RESOLUTION OENO 24/2003

MEASURE OF WINE ALCOHOLIC STRENGTH BY VOLUME BY DENSIMETRY USING HYDROSTATIC BALANCE

THE GENERAL ASSEMBLY,

CONSIDERING Article 5 of the International Convention for the Unification of Methods of Analysis and Appraisal of Wine of 13 October 1954,

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

DECIDES

To complete and substitute in Annex A of the Compendium of International Methods of Analysis of Wine and Musts, the method: “Determination of wine alcoholic strength by volume by densimetry using hydrostatic balance” by the following method:

1. METHOD OF MEASUREMENT

1.1. Strength and introduction

Measurement of alcoholic strength by volume should be determined before marketing notably to be in compliance with labelling rules.

Alcoholic strength by volume is equal to the number of litres of ethanol contained in 100 litres of wine measured at 20°C, referred to as “% vol.”.

1.2. Safety precaution

Respect safety measures concerning the use of distillation apparatuses, manipulation of hydro-alcoholic solutions and cleaning products.

1.3. Object and field of application

The method of measurement is densimetry using a hydrostatic balance.

In reference to regulatory provisions in force the trial temperature is set at 20°C.

1.4. Principle and definitions

The principle of this method involves firstly distilling wine volume by volume. The distilling method is described in the Compendium. Non volatile substances can be eliminated through distillation. Ethanol counterparts and ethanol found in esters are

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included in the alcoholic strength as they are found in the distillate.

Secondly, the volumetric weight of the distillate obtained is measured. The volumetric weight of a liquid at a given temperature is equal to the ratio of the weight over its volume: □=m/V, for wine, it is expressed in g/ml.

The alcoholic strength of wine can be measured by densimetry using a hydrostatic balance following the Archimedes principle by which any body plunged into a fluid undergoes a vertical push, from the bottom to the top, equal to the weight of the displaced fluid.

1.5. Reagents

Unless other wise indicated, only recognised analytical quality reagents should be used during the analysis with at least class 3 water corresponding to the definition of the standard ISO 3696:1987.

1.5.1. Solution for washing float device (sodium hydroxide , 30% m/v).

To prepare a 100 ml solution, weigh 30 g of sodium hydroxide and fill using 96% vol. ethanol.

1.6. Apparatus and material

current laboratory apparatus including:

1.6.1. Single-plate hydrostatic balance with 1 mg precision.

1.6.2. Floater with at least 20 ml volume, specifically adapted for the balance, suspended by a thread with a diameter less than or equal to 0.1 mm.

1.6.3. Cylindrical test tube with level indicator. The floater must entirely fill the test tube volume above the marker, only the slinging wire goes through the surface of the liquid. The cylindrical test tube should have an inside diameter at least above 6 mm of the floater.

1.6.4. Thermometer (or temperature measurement pipette) with degree and 10th of degree graduations, from 10°C to 40°C, calibrated to ± 0.05°C.

1.6.5. Calibrated weight by a recognized certification body.

1.7. Procedure

After each measurement, the floater and the test tube must be cleaned with distilled water, wiped with soft laboratory paper which doesn't loose its fibres and rinsed with solution whose volumetric weight is to be determined. These measurements must be carried out once the apparatus has reached a stable level in order to limit alcohol loss through evaporation.

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1.7.1. Balance calibration

While balances usually have internal calibration systems, hydrostatic balances must be calibrated with controlled weights by an official certification body.

1.7.2. Floater calibration

1.7.2.1. Fill cylindrical test tube up to marker with bidistilled water (or an equivalent purity, for example microfiltered water with a conductivity of 18.2 M□/cm), whose temperature between 15°C to 25°C, but preferably at 20°C.

1.7.2.2. Plunge the floater and the thermometer into the liquid, shake, note down the volumetric weight on the apparatus and, if necessary, adjust the reading in order for it to be equal to the water measurement temperature.

1.7.3. Control using a hydroalcoholic solution

1.7.3.1. Fill the cylindrical test tube up to the marker with a known titre of hydroalcoholic solution at a temperature between 15°C to 25°C, preferably at 20°C.

1.7.3.2. Plunge the floater and the thermometer into the liquid, shake, note down the volumetric weight on the apparatus (or the alcoholic strength if possible). The established alcoholic strength must be equal to the previously determined alcoholic strength.

Note 2: This alcoholic strength solution can be replaced by bidistilled water for floater calibration.

1.7.4. Measure volumetric weight of the distillate (or alcoholic strength if possible)

1.7.4.1. Pour the sample for the trial in the cylindrical test tube up to the marker level.

1.7.4.2. Plunge the floater and the thermometer into the liquid, shake, note down the volumetric weight on the apparatus (or the alcoholic strength if possible. Note the temperature if the volumetric mass is measured at t°C (□□t).

1.7.4.3. Correct □□t using a volumetric weight table □□t of hydroalcoholic mixtures [Table II of Annex II of the Compendium of methods of analysis of the OIV].

1.7.5. Clean the floater and cylindrical test tube.

1.7.5.1. Plunge the floater into the wash solution in the test tube.

1.7.5.2. Allow to soak 1 hour while turning the floater regularly.

1.7.5.3. Rinse with tap water, then with distilled water.

1.7.5.4. Wipe with soft laboratory paper which doesn't loose its fibres.

Carry out these operations when the floater is used for the first time and then on a

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regular basis when necessary.

1.7.6. Result

Using ρ_{20}, volumetric weight, calculate real alcoholic strength by using the table indicating volumetric alcoholic strength (% vol.) at 20°C according to volumetric weight at 20°C of hyrdoalcoholic mixtures. This is the international table adopted by the International Organisation of Legal Metrology in its recommendation number 22.

2. Comparison of measurements

Carried out using a hydrostatic balance with measurements obtained using an electronic density-meter (OIV Reference method, Annex A of the Compendium of International Methods of Analysis).

From samples whose alcoholic strength is between 4% vol. and 18% vol. the measurements of repeatability and reproducibility were performed after an inter-laboratory ring test. It is the comparison of the measurements of wine alcoholic strength of different samples using the hydrostatic balance and the electronic density-meter, including the repeatability and reproducibility values derived from pluri-annual intercomparison test trials performed on a large scale.

2.1. Samples:

Wines of different density and alcoholic strengths prepared monthly on an industrial scale, taken from a bottled stock stored under normal conditions, and supplied as anonymous products to laboratories.

2.2. Laboratories

laboratories participating into the monthly ring test organised by Unione Italiana Vini Verona, (Italy) according to ISO 5725 (UNI 9225) regulation and the 'International Protocol of Proficiency test for chemical analysis laboratories' established by AOAC, ISO and IUPAC (J. AOAC Intern., 1993, 74/4) and according to guidelines ISO 43 and ILAC G13. An annual report is supplied by the cited company to all participants.

2.3. Apparatus:

2.3.1. Electronic hydrostatic balance (whose precision allows to give the 5th decimal of density) eventually equipped with a data treatment device.

2.3.2. Electronic density-meter eventually equipped with an autosampler.

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2.4. Analyses

According to method validation rules (resolution OENO 6/99), each sample is analysed twice consecutively to determine the alcoholic strength.

2.5. Results

Table 1 shows the results of the measurements obtained by the laboratories using the hydrostatic balance.

Table 2 shows the results obtained by the laboratories using an electronic densimeter.

2.6. Evaluations of the results

2.6.1. The trial results were examined for evidence of individual systematic error ($p<0.025$) using Cochran's and Grubbs' tests successively, by procedures described in the internationally agreed ["Protocol for the Design, Conduct and Interpretation of Method-Performance Studies" Ed W Horwitz, Pure and Applied Chemistry, 1995, 67, (2), 331 343.].

2.6.2. Repeatability (r) and reproducibility (R)

Calculations for repeatability (r) and reproducibility (R) as defined by the protocol were carried out on the results remaining after the removal of outliers. When assessing a new method there is often no validated reference or statutory method with which to compare precision criteria, hence it is useful to compare the precision data obtained from collaborative trials with "predicted" levels of precision. These "predicted" levels are calculated from the Horwitz formula. Comparison of the trial results and the predicted levels indicate as to whether the method is sufficiently precise for the level of analyte being measured.

The predicted Horwitz value is calculated from the Horwitz formula.

$$RSD_R = 2^{(1.05 log C)}$$

where C = measured concentration of analyte expressed in decimals. (e.g. 1 g/100g = 0.01) [Horwitz, W., Analytical Chemistry, 1982, 54, 67A-76A.].

The Horrat value gives a comparison of the actual precision measured with the precision predicted by the Horwitz formula for the method and at that particular level of concentration of the analyte. It is calculated as follows:

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$$Ho_R = RSD_R(measured)/RSD_R(Horwitz)$$

2.6.1. Interlaboratory precision

A Horrat value of 1 usually indicates satisfactory inter-laboratory precision, whereas a value of more than 2 usually normally indicates unsatisfactory precision, i.e. one that is too variable for most analytical purposes or where the variation obtained is greater than that expected for the type of method employed. Hor is also calculated, and used to assess intra-laboratory precision, using the following approximation:

- RSD_r(Horwitz) = 0.66 RSD_R(Horwitz) (this assumes the approximation r = 0.66 R).

Table 3 shows the differences between the measurements obtained by laboratories using an electronic densimeter and those using a hydrostatic balance. Excluding the sample of 2000/3 with very low alcohol strength and for which both techniques show poor reproducibility, a very good concordance is generally observed for the other samples.

2.6.2. Fidelity parameters

Table 4 shows the averaged overall fidelity parameters computed from all monthly trials carried out from January 1999 until May 2001.
In particular:

- Repeatability (r)= 0.074 (% vol.) for the hydrostatic balance and 0.061 (% vol.) for electronic densitometry;
- Reproducibility (R)= 0.229 (% vol.) for the hydrostatic balance and 0.174 (% vol.) for electronic densimetry, this latter value is concordant to the value estimated for the electronic densimetry from the OIV Compendium of International Methods of Analysis;

2.7. Conclusion

The results concerning the determination of the alcoholic strength of a large range of wines show that the measurements carried out with the hydrostatic balance are concordant with those carried out by electronic densimetry using a flexion resonator and that the validation parameter values are similar for both methods.

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Legend:

mean: the mean of all the data used in the statistical analysis

n: total number of sets of data submitted

nc: number of results excluded from statistical analysis due to non-compliance

outliers: number of results excluded from statistical analysis due to determination as outliers by either Cochran’s or Grubbs’ tests

n_1: number of results used in statistical analysis

r: repeatability limit

Sr: the standard deviation of the repeatability

RSD_r: the relative standard deviation of the repeatability (S_r x 100/MEAN).

Ho_r: the HORRAT value for repeatability is the observed RSDr divided by the RSDr value estimated from the Horwitz formula using the approximation r = 0.66R

R: reproducibility limit

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S_R: the standard deviation of the reproducibility

Ho_R:the HORRAT value for reproducibility is the observed RSDR value divided by the RSDR value calculated from HoR = RSDR(measured)/RSDR

Table 1: Hydrostatic Balance (HB)

	MEAN	n	outliers	n1	r	sr	RSDr	Hor	R	sR	RSDR	HoR	no. of replicates	critical difference CrD95
1999/1	11.043	17	1	16	0.0571	0.0204	0.1846	0.1004	0.1579	0.0564	0.5107	0.18	2	0.1080
1999/2	11.247	14	1	13	0.0584	0.0208	0.1854	0.1011	0.1803	0.0644	0.5727	0.21	2	0.1241
1999/3	11.946	16	0	16	0.0405	0.0145	0.1211	0.0666	0.1593	0.0569	0.4764	0.17	2	0.1108
1999/4	7.653	17	1	16	0.0502	0.0179	0.2344	0.1206	0.1537	0.0549	0.7172	0.24	2	0.1057
1999/5	11.188	17	0	17	0.0871	0.0311	0.2780	0.1515	0.2701	0.0965	0.8622	0.31	2	0.1860
1999/6	11.276	19	0	19	0.0846	0.0302	0.2680	0.1462	0.2957	0.1056	0.9365	0.34	2	0.2047
1999/7	8.018	17	0	17	0.0890	0.0318	0.3964	0.2054	0.2573	0.0919	1.1462	0.39	2	0.1764
1999/9	11.226	17	0	17	0.0580	0.0207	0.1846	0.1423	0.2796	0.0999	0.8896	0.45	2	0.1956
1999/10	11.026	17	0	17	0.0606	0.0216	0.1961	0.1066	0.2651	0.0947	0.8588	0.31	2	0.1850
1999/11	7.701	16	1	15	0.0643	0.0229	0.2980	0.1535	0.2330	0.0832	1.0805	0.37	2	0.1616
1999/12	10.987	17	2	15	0.0655	0.0234	0.2128	0.1156	0.1258	0.0449	0.4089	0.15	2	0.0827
2000/1	11.313	16	0	16	0.0986	0.0352	0.3113	0.1699	0.2577	0.0920	0.8135	0.29	2	0.1754
2000/2	11.232	17	0	17	0.0859	0.0307	0.2731	0.1489	0.2535	0.0905	0.8060	0.29	2	0.1740
2000/3	0.679	10	0	10	0.0680	0.0243	3.5773	1.2783	0.6529	0.2332	34.3395	8.10	2	0.4604
2000/4	11.223	18	0	18	0.0709	0.0253	0.2257	0.1230	0.2184	0.0780	0.6951	0.25	2	0.1503
2000/5	7.439	19	1	18	0.0630	0.0225	0.3023	0.1549	0.1522	0.0544	0.7307	0.25	2	0.1029
2000/6	11.181	19	0	19	0.0536	0.0191	0.1710	0.0932	0.2783	0.0994	0.8890	0.32	2	0.1950
2000/7	10.858	16	0	16	0.0526	0.0188	0.1731	0.0939	0.1827	0.0653	0.6011	0.22	2	0.1265
2000/9	12.031	17	1	16	0.0602	0.0215	0.1787	0.0985	0.2447	0.0874	0.7263	0.26	2	0.1704
2000/10	11.374	18	0	18	0.0814	0.0291	0.2555	0.1395	0.2701	0.0965	0.8482	0.31	2	0.1866
2000/11	7.644	18	0	18	0.0827	0.0295	0.3863	0.1988	0.2289	0.0817	1.0694	0.36	2	0.1565
2000/12	11.314	19	1	18	0.0775	0.0277	0.2447	0.1336	0.2421	0.0864	0.7641	0.28	2	0.1667
2001/1	11.415	19	0	19	0.0950	0.0339	0.2971	0.1623	0.2410	0.0861	0.7539	0.27	2	0.1636
2001/2	11.347	19	0	19	0.0792	0.0283	0.2493	0.1361	0.1944	0.0694	0.6119	0.22	2	0.1316
2001/3	11.818	16	0	16	0.0659	0.0235	0.1990	0.1093	0.2636	0.0941	0.7965	0.29	2	0.1834
2001/4	11.331	17	0	17	0.1067	0.0381	0.3364	0.1836	0.1895	0.0677	0.5971	0.22	2	0.1229
2001/5	8.063	19	1	18	0.0782	0.0279	0.3465	0.1797	0.1906	0.0681	0.8442	0.29	2	0.1290

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Table 2: Electronic Densimetry (ED)

	MEAN n1	n	outliers	n1	r	sr	RSDr	Hor	R	sR	RSDR	HoR	no. of replicates	critical difference CrD95
D1999/1	11.019	18	1	17	0.0677	0.0242	0.2196	0.1193	0.1996	0.0713	0.6470	0.23	2	0.1370
D1999/2	11.245	19	2	17	0.0448	0.0160	0.1423	0.0776	0.1311	0.0468	0.4165	0.15	2	0.0900
D1999/3	11.967	21	0	21	0.0701	0.0250	0.2091	0.1151	0.1552	0.0554	0.4631	0.17	2	0.1040
D1999/4	7.643	19	1	18	0.0610	0.0218	0.2852	0.1467	0.1340	0.0479	0.6262	0.21	2	0.0897
D1999/5	11.188	21	3	18	0.0260	0.0093	0.0829	0.0452	0.2047	0.0731	0.6536	0.24	2	0.1442
D1999/6	11.303	21	0	21	0.0652	0.0233	0.2061	0.1125	0.1466	0.0523	0.4631	0.17	2	0.0984
D1999/7	8.026	21	0	21	0.0884	0.0316	0.3935	0.2039	0.1708	0.0610	0.7600	0.26	2	0.1124
D1999/9	11.225	17	0	17	0.0372	0.0133	0.1183	0.0645	0.1686	0.0602	0.5366	0.19	2	0.1178
D1999/10	11.011	19	0	19	0.0915	0.0327	0.2969	0.1613	0.1723	0.0615	0.5588	0.20	2	0.1129
D1999/11	7.648	21	1	20	0.0615	0.0220	0.2872	0.1478	0.1538	0.0549	0.7183	0.24	2	0.1043
D1999/12	10.999	16	1	15	0.0428	0.0153	0.1389	0.0755	0.2015	0.0720	0.6541	0.23	2	0.1408
D2000/1	11.248	22	1	21	0.0697	0.0249	0.2212	0.1206	0.1422	0.0508	0.4516	0.16	2	0.0944
D2000/2	11.240	19	3	16	0.0448	0.0160	0.1424	0.0776	0.1619	0.0578	0.5145	0.19	2	0.1123
D2000/3	0.526	12	1	11	0.0327	0.0117	2.2185	0.7630	0.9344	0.3337	63.4009	14.39	2	0.6605
D2000/4	11.225	19	1	18	0.0476	0.0170	0.1514	0.0825	0.1350	0.0482	0.4295	0.15	2	0.0924
D2000/5	7.423	21	0	21	0.0628	0.0224	0.3019	0.1547	0.2635	0.0941	1.2677	0.43	2	0.1836
D2000/6	11.175	23	2	21	0.0606	0.0217	0.1938	0.1056	0.1697	0.0606	0.5424	0.20	2	0.1161
D2000/7	10.845	21	5	16	0.0440	0.0157	0.1449	0.0786	0.1447	0.0517	0.4766	0.17	2	0.0999
D2000/9	11.983	22	1	21	0.0841	0.0300	0.2507	0.1380	0.2410	0.0861	0.7183	0.26	2	0.1651
D2000/10	11.356	22	1	21	0.0635	0.0227	0.1997	0.1090	0.1865	0.0666	0.5866	0.21	2	0.1280
D2000/11	7.601	27	0	27	0.0521	0.0186	0.2448	0.1258	0.1685	0.0602	0.7916	0.27	2	0.1162
D2000/12	11.322	25	1	24	0.0476	0.0170	0.1503	0.0820	0.1594	0.0569	0.5028	0.18	2	0.1102
D2001/1	11.427	29	0	29	0.0706	0.0252	0.2207	0.1206	0.1526	0.0545	0.4771	0.17	2	0.1020
D2001/2	11.320	29	1	28	0.0675	0.0241	0.2128	0.1161	0.1570	0.0561	0.4952	0.18	2	0.1057
D2001/3	11.826	34	1	33	0.0489	0.0175	0.1476	0.0811	0.1762	0.0629	0.5322	0.19	2	0.1222
D2001/4	11.339	31	2	29	0.0639	0.0228	0.2012	0.1099	0.1520	0.0543	0.4788	0.17	2	0.1026
D2001/5	8.058	28	0	28	0.0473	0.0169	0.2098	0.1088	0.2025	0.0723	0.8976	0.31	2	0.1412

Table 3: Comparison of results between hydrostatic balance and electronic densimetry

	MEAN (HB)	n	outliers	n1		MEAN (ED)	n	outliers	n1	¤TAV(HB-ED)
1999/1	11.043	17	1	16	D1999/1	11.019	18	1	17	0.024

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1999/2	11.247	14	1	13	D1999/2	11.245	19	2	17		0.002
1999/3	11.946	16	0	16	D1999/3	11.967	21	0	21		-0.021
1999/4	7.653	17	1	16	D1999/4	7.643	19	1	18		0.010
1999/5	11.188	17	0	17	D1999/5	11.188	21	3	18		0.000
1999/6	11.276	19	0	19	D1999/6	11.303	21	0	21		-0.028
1999/7	8.018	17	0	17	D1999/7	8.026	21	0	21		-0.008
1999/9	11.226	17	0	17	D1999/9	11.225	17	0	17		0.002
1999/10	11.026	17	0	17	D1999/10	11.011	19	0	19		0.015
1999/11	7.701	16	1	15	D1999/11	7.648	21	1	20		0.052
1999/12	10.987	17	2	15	D1999/12	10.999	16	1	15		-0.013
2000/1	11.313	16	0	16	D2000/1	11.248	22	1	21		0.065
2000/2	11.232	17	0	17	D2000/2	11.240	19	3	16		-0.008
2000/3	*0.679*	*10*	*0*	*10*	*D2000/3*	*0.526*	*12*	*1*	*11*	*	*0.153*
2000/4	11.223	18	0	18	D2000/4	11.225	19	1	18		-0.002
2000/5	7.439	19	1	18	D2000/5	7.423	21	0	21		0.016
2000/6	11.181	19	0	19	D2000/6	11.175	23	2	21		0.006
2000/7	10.858	16	0	16	D2000/7	10.845	21	5	16		0.013
2000/9	12.031	17	1	16	D2000/9	11.983	22	1	21		0.049
2000/10	11.374	18	0	18	D2000/10	11.356	22	1	21		0.018
2000/11	7.644	18	0	18	D2000/11	7.601	27	0	27		0.043
2000/12	11.314	19	1	18	D2000/12	11.322	25	1	24		-0.008
2001/1	11.415	19	0	19	D2001/1	11.427	29	0	29		-0.012
2001/2	11.347	19	0	19	D2001/2	11.320	29	1	28		0.027
2001/3	11.818	16	0	16	D2001/3	11.826	34	1	33		-0.008
2001/4	11.331	17	0	17	D2001/4	11.339	31	2	29		-0.008
2001/5	8.063	19	1	18	D2001/5	8.058	28	0	28		0.004
					Average difference/ ¤ TAV (HB-ED)						0.014
					standard deviation on difference						0.036
				*	round 2000/3 is not taken into account (very low grade)						

Table 4: Fidelity parameters

MEAN	Hydrostatic balance	Electronic densimeter

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n1	441	557
Weighted repeatability variance	0.309	0.267
r	0.074	0.061
sr	0.026	0.022
Weighted reproducibility variance	2.948	2.150
R	0.229	0.174
sR	0.082	0.062

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