using the matrix solution containing 1 g of caesium per litre (2.3.2). Aspirate

▼**B**

the diluted wine directly into the burner of the spectrophotometer, followed in succession by the standard solutions (2.3.2). Read off the absorbences. Repeat each measurement.

2.4. **Expression of results**

2.4.1. *Method of calculation*

Plot a graph giving the absorbence as a function of the sodium concentration in the standard solutions.

Record the absorbence obtained with the diluted wine on this graph and determine its sodium concentration C in milligrams per litre.

The sodium concentration in milligrams per litre of the wine will then be $20C$, expressed to the nearest whole number.

2.4.2. *Repeatability (r)*

$r = 1 + 0{,}024\ x_i$ mg/l.

x_i = concentration of sodium in the sample in mg/l.

2.4.3. *Reproducibility (R)*

$R = 2{,}5 + 0{,}05\ x_i$ mg/l.

x_i = concentration of sodium in the sample in mg/l.

▼**M9**

▼B

27. **POTASSIUM**

1. PRINCIPLE OF THE METHODS

1.1. **Reference method**

Potassium is determined directly in the diluted wine by atomic absorption spectrophotometry after the addition of an ionization suppression agent (caesium chloride) to prevent ionization of potassium.

1.2. **Usual method**

Potassium is determined directly in the diluted wine by flame photometry.

2. REFERENCE METHOD

2.1. **Reagents**

2.1.1. *Solution containing 1 g of potassium per litre:*

Use a standard commercial solution containing 1 g of potassium per litre. This solution may be prepared by dissolving 4,813 g of potassium hydrogen tartrate ($C_4H_5KO_6$) in distilled water and making up the volume to 1 litre.

2.1.2. *Matrix (model) solution:*

citric acid ($C_6H_8O_7 \cdot H_2O$)	3,5	g
sucrose ($C_{12}H_{22}O_{11}$)	1,5	g
glycerol ($C_3H_8O_3$)	5,0	g
anhydrous calcium chloride ($CaCl_2$)	50	mg
anhydrous magnesium chloride ($MgCl_2$)	50	mg
absolute alcohol (C_2H_5OH)	50	ml
water to	500	ml

2.1.3. *Caesium chloride solution containing 5 % caesium:*

dissolve 6,33 g of caesium chloride, CsCl, in 100 ml of distilled water.

2.2. **Apparatus**

2.2.1. Atomic absorption spectrophotometer, equipped with an air-acetylene burner.

2.2.2. Potassium hollow cathode lamp.

2.3. **Procedure**

2.3.1. *Preparation of sample*

Pipette 2,5 ml of wine (previously diluted by 1:10) into a 50-ml volumetric flask, add 1 ml of the caesium chloride solution (2.1.3) and make up to the mark with distilled water.

2.3.2. *Calibration*

Introduce 5,0 ml of the matrix solution (2.1.2) into each one of a set of 100-ml volumetric flasks and add 0, 2,0, 4,0, 6,0 and 8,0 ml respectively of the 1 g/l potassium solution (2.1.1)(previously diluted by 1:10). Add 2 ml of the caesium chloride solution (2.1.3) to each flask and make up to 100 ml with distilled water.

The standard solutions prepared in this way contain 0, 2, 4, 6 and 8 mg of potassium per litre respectively and each contains 1 g of caesium per litre. Keep these solutions in polyethylene bottles.

2.3.3. *Determination*

Set the wavelength to 769,9 nm. Zero the absorbence scale using the matrix solution containing 1 g of caesium per litre (2.3.2). Aspirate the diluted wine (2.3.1) directly into the burner of the spectrophot-

▼**B**

ometer, followed in succession by the standard solutions (2.3.2). Read off the absorbences. Repeat each measurement.

2.4. Expression of results

2.4.1. *Method of calculation*

Plot a graph giving the variation in absorbence as a function of the potassium concentration in the standard solutions.

Record the mean value of the absorbence obtained with the sample of diluted wine on this graph and determine its potassium concentration C in milligrams per litre.

The potassium concentration expressed in milligrams per litre of the wine to the nearest whole number will then be $F \times C$, where F is the dilution factor (here 200).

2.4.2. *Repeatability (r)*

$r = 35$ mg/l.

2.4.3. *Reproducibility (R)*

$R = 66$ mg/l.

2.4.4. *Other ways of expressing results*

In milliequivalents per litre: $0{,}0256 \times F \times C$.

In mg potassium hydrogen tartrate per litre: $4{,}813 \times F \times C$.

▼**M9**

▼**B**

28. **MAGNESIUM**

1. PRINCIPLE OF THE METHOD

Magnesium is determined directly on wine, suitably diluted, by atomic absorption spectrophotometry.

2. REAGENTS

2.1. **Concentrated standard solution containing 1 g magnesium per litre**

Use a standard commercial magnesium solution (1 g/l). This solution may be prepared by dissolving 8,3646 g of magnesium chloride ($MgCl_2.6H_2O$) in distilled water and making up to 1 litre.

2.2. **Dilute standard solution containing 5 mg magnesium per litre.**

Note:

Keep the standard magnesium solutions in polyethylene bottles.

3. APPARATUS

3.1. Atomic absorption spectrophotometer fitted with an air-acetylene burner.

3.2. Magnesium hollow cathode lamp.

4. PROCEDURE

4.1. **Preparation of sample**

Dilute the wine by 1:100 with distilled water.

4.2. **Calibration**

Place 5, 10, 15 and 20 ml of the dilute standard magnesium solution (2.2) into each one of a set of 100 ml volumetric flasks and make up to 100 ml with distilled water. The standard solutions prepared in this way contain 0,25, 0,50, 0,75 and 1,0 mg of magnesium per litre respectively. These solutions should be kept in polyethylene bottles.

4.3. **Determination**

Set the wavelength to 285 nm. Zero the absorbence scale using distilled water. Aspirate the diluted wine directly into the burner of the spectrophotometer, followed in succession by the standard solutions (4.2).

Read off the absorbences. Repeat each measurement.

5. EXPRESSION OF RESULTS

5.1. **Method of calculation**

Plot a graph of the variation in absorbence as a function of the magnesium concentration in the standard solutions. Record the mean value of the absorbence obtained with the diluted sample of wine on this graph and determine its magnesium concentration C in milligrams per litre.

The magnesium concentration in milligrams per litre of the wine to the nearest whole number will be 100 *C*.

5.2. **Repeatability (r)**

r = 3 mg/l.

5.3. **Reproducibility (R)**

R = 8 mg/l.

▼B

29. CALCIUM

1. PRINCIPLE OF THE METHOD

Calcium is determined directly on wine, suitably diluted, by atomic absorption spectrophotometry, after addition of an ionization suppression agent.

2. REAGENTS

2.1. **Standard solution containing 1 g calcium per litre**

Use a standard commercial calcium solution 1 g/l. This solution may be prepared by dissolving 2,5 g of calcium carbonate, $CaCO_3$, in a quantity of 1:10 (v/v) HCl sufficient to dissolve it completely and making up to one litre with distilled water.

2.2. **Dilute standard solution containing 50 mg calcium per litre**

Note:

Keep the standard calcium solutions in polyethylene bottles.

2.3. **Lanthanum chloride solution containing 50 g lanthanum per litre**

Dissolve 13,369 g of lanthanum chloride, $LaCl_3 \cdot 7H_2O$, in distilled water; add 1 ml of HCl diluted 1:10 (v/v) and make up to 100 ml.

3. APPARATUS

3.1. Atomic absorption spectrophotometer fitted with an air-acetylene burner.

3.2. Calcium hollow cathode lamp.

4. PROCEDURE

4.1. **Preparation of sample**

Place 1 ml of the wine, 2 ml of the lanthanum chloride solution (2.3) in a 20 ml volumetric flask and make up to the mark with distilled water. The wine, diluted by 1:20, contains 5 g lanthanum per litre.

Note:

For sweet wines, the concentration of 5 g lanthanum per litre is sufficient provided the dilution does not bring the sugar content to below 2,5 g per litre. For wines with higher concentrations of sugar, the lanthanum concentration should be increased to 10 g per litre.

4.2. **Calibration**

Place 0, 5, 10, 15 and 20 ml of the dilute standard calcium solution (2.2) respectively into a set of 100 ml volumetric flasks, add to each flask 10 ml of the lanthanum chloride solution (2.3) and make up to 100 ml with distilled water. The solutions prepared in this way contain 0, 2,5, 5,0, 7,5 and 10 mg of calcium per litre respectively and each contains 5 g of lanthanum per litre. These solutions should be kept in polyethylene bottles.

4.3. **Determination**

Set the wavelength to 422,7 nm. Zero the absorbence scale using the solution containing 5 g of lanthanum per litre (4.2). Aspirate the diluted wine directly into the burner of the spectrophotometer, followed in succession by the five standard solutions (4.2). Read the absorbences. Repeat each measurement.

5. EXPRESSION OF RESULTS

5.1. **Method of calculation**

Plot a graph giving the variation in absorbence as a function of the calcium concentration in the standard solutions.

Record the mean value of the absorbence obtained with the sample of diluted wine on this graph and determine its calcium concentra-

▼**B**

tion *C*. The calcium concentration in milligrams per litre of the wine to the nearest whole number will be 20 *C*.

5.2. Repeatability (r)

Concentration < 60 mg/l: r = 2,7 mg/l.

Concentration > 60 mg/l: r = 4 mg/l.

5.3. Reproducibility (R)

$R = 0{,}114x_i - 0{,}5$.

x_i = concentration in the sample in mg/l.

▼B

30. **IRON**

1. PRINCIPLE OF THE METHODS

Reference method

After suitable dilution of the wine and removal of alcohol, iron is determined directly by atomic absorption spectrophotometry.

Usual method

After digestion in 30 % hydrogen peroxide solution, the total iron, now in the Fe(III) state, is reduced to the Fe(II) state and is determined using the coloration produced by orthophenanthroline.

2. REFERENCE METHOD

2.1. **Reagents**

2.1.1. Concentrated standard iron solution containing 1 g Fe(III) per litre.

Use a standard commercial solution (1 g/l). This solution may be prepared by dissolving 8,6341 g of ferric ammonium sulphate ($FeNH_4(SO_4)_2 \cdot 12H_2O$) in distilled water slightly acidified with 1 M hydrochloric acid and making up to one litre.

2.1.2 Dilute standard iron solution containing 100 mg iron per litre.

2.2. **Apparatus**

2.2.1 Rotary evaporator with thermostatically controlled waterbath.

2.2.2. Atomic absorption spectrophotometer equipped with an air-acetylene burner.

2.2.3. Iron hollow cathode lamp.

2.3. **Procedure**

2.3.1. *Preparation of sample*

Remove the alcohol from the wine by reducing the volume of the sample to half its original volume using a rotary evaporator (50 to 60 ºC). Make up to the original volume with distilled water.

If necessary, dilute prior to the determination.

2.3.2. *Calibration*

Place 1, 2, 3, 4 and 5 ml of the solution containing 100 mg iron per litre (2.1.2) respectively into a set of 100 ml volumetric flasks and make up to 100 ml with distilled water. The solutions prepared in this way contain 1, 2, 3, 4 and 5 mg of iron per litre respectively.

These solutions should be kept in polyethylene bottles.

2.3.3. *Determination*

Set the wavelength to 248,3 nm. Zero the absorbence scale using distilled water. Aspirate the diluted sample directly into the burner of the spectrophotometer, followed in successionby the five standard solutions (2.3.2). Read off the absorbences. Repeat each measurement.

2.4. **Expression of results**

2.4.1. *Method of calculation*

Plot a graph giving the variation in absorbence as a function of the iron concentration in the standard solutions. Record the mean value of the absorbence obtained with the diluted wine sample on this this graph and determine its iron concentration *C.*

The iron concentration in milligrams per litre of the wine to one decimal place will be F.C, where F is the dilution factor.

▼M9

▼B

31. **COPPER**

1. PRINCIPLE OF THE METHOD

The method is based on the use of atomic absorption spectrophotometry.

2. APPARATUS

2.1. Platinum dish.

2.2. Atomic absorption spectrophotometer.

2.3. Copper hollow cathode lamp.

2.4. Gas supplies: air-acetylene or nitrous oxide/acetylene.

3. REAGENTS

3.1. Metallic copper.

3.2. Nitric acid, HNO_3, concentrated 65 %, ρ_{20} = 1,38 g/ml.

3.3. Dilute nitric acid, 1:2 (v/v).

3.4. Solution containing copper at 1 g/l.

Use a standard commercial copper solution. This solution may be prepared by weighing 1,000 g of metallic copper and transferring it without loss to a 1 000 ml volumetric flask. Add 1:2 (v/v) dilute nitric acid (3.3) in just sufficient quantity to dissolve the metal, add 10 ml of concentrated nitric acid (3.2) and make up to the mark with doubly distilled water.

3.5. Solution containing copper at 100 mg/l.

Introduce 10 ml of the solution prepared as in 3.4 into a 100 ml volumetric flask and make up to the mark with doubly distilled water.

4. PROCEDURE

4.1. **Preparation of sample and determination of copper**

If required, prepare a suitably dilute solution with doubly distilled water.

4.2. **Calibration**

Pipette 0,5, 1 and 2 ml of solution 3.5 (100 mg of copper per litre) into 100 ml volumetric flasks and make up to the volume with doubly distilled water: the solutions so obtained contain 0,5, 1 and 2 mg of copper per litre respectively.

4.3. Measure the absorbence at 324,8 nm. Set the zero with doubly distilled water. Measure directly the absorbence of successive standard solutions prepared in 4.2, Carry out in duplicate.

5. EXPRESSION OF RESULTS

5.1. **Method of calculation**

Plot a graph giving the variations in absorbence as a function of the copper concentrations in the standard solutions.

Using the measured absorbence of the samples, read off the concentration C in mg/l from the calibration curve.

If F is the dilution factor, the concentration of the copper present is given in milligrams per litre by $F \times C$. It is quoted to two decimal places.

Notes:

(a) Select the solutions for establishing the calibration curve and the dilutions of the sample appropriate to the sensitivity of the apparatus to be used and the concentration of the copper present in the sample.

(b) Proceed as follows when very low copper concentrations are expected in the sample to be analysed. Place 100 ml of the

▼**B**

sample in a platinum dish and evaporate on a waterbath at 100 ºC until it becomes syrupy. Add 2,5 ml of concentrated nitric acid (3.2) drop by drop, covering the bottom of the dish completely. Carefully ash the residue on an electric hotplate or over a low flame; then place the dish in a muffle furnace set at 500 ± 25 ºC and leave for about one hour. After cooling, moisten the ash with 1 ml of concentrated nitric acid (3.2) while crushing it with a glass rod; allow the mixture to evaporate and ash again as before. Place the dish in the muffle furnace again for 15 minutes; repeat the treatment with nitric acid at least three times. Dissolve the ash by adding 1 ml of concentrated nitric acid (3.2) and 2 ml of doubly distilled water to the dish and transfer to a 10 ml flask. Wash the dish three times using 2 ml of doubly distilled water each time. Finally, make up to the volume with doubly distilled water.

▼B

32. **CADMIUM**

1. Principle

The cadmium is determined directly in the wine by non-flame atomic absorption spectrophotometry.

2. APPARATUS

All the glassware must be washed prior to use in concentrated nitric acid heated to 70 to 80 ºC and rinsed in double distilled water.

2.1. Atomic absorption spectrophotometer equipped with a graphite oven, background correction and a multipotentiometer.

2.2. Cadmium hollow cathode lamp.

2.3. 5 µl micropipettes with special tips for atomic absorption measurements.

3. REAGENTS

The water used must be double distilled using borosilicate glass apparatus, or water of a similar purity. All reagents must be of recognized analytical reagent grade and, in particular, free of cadmium.

3.1. 85 % phosphoric acid (ρ_{20} = 1,71 g/ml).

3.2. Phosphoric acid solution obtained by diluting 8 ml of phosphoric acid with water to 100 ml.

3.3. A 0,02 M solution of di-sodium salt of ethylene diamine tetra-acetic acid (EDTA).

3.4. pH 9 buffer solution: dissolve 5,4 g of ammonium chloride in a few millilitres of water in a 100 ml volumetric flask, add 35 ml of ammonium hydroxide solution (ρ_{20} = 0,92 g/ml) diluted to 25 % (v/v) and make up to 100 ml with water.

3.5. Eriochrome black T: 1 % (w/w) solid solution in sodium chloride.

3.6. Cadmium sulphate ($CdSO_4 \cdot 8H_2O$).

The titre of the cadmium sulphate must be verified using the following method:

Weigh exactly 102,6 mg of the cadmium sulphate sample into a cylindrical vessel with some water and shake until dissolved; add 5 ml of the pH 9 buffer solution and approximately 20 mg of eriochrome black T. Titrate with the EDTA solution until the indicator begins to turn blue.

The volume of EDTA added must be equal to 20 ml. If the volume is slightly different, correct the weighed test portion of cadmium sulphate used in the preparation of the reference solution accordingly.

3.7. Cadmium reference solution at 1 g per litre.

Use a standard commercial grade solution. This solution may be obtained by dissolving 2,2820 g of cadmium sulphate in water and making up to one litre. Keep the solution in a borosilicate glass bottle with a ground glass stopper.

4. PROCEDURE

4.1. **Preparation of the sample**

Dilute the wine to 1:2 (v/v) with the phosphoric acid solution.

4.2. **Preparation of the calibration range of solutions**

Using the cadmium reference solution, prepare successive dilutions with titres of 2,5, 5, 10 and 15 µg of cadmium per litre respectively.

4.3. **Determination**

4.3.1. *Programming of oven (for guidance only):*

Drying at 100 ºC for 30 seconds

▼**B**

Mineralization at 900 ºC for 20 seconds

Atomization at 2 250 ºC for 2 to 3 seconds

Nitrogen flow (flushing gas): 6 litres/minute

Note:

At the end of the procedure, increase the temperature to 2 700 ºC to clean the oven.

4.3.2. *Atomic absorption measurements:*

Select wavelength 228,8 nm. Set the zero on the absorbence scale with double distilled water. Using a micropipette, introduce into the oven three 5-µl portions of each of the solutions in the calibration range and of the sample solution to be analysed. Record the absorbences measured. Calculate the mean absorbence value from the results for the three portions.

5. EXPRESSION OF RESULTS

5.1. **Method of calculation**

Draw the absorbence variation curve as a function of the concentrations of cadmium in the solutions in the calibration range. Variation is linear. Enter the mean absorbence value of the sample solution on the calibration curve, derive from it the cadmium concentration C. The cadmium concentration expressed in micrograms per litre of wine is equal to:

$2\,C$

▼B

33. **SILVER**

1. PRINCIPLE OF THE METHOD

The method is based on the use of atomic absorption spectrophotometry after ashing of the sample.

2. APPARATUS

2.1. Platinum dish.

2.2. Waterbath, thermostatically controlled to 100 ºC.

2.3. Furnace controlled to 500 to 525 ºC.

2.4. Atomic absorption spectrophotometer.

2.5. Silver hollow cathode lamp.

2.6. Gas supplies: air, acetylene.

3. REAGENTS

3.1. Silver nitrate, $AgNO_3$.

3.2. Nitric acid, HNO_3, concentrated 65 %, ρ_{20} = 1,38 g/ml.

3.3. Dilute nitric acid, 1:10 (v/v).

3.4. Solution containing silver at 1 g/l.

Use a standard commercial silver solution.

This solution may be prepared by dissolving 1,575 g of silver nitrate in dilute nitric acid and making up to a volume of 1 000 ml with dilute nitric acid (3.3).

3.5. Solution containing silver at 10 mg/l.

10 ml of the solution prepared as in 3.4 are diluted to 1 000 ml with dilute nitric acid.

4. PROCEDURE

4.1. **Preparation of sample**

Place 20 ml of the sample in a platinum dish and evaporate to dryness over a boiling waterbath. Ash in the furnace at 500 to 525 ºC. Moisten the white ash with 1 ml of concentrated nitric acid (3.2). Evaporate over a boiling waterbath, repeat the addition of 1 ml nitric acid (3.2) and evaporate a second time. Add 5 ml of dilute nitric acid (3.3) and heat slightly until dissolved.

4.2. **Calibration**

Pipette 2, 4, 6, 8, 10 and 20 ml of solution 3.5 (10 mg of silver per litre) respectively into a set of 100 ml volumetric flasks and make up to the mark with dilute nitric acid (3.3): the solutions so obtained contain 0,20, 0,40, 0,60, 0,80, 1,0 and 2,0 mg of silver per litre respectively.

4.3. Set the wavelength to 328,1 nm. Set zero using doubly distilled water. Measure the absorbence directly of successive standard solutions prepared in 4.2. Carry out in duplicate.

5. EXPRESSION OF RESULTS

5.1. **Method of calculation**

Plot a graph giving the variations in absorbence as a function of the silver concentrations in the standard solutions.

Using the measured absorbence of the sample, read off the concentration *C* in mg/l from the calibration curve.

The concentration of silver in the wine is given in milligrams per litre by 0,25 *C*. It is quoted to two decimal places.

Note:

Select the concentration of the solutions for the preparation of the calibration curve, the volume of the sample taken and the final

▼B

volume of the liquid to be appropriate to the sensitivity of the apparatus to be used.

▼B

34. ZINC

1. PRINCIPLE OF THE METHOD

After removal of alcohol, zinc is determined directly in the wine by atomic absorption spectrophotometry.

2. REAGENTS

The water used in borosilicate glass apparatus must be doubly distilled or of an equivalent degree of purity.

2.1. Standard solution containing 1 g of zinc per litre:

use a commercial standard zinc solution. This solution may be prepared by dissolving 4,3975 g of zinc sulphate. ($ZnSO_4 \times 7H_2O$) in water and making up the volume to one litre.

2.2. Dilute standard solution containing 100 mg of zinc per litre.

3. APPARATUS

3.1. Rotary evaporator with thermostatically controlled waterbath.

3.2. Atomic absorption spectrophotometer equipped with an air-acetylene burner.

3.3. Zinc hollow cathode lamp.

4. PROCEDURE

4.1. Preparation of sample

Remove the alcohol from 100 ml of the wine by reducing the volume of the sample to half its original volume using a rotary evaporator (50 to 60 ºC). Make up to the original volume of 100 ml with doubly distilled water.

4.2. Calibration

Place 0,5, 1, 1,5 and 2 ml of the solution containing 100 mg zinc per litre (2.2) into each one of a set of 100-ml volumetric flasks and make up to the mark with doubly distilled water. The solutions prepared in this way contain 0,5, 1, 1,5 and 2 mg of zinc per litre respectively.

4.3. Determination

Set the wavelength to 213,9 nm. Zero the absorbence scale using doubly distilled water. Aspirate the wine directly into the burner of the spectrophotometer, followed in succession by the four standard solutions. Read the absorbences. Repeat each measurement.

5. EXPRESSION OF RESULTS

5.1. Method of calculation

Plot a graph giving the variation in absorbence as a function of zinc concentration in the standard solutions. Record the mean value of the absorbence obtained with the diluted wine sample on this graph and determine its zinc concentration to one decimal place.

▼B

35. **LEAD**

1. PRINCIPLE

The lead is determined directly in the wine by non-flame atomic absorption spectrophotometry.

2. APPARATUS

All the glassware must be washed prior to use in concentrated nitric acid heated to 70 to 80 ºC and rinsed in double distilled water.

2.1. Atomic absorption spectrophotometer equipped with a graphite oven, non-specific absorption correction and a multipotentiometer.

2.2. Lead hollow cathode lamp.

2.3. 5 µl micropipettes with special tips for atomic absorption measurements.

3. REAGENTS

All reagents must be of recognized analytical reagent grade, and in particular, free of lead. The water used must be doubly distilled using borosilicate glass apparatus, or water of a similar purity.

3.1. 85 % phosphoric acid (ρ_{20} = 1,71 g/ml).

3.2. Phosphoric acid solution obtained by diluting 8 ml of phosphoric acid with water to 100 ml.

3.3. Nitric acid (ρ_{20} = 1,38 g/ml).

3.4. Lead solution at 1 g per litre.

Use a standard commercial grade solution. This solution may be obtained by dissolving 1,600 g of lead (II) nitrate, $Pb(NO_3)_2$ in nitric acid diluted to 1 % (v/v) and made up to one litre. Keep the solution in a borosilicate glass bottle with a ground glass stopper.

4. PROCEDURE

4.1. **Preparation of the sample**

Dilute the wine to 1:2 or 1:3 with the phosporic acid solution, depending on the presumed lead concentration.

4.2. **Preparation of the calibration range of solutions**

Using the lead reference solution, prepare successive solutions with titres of 2,5, 5, 10 and 15 µg of lead per litre respectively, by diluting with double distilled water.

4.3. **Determination**

4.3.1 *Programming of oven (for guidance only):*

Drying at 100 ºC for 30 seconds

Mineralization at 900 ºC for 20 seconds

Atomization at 2 250 ºC for 2 to 3 seconds

Nitrogen flow (flushing gas): 6 litres/minute.

Note:

At the end of the procedure, increase the temperature to 2 700 ºC to cleanse the oven.

4.3.2. *Measurements*

Select wavelength 217 nm. Set the zero on the absorbence scale with doubly distilled water. Using a micropipette, introduce into the programmed oven three 5-µl portions of each of the solutions in the calibration range and of the sample solution to be analysed. Record the absorbences measured. Calculate the mean absorbence value from the results for the three portions.

▼B

5. EXPRESSION OF RESULTS

5.1. Method of calculation

Draw the absorbence variation curve as a function of the concentrations of lead in the calibration range. The variation is linear. Carry over the mean absorbence value of the sample solution on the calibration curve, derive from it the lead concentration C. The lead concentration expressed in micrograms per litre of wine is equal to:

$C \times F$

Where

F = the dilution factor.

▼B

36. FLUORIDES

1. PRINCIPLE

The fluoride content of the wine, added to a buffer solution, is determined using a solid membrane selective electrode. The measured potential is proportionate to the logarithm of the activity of the fluoride ions in the medium being analysed, in accordance with the following equation: (1)

$E = E_o \pm S \log a_F$

Where

E = potential of the ion-selective electrode measured against the reference electrode in the medium being analysed;

$_o$ = standard potential of the sensor;

S = slope of the ion-selective electrode (Nernst factor). At 25 ºC the theoretical slope is equal to 59,2 mV;

a_F = activity of the fluoride ions in the solution being analysed.

2. APPARATUS

2.1. Fluoride-ion-selective crystal membrane electrode.

2.2. Reference electrode (calomel or Ag/AgCl).

2.3. Millivoltmeter (pH meter with extended scale in millivolts), accurate to 0,1 mV.

2.4. Magnetic stirrer with an insulating plate to protect the analysis solution from the heat of the motor. Stirring vessel covered with plastic (polythene or equivalent material).

2.5. Plastic beakers with a capacity of 30 or 50 ml, and plastic bottles (polythene or equivalent material).

2.6. Precision pipettes (pipettes graduated in microlitres or any other equivalent pipettes).

3. REAGENTS

3.1. Stock fluoride solution of 1 g/l.

Use standard commercial quality solution of 1 g/l. This solution can be prepared by dissolving 2,210 g of sodium fluoride (dried for three to four hours at 105 ºC) in distilled water. Make up to one litre with distilled water. The solution is kept in a plastic bottle.

3.2. Standard fluoride solutions of appropriate concentration are prepared by diluting the stock solution with distilled water and kept in plastic bottles. Solutions the fluoride content of which is in mg/l must not be prepared in advance.

3.3. Buffer solution, pH 5,5

10 g of trans-1,2-dicmiaocyclohexane tetra-acetic acid (CDTA) are added to water (about 50 ml); add a solution containing 58 g of sodium chloride and 29,4 g of trisodium citrate in 700 ml of distilled water. The CDTA is dissolved by adding approximately 6 ml of 32 % (m/v) sodium hydroxide solution.

Lastly, add 57 ml of acetic acid (ρ_{20} = 1,05 g/ml) and bring the pH to 5,5 with 32 % sodium hydroxide solution (about 45 ml). Leave to cool and make up to one litre with distilled water.

4. PROCEDURE

Preliminary comment:

Care should be taken to ensure that all the solutions remain at a temperature of 25 ºC (± 1 ºC) during measurement. (A deviation of more than 1 ºC causes a modification of about 0,2 mV.)

4.1. **Direct method**

Place a defined volume of wine in a plastic beaker with an equal volume of buffer solution.

The solution is stirred in an even and moderate manner. When the indicator is stable (stability is reached when the potential varies by

▼B

not more than 0,2 to 0,3 mV/three minutes), read the value of the potential in mV.

4.2. The known additions method

Stirring continuously, add a known volume of standard fluoride solution to the analysis medium using a precision pipette. When the indicator is stable, read the value of the potential in mV.

The concentration of the standard solution to be added is selected as follows:

(a) double or treble the fluoride concentration in the analysis medium;

(b) the volume of the analysis medium must remain practically constant (an increase in volume of 1 % or less).

(Condition (b) simplifies the calculations, see 5.)

The approximate concentration of the analysis medium is read on a calibration line drawn on a semi-logarithmic scale with the standard fluoride solutions with titres of 0,1, 0,2, 0,5, 1,0, 2,0 mg/l.

Note:

If the approximate concentration of the analysis medium lies outside the concentration range of the standard solutions, dilute the sample.

Example:

If the approximate fluoride content of the analysis medium (20 ml volume) is 0,25 mg/l F^-; the concentration must be increased by 0,25 mg/l. To do this, use the appropriate delivery pipette to add, for example, 0,20 ml (= 1 %) of a standard solution containing 25 mg/l F^- or 0,050 ml of a standard solution with 100 mg/l F^-.

5. CALCULATIONS

The fluoride content of the analysis medium expressed in mg/l is obtained by applying the following formula:

$$\mathrm{C}_F = \frac{V_a \times C_a}{V_o} \times \frac{1}{(\text{antilog } \Delta E/\ S) - 1}$$

C_F = fluoride concentration of the analysis medium (mg/l);

C_a = concentration of fluoride added (mg/l) to analysis medium (V_a);

V_o = initial volume of the analysis medium before overloading (ml);

V_a = volume of the overloaded solution (ml);

ΔE = difference between potentials E_1 and E_2 obtained in 4.1 and 4.2 (mV);

S = slope of the electrode in the analysis solution.

If V_a is very close to V_o (see 4.2), the following simplified formula is applied:

$$C_F = C_a \times \frac{1}{(antilog\ \Delta E/\ S) - 1}$$

The value obtained must be multiplied by the dilution factor arising from addition of the buffer solution.

▼B

37. CARBON DIOXIDE

1. PRINCIPLE OF METHODS

1.1. **Reference method**

1.1.1. *Still wines* (CO_2 over pressure ≤ 0,5 × 10^5 Pa) (1)

The volume of wine taken from the sample is cooled to around 0 ºC and mixed with a sufficient quantity of sodium hydroxide to give a pH of 10 to 11. Titration is carried out with an acid solution in the presence of carbonic anhydrase. The carbon dioxide content is calculated from the volume of acid needed to change the pH from 8,6 (bicarbonate form) to 4,0 (carbonic acid). A blank titration is carried out in the same conditions on decarbonated wine in order to take account of the volume of sodium hydroxide solution taken up by the wine acids.

1.1.2. *Sparkling and semi-sparkling wines*

The sample of wine to be analysed is cooled near to freezing point. After removal of a quantity to be used as a blank after decarbonation, the remainder of the bottle is made alkaline to fix all the carbon dioxide in the form of Na_2CO_3. Titration is carried out with an acid solution in the presence of carbonic anhydrase. The carbon dioxide content is calculated from the volume of acid solution needed to change the pH from 8,6 (bicarbonate form) to 4,0 (carbonic acid). A blank titration is carried out in the same conditions in decarbonated wine in order to take account of the volume of sodium hydroxide taken up by the wine acids.

1.2. **Usual method:** sparkling and semi-sparkling wines

Manometric method: the excess pressure of the carbon dioxide is measured directly in the bottle using an aphrometer.

2. REFERENCE METHOD

2.1. **Still wines** (CO_2 over pressure ≤ 0,5 × 10^5 Pa)

2.1.1. *Apparatus*

2.1.1.1. Magnetic stirrer.

2.1.1.2. pH meter.

2.1.2. *Reagents*

2.1.2.1. Sodium hydroxide solution, NaOH, 0,1 M.

2.1.2.2. Sulphuric acid solution, H_2SO_4, 0,05 M.

2.1.2.3. Carbonic anhydrase solution, 1 g/l.

2.1.3. *Procedure*

Cool the wine sample to approximately 0 ºC together with the 10 ml pipette used for sampling.

Place 25 ml of sodium hydroxide solution (2.1.2.1) in a 100 ml beaker; add two drops of aqueous solution of carbonic anhydrase (2.1.2.3). Introduce 10 ml of wine using the pipette cooled to 0 ºC.

Place the beaker on the magnetic stirrer, set up the pH electrode and stir moderately.

When the liquid has reached room temperature, titrate slowly with the sulphuric acid solution (2.1.2.2) until the pH reaches 8,6. Note the burette reading.

Continue titrating with the sulphuric acid (2.1.2.2) until the pH reaches 4,0. Let *n* ml be the volume used between pH 8,6 and 4,0.

Remove CO_2 from approximately 50 ml of the wine sample by agitation under vacuum for three minutes, the flask being heated in a waterbath to about 25 ºC.

Carry out the above procedure on 10 ml of the decarbonated wine. Let *n'* ml be the volume used.

---

(1) 10^5 pascal (Pa) = 1 bar.

▼B

2.1.4. *Expression of results*

1 ml of the titrated 0,1 M sodium hydroxide solution corresponds to 4,4 mg of CO_2.

The quantity of CO_2 in grams per litre of wine is given by the formula:

$0,44\ (n - n')$

It is quoted to two decimal places.

Note:

Where wines contain little CO_2 ($CO_2 < 1$ g/l), the addition of carbonic anhydrase to catalyse the hydration of CO_2 is unnecessary.

2.2. **Sparkling and semi-sparkling wines**

2.2.1. *Apparatus*

2.2.1.1. Magnetic stirrer.

2.2.1.2. pH meter.

2.2.2. *Reagents*

2.2.2.1. Sodium hydroxide, NaOH, 50 % (m/m).

2.2.2.2. Sulphuric acid solution, H_2SO_4, 0,05 M.

2.2.2.3. Carbonic anhydrase solution, 1 g/l.

2.2.3. *Procedure*

Mark the level of wine in the bottle and then cool until freezing begins. Allow the bottle to warm up slightly, while shaking, until ice crystals disappear. Remove the stopper rapidly and place 45 to 50 ml of wine in a measuring cylinder for blank titration. The exact volume removed, *v* ml, is determined by reading on the cylinder after it has returned to room temperature.

Immediately after the blank sample has been removed, add 20 ml of the sodium hydroxide solution (2.2.2.1) in the bottle with a capacity of 750 ml.

Wait until the wine has reached room temperature.

Place 30 ml of boiled distilled water and two drops of the carbonic anhydrase solution (2.2.2.3) into a 100 ml beaker. Add 10 ml of wine which has been made alkaline. Place the beaker on the magnetic stirrer, set up the electrode and magnetic rod and stir moderately.

Titrate with the sulphuric acid solution (2.2.2.2) slowly until the pH reaches 8,6. Note the burette reading.

Continue titrating slowly with the sulphuric acid (2.2.2.2) until the pH reaches 4,0. Let n ml be the volume used between pH 8,6 and 4,0.

Remove CO_2 from the *v* ml of wine placed on one side for the blank titration by agitating under vacuum for three minutes, the flask being heated in a waterbath at about 25 ºC. Remove 10 ml of decarbonated wine and add to 30 ml of boiled distilled water, add two to three drops of sodium hydroxide solution (2.2.2.1) to bring the pH to 10 to 11. Then follow the above procedure. Let *n′* ml be the volume of 0,5 M sulphuric acid added.

▼B

2.2.4. *Expression of results*

1 ml of 0,05 M sulphuric acid corresponds to 4,4 mg of CO_2.

Empty the bottle of wine which has been made alkaline and determine to within 1 ml the initial volume of wine by making up to the mark with water, say *V* ml.

The quantity of CO_2 in grams per litre of wine is given by the following formula:

$$0,44(n - n') \times \frac{(V - v + 20)}{V - v}$$

The result is quoted to two decimal places.

2.3. **Expression of results**

The excess pressure at 20 ºC ($Paph_{20}$) expressed in pascals is given by the formula:

$$Paph_{20} = \frac{Q}{1,951 \times 10^{-5}\ (0,86 - 0,01A)\ (1 - 0,00144S)} - Patm$$

where:

Q: CO_2 content in g/l of wine,

A: the alcoholic strength of wine at 20 ºC,

S: the sugar content of the wine in g/l,

*P*atm: the atmospheric pressure, expressed in pascals.

▼M9

▼B

4. RELATIONSHIP BETWEEN THE PRESSURE AND THE QUANTITY OF CARBON DIOXIDE CONTAINED IN A SEMI-SPARKLING WINE (1)

From the excess pressure at 20 ºC ($Paph_{20}$), the absolute pressure at 20 ºC ($Pabs_{20}$) is calculated using the formula:

$Pabs_{20} = Patm + Paph_{20}$

where Patm is the atmospheric pressure expressed in bars.

The quantity of carbon dioxide contained in a wine is given by the following relationships:

- in litres of CO_2 per litre of wine:

  $0,987 \times 10^{-5}\ Pabs_{20}\ (0,86 - 0,01A)\ (1 - 0,00144S)$,

- in grams of CO_2 per litre of wine:

  $1,951 \times 10^{-5}\ Pabs_{20}\ (0,86 - 0,01A)\ (1 - 0,00144S)$,

  where *A* is the alcoholic strength of the wine at 20 ºC,

  S is the sugar content of the wine in grams per litre.

---

(1) No account is taken of other gases present (O_2, N_2, etc.) in amounts that are too small to have any effect on the excess pressure.

▼B

Table I

Ratio of the excess pressure $Paph_{20}$ in a sparkling or semi-sparkling wine at 20 °C to the excess pressure $Paph_{t}$ at a temperature

0	1,85	13	1,24
1	1,80	14	1,20
2	1,74	15	1,16
3	1,68	16	1,13
4	1,64	17	1,09
5	1,59	18	1,06
6	1,54	19	1,03
7	1,50	20	1,00
8	1,45	21	0,97
9	1,40	22	0,95
10	1,36	23	0,93
11	1,32	24	0,91
12	1,28	25	0,88

▼**M7**

38. **CYANIDE DERIVATIVES**

(*Caution:* comply with safety measures for handling chemicals chloramine T, pyridine, potassium cyanide, hydrochloric acid, and phosphoric acid. Dispose of used products in the proper way, in compliance with environmental rules in force. Caution with hydrocyanic acid released during the distillation of acidified wine.)

1. PRINCIPLE

The total free hydrocyanic acid in the wine is released by acid hydrolysis and separated by distillation. After reacting with chloramine-T and pyridine, the glutaconic dialdehyde formed is determined by colorimetry on the basis of the blue coloration it gives with 1,3-dimethyl-barbituric acid.

2. APPARATUS

2.1. **Distillation apparatus**

Use the distillation apparatus described for determining the alcohol content of wine

2.2. 500-ml round-bottomed flask with standardised ground joints

2.3. Water bath thermostatically controlled at 20 ºC

2.4. Spectrophotometer allowing absorbance to be measured at wavelength of 590 nm

2.5. Glass cells or single-use cells with optical paths of 20 mm

3. REAGENTS

3.1. Phosphoric acid (H_3PO_4) at 25 % (m/v)

3.2. Chloramine-T solution ($C_7H_7ClNNa\ O_2S, 3H_2O$) 3 % (m/v)

3.3. Solution of 1,3-dimethyl-barbituric acid: dissolve 3,658 g 1,3-dimethyl-barbituric acid ($C_6H_8N_2O_3$) in 15 ml pyridine and 3 ml hydrochloric acid (ρ_{20}=1,19 g/ml) and add 50 ml distilled water

3.4. Potassium cyanide (KCN)

3.5. Potassium iodide (KI) solution at 10 % (m/v)

3.6. Silver nitrate solution ($AgNO_3$), 0,1 M

4. PROCEDURE

4.1. **Distillation**

Put 25 ml wine, 50 ml distilled water, 1 ml phosphoric acid (3.1) and a few glass beads into the 500-ml flask (2.2). Place flask immediately on the distillation apparatus. Use a tapering tube to conduct the distillate into a 50-ml calibrated flask containing 10 ml water. Immerse calibrated flask in iced water. Collect 30 to 35 ml of distillate (or around 45 ml liquid in total) in the calibrated flask.

Flush the tapering tube of the condenser with a few millilitres of distilled water, bring distillate to 20 ºC and fill to the calibration line with distilled water.

4.2. **Measurement**

Put 25 ml distillate in a 50-ml conical flask with a ground glass stopper, add 1 ml chloramine-T solution (3.2) and seal with the stopper. After exactly 60 seconds add 3 ml of 1,3-dimethyl-barbituric acid solution (3.3), seal with stopper and leave for 10 minutes. Then measure absorbance against a control (25 ml distilled water instead of 25 ml distillate) at wavelength of 590 nm in cells with optical paths of 20 mm.

5. DETERMINING THE CALIBRATION CURVE

5.1. **Argentometric titration of the potassium cyanide**

Dissolve around 0,2 g KCN (3.4), carefully measured, in 100 ml distilled water in a 300-ml calibrated flask. Add 0,2 ml of potassium iodide solution (3.5) and titrate with the 0,1 M silver nitrate solution (3.6) until a stable yellowish colouring is obtained.

Taking 1 ml of 0,1 M silver nitrate solution as corresponding to 13,2 mg KCN, calculate the concentration of the KCN sample.

5.2. **Standard curve**

5.2.1. *Preparation of standard solutions*

Having established the concentration of the KCN according to the procedure set out in 5.1, prepare a standard solution containing 30 ml/l hydrocyanic acid (30 ml HCN ≅ 72,3 ml KCN). Dilute the solution to 1/10.

Introduce 1,0 ml, 2,0 ml, 3,0 ml, 4,0 ml and 5,0 ml of the diluted sample solution into the 100-ml calibrated flasks and fill to the calibration line with distilled water. The solutions prepared contain 30 µg, 60 µg, 90 µg, 120 µg and 150 µg of hydrocyanic acid per litre respectively.

5.2.2. *Titration*

Take 25-ml samples of the solutions thus obtained and continue as indicated above at 4.1 and 4.2.

The values obtained for absorbance with regard to the standard solutions as a function of the corresponding hydrocyanic acid content should produce a straight line passing through the origin.

6. EXPRESSION OF RESULTS

The hydrocyanic acid is expressed in micrograms per litre (µg/l) with no decimal places.

6.1. **Method of calculation**

Read off the hydrocyanic acid content on the calibration curve. If the sample has been diluted, multiply the result by the dilution factor.

Repeatability (r) and reproducibility (R)

White wine = r = 3,1 µg/l or approximately 6 %· x_i
R = 12 µg/l or approximately 25 %· x_i
Red wine = r = 6,4 µg/l or approximately 8 %· x_i
R = 23 µg/l or approximately 29 %· x_i

x_i = average concentration of HCN in the wine
x_i = 48,4 µg/l for white wine
x_i = 80,5 µg/l for red wine.

▼B

39. **ALLYL ISOTHIOCYANATE**

1. PRINCIPLE OF THE METHOD

Any allyl isothiocyanate present in the wine is collected by distillation and identified by gas chromatography.

2. REAGENTS

2.1. Ethanol, absolute.

2.2. Standard solution: solution of allyl isothiocyanate in absolute alcohol containing 15 mg of allyl isothiocyanate per litre.

2.3. Freezing mixture consisting of ethanol and dry ice (temperature − 60 ºC).

3. APPARATUS

3.1. Distillation apparatus as shown in the figure overleaf. A stream of nitrogen is passed continuously through the apparatus.

3.2. Heating mantle, thermostatically controlled.

3.3. Flowmeter.

3.4. Gas chromatograph fitted with a flame spectrophotometer detector equipped with a selective filter for sulphur compounds (wavelength = 394 nm) or any other suitable detector.

3.5. Stainless steel chromatograph column of internal diameter 3 mm and length 3 m filled with Carbowax 20M at 10 % on Chromosorb WHP, 80 to 100 mesh.

3.6. Microsyringe, 10µl.

4. PROCEDURE

Put two litres of wine into the distillation flask, introduce a few millilitres of ethanol (2.1) into the two collecting tubes so that the porous parts of the gas dispersion rods are completely immersed. Cool the two tubes externally with the freezing mixture. Connect the flask to the collecting tubes and begin to flush the apparatus with nitrogen at a rate of three litres per hour. Heat the wine to 80 ºC with the heating mantle, distil and collect 45 to 50 ml of the distillate.

Stabilize the chromatograph. It is recommended that the following conditions are used:

— injector temperature: 200 ºC,

— column temperature: 130 ºC,

— helium carrier gas flow rate: 20 ml per minute.

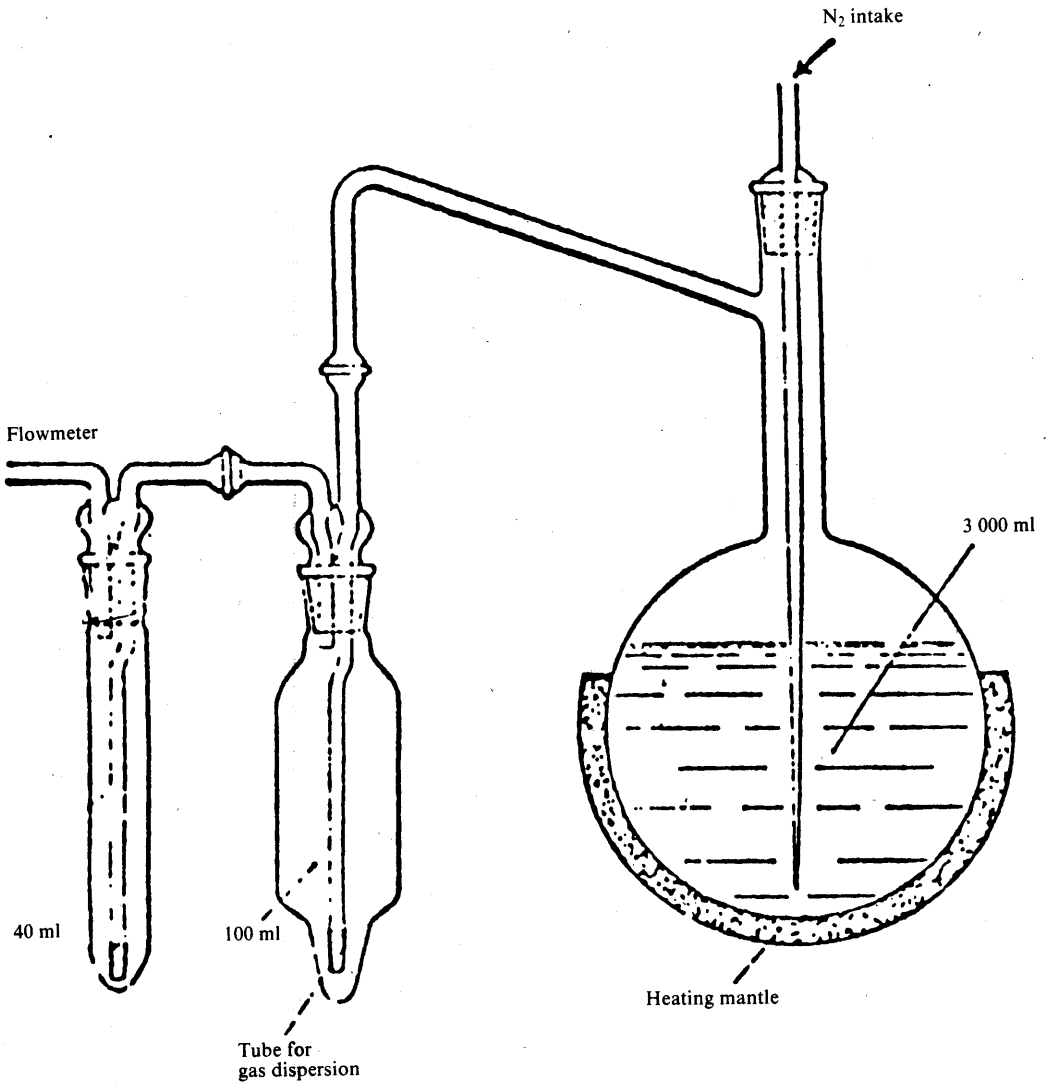
With the microsyringe, introduce a volume of the standard solution such that the peak corresponding to the allyl isothiocyanate can easily be identified on the gas chromatogram.

Similarly introduce an aliquot of the distillate into the chromatograph. Check that the retention time of the peak obtained corresponds with that of the peak of allyl isothiocyanate.

Under the conditions described above, compounds naturally present in the wine will not produce interfering peaks on the chromatogram of the sample solution.

▼**B**

Apparatus for distillation under a current of nitrogen



▼**B**

40. **CHROMATIC PROPERTIES**

1. WINES AND MUSTS

1.1. **Definitions**

The chromatic properties of a wine are defined as its luminosity and its chromaticity.

The *luminosity* is represented by the transmittance and it varies inversely with the colour intensity of the wine.

The *chromaticity* is represented by the dominant wavelength (which characterizes the tint) an the purity of the colour.

By convention, and for reasons of convenience, the chromatic properties of red and rosé wines are given as the colour intensity and the tint, in keeping with a procedure adopted as the usual method.

1.2. **Principle of the methods**

1.2.1. *Reference method*

This is a spectrophotometric method which makes it possible to determine the tristimulus values and three chromaticity coordinates necessary for the specification of the colour as laid down by the International Commission on Illumination (CIE).

1.2.2. *Usual method (applicable to red and rosé wines)*

This is a spectrophotometric method by which the chromatic properties are expressed by convention as follows:

The *colour intensity* is given by the sum of the absorbences at wavelengths af 420, 520 and 620 nm for radiation traversing a 1 cm optical path in the sample.

The *tint* is expressed by the ratio of the absorbences at 420 nm and 520 nm.

1.3. **Reference method**

1.3.1. *Apparatus*

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

1.3.1.2. Glass cells in pairs, with optical paths, b, equal to 0,1, 0,2, 0,5, 1,2 and 4 cm.

1.3.2. *Procedure*

1.3.2.1. Preparation of the sample

Cloudy wine must be clarified by centrifugation. The bulk of the carbon dioxide in young and sparkling wines must be removed by shaking under vacuum.

1.3.2.2. Measurements

The optical path, b, in the glass cell should be so chosen that the measured absorbence lies between 0,3 and 0,7.

The following guidance is given for the appropriate choice of the optical path: use cells of 2 (or 4) cm optical path for white wines, with 1 cm for rosé wines and with 0,1 cm (or 0,2 cm) for red wines.

The spectrophotometric measurements should be made using distilled water, in a cell with the same optical path, b, as reference liquid to establish the zero of the absorbence scale at wavelengths 445, 495, 550 and 625 nm.

The four corresponding absorbences for the wine should then be measured to three decimal places for the optical path, b. Let these be A_{445}, A_{495}, A_{550}, A_{625}.

1.3.3. *Calculations*

Together with Table I, use these values of the absorbences for the optical path, b cm to obtain the corresponding transmittances (T%). Let these be T_{445}, T_{495}, T_{550} and T_{625}.

- Calculate the tristimulus values X, Y and Z expressed as decimal fractions from the following expressions:

▼B

$X = 0{,}42\ T_{625} + 0{,}35\ T_{550} + 0{,}21\ T_{445}$

$Y = 0{,}20\ T_{625} + 0{,}63\ T_{550} + 0{,}17\ T_{495}$

$Z = 0{,}24\ T_{495} + 0{,}94\ T_{445}$

— Calculate the chromaticity coordinates x and y from:

$$x = \frac{X}{X+Y+Z} \qquad y = \frac{Y}{X+Y+Z}$$

1.3.4. *Expression of results*

1.3.4.1. The relative luminosity is given by the value of Y expressed as a percentage. (For complete darkness, $Y = 0\ \%$; for colourless liquids, $Y = 100\ \%$.)

1.3.4.2. The chromaticity is expressed by the dominant wavelength and the purity.

The determination of these two quantities makes use of the chromaticity diagram bounded by the spectral locus as given in Figure 1. The point O plotted in this diagram represents the white light source used and has the coordinates of a standard source, C, $x_o = 0{,}3101$ and $y_o = 0{,}3163$, representing daylight of average brightness.

— Dominant wavelength

Plot the point C with coordinates x, y on the chromaticity diagram.

If C is outside the triangle AOB, draw the straight line joining O to C and extend it to cut the spectral locus at the point S, which corresponds to the dominant wavelength.

If C is inside the triangle AOB, draw the straight line from C to O and extend it to intersect the spectral locus at a point corresponding to the wavelength of the colour complementary to that of the wine. This wavelength is denoted by its value followed by the letter C.

— Purity

If the point C is outside the triangle AOB, the purity is given as a percentage by the ratio:

$$100 \times \frac{\text{distance from C to O}}{\text{distance from O to S}}$$

If the point C is inside the triangle AOB, the purity is given as a percentage by the ratio:

$$100 \times \frac{\text{distance from C to O } (^1)}{\text{distance from O to P}}$$

where P is the point where the straight line OC cuts the line of purples (line AB).

Purity is also given directly by chromaticity diagrams from the known values of x and y (Figures 2, 3, 4, 5 and 6).

1.3.4.3. Results

The colour of a wine is completely defined by its luminosity, its chromaticity (expressed by the dominant wavelength) and its purity.

These should be indicated in the analysis report with the value of the optical path in which the measurements were made.

▼M9

---

(1) This distance must be given in a direction going from O to C.

▼**B**

TABLE 1

Transformation of absorbences to transmittances (T%)

Method of use

Find the first decimal figure of the absorbence in the first vertical column, and call its row R. Find the second decimal figure of the absorbence in the top horizontal row and call its column C. Read the figure in the box at the intersection of the row R and the column C. To calculate the transmittance, divide this figure by 10 if the absorbence is less than 1, by 100 if it lies between 1 and 2, and by 1 000 if it lies between 2 and 3.

Note:

The figure in the top right-hand corner of each box enables the third decimal figure of the absorbence to be taken into account by interpolation.

	0	1	2	3	4	5	6	7	8	9
0	1000^{23}	977^{22}	955^{22}	933^{21}	912^{21}	891^{20}	871^{20}	851^{19}	832^{19}	813^{19}
1	794^{18}	776^{18}	759^{17}	741^{17}	724^{16}	708^{16}	692^{16}	676^{15}	661^{15}	646^{15}
2	631^{14}	617^{14}	603^{14}	589^{14}	575^{13}	562^{13}	549^{13}	537^{12}	525^{12}	513^{12}
3	501^{11}	490^{11}	479^{11}	468^{11}	457^{10}	447^{9}	436^{9}	427^{10}	417^{10}	407^{9}
4	398^{9}	389^{9}	380^{9}	371^{8}	363^{8}	355^{8}	347^{8}	339^{8}	331^{7}	324^{8}
5	316^{7}	309^{7}	302^{7}	295^{7}	288^{6}	282^{7}	275^{6}	269^{6}	263^{6}	257^{6}
6	251^{6}	245^{5}	240^{6}	234^{5}	229^{5}	224^{5}	219^{5}	214^{5}	209^{5}	204^{5}
7	199^{4}	195^{5}	190^{4}	186^{4}	182^{4}	178^{4}	174^{4}	170^{4}	166^{4}	162^{4}
8	158^{3}	155^{4}	151^{3}	148^{4}	144^{4}	141^{3}	138^{3}	135^{3}	132^{3}	129^{3}
9	126^{3}	123^{3}	120^{3}	117^{2}	115^{3}	112^{2}	110^{3}	107^{2}	105^{3}	102^{2}

Example:

Absorbence	0,47	1,47	2,47	3,47
T %	33,9	3,4	0,3	0

Transmittances *T* % are to be expressed to the nearest 0,1 %.

▼B

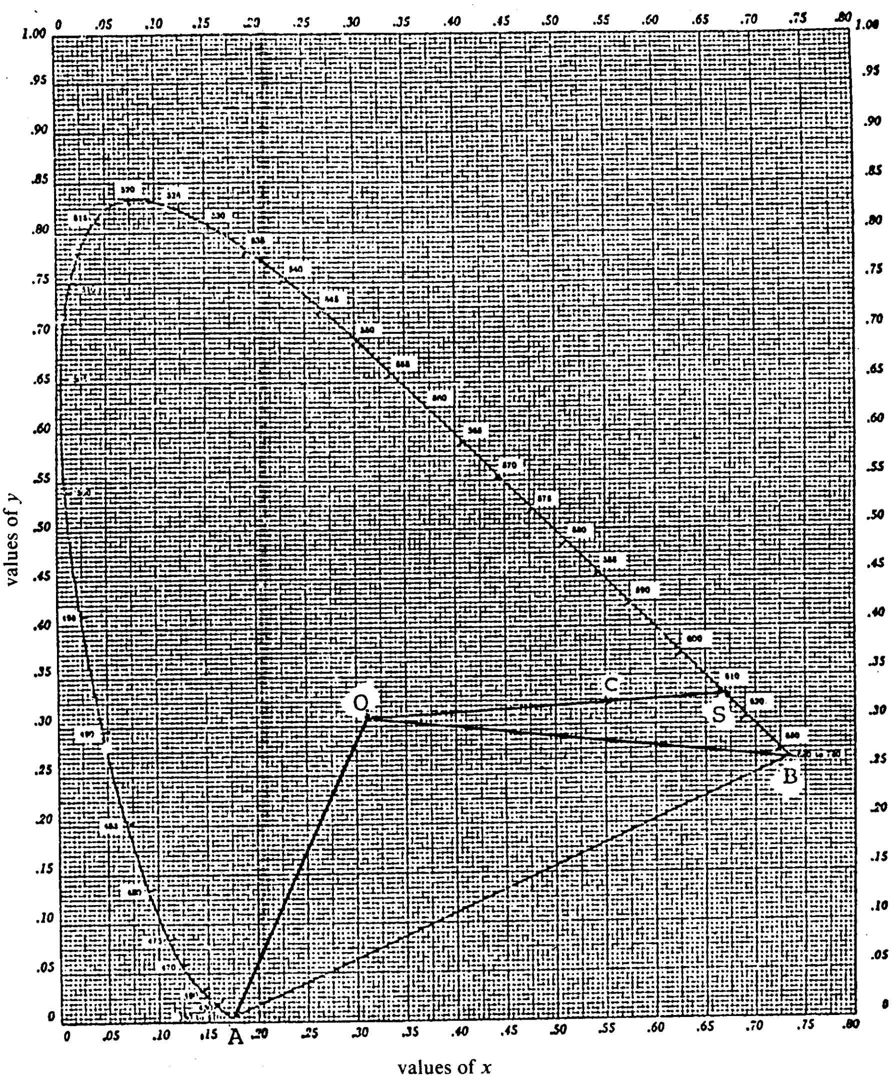


FIGURE 1

Chromaticity diagram including all colours in the spectrum

▼**B**

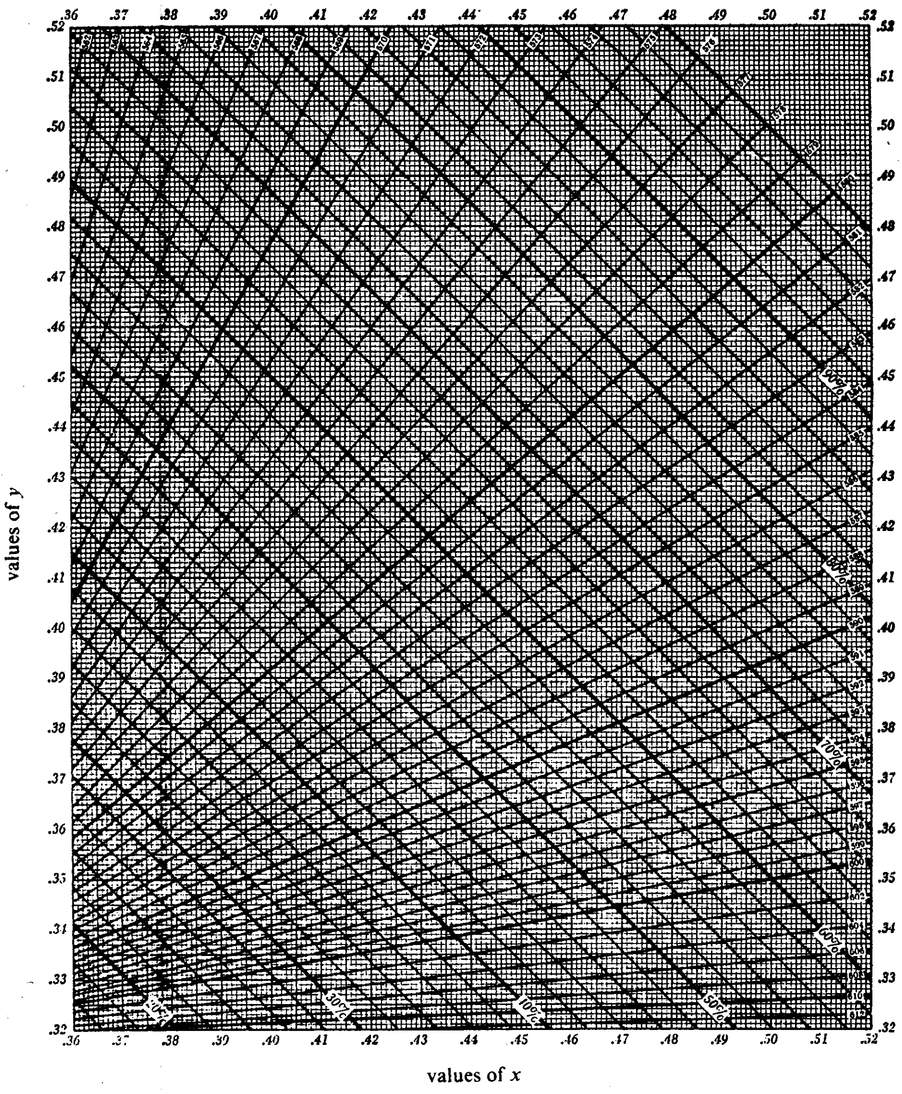


FIGURE 2

Chromaticity diagram for unadulterated red (bright red) wines and red-brown (brick red) wines

▼B

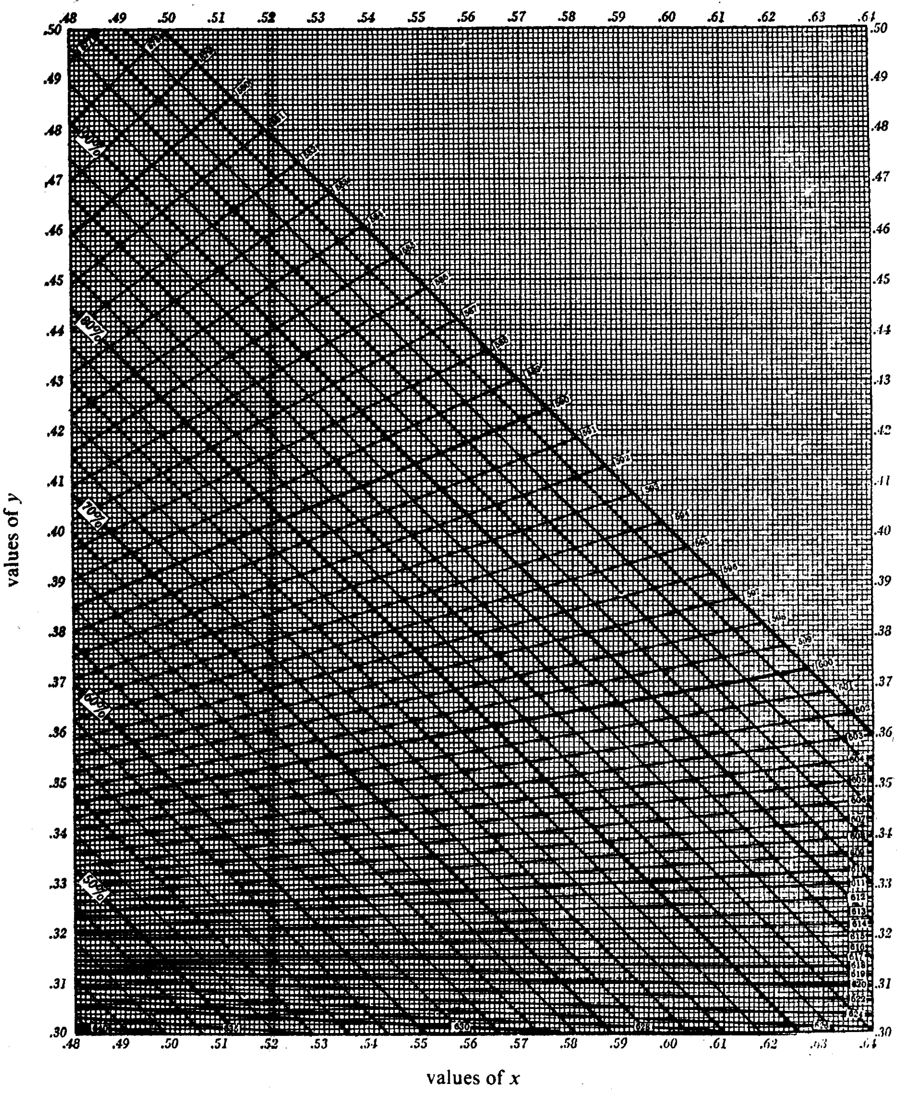


FIGURE 3

Chromaticity diagram for unadulterated red (bright red) wines and red-brown (brick red) wines

▼B

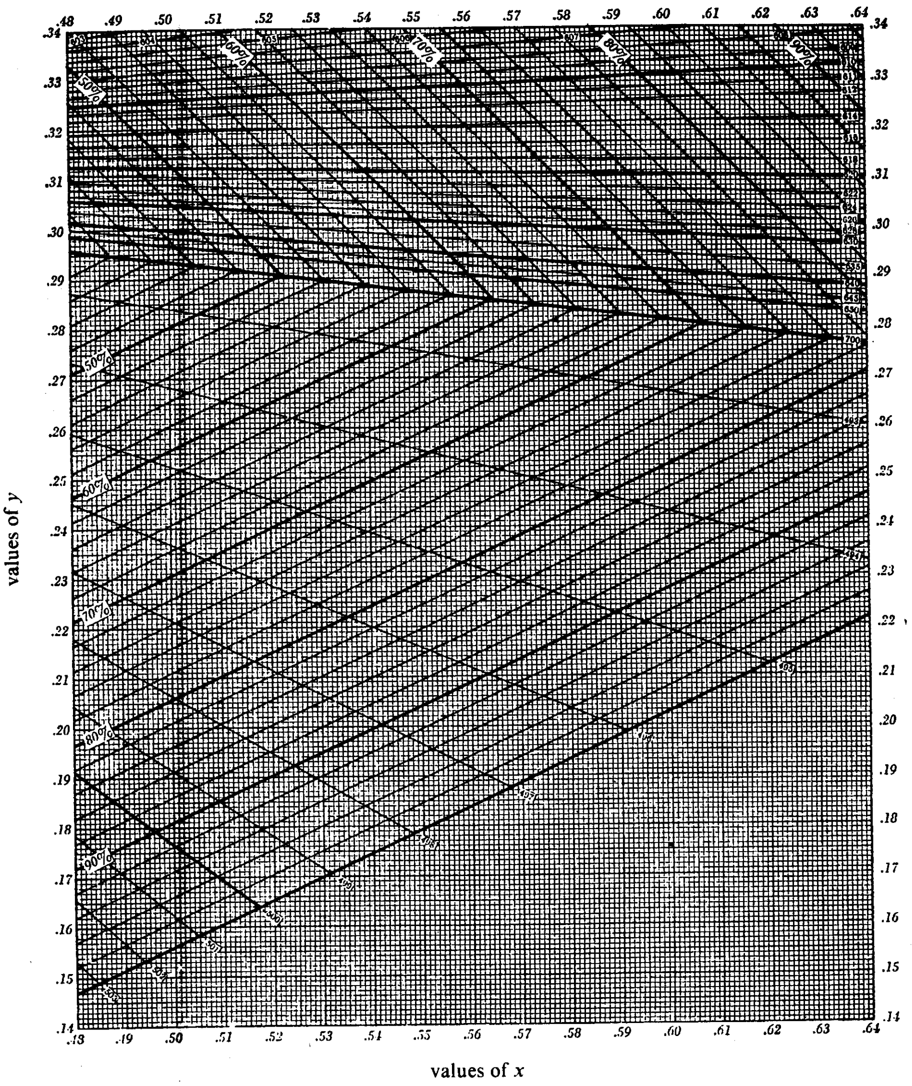


FIGURE 4

Chromaticity diagram for unadulterated red (bright red) wines and purple wines

▼B

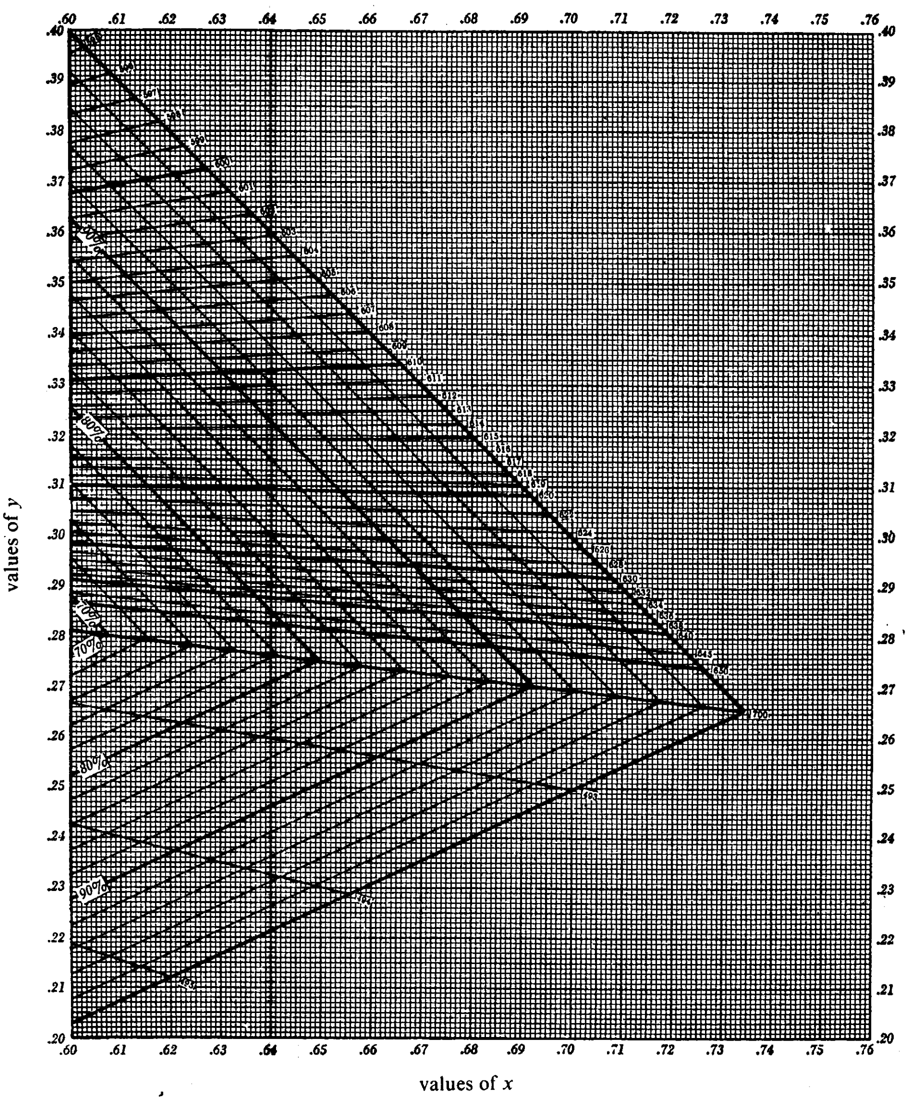


FIGURE 5

Chromaticity diagram for unadulterated red (bright red) wines and purple wines

▼B

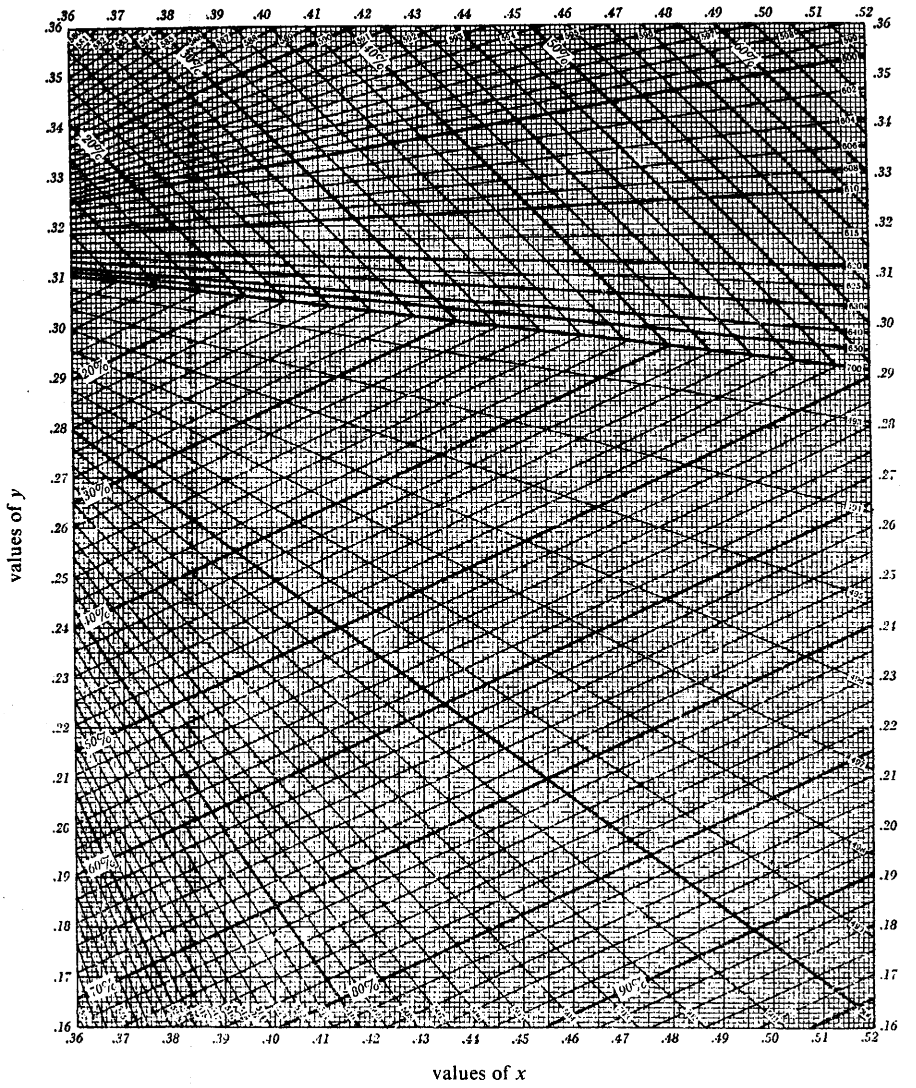


FIGURE 6

Chromaticity diagram for red-brown (brick red) wines and purple wines

▼B

2. RECTIFIED CONCENTRATED MUSTS

2.1. Principle of the method

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

2.2. Apparatus

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

2.2.2. Glass cells with optical paths of 1 cm.

2.2.3. Membrane filter of pore diameter 0,45 µm.

2.3. Procedure

2.3.1. *Preparation of the sample*

Use the solution with a sugar concentration of 25 % (m/m) (25º Brix) prepared as described in the chapter ‘pH’, section 4.1.2. Filter is through a membrane filter of pore diameter 0,45 µm.

2.3.2. *Determination of absorbence*

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

Measure the absorbence A at the same wavelength of the solution containing 25 % sugar (25º Brix) prepared as in 2.3.1 and placed in a cell with an optical path of 1 cm.

2.4. Expression of results

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

▼B

41. FOLIN-CIOCALTEU INDEX

1. DEFINITION

The Folin-Ciocalteu index is the result obtained from the application of the method described below.

2. PRINCIPLE OF THE METHOD

All the phenolic compounds contained in the wine are oxidized by the Folin-Ciocalteu reagent. This reagent is formed from a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$) which, after oxidation of the phenols, is reduced to a mixture of the blue oxides of tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}).

The blue coloration produced has a maximum absorption in the region of 750 nm, and it is proportional to the total quantity of phenolic compounds originally present.

3. REAGENTS

These must be of analytical reagent quality. The water used must be distilled or water of equivalent purity.

3.1. **Folin-Ciocalteu reagent**

This reagent is available commercially in a form ready for use. It may be prepared as follows: dissolve 100 g of sodium tungstate ($Na_2WO_4 \cdot 2H_2O$) and 25 g of sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) in 700 ml of distilled water. Add 50 ml of 85 % phosphoric acid (ρ_{20} = 1,71 g/ml) and 100 ml of concentrated hydrochloric acid (ρ_{20} = 1,19 g/ml). Bring to the boil and boil for 10 hours under reflux conditions. Then add 150 g of lithium sulphate ($Li_2SO_4 \cdot H_2O$) and a few drops of bromine and boil once more for 15 minutes. Allow to cool and make up to one litre with distilled water.

3.2. Anhydrous sodium carbonate, Na_2CO_3, made up into a 20 % m/v solution.

4. APPARATUS

Normal laboratory apparatus, particularly:

4.1. 100 ml volumetric flasks.

4.2. Spectrophotometer capable of operating at 750 nm.

5. PROCEDURE

5.1. **Red wine**

Introduce the following into a 100 ml volumetric flask (4.1) strictly in the order given:

1 ml of the wine, previously diluted 1:5,
50 ml of distilled water,
5 ml of Folin-Ciocalteu reagent (3.1),
20 ml of sodium carbonate solution (3.2).

Make up to 100 ml with distilled water.

Stir to homogenize. Wait 30 minutes for the reaction to stabilize. Determine the absorbence at 750 nm through a path length of 1 cm with respect to a blank prepared with distilled water in place of the wine.

If the absorbence is not around 0,3 an appropriate dilution should be made.

5.2. **White wine**

Carry out the same procedure with 1 ml of undiluted wine.

▼B

5.3. **Rectified concentrated must**

5.3.1. *Preparation of sample*

Use the solution with a sugar concentration of 25 % (m/m) (25º Brix) prepared as described in the chapter 'pH', section 4.1.2.

5.3.2. *Measurement*

Proceed as described for the case of red wine (5.1) using a 5 ml sample prepared as described in 5.3.1 and measuring the absorbence with respect to a control prepared with 5 ml of a 25 % (m/m) invert sugar solution.

6. EXPRESSION OF RESULTS

6.1. Method of calculation

The result is expressed in the form of an index obtained by multiplying the absorbence by 100 for red wines diluted 1:5 (or by the corresponding factor for other dilutions) and by 20 for white wines. For rectified concentrated musts, multiply by 16.

6.2. Repeatability

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 1.

Good repeatability of results is achieved by using scrupulously clean apparatus (volumetric flasks and spectrophotometer cells).

▼B

42. SPECIAL METHODS OF ANALYSIS FOR RECTIFIED CONCENTRATED GRAPE MUST

(a) TOTAL CATIONS

1. PRINCIPLE OF THE METHOD

The test sample is treated by a strongly acid cation exchanger. The cations are exchanged with H^+. Total cations are expressed by the difference between the total acidity of the effluent and that of the test sample.

2. APPARATUS

2.1. Glass column of internal diameter 10 to 11 mm and length approximately 300 mm, fitted with a drain tap.

2.2. pH meter with a scale graduated at least in 0,1 pH units.

2.3. Electrodes:

- glass electrode, kept in distilled water,
- calomel/saturated potassium chloride reference electrode, kept in a saturated solution of potassium chloride,
- or a combined electrode, kept in distilled water.

3. REAGENTS

3.1. Strongly acid cation exchange resin in H^+ form pre-swollen by soaking in water overnight.

3.2. Sodium hydroxide solution, 0,1 M.

3.3. Paper pH indicator.

4. PROCEDURE

4.1. **Preparation of sample**

Use the solution obtained by diluting the rectified concentrated must to 40 % (m/v) as described in the chapter ‘Total acidity’, section 5.1.2.

4.2. **Preparation of the ion exchange column**

Introduce into the column approximately 10 ml pre-swollen ion exchanger in H^+ form. Rinse the column with distilled water until all acidity has been removed, using the paper indicator to monitor this.

4.3. **Ion exchange**

Pass 100 ml of the rectified concentrated must solution prepared as in 4.1 through the column at the rate of one drop every second. Collect the effluent in a beaker. Rinse the column with 50 ml of distilled water. Titrate the acidity in the effluent (including the rinse water) with the 0,1 M sodium hydroxide solution until the pH is 7 at 20 ºC. The alkaline solution should be added slowly and the solution continuously shaken. Let n ml be the volume of 0,1 M sodium hydroxide solution used.

5. EXPRESSION OF RESULTS

The total cations are expressed in milliequivalents per kilogram of total sugar to one decimal place.

5.1. Calculations

- Acidity of the effluent expressed in milliequivalents per kilogram of rectified concentrated must:

  $E = 2{,}5n$
- Total acidity of the rectified concentrated must in milliequivalents per kilogram (see ‘Total acidity’, section 6.1.2): a
- Total cations in milliequivalents per kilogram of total sugars:

$$\frac{2{,}5n - a}{P} \times 100$$

▼B

Where

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

(b) CONDUCTIVITY

1. PRINCIPLE OF THE METHOD

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 ºC.

2. APPARATUS

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

2.2. Waterbath for bringing the temperature of samples to be analysed to approximately 20 ºC (20 ± 2 ºC).

3. REAGENTS

3.1. Demineralized water with specific conductivity below 2 µS cm^{-1} at 20 ºC.

3.2. Reference solution of potassium chloride.

Dissolve 0,581 g of potassium chloride, KCl, previously dried to constant mass at a temperature of 105 ºC, in demineralized water (3.1). Make up to one litre with demineralized water (3.1). This solution has a conductivity of 1 000 µS cm^{-1} at 20 ºC. It should not be kept for more than three months.

4. PROCEDURE

4.1. **Preparation of the sample to be analysed**

Use the solution with a total sugar concentration of 25 % (m/m) (25º Brix) as described in the chapter ‘pH’, section 4.1.2.

4.2. **Determination of conductivity**

Bring the sample to be analysed to 20 ºC by immersion in a waterbath. Maintain the temperature to within ± 0,1 ºC.

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 RESULTS

The result is expressed in microsiemens per cm (µS cm^{-1}) at 20 ºC to the nearest whole number for the 25 % (m/m) (25º Brix) solution of rectified concentrated must.

5.1. **Calculations**

If the apparatus is not provided with means for controlling the temperature, correct the measured conductivity using Table I. If the temperature is below 20 ºC, add the correction; if the temperature is above 20 ºC, subtract the correction.

TABLE I

Corrections to be made to the conductivity for temperatures different from 20 ºC (µS cm^{-1})

Conductivity	Temperature (ºC)									
	20,2 19,8	20,4 19,6	20,6 19,4	20,8 19,2	21,0 19,0	21,2 18,8	21,4 18,6	21,6 18,4	21,8 18,2	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

▼**B**

Conductivity	Temperature (°C)									
	20,2 19,8	20,4 19,6	20,6 19,4	20,8 19,2	21,0 19,0	21,2 18,8	21,4 18,6	21,6 18,4	21,8 18,2	22,0 (1) 18,0 (2)
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

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

(c) HYDROXYMETHYLFURFURAL (HMF)

1. PRINCIPLE OF THE METHODS

1.1. **Colorimetric method**

Aldehydes derived from furan, the main one being hydroxymethylfurfural, react with barbituric acid and paratoluidine to give a red compound which is determined by colorimetry at 550 nm.

1.2. **High-performance liquid chromatography (HPLC)**

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

2. COLORIMETRIC METHOD

2.1. **Apparatus**

2.1.1. Spectrophotometer for making measurements between 300 and 700 nm.

2.1.2. Glass cells with optical paths of 1 cm.

2.2. **Reagents**

2.2.1. *Barbituric acid, 0,5 % solution (m/v).*

Dissolve 500 mg of barbituric acid, $C_4O_3N_2H_4$, in distilled water and heat slightly over a waterbath at 100 °C. Make up to 100 ml with distilled water. The solution keeps for about a week.

2.2.2. *Paratoluidine solution, 10 % (m/v).*

Place 10 g of paratoluidine, $C_6H_4(CH_3)NH_2$, in a 100 ml volumetric flask; add 50 ml of isopropanol, $CH_3CH(OH)CH_3$, and 10 ml of glacial acetic acid, CH_3COOH (ρ_{20} = 1,05 g/ml). Make up to 100 ml with isopropanol. This solution should be renewed daily.

2.2.3. *Ethanal (acetaldehyde), CH_3CHO, 1 % (m/v) aqueous solution.*

Prepare just before use.

2.2.4. *Hydroxymethylfurfural, $C_6O_3H_6$, 1 g/l aqueous solution.*

Prepare successive dilutions containing 5, 10, 20, 30 and 40 mg/l. The 1 g/l and the diluted solutions must be freshly prepared.

2.3. **Procedure**

2.3.1. *Preparation of sample*

Use the solution obtained by diluting the rectified concentrated must to 40 % (m/v) as described in the chapter 'Total acidity', section 5.1.2. Carry out the determination on 2 ml of this solution.

▼B

2.3.2. *Colorimetric determination*

Into each of two 25 ml flasks *a* and *b* fitted with ground glass stoppers place 2 ml of the sample prepared as in 2.3.1. Place in each flask 5 ml of paratoluidine solution (2.2.2); mix. Add 1 ml of distilled water to flask *b* (control) and 1 ml barbituric acid (2.2.1) to flask *a*. Shake to homogenize. Transfer the contents of the flasks into spectrophotometer cells with optical paths of 1 cm. Zero the absorbence scale using the contents of flask *b* for a wavelength of 550 nm. Follow the variation in the absorbence of the contents of flask *a;* record the maximum value *A*, which is reached after two to five minutes.

Samples with hydroxymethylfurfural concentrations above 30 mg/l must be diluted before the analysis.

2.3.3. *Preparation of the calibration curve*

Place 2 ml of each of the hydroxymethylfurfural solutions with 5, 10, 20, 30 and 40 mg/l (2.2.4) into two sets of 25 ml flasks *a* and *b* and treat them as described in 2.3.2.

The graph representing the variation of absorbence with the hydroxymethylfurfural concentration in mg/l is a straight line passing through the origin.

2.4. **Expression of results**

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

2.4.1. *Method of calculation*

The hydroxymethylfurfural concentration *C* mg/l in the sample to be analysed is that concentration on the calibration curve corresponding to the absorbence *A* measured on the sample.

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

$$250 \times \frac{C}{P}$$

where

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

3. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

3.1. **Apparatus**

3.1.1. *High-performance liquid chromatograph equipped with:*

- a loop injector, 5 or 10 μl,
- spectrophotometer detector for making measurements at 280 nm,
- column of octadecyl-bonded silica (e.g. Bondapak C18 - Corasil, Waters Ass.),
- a recorder, possibly an integrator.

Flow rate of mobile phase: 1,5 ml/minute.

3.1.2. Membrane filtration apparatus, pore diameter 0,45 μm.

3.2. **Reagents**

3.2.1. Doubly distilled water.

3.2.2. Methanol, CH_3OH, distilled or HPLC quality.

3.2.3. Acetic acid CH_3COOH, (ρ = 1,05 g/ml).

3.2.4. Mobile phase: water-methanol (3.2.2)-acetic acid (3.2.3) previously filtered through a membrane filter (0,45 μm), (40:9:1 v/v).

This mobile phase must be prepared daily and outgassed befor use.

3.2.5. Reference solution of hydroxymethylfurfural, 25 mg/l (v/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 (3.2.2). Dilute this solution 1:10 with methanol (3.2.2) and filter through a membrane filter (0,45 µm).

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

3.3. **Procedure**

3.3.1. *Preparation of sample*

Use the solution obtained by diluting the rectified concentrated must to 40 % (m/v) as described in the chapter 'Total acidity', section 5.1.2, and filter it through a 0,45 µm membrane filter.

3.3.2. *Chromatographic determination*

Inject 5 (or 10) µl of the sample prepared as described in 3.3.1. and 5 (or 10) µl of the reference hydroxymethylfurfural solution (3.2.5) into the chromatograph. Record the chromatogram.

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

3.4. **EXPRESSION OF RESULTS**

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

3.4.1. *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:

$$250 \times \frac{C}{P}$$

where

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

(d) HEAVY METALS

1. PRINCIPLE OF THE METHODS

I. **Rapid method for evaluation of heavy metals**

Heavy metals are revealed in the suitably diluted rectified concentrated must by the coloration produced by the formation of sulphides. They are assessed by comparison with a standard lead solution corresponding to the maximum admissible concentration.

II. **Determination of lead content by atomic absorption spectrophotometry**

The chelate given by lead with ammonium pyrrolidinedithiocarbamate is extracted with methylisobutylketone and the absorbence measured at 283,3 nm. The lead content is determined by using known additional amounts of lead in a set of reference solutions.

2. RAPID METHOD FOR EVALUATION OF HEAVY METALS

2.1. **Reagents**

2.1.1. *Dilute hydrochloric acid, 70 % (m/v).*

Take 70 g of hydrochloric acid, HCl (ρ_{20} = 1,16 to 1,19 g/ml), and make up to 100 ml with water.

2.1.2. *Dilute hydrochloric acid, 20 % (m/v).*

Take 20 g of hydrochloric acid, HCl (ρ_{20} = 1,16 to 1,19 g/ml), and make up to 100 ml with water.

2.1.3. Dilute ammonia. Take 14 g of ammonia, NH_3 (ρ_{20} = 0,931 to 0,934 g/ml) and make up to 100 ml with water.

▼**B**

2.1.4. *pH 3,5 buffer solution.*

Dissolve 25 g of ammonium acetate CH_3COONH_4, in 25 ml of water and add 38 ml of dilute hydrochloric acid (2.1.1). Adjust the pH if necessary with the dilute hydrochloric acid (2.1.2) or the dilute ammonia (2.1.3) and make up to 100 ml with water.

2.1.5. Thioacetamide solution C_2H_5 SN, 4 % (m/v).

2.1.6. Glycerol solution, $C_3H_8O_3$, 85 % (m/v), ($n_D^{20\,°C}$ = 1,449 to 1,455).

2.1.7. *Thioacetamide reagent.*

To 0,2 ml of thioacetamide solution (2.1.5) add 1 ml of a mixture of 5 ml of water, 15 ml of 1 M sodium hydroxide solution and 20 ml of glycerol (2.1.6). Heat on a waterbath at 100 °C for 20 seconds. Prepare just before use.

2.1.8. *Solution containing 0,002 g/l of lead.*

Prepare a 1 g/l lead solution by dissolving 0,400 g of lead nitrate, $Pb(NO_3)_2$, in water and making up to 250 ml with water. At the time of use, dilute this solution with water to two parts in 1 000 (v/v) in order to obtain a 0,002 g/l solution.

2.2. Procedure

Dissolve a test sample of 10 g of the rectified concentrated must in 10 ml of water. Add 2 ml of the pH 3,5 buffer solution (2.1.4); mix. Add 1,2 ml of the thioacetamide reagent (2.1.7). Mix at once. Prepare the control under the same conditions by using 10 ml of the 0,002 g/l lead solution (2.1.8).

After two minutes, any brown coloration of the rectified concentrated must solution should not be more intense than that of the control.

2.3. Calculations

Under the conditions of the above procedure, the control sample corresponds to a maximum admissible heavy metal concentration expressed as lead of 2 mg/kg of rectified concentrated must.

3. DETERMINATION OF LEAD CONTENT BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

3.1. Apparatus

3.1.1. Atomic absorption spectrophotometer equipped with an air-acetylene burner.

3.1.2. Lead hollow cathode lamp.

3.2. Reagents

3.2.1. *Dilute acetic acid.*

Take 12 g of glacial acetic acid (ρ = 1,05 g/ml) and make up to 100 ml with water.

3.2.2. Solution of ammonium pyrrolidinedithiocarbamate, $C_5H_{12}N_2S_2$, 1 % (m/v).

3.2.3. Methylisobutylketone, $(CH_3)_2CHCH_2COCH_3$.

3.2.4. Solution containing 0,010 g/l of lead.

Dilute the 1 g/l lead solution (of 2.1.8) to 1 % (v/v).

3.3. Procedure

3.3.1. *Preparation of solution to be examined*

Dissolve 10 g of rectified concentrated must in a mixture of equal volumes of dilute acetic acid (3.2.1) and water, and make up to 100 ml with this mixture.

Add 2 ml of ammonium pyrrolidinedithiocarbamate solution (3.2.2) and 10 ml of methylisobutylketone (3.2.3). Shake for 30 seconds while protected from bright light. Leave the two layers to separate. Use the methylisobutylketone layer.

▼B

3.3.2. *Preparation of reference solutions*

Prepare three reference solutions containing, in addition to 10 g of rectified concentrated must, 1, 2 and 3 ml respectively of the solution containing 0,010 g/l of lead (3.2.4). Treat these in the same way as the solution to be examined.

3.3.3. *Control*

Prepare a control by proceeding under the same conditions as in 3.3.1, but without the addition of the rectified concentrated must.

3.3.4. *Determination*

Set the wavelength to 283,3 nm.

Atomize the methylisobutylketone from the control sample in the flame and zero the absorbence scale.

By operating with their respective solvent extracts, determine the absorbences of the solution to be examined and the reference solutions.

3.4. **Expression of results**

Express the lead content in milligrams per kilogram of rectified concentrated must to one decimal place.

3.4.1. *Calculations*

Plot the curve giving the variation in absorbence as a function of the lead concentration added to the reference solutions, zero concentration corresponding to the solution to be examined.

Extrapolate the straight line joining the points until it cuts the negative part of the concentration axis. The distance of the point of intersection from the origin gives the lead concentration in the solution to be examined.

(e) CHEMICAL DETERMINATION OF ETHANOL

This method is used for the determination of the alcoholic strength of low-alcohol liquids such as musts, concentrated musts and rectified concentrated musts.

1. PRINCIPLE OF THE METHOD

Simple distillation of the liquid. Oxidation of the ethanol in the distillate by potassium dichromate. Titration of the excess dichromate with an iron (II) solution.

2. APPARATUS

2.1. Use the distillation apparatus described in the chapter ‘Alcoholic strength by volume’, section 3.2.

3. REAGENTS

3.1. **Potassium dichromate solution.**

Dissolve 33,600 g of potassium dichromate, $K_2Cr_2O_7$, in sufficient quantity of water to make one litre of solution at 20 °C.

One millilitre of this solution oxidizes 7,8924 mg of alcohol.

3.2. **Iron (II) ammonium sulphate solution.**

Dissolve 135 g of iron (II) ammonium sulphate, $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6\,H_2O$, in sufficient quantity of water to make one litre of solution and add 20 ml of concentrated sulphuric acid, H_2SO_4 (ρ_{20} = 1,84 g/ml). This solution more or less corresponds to half its volume of dichromate solution when just prepared. Subsequently, it oxidizes slowly.

3.3. **Potassium permanganate solution.**

Dissolve 1,088 g of potassium permanganate, $KMnO_4$, in a sufficient quantity of water to make one litre of solution.

▼B

3.4. **Dilute sulphuric acid, 1:2 (v/v).**

A little at a time and stirring continuously, add 500 ml of sulphuric acid, H_2SO_4 (ρ_{20} = 1,84 g/ml) to 500 ml of water.

3.5. **Ferrous orthophenanthroline reagent.**

Dissolve 0,695 g of ferrous sulphate, $FeSO_4 \cdot 7\ H_2O$, in 100 ml of water, and add 1,485 g of orthophenanthroline monohydrate, $C_{12}H_8N_2 \cdot H_2O$. Heat to help the dissolution. This bright red solution keeps well.

4. PROCEDURE

4.1. **Distillation**

Place 100 g of rectified concentrated must and 100 ml of water in the distillation flask. Collect the distillate in a 100 ml volumetric flask and make up to the mark with water.

4.2. **Oxidation**

Take a flask with a ground glass stopper and with a widened neck enabling the neck to be rinsed without loss. In the flask, place 20 ml of the titrant potassium dichromate solution (3.1) and 20 ml of the 1:2 (v/v) dilute sulphuric acid (3.4) and shake. Add 20 ml of the distillate. Stopper the flask, shake, and wait at least 30 minutes, shaking occasionally. (This is the 'measurement' flask.)

Carry out the titration of the iron (II) ammonium sulphate solution (3.2) with respect to the potassium dichromate solution by placing in an identical flask the same quantities of reagents but replacing the 20 ml of distillate by 20 ml of distilled water. (This is the 'control' flask.)

4.3. **Titration**

Add four drops of the orthophenanthroline reagent (3.5) to the contents of the 'measurement' flask. Titrate the excess dichromate by adding to it the iron (II) ammonium sulphate solution (3.2). Stop adding the ferrous solution when the mixture changes from green-blue to brown.

To judge the end-point more precisely, change the colour of the mixture back from brown to green-blue with the potassium permanganate solution (3.3). Subtract a tenth of the volume of this solution used from the volume of the iron (II) solution added. Let the difference be n ml.

Proceed in the same way with the 'control' flask. Let n' ml be the difference here.

5. EXPRESSION OF RESULTS

The ethanol is expressed in grams per kilogram of sugar and is quoted to one decimal place.

5.1. **Method of calculation**

n' ml of ferrous solution reduces 20 ml of dichromate solution which oxidizes 157,85 mg of pure ethanol.

One millilitre of iron (II) solution has the same reducing power as

$$\frac{157,85}{n'} \text{ mg of ethanol.}$$

$n - n'$ ml of iron (II) solution have the same reducing power as

$$\frac{157,85\ (n - n')}{n'} \text{ mg ethanol}$$

Ethanol concentration in g/kg of rectified concentrated must is given by:

▼B

$$7,892 \times \frac{(n' - n)}{n}$$

Ethanol concentration in g/kg of total sugars is given by:

$$789,2 \times \frac{(n' - n)}{n' P}$$

where

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

(f) MESO-INOSITOL, SCYLLO-INOSITOL AND SUCROSE

1. PRINCIPLE

Gas chromatography of silylated derivatives.

2. REAGENTS

2.1. Internal standard: xylitol (aqueous solution of about 10 g/l to which a spatula tip of sodium azide is added)

2.2. Bis(trimethylsilyl)trifluoroacetamide — BSTFA — ($C_8H_{18}F_3NOSi_2$)

2.3. Trimethylchlorosilane (C_3H_9ClSi)

2.4. Pyridine p.A. (C_5H_5N)

2.5 Meso-inositol ($C_6H_{12}O_6$)

3. APPARATUS

3.1. Gas chromatograph equipped with:

3.2. Capillary column (e.g. in fused silica, coated with OV 1, film thickness of 0,15 µm, length 25 m and internal diameter of 0,3 mm)

Operating conditions:

- carrier gas: hydrogen or helium,
- carrier gas flow rate: about 2 ml/minute,
- injector and detector temperature: 300 ºC,
- programming of temperature: 1 minute at 160 ºC, 4 ºC per minute to 260 ºC, constant temperature of 260 ºC for 15 minutes,
- splitter ratio: about 1:20.

3.3. Integrator.

3.4. Microsyringe, 10 µl.

3.5. Micropipettes, 50, 100 and 200 µl.

3.6. 2 ml flask with Teflon stopper.

3.7. Oven.

4. METHOD OF OPERATION

An accurately weighed sample of about 5 g of rectified concentrated must is placed in a 50 ml flask. 1 µl of standard solution of xylitol (2.1) is added and water added to capacity. After mixing, 100 µl of solution is taken and placed in a flask (3.6) where it is dried under a gentle stream of air. 100 µl of absolute ethyl alcohol may be added if necessary to facilitate evaporation.

The residue is carefully dissolved in 100 µl of pyridine (2.4) and 100 µl of bis(trimethylsilyl)trifluoroacetamide (2.2) and 10 µl of trimethylchlorosilane (2.3) are added. The flask is closed with the Teflon stopper and heated at 60 ºC for one hour.

Draw off 0,5 µl of clear fluid and inject using a heated hollow needle in accordance with the stated splitter ratio.

5. CALCULATION OF RESULTS

5.1. A solution is prepared containing:

▼**B**

60 g/l of glucose, 60 g/l of fructose, 1 g/l of meso-inositol and 1 g/l of sucrose.

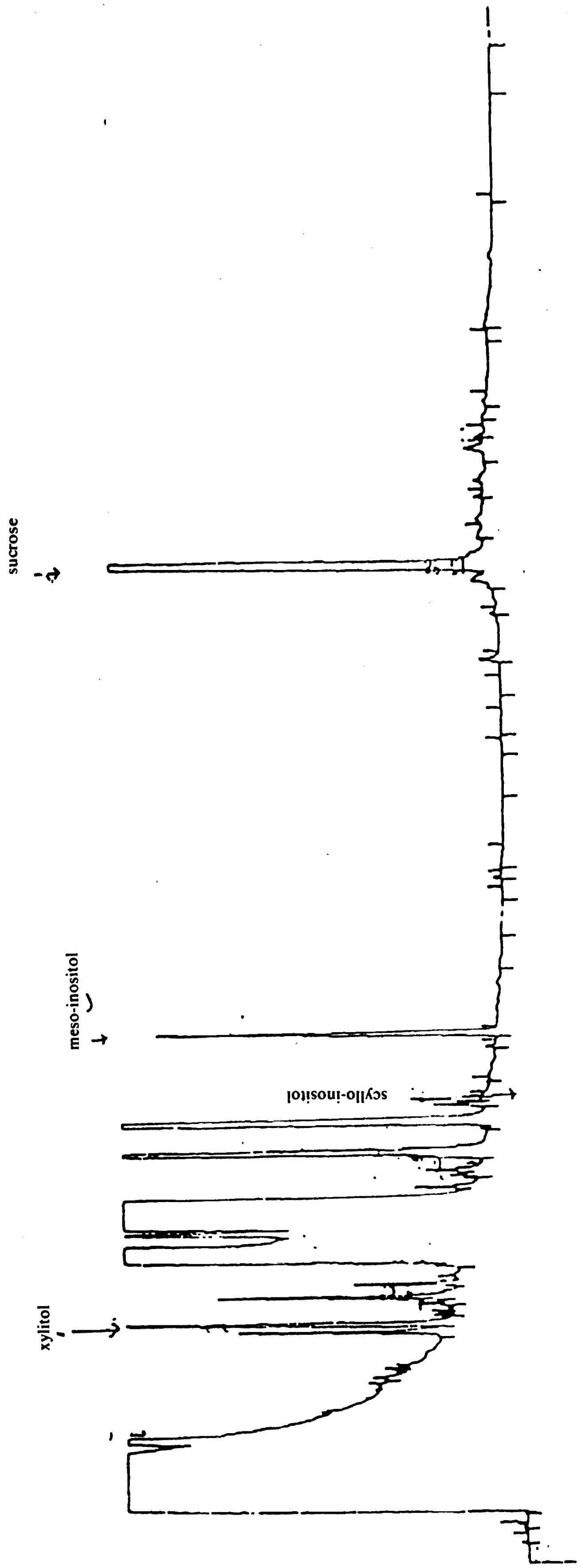
5 g of the solution is weighed and the procedure at 4 followed. The results for meso-inositol and sucrose with respect to xylitol are calculated from the chromatogram.

In the case of scyllo-inositol, which is not commercially available and has a retention time lying between the last peak of the anomeric form of glucose and the peak for meso-inositol (see diagram overleaf), the same result as for meso-inositol is taken.

6. EXPRESSION OF RESULTS

6.1. Meso-inositol and scyllo-inositol are expressed in milligrams per kilogram of sugar. Sucrose is expressed in grams per kilogram of must.

▼B



Chromatogram in gaseous phase of meso-inositol, scyllo-inositol and sucrose

▼M4

43. DETERMINATION OF THE ISOTOPIC RATIO $^{18}O/^{16}O$ OF THE WATER CONTENT IN WINES

I. DESCRIPTION OF THE METHOD

1. **Method objective**

The objective of the present method is to measure the isotopic ratio $^{18}O/^{16}O$ of waters of different origins. The isotopic ratio $^{18}O/^{16}O$ can be expressed in deviation δ ‰ in ratio to the value of isotopic ratio of the international reference V.SMOW:

$$\delta_i\ [\grave{e}0] = \left[\frac{R_i}{R_{SMOW}} - 1\right] \times 1\,000$$

2. **Principle**

The isotopic ratio $^{18}O/^{16}O$ is determined by mass spectrometry of isotopic ratios (MSIR) from ionic currents m/z 46 ($^{12}C^{16}O^{18}O$) and m/z 44 ($^{12}C^{16}O_2$) produced by carbon dioxide obtained after an exchange with the water in wine according to the reaction:

$C^{16}O_2 + H_2{}^{18}O \leftrightarrow C^{16}O^{18}O + H_2{}^{16}O$

The carbon dioxide in the gaseous phase is used for analysis.

3. **Reagents**

- Carbon dioxide for analysis
- SMOW (Standard Mean Ocean Water)
- GISP (Greenland Ice Sheet Precipitation)
- SLAP (Standard Light Arctic Precipitation)
- Reference water specific to the laboratory carefully standardized in relation to the reference sample of the International Agency of Atomic Energy in Vienna (IAEA).

4. **Laboratory equipment**

- mass spectrometer of isotopic ratios with an internal repeatability of 0,05 ‰
- triple collector for simultaneous recording of ions m/z 44, 45 and 46 or, by default, a double collector for measuring ions m/z 44 and 46
- thermostated system (± 0,5 °C) to carry out the equilibration between CO_2 and the water content in wine
- vacuum pump able to reach an internal pressure of 0,13 Pa
- phials for samples having 15 ml volume and a capillary annex tube with an interior diameter of about 0,015 mm
- Eppendorf pipette with plastic throw-away cone.

5. **Experimental determinations**

5.1. *Manual method*

Operational mode of the equilibration method

Introduction of the sample

- Take the Eppendorf pipette at the fixed volume of 1,5 ml, adapt a cone and pump the liquid to be analysed in order to insert it in a balloon flask. Then, place silicon grease around the neck of the balloon flask and attach the balloon flask to the valve while verifying that it is tightly shut,
- Repeat the operation for each balloon flask on the work ramp while introducing the laboratory's reference water into one of the balloons.

Degasing of the ramp

The two ramps are cooled down with liquid nitrogen, then the whole system is purged up to 0,1 mm Hg by opening the valves.

Then, shut the valves off and let it all heat up. The degasing cycle is repeated until there is no more pressure variation.

▼**M4**

Equilibration of the water and the CO_2

Cool the work ramps to − 70 °C (Liquid nitrogen and alcohol mix) to freeze the water and put it all in a vacuum. After stabilization of the vacuum, isolate the ramp by actioning the valve and purge the CO_2 introduction system. Insert the gaseous CO_2 into the work ramp and, after having isolated it from the rest of the system, introduce the ramp in a thermostated bath at 25 °C (± 0,5 °C) for 12 hours (one night). To optimize the necessary time for equilibration, it is advisable to prepare the samples at the end of the day and let the balance settle overnight.

Transfer of the CO_2 exchanged in the measuring cells

A sample holder which supports as many measuring cells as balloon flasks containing exchanged CO_2 is adapted on the empty line next to the work ramp. The empty cells are carefully purged and the exchanged gases contained in the ballons are transferred one after the other, into the measuring cells which have been cooled by liquid nitrogen. Then the measuring cells are allowed to heat up at room temperature.

5.2. *Use of an automatic exchange apparatus*

In order to carry out the equilibration, sample phials are filled with either 2 ml of wine or 2 ml of water (laboratory work reference) and cooled down to − 18 °C. The sample slides containing the frozen products are adapted to the equilibration system, and after having created a vacuum in the system, carbon dioxide is introduced at a pressure of 800 hPa.

The balance is reached at a temperature of 22 ± 0,5 °C after a minimum period of five hours and with moderate agitation. Since the equilibration duration depends on the phial's geometry, the optimum duration should be determined first for the system used.

Carbon dioxide contained in the phials is then transferred to the introduction chamber of the mass spectrometer by a capillary tube and the measurement is carried out according to a specific protocol for each kind of equipment.

6. Calculation and expression of the results

The relative difference δ′ of the ratio intensities of ions m/z 46 and 44 (I_{46}/I_{44}) between the sample and the reference is expressed in ‰ by means of the following equation:

$$\delta' \text{ sample} = \left[\frac{(I_{46}/I_{44}) \text{ sample}}{(I_{46}/I_{44}) \text{ reference}} - 1\right] \times 1\,000$$

The ^{18}O content of the sample compared to the reference V.SMOW on the V.SMOW/SLAP scale, is given by the relation:

$$\delta' 18O = \left[\frac{\delta' \text{ sample} - \delta' \text{ SMOW}}{\delta' \text{ SMOW} - \delta' \text{ SLAP}}\right] \times 55,5$$

The value accepted for SLAP is equal to − 55,5 ‰ compared to V.SMOW. The isotopic ratio of reference must be determined after each series of 10 measurements on unknown samples.

7. Fidelity

— the repeatability (r) is equal to 0,24 ‰

— the reproductibility (R) is equal to 0,50 ‰.

▼M7

44. DETERMINATION OF ETHYL CARBAMATE IN WINE: SELECTIVE DETECTION METHOD USING GAS CHROMATOGRAPHY/MASS SPECTROMETRY

(Applicable to the determination of ethyl carbamate for concentrations between 10 and 200 μg/l)

(*Caution:* comply with safety measures for handling chemicals, ethanol, acetone and carcinogenic products (ethyl carbamate and dichloromethane). Dispose of used solvents in the proper way, in compliance with environmental rules in force).

A. **Principle**

Propyl carbamate is added to a sample as an internal standard, the solution is diluted with water and placed in a 50 ml solid phase extraction column. Ethyl carbamate and propyl carbamate are eluted with dichloromethane.

The eluate is concentrated in a vacuum rotary evaporator. The concentrate is analysed by gas chromatography (GC). Detection is by mass spectrometry using fragmentometry in SIM (selected ion monitoring) mode.

B. **Apparatus and chromatographic conditions (example)**

(a) Gas chromatogram/mass spectrometer (GC/MS) and if necessary a sample filter and data treatment system or equivalent

Capillary column of fused silica 30 m (1) × 0,25 mm internal diameter, 0,25 μm of Carbowax 20M

Operation: injector 180 ºC, helium gas vector at 1 ml/minute at 25 ºC, injection by splitless method

Temperature programme: 40 ºC for 0,75 minutes, rising thereafter by 10 ºC/minute up to 60 ºC, then by 3 ºC/minute up to 150 ºC (2), rising to 220 ºC and maintaining that temperature for 4,25 minutes. Specific retention time for ethyl carbamate is 23 to 27 minutes, that for propyl carbamate is 27 to 31 minutes.

Gas chromatogram/spectrometer (GC/MS) interface: transfer line 220 ºC. Mass spectrometer parameters manually tuned with perfluorotributylamine and optimised for a lower mass sensitivity, SIM acquisition mode, solvent delay and acquisition start time 22 minutes, dwell time/ion 100 ms.

(b) Vacuum rotary evaporator or concentration system similar to Kuderna Danish.

(*NB:* the rate of recovery of ethyl carbamate from the test sample, C(g) must be between 90 and 110 % during the process.)

(c) Flask — pear-shaped, 300 ml, single ground neck

(d) Concentration tube — 4 ml, graduated, with a teflon-coated joint and a cork

C. **Reagents**

(a) Acetone — quality LC

(*NB:* check each batch before use in GC/MS for absence of response for m/z 62, 74 and 89 ions.)

(b) Dichloromethane

(*NB:* analyse each batch before use in GC/MS after 200-fold concentration, to check for absence of response for m/z 62, 74 and 89 ions.)

(c) Ethanol — anhydrous

(d) Ethyl carbamate (EC) standard solutions

1. Stock solution — 1,00 mg/ml. Weigh 100 mg EC (purity ≥ 99 %) in a 100 ml volumetric flask and dilute with acetone.
2. Standard working solution — 10,0 μg/ml. Transfer 1 ml of the stock EC solution to a 100 ml volumetric flask and dilute with acetone up to the mark.

---

(1) For certain particularly rich wines, a 50 m capillary column may be desirable.

(2) For certain particularly rich wines, a temperature programme of 2 ºC/minute may be desirable.

(e) Propyl carbamate (PC), standard solutions

1. Stock solution — 1,00 mg/ml. Weigh 100 mg PC (reagent grade) in a 100 ml volumetric flask and dilute with acetone up to the mark.
2. Standard working solution — 10,0 μg/ml. Transfer 1 ml of the stock PC solution to a 100 ml volumetric flask and dilute with acetone up to the mark.
3. Internal standard solution PC — 400 ng/ml. Transfer 4 ml of the standard PC working solution to a 100 ml volumetric flask and dilute with water up to the mark.

(f) Standard calibrated solutions EC-PC

Dilute EC standard working solution (d)(2) and PC standard working solution (e)(2), with dichloromethane to obtain:

1. (100 ng EC and 400 ng PC)/ml;
2. (200 ng EC and 400 ng PC)/ml;
3. (400 ng EC and 400 ng PC)/ml;
4. (800 ng EC and 400 ng PC)/ml;
5. (1 600 ng EC and 400 ng PC)/ml.

(g) Test sample — 100 ng EC/ml in 40 % of ethanol

Transfer 1 ml of EC standard working solution (d)(2) to a 100 ml volumetric flask and dilute with 40 % of ethanol up to the mark.

(h) Solid phase extraction column — disposable material, pre-packed with diatomaceous earth, capacity 50 ml

NB: Before analysis, check each batch of extraction columns for the recovery of EC and PC and the absence of response for ions of 62,74 and 89 m/z. Prepare 100 ng EC/ml of test sample (g). Analyse 5,00 ml of the test sample as described in D(a), E and F. The recovery of 90 to 110 ng of EC/ml is satisfactory. Absorbents whose particle diameter is irregular can lead to a slow flow which affects the recovery of EC and PC. If 90 to 110 % of the test sample value is not obtained after several trials, change the column or use a corrected calibration recovery curve to quantify EC. To obtain the corrected calibration curve, prepare standard solutions as described in (f) by using 40 % ethanol instead of dichloromethane.

Analyse 1 ml of the standard calibration solution as described in D, E and F.

Establish a new calibration curve by using the EC/PC ratio of the extracted standards.

D. Preparation of the test sample

Place the following volumes of test material in two separate 100 ml beakers:

(a) wines containing over 14 % vol of alcohol: 5,00 ± 0,01 ml;

(b) wines containing maximum 14 % vol of alcohol: 20,00 ± 0,01 ml.

To each beaker, add l ml of internal standard PC solution C(e)(3) and water, to obtain a total volume of 40 ml (or 40 g).

E. Extraction

Extraction should be carried out under an extractor hood, with adequate ventilation.

Transfer the sample prepared under heading D to the extraction column.

Rinse the beaker with 10 ml of water and transfer the rinsing water to the column.

Leave the liquid to absorb for four minutes. Elute with 2 × 80 ml of dichloromethane. Collect the eluate in a 300 ml conical flask.

Evaporate the eluate from 2 to 3 ml in a water bath rotary evaporator at 30 °C. (*NB:* do not allow to boil dry.)

Transfer the concentrated residue to a 4 ml graduated tube with a Pasteur pipette.

Rinse the flask with 1 ml of dichloromethane and transfer the rinsing liquid to the tube. Concentrate the sample to 1 ml under a weak nitrogen stream.

If necessary, transfer the concentrate to auto sampler flask for GC/MS analysis.

▼M7

F. **GC/MS analysis**

(a) Calibration curve

Inject 1 µl of each standard calibration solution C(f) into the GC/MS. Plot the graph of EC-PC area ratio for the m/z 62 ion response on the vertical axis and the quantity of EC in ng/ml on the horizontal axis (100, 200, 400, 800, 1 600 ng/ml).

(b) EC quantification

Inject 1 µl of concentrated extract prepared under E into the GC/MS system and calculate the EC-PC area ratio for the m/z 62 ion. Establish the concentration of EC (ng/ml) in the extract by using the internal standard calibration curve. Calculate the EC concentration in the test sample (ng/ml) by dividing the quantity of EC (ng) in the extract by the test sample volume (ml).

(c) Confirmation of EC identity

Determine whether the responses for the m/z 62, 74 and 89 ions appear during the period of EC retention. These responses are features of the main fragments $(M - C_2H_2)^+$ and $(M - CH_3)^+$ and molecular ion $(M)^+$ respectively. The presence of EC is confirmed if the relative ratios of these ions are within 20 % of the ratios for an EC standard. The extract may need to be further concentrated in order to obtain a sufficient response for the m/z 89 ion.

G. **Collaborative analysis**

The table shows individual results for the practical entrainment sample and for both types of wine.

The Cochran test led to the elimination of only one pair of results, for wine of alcoholic strength over 14 % vol and for wine of alcoholic strength of 14 % vol or less, from two different laboratories.

Relative reproducibility (RSD_R) tends to decrease as the concentration of ethyl carbamate increases.

Performance of the method for the determination of ethyl carbamate EC in alcoholic beverages by GC/MS

Sample	Average EC found (ng/ml)	Recovery of added EC (%)	S_r	S_R	RSD_r (%)	RSD_R (%)
Wines > 14 % vol	40		1,59	4,77	4,01	12,02
	80	89	3,32	7,00	4,14	8,74
	162	90	8,20	11,11	5,05	6,84
Wines ≤ 14 % vol	11		0,43	2,03	3,94	18,47
	25	93	1,67	2,67	6,73	10,73
	48	93	1,97	4,25	4,10	8,86

▼**M8**

45. DETERMINATION BY ISOTOPE MASS SPECTROMETRY OF THE $^{13}C/^{12}C$ RATIO IN WINE ETHANOL OR ETHANOL OBTAINED BY THE FERMENTATION OF MUSTS, CONCENTRATED MUSTS OR RECTIFIED CONCENTRATED MUSTS

1. FIELD OF APPLICATION

The method enables measurement of the $^{13}C/^{12}C$ isotope ratio in wine ethanol and ethanol obtained by fermentation of products of the vine (musts, concentrated musts, rectified concentrated musts).

2. REFERENCE STANDARDS

ISO:	5725:1994 'Accuracy (trueness and precision) of measurement methods and results: Basic method for the determination of repeatability and reproducibility of a standard measurement method'.
V-PDB:	Vienna-Pee-Dee Belemnite (R_{PDB} = 0,0112372).
Method 8 of the Annex to this Regulation:	'Detecting enrichment of grape musts, concentrated grape musts, rectified concentrated grape musts and wines by application of nuclear magnetic resonance of deuterium (SNIF-NMR).'

3. TERMS AND DEFINITIONS

$^{13}C/^{12}C$:	ratio of carbon 13 (^{13}C) to carbon 12 (^{12}C) isotopes for a given sample.
$\delta^{13}C$:	carbon 13 content (^{13}C) expressed in parts per 1 000 (‰).
SNIF-NMR:	fractionating the particular natural isotope under study by nuclear magnetic resonance.
V-PDB:	Vienna-Pee-Dee Belemnite. PDB is the primary reference material for measuring natural variations of carbon 13 isotope content, consisting of calcium carbonate from a Cretaceous belemnite guard from the Pee Dee Formation in South Carolina (USA). Its $^{13}C/^{12}C$ isotope ratio or R_{PDB} is 0,0112372. PDB reserves have been exhausted for a long time, but it has remained the primary reference for expressing natural variations of carbon 13 isotope content and against which the reference material available at the International Atomic Energy Agency (IAEA) in Vienna (Austria) is calibrated. Isotopic indications of naturally occurring carbon 13 are conventionally expressed in relation to V-PDB.
m/z:	mass-to-charge ratio.

4. PRINCIPLE

During photosynthesis, the assimilation of carbon dioxide by plants occurs via two principle forms of metabolism, the C_3 metabolism (Calvin cycle) and the C_4 metabolism (Hatch and Slack). These two photosynthesis mechanisms present a different type of isotope fractionation. Products of C_4 plants, such as sugars and alcohol derived from fermentation, have higher levels of carbon 13 than similar products of C_3 plants. Most plants, including vines and sugar beets, belong to the C_3 group. Sugar cane and maize belong to the C_4 group. Measuring the carbon 13 content enables the detection and evaluation of sugars of C_4 origin (sugar cane or maize isoglucose) added to grape products (grape musts, wines, etc.). The information on carbon 13 content combined with that obtained from SNIF-NMR enables the added quantities of mixtures of sugars or alcohols derived from C_3 and C_4 plants to be determined.

The carbon 13 content is determined on carbon dioxide produced during the complete combustion of the sample. The abundance of the principle isotopomers of masses 44 ($^{12}C^{16}O_2$), 45 ($^{13}C^{16}O_2$ and $^{12}C^{17}O^{16}O$) and 46 ($^{12}C^{16}O^{18}O$), resulting from the different possible combinations of isotopes ^{18}O, ^{17}O, ^{16}O, ^{13}C and ^{12}C, are determined from the ionic currents measured by three different collectors of a mass isotopic spectrometer. The contributions of isotopomers $^{13}C^{17}O^{16}O$ and $^{12}C^{17}O_2$ may be disregarded given their low levels. The ionic current for m/z = 45 is corrected for the contribution of $^{12}C^{17}O^{16}O$, which is calculated according to the current intensity measured for m/z = 46, while taking the relative abundance of ^{18}O and ^{17}O into account (Craig correction). Comparison with a reference calibrated against the international reference V-PDB permits calculation of carbon 13 content on the $\delta^{13}C$ relative scale.

▼**M8**

5. REAGENTS

The material and the consumables depend on the apparatus (point 6) used by the laboratory. The systems generally used are based on elemental analysers. These systems can be equipped to allow the introduction of samples placed in sealed metal capsules or the injection of liquid samples through a septum using a syringe.

Depending on the type of instrument used, the following reference materials, reagents, and consumables may be used:

— reference materials

— available from the IAEA:

Name	Materiel	$\delta^{13}C$ relative to V-PDB (9)
— IAEA-CH-6	Sucrose	- 10,4 ‰
— IAEA-CH-7	Polyethylene	- 31,8 ‰
— NBS22	Oil	- 29,7 ‰
— USGS24	Graphite	- 16,1 ‰

— available from the IRMM in Geel (B) (Institute for Reference Materials and Measurements):

Name	Material	$\delta^{13}C$ relative to V-PDB (9)
— CRM/BCR 656	Wine alcohol	- 26,93 ‰
— CRM/BCR 657	Glucose	- 10,75 ‰
— CRM/BCR 660	Hydroalcoholic solution (ASV 12 %)	- 26,72 ‰

— a standard working sample with a known $^{13}C/^{12}C$ ratio calibrated against international reference materials,

— the following is an indicative list of consumables for continuous-flow systems:

  — helium for analysis (CAS 07440-59-7),

  — oxygen for analysis (CAS 07782-44-7),

  — carbon dioxide for analysis, used as a secondary reference gas for the carbon 13 content (CAS 00124-38-9),

  — oxidation reagent for the furnace of the combustion system, for example copper (II) oxide for elemental analysis (CAS 1317-38-0),

  — a desiccant to eliminate water produced in combustion, for example anhydrone for elemental analysis (magnesium perchlorate) (CAS 10034-81-8) (This is not necessary for apparatuses equipped with a water elimination system using cryogenic traps or selectively permeable capillaries).

6. APPARATUS AND EQUIPMENT

6.1. **Isotope ratio mass spectrometer (IRMS)**

Isotope ratio mass spectrometer (IRMS) capable of determining the relative ^{13}C content of naturally occurring CO$_2$ gas with an internal accuracy of 0,05 ‰ or better expressed as a relative value (point 9). Internal accuracy here is defined as the difference between two measurements of the same sample of CO$_2$. The mass spectrometer used to measure isotope ratios is generally equipped with a triple collector to simultaneously measure intensities for $m/z = 44$, 45 and 46. The isotope ratio mass spectrometer must either be equipped with a dual inlet, to alternately measure the unknown sample and a reference sample, or use an integrated system that carries out the quantitative combustion of samples and separates the carbon dioxide from the other combustion products before measurement in the mass spectrometer.

6.2. **Combustion apparatus**

Combustion apparatus able to quantitatively convert ethanol into carbon dioxide and eliminate all other combustion products including water, without any isotopic fractionation. The apparatus may be either a continuous-flow system integrated with the mass spectrometry apparatus (point

▼**M8**

6.2.1) or a separate combustion system (point 6.2.2). The apparatus must permit an accuracy of at least that indicated in (point 11).

6.2.1. *Continuous-flow systems*

These comprise either an elemental analyser or a gas chromatograph with an online combustion system.

The following laboratory equipment is needed for systems equipped for the introduction of samples contained in metal capsules:

- calibrated microsyringe or micropipette with appropriate tips,
- balance with µg accuracy or better,
- tweezers for encapsulation,
- tin capsules for liquid samples,
- tin capsules for solid samples.

Note:

in order to reduce the risk of evaporation of ethanol samples, an absorbent material (for example Chromosorb W 45-60 mesh) may be placed in the capsules, it first having been verified by means of a measurement without a sample that is does not contain a significant quantify of carbon likely to affect the results.

The following laboratory equipment is needed when using an elemental analyser equipped with a liquid injector or in the case of a combustion chromatography preparation system:

- syringe for liquids,
- flasks equipped with airtight closing systems and inert septa.

The laboratory equipment indicated in the above lists are examples and may be replaced by other equipment of equivalent performance depending on the type of combustion and mass spectrometry apparatus used by the laboratory.

6.2.2. *Separate preparation system*

The samples of carbon dioxide resulting from the combustion of the samples to be analysed and the reference sample are collected in bulbs which are then placed in the dual inlet of the spectrometer for isotopic analysis. Several combustion apparatuses described in the literature may be used:

- closed combustion system filled with circulating oxygen,
- elemental analyser with helium and oxygen flow,
- sealed glass bulb filled with copper (II) oxide as an oxidation agent.

7. PREPARATION OF SAMPLES FOR TESTS

The ethanol must be extracted from the wine before isotopic testing. This is carried out by distilling wine as described in point 3.1 of method No 8 (SNIF-NMR).

In the case of grape musts, concentrated grape musts and rectified concentrated grape musts, the sugars must be fermented in ethanol first as described in point 3.2 of method No 8.

8. PROCEDURE

All steps of the preparation must be carried out without any significant ethanol loss through evaporation that would change the isotopic composition of the sample.

The following description refers to the procedures generally used for ethanol sample combustion using commercial automated combustion systems. All other methods that ensure that all of the ethanol sample is converted into carbon dioxide without any loss of ethanol through evaporation may be used for the preparation of carbon dioxide for isotopic analysis.

Experimental procedure based on the use of an elemental analyser:

(a) placing the samples in capsules:

  - use capsules, tweezers and a preparation tray, all of which must be clean,
  - take an appropriate-sized capsule using the tweezers,
  - introduce an appropriate amount of liquid into the capsule using a micropipette,

▼M8

— *Note:*

3,84 mg of absolute ethanol or 4,17 mg of distillate with an alcohol strength of 92 % m/m are necessary to obtain 2 mg of carbon. The appropriate quantity of distillate must be calculated on that basis, according to the quantity of carbon necessary given the sensitivity of the mass spectrometry apparatus,

— close the capsule with the tweezers,

— each capsule must be completely sealed. If not, it must be discarded and a new capsule prepared,

— two capsules must be prepared for every sample,

— place the capsules in the appropriate place on the tray of the automatic sampler of the elemental analyser. Every capsule must be carefully identified by a serial number,

— systematically place capsules containing working references at the beginning and the end of the sample series,

— regularly insert control samples in the sample series;

(b) checking and adjusting the elemental analysis and mass spectrometry apparatus:

— adjust the temperature of the elemental analyser furnaces and the helium and oxygen flows for optimal combustion of the sample,

— check the elemental analysis and mass spectrometry system for leaks (for example by checking the ionic current where m/z = 28 for N_2),

— adjust the mass spectrometer to measure the ionic currents where m/z = 44, 45 and 46,

— check the system using known control samples before starting to measure the samples;

(c) carrying out a series of measurements

The samples placed on the automatic sampler of the elemental analyser (or of the chromatograph) are introduced in turn. The carbon dioxide from each sample combustion is eluted towards the mass spectrometer which measures the ionic currents. The interfaced computer records the ionic currents and calculates the δ value for each sample (point 9).

9. CALCULATION

The purpose of the method is to measure the $^{13}C/^{12}C$ isotope ratio ofethanol extracted from wine or from products derived from grapes following fermentation. The $^{13}C/^{12}C$ isotope ratiocan be expressed by its deviation from a working reference. The isotopic deviation of carbon 13 ($\delta\ ^{13}C$) is then calculated on a delta scale per thousand (δ/1 000) by comparing the results obtained for the sample to be measured with those for a working reference previously calibrated on the basis of the primary international reference (V-PDB). The $\delta\ ^{13}C$ values are expressed in relation to the working reference as follows:

$$\delta^{13}C_{sam/ref}\ ‰ = 1\ 000 \times (R_{sam}-R_{ref})/R_{ref}$$

where R_{sam} and R_{ref} are respectively the $^{13}C/^{12}C$ isotope ratios of the sample and of the carbon dioxide used as the reference gas.

The $\delta\ ^{13}C$ values are expressed in relation to V-PDB as follows:

$$\delta^{13}C_{sam/V\text{-}PDB}\ ‰ = \delta^{13}C_{sam/ref} + \delta^{13}C_{ref/V\text{-}PDB} + (\delta^{13}C_{sam/ref} \times \delta^{13}C_{ref/V\text{-}PDB})/1\ 000,$$

where $\delta^{13}C_{ref/V\text{-}PDB}$ is the previously determined isotopic deviation of the working reference from V-PDB.

Small variations may occur while measuring on line due to changes in the instrumental conditions. In this case the $\delta\ ^{13}C$ values of the samples must be corrected according to the difference in the measured $\delta^{13}C$ value of the standard working sample and its true value, previously calibrated against V-PDB by comparison with one of the international reference materials. Between two measurements of the standard working sample, the variation, and therefore the correction to be applied to the results obtained from the samples, may be assumed to be linear. The standard working sample must be measured at the beginning and at the end of all sample series. A correction can then be calculated for each sample using linear interpolation.

▼**M8**

10. QUALITY ASSURANCE AND CONTROL

Check that the ^{13}C value for the working reference does not differ by more than 0,5 ‰ from the admissible value. If not, the spectrometry apparatus settings should be checked and, if necessary, adjusted.

For each sample, check that the difference in the results for two capsules measured successively is less than 0,3 ‰. The final result for a given sample is the average value for the two capsules. If the deviation is greater than 0,3 ‰, the measurement must be repeated.

Checks on correct measurement can be based on the ionic current where $m/z = 44$, which is proportional to the quantity of carbon injected into the elemental analyser. Under standard conditions, the ionic current should be almost constant for the samples analysed. A significant deviation could be indicative of ethanol evaporation (for example an imperfect seal on a capsule) or instability of the elemental analyser or mass spectrometer.

11. PERFORMANCE CHARACTERISTICS OF THE METHOD (Accuracy)

An initial collaborative study (point 11.1) was carried out on distillates containing alcohol of vinous origin and cane and beet alcohol, as well as different mixtures of alcohol of those three origins. Since this study did not take into account the distillation procedure, further information from other interlaboratory studies on wine (point 11.2) and, in particular, series of proficiency tests (point 11.3) for isotopic measurements were also considered. The results show that under satisfactory conditions, and in particular those for measurement using SNIF-NMR, the different distillation systems do not produce significant variation in the determination of the $\delta^{13}C$ value of wine ethanol. The accuracy parameters observed for wine are almost identical to those obtained in the joint study on distillates (point 11.1).

11.1. **Joint study on distillates**

Year of interlaboratory tests:	1996
Number of laboratories:	20
Number of samples:	Six samples in double-blind comparison
Analyte:	$\delta\ ^{13}C$ of ethanol

Sample code	Alcohol of vinous origin	Beet alcohol	Sugar cane alcohol
A & G	80 %	10 %	10 %
B & C	90 %	10 %	0 %
D & F	0 %	100 %	0 %
E & I	90 %	0 %	10 %
H & K	100 %	0 %	0 %
J & L	0 %	0 %	100 %

Samples	A/G	B/C	D/F	E/I	H/K	J/L
Number of laboratories retained after eliminating aberrant results	19	18	17	19	19	19
Number of results accepted	38	36	34	38	38	38
Average value ($\delta\ ^{13}C$) ‰	- 25,32	- 26,75	- 27,79	- 25,26	- 26,63	- 12,54
Sr^2	0,0064	0,0077	0,0031	0,0127	0,0069	0,0041

▼M8

Samples	A/G	B/C	D/F	E/I	H/K	J/L
Repeatability standard deviation (Sr) ‰	0,08	0,09	0,06	0,11	0,08	0,06
Limit of repeatability r (2,8 × S_r) ‰	0,22	0,25	0,16	0,32	0,23	0,18
S_R^2	0,0389	0,0309	0,0382	0,0459	0,0316	0,0584
Reproducibility standard deviation (S_R) ‰	0,20	0,18	0,20	0,21	0,18	0,24
Limit of reproducibility R (2,8 × S_R)	0,55	0,49	0,55	0,60	0,50	0,68

11.2. Interlaboratory study on two wines and one alcohol

Year of interlaboratory tests:	1996
Number of laboratories:	14 for distillation of wine of which seven also measured $\delta^{13}C$ of wine ethanol,
	Eight for measuring $\delta^{13}C$ of alcohol sample,
Number of samples:	Three (white wine of 9,3 % ASV, white wine of 9,6 % ASV and alcohol of strength 93 % m/m).
Analyte:	$\delta^{13}C$ of ethanol

Samples	Red wine	White wine	Alcohol
Number of laboratories	7	7	8
Number of results accepted	7	7	8
Average value ($\delta^{13}C$) ‰	- 26,20	- 26,20	- 25,08
Reproducibility variance S_R^2	0,0525	0,0740	0,0962
Reproducibility standard deviation (S_R) ‰	0,23	0,27	0,31
Limit of reproducibility R (2,8 × S_R) ‰	0,64	0,76	0,87

Different distillation systems were used by the participating laboratories. The isotopic determinations ($\delta^{13}C$) carried out in a single laboratory on the whole number of distillates returned by the participants do not reveal any aberrant values or values that differ significantly from the average values. The variation in results ($S^2 = 0,0059$) is comparable to the repeatability variances Sr^2 in the joint study on distillates (point 11.1).

11.3. Results of the exercises carried out to monitor proficiency in performing isotopic tests

Since December 1994, international proficiency tests for the determination of isotopic measurements for wine and alcohol (distillates of 96 % ASV) have been regularly organised. The results enable participating laboratories to check the quality of their analyses. Statistical results permit appreciation of the variation in measurements under conditions of reproducibility and therefore an estimation of the parameters of variance and the limit of reproducibility. The results obtained for the determination

▼**M8**

of δ ^{13}C for wine and distillate ethanol are summarised in the following table:

Date	Wines				Distillates			
	N	S_R	S^2_R	R	N	S_R	S^2_R	R
December 1994	6	0,210	0,044	0,59	6	0,151	0,023	0,42
June 1995	8	0,133	0,018	0,37	8	0,147	0,021	0,41
December 1995	7	0,075	0,006	0,21	8	0,115	0,013	0,32
March 1996	9	0,249	0,062	0,70	11	0,278	0,077	0,78
June 1996	8	0,127	0,016	0,36	8	0,189	0,036	0,53
September 1996	10	0,147	0,022	0,41	11	0,224	0,050	0,63
December 1996	10	0,330	0,109	0,92	9	0,057	0,003	0,16
March 1997	10	0,069	0,005	0,19	8	0,059	0,003	0,16
June 1997	11	0,280	0,079	0,78	11	0,175	0,031	0,49
September 1997	12	0,237	0,056	0,66	11	0,203	0,041	0,57
December 1997	11	0,127	0,016	0,36	12	0,156	0,024	0,44
March 1998	12	0,285	0,081	0,80	13	0,245	0,060	0,69
June 1998	12	0,182	0,033	0,51	12	0,263	0,069	0,74
September 1998	11	0,264	0,070	0,74	12	0,327	0,107	0,91
Weighted average		0,215	0,046	0,60		0,209	0,044	0,59

N: number of participating laboratories.

11.4. Limits of repeatability and reproducibility

On the basis of the data from the different interlaboratory tests given in the above tables, the following limits of repeatability and reproducibility can be established for this method, including the distillation stage:

limit of repeatability r: 0,24

limit of reproducibility R: 0,6.